UNIVERSITY OF
LIVERPOOL

New and Established Drug Targets for Malaria Chemotherapy from Lead Optimisation of 2-Pyridyl Quinolone *Pf*NDH2 Inhibitors to Semi-synthetic Pyrrole Mannich Base Artemisinin Derivatives

Thesis submitted in the accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by

Suet Ching Leung

Jun 2012

Declaration

This thesis is the result of my own work. The material contained in the thesis has not been presented, nor is currently being presented, either wholly or in part for any other degree or other qualification.

Suet Ching Leung

This research was carried out in the Department of Chemistry at The University of Liverpool.

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Publications

1. **Suet C. Leung**, Peter Gibbons, Richard Amewu, Gemma L. Nixon, Chandrakala Pidathala, W. David Hong, Bénédicte Pacorel, Neil Berry, Raman Sharma, Paul A. Stocks, Abhishek Srivastava, Alison E. Shone, Sitthivut Charoensutthivarakul, Lee Taylor, Olivier Berger, Alison Mbekeani, Alasdair Hill, Nicholas E. Fisher, Ashley J. Warman, Giancarlo A. Biagini, Stephen A. Ward and Paul M. O'Neill Identification, Design and Biological Evaluation of Heterocyclic Quinolones Targeting *Plasmodium falciparum* Type II NADH: Quinone Oxidoreductase (*Pf*NDH2), *Journal of Medicinal Chemistry*, **2012**, *55* (5), 1844-1857.

2. Chandrakala Pidathala, Richard Amewu, Bénédicte Pacorel, Gemma L. Nixon, Peter Gibbons, W. David Hong, **Suet C. Leung**, Neil G. Berry, Raman Sharma, Paul A. Stocks, Abhishek Srivastava, Alison E. Shone, Sitthivut Charoensutthivarakul, Lee Taylor, Olivier Berger, Alison Mbekeani, Alasdair Hill, Nicholas E. Fisher, Ashley J. Warman, Giancarlo A. Biagini, Stephen A. Ward and Paul M. O'Neill Identification, Design and Biological Evaluation of Bisaryl Quinolones Targeting *Plasmodium falciparum* Type II NADH: Quinone Oxidoreductase (*Pf*NDH2), *Journal of Medicinal Chemistry*, **2012**, *55* (5), 1831-1843.

3. Giancarlo A. Biagini, Nicholas Fisher, Alison E. Shone, Murad A. Mubaraki, Abhishek Srivastava, Alasdair Hill, Thomas Antoine, Ashley J. Warman, Jill Davies, Chandrakala Pidathala, Richard K. Amewu, **Suet C. Leung**, Raman Sharma, Peter Gibbons, David W. Hong, Bénédicte Pacorel, Alexandre S. Lawrenson, Sitthivut Charoensutthivarakul, Lee Taylor, Olivier Berger, Alison Mbekeani, Paul A. Stocks, Gemma L. Nixon, James Chadwick, Janet Hemingway, Michael J. Delves, Robert E. Sinden, Anne-Marie Zeeman, Clemens H.M. Kocken, Neil G. Berry, Paul M. O'Neill and Stephen A. Ward Generation of quinolone antimalarials targeting the *Plasmodium falciparum* mitochondrial respiratory chain for the treatment and prophylaxis of malaria, *PNAS*, **2012**, *109* (21), 8298-8303.

4. Robin Cowley, **Suet Leung**, Nicholas Fisher, Mohammed Al-Helal, Neil G. Berry, Alexandre S. Lawrenson, Raman Sharma, Alison E. Shone, Stephen A. Ward, Giancarlo A. Biagini and Paul M. O'Neill The development of quinolone esters as novel antimalarial agents targeting the *Plasmodium falciparum* bc_1 protein complex, *MedChemComm*, **2012**, *3*, 39-44.

5. Bénédicte Pacorel, **Suet C. Leung**, Andrew V. Stachulski, Jill Davies, Livia Vivas, Hollie Lander, Stephen A. Ward' Marcel Kaiser, Reto Brun and Paul M. O' Neill Modular Synthesis and In Vitro and In Vivo Antimalarial Assessment of C-10 Pyrrole Mannich Base Derivatives of Artemisinin, *Journal of Medicinal Chemistry*, **2010**, *53* (2), 633-640.

Abstract

The rapid development of resistance to currently deployed antimalarial drugs has raised the desperate need for new chemotherapies, preferably with novel therapeutic target. This thesis explores the synthesis of novel quinolone compounds and artemisinin derivatives targeting the mitochondrial electron transfer chain (ETC) and the haemoglobin degradation of *Plasmodium falciparum* respectively.

The respiratory chain of the human malaria is an attractive target for antimalarial drugs. It is believed that the collapse of the mitochondrial potential will shut down the metabolism and malaria parasite *de novo* synthesis of pyrimidines, ultimately leading to the death of parasite. The bc_1 (Complex III) inhibitors are being studied by many scientists, with recent studies targeting *Pf*NDH2 due to its potential as a therapeutic target (Humans lack this enzyme in the respiratory chain).

Following the hit to lead optimisation of chemical name here CK-2-68 against the *Pf*NDH2 enzyme in the group, a series of quinolones were designed to improve the ClogP and aqueous solubility. Analogues in this series were synthesised in less than six-step. Our strategy for reducing the ClogP of the original series involves the incorporation of heterocycles into the C and D rings of the side chain. Work describes in this thesis was principally cover the 2-pyridyl series of compounds. Two of these analogues have IC_{50} values in the nanomolar range versus *Pf*NDH2 enzyme and 3D7 strain of *Plasmodium falciparum*. Further *in vivo* studies showed that these two analogues have notable ED_{50}/ED_{90} against *Plasmodium berghei* (NS Strain) following oral administration.

A series of 6-substituted quinolone esters and pyrrolidine-fused quinolones were also prepared for targeting bc_1 complex. They were tested *in vitro* to explore their structure-activity relationship (SAR). The 6-substituted quinolone ester were synthesised in four steps employing the Gould-Jacobs method. It was noticed that the 3-ester functionality and its steric size are essential for good activity. The 6-substituted quinolone esters possess moderate antimalarial activity with the lead analogue IC_{50} of 40.4 nM. The 6-substituted quinolone esters were compared head to head with the 7-series of analogues. In an attempt to enhance solubilities, pyrrolidine-fused quinolones were synthesised using Winterfeldt oxidation. Although the compounds were poorly soluble, the potent *in vitro* result of one of the analogues underlines the potential of the template for further study.

Artemisinin and its semi-synthetic derivatives are the most effective drugs in malarial chemotherapy. Despite of their high therapeutic indices, they have poor bioavailability and short half-lives in general. To improve the aqueous solubility and metabolic stability, a series of semi-synthetic C-10 pyrrole Mannich artemisinin derivatives were prepared in 2 steps from dihydroartemisinin. These analogues have demonstrated nanomolar antimalarial activity against the 3D7 strain and K1 strain of *Plasmodium falciparum in vitro* with high therapeutic indices. Further *in vivo* studies showed that three of the analogues have excellent ED_{50}/ED_{90} indicating their overall *in vitro* and *in vivo* drug profiles are superior to those of clinically used artemether and artesunate.

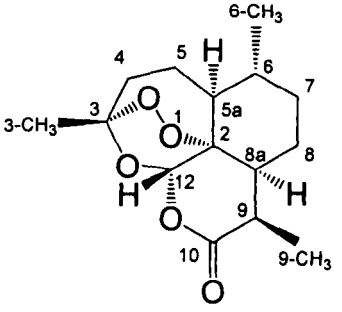
Definitions and Abbreviations

AcOH	-	Acetic acid
AIBN	-	2,2'-Azobis-(isobutyro) nitrile
Anal.	-	Analysis
approx.	-	Approximately
bc_1	-	Complex III of mitochondrial electron transfer chain
cat.	-	Catalytic
CI	-	Chemical ionisation
ClogP	-	Calculated partition coefficient, an indicator of hydrophobicity
Cytb	-	Cytochrome b
DCM	-	Dichloromethane
DDT	-	Dichlorodiphenyltrichloroethane
DHA	-	Dihydroartemisinin
DMAP	-	4-(Dimethylamino)pyridine
DMSO	-	Dimethylsulfoxide
ED_{50}	-	Dose of drug which produces 50% of its maximum response or effect
ED_{90}	-	Dose of drug which produces 90% of its maximum response or effect
Equiv./eq.	-	Molar equivalent
ESI	-	Electrospray ionization
ETC	-	Electron transport chain
EtOAc	-	Ethyl acetate
EtOH	-	Ethanol
g	-	gram(s)
h	-	hour(s)
HRMS	-	High resolution mass spectrometry

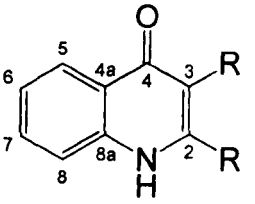
IC_{50}	-	Concentration of a drug that is required for 50% inhibition in vitro
m	-	*meta*
M^+/M^-	-	Molecular ion
MeOH	-	Methanol
min	-	Minute(s)
mL	-	Milliltre(s)
mol	-	mole(s)
mp	-	Melting point
MS	-	Mass spectrometry
MSD	-	Mean survival time in days
n-BuOH	-	n-Butanol
nM	-	nanomolar
NMO	-	*N*-methylmorpholine *N*-oxide
o	-	*ortho*
p	-	*para*
PCC	-	Pyridinium chlorochromate
Pd/C	-	Palladium on carbon
$PdCl_2(dppf)$	-	[1,1' bis(diphenylphosphino)ferrocene]-dichloropalladium(II)
$Pd(PPh_3)_4$	-	tetrakis(triphenylphosphine)palladium(0)
*Pf*NDH2	-	Type II Complex I of mitochondrial electron transfer chain in *Plasmodium falciparum*
PTSA	-	*p*-Toluenesulfonic acid
TFA	-	Trifluoroacetic acid
THF	-	Tetrahydrofuran
TI	-	Therapetic Index
tlc	-	Thin Layer Chromatography

TPAP	-	Tetrapropylammonium perruthenate
R_f	-	Retention factor
r.t.	-	room temperature
WHO	-	World Health Organisation
^{13}C NMR	-	Carbon-13 Nuclear Magnetic Resonance
^{1}H NMR	-	Proton Nuclear Magnetic Resonance
α	-	Alpha
β	-	Beta
γ	-	Gamma
δ	-	Chemical Shift (parts per million)
3D7	-	Chloroquine sensitive strain (whole cell parasite) of *Plasmodium falciparum*

Numbering schemes used throughout this thesis:



Artemisinin



Quinolone

Content

Chapter 1

General Introduction

Chapter 1

1.1 About Malaria

The first symptoms of malaria are flu-like and include fever, headache, chills and vomiting. However, unlike flu, malaria is a serious and sometimes fatal mosquito-borne infectious disease to man. If the infection is not treated promptly, severe complications can develop by destroying the red blood cells and disrupting the blood supply to vital organs, which leads to serious organ failures and death.

The World Health Organisation (WHO) estimates that in 2009, malaria contributed to 225 million cases and nearly 1 million deaths worldwide.[1] Over 80% of the malaria deaths occur in Africa, mostly among children and pregnant women. Approximate half of the global population live in the 109 malaria-endemic countries and territories at risks of infection.[1-2] The highest transmission is found in tropical and subtropical areas (Figure 1), thus making malaria one of the leading causes of death in Africa, together with HIV/AIDS and tuberculosis among all infectious diseases.

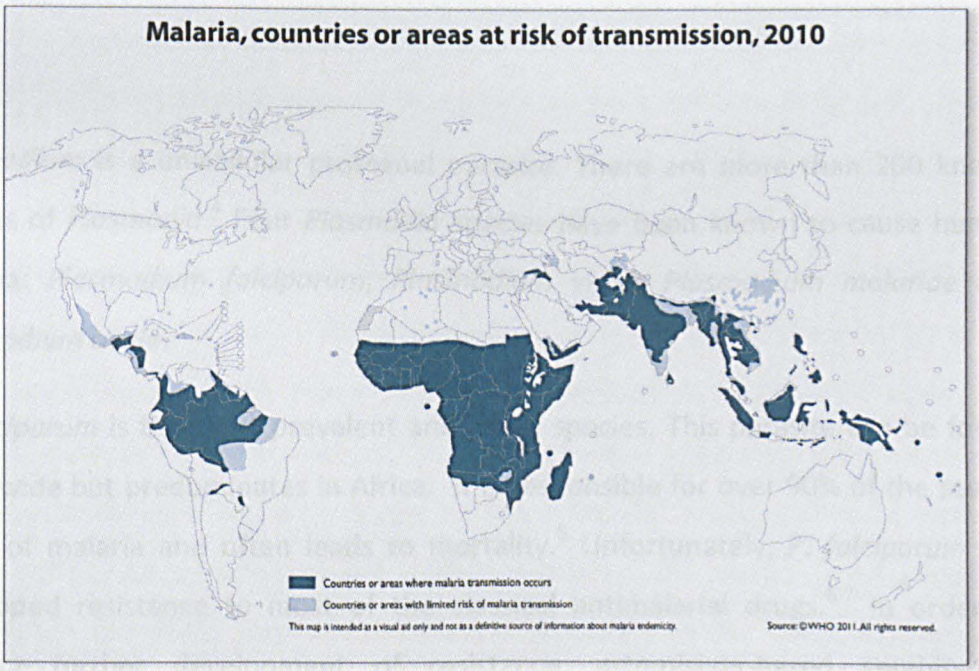


Figure 1. Malaria, countries or areas at risk of transmission, 2010.[1]

1.2 The *Plasmodium* parasites and *Anopheles* mosquitoes

Malaria is caused by the parasite of the genus *Plasmodium* and is transmitted to people through the bites of infected female mosquitoes of the genus *Anopheles* (Figure 2).

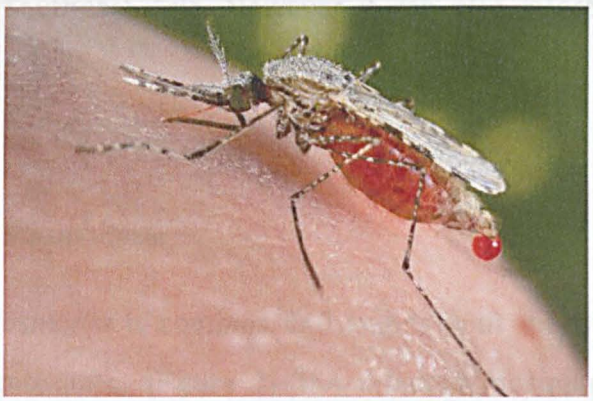


Figure 2. A Female *Anopheles* mosquito.[3]

Plasmodium is a unicellular protozoal parasite. There are more than 200 known species of *Plasmodia*.[4] Four *Plasmodia* species have been known to cause human malaria: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*.

P. falciparum is the most prevalent and lethal species. This parasite can be found worldwide but predominates in Africa. It is responsible for over 90% of the severe cases of malaria and often leads to mortality.[5] Unfortunately, *P. falciparum* has developed resistance to most of the classical antimalarial drugs.[6-7] In order to prevent further development of resistance, artemisinin-based combination therapies (ACTs) are currently used as the first-line treatment of *P. falciparum* malaria.[8]

P.vivax is the second most common species which is mainly found in Asia, Latin America and some parts of Africa.[9-10] However, it is not as virulent as *P. falciparum* even though it has the greatest geographical distribution.

Malaria caused by *P. malariae* and *P. ovale* is generally less complicated and milder. However, chronic infection with *P. malariae* can lead to nephrotic syndrome which can be fatal.[11] Recently, an increasing number of human infections with *Plasmodium knowlesi* have been reported.[12-13] *P. Knowlesi* is a monkey malaria which is found in Southeast Asia. However it can also infect humans, causing zoonotic malaria.[14]

1.3 The life cycle of *Plasmodium*

The life cycle of *Plasmodia* is complex and begins with the bite of an infected female *Anopheles* mosquito. (Figure 3) The infected mosquito first injects the sporozoites from the salivary glands into the human host during the blood meal. The sporozites travel to the liver through the bloodstream within 2 hours and invade the hepatocytes (liver cells). Once in the liver cell, the sporozites undergo asexual multiplication into tens of thousands of merozoites in 6-14 days. This is dependent on the parasite species. The merozoites eventually break from their host hepatocytes and infect other red blood cells (erythrocytes) where they utilise the host hemoglobin as a food source and undergo further multiplications and develop into a schizont in 48-72 hours. The released merozoites go on to further infect more erythrocytes. Some of the merozoites at this stage differentiate into male and female gametocytes. After another female *Anopheles* mosquito takes up these gametocytes during a blood meal, the gametocytes will begin a different cycle of growth and sexual reproduction in the digestive tract of the mosquito. After around 14 days, the sporozoite forms of the parasite migrate into the mosquito's salivary gland and are ready for another human infection.

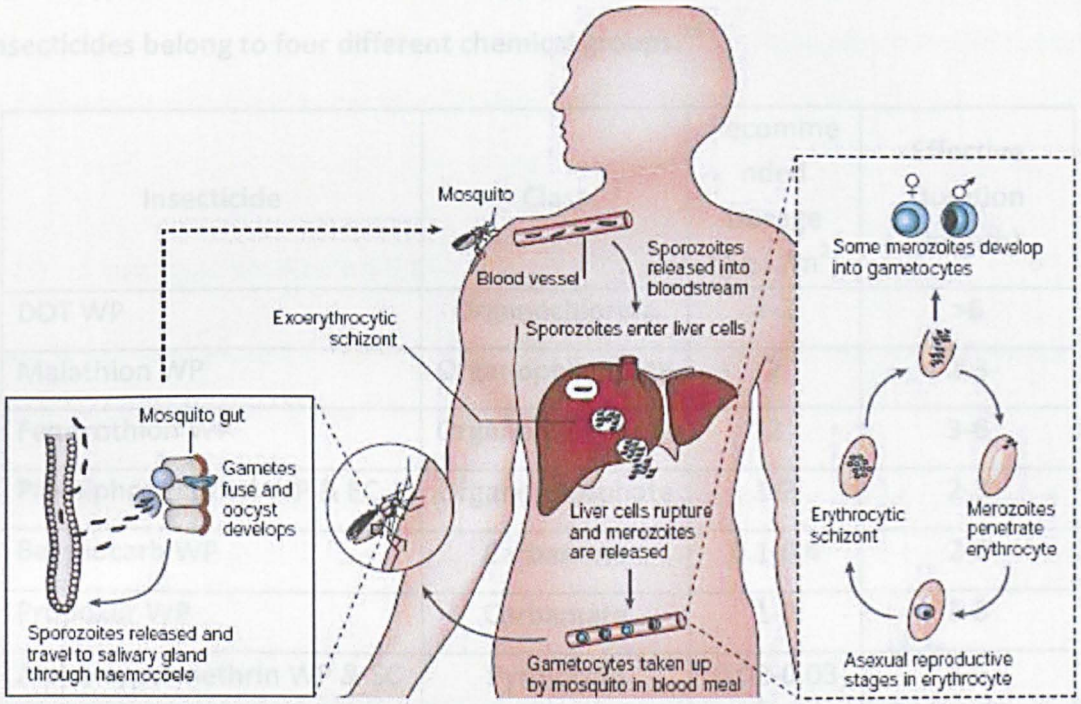


Figure 3. The life cycle of *Plasmodium falciparum*.[9]

1.4 Vector control of Malaria

As mentioned above, almost half of the world population live in the risk area. In order to prevent the transmission, there are a number of vector control approaches involving the use of DDT and pyrethroids in endemic areas. This is described in detail in the next section.

1.4.1 Indoor residual spraying (IRS)

Indoor residual spraying is a widely used method towards the control of the adult mosquitoes entering houses and dwellings. IRS is the spraying of chemical insecticides on the inside surfaces of the houses and animal shelters to kill the adult mosquitoes which land or rest on these surfaces.[15] This method can shorten the life

span of mosquitoes and reduce their density, therefore to reduce the transmission of malaria.

Currently, there are 12 insecticides approved by WHO for IRS (Table 1). These insecticides belong to four different chemical groups.[16]

Insecticide	Class	Recommended Dosage (g a.i./m^2)	Effective Duration (month)
DDT WP	Organochlorine	1-2	>6
Malathion WP	Organophosphate	2	2-3
Fenitrothion WP	Organophosphate	2	3-6
Pirimiphos-methyl WP & EC	Organophosphate	1-2	2-3
Bendiocarb WP	Carbamate	0.1-0.4	2-6
Propoxur WP	Carbamate	1-2	3-6
Alpha-cypermethrin WP & SC	Pyrethroid	0.02-0.03	4-6
Bifenthrin WP	Pyrethroid	0.025-0.05	3-6
Cyfluthrin WP	Pyrethroid	0.02-0.05	3-6
Deltamethrin WP, WG	Pyrethroid	0.02-0.025	3-6
Etofenprox WP	Pyrethroid	0.1-0.3	3-6
Lambad-cyhalothrin WP, CS	Pyrethroid	0.02-0.03	3-6

Table 1. The list of insecticides approved by WHO.[16]

Among the 12 insecticides, DDT (**D**ichloro**d**iphenyl**t**richloroethane) has been considered the most cost effective. It was used extensively after the WHO commenced a program to attempt to eradicate malaria worldwide in 1955.[15, 17] DDT succeeded in the elimination of malaria in the Europe, Taiwan and the United States. However, it has demonstrated both acute and chronic toxicity[18-21], can cause damage to the environment and accumulate in the food chain.[22-23] Research

suggested that DDT may cause diabetes[24-25] and cancers[21, 26-27], and this resulted in a ban in the majority of areas where this insecticide was used.

Due to the appearance of the resistance to pyrethroids and organophosphates in some Anopheles species in South Africa[28-29], DDT is reintroduced with strict conditions to restore the control of malaria.[15, 30-31]

1.4.2 Insecticide-treated materials

Insecticide-treated nets (ITNs) and other materials (e.g. bedclothes) are widely used as personal protection from the malarial infection. [32-34]

ITNs were developed 3 decades ago for malaria prevention and they are one of the effective interventions. A review concluded that ITNs reduce the number of malaria infections by 50%.[35-36]

Insecticides are used to treat bednets and other clothings, the nets can act as a physical barrier to the vector mosquitoes, and the insecticides can also kill the mosquitoes that come into contact with the net, therefore the vector population can be reduced and so the risk of malaria. However, these insecticide-treated nets have to be re-treated every 6-12 months generally to maintain their effectiveness. Therefore, long-lasting insecticide-treated nets (LLINs) were developed recently.[37] LLTNs can last for 3 years without re-treatment, thus providing long-lasting and effective protection.

1.4.3 Other methods

Apart from the IRS and ITN, there are other vector control methods. Larval control is an intervention to reduce the vector populations by preventing breeding.[38] Source reduction is another method which destroys the mosquito breeding sites. There are also other potential biological control interventions, for example, by using fungi[39-40] and bacteria[41] to kill the mosquitoes. Scientists are also experimenting with the newer approaches of modifying the genes of the malaria

vectors[42] to develop mosquitoes that are refractory to the parasite and introducing the sterile male mosquitoes[43-44] into the environment.

Despite the availability of vector control measurements, the eradication of malaria continues to be a great challenge, especially in some poor nations. Apart from the cultural and economical issues, environmental conditions and improper use of insecticides contribute to the emergence of resistant mosquitoes, making vector elimination extremely challenging. [45]

1.5 Vaccines

Vaccines are effective tools against infectious diseases. They have contributed to the eradication of smallpox, a lethal infectious disease unique to humans. They also have reduced other diseases, such as rubella, mumps and typhoid. Although there is a great potential for vaccines against malaria, there is still currently no effective malaria vaccine on the market. The development of malaria vaccines has been progressed in the last decade through different approaches, there are still several obstacles for scientists to overcome.[46-47] The key obstacles include the antigenic diversity of the parasite, the lack of predictive animal models and the lack of developers and effective markets etc.[47-48] Due to the complex process, only one vaccine candidate, RTS, S/AS01, developed by GlaxoSmithKline (GSK)[48] with close collaboration of Walter Reed Army Institute of Research, is progressing through a Phase 3 clinical trial[49]. RTS, S/AS01 has demonstrated good protections among the vaccinees in both Phase I and Phase II trials, thus it could become the very first licensed malaria vaccine. Since RTS, S/AS01 vaccine is a circumsporozoite protein (CSP) from *P. falciparum* that fused to the hepatitis B surface antigen, it will be a joint vaccine that can fight against malaria and hepatitis B. However, it is still years away as the trial data will only be available for decision making in 2015. Although over 30 other malaria vaccine projects have reached the clinical evaluation stage, they are at least 5 years behind RTS, S/AS01 in their development.[48]

1.6 Antimalarial agents

Malaria can be fatal especially when caused by *P. falciparum* since hypoglycaemia, hemoglobinuria, acute kidney failure and cerebral malaria can develop.[50] Thus prompt treatment should be started once the infection is diagnosed to avoid its progression to severe condition.

Artemisinin (qinghaosu, from *Artemisia annua*) is an herbal medicine used traditionally in the treatment of malaria fever for more than a thousand years in China even though the active ingredient was only isolated in 1971 (Figure 4).[51] Also Quinine (from *cinchona* bark) has been used to treat malaria for over 300 years.[52] Additionally, to date, a number of chemical classes of antimalarial drugs with different parasitic targets are developed and available on the market for both chemoprophylaxis and treatment.[53-54] (Figure 5 and Table 2)

(a) (b)

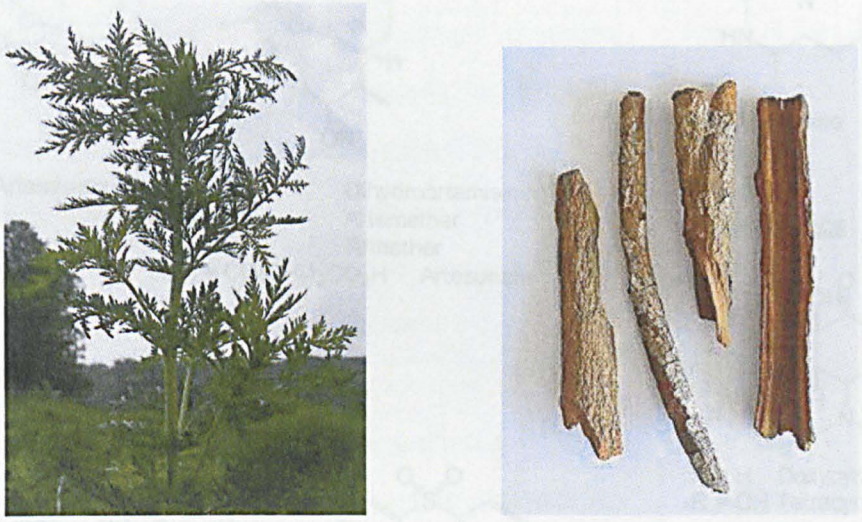
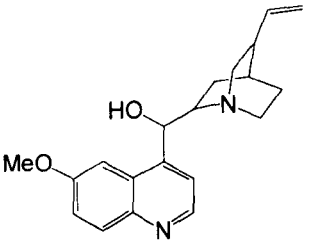
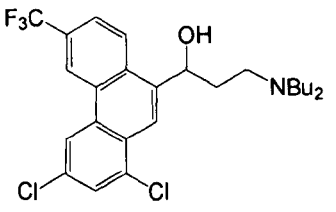


Figure 4. (a) *Artemisia annua*. (b) *Cinchona* bark. (Pictures from wikipedia.org)[55-56]

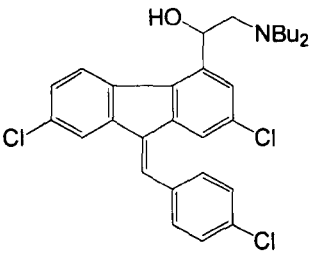
Arylaminoalcohols and 4-Quinolines



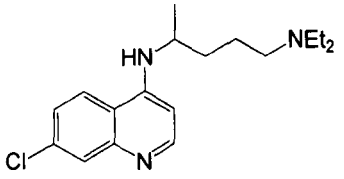
Quinine



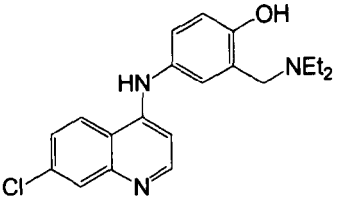
Halofantrine



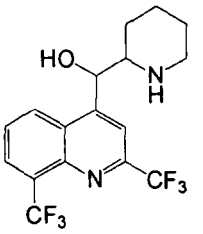
Lumefantrine



Chloroquine

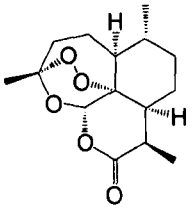


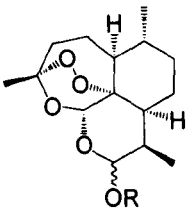
Amodiaquine



Mefloquine

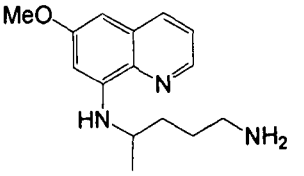
Artemisinin and its derivatives





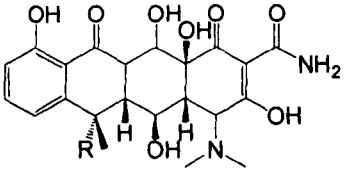
Artemisinin R = OH Dihydroartemisinin
R(β) = Me Artemether
R(β) = Et Arteether
R(α) = $CO(CH_2)_2CO_2H$ Artesunate

8-Aminoquinolone



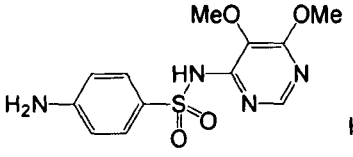
Primaquine

Antibiotics

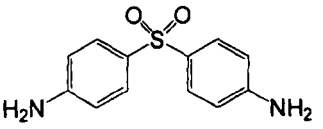


R = H Doxycycline
R = OH Tetracycline

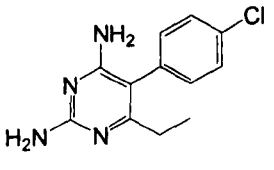
Antifolates



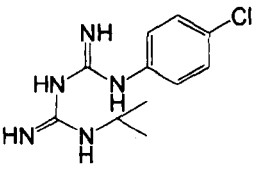
Sulfadoxine



Dapsone

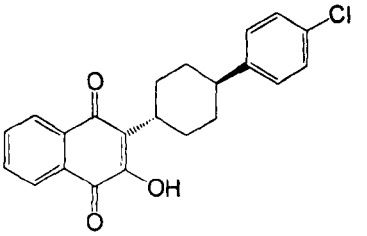


Pyrimethamine



Proguanil

bc$_1$ inhibitor



Atovaquone

Figure 5. The structures of some existing antimalarial drugs.

Table 2. A list of some common antimalarial drugs and their side effects. [57-61]

Class	Medication	Usage	Side effect(s)
Quinolines	Chloroquine (CQ)	Treatment of non-*falciparum* infection and chemoprophylaxis in regions where CQ remains effective	Headache, various skin eruptions and gastrointestinal disturbances
	Mefloquine (MQ) (4-methanolquinoline)		Similar to chloroquine
	Amodiaquine (AQ)	Treatment of non-severe *falciparum* infection	Gastrointestinal disturbances, headache, dizziness, insomnia
Arylaminoalcohols	Quinine	Treatment of severe malaria, multidrug-resistant *P. falciparum* and malaria during 1st trimester of pregnancy	Tinnitus, headache, nausea, dizziness and dysphoria
	Halofantrine	Treatment of suspected multidrug-resistant *falciparum*	Risk of fatal cardiotoxicity
	Lumefantrine	Therapy	Nausea, abdominal discomfort, headache and dizziness
8-aminoquinoline	Primaquine	Treatment of *P. vivax* infection	Abdominal pain

The Peroxides	Artemisinin	Therapy of multidrug-resistant *P. falciparum* malaria	Mild gastrointestinal disturbances, tinnitus, neutropenia etc. *Blood schizontocide
	Artemether		
	Arteether		
	Artesunate		
Antifolates	Proguanil (Biguanide)	Prophylaxis	Mild gastric intolerance, hair loss
	Pyrimethamine (Diaminopyrimidine)	Therapy and prophylaxis, with sulfonamides	Skin rushes, abdominal pain. *Inhibitor of plasmodial dihydrofolate reductase, blood schizontocide
	Sulfadoxine		Nausea, anorexia and diarrhoea. *Competitive inhibitors of dihydropteroate synthase
Antimicrobial	(Tetracycline) Doxycyline	Treatment and prophylaxis	GI effects, anorexia, hypersensitivity reactions
Inhibitor of respiratory chain	Atovaquone		Skin rashes, headache, insomnia, nausea. *Interferes with cytochrome electron transport

1.6.1 Antimalarial targets

It is believed that the arylamino alcohols and 4-aminoquinolines act mostly during the blood stages of the parasite's life cycle. Within malaria parasite, host hemoglobin is broken down by a number of protease enzymes to give peptides and amino acids for parasite growth.[62-63] During the parasite's digestion of host hemoglobin in the food vacuole, ferric heme (ferriprotoporphyrine IX or Fe(II)PP IX) is also generated which is a toxic to the parasite. However, the parasite is able to detoxify heme by dimerisation to form insoluble non-toxic hemazoin. It has been suggested that the quinolines binds to ferric heme therefore preventing its dimerisation into non-toxic hemazoin by the parasite, resulting the accumulation of heme and subsequently the cytotoxicity to the parasite. [11, 64](Figure 6)

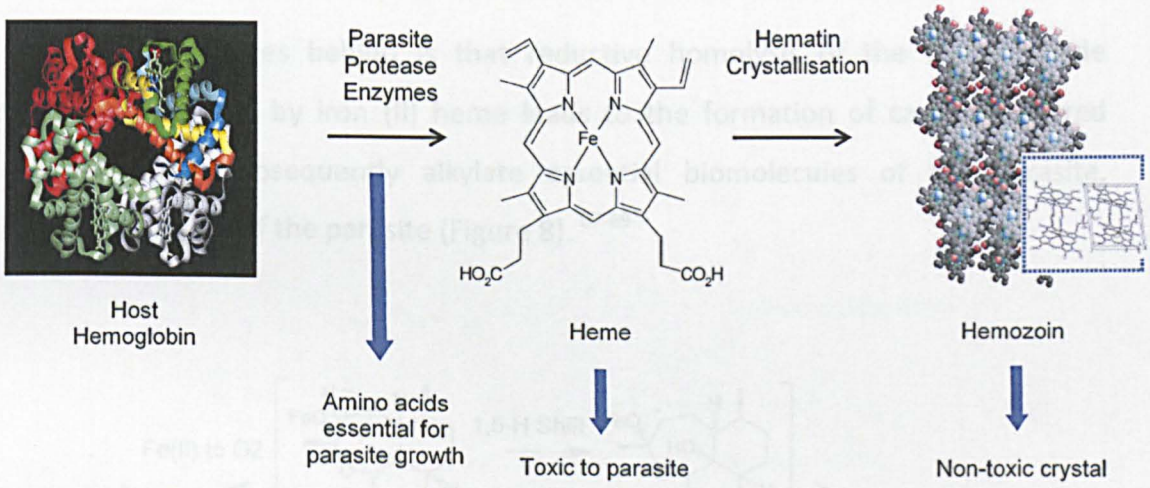


Figure 6. The degradation of hemoglobin and detoxification of heme by malaria parasite.[62]

The mechanism of action of primaquine and other 8-aminoquinolines is still unclear.[11] However, primaquine has demonstrated activity against the liver and the sexual blood stages of different plasmodia. [65-66]

The debate of the mechanism of action of the artemisinins and related peroxides is still continuing.[62] It has been demonstrated the endoperoxide bridge is essential for the activity (Figure 7).

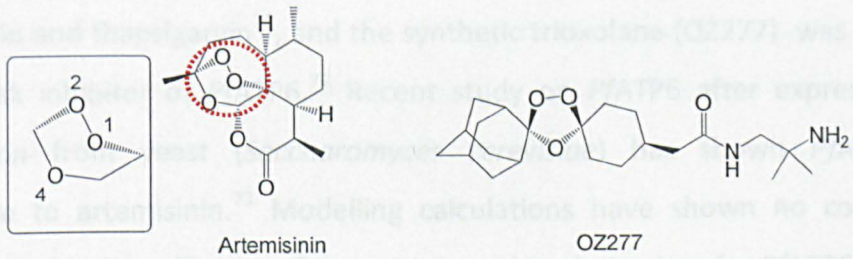


Figure 7. The 1,2,4-trioxane pharmacophore of Artemisinin and the synthetic trioloxane OZ277.

One of the theories behind is that reductive homolysis of the endoperoxide function promoted by iron (II) heme leads to the formation of carbon-centered radicals which subsequently alkylate essential biomolecules of the parasite, resulting in death of the parasite (Figure 8). [67-69]

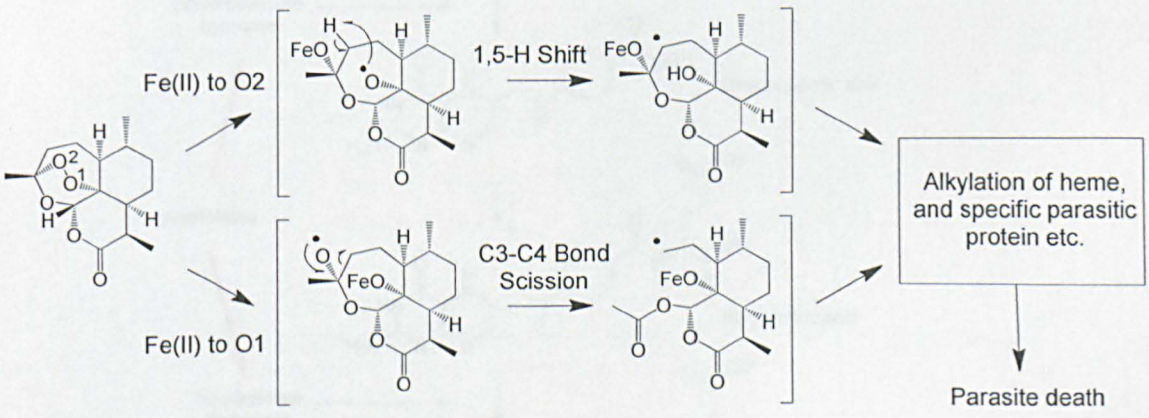


Figure 8. Homolytic bioactivation of the endoperoxide bridge.

Another theory of their antimalarial activity is due to the specific inhibition of *Pf*ATP6[70], which is an enzyme located on the endoplasmic reticulum and orthologous to mammalian Ca^{2+} transporting ATP-ases (SERCA), in similar way to thapsigargin (a highly selective inhibitor of SERCA). However, later research studies contradicted this suggestion indicated that there was no antagonism between artemisinin and thapsigargin[71], and the synthetic trioxolane (OZ277) was shown to be a weak inhibitor of *Pf*ATP6.[72] Recent study on *Pf*ATP6 after expression and purification from yeast (*Saccharomyces cerevisiae*) has shown *Pf*ATP6 was insensitive to artemisinin.[73] Modelling calculations have shown no correlations between the binding affinities of artemisinin and its derivatives for *Pf*ATP6 and their *in vitro* antimalarial activity.[74-75]

Tetrahydrofolic acid is essential for the biosynthesis of nucleobases and some amino acids.[11, 76] While humans depend on intake of pre-formed dihydrofolic acid through the diet and convert it to tetrahydrofolic acid, the parasite can synthesise dihydrofolic acid (Figure 9).[77-78]

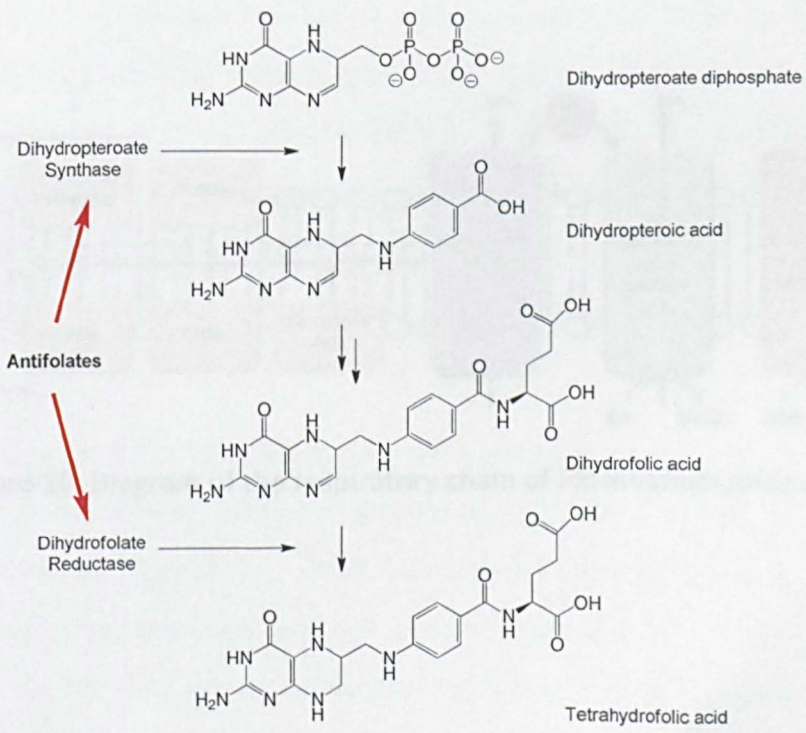


Figure 9. The simplified folate pathway and the targets of antifolates.

Therefore, antifolates target the two enzymes of the biosynthetic pathway of tetrahydrofolate, the dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR).[11, 79]

Also, several antibiotics effect as antimalarials by inhibiting the protein synthesis and/or the apicoplast. However, due to the slow actions of the antibiotics, they are used as a combination with other antimalarials. [11, 80]

Atovaquone, which is the only inhibitor of the respiratory chain of the parasite on the market currently (Figure 10)[81-82], binds to the Q_o site of the cytochrome bc_1 complex of the mitochondrial electron transport chain and results in the collapse of the mitochondrial membrane potential, the respiratory processes and ultimately the death of the parasite. This mitochondrial electron transport chain is the main target being looked at in this thesis, therefore more information on the respiratory chain will be explained in detail in later chapters.

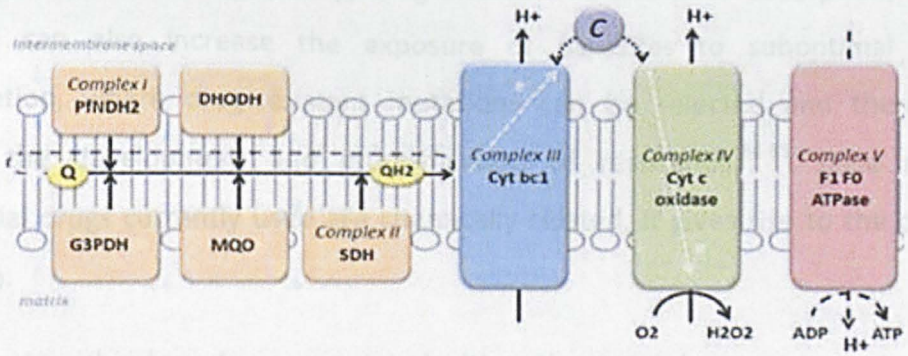


Figure 10. Diagram of the respiratory chain of *Plasmodium falciparum*.[83]

1.7 Drug resistance

Despite the numerous antimalarial drugs available in the market, drug resistance has widely and rapidly spread over the world in the last few decades. According to the WHO, antimalarial drug resistance is "the ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in dose equal to or higher than those usually recommended, but within the limits of tolerance of the subject, provided drug exposure at the site of action is adequate."[53]

Resistance to antimalarial drugs can be caused by genetic mutations or gene amplifications in malaria parasites that confer reduced sensitivity to one or a class of structurally related drugs.[57] The emergence and spread of the resistance in *Plasmodium* is contributed by various factors in different aspects including drug pressure, human behaviour, the strain of parasite and economics.[6, 10, 57]

The pharmacokinetics of drug is one of the key aspects in the development of resistance. Long terminal elimination half-life of a drug may lead to the prolonged low plasma concentration. Mismatched pharmacokinetics in combination therapy can lead to the "unprotected" period of a drug in synergy.[57] Other factors such as incorrect dosing, poor drug quality, drug interactions and poor absorption, in an individual can also increase the exposure of parasites to suboptimal drug concentration, where drug-resistant mutations can be selected and therefore enhance the development and intensification of resistance.[84-85] Since many antimalarial drugs currently used are chemically related, it gives rise to the cross-resistance.

Parasite factors that have been associated with antimalarial drug resistance include the intrinsic frequency with which the genetic changes occur and the fitness cost of the resistance mechanism.[53, 86] Human factors include misdiagnosis, improper use of antimalarial drugs and probably the immune response of the host.[7] The low effectiveness of the immune system in clearing parasite residuum after treatment could increase the survival of the parasites and facilitate the resistance development and its spread.

Antimalarial drug	Introduced	First report of resistance	Difference/effectiveness (years)
Quinine	1632	1910	278
Chloroquine	1945	1957	12
Proguanil	1948	1949	1
Sulfadoxine-pyrimethamine	1967	1967	<1
Mefloquine	1977	1982	5
Atovaquone	1996	1996	<1

Table 3. First report of resistance to some common antimalarial drugs, data taken from Wongsrichanalai *et al.*[7]

Research has shown that resistance to antimalarial drugs has been developed in *P. falciparum*, *P. malariae* and *P. vivax*.[87-89] *P. falciparum* has even shown resistance to all currently used drugs (Table 3), including artemisinin recently[90-92], even through the geographical distribution and rates of spread to a single drug vary. (Figure 11) *P. vivax* resistance to chloroquine and primaquine have also reported.[6]

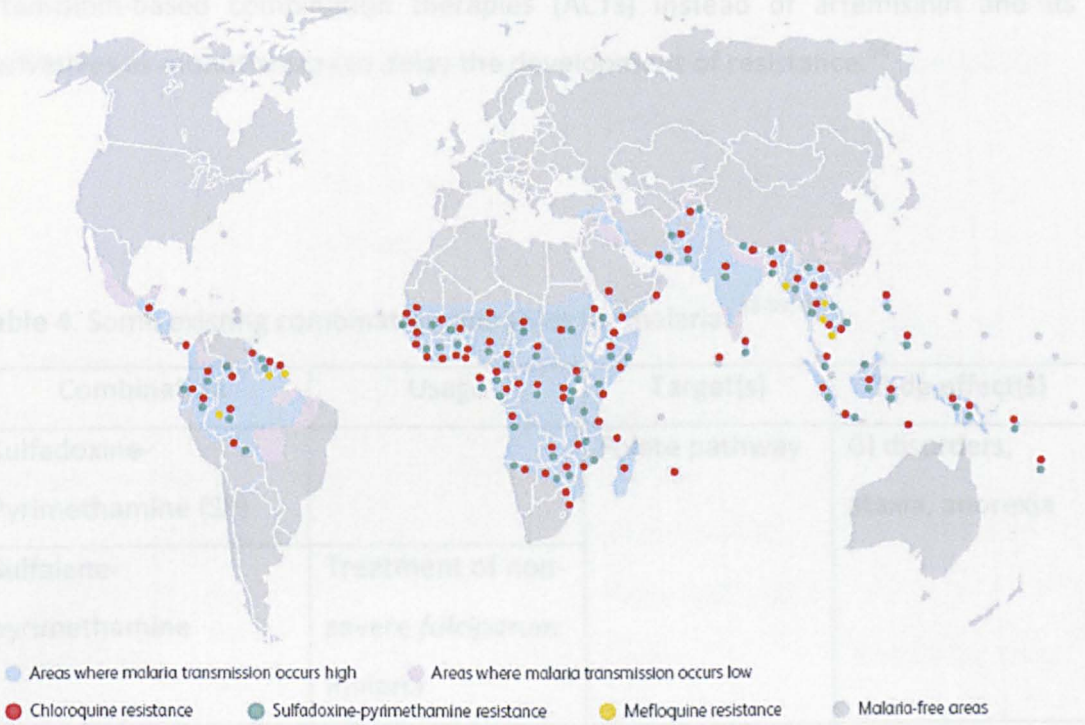


Figure 11. Drug resistance to *Plasmodium falciparum* from stuides up to 2004. (Picture for UK Collaborative on Development Sciences, 2010)[93]

1.7.1 Prevention of drug resistance by using combination therapy

There are a number of interventions to prevent the development and the spread of drug resistance – reducing overall drug pressure through more restrictive drug use, improving the way drugs are used through proper diagnosis and follow-up practice, and using combination therapy.[57]

The rationale of combination treatment is well established in tuberculosis, leprosy and HIV therapy. Combination therapy is a combination of two or more different classes of drug with different mechanisms of action.[53] It is used to exploit the synergistic effect of individual drug. It can improve the efficacy and medication compliance, shorten the treatment, and to prevent emerge of simultaneous mutation in the parasite and thus the development of resistance to individual drug in the combination. To avoid the threat of resistance of *P. falciparum* to monotherapies, and to improve cure rates, WHO recommends the use of

artemisinin-based combination therapies (ACTs) instead of artemisinin and its derivatives as monotherapy to delay the development of resistance.[86]

Table 4. Some existing combination therapies for malaria. [58-59, 94]

Combination	Usage	Target(s)	Side effect(s)
Sulfadoxine-Pyrimethamine (SP)		Folate pathway	GI disorders, ataxia, anorexia
Sulfalene-pyrimethamine	Treatment of non-severe *falciparum* malaria		
Atovaquone-proguanil (Malarone™)	Treatment of multidrug resistant *falciparum* malaria and prophylaxis	Cytochrome bc_1 and folate pathway	Headache, abdominal effects, anorexia, coughing
Chloroquine + sulfadoxine-pyrimethamine	Treatment of *vivax* malaria and non-severe *falciparum* malaria	Folate pathway and heme detoxification	As for CQ and SP
Chloroquine + sulfalene-pyrimethamine			
Quinine + sulfadoxine-pyrimethamine			As for quinine and SP
Quinine + doxcycline	Treatment of *vivax* malaria and non-severe *falciparum* malaria	Aminoacyl-tRNA binding and heme detoxification	As for quinine and doxycycline

Artesunate + mefloquine	Treatment of multidrug resistant *falciparum* malaria	Heme alkylation, protein alkylation and Heme detoxification	As for artesunate and mefloquine
Artesunate + sulfadoxine-pyrimethamine		Heme alkylation, protein alkylation and folate pathway	
Artemether-lumefantrine (Coartem™, Riamel™)	Treatment of non-severe *falciparum* malaria thought to be CQ and SP resistant	Heme alkylation, protein alkylation and heme detoxification	Headache, sleep disorders, GI disorders, asthenia

1.8 Aims of this thesis

The artemisinin-based combination therapies (ACTs) are still highly effective against malaria infections and considerable efforts to prevent the development of drug resistance have been made in the endemic regions. However, current treatments rely to a large extent on ACTs, it would be a disaster if the resistance detected on the Cambodia-Thai border to artemisinins[92] emerges and spreads. Therefore new antimalarial therapies with new targets compared to clinically used drugs are urgently needed.[95-96]

In response to the search of novel drugs for both known and new targets, the aims of the research were to synthesise novel antimalarial compounds which are structurally based on the quinolone pharmacophore targeting the *Pf*NDH2 (a new target) and cytochrome bc_1 of the parasite respectively, and to investigate their antimalarial activities. In addition, it was our aim to synthesise a series of C-10 pyrrole mannich base derivatives of artemisinin with improved activity and stability over the first generation artemisinins.

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Chapter 2

Novel quinolones targeting *Pf*NDH2 enzyme

Chapter 2

2.1 Introduction to *Pf*NDH2

Most eukaryotic cells have mitochondria, where the mitochondrial electron transport chain (ETC) is central to the adenosine triphosphate (ATP) synthesis and metabolic pathways.[1-2] The mitochondrial electron transport chain in eukaryotic cells consists of 4 enzyme complexes located in the inner mitochondrial membrane. They are NADH:ubiquinone oxidoreductase (Complex I), succinate dehydrogenase (Complex II), cytochrome bc_1 complex (Complex III) and cytochrome *c* oxidase (Complex IV).[3]

Malaria parasites have a primary ATP source through glycolysis rather than the oxidative metabolism pathway present in many eukaryotic cells. The malaria genome has revealed that although Complex II to IV are conserved in *P. falciparum's* ETC[4], it also contains an alternative complex I (*Pf*NDH2 or Type II NADH: dihydrogenase) instead of the conventional one.[5-6]

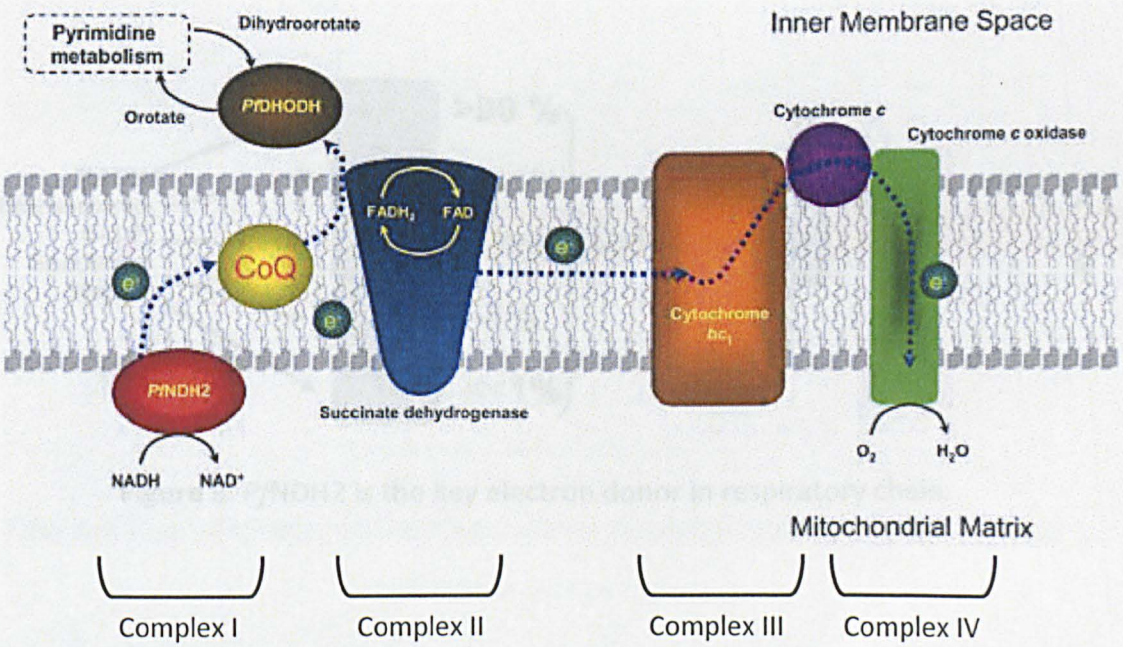


Figure 1. Mitochondrial electron transport chain enzymes in *Plasmodium falciparum*. Picture adapted from http://sites.huji.ac.il/malarial.

*Pf*NDH2 is a single subunit 52-kDa, non-protonmotive and rotenone (a classical inhibitor of Complex I, Figure 2) insensitive enzyme that is involved in the redox reaction of NADH oxidation with subsequent quinol production.[7] It catalyses the electron transfer from NADH to ubiquinone in a "ping-pong" mechanism to supply the oxidised NADH (NAD(P)H or NAD^+) for reductive metabolic pathways such as glycolysis and the tricarboxylic acid cycle.[4, 7-8]

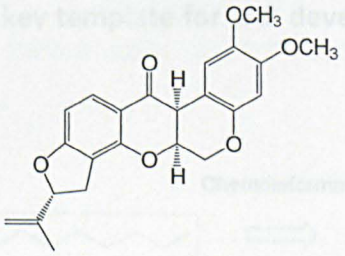


Figure 2. Rotenone

*Pf*NDH2 is a principle electron donor to the mitochondrial ETC, linking fermentative metabolism to the generation of mitochondrial electrochemical membrane potential ($\Delta\Psi m$), an essential function for parasite viability.[5, 7]

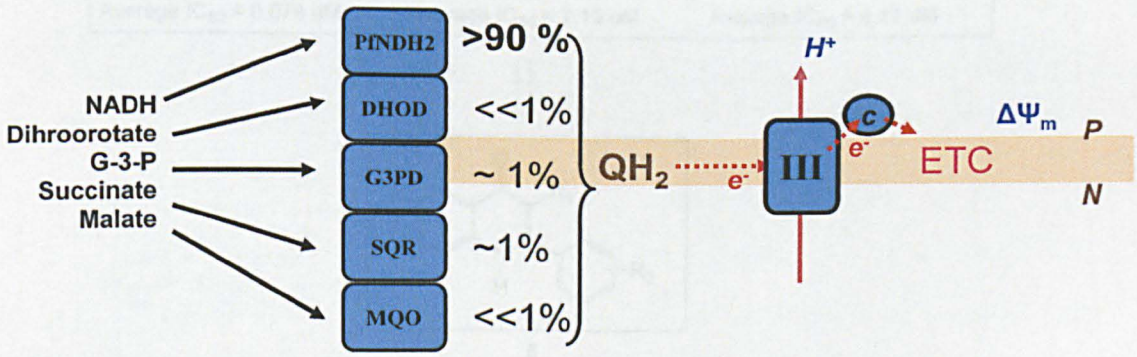


Figure 3. *Pf*NDH2 is the key electron donor in respiratory chain.

Targeting the mitochondrial ETC of human malaria has proven to be a successful chemotherapeutic strategy by atovaquone, which inhibiting the bc_1 complex.[9-10] Additionally, type II NADH:dihydrogenase is absent from human mitochondria. Therefore, targeting *Pf*NDH2 is an attractive and promising strategy in the development of antimalarial drugs.

2.2 From HDQ to initial hit molecules – aim

*Pf*NDH2 has one known inhibitor, hydroxyl-2-dodecyl-4-(1H)-quinolone (HDQ)[11] (**1**), and this was used along with a range of ligand-based chemoinformatics methods[12-16] in the rational selection of approximately 17000 compounds that were predicted to possess activity against *Pf*NDH2 for High-Throughput screening (HTS). Several distinct chemotypes were identified and briefly examined. This led to the selection of the quinolone core as the key template for SAR development due to its HDQ-like structure (Figure 4).

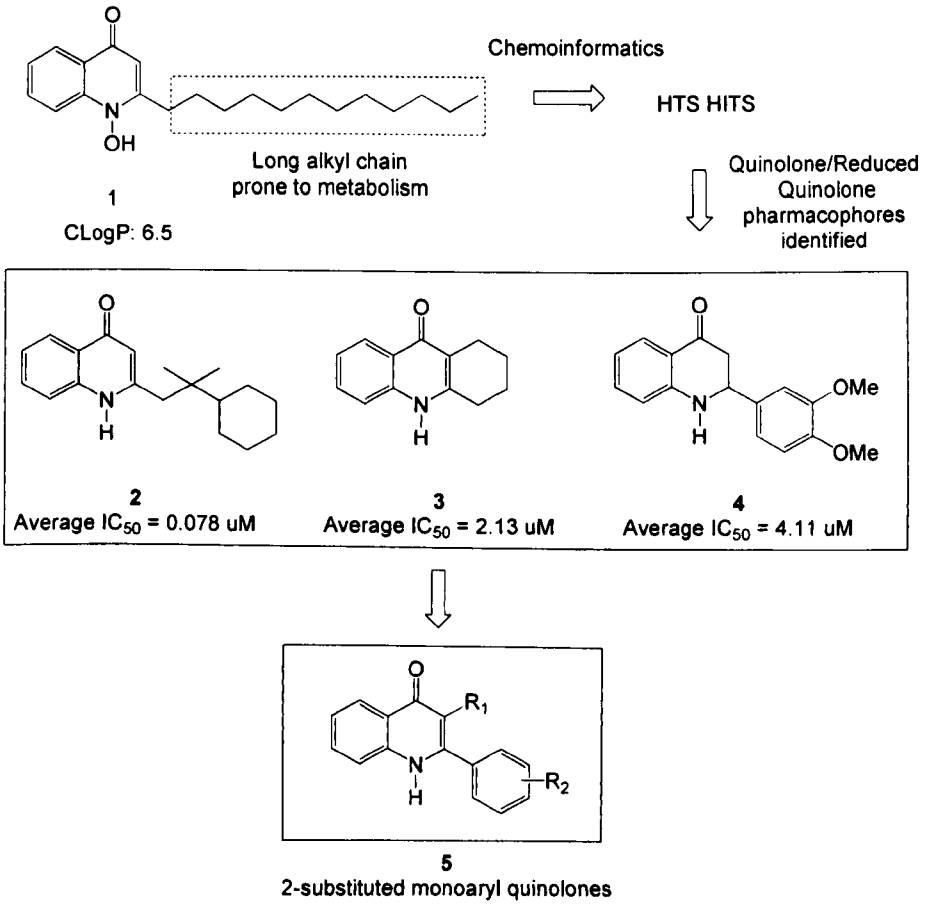


Figure 4. High-Throughput screening (HTS) to the initial template – 2-substituted monoaryl quinolones.

Initial studies within the group focused on the 2-subsitituted monoaryl quinolones (**5**) however it was quickly found that activity below 500nM against the 3D7 strain of *P. falciparum* was not achievable. Consideration of the inhibitory activity of HDQ

against *Pf*NDH2 and the metabolic instability of its long alkyl moiety led to the investigation of biaryl/phenoxyl biaryl as the side chain. This progression improved both antimalarial and *Pf*NDH2 activity as exemplified by RKA070 (**6**) and RKA073 (**7**) (Figure 5). The next structural modification was the introduction of a methyl substituent at the 3-position. The methyl group twists the 2-aryl side chain, altering the torsion angle leading to a reduction in aggregation and better overall solubility.[17] This optimisation led to the generation of over 60 compounds as exemplified by CK-2-68 (**10**) with activity of 31nM against 3D7 and 16nM against *Pf*NDH2.

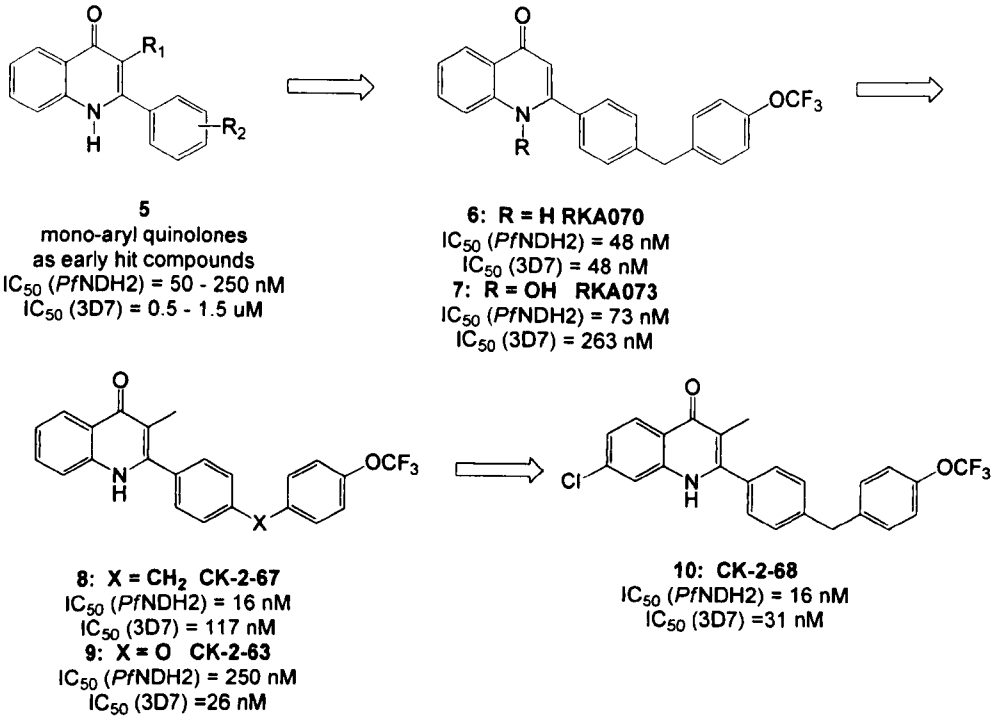


Figure 5. The Optimisation of the monoaryl quinolones to CK-2-68 (**10**).

From the preliminary animal studies, it was apparent that ClogP needed to be further reduced and aqueous solubility needed to be improved in order to administer the compound in a suitable vehicle.

Therefore, this study focused on the optimisation of CK-2-68 (**10**) by employing different medicinal chemistry approaches, such as incorporation of a pyridine group and the use of protonatable groups within the side chain, to reduce ClogP and improve solubility (Figure 6).

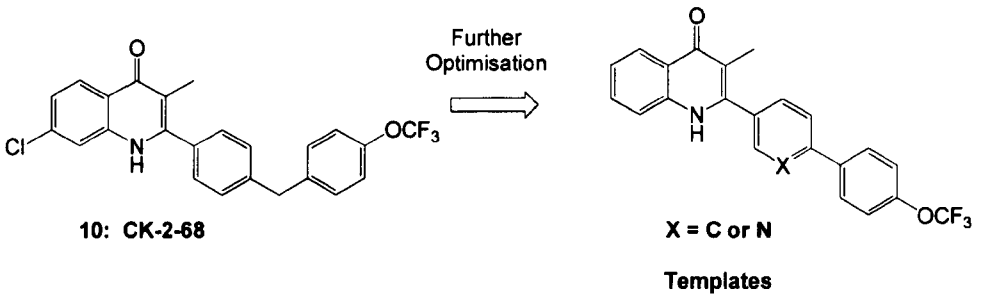


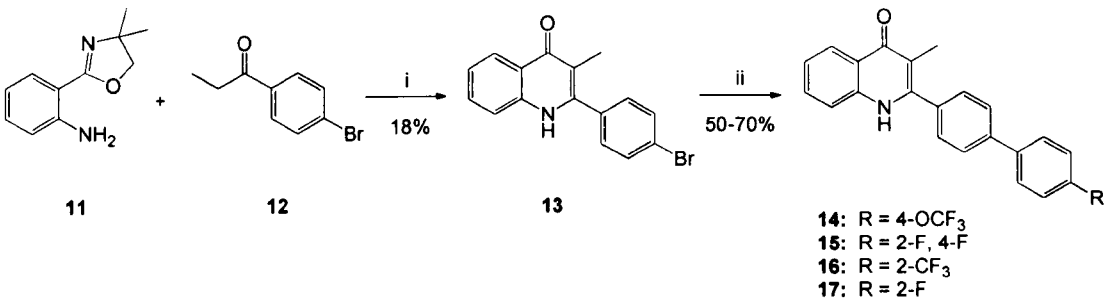
Figure 6. The Templates investigated in this thesis.

2.3 Results and discussion

2.3.1 Synthesis

The incorporation of a heterocycle into the side chain reduces the ClogP of the parent compound and hence improves aqueous solubility.[18-19] Access to a library of side chain heterocyclic analogues would be facilitated if there was no linker between the two phenyl rings within the side chain.

For this reason, the analogue of CK-2-68 without the 7-Cl substitution and any linker between the aryl rings **14** was first synthesised together with different substituents on the terminal ring to determine if activity could be maintained. It can be seen from Table 1 in the next section that antimalarial activity is maintained. The synthesis of **14** and quinolones **15-17** is outlined below (Scheme 1).



Scheme 1. *Reagents and conditions*: i) PTSA (cat.), n-BuOH, 130°C, o/n; ii) Corresponding boronic acid, $PdCl_2(dppf)$, K_2CO_3, 1,4-dioxane, 100°C, 1 d.

The common intermediate, quinolone **13** was formed from refluxing oxazoline **11** (its synthesis is detailed later) and commercially available 4'-bromopropiophenone **12** with a catalytic amount of PTSA in n-butanol.[20] Quinolone **13** was reacted with appropriate boronic acids under the Suzuki conditions to give Quinolones **14-17** in 50-70% yields (Scheme 1). This diversity-point of the synthetic route (with the Suzuki reaction performed after the quinolone formation) is advantageous since it enables facile the substitution on the terminal ring system.

The proposed mechanism for the quinolone **13** formation from oxazoline **11** and ketone **12** is outlined as below (Figure 7).[20]

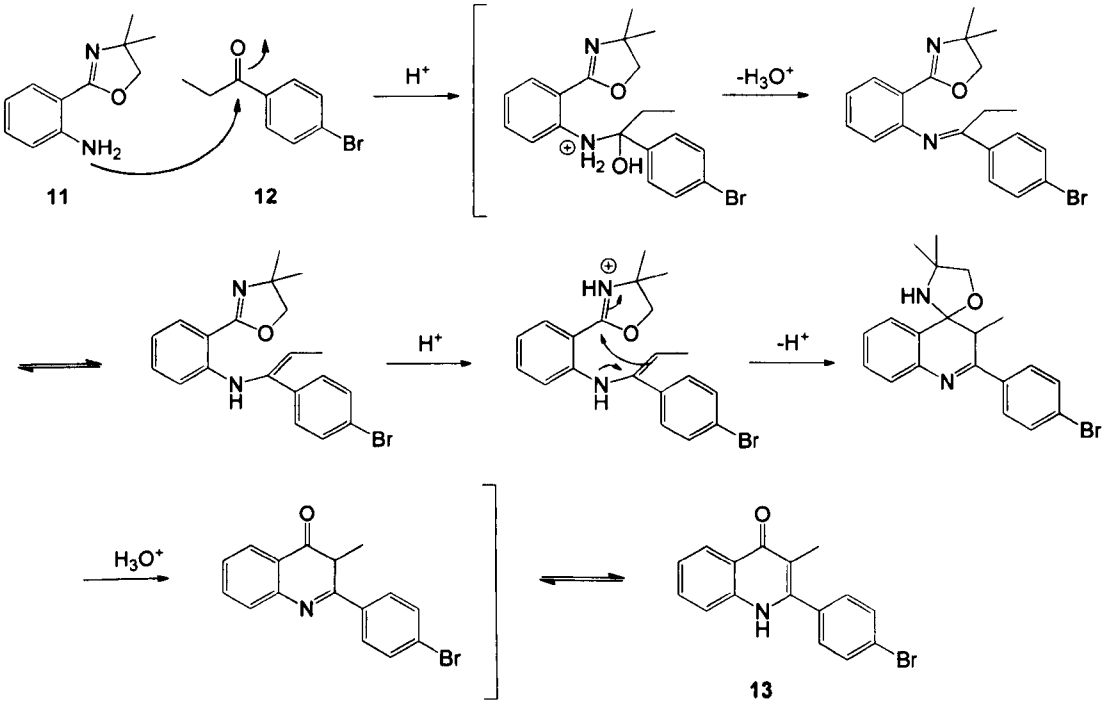


Figure 7. The proposed mechanism for the cyclisation of oxazoline and ketone.[20]

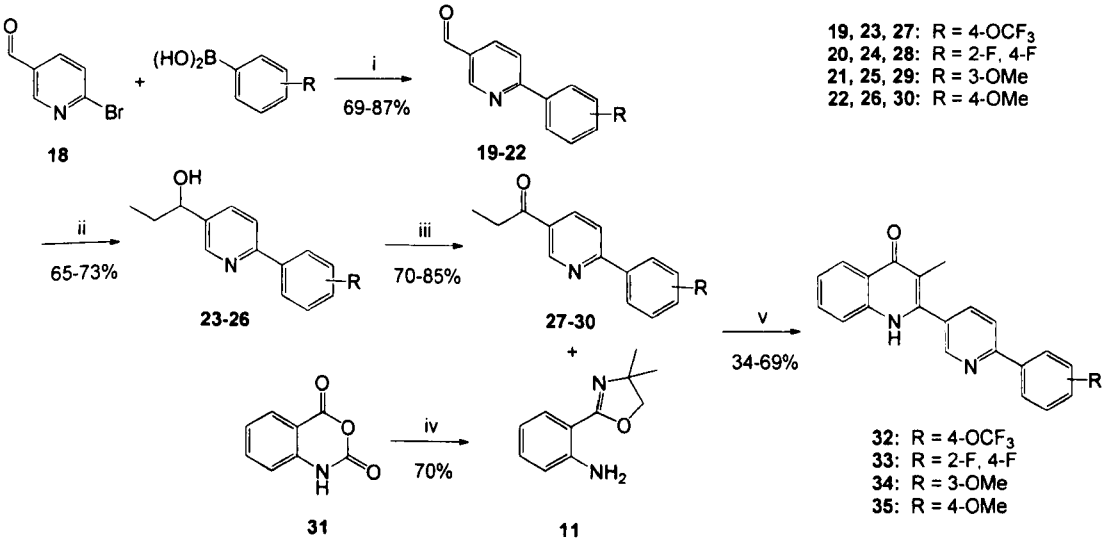
Interestingly, although the reaction, developed by Luo *et al*,[20] to prepare the 4-quinolone is promising, the yield was disappointing (10%) when the same conditions were applied to the synthesis of quinolone **13**. Therefore, we decided to investigate the effect of varying the catalytic acid used in the quinolone formation step whilst maintaining the other conditions outlined in the synthesis of **13**.

Acid	Amount (mol%)	Yield (%)
PTSA	10	18
PTSA	20	20
Methanesulfonic acid	20	45
Trifluoromethanesulfonic acid	20	68

Performing the cyclisation with catalytic amount of methanesulfonic acid gave 20 – 45% yields while 20 mol% trifluoromethanesulfonic acid gave an acceptable 68%

yield. Therefore, trifluoromethanesulfonic acid became the catalyst of choice for further quinolone formations.

After the positive outcome of the 2-biaryl quinolone, the incorporation of a pyridine ring into the side chain and terminal aryl ring substituents were then investigated. The synthesis of these compounds is outlined below. (Scheme 2)

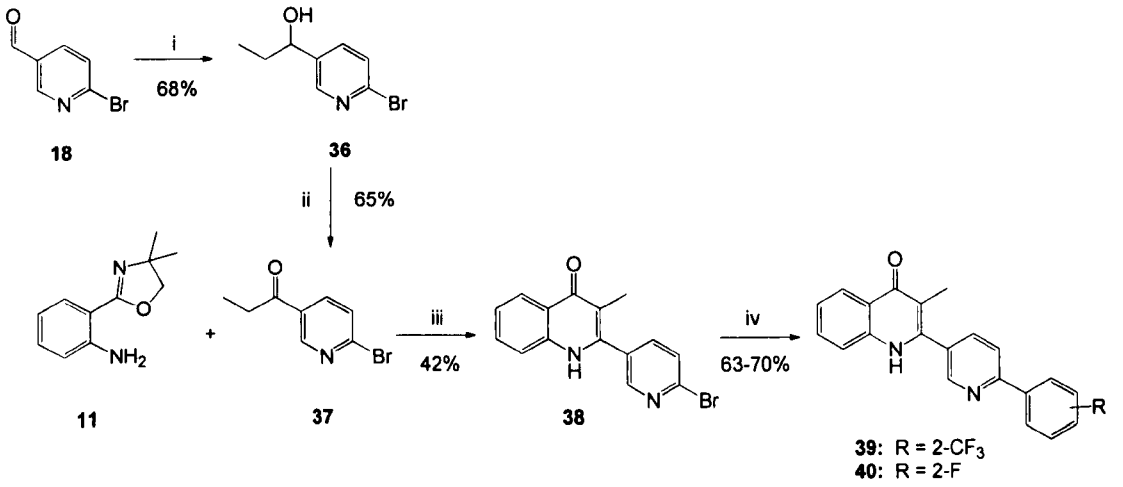


Scheme 2. *Reagents and conditions*: i) $Pd(PPh_3)_4$, K_2CO_3, THF-H_2O, 80°C, 24 h; ii) EtMgBr, THF, 0°C, 1h; iii) PCC, DCM, r.t., 4 h; iv) 2-amino-2-methylpropanol, $ZnCl_2$, chlorobenzene, 140°C, 24 h; v) trifluoromethanesulfonic acid (20 mol%), n-BuOH, reflux, 1 d.

Suzuki coupling of the commercially available 6-bromopyridine-3-carboxaldehyde **18** with an appropriate substituted phenylboronic acid afforded the biaryl pyridine aldehyde **19-22** in high yields. The aldehydes **19-22** were then converted to the alcohols **23-26** by treatment with ethylmagnesium bromide in anhydrous THF at 0°C for 1 hour and subsequent oxidation by PCC in dichloromethane to give the ketone side chain **27-30** in 70-85 % yields. Oxazoline **11** was prepared from isatoic anhydride **31** and 2-amino-2-methylpropanol with catalytic zinc chloride in chlorobenzene under anhydrous conditions in 70% yield. Reaction of oxazoline **11**

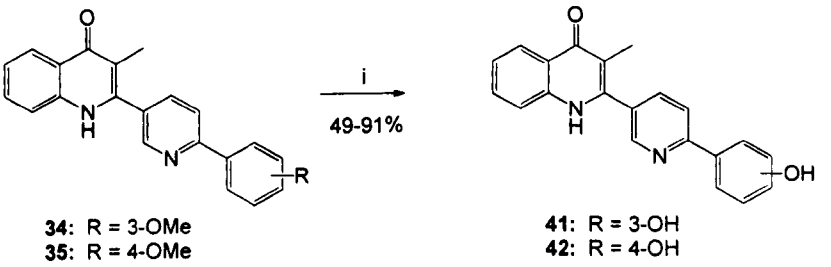
with the ketone **27-30** in the presence of 20 mol % trifluoromethanesulfonic acid afforded the desired quinolones **32-35** in 34-69% yield.

Quinolone **38** was also synthesised as the precursor to **39** and **40**, utilising the same reaction conditions as described in Scheme 1. (Scheme 3)



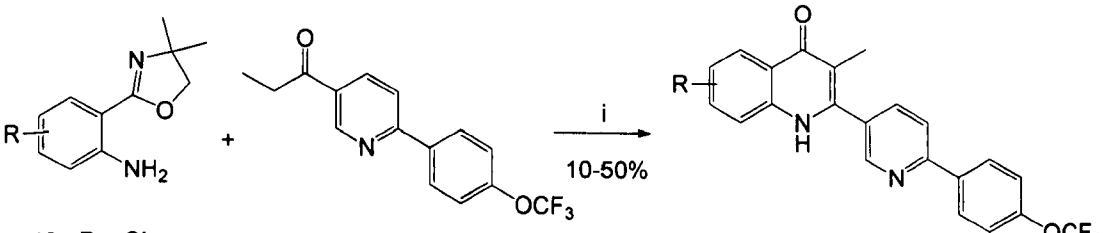
Scheme 3. *Reagents and conditions*: i) EtMgBr, THF, 0°C, 1h; ii) PCC, DCM, r.t., 4 h; iii) Trifluoromethanesulfonic acid (20 mol%), n-BuOH, 130°C, o/n; iv) Boronic acid, $PdCl_2(dppf)$, K_2CO_3, 1,4-dioxane, 100°C, 1 d.

Quinolones **41** and **42**, both containing a hydroxyl group at the terminal ring, were prepared from **34** and **35**. Demethylation was performed on **34** and **35** respectively with 1.0 M boron tribromide solution in dichloromethane at room temperature overnight (Scheme 4).



Scheme 4. *Reagents and conditions*: i) 1.0 M BBr_3 in DCM, r.t., 24 h.

In order to investigate the effect of substitution in the quinolone A ring, analogues with different substitutions at the 7 (and 6) position corresponding to quinolone **32** were synthesised (Scheme 5). **32** was chosen as the basic core for study since it was the most potent analogue among the series. The substituents were selected with reference to the Craig plot[21] and Topliss operational scheme[22-23], based on physiochemical parameters such as electronics, sterics and hydrophobicity effects. The preparation of those analogues **50-56** was similar but using different substituted oxazoline to couple with the ketone.



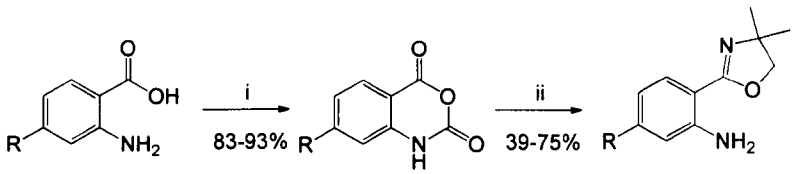
43: R = Cl
44: R = CF_3
45: R = F
46: R = OMe
47: R = SO_2Me
48: R = 6-Cl, 7-F
49: R = 6-F, 7-Cl

27

50: R = Cl
51: R = CF_3
52: R = F
53: R = OMe
54: R = SO_2Me
55: R = 6-Cl, 7-F
56: R = 6-F, 7-Cl

Scheme 5. *Reagents and conditions*: i) Trifluoromethanesulfonic acid (20 mol%), n-BuOH, reflux, 1 d.

The required substituted oxazolines **43-47** were not commercially available and therefore had to be synthesised (Scheme 6). Slow addition of trichloromethyl chloroformate (diphosgene) to appropriately substituted anthranillic acids **58-61** in potassium hydroxide solution at 0°C gave the corresponding isatoic anhydride **63-66**. Potassium hydroxide solution is preferable over organic solvents such as THF used in literature[24-26] as the solid product would be easily isolated by filtration. Subsequently, these intermediates were converted into the oxazolines **43-47** by reacting with 2-amino-2-methylpropanol.



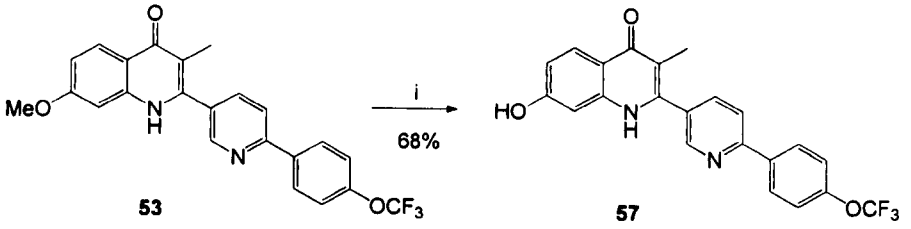
58: R = CF_3
59: R = F
60: R = OMe
61: R = SO_2Me

62: R = Cl
63: R = CF_3
64: R = F
65: R = OMe
66: R = SO_2Me

43: R = Cl
44: R = CF_3
45: R = F
46: R = OMe
47: R = SO_2Me

Scheme 6. *Reagents and conditions*: i) diphosgene, aq. KOH, 0°C, 2 - 4 h; ii) 2-amino-2-methylpropanol, $ZnCl_2$, chlorobenzene, 140°C, 24 h.

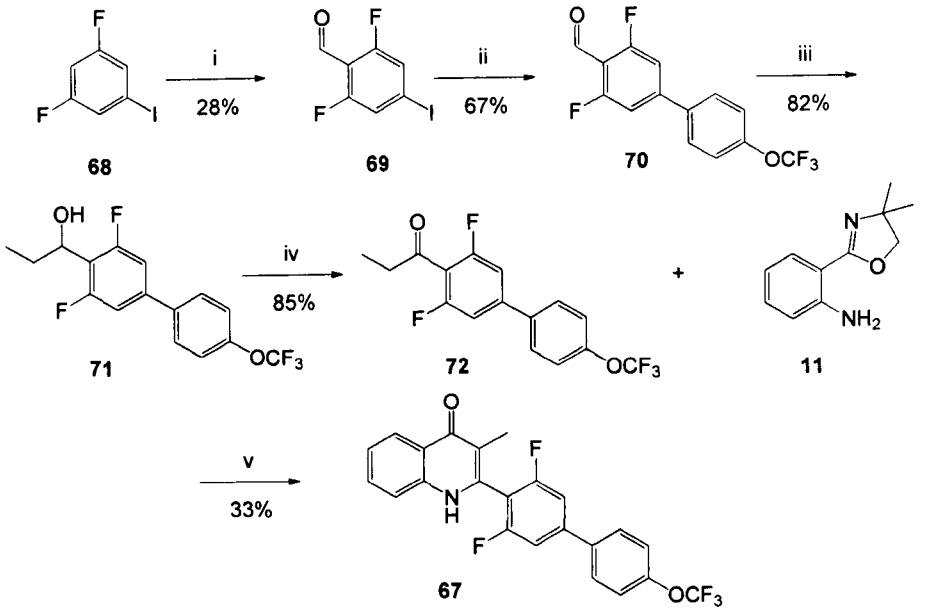
In the case of the hydroxyl substitution (**57**), it was prepared via the methoxy analogue **53**. Unfortunately, by use of demethylation condition used in Scheme 4, the reaction progressed slowly probably due to the poor solubility of **53** in dichloromethane. To overcome this, removal of the methyl group was achieved by refluxing **53** with boron tribromide in toluene for 18 h. (Scheme 7)



53 **57**

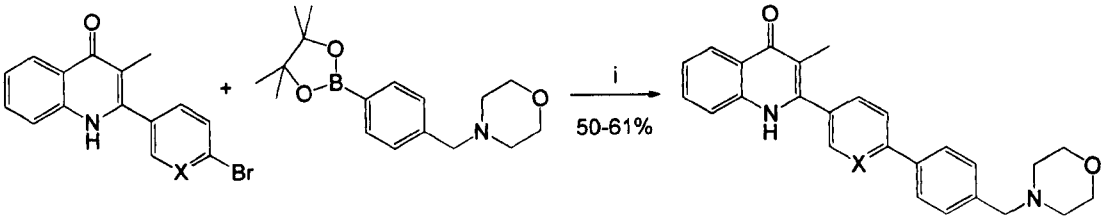
Scheme 7. *Reagents and conditions*: i) 1.0 M BBr_3 in THF, THF-Toluene, reflux, 18 h.

Difluoro-substitution of the first phenyl ring of the side chain (**67**) was also investigated. The difluoro-substituent was incorporated with the intent to further reduce the aggregation and enhance the solubility in a similar way to the incorporation of a methyl group at the 3-position of the quinolone. The synthetic route to **67** is illustrated in Scheme 8. The aldehyde **69** was commercially unavailable and thus prepared from reacting 3,5-difluoroiodobenzene **68** with n-butyl lithium and 2,2,6,6-tetramethyl piperidine in anhydrous THF at -78°C. The lithiated species was trapped with DMF, and hydrolysed to the desired aldehyde **69**.



Scheme 8. *Reagents and Conditions*: i) (a) nBuLi, 2,2,6,6-tetramethyl piperidine, THF, -78°C, 2 h; (b) DMF, 2 h, r.t.; ii) 4-(trifluoromethoxy)benzene boronic acid, $Pd(PPh_3)_4$, K_2CO_3, THF-H_2O, o/n; (iii) EtMgBr, THF, 0°C, 1 h; (iv) PCC, DCM, r.t., 4 h; (v) trifluoromethanesulfonic acid (20 mol%), n-BuOH, reflux, 1 d.

Investigations then focused on the possibility of formulating the series as salt and improving aqueous solubility by introducing the morpholine group at the terminal ring.[27-28] Quinolones with extended morpholine side chains (**74** and **75**) were thus synthesised using similar chemistry to that employed in Scheme 1. Quinolone **13** or **38** was reacted with 4-(4-morpholinomethyl)phenylboronic acid pinacol ester to give morpholine quinolones **74** and **75** in 50% and 61% yields (Scheme 9).



Scheme 9. *Reagents and conditions*: i) $PdCl_2(dppf)$, K_2CO_3, 1,4-Dioxane-H_2O, 100°C, 1 d.

Simultaneously, a set of quinolones (**76-79**) containing an extended ethoxy morpholine and piperidine were designed to further the SAR and to determine the side chain length which could be tolerated (Figure 8).

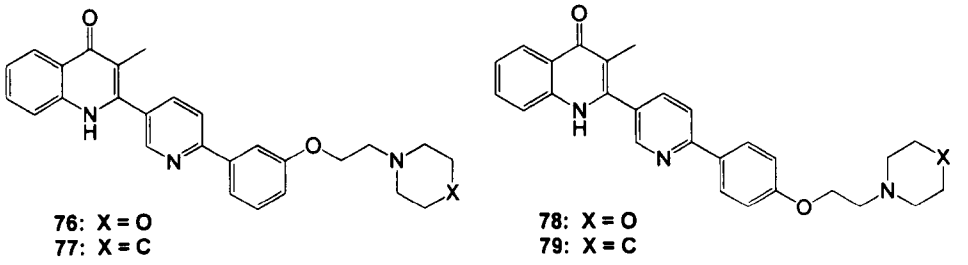
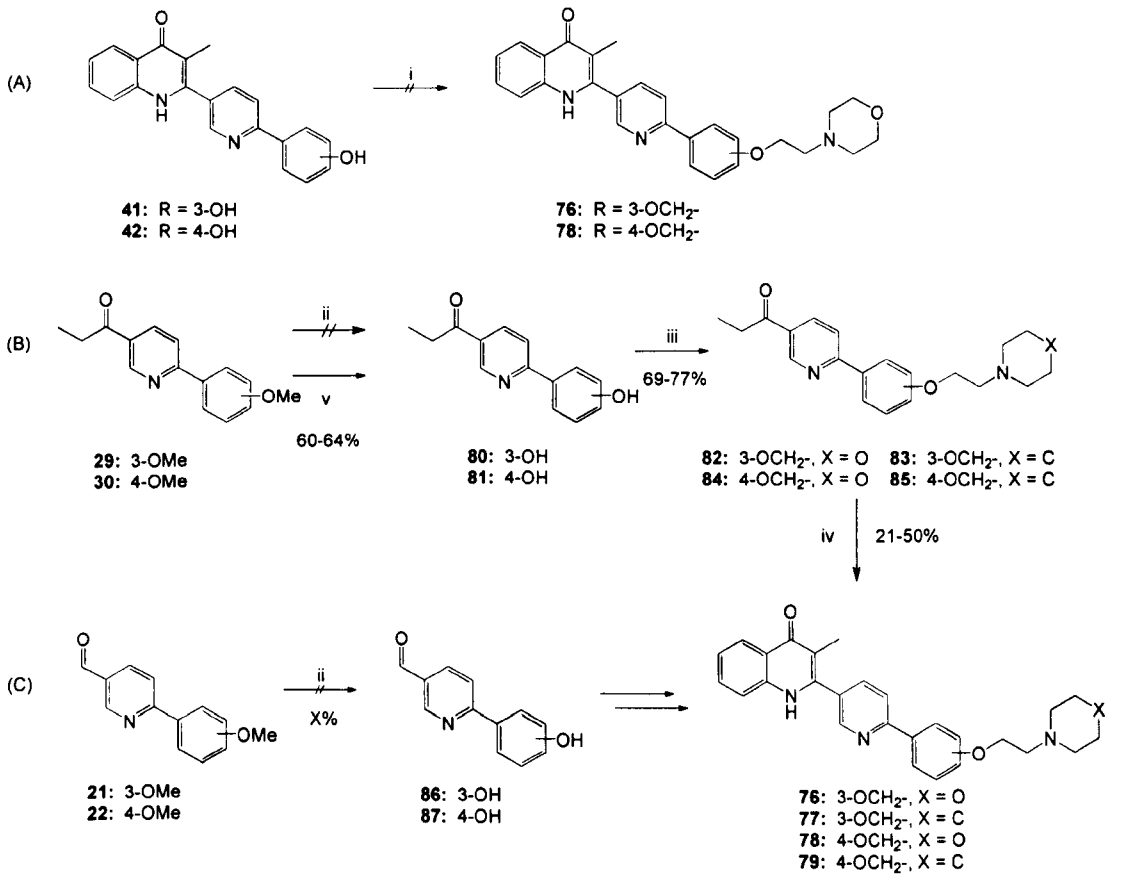


Figure 8. Quinolones containing an extended ethoxy morpholine and piperidine side chain.

Firstly, the synthetic route to quinolones **76-79** was set out as route A in Scheme 10 However, alkylation of quinolone **41** with 4-(2-chloroethyl) morpholine with potassium carbonate in DMF at reflux was not favourable due to the competing alkylation on the oxygen at the 4-position of the quinolone under these basic conditions. Difficulty was also experienced during the separation and purification of the desired product by column chromatography due to its polarity.

Unfortunately, via route B, attempts to *O*-demethylate **29-30** using BBr_3 in DCM at <0 °C, after the oxidation of alcohol, were also unsuccessful. Under different sets of conditions, demethylation on the aldehyde **21-22** was not accomplished in acceptable yield. After repeating these BBr_3 demethylations several times, it was noticed that the reactions were temperature-sensitive and thus the yields varied greatly. A large number of by-products could be seen on tlc even with precise control of temperature (-10°C) and shortened reaction period (from 2h to 30 min). Further lowering the temperature led to slow rates of reaction and poor yields. The problem may have been due to the presence of the pyridine lone pair on nitrogen which is capable of coordinating with BBr_3.



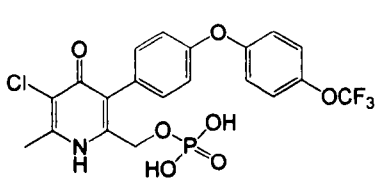
Scheme 10. *Reagents and conditions*: i) 4-(2-chloroethyl)morpholine, K_2CO_3, DMF, reflux, 4 h; ii) BBr_3 (1.0 M in THF), THF, < 0°C, 30 min – 2 h; iii) 4-(2-chloroethyl)morpholine hydrochloride or 1-(2-chloroethyl)piperidine hydrochloride, K_2CO_3, acetone, 70 °C, o/n; iv) oxazoline **11**, trifluoromethanesulfonic acid (30 mol %), n-BuOH, reflux, 1 d; v) 2-(diethylamino)ethanethiol hydrochloride, DMF, Na^tOBu, 1 h.

We decided to investigate other ether cleavage reagents and abandon the use of BBr_3. During the search of other literature methods and reagents available, a chemical, 2-(diethylamino)ethanethiol, drew our attention. This compound is available as a HCl salt and was discovered by Magano *et al* for the deprotection of aromatic methyl ethers.[29] It was chosen for the following reasons: the reaction can be done with reflux temperature in DMF, the ease of workup and the by-product 2-(diethylamino)ethyl methyl sulphide can be removed into aqueous phase. Finally, route B was kept but 2-(diethylamino)ethanethiol with sodium *tert*-butoxide in

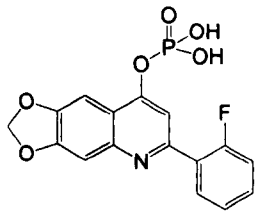
DMF were used instead of BBr_3 in DCM to give the alcohol **80-81** in 60-64% yields. Nucleophilic substitution to 4-(2-chloroethyl)morpholine or 1-(2-chloroethyl)piperidine using potassium carbonate in acetone produced the side chains **82-85** in 69-77% yields. Cyclisation with oxazoline **11** in n-butanol in the presence of 30 mol% trifluoromethanesulfonic acid gave the desired quinolones **76-79** in 21-50% yields (Scheme 10).

Whilst medicinal chemistry manipulation of the core template was our primary strategy to maximise solubility and activity, prodrug approaches were also briefly examined. Prodrugs are typically pharmacologically inactive but undergo an enzymatic or chemical transformation *in vivo* to release the active parent drug which can then exert its desired effect. Prodrugs have been useful to overcome the limitations of the parent drug for examples poor membrane permeability, solubility, short duration of action and bad taste.[30-31] Prodrug strategies have been successful in improving the clinical performance of oestrone[32], ampicillin[33], chloramphenicol[34], enalaprilat[35-36] etc.

Recently, GSK has adopted this strategy in their antimalarial pyridone (GSK932121) programme. Their pyridine based phosphate prodrugs (Figure 9, **88**) have demonstrated excellent improvement in *in vivo* antimalarial activity and exposure profiles.[37] Chou *et al* have also successfully developed a novel water-soluble phosphate prodrug of the 2-arylquinolone (CHM-1-P-Na, **89**) as an antitumor agent, which could overcome the relatively low hydrophilicity of the parent drug.[38]



88: GSK932121

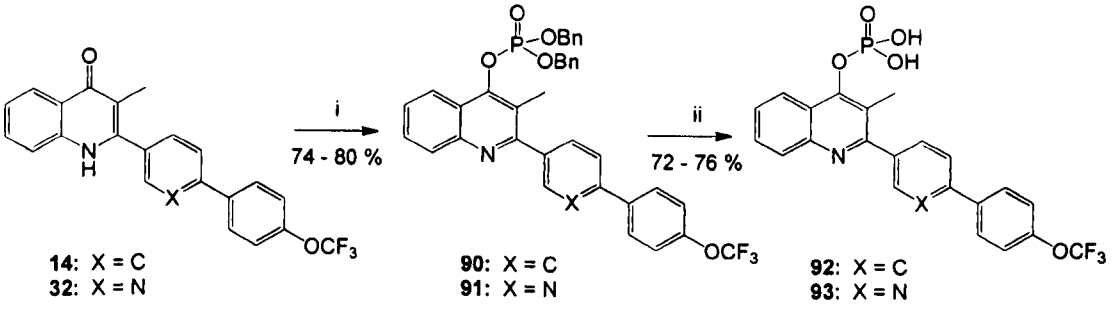


89: CHM-1-P-Na

Figure 9. The pyridine prodrug GSK932121 by GSK[37] and the 2-arylquinolone CHM-1-P-Na by Chou[38].

Phosphate ester prodrugs are the common strategy to improve the aqueous solubility of drugs containing hydroxyl or amine functionalities. In general, due to the presence of dianionic moiety on phosphate prodrugs, they are highly ionized at physiological pH and therefore highly soluble in water. They are chemically stable yet undergo enzymatic cleavage at the gut wall by membrane-bound alkaline phosphatases to produce high concentrations of the parent drug in the systemic circulation.[30, 39]

Encouraged by the GSK and Chou studies, quinolones **14** and **32** were selected for the prodrug study as they demonstrated good *in vitro* antimalarial activities and selectivity against *Pf*NDH2. (Scheme 11) Quinolones **14** and **32** were initially dissolved in anhydrous THF and deprotonated by sodium hydride at 0°C, which were then treated with tetrabenzyl pyrophosphate to afford the corresponding phosphonate esters **90-91** in 74 – 80% yield. Deprotection of the benzyl group by hydrogenation using 10% Pd/C gave the phosphate pro-drugs **92-93** in 72 – 76% yields.

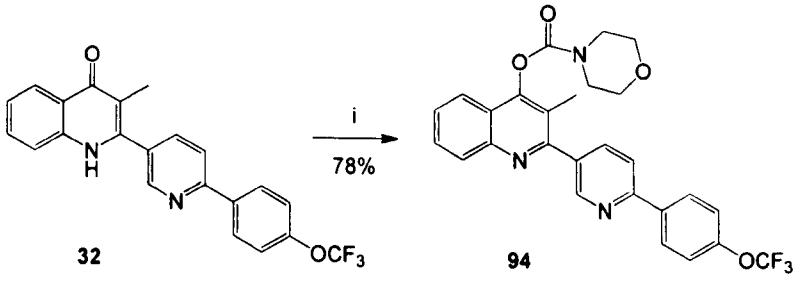


Scheme 11. *Reagents and conditions*: i) Tetrabenzyl pyrophosphate, NaH, THF, 2 h; ii) 10% Pd/C, H_2, MeOH, 10 min.

Although the phosphate prodrug **93** achieved 100% parasite kill at 20 mg/Kg *in vivo* when it was dosed in a sodium carbonate solution, it was noticed that the stability of the phosphate prodrug decreased. Decomposition of the phosphate prodrug could be observed in protic solvents. This compound is readily hydrolysed back to **32** in methanol where the quinolone is the most stable form.

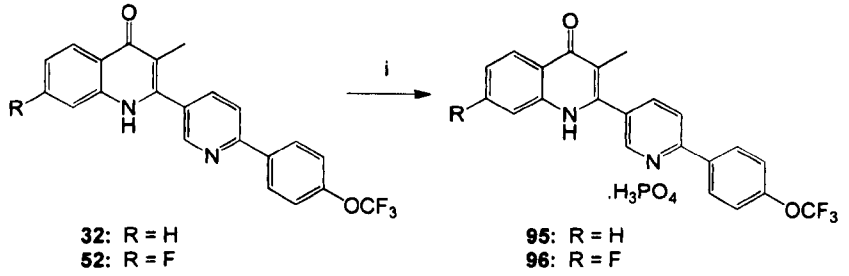
Carbamate prodrugs were also studied. Aside from the phosphate ester, carbamate is one of the popular types of prodrug to enhance the aqueous solubility and bioavailability.[40-42] The carbarmate prodrug can be bio-converted back to the parent drugs by esterases.[40, 43-44] Irinotecan[45], duocarmycin, camptothecin, entacapone and 3-PPP are the examples of carbamate prodrugs[44] currently on the market.

To synthesise the carbamate prodrug **94**, quinolone **32** was reacted with morpholine carbonyl chloride and potassium *tert*-butoxide in THF. (Scheme 12)



Scheme 12. *Reagents and conditions*: i) morpholine carbonyl chloride, K^{t}OBu, THF, r.t., 4 h.

Phosphoric acid salts of quinolones **32** and **54** were also prepared by treating quinolones with 1.5 equivalent of 85% phosphoric acid in ethanol (Scheme 13). Evaporation of most of the solvents followed by the trituration with diethyl ether gave the salts as solid.



Scheme 13. *Reagents and conditions*: i) 85% H_3PO_4, EtOH, r.t. 2 h.

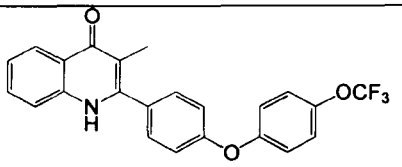
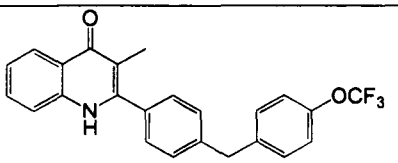
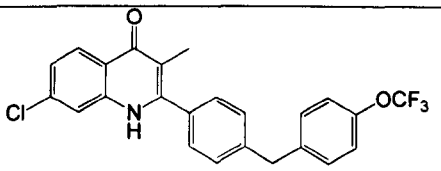
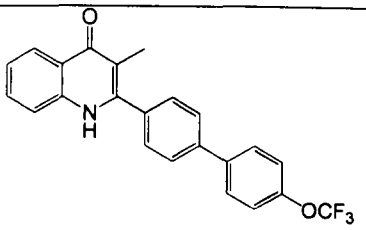
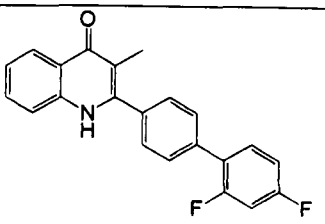
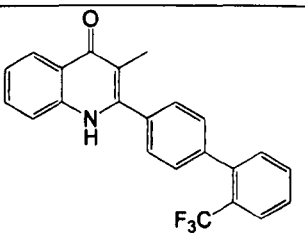
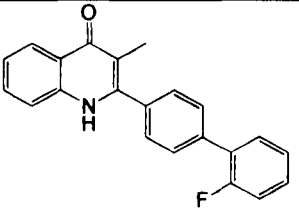
2.3.2 Antimalarial activity and SAR studies

In vitro antimalarial activity

Antimalarial activities of all the quinolone compounds were assayed against the 3D7 strain (chloroquine sensitive) of *Plasmodium falciparum* and NDH2 enzyme respectively at the Liverpool School of Tropical Medicine. The IC_{50} values were calculated by using the four-parameter logistic method (Grafit program; Erithacus Software, United Kingdom) and recorded in Table 1-3. Due to the time consuming nature of the assay and large amounts of parasites required, only a small selection of the quinolones prepared were tested against the *Pf*NDH2 enzyme. Any compounds with antimalarial activity (IC_{50}) higher than 1000 nM *in vitro* were not tested further for exact IC_{50} values. In addition, the ClogP values were calculated by ALOGPS 2.1 programme.[46-47]

Table 1 shows the antimalarial activities for the bisaryl compounds. In general, the bisaryl quinolones with no linker perform reasonably well compared to CK-2-63 (**9**), CK-2-67 (**8**) and CK-2-68 (**10**) in the previous studies within the group. The exception was quinolone **67** which difluoro-substitution is clearly having a profoundly negative effect – this may be related to conformation of the side chain. From these studies, 4-OCF_3 group is the optimal terminal group on the side chain. It is observed that the activity drops when the ClogP is lowered, suggesting certain hydrophobicity is required for binding to the biological target. Given these molecules are competitive antagonists of ubiquinone, it seems likely that the biaryl side chain occupies the receptor that normally accommodates the liphophilic tail of the ubiquinone co-factor.

Table 1. *In vitro* antimalarial activities of bisaryl quinolones against 3D7 strain of *P. falciparum*.

	Structure	IC_{50} 3D7 (nM)	IC_{50} *Pf*NDH2 (nM)	ClogP
CK-2-63 **(9)**		26±1	10	6.0 ± 1.0
CK-2-67 **(8)**		117±27	16	6.3 ± 0.9
CK-2-68 **(10)**		36±5	16	6.9 ± 0.9
14		59±9	<1	6.1 ± 0.8
15		213±33	-	5.4 ± 0.4
16		96±17	8.1	5.9 ± 0.5
17		471±75	-	5.3 ± 0.4

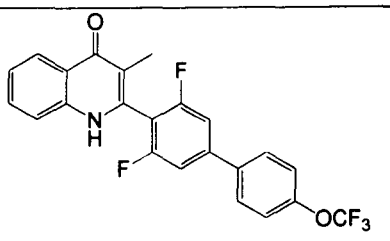
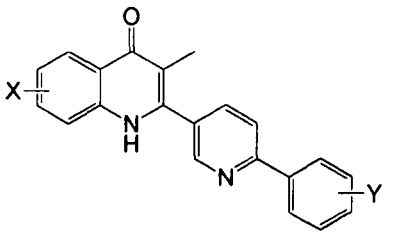
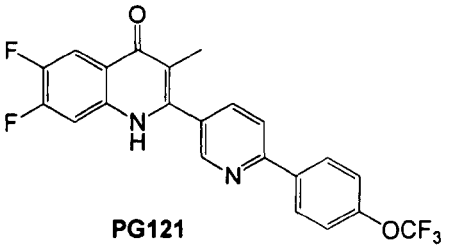
67		940±50	-	6.4 ± 0.8

Table 2 below shows the *in vitro* antimalarial activity of the bicyclic pyridine quinolones and the differently substituted quinolone **32** analogues. The same trend as in the bisaryl quinolones is observed in quinolones **32-35** and **39-42** since 4-OCF_3 is the optimal terminal substituent. However, the presence of an hydroxyl group in the terminal side chain in **34** and **35** results in a loss in the activity, suggesting that the binding site around the side chain is hydrophobic as noted above.

The quinolones (**50-57**) based on **32** were synthesised with the intent of investigating the effect of varying the substituents with different steric or electronic withdrawing properties at the 7-position of the quinolone ring. In general, with reference to **32**, most of the substitutions on the quinolone ring provided no favourable effects in terms of enhancement of antimalarial activity. Some smaller groups such as F, Cl and OH are still well tolerated with the 7-F (**52**) almost as potent as the parent template **32**. **52** has improved activity selectivity (4.2 nM) against *Pf*NDH2 when compared with **32** (15 nM). Unlike the previous studies in the group, disubstitutions at 6- and 7- position in this series have no potential synergistic effects on phenotypic activity. Instead, disubstituted quinolones such as **55** and **56** and **PG121** are less potent. Larger electron-withdrawing substituents, such as CF_3 and SO_2Me also lead to a drop in activity into the micromolar range as seen in **51** and **54** (IC_{50}s > 1000nM). These observations highlight the steric hindrance in the binding region, and conclude that 6 and 7 positions are less suitable for substitution or modification as it will reduce the antimalarial activity.

Table 2. *In vitro* antimalarial activities of bicyclic pyridine quinolones against the 3D7 strain of *P. falciparum*.



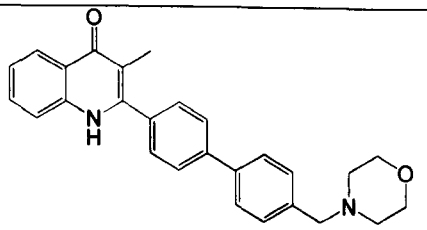
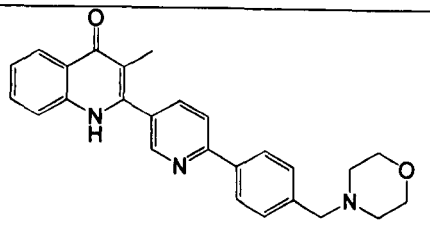
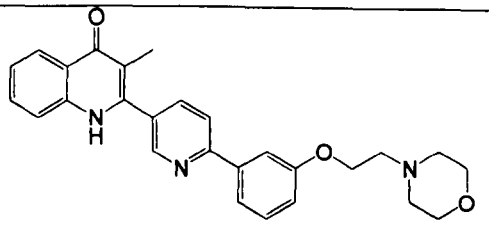
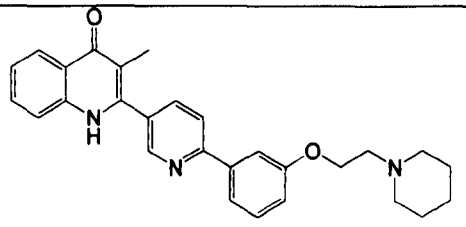


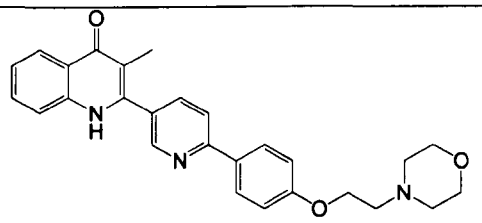
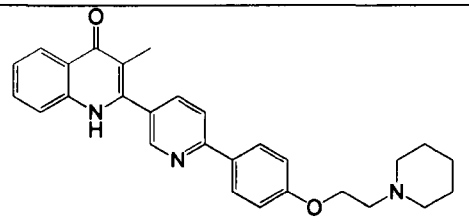
PG121

Compound	X	Y	IC_{50} 3D7 (nM)	IC_{50} *Pf*NDH2 (nM)	ClogP
32	H	4-OCF_3	54±6	15	5.1 ± 0.9
33	H	2-F, 4-F	219±62	89	4.4 ± 0.4
34	H	3-OMe	556±41	-	4.1 ± 0.5
35	H	4-OMe	223±14	-	4.1 ± 0.5
39	H	2-CF_3	109±13	352	4.9 ± 0.4
40	H	2-F	151±22	131	4.3 ± 0.4
41	H	3-OH	>1000	-	3.8 ± 0.5
42	H	4-OH	>1000	-	3.8 ± 0.5
50	7-Cl	4-OCF_3	373±74	-	5.7 ± 1.0
51	7-CF_3	4-OCF_3	>1000	-	5.9 ± 1.0
52	7-F	4-OCF_3	75±9	4.2	5.3 ± 0.9
53	7-OMe	4-OCF_3	240±52	384	5.0 ± 1.0
54	7-SO_2Me	4-OCF_3	>1000	-	4.3 ± 1.0
55	6-Cl, 7-F	4-OCF_3	390±20	-	5.8 ± 1.0
56	6-F, 7-Cl	4-OCF_3	390±80	-	5.7 ± 1.1
57	7-OH	4-OCF_3	202±79	-	4.7 ± 1.0
PG121	6-F, 7-F	4-OCF_3	334±68	-	5.3 ± 1.0

Subsequently, a set of quinolones containing an extended amine **74-79** were developed to enhance the aqueous solubility. Quinolone **74** is still moderately potent with the IC_{50} at 496 nM. (Table 3) **75** and Quinolones containing extended ethoxy morpholine (**76** and **78**) or piperidine (**77** and **79**) were inactive. It is believed that the presence of the amino group together with the pyridine ring decreases the ClogP to a point where the compound does not bind sufficient well within the hydrophobic active site.

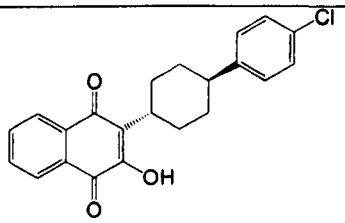
Table 3. *In vitro* antimalarial activities of quinolones with an extended amine side chain against 3D7 strain of *P. falciparum*.

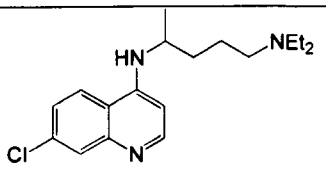
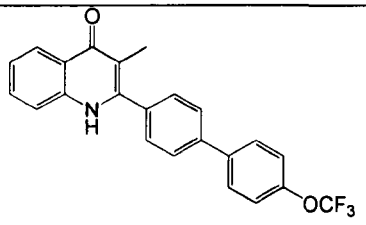
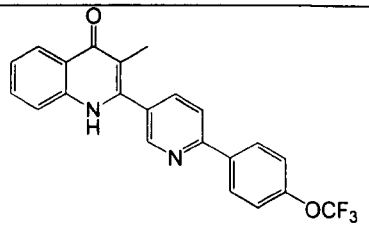
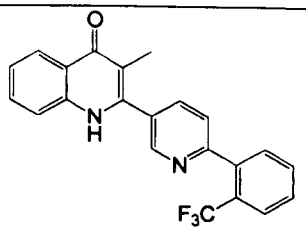
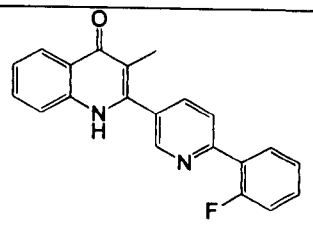
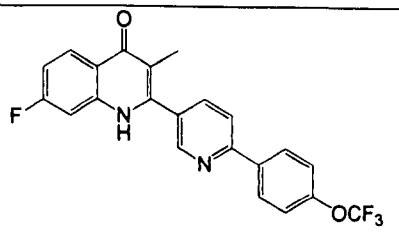
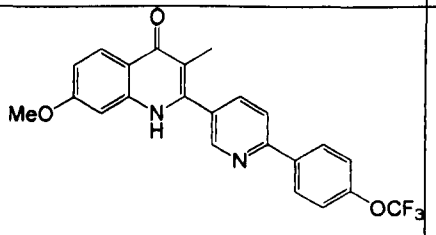
Compound	Structure	IC_{50} 3D7 (nM)	ClogP
74		496±30	4.6 ± 0.5
75		>1000	3.7 ± 0.4
76		>1000	3.7 ± 0.5
77		>1000	4.9 ± 0.6

78		>1000	3.7 ± 0.5
79		>1000	4.9 ± 0.6

A selection of quinolones were also tested against the atovaquone-resistant TM90C2B strain of *P. falciparum* containing the cytochrome *b* mutation *Y268S* at Liverpool School of Tropical Medicine, and against the chloroquine-resistant W2 strain of *P.falciparum* by Rosenthal group at University of California, San Francisco. The tested compounds (**14**, **32**, **39** and **40**) have demonstrated better activity in nanomolar range compared to atovaquone which has an IC_{50} of 12μM against the TM90C2B strain. (Table 4) The tested compounds (**32**, **39**, **40**, **52** and **53**) are also active in nanomolar range against the W2 strain. Quinolones **32** and **52** have similar potency to chloroquine against the W2 strains. Quinolone **53** demonstrates better activity against the same strain with IC_{50} of 14 nM, compared to chloroquine with IC_{50} of 44 nM.

Table 4. *In vitro* antimalarial activities of selected quinolones against TM90C2B strain and W2 strain of *P. falciparum*.

Compound	**Structure**	**IC_{50} TM90C2B (nM)**	**IC_{50} W2 (nM)**	**IC_{50} 3D7 (nM)**
Atovaquone		12,000	-	-

Compound	Structure	IC_{50} TM90C2B (nM)	IC_{50} W2 (nM)	IC_{50} 3D7 (nM)
Chloroquine		-	44±1	-
14		326±42	-	59±9
32		156±22	49±12	54±6
39		381±144	88±22	109±13
40		324±76	120±6	151±22
52		183±22	69±9	75±9
53		-	14±2	240±52

In vivo antimalarial activity

The initial lead from the sets of bisaryl quinolones and pyridinyl quinolones were **14** and **32**. Quinolone **14** and its phosphate prodrug **92**, together with **32**, the phosphate prodrug, the morpholine prodrug and phosphate salt of **32** (**93**, **94**, **95**) were selected and further tested for *in vivo* activity using Peters' Standard 4-day suppressive test[48] in male CD-1 mice infected with *Plasmodium berghei* (NS strain), through oral administration. Table 5 shows the average percentage clearance of the tested quinolones on the parasite at 20 mg/Kg.

Table 5. *In vivo* Peters' Standard 4-day suppressive test – oral administration at 20 mg/kg.

	% Average Parasite Clearance		
Vehicle / Compound	SSV[+]	DET[++]	Na_2CO_3
Atovaquone	100	100	-
14	-	-	34*
32	87.5	100	-
92	-	-	100
93	-	-	100
94	100	-	-
95	100	-	-

[+] SSV: 0.5% sodium carboxymethylcellulose, 0.5% BnOH, 0.4% Tween 80 in 0.9% aqueous NaCl.

[++] DET: 5% DMSO and 5% EtOH in tetraglycol.

* Variable response across group with range from 0-92.5% kill.

14 displayed a variable response across the tested groups from 0 – 92.5% parasite clearance, it is possibly due to the poor absorption of the compound in the gut. **32** experienced solubility problems with the use of nonsolubilising SSV (standard suspension vehicle which comprises of 0.5% sodium carboxymethylcellulose, 0.5% benzyl alcohol, 0.4% Tween 80 in 0.9% aqueous NaCl) as the vehicle, therefore the compound had to be dosed as suspension and this may have reduced % parasite kill.

However, the use of DET (5% DMSO and 5% EtOH in tetraglycol) as the vehicle, where **32** is fully dissolved, confirmed that **32** demonstrated 100% inhibition on the parasitemia growth at 20mg/kg dosing even though the use of SSV was preferred in the full ED_{50}/ED_{90} determination. The phosphate prodrugs **92** and **93** were successfully dosed in sodium carbonate solution and 100% parasite clearance was also observed.

Moreover, the morpholine carbamate prodrug **94** and the phosphate salt **95** displayed 100% parasite kill at the same dose.

Salt formulation was preferable over the prodrug approach since it does not require any enzymatic conversion *in vivo* and it is more stable, soluble and easier to synthesise and handle. Moreover, SSV can be used as the drug vehicle to give a better ED_{50}/ED_{90} determination. Formulation as the phosphate salt was therefore employed in the further *in vivo* test to determine the ED_{50}/ED_{90} values. Some of the most active quinolones were also selected and tested further for ED_{50}/ED_{90}. The results are shown in table 6.

Table 6. *In vivo* (oral) antimalarial activities of **32** and **52** against *Plasmodium berghei.*

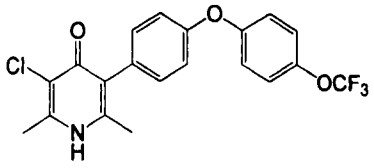
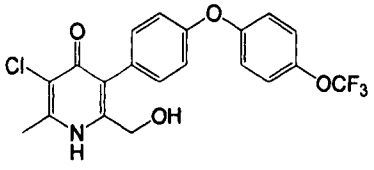
Compound	ED_{50} (mg/kg)	ED_{90} (mg/kg)
Atovaquone	0.07	0.11
Chloroquine	3.3	4.6
Artemether	3.1	5.8
32	12.75	27.3
94	10.37	15.17
95	1.87	4.72
96	2.6	6.5

Formulation as the phosphate salt significantly improved the potency *in vivo* as demonstrated by **95** with ED_{50}/ED_{90} values of 1.87/4.72 mg/kg compared to the parent **32** with ED_{50}/ED_{90} values of 12.75/27.3 mg/kg. Morpholine carbamate

prodrug (**94**) has also enhanced the *in vivo* activity (ED_{50}/ED_{90} = 10.37/15.17 mg/kg). Phosphate salt of **52** (**96**) also displayed a good *in vivo* activity with the ED_{50}/ED_{90} values of 2.6/6.5 mg/kg. As a comparison, chloroquine against the same strain had an ED_{50}/ED_{90} of 3.3/4.6 mg/kg while artemether showed the ED_{50}/ED_{90} at 3.1/5.8 mg/kg. The tested quinolone compounds **95** and **96** are of similar potency to the clinically used drug artemether.

The *in vivo* results together with the solubility measurements, demonstrated that the incorporation of a pyridine ring in the side chain (**32**) enhances the solubility (Table 7). Quinolone **32** has also demonstrated a better solubility than atovaquone and the GSK pyridines GW844520 and GSK9321121A, especially at the low pH. The solubility and *in vivo* activity are further improved when formulated as the phosphate salt as exemplified by **32** and **95**.

Table 7. The solubility measurements of selected quinolones at different pH. Solubility measured by BioFocus*.

Compound	Solubility (μM)		
	pH 7.4	**pH 4.5**	**pH 1**
Atovaquone	<0.01	-	<0.01
GW844520 	0.02	-	0.2
GSK9321121A 	1.0	-	2.7
CK-2-67 **(8)**	0.03	0.02	0.1
32	0.04	0.08	18
95	0.08	0.12	42

* BioFocus, Chesterford Research Park, Saffron Walden, UK.

Human Liver Microsomal Incubations

Encouraged by the *in vivo* activity, *in vitro* metabolism stabilities with human liver microsomes (1 mg/mL) were carried out for **32** and **52** at a concentration of 1 μM in the presence of NADPH for 0, 10, 30 and 60 mins. More than 60% of both of the test quinolones were still present after 60 min incubation. Their *in vitro* half-lives and intrinsic clearance values are recorded in the table below.

Compound	*In vitro* half-life t ½ min	Intrinsic clearance mL/min/kg
32	96.7	1.78
52	130.0	1.32

Table 8. Metabolic stability of **32** and **52** in Human Liver Microsomes.

***In vivo* pharmacokinetic parameters (rat)**

In vivo pharmacokinetic parameters of the morpholine prodrug (**94**) and the phosphate salt (**95**) were also studied. Parent compound **32** concentration in plasma samples were analysed by LC-MS/MS and the pharmacokinetic values were determined using PKsolutions2.0 software. Upon oral dosing in the rat of the morpholine pro-drug **94** (20 mg/kg), the observed pharmacokinetic parameters in plasma for the parent compound **32**, were: C_{max} 8.1 μg/mL, T_{max} 7.0 h, half life (T ½) of 20.3 h, a volume of distribution V_d of 2875.6 mL/kg, an area under the curve of $AUC_{0\text{-}t}$ 167.2 μg.h/mL and a calculated total clearance Cl_T was 98.0 mL/h/kg (Figure 10).

The pharmacokinetic features of **95** were also favourable, the observed parameters for the parent compound **32**, following oral administration (5 mg/kg) were: C_{max} 3.1 μg/mL, T_{max} 7.0 h, T ½ 10.6 h, V_d 1261.4 mL/kg, $AUC_{0\text{-}t}$ 57.9 μg.h/mL and a Cl_T of 82.1 mL/h/kg (Figure 11). These pharmacokinetic parameters are consistent with a once-daily oral dosing target product profile.

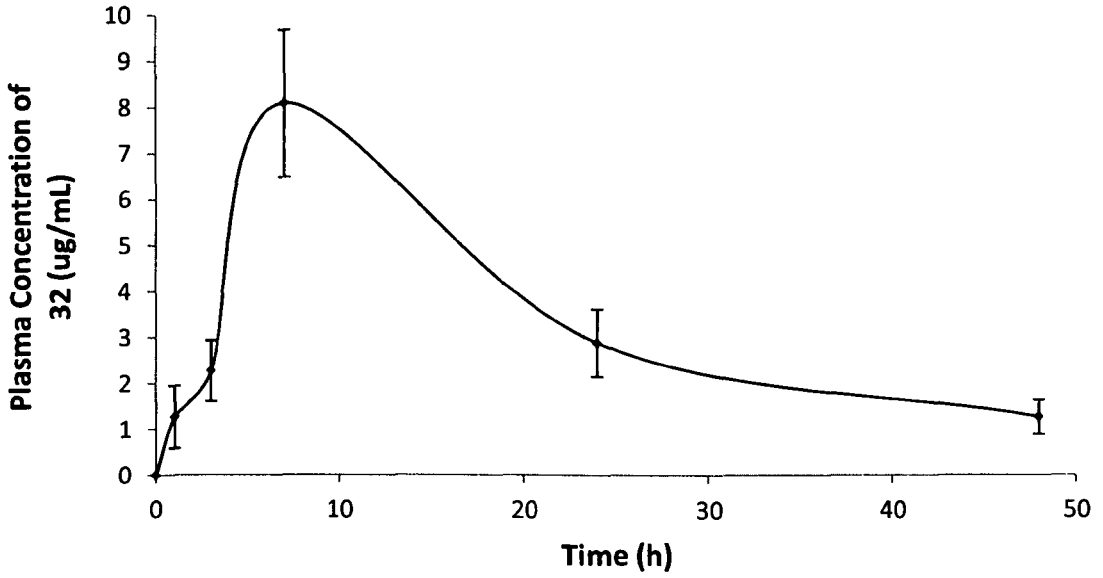


Figure 10. Plasma Concentration-Time curve of **32** in male *Wistar* rats after administration of the pro-drug **94** to (**20 mg/kg** (n=4)).

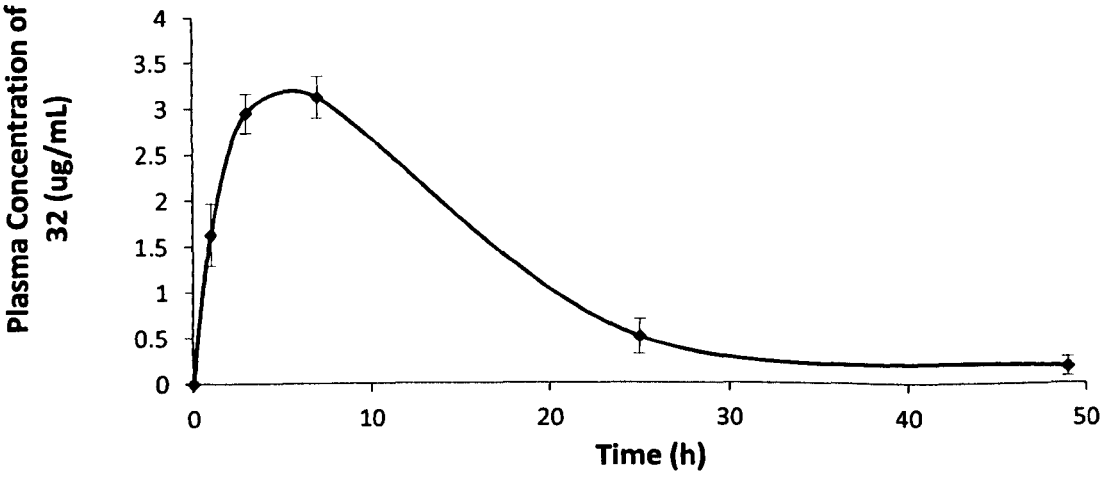


Figure 11. Plasma Concentration-Time profile of **32** in male *Wistar* rats after administration of **95** (**5 mg/kg** (n=4)).

2.4 Nanoparticle formulation

Aqueous nanodispersion is one of the latest strategies to overcome insolubility of the organic compounds in aqueous environments in order to improve drug solubility and subsequently the bioavailability and other pharmacokinetic properties of the drug.[49-50] Not only limited to pharmaceutical products, this technique has also been applied in various consumer products.[51-53] Rannard *et al*[49] work on aqueous nanodispersions of Triclosan (an antibacterial and antifungal agent used in many consumer products such as toothpastes and mouth washes) has exhibited enhanced activity than organic/aqueous solutions of Triclosan.

In light of the advantages of nanodispersion, collaboration work with Rannard group at the University of Liverpool had led to the development of a nanoparticle formulation for **32**. Initial screening of nanoformulation of **32** at 10% drug loading identified 3 possible combinations of excipients to formulate **32** as nanoparticles (Figure 12). The excipients applied in the screening are common additive materials used in pharmaceutical industries, their cost and safety properties are well established.

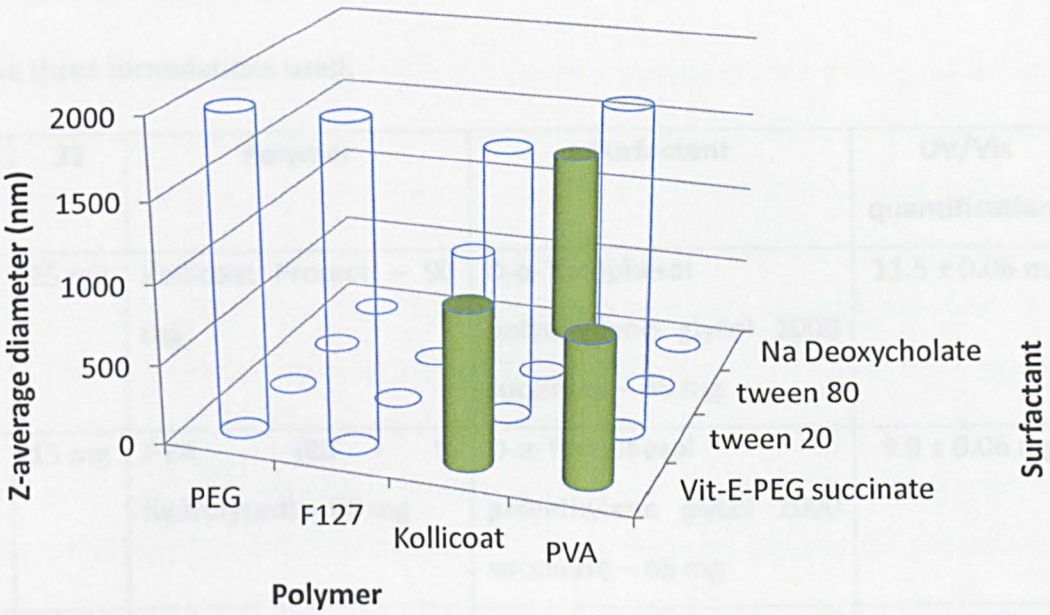


Figure 12. Initial screening interpretation.

The formulations were dispersed at 1mg/mL with regards to the active content and analysed by dynamic light scattering (DLS). The 3D bar charts represent a formulation screen in which the polymer and surfactant are varied. A green bar represents 'good' dispersion of the sample (no visible lumps); less than 1000 nm diameter; Polydispersity index (PDI) less than 0.5 and less than 10% standard deviation between measurements. A Clear bar represents the sample did not meet the requirements. Data provided by Dr Thomas McDonald, Rannard Group, Department of Chemistry, University of Liverpool.

Repeating the method* using the identified 3 formulas successfully prepared 150 mg of nanoformulated **32** for each formulation for *in vivo* studies. Preliminary *in vivo* Peters' 4 day suppressive test has demonstrated that formulation 2 showed 30% kill using water as the dosing vehicle at only 1.3 mg/kg, indicating an impressive improvement in drug solubility and bioavailability compared to the parent compound which has to be dosed in SSV for *in vivo* tests.

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* Method details: The nanoformulation was prepared by dissolving **32** in DMSO followed by addition to the aqueous solution of required polymer and surfactant. The dispersion was then vortexed to ensure mixing and followed by freeze-dried for 42 hours.

The three formulations used:

	32	Polymer	Surfactant	UV/Vis quantification[a]
1	15 mg	Kollicoat Protect – 90 mg	D-α-Tocopherol polyethylene glycol 1000 succinate – 45 mg	11.5 ± 0.06 mg
2	15 mg	PVA (80 % hydrolysed) – 90 mg	D-α-Tocopherol polyethylene glycol 1000 succinate – 45 mg	9.9 ± 0.06 mg
3	15 mg	Kollicoat Protect – 90 mg	sodium deoxycholate – 45 mg	11.4 ± 0.06 mg

[a] The active content in the dispersion

All three samples had mean particle diameters between 700-800 nm and zeta potentials between -3 to -6 mV.

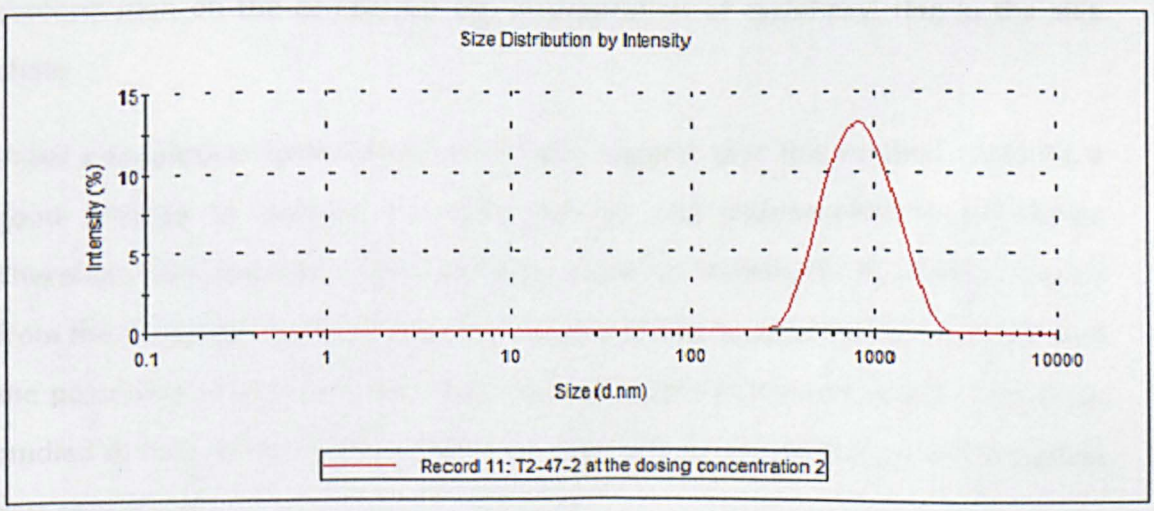


Figure 13. The size distribution of formulation 2. Graph provided by Dr Thomas McDonald.

2.5 Conclusions and future work

In summary, this chapter highlights the optimisation from CK-2-68 and the 4-6 step synthesis of a series of novel quinolones with bicyclic side chain, possessing potent antimalarial *in vitro* activities. Several of the compounds also showed selectively against the novel *Pf*NDH2 enzymatic target. Figure 14a summarises our medicinal chemistry strategy that has seen the development of two excellent lead compounds from the starting point HDQ by iterative, cycles of drug synthesis and *in vitro* antimalarial evaluation. Figure 14b depicts the target zones in light blue and it can be seen from this radar plot how our two molecules map onto the desired regions. Both **32** (SL-2-25) and **52** (SL-2-64) have appropriate polar surface area (PSA), molecular weight, hydrogen bond acceptors/donors and rotable bonds with a ClogP below 5. These Lipinski-like properties[54-55] have imparted excellent drug-like properties which include good oral activity and high metabolic stability.

Further work would focus on the SAR to discover the optimal ClogP windows for antimalarial activity. Work should also focus on the salt screening on the lead compound to find suitable counter ions to enhance the solubility, drug delivery and other properties, and also looking at the effect on solubility by changing the conformation on the compound, eg, incorporation of cyclohexyl ring in the side chain.

Initial nanoparticle formulation results also suggest that this method could be a good strategy to improve the bioavailability and pharmacokinetic properties. Therefore, considerable effort should be made to investigate this further. Apart from the complete *in vivo* profile, the stability of the nanoformulated material and the possibility of increasing the drug loading to 70% in the formulation should be studied to have better understanding on their effects and to lower the production cost respectively.

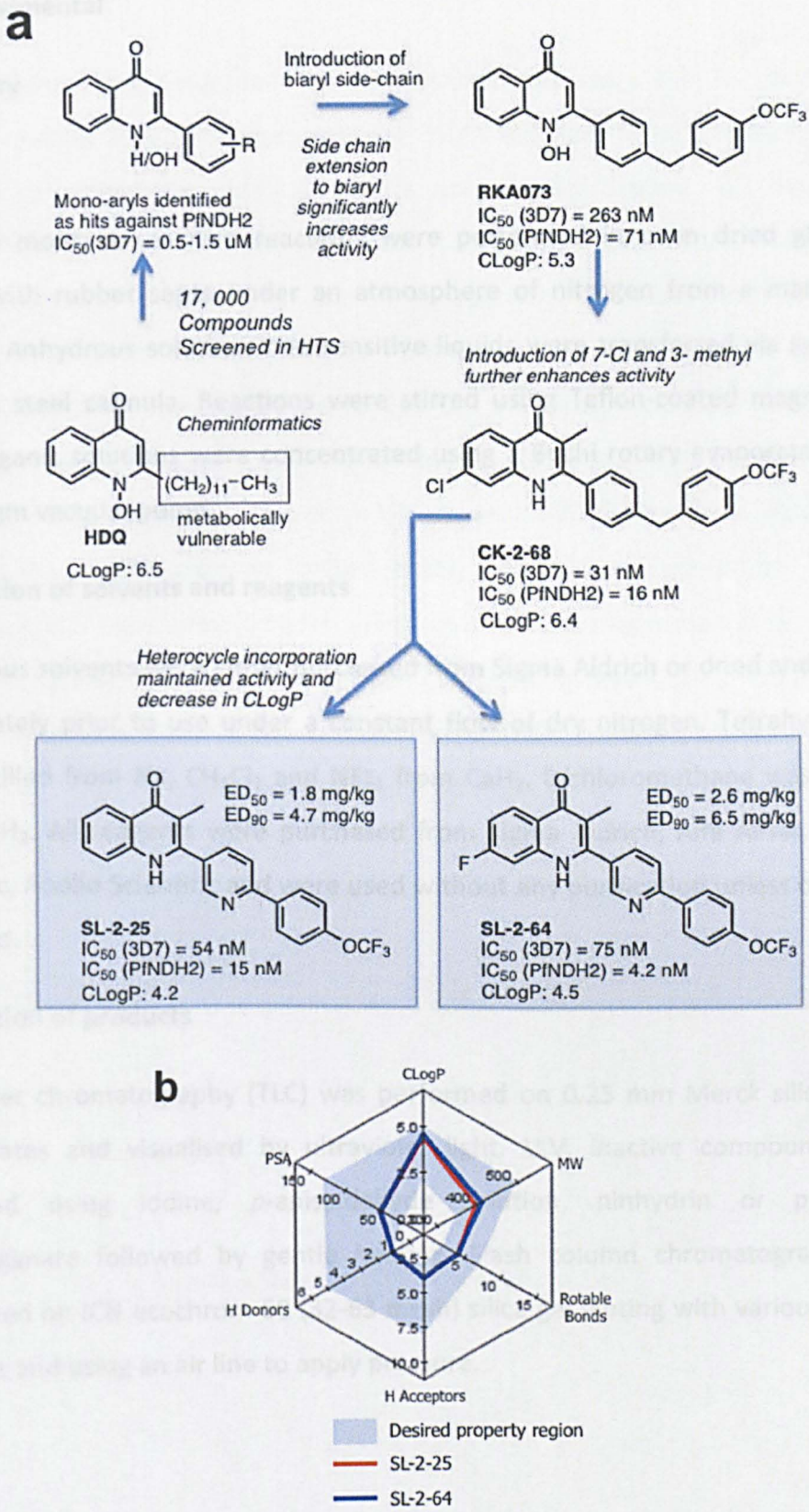


Figure 14. (a) Optimisation from High-throughput screen via CK-2-68 to **32** (SL-2-25) and **52** (SL-2-64). (b) Radar plot of physiochemical properties of **32** (SL-2-25, red line) and **52** (SL-2-64, blue line).

2.6 Experimental

Chemistry

General

Air- and moisture-sensitive reactions were performed in oven dried glassware sealed with rubber septa under an atmosphere of nitrogen from a manifold or balloon. Anhydrous solutions and sensitive liquids were transferred via syringe or stainless steel cannula. Reactions were stirred using Teflon-coated magnetic stir bars. Organic solutions were concentrated using a Buchi rotary evaporator with a diaphragm vacuum pump.

Purification of solvents and reagents

Anhydrous solvents were either purchased from Sigma Aldrich or dried and distilled immediately prior to use under a constant flow of dry nitrogen. Tetrahydrofuran was distilled from Na, CH_2Cl_2 and NEt_3 from CaH_2. Dichloromethane was distilled from CaH_2. All reagents were purchased from Sigma Aldrich, Alfa Aesar, Frontier Scientific, Apollo Scientific and were used without any purification unless otherwise indicated.

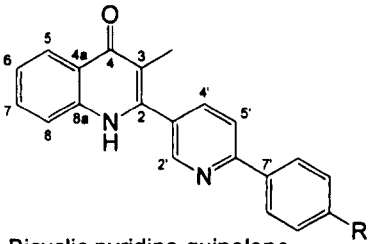
Purification of products

Thin layer chromatography (TLC) was performed on 0.25 mm Merck silica gel 60 F254 plates and visualised by ultraviolet light. U.V. inactive compounds were visualised using iodine, *p*-anisaldehyde solution, ninhydrin or potassium permanganate followed by gentle heating. Flash column chromatography was performed on ICN ecochrom 60 (32-63 mesh) silica gel eluting with various solvent mixtures and using an air line to apply pressure.

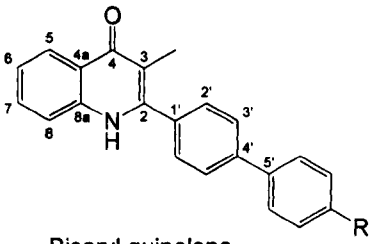
Analysis

Melting points were determined by a Gallenkamp apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker AMX 400 (400 MHz) spectrometer and reported as chemical shift on parts per million (ppm, δ) relative to tetramethylsilane as the internal reference, integration, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q =quartet, sex = sextet, m = multiplet), coupling constant (J, Hz), assignment. ^{13}C NMR spectra were recorded on Bruker AMX400 (100 MHz) spectrometer and reported in terms of chemical shift (ppm, δ) relative to residual solvent peak. Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded on a VG analytical 7070E machine, Fisons TRIO spectrometers using electron ionisation (EI) and chemical ionisation (CI), and Micromass LCT mass spectrometer using electron spray ionisation (ESI). All mass values are within error limits of ±5 ppm. Elemental analyses (%C, %H, %N) were either determined by the University of Liverpool Microanalysis Laboratory or the London Metropolitan University Elemental Analysis Service. Reported percentages are within error limits of ±0.5 %.

Numbering



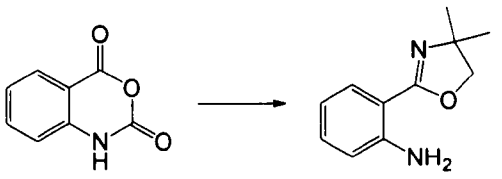
Bicyclic pyridine quinolone



Bisaryl quinolone

Synthesis

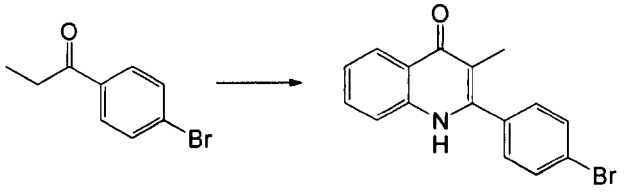
Preparation of **2-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)aniline 11.**



31 **11**

Isatoic anhydride (10 g, 61 mmol) was suspended in anhydrous chlorobenzene (100 mL) under nitrogen. 2-Methyl-2-amino-1-propanol (8.1 mL, 85 mmol) was added to the suspension followed by anhydrous $ZnCl_2$ (0.8 g, 6.1 mmol), and the mixture was heated to reflux for 24 h. After the reflux period it was cooled to room temperature. The solvent was removed under reduced pressure and the residue was added to ethyl acetate and the resulting solution was washed with brine. The aqueous layer was extracted with ethyl acetate (50 mL x 2) and the combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure to give the crude product. Purification by column chromatography using 10% ethyl acetate in hexane gave the title compound (8.16 g, 70 %) as a white solid: R_f = 0.53, 20% ethyl acetate in hexane; mp 111 – 112°C (lit.[56] 103–105°C).; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (dd, *J* = 7.9, 1.6 Hz, 1H, H-3), 7.19 (ddd, *J* = 8.5, 7.2, 1.6 Hz, 1H, H-5), 6.69 (dd, *J* = 8.2, 0.9 Hz, 1H, H-6), 6.65 (ddd, *J* = 8.2, 7.2, 1.1 Hz, 1H, H-4), 6.08 (s, 2H), 3.99 (s, 2H, CH_2), 1.36 (s, 6H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.50, 148.89, 132.29, 129.87, 116.43, 116.01, 109.68, 77.79, 68.17, 29.12; IR ν_{max} (neat)/cm^{-1} 3442.3, 3284.2, 2977.6, 2931.3, 1631.5, 1610.3, 1564.0, 1456.0 and 1159.0; MS (CI) $C_{11}H_{15}N_2O$ $[M+H]^+$ m/z 191.3; Anal. $C_{11}H_{14}N_2O$ requires C 69.45%, H 7.42%, N 14.73%, found C 69.51%, H 7.52%, N 14.69%.

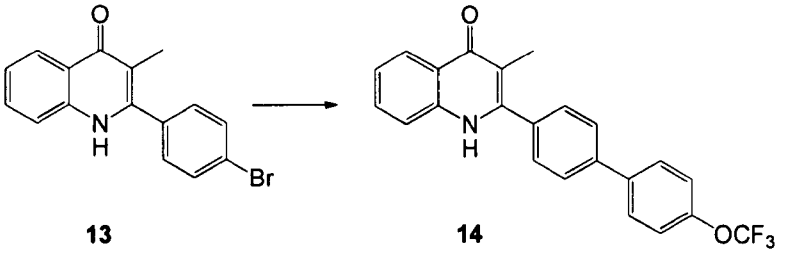
Preparation of **2-(4-Bromophenyl)-3-methylquinolin-4(1H)-one 13.**



12 **13**

To a mixture of **11** (2.03 g, 10.67 mmol) and 4'-bromopropiophenone **12** (2.27 g, 10.67 mmol) in anhydrous n-butanol (20 mL) were added trifluoromethanesulfonic acid (0.18 mL, 2.13 mmol, 0.2 equiv). The mixture was heated to 130°C for 24 h (followed by tlc). The reaction was cooled and the solvent was removed under reduced pressure. Sat. $NaHCO_3$ (aq) was added and the resulting aqueous solution was extracted with ethyl acetate (x 3), the combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered and concentrated to a yellow solid. The crude product was triturated with diethyl ether to give the title compound (2.5 g, 70 %) as a white solid: R_f = 0.24, 50% ethyl acetate in hexane; mp 251 – 253 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.61 (s, 1H, N-H), 8.12 (d, *J* = 7.7 Hz, 1H, H-5), 7.82 – 7.76 (m, 2H, H-3'), 7.66 – 7.60 (m, 1H, H-7), 7.60 – 7.56 (m, 1H, H-8), 7.56 – 7.50 (m, 2H, H-2'), 7.30 (ddd, *J* = 8.1, 6.5, 1.5 Hz, 1H, H-6), 1.88 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.63 (C=O), 146.56, 139.42, 134.15, 131.50 (C-3'), 131.33, 131.14 (C-2'), 124.92, 123.02, 122.83, 122.72, 118.09, 114.43, 12.02 (CH_3); HRMS (ESI) $C_{16}H_{13}NO^{79}Br$ $[M+H]^+$ requires 314.0181, found 314.0179 (100 %); $C_{16}H_{13}NO^{81}Br$ $[M+H]^+$ requires 316.0160, found 316.0154 (97.2 %); Anal. $C_{16}H_{12}NOBr$ requires C 61.17%, H 3.85%, N 4.46%, found C 61.04%, H 4.00%, N 4.84%.

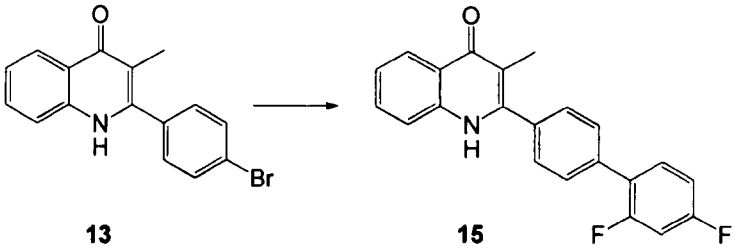
Preparation of **3-Methyl-2-(4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)quinolin-4(1H)-one 14.**



13 **14**

13 (0.25 g, 0.81 mmol), $PdCl_2$(dppf) (0.03 g, 0.04 mmol, 0.05 equiv) and anhydrous potassium carbonate (0.34 g, 2.42 mmol, 3 equiv) in anhydrous 1,4-dioxane (10 mL) were stirred under nitrogen environment at room temperature for 10 minutes. 4-Trifluoromethoxyphenylboronic acid (0.33 g, 1.61 mmol, 2 equiv) was added and then the reaction system was degassed and refilled with N_2 3 times. The mixture was heated to 100°C for 24 h (followed by tlc) and the mixture was cooled, diluted with 50% ethyl acetate in hexane, and filtered through a pad of magnesium sulphate-silica. The filter pad was washed with 2% methanol in ethyl acetate. The filtrate was concentrated to give a solid and the crude product was purified by column chromatography using 80% ethyl acetate in hexane to give the title compound (0.22 g, 70 %) as a white solid: R_f = 0.48, 80% ethyl acetate in hexane; mp 295 – 296 °C; ^{1}H NMR (400 MHz, DMSO) δ 11.75 (s, 1H, N-H), 8.15 (d, *J* = 7.9 Hz, 1H, H-5), 7.91 (dt, *J* = 5.2, 3.0 Hz, 4H, ArH'), 7.69 (d, *J* = 8.3 Hz, 2H, ArH'), 7.65 – 7.60 (m, 2H, H-7 + H-8), 7.52 (d, *J* = 8.0 Hz, 2H, ArH'), 7.31 (ddd, *J* = 8.1, 5.4, 2.6 Hz, 1H, H-6), 1.95 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.97 (C=O), 148.56, 147.80, 140.14, 139.89, 139.04, 135.14, 131.51, 130.09 (C-2'), 129.15 (C-6'), 127.26 (C-3'), 125.29, 123.51, 122.95, 121.97 (C-7'), 121.77, 118.76, 114.72, 12.63 (CH_3); IR ν_{max} (neat)/cm^{-1} 2979.5, 2886.9, 1627.6 1587.1, 1554.3, 1500.4, 1440.6, 1249.7 and 1159.0. HRMS (ESI) $C_{23}H_{17}NO_2F_3$ $[M+H]^+$ requires 396.1211, found 396.1198; Anal. $C_{23}H_{16}NO_2F_3$ requires C 69.87%, H 4.08%, N 3.54%, found C 69.71%, H 4.04%, N 3.59%.

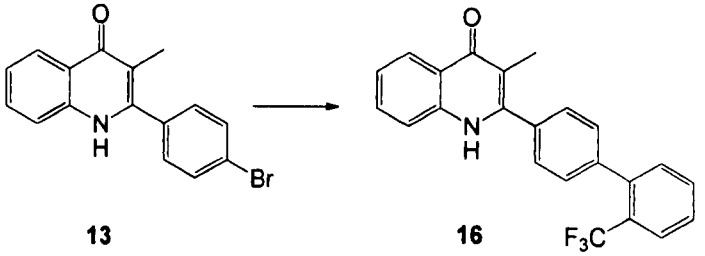
Preparation of **2-(2',4'-Difluoro-[1,1'-biphenyl]-4-yl)-3-methylquinolin-4(1H)-one 15.**



13 **15**

15 was prepared from 2,4-difluorophenylboronic acid (0.26 g, 1.65 mmol) according to the procedure for the preparation of **14**. Purification by flash column chromatography using 80% ethyl acetate in hexane gave **15** (0.18 g, 65%) as a white solid: R_f = 0.56, ethyl acetate; mp > 315°C; ^{1}H NMR (400 MHz, DMSO) δ 11.66 (s, 1H, N-H), 8.14 (d, *J* = 7.8 Hz, 1H, H-5), 7.77 – 7.71 (m, 2H, H-3'), 7.71 – 7.65 (m, 3H, H-7 + H-2'), 7.65 – 7.60 (m, 2H, H-8 + H-10'), 7.49 – 7.40 (m, 1H, H-7'), 7.34 – 7.29 (m, 1H, H-6), 7.29 – 7.22 (m, 1H, H-9'), 1.95 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 177.06 (C=O), 160.89 (C-F), 160.77 (C-F), 147.51, 139.88, 135.61, 134.94, 132.35 (C-2'), 131.68, 129.72 (C-3'), 129.30, 128.53, 125.30 (C-5), 124.59, 124.49, 123.43 (C-6), 123.08, 118.53 (C-10'), 114.81, 112.65 (d, *J*=24.8 Hz, C-9'), 105.05 (t, *J*=26.5 Hz, C-7'), 12.57 (CH_3); IR ν_{max} (neat)/cm^{-1} 2977.6, 2886.9, 1637.3 (C=O), 1589.1, 1471.4, 1259.3, 1145.5 and 966.2; HRMS (ESI) $C_{22}H_{16}NOF_2$ $[M+H]^+$ requires 348.1200, found 348.1183; Anal. $C_{22}H_{15}NOF_2$ requires C 76.07%, H 4.35%, N 4.03%, found C 76.16%, H 4.29%, N 3.97%.

Preparation of **3-Methyl-2-(2'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)quinolin-4(1H)-one 16.**

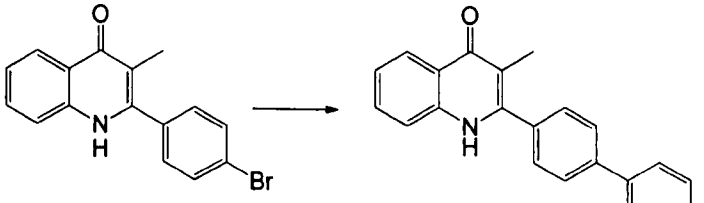


13 **16**

16 was prepared from 2-(trifluoromethyl)phenylboronic acid (0.31 g, 1.62 mmol) according to the procedure for the preparation of **14**. Purification by flash column

chromatography using 80% ethyl acetate in hexane gave **16** (0.151 g, 50%) as a pale pink solid: R_f = 0.6, ethyl acetate; mp 270 – 271°C; ^{1}H NMR (400 MHz, DMSO) δ 11.71 (s, 1H, N-H), 8.21 – 8.07 (m, 1H, H-5), 7.90 (d, *J* = 7.7 Hz, 1H, H-10'), 7.78 (t, *J* = 7.5 Hz, 1H, H-7'), 7.72 – 7.59 (m, 5H, H-7 + H-8 + H-3' + H-9'), 7.54 (d, *J* = 8.1 Hz, 2H, H-2'), 7.48 (d, *J* = 7.5 Hz, 1H, H-8'), 7.32 (ddd, *J* = 8.1, 6.4, 1.6 Hz, 1H, H-6), 1.94 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 177.07 (C=O), 147.51, 140.68, 140.33, 139.93, 134.85, 132.83 (C-7'), 132.53 (C-8), 131.65, 129.24 (C-2'), 129.01 (C-3'), 128.77, 127.22, 126.57, 125.29, 123.46 (C-6), 123.21, 123.05, 118.59, 114.90, 12.64 (CH_3); HRMS (ESI) $C_{23}H_{17}NOF_3$ $[M+H]^+$ requires 380.1262, found 380.1262; $C_{23}H_{16}NOF_3{}^{23}Na$ $[M+Na]^+$ requires 402.1082, found 402.1089; Anal. $C_{23}H_{16}NOF_3$ requires C 72.82%, H 4.25%, N 3.69%, found C 73.16%, H 4.03%, N 3.59%.

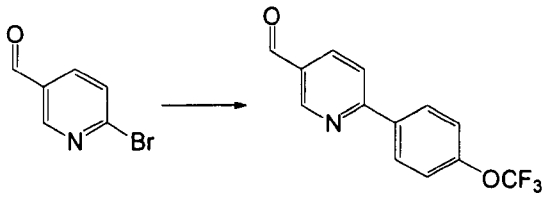
Preparation of **2-(2'-Fluoro-[1,1'-biphenyl]-4-yl)-3-methylquinolin-4(1H)-one 17.**



13 **17**

17 was prepared from **13** (85.4 mg, 0.27 mmol) and 2-fluorophenylboronic acid (85 mg, 0.60 mmol, 2.25 equiv) according to the procedure for the preparation of **14**. Purification by column chromatography using 5% methanol in dichloromethane gave **17** (55 mg, 62%) as a brown solid: R_f = 0.4, 5% methanol in dichloromethane; mp 320 – 322°C; ^{1}H NMR (400 MHz, DMSO) δ 11.67 (s, 1H, N-H), 8.14 (d, *J* = 8.0 Hz, 1H, H-5), 7.77 (dd, *J* = 8.2, 1.4 Hz, 2H, H-2'), 7.72 – 7.67 (m, 2H, H-3'), 7.67 – 7.61 (m, 3H, H-7 + H-9' + H-10'), 7.53 – 7.45 (m, 1H, H-7'), 7.42 – 7.34 (m, 2H, H-8 + H-8'), 7.31 (ddd, *J* = 8.1, 5.1, 2.9 Hz, 1H, H-6), 1.95 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 177.06 (C=O), 158.31, 147.56, 139.88, 136.44, 134.89, 131.67, 131.18, 129.66 (C-2'), 129.32, 127.95, 125.52, 125.31, 123.44, 123.06, 118.52, 116.72, 116.50, 114.81, 12.57 (CH_3); HRMS (ESI) $C_{22}H_{17}NOF$ $[M+H]^+$ requires 330.1294, found 330.1297.

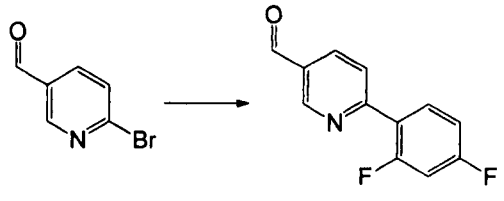
Preparation of **6-(4-(Trifluoromethoxy)phenyl)nicotinaldehyde 19.**



18 **19**

To potassium carbonate (12.2 g, 88.27 mmol) in distilled water (30 mL) was added anhydrous tetrahydrofuran (80 mL) under nitrogen. 6-Bromopyridine-3-carboxaldehyde (5.5 g, 29.57 mmol) was added followed by tetrakis(triphenylphosphine)palladium (0) (2.73 g, 2.36 mmol). The mixture was allowed to stir at room temperature for 5 min under N_2. 4-(Trifluoromethoxy)benzeneboronic acid (6.77 g, 32.88 mmol) was added. The resulting mixture was heated to 80°C for 24 h (followed by tlc) and then allowed to cool to room temperature. The mixture was extracted with ethyl acetate (x 3), washed with brine, dried over $MgSO_4$, filtered and concentrated to an oil. The crude product was purified by column chromatography using 10% ethyl acetate in hexane to give the title compound (6.9 g, 87 %) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.16 (s, 1H, C<u>H</u>O), 9.14 (d, *J* = 1.6 Hz, 1H, H-2), 8.25 (dd, *J* = 8.2, 2.2 Hz, 1H, H-4), 8.19 – 8.09 (m, 2H, H-8), 7.90 (d, *J* = 8.2 Hz, 1H, H-5), 7.36 (d, *J* = 8.1 Hz, 2H, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.73 (<u>C</u>HO), 161.06, 152.79 (C-2), 151.25, 137.15, 136.88, 130.45, 129.56 (C-8), 122.11, 121.57 (C-9), 120.88, 119.54; HRMS (ESI) $C_{13}H_9NO_2F_3$ $[M+H]^+$ requires 268.0585, found 268.0592.

Preparation of **6-(2,4-Difluorophenyl)nicotinaldehyde 20.**

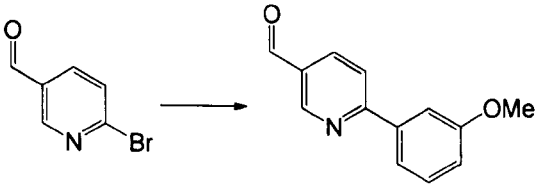


18 **20**

20 was prepared from 2,4-difluorophenylboronic acid (0.87 g, 5.5 mmol) according to the procedure for the preparation of **19**. Purification by flash column

chromatography using 5% ethyl acetate in hexane gave **20** (0.8 g, 69%) as a white solid; MP 84°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H, CHO), 9.15 (d, *J* = 2.1 Hz, 1H, H-2), 8.24 (dd, *J* = 8.2, 2.2 Hz, 1H), 8.15 (td, *J* = 8.8, 6.6 Hz, 1H, H-12), 7.97 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.11 – 7.02 (m, 1H, H-11), 6.96 (ddd, *J* = 11.3, 8.7, 2.5 Hz, 1H, H-9); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.77 (C=O), 164.46 (dd, *J* = 253.3, 12.3 Hz, C-8), 161.50 (dd, *J* = 254.5, 12.2 Hz, C-10), 157.73 (C-6), 152.37 (C-2), 136.91 (C-4), 133.11, 130.29, 124.67, 122.92 (C-7), 112.71 (C-11), 105.78 (C-9); MS (CI) $C_{12}H_8NOF_2$ $[M+H]^+$ m/z 220.3; Anal. $C_{12}H_7NOF_2$ requires C 65.76%, H 3.22%, N 6.39%, found C 65.82%, H 3.26%, N 6.27%.

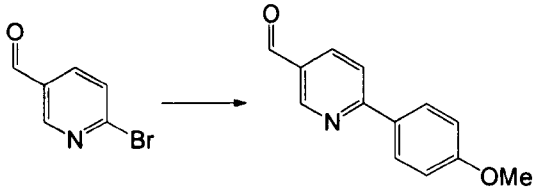
Preparation of **6-(3-Methoxyphenyl)nicotinaldehyde 21.**



18 **21**

21 was prepared from 3-methoxybenzeneboronic acid (2.69 g, 17.7 mmol) according to the procedure for the preparation of **19**. Purification by column chromatography using 30% ethyl acetate in hexane gave **21** (2.90 g, 76 %) as a pale yellow solid: R_f = 0.58, 50 % ethyl acetate in hexane; mp 74°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.15 (s, 1H, CHO), 9.13 (d, *J* = 2.0 Hz, 1H, H-2), 8.24 (dd, *J* = 8.3, 2.2 Hz, 1H, H-4), 7.91 (d, *J* = 8.3 Hz, 1H, H-5), 7.71 – 7.66 (m, 1H, H-8), 7.64 (d, *J* = 7.7 Hz, 1H, H-12), 7.43 (t, *J* = 8.0 Hz, 1H, H-11), 7.05 (dd, *J* = 8.2, 2.5 Hz, 1H, H-10), 3.92 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.51 (C=O), 161.98, 160.18, 152.35 (C-2), 139.40, 136.53 (C-4), 130.00 (C-11), 129.93, 120.78 (C-5), 119.89 (C-12), 116.49 (C-10), 112.57 (C-8), 55.45 (OCH_3); MS (CI) $C_{13}H_{12}NO_2$ $[M+H]^+$ m/z 214.2.

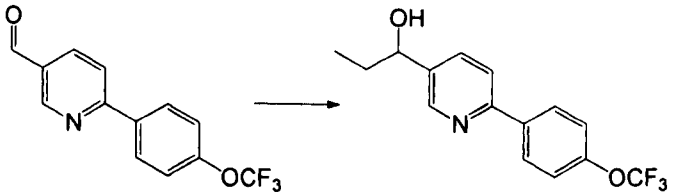
Preparation of **6-(4-Methoxyphenyl)nicotinaldehyde 22.**



18 **22**

22 was prepared from 4-methoxybenzeneboronic acid (2.69 g, 17.7 mmol) according to the procedure for the preparation of **19**. Purification by column chromatography using 30% ethyl acetate in hexane gave **22** (2.92 g, 77 %) as a white solid: R_f = 0.56, 50 % ethyl acetate in hexane; MP 106 – 107°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.11 (s, 1H, CHO), 9.08 (dd, *J* = 2.2, 0.8 Hz, 1H, C-2), 8.19 (dd, *J* = 8.3, 2.2 Hz, 1H, H-4), 8.12 – 8.03 (m, 2H, H-8), 7.85 (d, *J* = 8.3 Hz, 1H, H-5), 7.08 – 6.98 (m, 2H, H-9), 3.89 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.91(C=O), 162.23, 162.06, 153.00, 136.76, 130.89, 129.68, 129.49 (C-8), 120.08, 114.81 (C-9), 55.86; MS (CI) $C_{13}H_{12}NO_2$ $[M+H]^+$ m/z 214.2; Anal. $C_{13}H_{11}NO_2$ requires C 73.23%, H 5.20%, N 6.57%, found C 72.91%, H 5.20%, N 6.48%.

Preparation of **1-(6-(4-(Trifluoromethoxy)phenyl)pyridin-3-yl)propan-1-ol 23.**

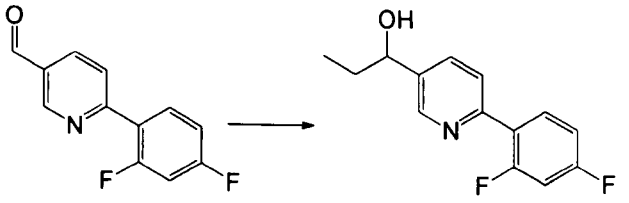


19 **23**

19 (6.21 g, 23.2 mmol) in anhydrous tetrahydrofuran (15 mL) was cooled to 0°C and ethylmagnesium bromide (1.0 M in THF) (30 mL, 28 mmol, 1.2 equiv) was added and the yellow solution was allowed to stir at 0°C for 1 h. The reaction was warmed to room temperature and 2N HCl (aq) (approx. 30 mL) was added to quench the reaction followed by water. The reaction mixture was extracted with ethyl acetate (x 3), washed with brine, dried over $MgSO_4$, filtered and concentrated to yellow oil. The crude product was purified by column chromatography using 25% ethyl acetate in hexane to give the title compound (5.02 g, 73 %) as a colourless oil: R_f = 0.38,

50% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, *J* = 2.1 Hz, 1H, H-2), 8.06 – 7.95 (m, 2H, H-8), 7.76 (dd, *J* = 8.2, 2.2 Hz, 1H, H-4), 7.67 (d, *J* = 8.2 Hz, 1H, H-5), 7.30 (d, *J* = 8.1 Hz, 2H, H-9), 4.68 (t, *J* = 6.5 Hz, 1H, C<u>H</u>OH), 2.56 (s, 1H, OH), 1.96 – 1.71 (m, 2H, CH_2), 0.95 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.66, 150.24, 148.30 (C-2), 138.99, 138.10, 135.04, 128.72 (C-8), 121.46 (C-9), 120.88 (d, J = 257 Hz, OCF_3), 120.65, 73.76 (<u>C</u>HOH), 32.29 (CH_2), 10.30 (CH_3); HRMS (ESI) $C_{15}H_{15}NO_2F_3$ $[M+H]^+$ requires 298.1055, found 298.1043; Anal. $C_{15}H_{14}NO_2F_3$ requires C 60.06%, H 4.75%, N 4.71%, found C 60.41%, H 4.81%, N 4.77%.

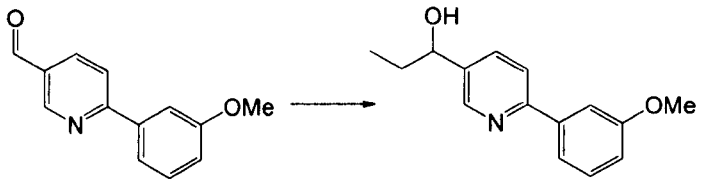
Preparation of **1-(6-(2,4-Difluorophenyl)pyridin-3-yl)propan-1-ol 24.**



20 **24**

24 was prepared from **20** (0.76 g, 3.44 mmol) according to the procedure for the preparation of **23**. Purification by column chromatography using 25% ethyl acetate in hexane gave **24** (0.56 g, 65 %) as a yellow oil: R_f = 0.44, 50% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H, H-2), 8.00 (td, *J* = 8.8, 6.7 Hz, 1H, H-12), 7.77 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.74 (ddd, *J* = 8.2, 2.0, 1.0 Hz, 1H), 7.04 – 6.97 (m, 1H, H-11), 6.92 (ddd, *J* = 11.3, 8.8, 2.5 Hz, 1H, H-9), 4.72 (td, *J* = 6.6, 3.6 Hz, 1H, C<u>H</u>OH), 2.02 – 1.95 (br. s, 1H, OH), 1.95 – 1.75 (m, 2H, CH_2), 0.98 (t, *J* = 7.4 Hz, 3H, CH_3); IR ν_{max} (neat)/cm^{-1} 3182.0 (O-H), 2923.6, 2854.1, 1619.9, 1598.7, 1510.0, 1475.3, 1147.4, 1105.0, 970.0 and 819.6; HRMS (ESI) $C_{14}H_{14}NOF_2$ $[M+H]^+$ requires 250.1043, found 250.1049.

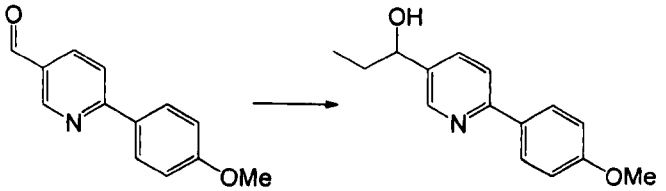
Preparation of **1-(6-(3-Methoxyphenyl)pyridin-3-yl)propan-1-ol 25.**



21 **25**

25 was prepared from **21** (2.32 g, 10.88 mmol) according to the procedure for the preparation of **23**. Purification by column chromatography using 50% ethyl acetate in hexane gave **25** (1.90 g, 72 %) as a pale yellow solid: R_f = 0.22, 50% ethyl acetate in hexane; mp 82°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (d, *J* = 2.1 Hz, 1H, H-2), 7.78 (dd, *J* = 8.2, 2.1 Hz, 1H), 7.72 (dd, *J* = 8.2, 0.7 Hz, 1H), 7.59 (dd, *J* = 2.4, 1.7 Hz, 1H), 7.57 – 7.50 (m, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 6.97 (ddd, *J* = 8.2, 2.6, 0.9 Hz, 1H), 4.72 (t, *J* = 5.5 Hz, 1H, C<u>H</u>OH), 3.90 (s, 3H, OCH_3), 1.94 (d, *J* = 2.8 Hz, 1H, OH), 1.93 – 1.72 (m, 2H, CH_2), 0.97 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.47, 156.98, 148.19 (C-2), 141.02, 138.57, 134.83 (C-4), 130.15 (C-11), 120.89 (C-5), 119.65 (C-12), 115.47 (C-10), 112.30 (C-8), 73.98, 55.80, 32.31, 10.37; HRMS (CI) $C_{15}H_{18}NO_2$ $[M+H]^+$ requires 244.1332, found 244.1333; Anal. $C_{15}H_{17}NO_2$ requires C 74.05%, H 7.04%, N 5.76%, found C 73.83%, H 7.12%, N 5.49%.

Preparation of **1-(6-(4-Methoxyphenyl)pyridin-3-yl)propan-1-ol 26.**

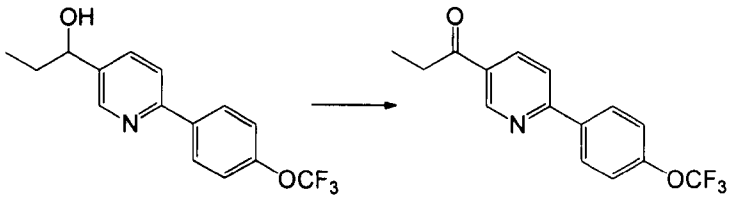


22 **26**

26 was prepared from **22** (2.62 g, 12.33 mmol) according to the procedure for the preparation of **23**. Purification by column chromatography using 50% ethyl acetate in hexane gave **26** (2.05 g, 69 %) as a pale yellow solid: R_f = 0.31, 50% ethyl acetate in hexane; mp 91 – 92°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (d, *J* = 2.2 Hz, 1H, H-2), 8.02 – 7.92 (m, 2H, H-8), 7.74 (dd, *J* = 8.2, 2.3 Hz, 1H, H-4), 7.67 (dd, *J* = 8.2, 0.7 Hz, 1H, H-5), 7.04 – 6.96 (m, 2H, H-9), 4.69 (td, *J* = 6.7, 3.0 Hz, 1H, C<u>H</u>OH), 3.87 (s, 3H, OCH_3), 1.93 (d, *J* = 3.8 Hz, 1H, OH), 1.91 – 1.73 (m, 2H, CH_2), 0.96 (t, *J* = 7.4 Hz, 3H,

CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.50, 160.82, 156.92, 148.14 (C-2), 134.78, 132.19, 128.51 (C-8), 120.00, 114.53 (C-9), 74.04, 55.77, 32.25, 10.39; MS (CI) $C_{15}H_{18}NO_2$ $[M+H]^+$ m/z 244; Anal. $C_{15}H_{17}NO_2$ requires C 74.05%, H 7.04%, N 5.76%, found C 74.03%, H 7.16%, N 5.63%.

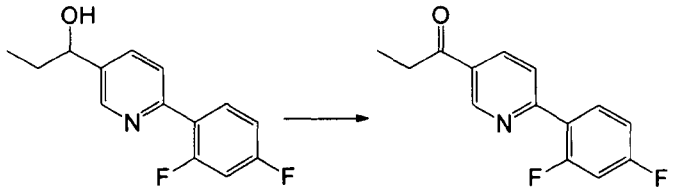
Preparation of **1-(6-(4-(Trifluoromethoxy)phenyl)pyridin-3-yl)propan-1-one 27.**



23 **27**

To **23** (4.93 g, 16.6 mmol) in dry dichloromethane (40 mL) was added pyridinium chlorochromate (5.47 g, 25.4 mmol, 1.5 equiv). The orange mixture was allowed to stir at room temperature for 2.5 h (followed by tlc). The dark mixture was then diluted with diethyl ether (40 mL) and filtered through a pad of silica to remove the reagent and washed with diethyl ether. The colourless filtrate was concentrated to give the title compound (4.12 g, 85 %) as a white solid: R_f = 0.78, 50% ethyl acetate in hexane; mp 120 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 9.24 (d, *J* = 2.2 Hz, 1H, H-2), 8.32 (dd, *J* = 8.3, 2.2 Hz, 1H, H-4), 8.18 – 8.04 (m, 2H, H-8), 7.83 (dd, *J* = 8.3, 0.6 Hz, 1H, H-5), 7.35 (d, *J* = 8.1 Hz, 2H, H-9), 3.07 (q, *J* = 7.2 Hz, 2H, CH_2), 1.28 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.55 (C=O), 159.63, 151.00, 150.15 (C-2), 137.15, 136.80, 130.98, 129.30 (C-8), 121.54 (C-9), 120.84 (q, *J* = 257 Hz, OCF_3), 120.46, 32.60 (CH_2), 8.35 (CH_3); HRMS (ESI) $C_{15}H_{13}NO_2F_3$ $[M+H]^+$ requires 296.0898, found 296.0901; Anal. $C_{15}H_{12}NO_2F_3$ requires C 61.02%, H 4.10%, N 4.74%, found C 61.42%, H 4.23%, N 4.40%.

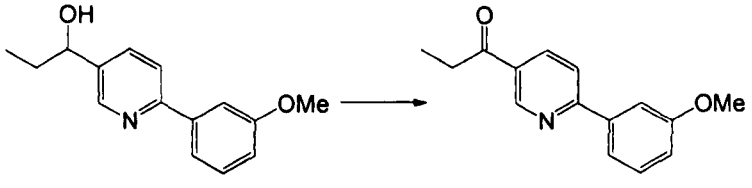
Preparation of **1-(6-(2,4-Difluorophenyl)pyridin-3-yl)propan-1-one 28.**



24 **28**

28 was prepared from **24** (0.55 g, 2.19 mmol) according to the procedure for the preparation of **27**. Filtration through a pad of silica gave **28** (0.38 g, 70 %) as a white solid: R_f = 0.78, 50% ethyl acetate in hexane; mp 84 – 85°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (d, *J* = 1.6 Hz, 1H, H-2), 8.30 (dd, *J* = 8.3, 2.3 Hz, 1H, H-4), 8.11 (td, *J* = 8.9, 6.6 Hz, 1H, H-12), 7.89 (dd, *J* = 8.3, 1.4 Hz, 1H, H-5), 7.09 – 7.01 (m, 1H, H-11), 6.95 (ddd, *J* = 11.3, 8.7, 2.5 Hz, 1H, H-9), 3.06 (q, *J* = 7.2 Hz, 2H, CH_2), 1.28 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.61 (C=O), 164.22 (dd, *J* = 252.9, 12.2 Hz, C-8), 161.37 (dd, *J* = 254.1, 12.0 Hz, C-10), 156.31, 149.96 (C-2), 136.40 (C-4), 132.88 (C-12), 130.85, 124.25 (C-5), 123.16, 112.60 (C-11), 105.03 (C-9), 32.61 (CH_2), 8.34 (CH_3); HRMS (ESI) $C_{14}H_{12}NOF_2[M+H]^+$ requires 248.0887, found 248.0892; Anal. $C_{14}H_{11}NOF_2$ requires C 68.01%, H 4.48%, N 5.67%, found C 68.15%, H 4.34%, N 5.65%.

Preparation of **1-(6-(3-Methoxyphenyl)pyridin-3-yl)propan-1-one 29.**

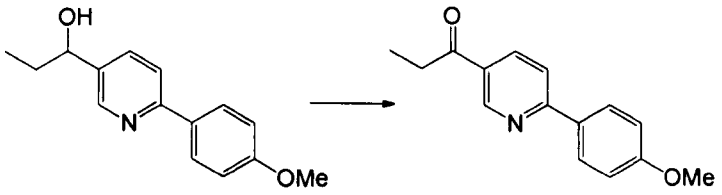


25 **29**

29 was prepared from **25** (2.09 g, 8.6 mmol) according to the procedure for the preparation of **27**. Purification by column chromatography using 50% ethyl acetate in hexane gave **29** (1.76 g, 85 %) as a white solid: R_f =0.67, 5% ethyl acetate in dichloromethane; mp 58 – 60°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (d, *J* = 1.7 Hz, 1H, H-2), 8.30 (dd, *J* = 8.3, 2.2 Hz, 1H, H-4), 7.84 (d, *J* = 8.3 Hz, 1H, H-5), 7.69 – 7.65 (m, 1H, H-8), 7.61 (d, *J* = 7.8 Hz, 1H, H-12), 7.41 (t, *J* = 8.0 Hz, 1H, H-11), 7.03 (ddd, *J* = 8.1, 2.5, 0.7 Hz, 1H, H-10), 3.91 (s, 3H, OCH_3), 3.06 (q, *J* = 7.2 Hz, 2H, CH_2), 1.27 (t, *J* =

7.2 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.67 (C=O), 160.90 (C-6), 160.58 (C-9), 150.02 (C-2), 140.04 (C-7), 136.63 (C-4), 130.85 (C-3), 130.32 (C-11), 120.74 (C-5), 120.09 (C-12), 116.52 (C-8), 112.81 (C-10), 55.84 (OCH_3), 32.56 (CH_2), 8.39 (CH_3); MS (CI) $C_{15}H_{16}NO_2$ $[M+H]^+$ m/z 242.3; Anal. $C_{15}H_{15}NO_2$ requires C 74.67%, H 6.27%, N 5.81%, found C 74.21%, H 6.42%, N 5.70%.

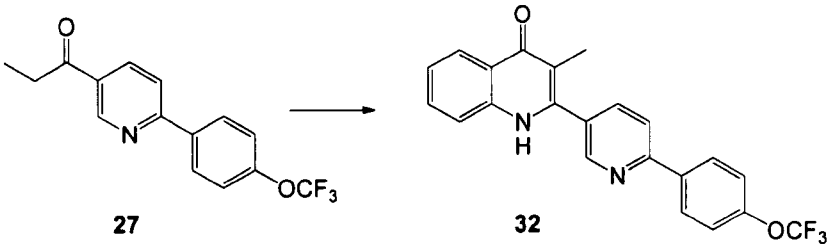
Preparation of **1-(6-(4-Methoxyphenyl)pyridin-3-yl)propan-1-one 30.**



26 **30**

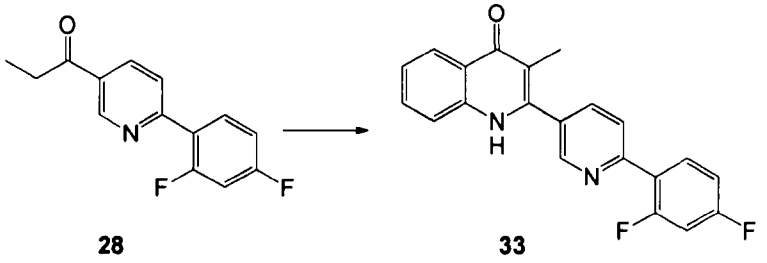
30 was prepared from **26** (2.0 g, 8.22 mmol) according to the procedure for the preparation of **27**. Purification by column chromatography using 30% ethyl acetate in hexane gave **30** (1.82 g, 74 %) as a white solid: R_f = 0.64, 50% ethyl acetate in hexane; mp 137 – 139°C; ^1H NMR (400 MHz, $CDCl_3$) δ 9.20 (dd, *J* = 2.3, 0.8 Hz, 1H, H-2), 8.27 (dd, *J* = 8.4, 2.3 Hz, 1H, H-4), 8.09 – 7.98 (m, 2H, H-8), 7.78 (dd, *J* = 8.4, 0.8 Hz, 1H, H-5), 7.14 – 6.95 (m, 2H, H-9), 3.89 (s, 3H, OCH_3), 3.05 (q, *J* = 7.2 Hz, 2H, CH_2), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.71 (C=O), 161.73, 160.77, 150.13 (C-2), 136.55 (C-4), 131.16, 130.09, 129.18 (C-8), 119.67 (C-5), 114.72 (C-9), 55.83 (OCH_3), 32.47 (CH_2), 8.44 (CH_3); MS (CI) $C_{15}H_{16}NO_2$ $[M+H]^+$ m/z 242; Anal. $C_{15}H_{15}NO_2$ requires C 74.67%, H 6.27%, N 5.81%, found C 74.44%, H 6.53%, N 5.64%.

Preparation of **3-Methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one 32.**



To **11** (1.15 g, 6.0 mmol) and **27** (1.78 g, 6.0 mmol) in anhydrous n-Butanol (20 mL) were added trifluoromethanesulfonic acid (0.1 mL, 1.2 mmol, 0.2 equiv). The yellow mixture was heated to 130°C for 24 h (followed by tlc). The reaction was cooled and the solvent was removed under reduced pressure. Sat. $NaHCO_3$ (aq) was added and the resulting aqueous solution was extracted with ethyl acetate (x 3), washed with water and brine, dried over $MgSO_4$, filtered and concentrated to a yellow solid. The crude product was purified by column chromatography using 5% methanol in ethyl acetate to give the title compound (1.64 g, 69 %) as a white solid: R_f = 0.31, 80% ethyl acetate in hexane; mp 277-278°C; ^{1}H NMR (400 MHz, DMSO) δ 11.76 (s, 1H, N-H), 8.90 (d, *J* = 2.1 Hz, 1H, H-2'), 8.37 – 8.31 (m, 2H, H-8'), 8.25 (d, *J* = 8.2 Hz, 1H, H-5'), 8.17 (dd, *J* = 8.2, 2.2 Hz, 1H, H-4'), 8.15 (dd, *J* = 7.0, 1.5 Hz, 1H, H-5), 7.65 (ddd, *J* = 8.2, 6.8, 1.4 Hz, 1H, H-7), 7.60 (d, *J* = 7.7 Hz, 1H, H-8), 7.55 (d, *J* = 8.2 Hz, 2H, H-9'), 7.33 (ddd, *J* = 8.0, 6.8, 1.2 Hz, 1H, H-6), 1.96 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.98 (C=O), 155.52, 149.75(C-2'), 144.79, 139.95, 138.49, 137.46, 131.83, 130.30, 129.34, 129.17 (C-8'), 125.37, 123.50, 123.22, 121.70 (C-9'), 120.35, 119.18, 118.51, 115.59, 12.39 (CH_3); IR ν_{max} (neat)/cm^{-1} 2979.5, 2886.9, 1627.6 (C=O), 1604.5, 1562.1, 1481.1, 1442.5, 1267.0, 1209.2 and 1155.2; HRMS (ESI) $C_{22}H_{16}N_2O_2F_3$ $[M+H]^+$ requires 397.1164, found 397.1173. Anal. $C_{22}H_{15}N_2O_2F_3$ requires C 66.67%, H 3.81%, N 7.07%, found C 66.77%, H 3.73%, N 6.98%.

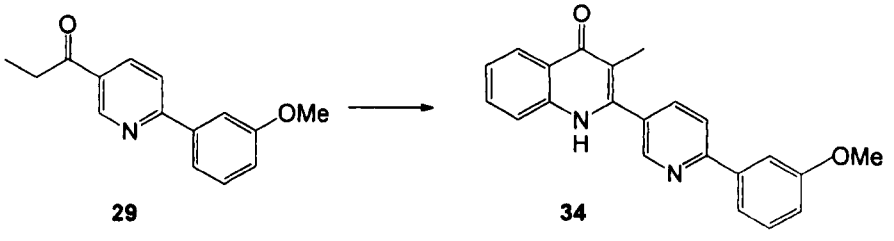
Preparation of **2-(6-(2,4-Difluorophenyl)pyridin-3-yl)-3-methylquinolin-4(1H)-one 33.**



28 **33**

33 was prepared from **11** (0.19 g, 1.0 mmol) and **28** (0.25 g, 1.0 mmol) according to the procedure for the preparation of **32**. Trituration with diethyl ether gave **33** (0.12 g, 34 %) as a very pale yellow solid: R_f = 0.24, 80% ethyl acetate in hexane; mp 312 – 313°C; ^{1}H NMR (400 MHz, DMSO) δ 11.78 (s, 1H, N-H), 8.93 (d, *J* = 1.6 Hz, 1H, H-2'), 8.22 – 8.12 (m, 2H, H-5 + H-4'), 8.12 – 8.04 (m, 1H, H-12'), 7.99 (d, *J* = 6.9 Hz, 1H, H-5'), 7.69 – 7.56 (m, 2H, H-7 + H-8), 7.48 (ddd, *J* = 11.6, 9.5, 2.4 Hz, 1H, H-11'), 7.34 (d, *J* = 7.3 Hz, 1H, H-6), 7.29 (dd, *J* = 8.5, 2.2 Hz, 1H, H-9'), 1.95 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.97 (C=O), 162.04, 159.38, 152.63, 149.86 (C-2'), 144.68, 139.96, 138.13 (C-4'), 132.72 (C-12'), 131.85 (C-7), 130.23, 125.37 (C-5), 123.85 (C-5'), 123.77, 123.50 (C-6), 123.25, 118.52 (C-8), 115.61, 112.72 (C-9'), 105.21 (C-11'), 12.39 (CH_3); IR ν_{max} (neat)/cm^{-1} 2979.5, 2886.9, 1612.2, 1598.7, 1562.1, 1479.1, 1444.9, 1376.9, 1261.2, 1145.5, 1008.6 and 968.1; HRMS (ESI) $C_{21}H_{15}N_2OF_2$ $[M+H]^+$ requires 349.1152, found 349.1153; Anal. $C_{21}H_{14}N_2OF_2$ requires C 72.41%, H 4.05%, N 8.04%, found C 72.34%, H 3.97%, N 7.96%.

Preparation of **3-Methyl-2-(6-(m-tolyl)pyridin-3-yl)quinolin-4(1H)-one 34.**

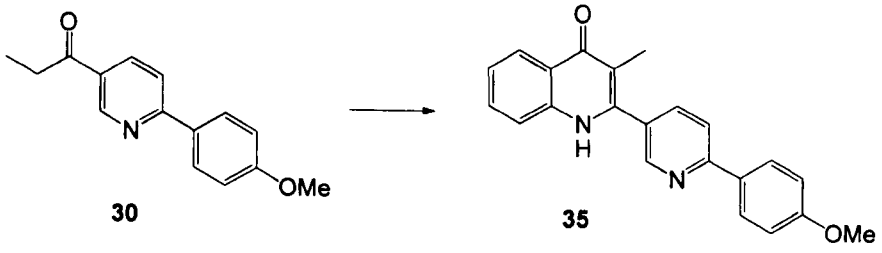


29 **34**

34 was prepared from **11** (0.381 g, 2.0 mmol) and **34** (0.485 g, 2.0 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using ethyl acetate gave **34** (0.4 g, 58 %) as a white solid: R_f = 0.4, 70% ethyl acetate

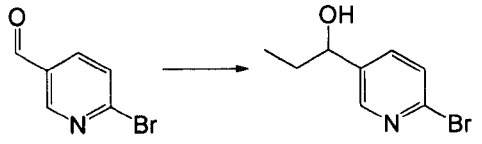
in hexane; mp 239 – 240°C; ^{1}H NMR (400 MHz, DMSO) δ 11.76 (s, 1H, N-H), 8.88 (d, *J* = 1.7 Hz, 1H, H-2'), 8.22 (d, *J* = 8.1 Hz, 1H, H-5'), 8.14 (m, 2H, H-5 + H-4'), 7.77 (m, 2H, H-8 + H-8'), 7.64 (m, 2H, H-7 + H-12'), 7.47 (t, *J* = 7.9 Hz, 1H, H-11'), 7.37 – 7.29 (m, 1H, H-6'), 7.08 (dd, *J* = 8.2, 1.9 Hz, 1H, H-10'), 3.87 (s, 3H, OCH_3), 1.96 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 202.11, 176.96, 160.16, 156.69, 149.58, 144.92, 139.94, 139.75, 138.27, 131.83, 130.42, 130.06, 125.37, 123.22, 120.31, 119.44, 118.52, 115.82, 115.54, 112.21, 55.58, 12.42; IR ν_{max} (neat)/cm^{-1} 2979.5, 2912.0, 1631.5, 1600.6, 1544.7, 1504.2, 1444.4, 1392.4 and 1367.3; HRMS (ESI) $C_{22}H_{19}N_2O_2$ $[M+H]^+$ requires 343.1447, found 343.1451; Anal. $C_{22}H_{18}N_2O_2$ requires C 77.17%, H 5.30%, N 8.18%, found C 77.26%, H 5.40%, N 8.15%.

Preparation of **2-(6-(4-Methoxyphenyl)pyridin-3-yl)-3-methylquinolin-4(1H)-one 35.**



35 was prepared from **11** (0.4737 g, 2.5 mmol) and **30** (0.601 g, 2.5 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using ethyl acetate gave **35** (0.36 g, 52 %) as a white solid: R_f = 0.38, ethyl acetate; MP 288°C; ^{1}H NMR (400 MHz, MeOD) δ 8.76 (dd, *J* = 2.1, 1.0 Hz, 1H, H-2'), 8.31 (dd, *J* = 8.3, 1.1 Hz, 1H, H-5), 8.09 – 7.95 (m, 4H, H-4'+H-5'+H-8'), 7.70 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H, H-7), 7.62 (d, *J* = 8.2 Hz, 1H, H-8), 7.42 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H, H-6), 7.15 – 7.05 (m, 2H, H-9'), 3.89 (s, 3H, OCH_3), 2.11 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.60 (C=O), 160.59 (C-10'), 156.35 (C-2'), 149.07, 144.72, 139.54, 137.72, 131.39, 130.34, 128.67, 128.16 (C-8'), 124.96, 123.08, 122.79, 118.77, 118.10, 115.09, 114.29 (C-9'), 55.27 (OCH_3), 12.03 (CH_3); HRMS (ESI) $C_{22}H_{19}N_2O_2$ $[M+H]^+$ requires 343.1447, found 343.1450; Anal. $C_{22}H_{18}N_2O_2$ requires C 77.17%, H 5.30%, N 8.18%, found C 77.33%, H 5.42%, N 8.13%.

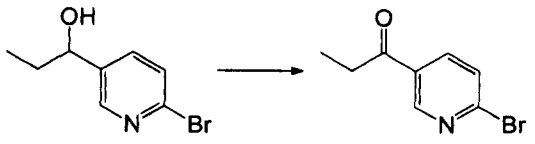
Preparation of **1-(6-Bromopyridin-3-yl)propan-1-ol 36.**



18 **36**

36 was prepared from 6-bromopyridine-3-carboxaldehyde **18** (2.89 g, 15.5 mmol) according to the procedure for the preparation of **23**. Purification by column chromatography using 25% ethyl acetate in hexane gave **36** (2.45, 68 %) as a yellow oil: R_f = 0.47, 50% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, *J* = 2.5 Hz, 1H, H-2), 7.69 – 7.52 (m, 1H, H-4), 7.47 (d, *J* = 8.2 Hz, 1H, H-5), 4.69 – 4.61 (m, 1H, CHOH), 1.95 – 1.63 (m, 2H, CH_2), 0.94 (t, *J* = 7.4 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.65 (C-2), 141.42 (C-6), 139.48 (C-3), 136.72 (C-4), 128.33 (C-5), 73.30 (CHOH), 32.38 (CH_2), 10.14 (CH_3); HRMS (CI) $C_8H_{11}NOBr$ $[M+H]^+$ requires 216.0019, found 216.0020; Anal. $C_8H_{10}NOBr$ requires C 44.47%, H 4.66%, N 6.48%, found C 44.56%, H 4.89%, N 6.94%.

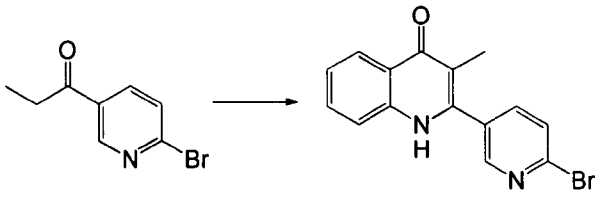
Preparation of **1-(6-Bromopyridin-3-yl)propan-1-one 37.**



36 **37**

37 was prepared from **36** (2.41 g, 11.15 mmol) according to the procedure for the preparation of **27**. Purification by column chromatography using 50% ethyl acetate in hexane gave **37** (3.12 g, 65 %) as a white solid: R_f = 0.47, ethyl acetate; mp 98 – 99°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (d, *J* = 2.1 Hz, 1H, H-2), 8.08 (dd, *J* = 8.3, 2.5 Hz, 1H, H-4), 7.61 (dd, *J* = 8.3, 0.6 Hz, 1H, H-5), 3.00 (q, *J* = 7.2 Hz, 2H, CH_2), 1.25 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.84 (C=O), 150.41 (C-2), 147.09 (C-6), 137.97 (C-4), 131.62 (C-3), 128.83 (C-5), 32.68 (CH_2), 8.18 (CH_3); MS (CI) $C_8H_9NO^{79}Br$ $[M+H]^+$ m/z 214.3, $C_8H_9NO^{81}Br$ $[M+H]^+$ m/z 216.2; Anal. C_8H_8NOBr requires C 44.89%, H 3.77%, N 6.54%, found C 45.06%, H 3.75%, N 6.50%.

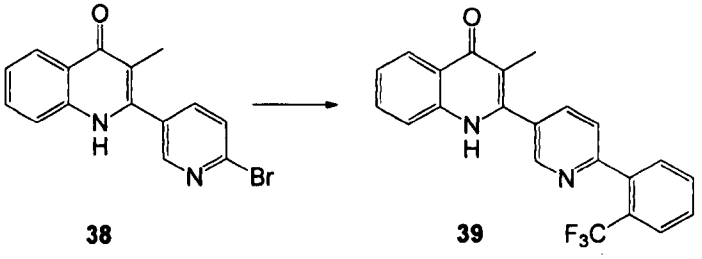
Preparation of **2-(6-Bromopyridin-3-yl)-3-methylquinolin-4(1H)-one 38.**



37 **38**

38 was prepared from **11** (1.41 g, 7.44 mmol) and **37** (1.60 g, 7.44 mmol) and trifluoromethanesulfonic acid (0.37 g, 1.95 mmol, 0.25 equiv) according to the procedure for the preparation of **32**. Purification by column chromatography using 5% methanol in ethyl acetate to give the title compound (0.99 g, 42 %) as a white solid: R_f = 0.40, ethyl acetate; mp 283-284°C; ^{1}H NMR (400 MHz, DMSO) δ 11.74 (s, 1H, N-H), 8.64 (d, *J* = 2.4 Hz, 1H, H-2'), 8.13 (d, *J* = 7.6 Hz, 1H, H-5), 8.02 (dd, *J* = 8.2, 2.4 Hz, 1H, H-4'), 7.90 (d, *J* = 8.2 Hz, 1H, H-5'), 7.65 (t, *J* = 7.5 Hz, 1H, H-7), 7.56 (d, *J* = 8.2 Hz, 1H, H-8), 7.33 (t, *J* = 7.5 Hz, 1H, H-6), 1.89 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.90 (C=O), 150.61 (C-2'), 143.78, 142.56, 140.46, 139.88, 131.93, 130.91, 128.27, 125.38, 123.47, 123.31, 118.48, 115.68, 12.23 (CH_3); HRMS (ESI) $C_{15}H_{12}N_2O^{79}Br$ $[M+H]^+$ requires 315.0133 (100 %), $C_{15}H_{12}N_2O^{81}Br$ $[M+H]^+$ requires 317.0113, found 317.0110 (92 %).

Preparation of **3-Methyl-2-(6-(2-(trifluoromethyl)phenyl)pyridin-3-yl)quinolin-4(1H)-one 39.**

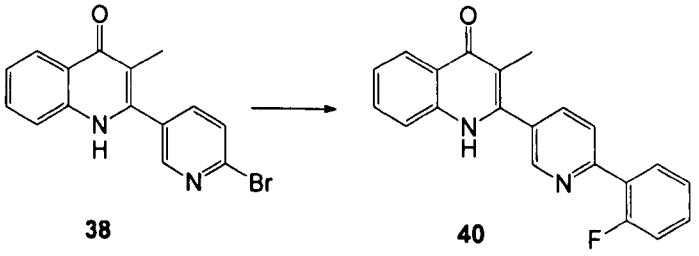


38 **39**

39 was prepared from **38** (0.25 g, 0.79 mmol) and 2-(trifluoromethyl)phenylboronic acid (0.30 g, 1.59 mmol, 2 equiv) according to the procedure for the preparation of **14**. Trituration by diethyl ether gave **39** (0.21 g, 70%) as a pink solid: R_f = 0.2, 5% methanol in dichloromethane; mp 279 – 280°C; ^{1}H NMR (400 MHz, DMSO) δ 11.82 (s, 1H, N-H), 8.88 (d, *J* = 1.7 Hz, 1H, H-2'), 8.17 – 8.15 (m, 2H, H-5 + H-4'), 7.93 (d, *J* = 7.8 Hz, 1H, H-12'), 7.83 (t, *J* = 7.4 Hz, 1H, H-11'), 7.77 – 7.70 (m, 2H, H-8 + H-5'),

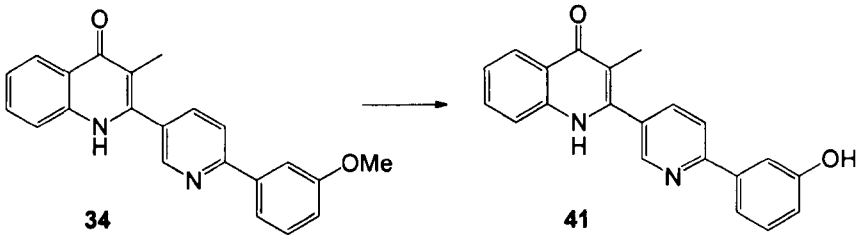
7.69 – 7.61 (m, 3H, H-7 + H-9' + H-10'),, 7.34 (ddd, *J* = 8.1, 6.0, 2.0 Hz, 1H, H-6), 1.94 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.98 (C=O), 158.29, 149.02 (C-2'), 144.72, 139.99, 139.49, 137.62 (C-4'), 132.84, 132.09 (C-11), 131.85, 130.24, 129.57, 127.43, 127.13, 126.87, 125.87, 125.36 (C-5), 123.67, 123.52, 123.25 (C-6), 118.56, 115.68, 12.44 (CH_3); HRMS (ESI) $C_{22}H_{16}N_2OF_3$ $[M+H]^+$ requires 381.1215, found 381.1197; Anal. $C_{22}H_{15}N_2OF_3$ requires C 69.47%, H 3.97%, N 7.36%, found C 69.61%, H 4.02%, N 7.46%.

Preparation of **2-(6-(2-Fluorophenyl)pyridin-3-yl)-3-methylquinolin-4(1H)-one 40.**



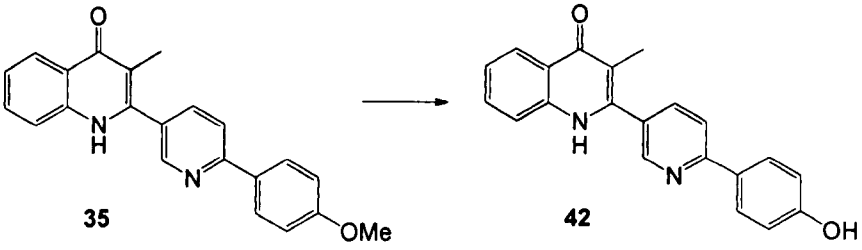
40 was prepared from **38** (65 mg, 0.21 mmol) and 2-fluorophenylboronic acid (69 mg, 0.49 mmol, 2.5 equiv) according to the procedure for the preparation of **14**. Purification by column chromatography using 5% methanol in dichloromethane gave **40** (43 mg, 63%) as a brown solid: R_f = 0.35, 5% methanol in dichloromethane; mp 269 – 270°C; ^1H NMR (400 MHz, DMSO) δ 11.79 (s, 1H, N-H), 8.94 (d, *J* = 1.7 Hz, 1H, H-2'), 8.20 – 8.13 (m, 2H, H-5 + H-4'), 8.08 – 7.99 (m, 2H, H-5' + H-12'), 7.66 (ddd, *J* = 8.2, 6.8, 1.4 Hz, 1H, H-7), 7.60 (d, *J* = 7.6 Hz, 1H, H-8), 7.56 (ddd, *J* = 7.1, 4.4, 1.9 Hz, 1H, H-10'), 7.43 (dd, *J* = 4.7, 3.7 Hz, 1H, H-11'), 7.40 (d, *J* = 7.9 Hz, 1H, H-9'), 7.34 (ddd, *J* = 8.0, 6.8, 1.2 Hz, 1H, H-6), 1.96 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.97 (C=O), 161.36 (d, *J*=248.9 Hz, C-8'), 153.46, 149.84 (C-2'), 144.74, 139.95, 138.00 (C-4'), 131.86 (C-7), 131.28 (C-10'), 130.21 (C-12'), 126.80, 126.69, 125.38 (C-5), 124.09 (C-11'), 124.01, 123.50 (C-5'), 123.26 (C-6), 118.52, 116.83 (d, *J*=22.5 Hz,C-9'), 115.60, 12.40 (CH_3); HRMS (ESI) $C_{21}H_{16}N_2OF$ $[M+H]^+$ requires 331.1247, found 331.1239; Anal. $C_{21}H_{15}N_2OF$ requires C 76.35%, H 4.58%, N 8.48%, found C 76.24%, H 4.45%, N 8.38%.

Preparation of **2-(6-(3-Hydroxyphenyl)pyridin-3-yl)-3-methylquinolin-4(1H)-one 41.**



34 (120 mg, 0.35 mmol) in anhydrous dichloromethane (20 mL) was cooled to -10°C. Boron tribromide 1.0 M in dichloromethane (1.00 mL, 1 mmol) was added slowly. The mixture was allowed to stir at -10°C for 2 h, then allowed to stir at room temperature overnight. The reaction was quenched with ice-water and was further diluted with dichloromethane. The solid was filtered and washed with water followed by ethyl acetate to give the title compound (105 mg, 91 %) as a white solid: R_f = 0.28, ethyl acetate; ^{1}H NMR (400 MHz, MeOD) δ 8.87 – 8.78 (m, 1H, H-2'), 8.33 (dd, *J* = 8.3, 1.0 Hz, 1H, H-5), 8.12 (dd, *J* = 8.2, 2.3 Hz, 1H, H-4'), 8.06 (d, *J* = 8.2 Hz, 1H, H-5'), 7.73 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H, H-7), 7.65 (d, *J* = 8.3 Hz, 1H, H-8), 7.59 – 7.52 (m, 2H, H-8' + H-12'), 7.46 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H, H-6), 7.40 – 7.30 (m, 1H, H-11'), 6.99 – 6.87 (m, 1H, H-10'), 2.13 (s, 3H, CH_3); ^{13}C NMR (101 MHz, MeOD) δ 195.17, 174.53, 172.79, 158.37, 148.54, 143.73, 139.65, 139.40, 138.12, 132.08, 129.73, 129.47, 124.79, 123.92, 120.57, 118.09, 117.91, 116.57, 116.17, 113.67, 11.20; HRMS (ESI) $C_{21}H_{17}N_2O_2$ $[M+H]^+$ requires 329.1290, found 329.1291.

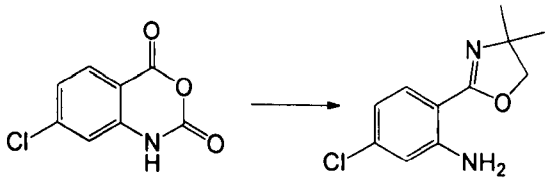
Preparation of **2-(6-(4-Hydroxyphenyl)pyridin-3-yl)-3-methylquinolin-4(1H)-one 42.**



42 was prepared from **35** (120 mg, 0.35 mmol) according to the procedure for the preparation of **41**. Purification by column chromatography using 5% methanol in dichloromethane gave **42** (56 mg, 49%) as a yellow solid: R_f = 0.07, 5% methanol in

dichloromethane; mp 283 – 284°C; ^{1}H NMR (400 MHz, Acetone) δ 8.74 (dd, *J* = 2.2, 0.9 Hz, 1H, H-2'), 8.35 – 8.26 (m, 1H, H-5), 8.03 (dd, *J* = 8.3, 2.3 Hz, 1H, H-4'), 7.99 (dd, *J* = 8.3, 0.9 Hz, 1H, H-5'), 7.99 – 7.94 (m, 2H, H-8'), 7.70 (ddd, *J* = 8.4, 6.9, 1.5 Hz, 1H, H-7), 7.62 (ddd, *J* = 8.5, 1.1, 0.6 Hz, 1H, H-8), 7.42 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H, H-6), 6.97 – 6.88 (m, 2H, H-9'), 2.12 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.98 (C=O), 159.47, 157.12, 149.39 (C-2'), 138.00, 131.76, 129.20, 128.70, 128.64 (C-8'), 125.36, 123.48, 123.16, 118.79, 118.50, 116.05 (C-9'), 115.45, 12.45 (CH_3); HRMS (ESI) $C_{21}H_{17}N_2O_2$ $[M+H]^+$ requires 329.1290, found 329.1284.

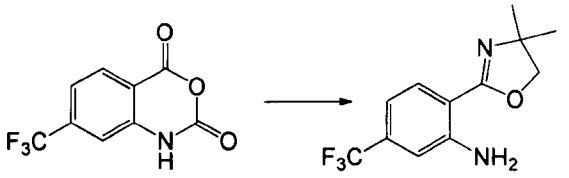
Preparation of **5-Chloro-2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)aniline 43.**



62 **43**

43 was prepared from 4-chloroisatoic anhydride **62** (1.97 g, 10 mmol) according to the procedure for the preparation of **11**. Purification by column chromatography using 10% ethyl acetate in hexane gave **43** (1.30 g, 58 %) as a white solid: mp 84 – 85°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (d, *J* = 8.5 Hz, 1H, H-3), 6.68 (d, *J* = 2.0 Hz, 1H, H-6), 6.61 (dd, *J* = 8.5, 2.0 Hz, 1H, H-4), 6.20 (s, 2H, NH_2), 3.99 (s, 2H, CH_2), 1.36 (s, 6H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.87 (C-O), 149.72, 137.93, 131.13 (C-3), 116.65 (C-4), 115.29 (C-6), 108.28, 68.31 (CH_2), 66.28 ($\underline{C}(CH_3)_2$), 29.09 (CH_3); HRMS (CI) $C_{11}H_{14}N_2OCl$ $[M+H]^+$ requires 225.0789, found 225.0790; Anal. $C_{11}H_{13}N_2OCl$ requires C 58.80%, H 5.83%, N 12.47%, found C 59.04%, H 5.85%, N 12.44%.

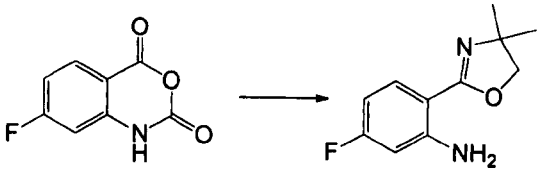
Preparation of **2-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)-5-(trifluoromethyl)aniline 44.**



63 **44**

44 was prepared from **63** (0.91 g, 3.94 mmol) according to the procedure for the preparation of **11**. Purification by column chromatography using 40% ethyl acetate in hexane gave a white solid (0.69 g, 68 %): mp 100 – 102°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.2 Hz, 1H, H-3), 6.92 (s, 1H, H-6), 6.86 (d, *J* = 8.3 Hz, 1H, H-4), 6.31 (s, 2H, NH_2), 4.02 (s, 2H, CH_2), 1.38 (s, 6H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.66, 148.77 (C-1), 133.74 (q, *J* = 32.0 Hz, C-5), 130.60 (C-3), 124.27 (q, *J* = 272.6 Hz, CF_3), 112.46 (C-6), 112.45, 112.14 (C-4), 77.93 ($\underline{C}(CH_3)_2$), 68.49 (OCH_2), 29.05 (CH_3); HRMS (ESI) $C_{12}H_{14}N_2OF_3$ $[M+H]^+$ requires 259.1058, found 259.1065.

Preparation of **2-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)-5-fluoroaniline 45.**

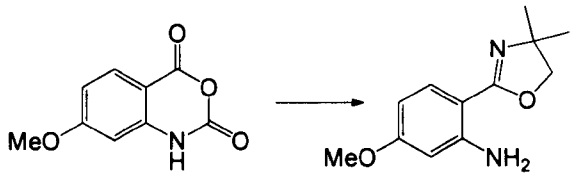


64 **45**

45 was prepare from **64** (2 g, 11 mmol) according to the procedure for the preparation of **11**. Purification by column chromatography using ethyl acetate in hexane gave **45** (1.37 g, 60 %) as a pale yellow solid: R_f = 0.67, 20% ethyl acetate in hexane; mp 76 – 77°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, *J* = 9.3, 6.6 Hz, 1H, H-3), 6.38 – 6.32 (m, 2H, H-4 + H-5), 6.26 (s, 2H, NH_2), 3.98 (s, 2H, CH_2), 1.36 (s, 6H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.59 (d, *J* = 248.3 Hz, C-F), 161.86 ($\underline{C}$=N), 150.84, 132.05 (C-3), 106.29, 103.99, 101.77, 77.44 (OCH_2), 68.20 ($\underline{C}(CH_3)_2$), 29.11 (CH_3); IR ν_{max} (neat)/cm^{-1} 3390.2, 3251.3, 2977.6, 2906.2, 1637.3, 1591.0, 1463.7, 1270.9 and 1051.0; HRMS (CI) $C_{11}H_{14}N_2OF$ $[M+H]^+$ requires 209.1085, found 209.1084; Anal.

$C_{11}H_{13}N_2OF$ requires C 63.45%, H 6.29%, N 13.45%, found C 63.43%, H 6.34%, N 13.38%.

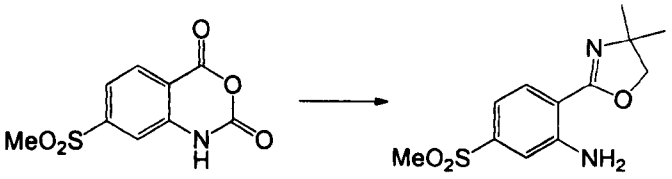
Preparation of **2-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)-5-methoxyaniline 46.**



65 **46**

46 was prepare from **65** (2.63 g, 13.6 mmol) according to the procedure for the preparation of **11**. Purification by column chromatography using ethyl acetate in hexane gave **46** (2.25 g, 75%) as a white solid: R_f = 0.48, 20% ethyl acetate in hexane; mp 92°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (d, *J* = 8.8 Hz, 1H, H-3), 6.24 (dd, *J* = 8.8, 2.5 Hz, 1H, H-4), 6.17 (d, *J* = 2.4 Hz, 1H, H-6), 6.13 (s, 2H, NH_2), 3.96 (s, 2H, OCH_2), 3.78 (s, 3H, OCH_3), 1.35 (s, 6H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.97, 162.29 (C-5), 150.58 (C-1), 131.40 (C-3), 103.86 (C-6), 103.52, 99.66 (C-4), 77.62 ($\underline{C}(CH_3)_2$), 68.01 (OCH_2), 55.52 (OCH_3), 29.17 (CH_3); IR ν_{max} (neat)/cm^{-1} 3398.0, 3251.4, 2975.6, 2894.6, 1635.3, 1600.6, 1365.4, 1270.9, 1214.9 and 1029.8; MS (CI) $C_{12}H_{17}N_2O_2$ $[M+H]^+$ m/z 221.2; Anal. $C_{12}H_{16}N_2O_2$ requires C 65.43%, H 7.32%, N 12.72%, found C 65.41%, H 7.38%, N 12.93%.

Preparation of **2-(4,4-Dimethyl-4,5-dihydrooxazol-2-yl)-5-(methylsulfonyl)aniline 47.**

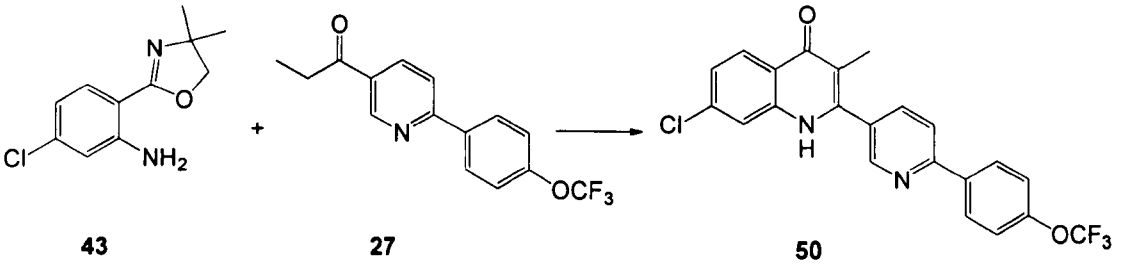


66 **47**

47 was prepared from **66** (0.90 g, 3.73 mmol) according to the procedure for the preparation of **11**. Purification by column chromatography using ethyl acetate in hexane gave **47** (0.39 g, 39 %) as a pale yellow solid: R_f = 0.07, 80% ethyl acetate in hexane; mp 153 – 154°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, *J* = 8.3 Hz, 1H, H-3), 7.24 (d, *J* = 1.8 Hz, 1H, H-5), 7.13 (dd, *J* = 8.3, 1.8 Hz, 1H, H-4), 6.47 (s, 2H, NH_2), 4.04

(s, 2H, CH_2), 3.03 (s, 3H, CH_3), 1.38 (s, 6H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.39, 149.18 (C-1), 143.22, 131.27 (C-3), 114.27 (C-4), 113.73 (C-6), 113.45, 78.03($\underline{C}(CH_3)_2$), 68.65 (OCH_2), 44.67 ($SO_2\underline{C}H_3$), 29.03 (CH_3); HRMS (ESI) $C_{12}H_{16}N_2O_3{}^{23}NaS$ $[M+Na]^+$ requires 291.0779, found 291.0781; Anal. $C_{12}H_{16}N_2O_3S$ requires C 53.71%, H 6.01%, N 10.44%, found C 53.94%, H 6.02%, N 10.45%.

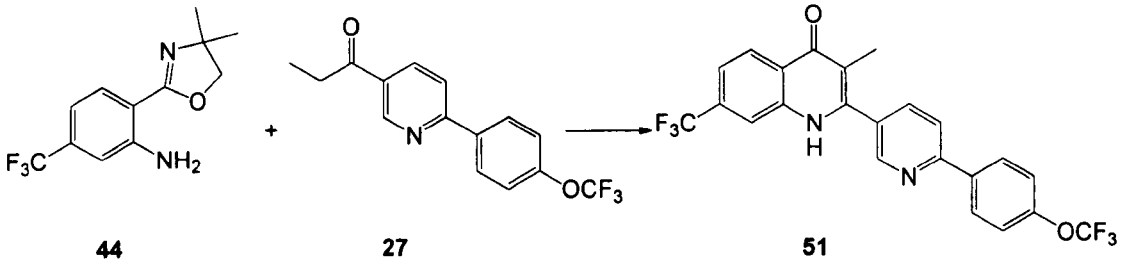
Preparation of **7-Chloro-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one** **50.**



43 **27** **50**

50 was prepared from **43** (0.23 g, 1.0 mmol) and **27** (0.30 g, 1.0 mmol) according to the procedure for the preparation of **32**. Trituration with diethyl ether gave **50** (0.13 g, 30 %) as a white solid: mp 317 – 318°C; ^1H NMR (400 MHz, DMSO) δ 11.82 (s, 1H, N-H), 8.91 (s, 1H, H-2'), 8.34 (d, *J* = 8.7 Hz, 2H, H-8'), 8.26 (d, *J* = 8.1 Hz, 1H, H-5'), 8.21 – 8.05 (m, 2H, H-5 + H-4'), 7.61 (s, 1H, H-8), 7.55 (d, *J* = 8.2 Hz, 2H, H-9'), 7.35 (dd, *J* = 8.7, 1.5 Hz, 1H, H-6), 1.95 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.09 (C=O), 155.30, 149.31 (C-2'), 144.76, 140.21, 138.07, 136.99, 136.05, 129.62, 128.80 (C-8'), 127.42, 123.26, 121.66, 121.30 (C-9'), 120.03, 118.78, 118.01, 117.13, 116.04, 11.91 (CH_3); IR ν_{max} (neat)/cm^{-1} 2979.5, 2886.9, 1627.6 (C=O), 1600.6, 1556.3, 1477.2, 1380.8, 1255.4 and 1155.1; HRMS (ESI) $C_{22}H_{15}N_2O_2F_3{}^{35}Cl$ $[M+H]^+$ requires 431.0774, found 431.076 (100 %), $C_{22}H_{15}N_2O_2F_3{}^{37}Cl$ $[M+H]^+$ requires 433.0745, found 433.0757 (32 %); Anal. $C_{22}H_{14}N_2O_2F_3$ requires C 61.33%, H 3.28%, N 6.50%, found C 61.43%, H 3.28%, N 6.41%.

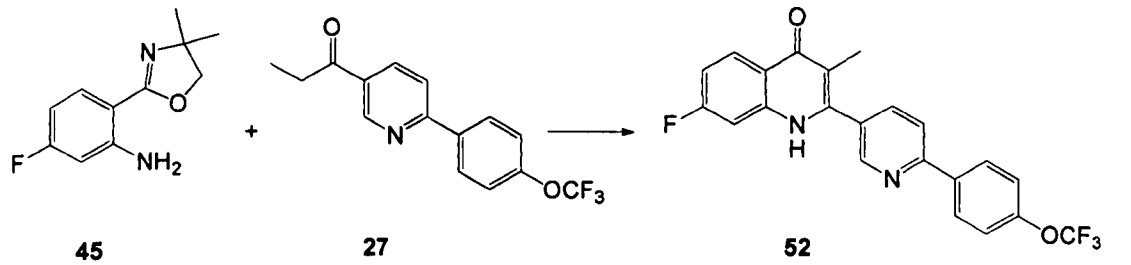
Preparation of **3-Methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)-7-(trifluoromethyl)quinolin-4(1H)-one 51.**



44 **27** **51**

51 was prepared from **44** (0.26 g, 1.0 mmol) and **27** (0.30 g, 1.0 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using ethyl acetate gave **51** (48 mg, 10 %) as a white solid: R_f = 0.22, 35% ethyl acetate in hexane; mp 322 – 324°C; ^{1}H NMR (400 MHz, DMSO) δ 12.07 (s, 1H, N-H), 8.94 (d, *J* = 2.0 Hz, 1H, H-2'), 8.35 (d, *J* = 8.7 Hz, 3H, H-5 + H-8'), 8.27 (d, *J* = 8.2 Hz, 1H, H-5'), 8.20 (dd, *J* = 8.2, 2.1 Hz, 1H, H-4'), 7.96 (s, 1H, H-8), 7.61 (d, *J* = 8.5 Hz, 1H, H-6), 7.55 (d, *J* = 8.4 Hz, 2H, H-9'), 1.99 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.38 (C=O), 155.79, 149.74 (C-2'), 145.80, 139.40, 138.48 (C-4'), 137.37, 131.74, 131.42, 129.90, 129.22 (C-8'), 127.40, 125.53, 125.27, 121.72 (C-9'), 120.44 (C-5'), 119.18, 118.75 (C-6), 117.18 (C-8), 116.15, 12.41 (CH_3); HRMS (ESI) $C_{23}H_{15}N_2O_2F_6$ $[M+H]^+$ requires 465.1038, found 465.1039; Anal. $C_{23}H_{14}N_2O_2F_6$ requires C 59.49%, H 3.04%, N 6.03%; found C 59.58%, H 2.89%, N 5.95%.

Preparation of **7-Fluoro-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one 52.**

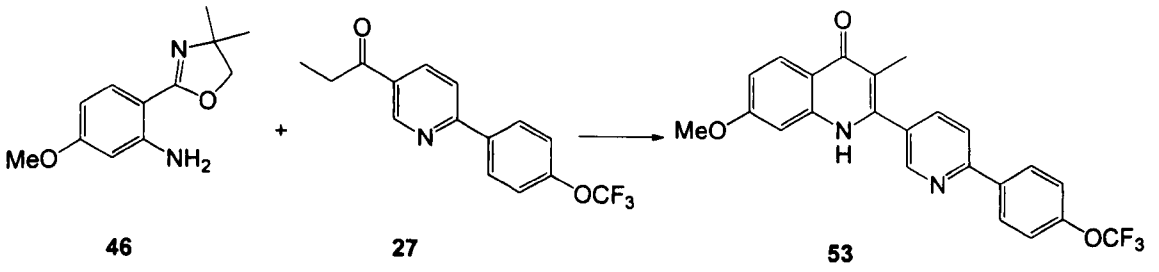


45 **27** **52**

52 was prepared from **45** (0.21 g, 1.0 mmol) and **27** (0.30 g, 1.0 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 5% methanol in dichloromethane gave **52** (120 mg, 30 %) as a pale yellow

solid: R_f = 0.2, 5% methanol in dichloromethane; mp 317 – 319°C; ^{1}H NMR (400 MHz, DMSO) δ 11.83 (s, 1H, N-H), 8.90 (d, *J* = 1.8 Hz, 1H, H-2'), 8.39 – 8.31 (m, 2H, H-8'), 8.24 (t, *J* = 8.7 Hz, 1H, H-5'), 8.22 – 8.11 (m, 2H, H-5 + H-4'), 7.55 (d, *J* = 8.2 Hz, 2H, H-9'), 7.30 (dd, *J* = 10.1, 2.4 Hz, 1H, H-8), 7.20 (td, *J* = 8.8, 2.5 Hz, 1H, H-6), 1.95 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.06 (C=O), 162.47 (C-7), 155.23, 149.31 (C-2'), 144.77, 140.84, 140.71, 138.07, 137.00, 129.66, 128.79 (C-8'), 128.49, 128.39, 121.34 (C-9'), 120.19, 120.01, 115.59, 111.85 (d, *J* = 23.2 Hz, C-6), 102.93 (d, *J* = 25.0 Hz, C-8), 11.88 (CH_3); IR ν_{max} (neat)/cm^{-1} 2979.5, 2886.9, 1600.6, 1560.1, 1513.8, 1475.3, 1380.8, 1255.4, 1153.2 and 954.6; HRMS (ESI) $C_{22}H_{14}N_2O_2F_4{}^{23}Na$ $[M+Na]^+$ requires 437.0889, found 437.0905; Anal. $C_{22}H_{14}N_2O_2F_4$ requires C 63.77%, H 3.41%, N 6.76%, found C 63.63%, H 3.29%, N 6.82%.

Preparation of **7-Methoxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one 53.**

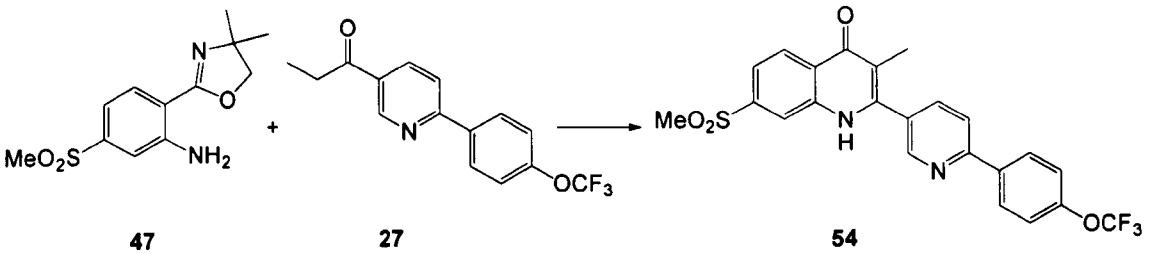


46 **27** **53**

53 was prepared from **46** (0. 18 g, 0.82 mmol) and **27** (0.24 g, 0.82 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 5% methanol in ethyl acetate gave **53** (83 mg, 23 %) as a white solid: R_f = 0.18, 5% methanol in dichloromethane; mp 324 – 325°C; ^{1}H NMR (400 MHz, DMSO) δ 11.60 (s, 1H, N-H), 8.88 (d, *J* = 2.1 Hz, 1H, H-2'), 8.34 (d, *J* = 8.8 Hz, 2H, H-8'), 8.24 (d, *J* = 8.3 Hz, 1H, H-5'), 8.15 (dd, *J* = 8.2, 2.3 Hz, 1H, H-4'), 8.04 (d, *J* = 8.9 Hz, 1H, H-5), 7.55 (d, *J* = 8.3 Hz, 2H, H-9'), 6.99 (d, *J* = 2.2 Hz, 1H, H-8), 6.93 (dd, *J* = 9.0, 2.4 Hz, 1H, H-6), 3.84 (s, 3H, OCH_3), 1.93 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.14 (C=O), 161.68 (C-7), 155.05, 149.33 (C-2'), 143.88, 141.29, 138.03 (C-4'), 137.06, 129.94, 128.76 (C-8'), 126.88 (C-5), 121.30 (C-9'), 119.95 (C-5'), 118.78, 117.65, 114.84, 113.17 (C-6), 98.67 (C-8), 55.33 (OCH_3), 11.89 (CH_3); HRMS (ESI)

$C_{23}H_{18}N_2O_3F_3$ $[M+H]^+$ requires 427.1270, found 427.1280; Anal. $C_{23}H_{17}N_2O_3F_3$ requires C 64.79%, H 4.02%, N 6.57%, found C 64.65%, H 3.88%, N 6.47%.

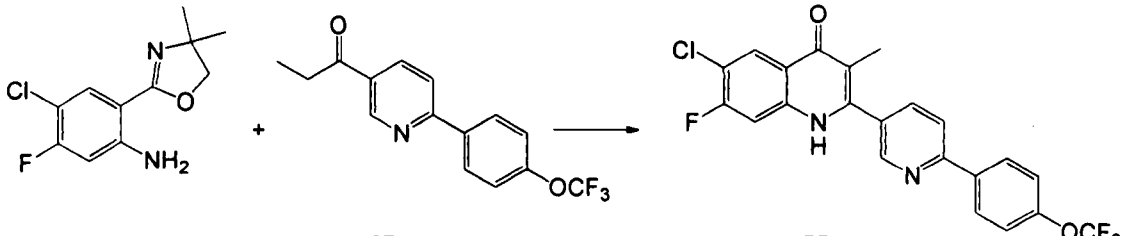
Preparation of **3-Methyl-7-(methylsulfonyl)-2-(6-(4-(trifluoromethoxy)phenyl) pyridin-3-yl)quinolin-4(1H)-one 54.**



47 **27** **54**

54 was prepared from **47** (0.13 g, 0.5 mmol) and **27** (0.15 g, 0.5 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography by 10% methanol in dichloromethane gave **54** (0.08 g, 34 %) as a white solid: R_f = 0.44, 80% ethyl acetate in hexane; MP 334°C; ^{1}H NMR (400 MHz, DMSO) δ 12.17 (s, 1H, N-H), 8.93 (dd, *J* = 2.3, 0.8 Hz, 1H, H-2'), 8.37 (d, *J* = 9.2 Hz, 1H, H-5), 8.37 – 8.32 (m, 2H, H-8'), 8.27 (dd, *J* = 8.3, 0.6 Hz, 1H, H-8), 8.23 – 8.17 (m, 2H, H-4'+ H-5'), 7.81 (dd, *J* = 8.5, 1.7 Hz, 1H, H-6), 7.55 (d, *J* = 8.0 Hz, 2H, H-9'), 3.31 (s, 3H, CH_3), 2.00 (s, 3H, CH_3); IR ν_{max} (neat)/cm^{-1} 2979.5, 2886.9, 1627.6, 1598.7, 1558.2, 1477.2, 1461.8, 1382.7, 1253.5 and 1151.3; HRMS (ESI) $C_{23}H_{18}N_2O_4F_3S$ $[M+H]^+$ requires 475.0939, found 475.0922; Anal. $C_{23}H_{17}N_2O_4F_3S$ requires C 58.22%, H 3.61%, N 5.90%, found C 58.10%, H 3.55%, N 5.85%.

Preparation of **6-Chloro-7-fluoro-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl) pyridin-3-yl)quinolin-4(1H)-one 55.**

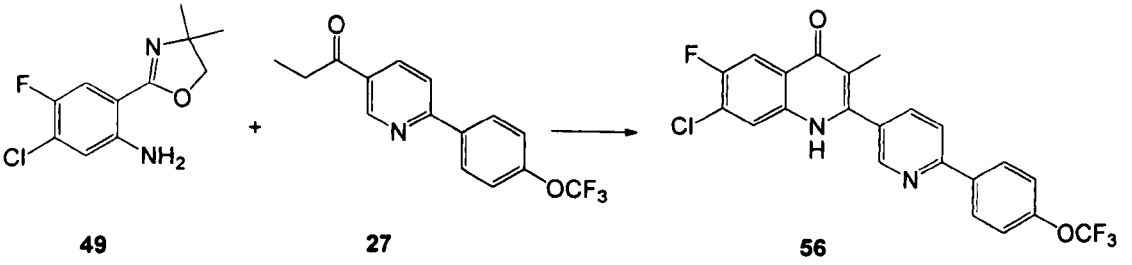


48 **27** **55**

55 was prepared from 4-chloro-2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-5-fluoroaniline **48** (181 mg, 0.75 mmol) and **27** (221 mg, 0.75 mmol) according to the

procedure for the preparation of **32**. Purification by column chromatography using 80% ethyl acetate in hexane gave **55** (132 mg, 40 %) as a yellow solid: R_f = 0.27, 40% ethyl acetate in hexane; mp >340°C; ^{1}H NMR (400 MHz, DMSO) δ 11.99 (s, 1H, N-H), 8.91 (d, *J* = 1.8 Hz, 1H, H-2'), 8.37 – 8.31 (m, 2H, H-8'), 8.26 (d, *J* = 8.2 Hz, 1H, H-5), 8.21 (d, *J* = 8.2 Hz, 1H, H-5'), 8.17 (dd, *J* = 8.2, 2.3 Hz, 1H, H-4'), 7.55 (d, *J* = 8.2 Hz, 2H, H-9'), 7.49 (d, *J* = 10.0 Hz, 1H, H-8), 1.95 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 175.40 (C=O), 155.75, 149.69 (C-2'), 145.57, 139.68, 138.45, 137.36 (C-4'), 132.99, 129.82, 129.21 (C-8'), 127.62 (C-5'), 123.07, 121.71 (C-9'), 121.05, 120.41 (C-5), 119.17, 116.42, 116.11, 105.46 (C-8), 12.30 (CH_3); IR ν_{max} (neat)/cm^{-1} 2977.6, 2890.8, 1633.41 (C=O), 1498.4, 1471.4, 1247.7, 1207.2 and 1162.9; HRMS (ESI) $C_{22}H_{14}N_2O_2F_4{}^{35}Cl$ $[M+H]^+$ requires 449.0680, found 449.0677 (100%), $C_{22}H_{14}N_2O_2F_4{}^{37}Cl$ $[M+H]^+$ requires 451.0650, found 451.0661 (35%); Anal. $C_{22}H_{13}N_2O_2F_4Cl$ requires C 58.88%, H 2.92%, N 6.24%, found C 58.99%, H 2.80%, N 6.15%.

Preparation of **7-Chloro-6-fluoro-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl) pyridin-3-yl)quinolin-4(1H)-one 56.**

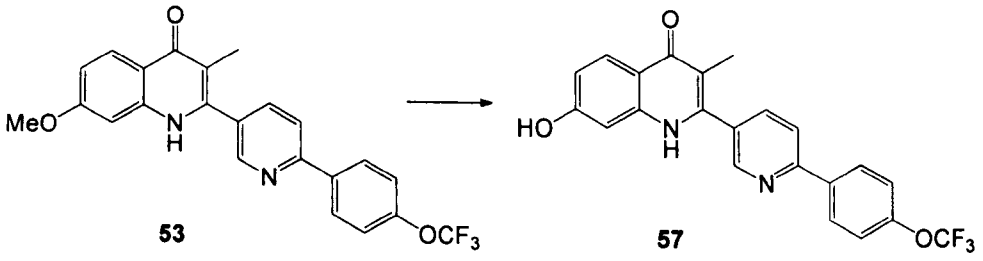


49 **27** **56**

56 was prepared from 5-chloro-2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-4-fluoroaniline **49** (170 mg, 0.70 mmol) and **27** (206 mg, 0.70 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 80% ethyl acetate in hexane gave **56** (156 mg, 50 %) as a yellow solid: R_f = 0.18, 40% ethyl acetate in hexane; mp >351°C; ^{1}H NMR (400 MHz, DMSO) δ 11.97 (s, 1H, N-H), 8.91 (d, *J* = 2.2 Hz, 1H, H-2'), 8.39 – 8.29 (m, 2H, H-8'), 8.26 (d, *J* = 8.2 Hz, 1H, H-5'), 8.17 (dd, *J* = 8.2, 2.2 Hz, 1H, H-4'), 7.96 (d, *J* = 9.6 Hz, 1H, H-5), 7.77 (d, *J* = 6.3 Hz, 1H, H-8), 7.55 (d, *J* = 8.4 Hz, 2H, H-9'), 1.95 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 175.74 (C=O), 155.76, 154.77, 152.34, 149.69 (C-2'), 145.41, 138.47, 137.36 (C-4'),

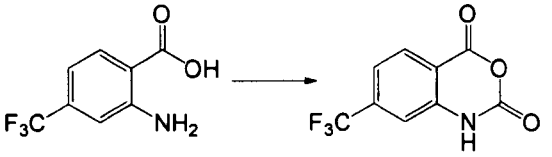
136.79, 129.89, 129.21 (C-8'), 124.82, 124.61, 123.10, 121.71 (C-9'), 120.43, 119.17, 115.71, 111.30 (C-5), 12.33 (CH_3); IR ν_{max} (neat)/cm^{-1} 3064.3, 2919.7, 1635.3 (C=O), 1500.4, 1481.1, 1376.9, 1241.9 and 1168.6; HRMS (ESI) $C_{22}H_{14}N_2O_2F_4{}^{35}Cl$ $[M+H]^+$ requires 449.0680, found 449.0671 (100 %), $C_{22}H_{14}N_2O_2F_4{}^{37}Cl$ $[M+H]^+$ requires 451.0650, found 451.0654 (35 %). Anal. $C_{22}H_{13}N_2O_2F_4Cl$ requires C 58.88%, H 2.92%, N 6.24%, found C 58.76%, H 2.85%, N 6.34%.

Preparation of **7-Hydroxy-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one 57.**



53 (18.3 mg, 0.04 mmol) in anhydrous toluene (10 mL) was cooled to 0°C. Boron tribromide 1.0 M in dichloromethane (0.13 mL, 0.12 mmol, 3 equiv) was added. The mixture was allowed to stir at room temperature for ½ h then was heated to reflux for 18 h. The mixture was cooled to 0°C and methanol was added. The solution was evaporated and the residue triturated with diethyl ether. The crude product was purified by column chromatography using 10% methanol in dichloromethane to give the title compound (12 mg, 68 %) as a white solid: R_f = 0.21, 5% methanol in dichloromethane; ^1H NMR (400 MHz, MeOD) δ 8.81 (dd, *J* = 2.2, 1.0 Hz, 1H, H-2), 8.33 – 8.20 (m, 2H, H-8'), 8.17 – 8.13 (m, 1H, H-5), 8.11 (dd, *J* = 8.2, 1.0 Hz, 1H, H-5'), 8.08 (dd, *J* = 8.2, 2.2 Hz, 1H, H-4'), 7.45 (dd, *J* = 8.9, 0.9 Hz, 2H, H9'), 6.92 (dd, *J* = 8.9, 2.3 Hz, 1H, H-8), 6.89 (m, 1H, H-6), 2.06 (s, 3H, CH_3); ^{13}C NMR (101 MHz, MeOD) δ 199.27, 178.62, 161.39, 156.56, 155.27, 150.26, 149.03, 145.49, 141.82, 137.97, 137.26, 130.10, 128.66, 126.87, 120.95, 120.26, 117.19, 115.18, 114.82, 112.86, 100.31, 11.01; HRMS (ESI) $C_{22}H_{16}N_2O_3F_3$ $[M+H]^+$ requires 413.1113, found 412.1102.

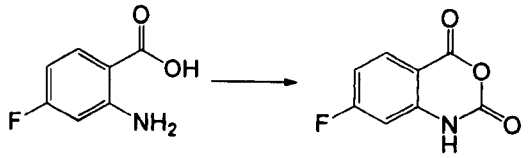
Preparation of **7-(Trifluoromethyl)-1H-benzo[d][1,3]oxazine-2,4-dione 63.**



58 **63**

2-Amino-4-trifluoromethylbenzoic acid **58** (1.0 g, 4.88 mmol) in KOH(aq) (0.58 g, 10.3 mmol, in 10 mL water) was cooled to 0°C. Trichloromethyl chloroformate (0.88 mL, 7.3 mmol, 1.5 equiv) was slowly added to the reaction. The mixture was warmed to room temperature and was allowed to stir for 4 h. The solid was filtered and washed with water. The solid was dissolved in ethyl acetate, dried over $MgSO_4$, filtered, concentrated to give the title compound (0.93 g, 83 %) as a pale yellow solid: mp 232 – 234°C; ^{1}H NMR (400 MHz, DMSO) δ 11.99 (s, 1H, N-H), 8.13 (d, *J* = 8.2 Hz, 1H, H-5), 7.57 (d, *J* = 8.2 Hz, 1H, H-6), 7.40 (s, 1H, H-8); ^{13}C NMR (101 MHz, DMSO) δ 159.38 (C=O), 147.04 (C=O), 142.14, 135.88 (q, *J* = 32.4 Hz, C-7), 130.84 (C-5), 123.38 (q, *J* = 273.3 Hz, CF_3), 119.74 (q, *J* = 3.3 Hz, C-6), 114.29, 112.37 (q, *J* = 4.0 Hz, C-8); MS (CI) $C_9H_8N_2O_3F_3$ $[M+NH_4]^+$ m/z 249.2.

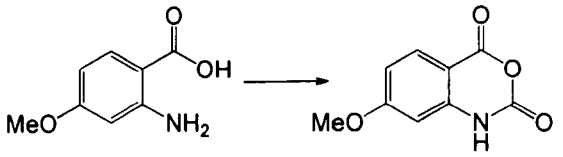
Preparation of **7-Fluoro-1H-benzo[d][1,3]oxazine-2,4-dione 64.**



59 **64**

64 was prepared from 2-Amino-4-fluorobenzoic acid **59** (2.50 g, 16.11 mmol) according to the procedure for the preparation of **63**. Filtration of the mixture gave **64** (2.27 g, 78 %) as a pale brown solid: mp 234 – 236°C; ^{1}H NMR (400 MHz, DMSO) δ 11.89 (s, 1H, N-H), 8.01 (dd, *J* = 8.8, 6.0 Hz, 1H, H-5), 7.12 (td, *J* = 8.8, 2.4 Hz, 1H, H-6), 6.89 (dd, *J* = 9.7, 2.4 Hz, 1H, H-8); ^{13}C NMR (101 MHz, DMSO) δ 167.14 (d, *J* = 254.3 Hz, C-F), 159.30 (C=O), 147.38 (C=O)), 144.08 (C-N), 132.75 (C-5), 111.90 (C-6), 107.76 (C-CO), 102.16 (C-8); HRMS (CI) $C_8H_5NO_3F$ $[M+H]^+$ requires 182.0248, found 182.0245; Anal. $C_8H_4NO_3F$ requires C 53.05%, H 2.23%, N 7.73%, found, C 53.17%, H 2.15%, N 7.67%.

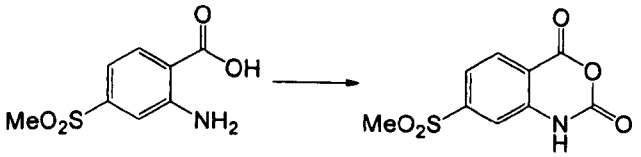
Preparation of **7-Methoxy-1H-benzo[d][1,3]oxazine-2,4-dione 65.**



60 **65**

65 was prepared from 2-Amino-4-methoxybenzoic acid (2.61 g, 15.6 mmol) according to the procedure for the preparation of **63**. Filtration of the mixture gave **65** (2.79 g, 93 %) as a grey solid: mp 204 – 206°C; ^{1}H NMR (400 MHz, DMSO) δ 11.66 (s, 1H, N-H), 7.84 (d, *J* = 8.8 Hz, 1H, H-5), 6.84 (dd, *J* = 8.8, 2.4 Hz, 1H, H-6), 6.59 (d, *J* = 2.4 Hz, 1H, H-8), 3.86 (s, 3H, OCH_3); ^{13}C NMR (101 MHz, DMSO) δ 166.23 (C-7), 159.58 (C=O), 147.77 (C=O), 143.98, 131.42 (C-5), 112.01 (C-8), 103.25, 98.89 (C-6), 56.30 (OCH_3); MS (CI) $C_9H_{11}N_2O_4$ $[M+H+NH_3]^+$ m/z 211.2; Anal. $C_9H_7NO_4$ requires C 55.96%, H 3.65%, N 7.25%, found C 55.93%, H 3.61%, N 7.27%.

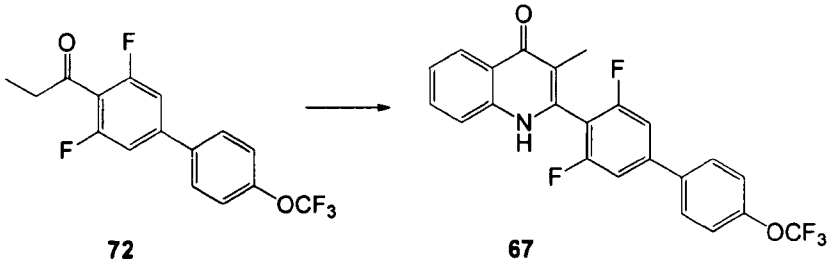
Preparation of **7-(Methylsulfonyl)-1H-benzo[d][1,3]oxazine-2,4-dione 66.**



61 **66**

66 was prepared from 2-amino-4-(methylsulphonyl)benzoic acid **61** (1.0 g, 4.67 mmol) according to the procedure for the preparation of **63**. Filtration of the mixture gave **66** (0.97 g, 86%) as a yellow solid: mp 235 - 236°C; ^{1}H NMR (400 MHz, DMSO) δ 12.04 (s, 1H, N-H), 8.16 (d, *J* = 8.2 Hz, 1H, H-5), 7.74 (dd, *J* = 8.2, 1.6 Hz, 1H, H-6), 7.62 (d, *J* = 1.7 Hz, 1H, H-8), 3.32 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 159.47 (C=O), 147.69, 147.13, 142.22, 130.88 (C-5), 121.33 (C-6), 114.81, 114.27 (C-8), 43.34 (CH_3); Anal. $C_9H_7NO_5S$ requires C 44.81%, H 2.92%, N 5.81%, found C 44.47%, H 2.91%, N 5.85%.

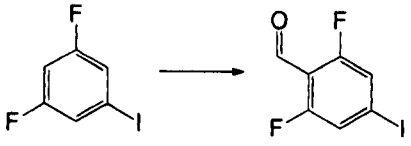
Preparation of **2-(3,5-Difluoro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)-3-methylquinolin-4(1H)-one 67.**



72 **67**

67 was prepared from **11** (0.138 g, 0.72 mmol) and **72** (0.231 g, 0.70 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 80% ethyl acetate in hexane gave **67** (98 mg, 33 %) as a yellow solid: R_f = 0.24, 40% ethyl acetate in hexane; mp 297 – 298°C; ^{1}H NMR (400 MHz, DMSO) δ 12.00 (s, 1H, N-H), 8.16 (dd, *J* = 8.1, 1.2 Hz, 1H, H-5), 8.04 – 7.99 (m, 2H, H-6'), 7.85 – 7.78 (m, 2H, H-3'), 7.67 (ddd, *J* = 8.4, 7.0, 1.5 Hz, 1H, H-7), 7.57 – 7.52 (m, 3H, H-8 + H-7'), 7.41 – 7.27 (m, 1H, H-6), 1.86 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.74 (C=O), 161.27, 158.72, 149.32, 143.61, 140.06, 136.64, 136.08, 132.15 (C-7), 129.54 (C-6'), 125.43 (C-5), 123.52 (C-6), 123.47, 122.00 (C-7'), 118.36 (C-8), 117.71, 111.01 (C-3'), 110.76 (C-3'), 12.12 (CH_3); IR ν_{max} (neat)/cm^{-1} 2977.6, 2885.0, 1639.2 (C=O), 1498.4, 1251.6, 1222.7, 1160.9, 1035.6 and 1014.3; HRMS (ESI) $C_{23}H_{15}NO_2F_5$ $[M+H]^+$ requires 432.1023, found 432.1018; Anal. $C_{23}H_{14}NO_2F_5$ requires C 64.04%, H 3.27%, N 3.25%, found C 64.15%, H 3.16%, N 3.15%.

Preparation of **2,6-Difluoro-4-iodobenzaldehyde 69.**

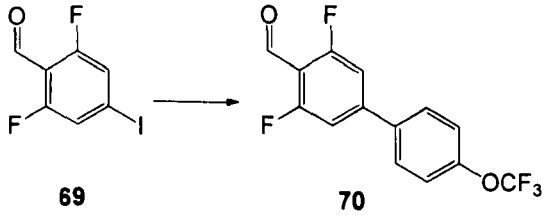


68 **69**

To a solution of 2,2,6,6-tetramethyl piperidine **68** (1.41 g, 9.98 mmol) in anhydrous tetrahydrofuran (50 mL) was added n-butyl lithium (4 mL) at -20°C. The light yellow solution was allowed to stir at -20°C for ½ h. The mixture was cooled to -78°C and 3,5-difluoroiodobenzene (2.10 g, 8.75 mmol) was added. The red brown mixture

was allowed to stir for further 2 h. Anhydrous DMF (1.6 mL, 21.8 mmol, 2.5 equiv) was added to the mixture and the yellow mixture was allowed to warm up and stir at room temperature for another 2 h. The solvent was removed under reduced pressure and the resulting residue was extracted with diethyl ether (x 3), washed with water, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography using 10 % ethyl acetate in hexane to give the title compound (0.65 g, 28 %) as a clear liquid: R_f = 0.70, 10% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.29 (s, 1H, CHO), 7.43 (d, *J* = 7.6 Hz, 2H).

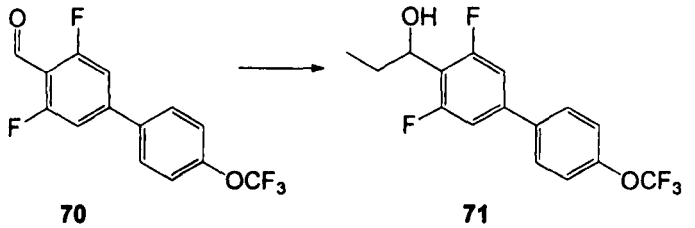
Preparation of **3,5-Difluoro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-carbaldehyde 70.**



69 **70**

70 was prepared from **69** (0.4 g, 1.49 mmol) according to the procedure for the preparation of **19**. Purification by column chromatography using 10% ethyl acetate in hexane gave **70** (0.30 g, 67 %) as a clear oil: R_f = 0.56, 10% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.38 (s, 1H, CHO), 7.67 – 7.58 (m, 2H, H-2' + H-6'), 7.39 – 7.32 (m, 2H, H-3' + H-5'), 7.24 – 7.14 (m, 2H, H-2 + H-6); HRMS (ESI) $C_{15}H_{11}O_3F_5{}^{23}Na$ $[M+Na]^+$ requires 357.0526, found 356.0524;

Preparation of **1-(3,5-Difluoro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)propan-1-ol 71.**

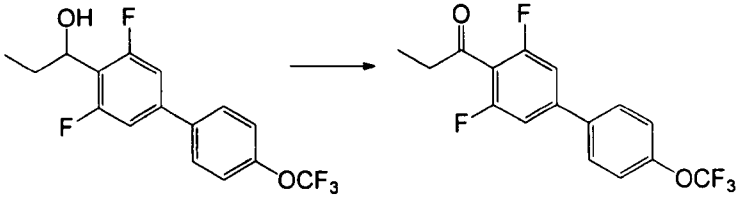


70 **71**

71 was prepared from **70** (0.30 g, 0.99 mmol) according to the procedure for the preparation of **23**. Purification by column chromatography using 20 % ethyl acetate in hexane gave **71** (0.27 g, 82 %) as a yellow oil: R_f = 0.44, 20% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 – 7.53 (m, 2H, H-2' + H-6'), 7.31 (dd, *J* =

8.8, 0.8 Hz, 2H, H-3' + H-5'), 7.13 – 7.03 (m, 2H, H-2 + H-6), 4.99 (dt, *J* = 8.7, 7.3 Hz, 1H, C<u>H</u>OH), 2.20 (dt, *J* = 9.0, 2.3 Hz, 1H, OH), 2.12 – 1.79 (m, 2H, CH_2), 0.99 (t, *J* = 7.4 Hz, 3H, CH_3); HRMS (CI) $C_{16}H_{13}F_5O_2$ $[M]^+$ requires 332.0830, found 332.0822; $C_{16}H_{12}F_5O_2$ $[M-H]^+$ requires 331.0752, found 331.0743.

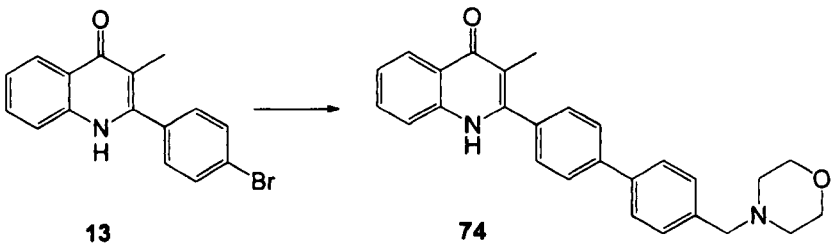
Preparation of **1-(3,5-Difluoro-4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)propan-1-one 72.**



71 **72**

72 was prepared from **71** (0.273 g, 0.8 mmol) according to the procedure for the preparation of **27**. Purification by column chromatography using 50% ethyl acetate in hexane gave **72** (0.23 g, 85 %) as a pale yellow oil: R_f =0.69, 20% ethyl acetate in hexane; ^1H NMR (400 MHz, $CDCl_3$) δ 7.63 – 7.55 (m, 2H, H-2' + H-6'), 7.38 – 7.30 (m, 2H, H-3' + H-5'), 7.19 – 7.11 (m, 2H, H-2 + H-6), 2.93 (q, *J* = 7.3 Hz, 2H, CH_2), 1.23 (t, *J* = 7.2 Hz, 3H, CH_3); HRMS (ESI) $C_{16}H_{11}O_2F_5{}^{23}Na$ $[M+Na]^+$ requires 353.0577, found 353.0583.

Preparation of **3-Methyl-2-(4'-(morpholinomethyl)-[1,1'-biphenyl]-4-yl)quinolin-4(1H)-one 74.**

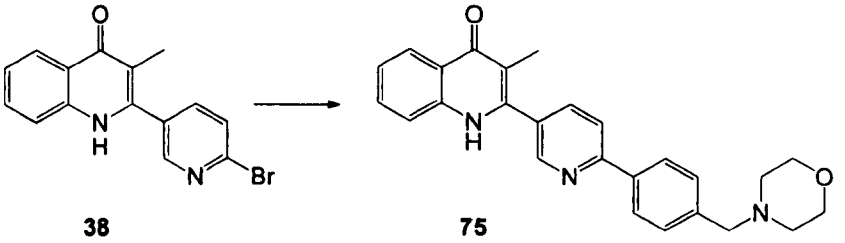


13 **74**

13 (0.157 g, 0.50 mmol) $PdCl_2$(dppf) (0.03 g, 0.04 mmol, 0.05 equiv) and anhydrous potassium carbonate (0.21 g, 1.50 mmol, 3 equiv) in anhydrous 1,4-dixoane (13.5 mL) and water (1.5 mL) were allowed to stir under nitrogen environment at room temperature for 10 minutes. 4-(4-Morpholinomethyl)phenylboronic acid pinacol ester (0.249 g, 0.82 mmol) was added and then the reaction system was degassed

and refilled with N_2 3 times. The mixture was heated to 100°C for 24 h (followed by tlc) and the mixture was cooled, diluted with 50% ethyl acetate in hexane, and filtered through a pad of magnesium sulphate-silica. The filter pad was washed with 2% methanol in ethyl acetate. The filtrate was concentrated to give a solid and the crude product was purified by column chromatography using 10% methanol in acetate to give the title compound (0.125 g, 61 %) as a brown solid: R_f = 0.22, 5% methanol in ethyl acetate; mp 258 – 260°C; ^{1}H NMR (400 MHz, DMSO) δ 11.65 (s, 1H, N-H), 8.14 (d, *J* = 8.1 Hz, 1H, H-5), 7.95 – 7.84 (m, 2H, Ar'-H), 7.74 (d, *J* = 8.2 Hz, 2H, Ar'-H), 7.69 – 7.64 (m, 2H, Ar'-H), 7.62 (m, 1H, H-7), 7.45 (d, *J* = 8.2 Hz, 2H, Ar'-H), 7.38 (d, *J* = 8.2 Hz, 1H, H-8), 7.31 (ddd, *J* = 8.1, 4.9, 3.1 Hz, 1H, H-6), 3.69 – 3.58 (m, 4H, OC$\underline{H}_2$), 3.53 (s, 2H, CH_2), 2.39 (m, 4H, NC$\underline{H}_2CH_2$), 1.95 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 177.06 (C=O), 147.67, 141.27, 139.88, 138.38, 138.10, 134.35, 131.64, 130.01 (C-2'), 129.99 (C-7'), 127.03 (C-3'), 127.00 (C-6'), 126.74, 125.31, 123.43, 118.52, 114.77, 66.58 (OCH_2), 62.40 (NCH_2), 53.57 (N$\underline{C}H_2CH_2$), 12.62 (CH_3); HRMS (ESI) $C_{27}H_{27}N_2O_2$ $[M+H]^+$ requires 411.2073, found 411.2071; Anal. $C_{27}H_{26}N_2O_2$ requires C 79.00%, H 6.38%, N 6.82%, found C 78.80%, H 6.38%, N 6.82%.

Preparation of **3-Methyl-2-(6-(4-(morpholinomethyl)phenyl)pyridin-3-yl)quinolin-4(1H)-one 75.**

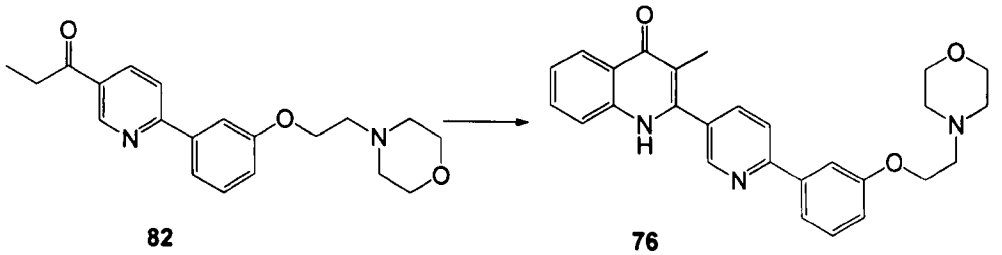


38 **75**

75 was prepared from **38** (0.163 g, 0.47 mmol) and 4-(4-morpholinomethyl)phenylboronic acid pinacol ester (0.265 g, 0.87 mmol) according to the procedure for the preparation of **74**. Purification by column chromatography using 10% methanol in ethyl acetate gave **75** (0.106 g, 50 %) as a yellow solid: R_f = 0.13, 5% methanol in ethyl acetate; mp 272 – 274°C; ^{1}H NMR (400 MHz, DMSO) δ 11.77 (s, 1H, N-H), 8.87 (d, *J* = 2.2 Hz, 1H, H-2'), 8.23 – 8.07 (m, 5H), 7.71 – 7.57 (m, 2H), 7.48 (d, *J* = 8.2 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 1H), 3.63 – 3.57 (m, 4H, OC$\underline{H}_2$), 3.55

(s, 2H, CH_2), 2.40 (m, 4H, $NC\underline{H}_2CH_2$), 1.96 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 176.98 (C=O), 156.85, 149.62 (C-2'), 144.98, 139.95, 138.26, 137.09, 131.81, 129.80 (C-8'), 127.01 (C-9'), 125.37, 123.49, 123.21, 119.93, 118.52, 115.53, 66.57 (OCH_2), 62.42 (NCH_2), 53.58 ($N\underline{C}H_2CH_2$), 12.43 (CH_3); HRMS (ESI) $C_{26}H_{26}N_3O_2$ $[M+H]^+$ requires 412.2025, found 412.2043; Anal. $C_{26}H_{25}N_3O_2$ requires C 75.89%, H 6.12%, N 4.21%, found C 75.65%, H 6.23%, N 3.97%.

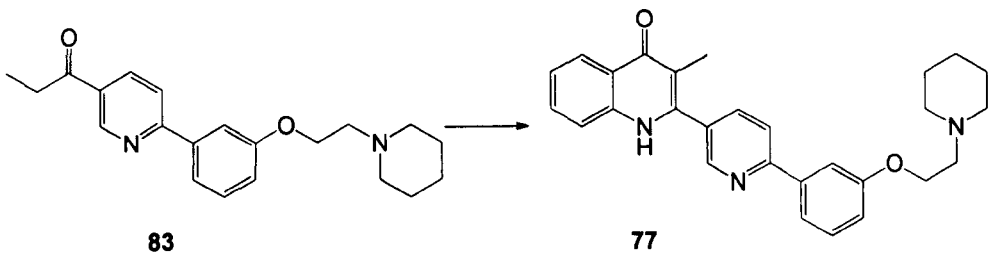
Preparation of **3-Methyl-2-(6-(3-(2-morpholinoethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one 76.**



82 **76**

76 was prepared from **11** (131 mg 0.69 mmol) and **82** (235 mg, 0.69 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 10% methanol in dichloromethane gave **76** (152 mg, 50 %) as a white solid: R_f = 0.27, 5% methanol in dichloromethane; MP 232- 234°C; ^1H NMR (400 MHz, $CDCl_3$) δ 10.67 (s, 1H, N-H), 8.61 (d, *J* = 1.6 Hz, 1H, H-2'), 8.18 (d, *J* = 7.5 Hz, 1H, H-5), 7.69 (d, *J* = 8.3 Hz, 1H, H-8'), 7.65 – 7.43 (m, 5H), 7.37 (t, *J* = 7.9 Hz, 1H, H-11'), 7.31 – 7.21 (m, 1H, H-6), 6.98 (dd, *J* = 8.0, 1.9 Hz, 1H, H-10'), 4.16 (t, *J* = 5.5 Hz, 2H, OCH_2), 3.73 – 3.64 (m, 4H, OCH_2), 2.80 (t, *J* = 5.5 Hz, 2H, NCH_2), 2.61 – 2.46 (m, 4H, NCH_2), 1.94 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.79 (C=O), 159.61, 157.73, 149.41 (C-2'), 145.03, 140.02, 139.91, 137.48, 132.17, 130.34 (C-11'), 130.06, 126.21 (C-5), 124.10, 123.94, 120.13, 119.82 (C-12'), 118.23, 117.21, 116.21 (C-10'), 113.64, 67.20 (OCH_2), 65.98 ($ArO\underline{C}H_2$), 58.10 ($ArOCH_2\underline{C}H_2N$), 54.45 (NCH_2), 12.72 (CH_3); IR ν_{max} (neat)/cm^{-1} 2977.6, 2886.9, 1677.8 (C=O), 1629.6, 1591.0, 1477.2, 1438.7, 1376.9 and 1251.6; HRMS (ESI) $C_{27}H_{28}N_3O_3$ $[M+H]^+$ requires 442.2131, found 442.2138; Anal. $C_{27}H_{27}N_3O_3$ requires C 73.45%, H 6.16%, N 9.52%, found C 73.09%, H 6.26%, N 9.19%.

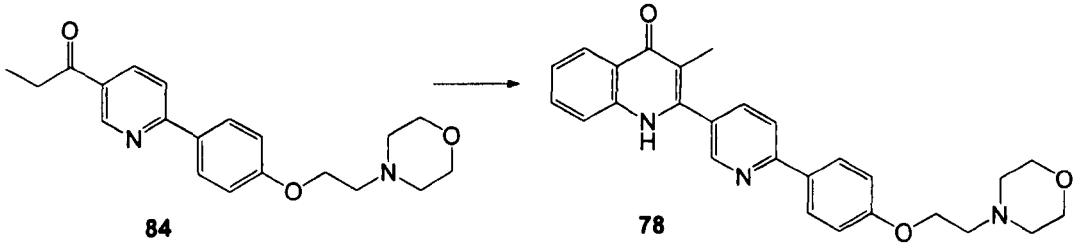
Preparation of **3-Methyl-2-(6-(3-(2-(piperidin-1-yl)ethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one 77.**



83 **77**

77 was prepared from **11** (68 mg 0.36 mmol) and **83** (120.6 mg, 0.36 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 15% methanol in dichloromethane gave **77** (31 mg, 21 %) as a white solid: R_f = 0.49, 20% methanol in dichloromethane; mp 193 – 194°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.88 (s, 1H, N-H), 8.58 (d, *J* = 1.9 Hz, 1H, H-2'), 8.19 (dd, *J* = 8.2, 1.0 Hz, 1H, H-5), 7.90 (d, *J* = 8.3 Hz, 1H, H-8), 7.61 – 7.51 (m, 2H, H-4' + H-7), 7.51 – 7.43 (m, 3H, H-5' + H-8' + H-11'), 7.37 (t, *J* = 7.9 Hz, 1H, H-10'), 7.25 (t, *J* = 7.4 Hz, 1H, H-6), 6.96 (dd, *J* = 8.1, 2.1 Hz, 1H, H-9'), 4.22 (t, *J* = 5.5 Hz, 2H, $ArOC\underline{H}_2$), 2.93 (t, *J* = 5.4 Hz, 2H, $ArOCH_2C\underline{H}_2N$), 2.66 (d, *J* = 16.1 Hz, 4H, NCH_2), 1.93 (d, *J* = 3.2 Hz, 3H, CH_3), 1.80 – 1.58 (m, 4H, $NCH_2C\underline{H}_2$), 1.54 – 1.41 (m, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.72 (C=O), 159.28, 157.16, 149.61 (C-2'), 145.51, 140.25, 140.09, 137.74, 131.95, 130.32, 129.90, 125.78, 124.05, 123.78, 119.93, 119.78, 118.84, 116.80, 116.35, 113.13, 66.25 (OCH_2), 57.95, 55.28, 25.26 ($OCH_2\underline{C}H_2N$), 23.80 ($NCH_2CH_2\underline{C}H_2$), 12.72 ($CH_3$); HRMS (ESI) $C_{28}H_{30}N_3O_2$ $[M+H]^+$ requires 440.2338, found 440.2357.

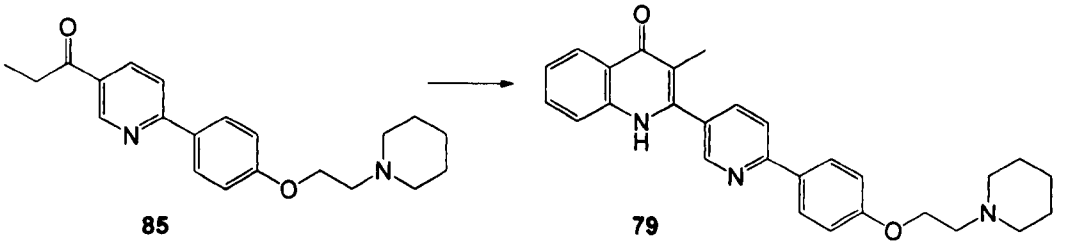
Preparation of **3-Methyl-2-(6-(4-(2-morpholinoethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one 78.**



84 **78**

78 was prepared from **11** (139 mg 0.73 mmol) and **84** (249 mg, 0.73 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 10% methanol in dichloromethane gave **78** (158 mg, 49 %) as a white solid: R_f = 0.36, 15% methanol in dichloromethane; mp 263 – 264°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.17 (s, 1H, N-H), 8.61 (d, *J* = 1.7 Hz, 1H, H-2'), 8.24 (d, *J* = 8.1 Hz, 1H, H-5), 7.89 (d, *J* = 8.9 Hz, 2H, H-8'), 7.63 (d, *J* = 2.3 Hz, 1H, H-4'), 7.62 – 7.51 (m, 3H, H-5' + H-7 + H-8), 7.35 – 7.26 (m, 1H, H-6), 7.01 – 6.94 (m, 2H, H-9'), 4.15 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.78 – 3.68 (m, 4H, OCH_2), 2.82 (t, *J* = 5.7 Hz, 2H, NCH_2), 2.66 – 2.52 (m, 4H, NCH_2), 1.98 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.85 (C=O), 160.58, 157.85, 149.33 (C-2'), 145.03, 139.81, 137.32 (C-4'), 132.18, 131.28, 129.19, 128.72 (C-8'), 126.35 (C-5), 124.12, 123.92, 119.26, 118.06, 117.28, 115.29 (C-9'), 67.31 ($O\underline{C}H_2$), 66.32 ($ArOCH_2$), 57.99 ($ArOCH_2\underline{C}H_2$), 54.52 (NCH_2), 12.74 (CH_3); IR ν_{max} (neat)/cm^{-1} 2977.6, 2888.8, 1627.6 (C=O), 1602.6, 1554.3, 1479.1, 1452.1, 1253.5 and 116.6; HRMS (ESI) $C_{27}H_{28}N_3O_3$ $[M+H]^+$ requires 442.2131, found 442.2126; Anal. $C_{27}H_{27}N_3O_3$ requires C 73.45%, H 6.16%, N 9.52%, found C 73.28%, H 6.17%, N 9.42%.

Preparation of **3-Methyl-2-(6-(4-(2-(piperidin-1-yl)ethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one 79.**

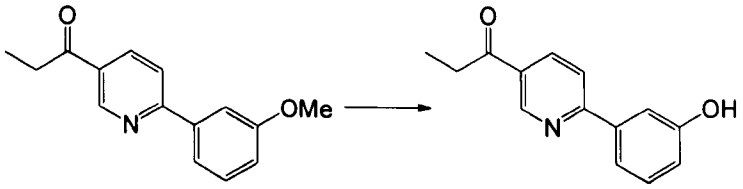


85 **79**

79 was prepared from **11** (96.9 mg 0.51 mmol) and **85** (173 mg, 0.51 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 20% methanol in dichloromethane gave **79** (88 mg, 40 %) as a white solid: R_f = 0.31, 15% methanol in dichloromethane; mp 234 – 235°C; ^{1}H NMR (400 MHz, $CDCl_3$+MeOD) δ 8.74 (dd, *J* = 2.2, 0.7 Hz, 1H, H-2'), 8.35 (dd, *J* = 8.2, 1.0 Hz, 1H, H-5), 8.00 – 7.94 (m, 2H, H-8'), 7.87 (dd, *J* = 8.2, 2.3 Hz, 1H, H-4'), 7.80 (d,

J = 8.2 Hz, 1H, H-5'), 7.61 (ddd, *J* = 8.3, 6.9, 1.4 Hz, 1H, H-7), 7.53 (d, *J* = 8.0 Hz, 1H, H-8), 7.36 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H, H-6), 7.07 – 6.96 (m, 2H, H-9'), 4.30 (t, *J* = 5.3 Hz, 2H, $ArOC\underline{H}_2$), 3.02 (s, 2H, $ArOCH_2C\underline{H}_2$), 2.77 (s, 4H, NCH_2), 2.10 (d, *J* = 3.5 Hz, 3H, CH_3), 1.86 – 1.71 (m, 4H, $NCH_2C\underline{H}_2$), 1.54 (s, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$+MeOD) δ 179.15 (C=O), 164.18, 158.05, 149.11 (C-2'), 145.39, 139.88, 138.05 (C-4'), 132.18 (C-7), 131.46, 129.33, 128.87 (C-8'), 128.08, 126.03 (C-5), 123.98 (C-6), 119.89 (C-5'), 118.02, 117.06 (C-8), 115.29 (C-9'),64.87 ($ArO\underline{C}H_2$), 57.26 ($ArOCH_2\underline{C}H_2$), 55.09 (NCH_2), 25.06 ($NCH_2\underline{C}H_2$), 23.63 (CH_2), 12.59 (CH_3); HRMS (ESI) $C_{28}H_{30}N_3O_2$ $[M+H]^+$ requires 440.2338, found 440.2341.

Preparation of **1-(6-(3-Hydroxyphenyl)pyridin-3-yl)propan-1-one 80.**

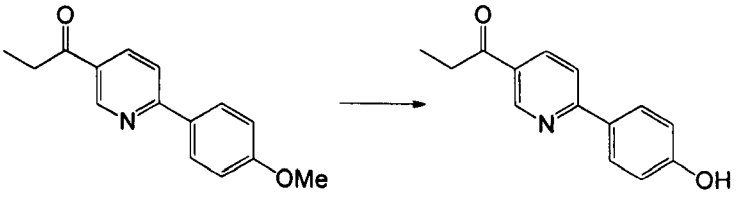


29 **80**

2-(Diethylamino)ethanethiol hydrochloride (0.34 g, 2.0 mmol, 1.2 equiv) in anhydrous DMF (4 mL) was cooled to 0°C. Sodium *tert*-butoxide (0.40 g, 4.16 mmol, 2.52 equiv) was added. After 5 min, the ice bath was removed and the white suspension was allowed to warm to room temperature. After 15 min, **29** (0. 40 g, 1.66 mmol) was added. The resulting dark mixture was heated to reflux for 2 h. The mixture was cooled to room temperature, and the flask was placed in an ice water bath. The mixture was acidified to pH 1 by 1 N HCl (aq) and diluted with water. The aqueous phase was extracted with ethyl acetate (x 3), and the combined organic extracts were washed with water (x 3) and saturated brine, dried over $MgSO_4$, filtered and concentrated. The crude product was purified by column chromatography using 2% methanol in dichloromethane to give the title compound (0.23 g, 60 %) as a yellow solid: R_f = 0.2, 15% ethyl acetate in dichloromethane; mp 151 – 152°C; ^1H NMR (400 MHz, MeOD) δ 9.16 (dd, *J* = 2.2, 0.7 Hz, 1H, H-2), 8.38 (dd, *J* = 8.4, 2.3 Hz, 1H, H-4), 7.93 (dd, *J* = 8.4, 0.8 Hz, 1H, H-5), 7.54 – 7.41 (m, 2H, H-8 + H-12), 7.37 – 7.28 (m, 1H, H-11), 6.91 (ddd, *J* = 8.1, 2.2, 1.2 Hz, 1H, H-10), 3.11 (q, *J* =

7.2 Hz, 2H, CH_2), 1.22 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, MeOD) δ 199.81 (C=O), 160.72, 157.86, 148.95, 139.44, 136.56, 130.62, 129.65, 120.53, 118.27, 116.78, 113.83, 31.57 (CH_2), 6.83 (CH_3); IR ν_{max} (neat)/cm^{-1} 3351.7 (O-H), 2979.5, 2890.8, 1668.1 (C=O), 1592.9, 1562.1, 1450.2 and 1232.3; HRMS (CI) $C_{14}H_{14}NO_2$ $[M+H]^+$ requires 228.1019, found 228.1023; Anal. $C_{14}H_{13}NO_2$ requires C 73.99%, H 5.77%, N 6.16%, found C 73.59%, H 5.82%, N 6.02%.

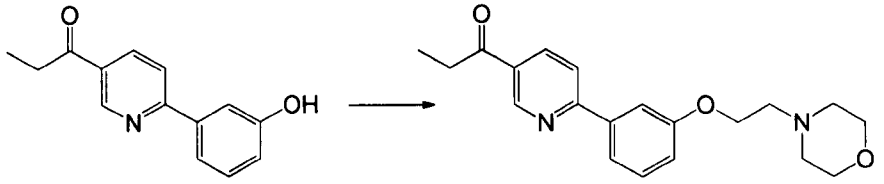
Preparation of **1-(6-(4-Hydroxyphenyl)pyridin-3-yl)propan-1-one 81.**



30 **81**

81 was prepared from **30** (0.35 g, 1.45 mmol) according to the procedure for the preparation of **80**. Purification by column chromatography using 2% methanol in dichloromethane gave **81** (0.21 g, 64 %) as a yellow solid: R_f = 0.51, 50% ethyl acetate in hexane; mp 164 – 165°C; ^1H NMR (400 MHz, $CDCl_3$) δ 9.20 (dd, *J* = 2.3, 0.8 Hz, 1H, H-2), 8.27 (dd, *J* = 8.4, 2.3 Hz, 1H, H-4), 8.09 – 7.89 (m, 2H, H-8), 7.77 (dd, *J* = 8.4, 0.8 Hz, 1H, H-5), 6.93 (d, *J* = 8.9 Hz, 2H, H-9), 5.69 (s, 1H, OH), 3.04 (q, *J* = 7.2 Hz, 2H, CH_2), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.67, 160.77, 158.05, 150.02, 145.53, 136.68, 130.19, 129.48, 119.84, 116.29, 32.47, 8.43; IR ν_{max} (neat)/cm^{-1} 3108.7 (O-H), 2977.6, 2888.8, 1681.6 (C=O), 1589.1, 1552.4, 1484.9, 1436.7, 1228.4 and 1170.6; MS (CI) $C_{14}H_{14}NO_2$ $[M+H]^+$ m/z 228.3; Anal. $C_{14}H_{13}NO_2$ requires C 73.99%, H 5.77%, N 6.16%, found C 73.63%, H 5.95%, N 5.95%.

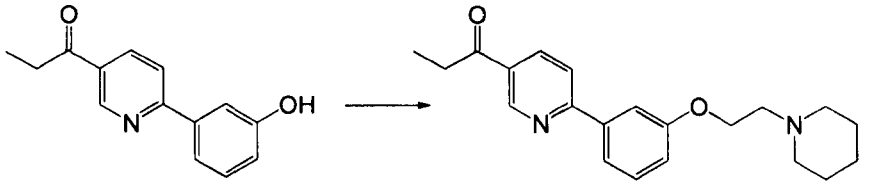
Preparation of **1-(6-(3-(2-Morpholinoethoxy)phenyl)pyridin-3-yl)propan-1-one 82.**



80 **82**

To a solution of **80** (0.2 g, 0.88 mmol) in dry acetone (10 mL) was added potassium carbonate (0.49 g, 3.55 mmol, 4 equiv). The yellow mixture was allowed to stir for 15 min and 4-(2-chloroethyl)morpholine hydrochloride (0.33 g, 1.76 mmol, 2 equiv) was added. The mixture was stirred for another 10 min, then was heated to 60 °C for 30 h. The reaction was allowed to cool, the mixture was filtered, washed with cool acetone. The filtrate was concentrated under reduced pressure and the crude product was purified by column chromatography using 5% methanol in dichloromethane to give the title compound (0.21 g, 70 %) as a white solid: R_f = 0.36, 5% methanol in dichloromethane; mp 106 – 107°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (d, *J* = 1.6 Hz, 1H, H-2), 8.30 (dd, *J* = 8.3, 2.2 Hz, 1H, H-4), 7.83 (d, *J* = 8.3 Hz, 1H, H-5), 7.68 (s, 1H, H-8), 7.61 (d, *J* = 7.8 Hz, 1H, H-12), 7.41 (t, *J* = 7.9 Hz, 1H, H-11), 7.03 (dd, *J* = 8.1, 2.0 Hz, 1H, H-10), 4.22 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.81 – 3.71 (m, 4H, OCH_2), 3.06 (d, *J* = 7.2 Hz, 2H, $C\underline{H}_2CH_3$), 2.85 (t, *J* = 5.6 Hz, 2H, NCH_2), 2.73 – 2.57 (m, 4H, NCH_2), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.09, 160.83, 150.03, 140.07, 136.62, 130.33, 120.69, 120.26, 117.73, 116.97, 113.69, 67.36, 66.36, 58.09, 54.52, 32.56, 8.39; IR ν_{max} (neat)/cm^{-1} 2977.6, 2888.8, 1685.5 (C=O), 1585.2, 1461.8, 1450.2, 1382.7, 1295.9, 1228.4, 1116.6, 1037.5 and 950.7; HRMS (ESI) $C_{20}H_{25}N_2O_3$ $[M+H]^+$ requires 341.1865, found 341.1879; Anal. $C_{20}H_{24}N_2O_3$ requires C 70.56%, H 7.11%, N 8.23%, found C 70.19%, H 7.31%, N 8.09%.

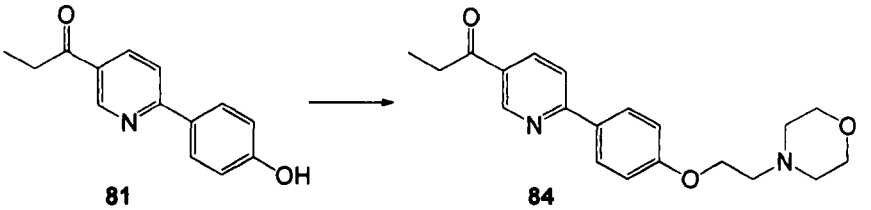
Preparation of **1-(6-(3-(2-(Piperidin-1-yl)ethoxy)phenyl)pyridin-3-yl)propan-1-one 83.**



80 **83**

83 was prepared from **80** (0.2 g, 0.88 mmol) and 4-(2-chloroethyl) piperidine hydrochloride (0.324 g, 1.76 mmol) according to the procedure for the preparation of **82**. Purification by column chromatography using 10% methanol in dichloromethane gave **83** (0.21 g, 69 %) as a yellow oil: R_f = 0.13, 5% methanol in dichloromethane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (d, *J* = 1.9 Hz, 1H, H-2), 8.28 (dd, *J* = 8.3, 2.3 Hz, 1H, H-4), 7.82 (d, *J* = 8.3 Hz, 1H, H-5), 7.69 – 7.64 (m, 1H, H-8), 7.61 (d, *J* = 7.8 Hz, 1H, H-12), 7.39 (d, *J* = 7.9 Hz, 1H, H-11), 7.02 (dd, *J* = 8.1, 2.1 Hz, 1H, H-10), 4.23 (t, *J* = 5.9 Hz, 2H, OCH_2), 3.05 (q, *J* = 7.2 Hz, 2H, $C\underline{H}_2CH_3$), 2.86 (t, *J* = 5.9 Hz, 2H, $OCH_2C\underline{H}_2$), 2.58 (s, 4H, NCH_2), 1.64 (dt, *J* = 11.2, 5.6 Hz, 4H, $NCH_2C\underline{H}_2$), 1.53 – 1.40 (m, 2H, CH_2), 1.27 (t, *J* = 5.8 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.64 (C=O), 160.85, 159.74, 150.00(C-2), 140.01, 136.58, 130.83, 130.29, 120.66, 120.16, 116.91, 113.72, 66.29 (OCH_2), 58.18, 55.32 (NCH_2), 32.53, 26.11 ($NCH_2\underline{C}H_2$), 24.44, 8.37 (CH_3); HRMS (ESI) $C_{21}H_{27}N_2O_2$ $[M+H]^+$ requires 339.2073, found 339.2086.

Preparation of **1-(6-(4-(2-Morpholinoethoxy)phenyl)pyridin-3-yl)propan-1-one 84.**

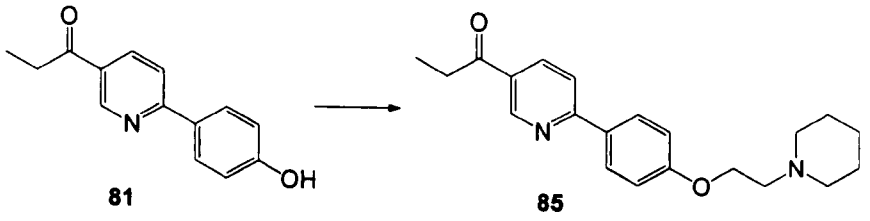


81 **84**

84 was prepared from **81** (0.23 g, 0.99 mmol) and 4-(2-chloroethyl)morpholine hydrochloride (0.37 g, 1.98 mmol) according to the procedure for the preparation of **82**. Purification by column chromatography using 10% methanol in dichloromethane gave **84** (0.25 g, 74 %) as a white solid: R_f = 0.42, 10% methanol in

ethyl acetate; mp 107 – 108°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (dd, *J* = 2.3, 0.7 Hz, 1H, H-2), 8.26 (dd, *J* = 8.4, 2.3 Hz, 1H, H-4), 8.06 – 7.99 (m, 2H, H-8), 7.77 (dd, *J* = 8.4, 0.8 Hz, 1H, H-5), 7.05 – 6.99 (m, 2H, H-9), 4.19 (t, *J* = 5.7 Hz, 2H, $C\underline{H}_2CH_3$), 3.82 – 3.70 (m, 4H, OCH_2), 3.04 (q, *J* = 7.2 Hz, 2H, OCH_2), 2.84 (t, *J* = 5.7 Hz, 2H, NCH_2), 2.68 – 2.52 (m, 4H, NCH_2), 1.27 (t, *J* = 7.2 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.67 (C=O), 160.85 (C-10), 160.70 (C-6), 150.12 (C-2), 136.56 (C-4), 131.35, 130.14, 129.18 (C-8), 119.67, 115.33 (C-9), 67.32 (OCH_2), 66.34 ($ArO\underline{C}H_2$), 57.98 ($ArOCH_2\underline{C}H_2N$), 54.53 (NCH_2), 32.46 (CH_2), 8.44 (CH_3); HRMS (ESI) $C_{20}H_{25}N_2O_3$ $[M+H]^+$ requires 341.1865, found 341.1877; Anal. $C_{20}H_{24}N_2O_3$ requires C 70.57%, H 7.11%, N 8.23%, found C 70.47%, H 7.14%, N 8.19%.

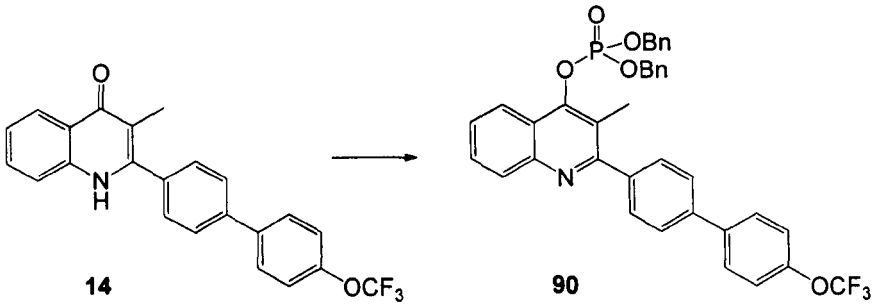
Preparation of **1-(6-(4-(2-(Piperidin-1-yl)ethoxy)phenyl)pyridin-3-yl)propan-1-one 85.**



85 was prepared from **81** (0.2 g, 0.88 mmol) and 4-(2-chloroethyl)piperidine hydrochloride (0.324 g, 1.76 mmol) according to the procedure for the preparation of **82**. Purification by column chromatography using 10% methanol in dichloromethane gave **85** (0.23 g, 77 %) as a pale yellow solid: R_f =0.56, 15% methanol in dichloromethane; mp 111 – 113°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.20 (dd, *J* = 2.2, 0.7 Hz, 1H, H-2), 8.26 (dd, *J* = 8.4, 2.3 Hz, 1H, H-4), 8.10 – 7.94 (m, 2H, H-8), 7.77 (dd, *J* = 8.4, 0.7 Hz, 1H, H-5), 7.07 – 6.97 (m, 2H, H-9), 4.20 (t, *J* = 6.0 Hz, 2H, OCH_2), 3.04 (q, *J* = 7.2 Hz, 2H, $C\underline{H}_2CH_3$), 2.84 (t, *J* = 5.5 Hz, 2H, $OCH_2C\underline{H}_2$), 2.56 (s, 4H, NCH_2), 1.64 (dt, *J* = 11.1, 5.6 Hz, 4H, $NCH_2C\underline{H}_2$), 1.47 (dd, *J* = 11.3, 6.3 Hz, 2H, CH_2), 1.26 (dd, *J* = 8.4, 6.1 Hz, 4H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.69 (C=O), 160.95, 160.76, 150.11 (C-2), 136.54, 131.19, 130.08, 129.15 (C-8), 119.65, 115.34 (C-9), 66.43 (OCH_2), 58.21 ($OCH_2\underline{C}H_2N$), 55.48 (NCH_2), 32.45 (CH_2), 26.24 ($NCH_2\underline{C}H_2$), 24.50

($NCH_2CH_2\underline{C}H_2$), 8.44 ($CH_3$); HRMS (ESI) $C_{21}H_{27}N_2O_2$ $[M+H]^+$ requires 339.2073, found 339.2084.

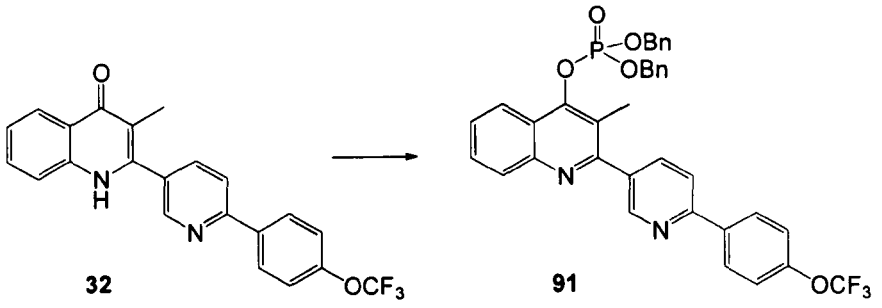
Preparation of **Dibenzyl(3-methyl-2-(4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)quinolin-4-yl) phosphate 90.**



14 **90**

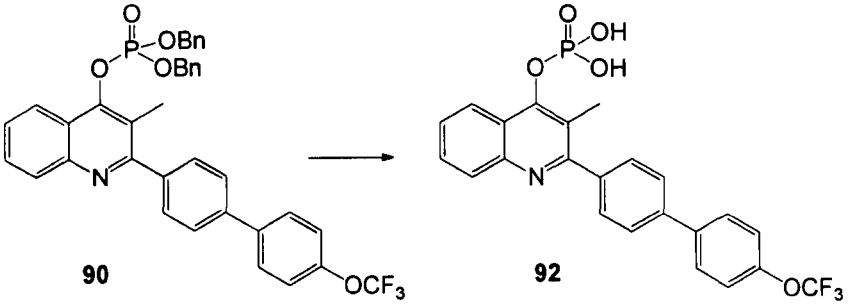
14 (57.5 mg, 0.15 mmol) in anhydrous tetrahydrofuran (7 mL) was cooled to 0°C and sodium hydride (60% in mineral oil) (18 mg, 0.75 mmol, 2.5 equiv) was added. The mixture was allowed to stir for 1 h under nitrogen. Tetrabenzyl pyrophosphate (77 mg, 0.14 mmol) was added and the mixture was stirred for another 1 h at room temperature. The mixture was filtered and the solid was washed with dichloromethane. The combined filtrate was concentrated and the residue was dissolved in dichloromethane, washed with saturated $NaHCO_3$ (aq), dried over $MgSO_4$, filtered and concentrated to give a pale yellow solid. The crude product was purified by column chromatography using 35% ethyl acetate in hexane to give the title compound (84.5 mg, 89%) as a colourless oil: R_f = 0.87, 80% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 7.8 Hz, 1H, H-5), 8.10 (d, *J* = 8.4 Hz, 1H, H-8), 7.73 – 7.68 (m, 1H, H-7), 7.68 – 7.63 (m, 4H, ArH), 7.63 – 7.58 (m, 2H, ArH), 7.51 (ddd, *J* = 8.1, 6.9, 1.0 Hz, 1H, H-6), 7.37 – 7.27 (m, 12H, ArH), 5.15 (dd, *J* = 8.8, 1.7 Hz, 4H, $OC\underline{H}_2Ph$), 2.43 (d, *J* = 1.6 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.78 (C-2), 149.27, 148.19, 140.29, 140.21, 139.93, 135.62, 135.55, 131.86, 131.15, 130.05, 129.86, 129.68, 129.24, 129.03 (Ar''-C x 4), 128.94, 128.48 (Ar''-C x 4), 127.44, 127.19, 122.50, 122.13, 121.73, 121.16, 116.21, 70.96 (CH_2), 70.90 (CH_2), 15.18 (CH_3); ^{31}P NMR (162 MHz, $CDCl_3$) δ -5.84; HRMS (ESI) $C_{37}H_{30}NO_5F_3P$ $[M+H]^+$ requires 656.1814, found 656.1804; Anal. $C_{37}H_{29}NO_5F_3P$ requires C 67.78%, H 4.46%, N 2.14%, found C 67.85%, H 4.35%, N 2.08%.

Preparation of **Dibenzyl (3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4-yl) phosphate 91.**



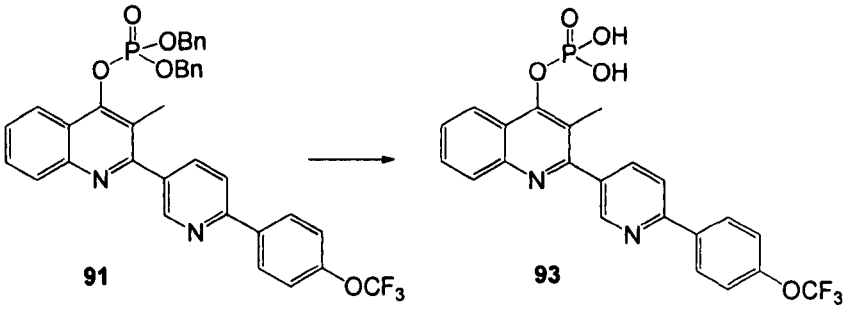
91 was prepared from **32** (120 mg, 0.30 mmol) according to the procedure for the preparation of **90**. Purification by column chromatography using 25% ethyl acetate in hexane gave **91** (165 mg, 74 %) as a white solid: R_f = 0.67, 60% ethyl acetate in hexane; mp 106°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (d, *J* = 1.6 Hz, 1H, H-2'), 8.18 (d, *J* = 8.2 Hz, 1H, H-5), 8.14 – 8.05 (m, 3H, H-8 + H-8'), 7.97 (dd, *J* = 8.2, 2.3 Hz, 1H, H-4'), 7.84 (d, *J* = 8.2 Hz, 1H, H-5'), 7.72 (ddd, *J* = 8.4, 6.9, 1.3 Hz, 1H, H-7), 7.60 – 7.51 (m, 1H, H-6), 7.41 – 7.25 (m, 12H, ArH + H-9'), 5.15 (dd, *J* = 9.0, 3.7 Hz, 4H, CH_2), 2.44 (d, *J* = 1.6 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.50, 158.75, 156.15, 155.86, 150.36, 143.41, 138.04, 137.99, 135.53, 135.46, 135.22, 130.13, 129.67, 129.30 (C-8'), 129.04 (ArC x 4), 128.98 (C-9'), 128.51 (ArC x 4), 127.57, 122.63, 122.30, 121.56, 121.27, 120.29, 71.05 (OCH_2), 70.99 (OCH_2), 15.06 (CH_3); ^{31}P NMR (162 MHz, $CDCl_3$) δ -5.85; HRMS (ESI) $C_{36}H_{29}N_2O_5F_3P$ $[M+H]^+$ requires 657.1766, found 657.1772; Anal. $C_{36}H_{28}N_2O_5F_3P$ requires C 65.85%, H 4.30%, N 4.27%, found C 65.93%, H 4.20%, N 4.18%.

Preparation of **3-Methyl-2-(4'-(trifluoromethoxy)-[1,1'-biphenyl]-4-yl)quinolin-4-yl dihydrogen phosphate 92.**



90 (130 mg, 0.20 mmol) in anhydrous methanol (10 ml) was submitted to hydrogenation in the presence of 10% palladium on carbon (64 mg) at room temperature for 10 min. The catalyst was filtered through a pad of celite and washed with methanol. The filtrate was evaporated to give the title compound (94.3 mg, 76%) as a pale yellow solid: (No further purification was required) mp 201 – 202°C; ^{1}H NMR (400 MHz, DMSO) δ 11.66 (s, 2H, OH), 8.14 (d, *J* = 7.8 Hz, 1H), 8.02 (d, *J* = 12.0 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 2H), 7.85 (s, 1H), 7.69 (ddt, *J* = 32.1, 27.6, 11.3 Hz, 4H), 7.51 (d, *J* = 8.3 Hz, 2H), 7.37 – 7.26 (m, 1H), 2.45 (s, 3H, CH_3); ^{31}P NMR (162 MHz, DMSO) δ -1.16; HRMS (ESI) $C_{23}H_{16}NO_5F_3P$ $[M-H]^-$ requires 474.0718, found 474.0704.

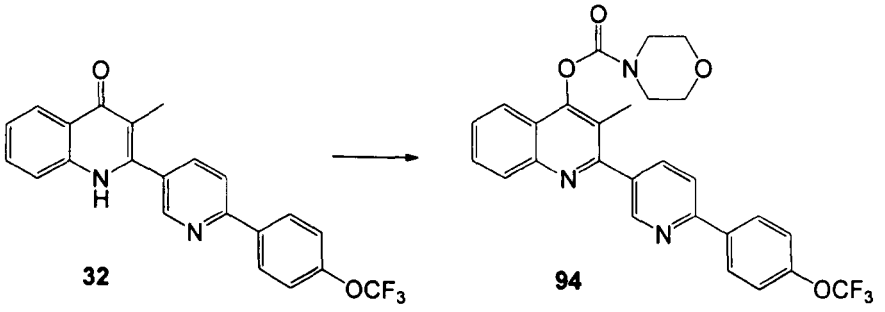
Preparation of **3-Methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4-yl dihydrogen phosphate 93.**



93 was prepared from **91** (144.4 mg, 0.22 mmol) according to the procedure for the preparation of **92**. Evaporation of the solvent gave **93** (76.2 mg, 72 %) as a pale

yellow solid: (No further purification was required) mp 257 – 258°C; ^{1}H NMR (400 MHz, DMSO) δ 11.78 (s, 2H, OH), 8.92 (s, 1H, H-2'), 8.42 – 8.22 (m, 3H, H5 + H-8'), 8.16 (m, 2H, H-4' + H-5'), 8.02 (d, *J* = 8.4 Hz, 1H, H-8), 7.76 (t, *J* = 7.6 Hz, 1H, H-7), 7.69 – 7.60 (m, 1H, H-6), 7.53 (d, *J* = 8.2 Hz, 2H, H-9'), 2.46 (s, 3H, CH_3); ^{31}P NMR (162 MHz, DMSO) δ -1.17, -5.89; HRMS (ESI) $C_{22}H_{15}N_2O_5F_3P$ $[M-H]^-$ requires 475.0671, found 475.0665.

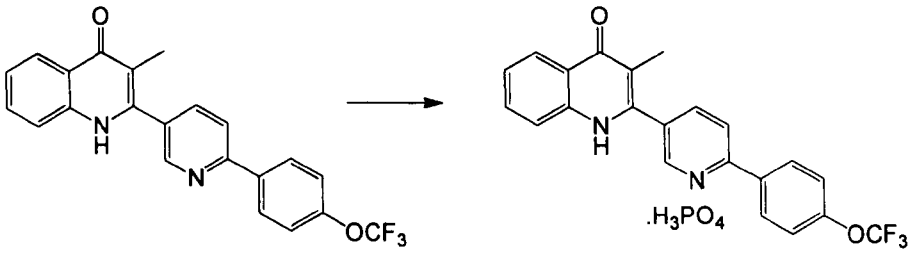
Preparation of **3-Methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4-yl morpholine-4-carboxylate 94.**



To **32** (124 mg, 0.31 mmol) in anhydrous tetrahydrofuran was added potassium *tert*-butoxide (52.7 mg, 0.47 mmol) at room temperature. The mixture was allowed to stir for ½ h. 4-Morpholinecarbonyl chloride (0.05 mL, 0.41 mmol) was added and the mixture was stirred for further 2 h. The reaction was quenched with brine, extracted with ethyl acetate, dried over Na_2SO_4, filtered and concentrated to an oil. The crude product was purified by column chromatography using 20 % ethyl acetate in hexane to give the title compound (125 mg, 78 %) as a white solid: R_f = 0.49, 70% ethyl acetate in hexane; mp 150 – 151°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (dd, *J* = 2.2, 0.8 Hz, 1H, H-2'), 8.15 (d, *J* = 8.4 Hz, 1H, H-5), 8.13 – 8.10 (m, 2H, H-8'), 8.08 (dd, *J* = 8.1, 2.3 Hz, 1H, H-4'), 7.91 – 7.81 (m, 2H, H-8 + H-5'), 7.73 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H, H-6), 7.60 (ddd, *J* = 8.2, 6.9, 1.1 Hz, 1H, H-7), 7.36 (d, *J* = 8.0 Hz, 2H, H-9'), 3.91 – 3.89 (m, 4H, OCH_2), 3.86 – 3.80 (m, 2H, NCH_2), 3.73 – 3.62 (m, 2H, OCH_2), 2.40 (s, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.51, 156.16, 153.69, 152.41, 150.48, 150.37 (C-2'), 148.27, 138.14, 137.99, 135.26, 130.06 (C-6), 128.97 (C-8'), 127.79 (C-7), 122.88, 122.18, 122.06, 121.56 (C-9'), 121.18, 120.41, 119.62, 67.15 (OCH_2), 45.70 (NCH_2), 44.90 (NCH_2), 14.33 (CH_3); IR ν_{max} (neat)/cm^{-1} 3074.0,

2925.5, 1729.8, 1261.2, 1232.3, 1207.2 and 1162.9; HRMS (ESI) $C_{27}H_{23}N_3O_4F_3$ $[M+H]^+$ requires 510.1641, found 510.1637; Anal. $C_{27}H_{22}N_3O_4F_3$ requires C 63.65%, H 4.35%, N 8.25%, found C 63.73%, H 4.23%, N 8.17%.

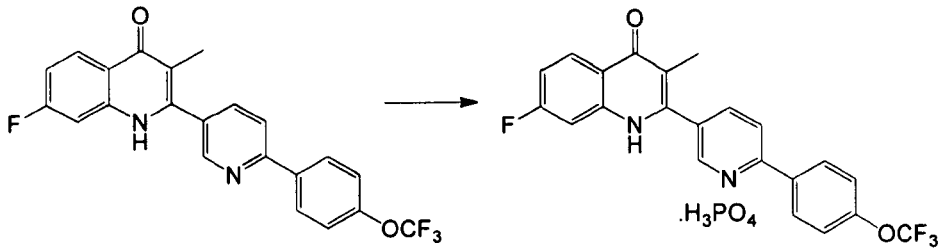
Preparation of **phosphate salt of 3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one (32) 95.**



32 **95**

To a solution of **32** (97 mg, 0.25 mmol) in ethanol (20 mL) was added 85% phosphoric acid (0.03 mL, 2 equiv). The clear solution was allowed to stir at room temperature for ~ 1 h and then the solvent was removed on rotary until 2-3 mL left. Diethyl ether (~20 mL) was added and the solid was filtered. The solid was further washed with diethyl ether and dried under high vacuum to give the title compound (quantitative yield) as a pale yellow solid: ^{1}H NMR (400 MHz, MeOD) δ 8.85 (dd, *J* = 1.9, 1.2 Hz, 1H, H-2'), 8.32 (dd, *J* = 8.3, 0.9 Hz, 1H, H-5), 8.27 – 8.21 (m, 2H, H-8'), 8.14 – 8.11 (m, 2H, H-4' + H-5'), 7.74 – 7.66 (m, 1H, H-7), 7.62 (d, *J* = 8.3 Hz, 1H, H-8), 7.49 – 7.39 (m, 3H, H-6 + H-9'), 2.11 (s, 3H, CH_3).

Preparation of **phosphate salt of 7-fluoro-3-methyl-2-(6-(4-(trifluoromethoxy)phenyl)pyridin-3-yl)quinolin-4(1H)-one(52) 96.**



52 **96**

96 was prepared from **52** (80 mg, 0.19 mmol) according to the procedure for the preparation of **92**. Filtration of the mixture gave **96** (quantitative yield) as a pale yellow solid: ^{1}H NMR (400 MHz, MeOD) δ 8.85 (dd, *J* = 2.0, 1.1 Hz, 1H, H-2'), 8.34 (dd, *J* = 9.1, 6.1 Hz, 1H, H-5), 8.30 – 8.20 (m, 2H, H-8'), 8.18 – 8.08 (m, 2H, H-4' + H-5'), 7.52 – 7.40 (m, 2H, H-9'), 7.28 (dd, *J* = 9.8, 2.3 Hz, 1H, H-8), 7.19 (td, *J* = 8.8, 2.4 Hz, 1H, H-6), 2.09 (s, 3H, CH_3).

Biology

Parasite Culture

Plasmodium blood stage cultures[57] and drug sensitivity[58] were determined by established methods. IC_{50}s (50% inhibitory concentrations) were calculated by using the four-parameter logistic method (Grafit program; Erithacus Software, United Kingdom)

High Throughput Screening (HTS)

*Pf*NDH2 activity was measured using an end-point assay in a 384 well plate format. Final assay concentrations used were; 200 µM NADH, 10 mM KCN, 1 µg/ml F571 membrane[11] and 20 µM decylubiquinone (dQ). A pre read at 340 nm was obtained prior to the addition of dQ to initiate the reaction followed by a post read at 1 min. HDQ was used as positive control at 5 µM. The agreed QC pass criteria was $Z' > 0.6$ and signal/background >10. Compounds were selected by the described chemoinformatics algorithms from the Biofocus DPI compound library (Galapagos Company).

Enzymology

P. falciparum cell-free extracts were prepared from erythrocyte-freed parasites as described previously[59] and recombinant *Pf*NDH2 was prepared from the *E.coli* heterologous expression strain F571.[11] *Pf*NDH2 and bc1 activities were measured as described previously.[11, 59]

Pharmacology

In vivo efficacy studies were measured against *P. berghei* (NS-Strain) in the standard a 4-day test.[48] All in vivo studies were approved by the appropriate institutional animal care and use committee and conducted in accordance with the International Conference on Harmonization (ICH) Safety Guidelines.

2.7 References

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Chapter 3

Novel quinolones targeting *Plasmodium falciparum* bc_1 complex

Chapter 3

3.1 Introduction to cytochrome bc_1 complex

As introduced in Chapter 2, the mitochondrial electron transport chain is a crucial component for several linked metabolic pathways.[1-2] The cytochrome bc_1 complex (ubiquinol: cytochrome *c* oxidoreductase or Complex III) is one of the key enzymes in the ETC and has proved to be a validated drug target.[3] This chapter refers to the second aim of this thesis, the synthesis of novel quinolones that target the cytochrome bc_1 complex of the parasite and their antimalarial activity.

Cytochrome bc_1 complex is the only enzyme common to almost all respiratory electron transport chains.[4] It catalyses the transfer of an electron from ubiquinol (**1**) to cytochrome *c* (Figure 1).[5] This electron transfer leads to the translocation of protons across the inner mitochondrial membrane generating an electrochemical gradient that is vital for metabolism within the organism and the transport process across the mitochondrial membrane. Since parasites are unable to scavenge used pyrimidine bases, they rely on the *de novo* pyrimidine biosynthesis to generate uridine monophosphate (UMP), an essential precursor for the synthesis of nucleic acids and proteins.[4, 6] Collapse of the mitochondrial membrane potential will then shutdown mitochondrial metabolism, resulting in the death of the parasite.[7-8] Cytochrome bc_1 complex therefore represents an attractive target for antimalarial drug development.

The bc_1 complex is a homodimeric transmembrane protein consisting of 11 different polypeptides in *Plasmodium falciparum*.[9-10] The catalytic core contains three subunits: cytochrome *b* (43kDa) which contain two *b*-type hemes (a high potential heme b_h and a low potential heme b_l), cytochrome c_1 (27 kDa) and the Rieske iron-sulfur protein ([2Fe2S] ISP, 21 kDa) which is involved in the protonmotive Q cycle mechanism.[4] (Figure 1)

The protonmotive catalytic mechanism (or Q cycle) of the bc_1 complex has been reviewed extensively in the literature.[5, 11] The Q cycle hypothesis was first proposed by Mitchell [12] ,with its modified version widely accepted.[3-4]

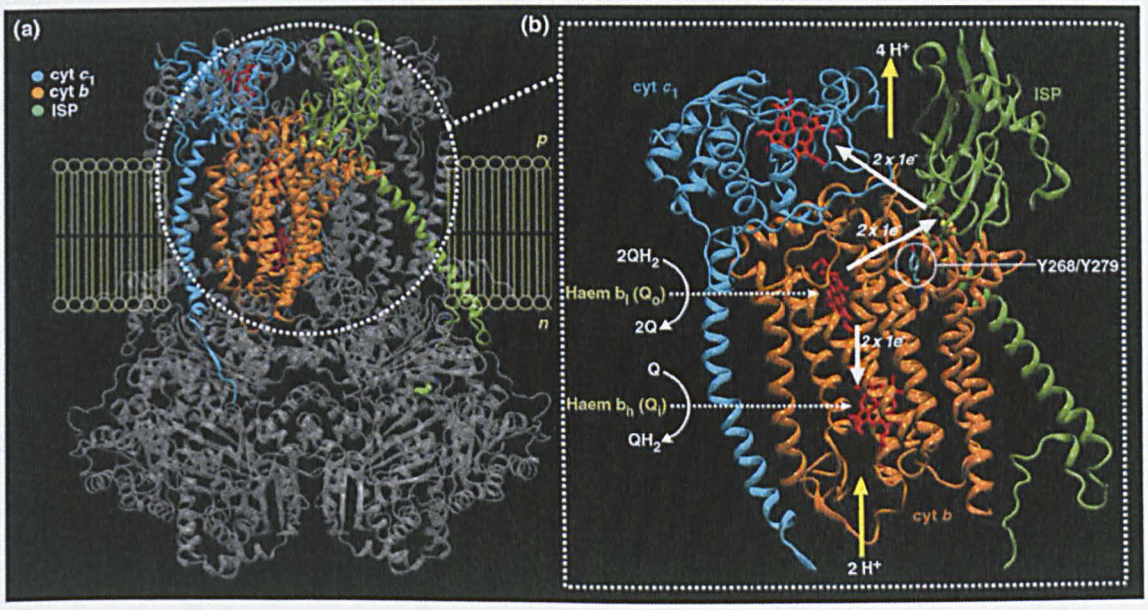


Figure 1. The structure and Q-cycle mechanism of the catalytic core of the cytochrome bc_1 complex.[3]

In general, the Q cycle requires two separate quinone binding sites - (the quinol oxidation site Q_o and the quinone reduction site Q_i) within cytochrome *b*.[11-12] Ubiquinol (**1**, produced by dehydrogenases, Complex II), binds to the Q_o site, where it is oxidised to release two protons and two electrons into the intermembrane space. Each electron transfers to different pathways and reduces different acceptors. One electron reduces the iron-sulfur cluster of the Rieske protein, and another electron reduces heme b_l. Subsequently, heme b_l reduces heme b_h which is also located within cytochrome *b*. Heme b_h further recycles the electron by reducing ubiquinone (**2**) to ubiquinol (**1**) at the Q_i site. The reduced ISP then undergoes a conformational shift, in which the histidine acceptor residue at the cytoplasmic domain of the ISP rotates. This results in the contact of the iron-sulfur cluster with the heme group of cytochrome c_1, which is followed by the transfer of an electron. Reduced ISP is then oxidised by soluble cytochrome *c*, releasing an electron to

cytochrome *c* oxidase (Complex IV). Overall, two ubiquinol molecules are oxidised at the Q_o site while one ubiquinone molecule is reduced at the Q_i site. The net translocation of two protons from the negative to the positive side of the mitochondrial membrane therefore results an electrochemical gradient (Figure 1).

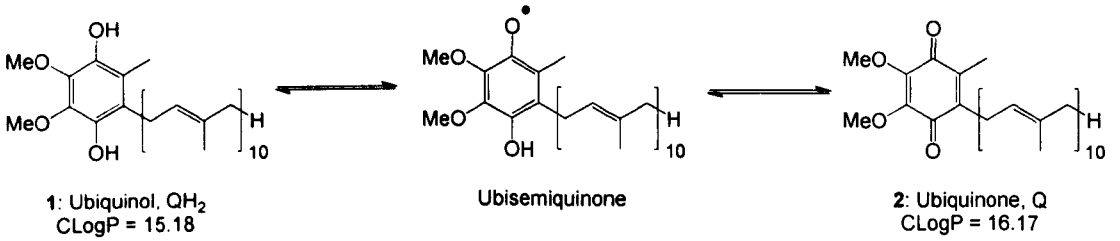


Figure 2. Redox reactions of ubiquinone.

3.2 Mechanism of action of the known bc_1 inhibitor Atovaquone

This attractive drug target has led to the development of a number of bc_1 inhibitors. The inhibitors can be classified into different groups, depending on their mode and site of action.[10] The natural antibiotic antimycin (**3**) binds to the Q_i site and inhibits the electron transfer from heme b_H to ubiquinone (Figure 3).[13-14] Myxothiazol (**4**) and stigmatellin (**5**) are both specific inhibitors to the Q_o site.[10] However, myxothiazol inhibits the electron transfer from ubiquinol to the ISP while stigmatellin blocks the electron transfer from the ISP to cytochrome c_1.[10]

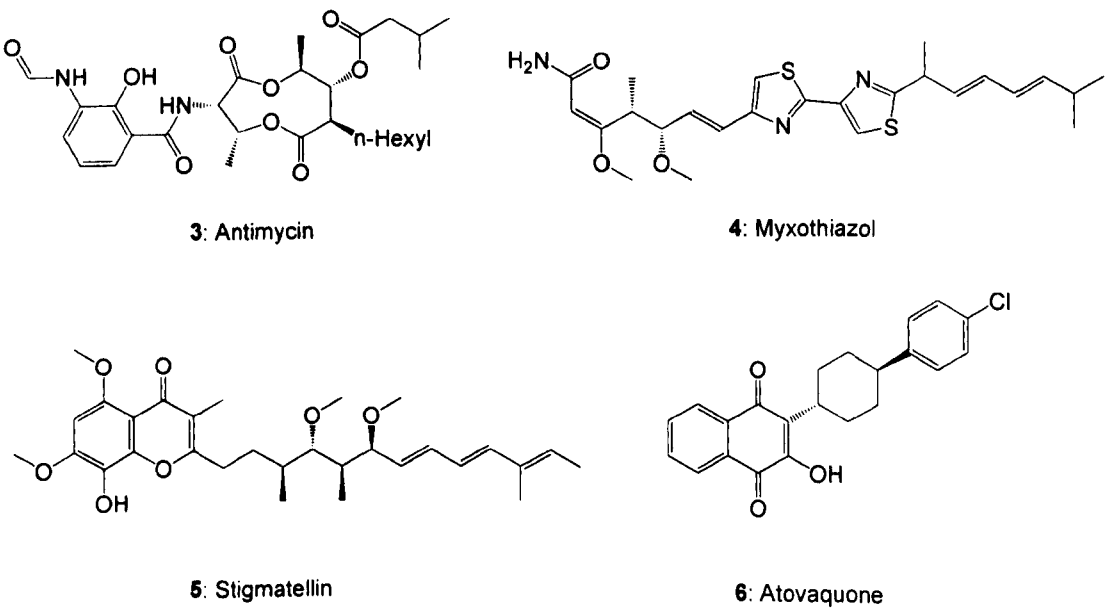


Figure 3. Current bc_1 inhibitors.

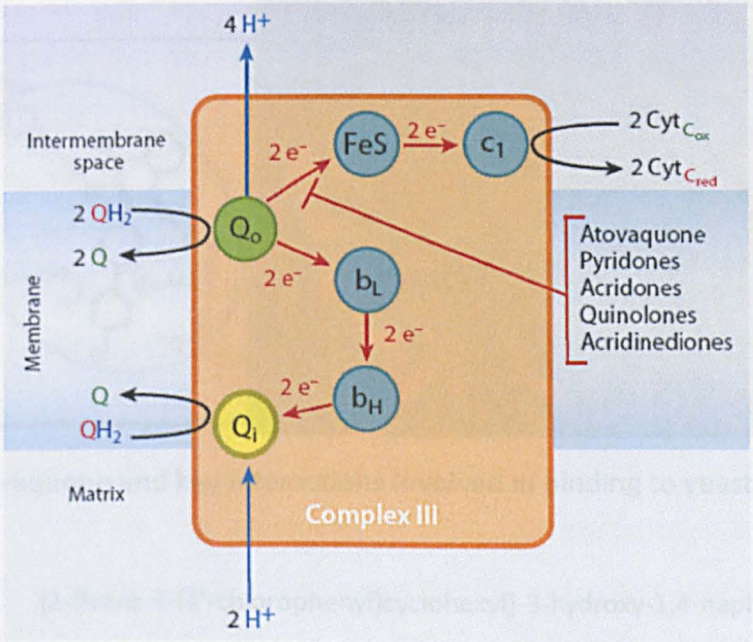


Figure 4. Complex III reactions targets by drug candidates. Picture adapted from Vaidya *et al.*[1]

Atovaquone (**6**) is presently the only antimalarial drug targeting the cytochrome bc_1 complex in clinical use.[15] Although the crystal structure of bc_1 complex from malarial parasites is currently unavailable, the binding of atovaquone to the bc_1 complex has been modelled and studied in *Saccharomyces cerevisiae*, a yeast which shares high sequence homology with *P. falcipaurm*.[3] Docking studies have shown that atovaquone, a lipophilic hydroxynapthoquinolone analogue of ubiquinone, acts as a competitive inhibitor of ubiquinol. It binds to the Q_o site where it interacts directly with the Rieske protein through the polar head group, locking the conformation of Rieske complex in its cytochrome *b*-binding conformer.[3, 15] This prevents the ISP mobilization to cytochrome c_1 and consequently the electron transfer, thus collapsing the mitochondrial membrane potential.[16] (Figures 4 and 5)

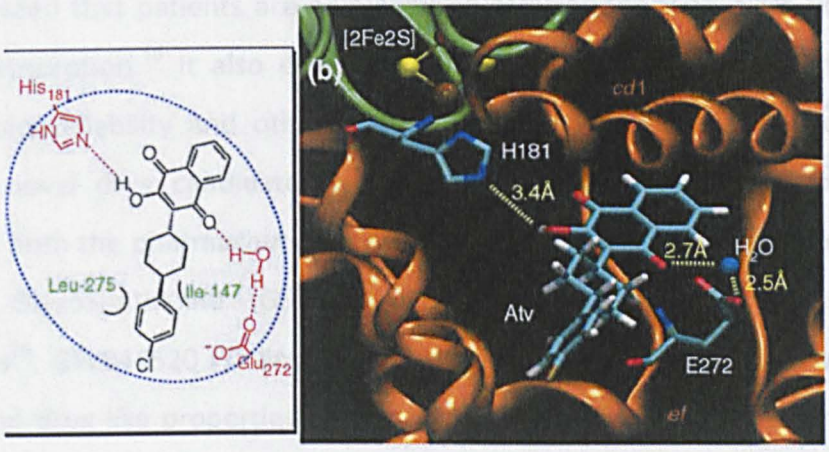
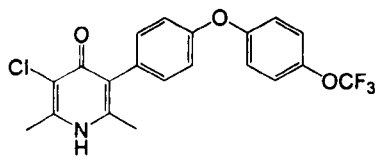


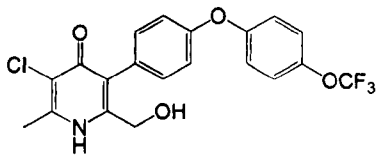
Figure 5. Atovaquone and key interactions involved in binding to yeast bc_1 Q_o site.[3]

Atovaquone (2-[trans-4-(4'-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquionone) was developed at the Wellcome Research Laboratories in 1980s while hydroxynaphthoquinones were studied as potential antimalarials from the 1940s.[16-17] Atovaquone has a board spectrum antiparasitic activity.[18] It is used against *Pneumocystis jiroveci* (*Pneumocystis carinii*) and toxoplasmosis. It also displays excellent antimalarial activity, with IC_{50} values between 0.56 and 4.53 nM in laboratory strains,[16, 19] as well as activity against chloroquine-resistant parasites. Unfortunately, the application of atovaquone as a single agent showed rapid emergence of resistance, resulting in a 30% treatment failure.[20] Resistance mutations decreased the sensitivity of atovaquone by several hundred-fold.[17] To address this concern, and to improve the efficiency, atovaquone is strictly used in combination with the synergistic agent proguanil under the brand name Malarone ™.[16] However, its costly price has limited its widespread use. The mechanism of synergistic action of atovaquone and proguanil is still unknown. However, once a strain has developed resistance to atovaquone, it will also be resistant to Malarone ™.[16]

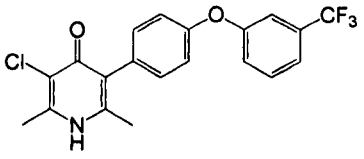
Despite its excellent antimalarial activity and metabolic stability, atovaquone displays poor aqueous solubility and poor bioavailability.[21] It has been recommended that patients are administered atovaquone with a fat rich meal to improve absorption.[16] It also exhibits high plasma protein binding. Attempts to improve bioavailability and other pharmacokinetic properties have generated a series of novel drug candidates which are active against atovaquone-resistant malaria in both the pharmaceutical industry and academic research groups.[22-25] For example, GlaxoSmithKline (GSK) has developed a range of 4(1*H*)-pyridone derivatives[26], GW844520 (**7**) and GSK932121 (**8**), which possess good antimalarial activity and drug-like properties (Figure 6). Unfortunately, despite their promising activities, development has been discounted due to toxicity issues observed in clinical trials, possibly as a consequence of off target mammalian bc_1 inhibition.[3] GSK developed an alternative pyridone analogue (**9**), which was selected for further exploration because of its significant different ADME profile and excellent antimalarial activity.[4, 26]



7: GW844520
IC_{50} *Pfbc*$_1$ = 2 nM



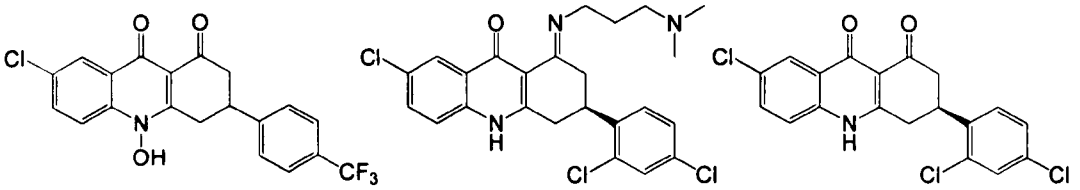
8: GSK932121
ED_{50} *PfV1/S* = < 1 mg/Kg



9
IC_{50} *Pf*3D7A = 5 nM

Figure 6. GSK pyridines series as bc_1 inhibitors.

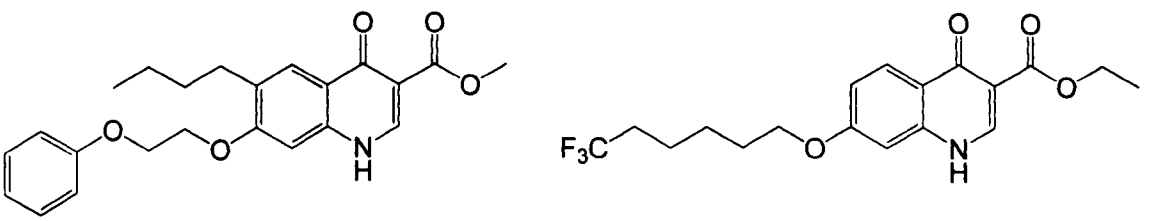
The Walter Reed Army Institute of Research developed a number of acridinediones as potent bc_1 inhibitors – floxacrine (**10**), WR243251 (**11**), and WR249685 (**12**) (Figure 7).[3-4] Both floxacrine and WR249685 are shown to be inhibitors of the parasitic mitochondrial bc_1 complex apart from their heme-binding properties.[7] WR249685 was also found to possess high selectivity against the Q_o site of the *Plasmodium falciparum* bc_1 complex (IC_{50} = 3 nM).[7]



10: Floxacrine **11**: WR243251 **12**: WR249685

Figure 7. Structures of WR249685, WR243251 and floxacrine.

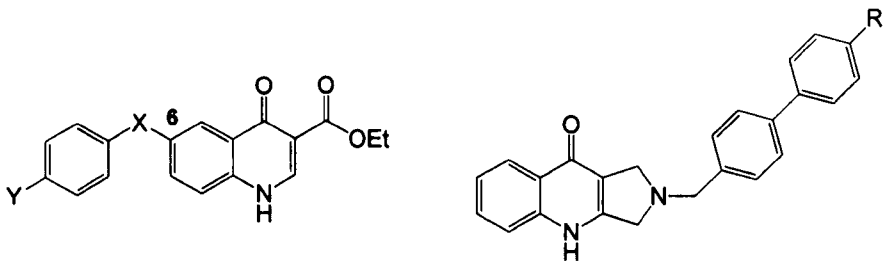
Following a study of quinolone esters (eg. ICI 56780, **13**) by Ryley and Peters[27], Riscoe *et al* synthesised a number of endochin-like quinolones with potency against *P. falciparum*[22-23] (eg. **14** in Figure 8). The quinolones have also been shown to target the Q_o site of bc_1 complex[23]. Quinolone **14** was found to possess an IC_{50} value of 1.2 nM against chloroquine-sensitive *P. falciparum*. Guy *et al* also synthesised a series of 4-oxo-3-carboxyl quinolones which displayed good antimalarial activity.[24]



13: ICI 56780 **14**

Figure 8. Structures of ICI 56780 (**13**) and **14** as bc_1 inhibitors.

The aim of this study was divided into two parts. In the first part, we explored the Structure-Activity Relationship (SAR) of a series of 6-substituted quinolones, based on Template I. In the second part, we explored the effect of the longer side chain on a tricyclic quinolone as shown in Template II (Figure 9).



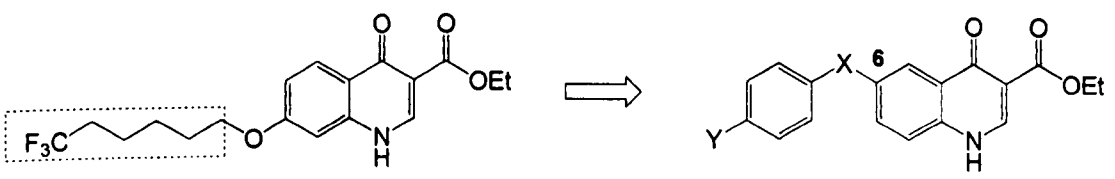
Template I Template II

Figure 9. Templates I and II.

3.3 Results and discussion

3.3.1 Quinolone esters

Since the antimalarial quinolones identified by Riscoe *et al* [22-23] consisted of long aliphatic or perfluorinated alkyl side chains (which are likely to be prone to metabolism), aromatic substituted quinolones were therefore designed to provide a more drug-like template. SAR studies were conducted to determine if the template was suitable for further development (Figure 10).



Prone to metabolism and not drug-like

14 **Template I**

Figure 10. Optimisation of quinolone template.

The 3-ester functionality was proposed to act as the polar head group, binding to the Q_o site in a similar manner to other *bc_1* inhibitors. The aromatic substituents would sit in a hydrophobic channel in a similar way to the naturally occurring inhibitor stigmatellin **5** (Figure 11). Different aromatic substituents were designed to explore the structure activity relationship of the hydrophobic pocket.

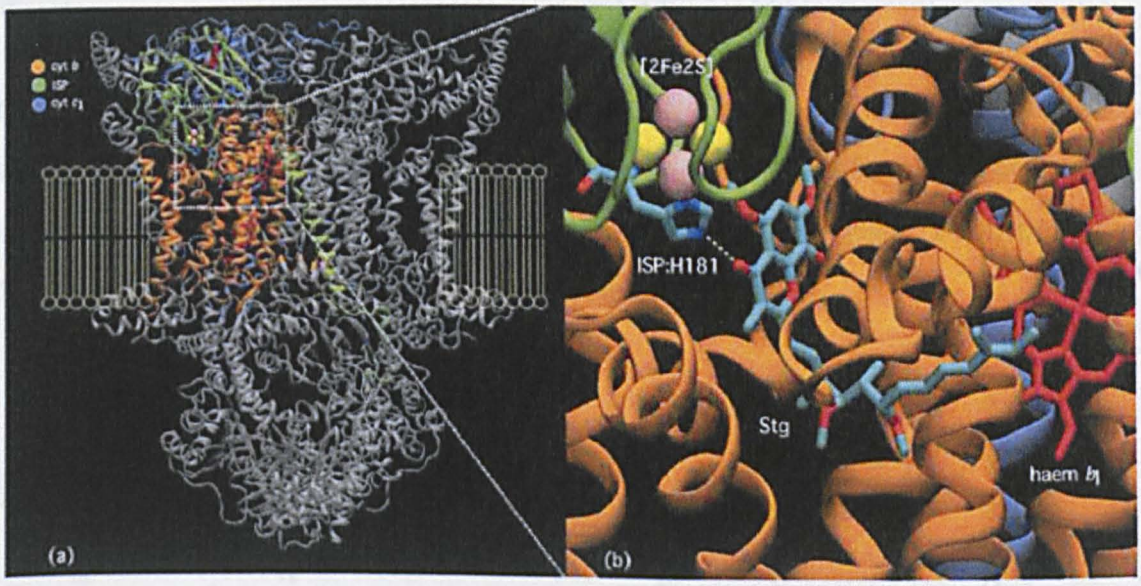
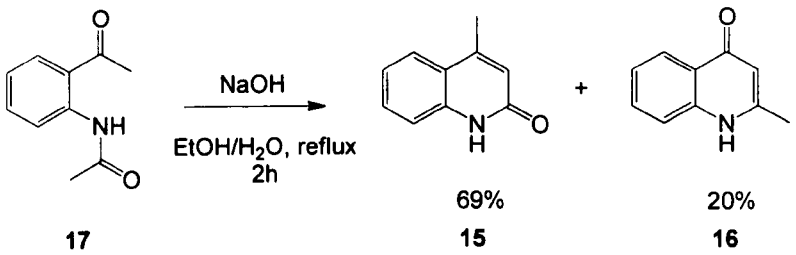


Figure 11. Stigmatellin (**5**) bound in the Q_o site of yeast *bc_1* (3CX5.PDB)[28]

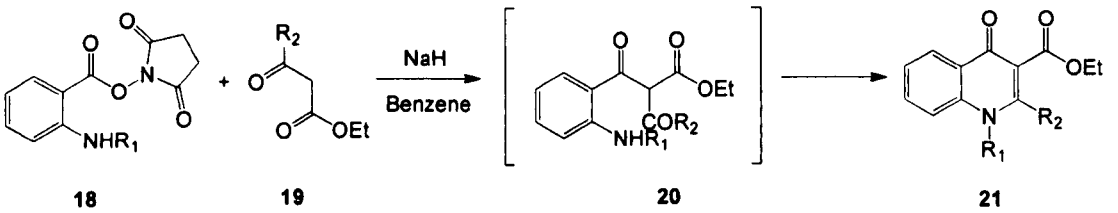
3.3.1.1 Synthesis

Many literature methods have been reported for the synthesis of 4-quinolones.[29-30] Different reactions can be used, depending on the formation of the bond that leads to ring closure and the substitution pattern required on the 4-quinolone template. The method mentioned in Chapter 2 is one of the reactions for the production of 4-quinolones from appropriate ketones and oxazoline, involving a catalytic amount of *p*-toluenesulfonic acid (or triflic acid) in n-butanol.[31] Camps discovered a convenient method to synthesise 2- or 4-quinolones (**15** or **16**) from acetyl-ortho-amidoacetophenone **17** in 1988.[32-34]



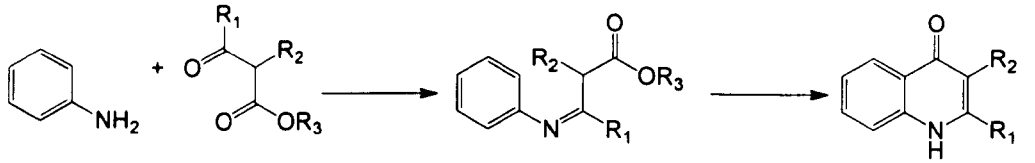
Scheme 1. Camps-cyclisation.

Mitsos[35] *et al* had described the reaction of *N*-hydroxy-succinimide esters of anthranilic acids **18** with anions of β-keto esters **19** to form a C-acylated intermediate **20** which spontaneously cyclises to give 2-substituted 3-ethoxycarbonylquinolin-4-ones **21** in good yields (Scheme 2).



Scheme 2. The synthesis of quinolone via *N*-hydroxy-succinimide ester of anthranilic acid.

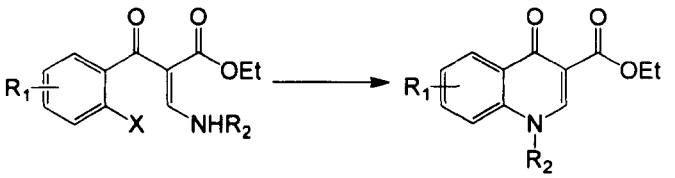
Another method used to synthesise quinolones is the Conrad-Limpach reaction (Scheme 3).[30, 36-38] This generates a 2-aryl-4-quinolone **22** though the condensation of (substituted) aniline **23** with β-keto esters **24**, followed by the cyclisation of the enamine intermediate **25** in the presence of polyphosphoric acid in diphenyl ether or other high boiling solvents at high temperatures. A similar method, known as the Gould-Jacobs approach[39-40], can be used to form the 3-ester functionalized substitution on the resulting 4-quinolone. During the reaction, a β-keto ester is replaced by diethyl ethoxymethylene malonate to couple with aniline.



23 **24** **25** **22**

Scheme 3. The Conrad-Limpach reaction.

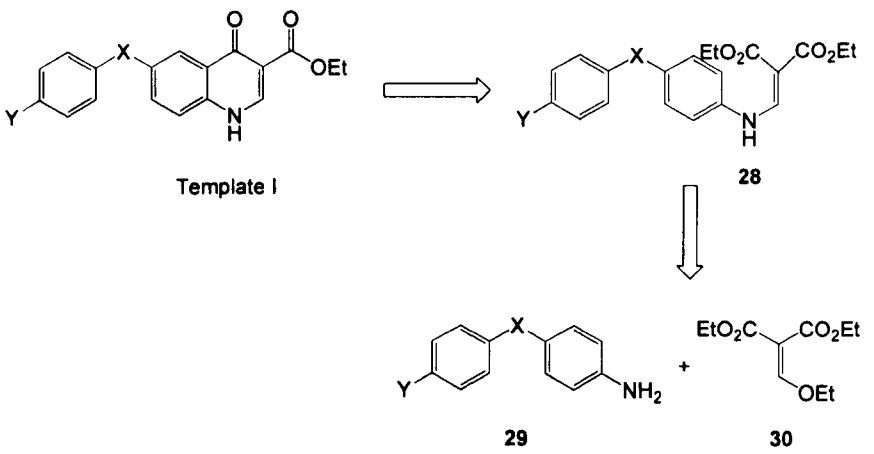
Cyclisation of aminovinyl phenyl ketones **26** using various reagents, such as sodium ethoxide in ethanol, potassium fluoride in DMF, and sodium hydride in dioxane, can also give the 4-quinolones **27**.[29]



26 **27**

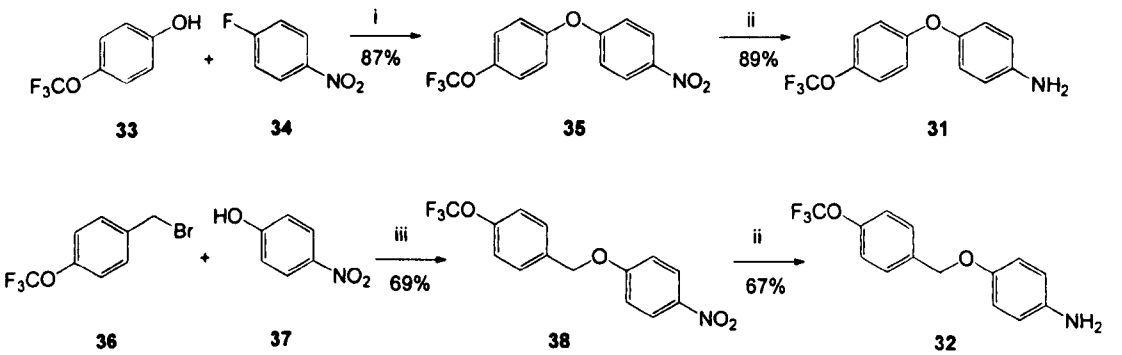
Scheme 4. The cyclisation of aminovinyl phenyl ketones to quinolones.

In addition to the methods mentioned above, there are many alternative cyclisation reactions that can be used to generate the 4-quinolones.[29, 41] Since the main aim of this synthesis was to obtain 6-aryl-4-quinolone with an ethyl ester group at the 3-position, the Gould-Jacobs was the most appropriate. In a retrosynthesis study (Scheme 5), a disconnection at the C-C bond gives an enamine intermediate **28**, which can be produced by the condensation of *para*-substituted aniline **29** with diethyl ethoxymethylene malonate **30**. The requisite *para*-substituted anilines, that are not commercially available, could be synthesised by different methods, depending on the aryl linkers required.



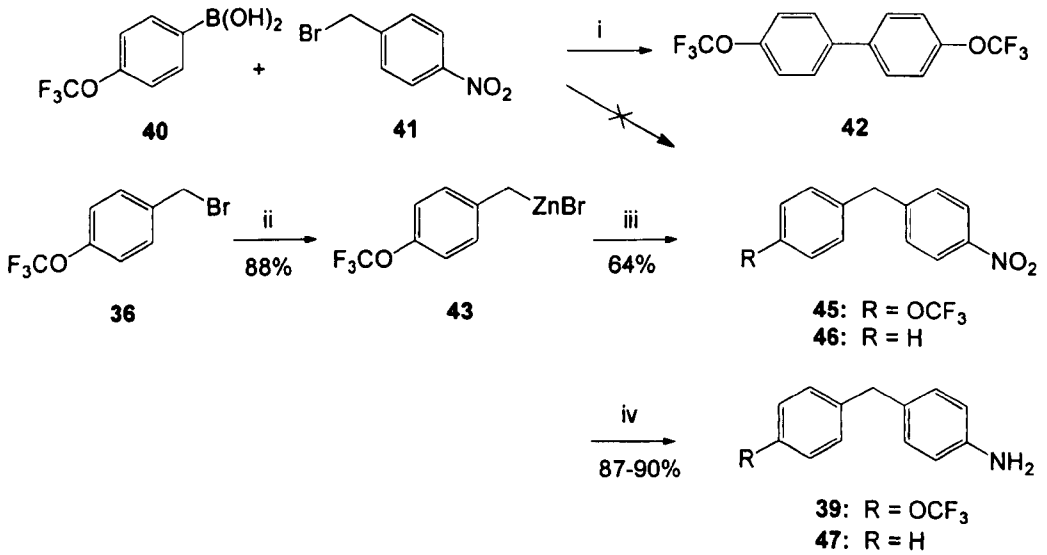
Scheme 5. Retrosynthetic analysis of quinolone esters.

Variation of the substituents on the aniline allowed us to explore the structure activity relationships at the 6-position of the quinolone ring system. 4-((4'-Trifluoromethoxy)phenoxy)aniline (**31**) and 4-((4'-trifluoromethoxy)benzoxy) aniline (**32**) were synthesized by nucleophilic substitution using appropriately substituted phenols and aryl halides in the presence of potassium or cesium carbonate, followed by hydrogenation of the nitro intermediates using 10% palladium on carbon in ethanol (Scheme 6).



Scheme 6. *Reagents and conditions*: i) K_2CO_3, 18-crown-6, DMF, r.t., 24 h; ii) 10% Pd/C, EtOH, H_2, r.t., 24 h; iii) Cs_2CO_3, MeCN, reflux.

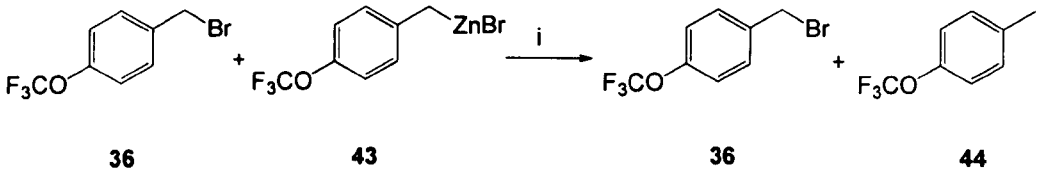
Attempts to synthesise a biaryl intermediate with a methylene linker **39** by a Suzuki coupling of (4-trifluoromethoxy)phenylboronic acid **40** and 4-nitrobenzyl bromide **41** were unsuccessful (Scheme 7). Only the homo coupling product **42** was obtained. It is believed that the boronic acid is very reactive compared to the benzyl bromide. An alternative approach by using a Neigishi coupling was employed where (4-trifluoromethoxy)benzyl bromide **36** was first converted into (4-trifluoromethoxy)benzylzinc bromide **43** by direct inserting of zinc dust which is activated by 1,2-dibromoethane and TMSCl in THF under extremely anhydrous environment. The converted product in resulting solution was used directly in the Neigishi coupling.



Scheme 7. *Reagents and conditions*: i) $Pd(PPh_3)_4$ (5 mol%), K_3PO_4 (4 equiv), DME, reflux, o/n or $PdCl_2$ (2 mol%), K_2CO_3 (2.5 equiv), Acetone-H_2O, r.t., o/n; ii) Zn dust (1.2 equiv), 1,2-dibromoethane (0.05 equiv), TMSCl (0.04 equiv), THF, 0°C, 3 h; iii) $Pd(OAc)_2$ (5 mol%), P(*o*-tolyl)$_3$ (0.1 equiv), THF, r.t., o/n; iv) $SnCl_2.2H_2O$, EtOAc, reflux, 2h.

To analyse the percentage conversion, a small amount of the resulting solution was treated with 1M hydrochloric acid to hydrolyse the benzylic zinc bromide **43** to **44**, with the unconverted benzyl bromide unaffected. Observation of the chemical shift

intensities corresponding to the methyl group of **44** and the methylene of benzyl bromide were then used to calculate the percentage conversion (88%, Scheme 8).

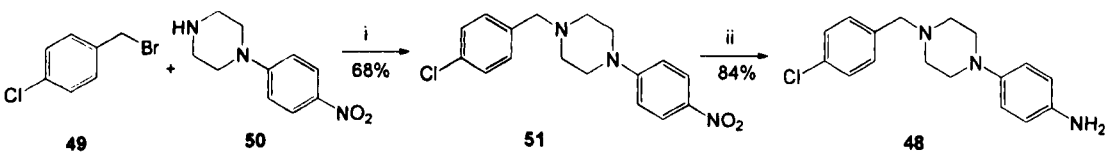


36 **43** **36** **44**

Scheme 8. *Reagents and conditions*: i) 1M HCl (aq), EtOAc.

Neigishi coupling of (4-trifluoromethoxy)benzylzinc bromide **43** and 4-iodonitrobenzene in the presence of $Pd(OAc)_2$ and tri(*o*-tolyl)phosphine proceeded smoothly at room temperature, generating the intermediate **45** in 64% yield. It was then reduced to the corresponding aniline **39** by heating with tin(II) chloride. Similar analogue **47** without the trifluoromethoxy substituent was also prepared in the same way (Scheme 7).

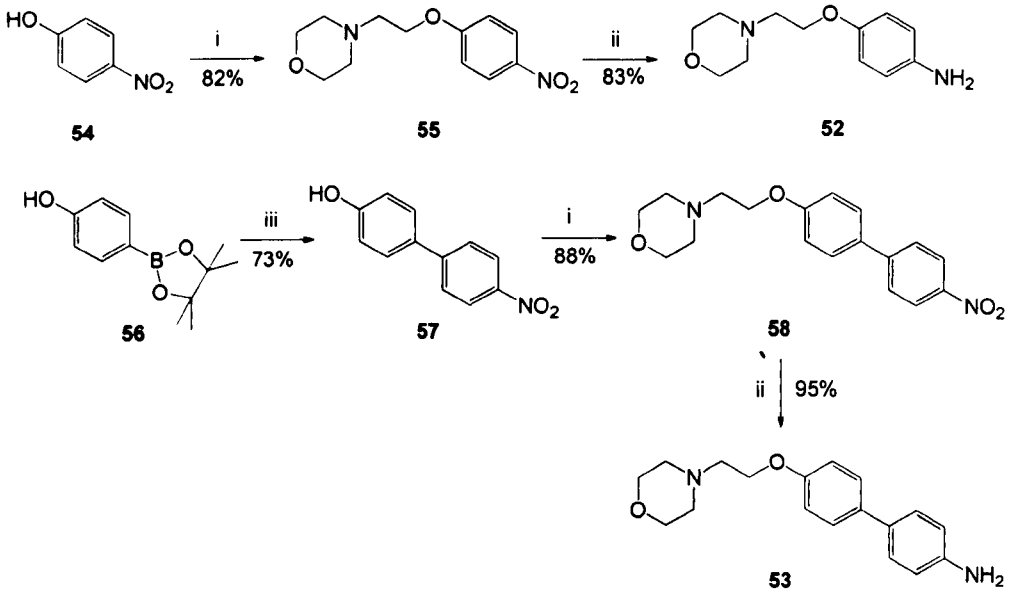
In an attempt to improve aqueous solubility, an analogue containing an amino group on the side chain (Scheme 9) was designed. The substituted aniline **48** was successfully prepared in 2 steps. 4-Chlorobenzyl bromide **49** was reacted with 1-(4-nitrophenyl)piperazine **50** under basic conditions to give the intermediate **51** in 68% yield. The mediated reduction of the nitro group by tin(II) chloride dihydrate gave the corresponding aniline **48** in 84% yield.



49 **50** **51** **48**

Scheme 9. *Reagents and conditions*: i) K_2CO_3, MeCN, reflux, 5h; ii) $SnCl_2.2H_2O$, EtOAc, reflux, 2h.

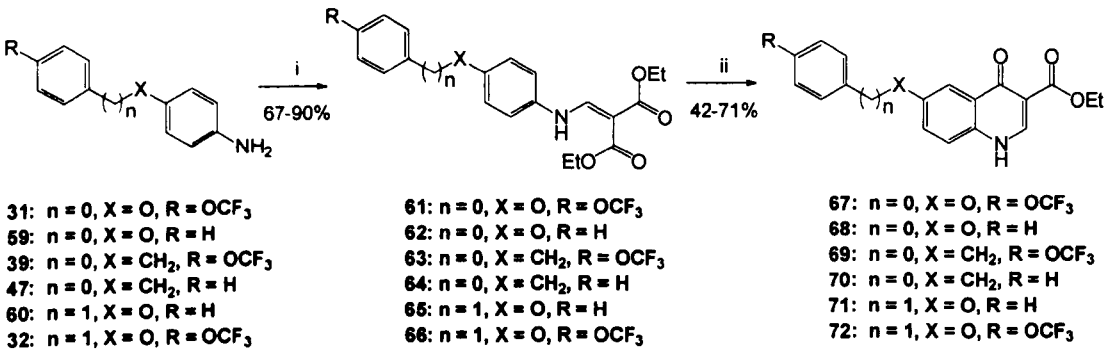
In addition to aryl substitutions, alkylamino side chains were also designed in an attempt to improve the solubility of the quinolone. Furthermore, it is intended to provide the conformational flexibility to twist around in the hydrophobic pocket in the target site of the mutant and give the chance to bind to the target (Scheme 10). Substituted aniline **52** was prepared from the reaction of 4-hydronitrobenzene and *N*-(2-chloroethyl)morpholine, followed by the hydrogenation in excellent yields. The longer alkylamino side chain **53** was synthesised from the Suzuki coupling of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) phenol with 4-iodonitrobenzene using 10% palladium on carbon as catalyst, followed by the substitution and subsequently hydrogenation.



Scheme 10. *Reagents and conditions*: i) *N*-(2-chloroethyl)morpholine (1.5 equiv), K_2CO_3 (2 equiv), DMF, 80°C, 3 h; ii) 10% Pd/C (0.02 equiv), EtOH, o/n; iii) 4-iodonitrobenzene, 10% Pd/C (0.015 equiv), Na_3PO_4 (2.6 equiv), 50% $^iPrOH/H_2O$, 50°C, 3 h.

The synthesis of a small library of 6-substituted-4-quinolones containing an ethyl ester at the 3-position was achieved using Gould-Jacobs's method (Scheme 11). Several *para*-substituted anilines were heated in diethyl ethoxymethylene malonate for 4 hours to give the enamine intermediates **61-66** in 67-90% yields. The enamines

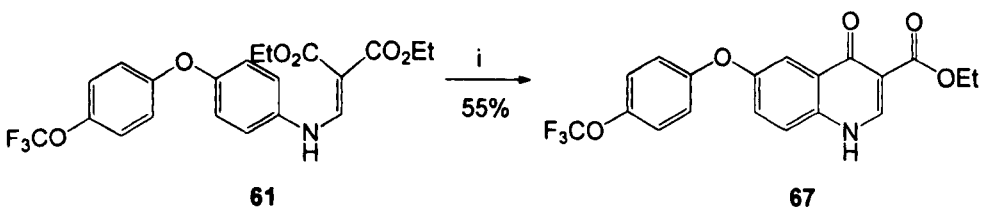
were then cyclised to form the corresponding quinolones **67-72** in 42-71% yields. However, due to the poor solubility of the quinolone esters, for example quinolone **71**, clear NMR spectra in DMSO-d^6 or MeOD proved to be somewhat difficult. Some quinolones were only dissolved in DMSO or methanol in the presence of trifluoroacetic acid.



Scheme 11. *Reagents and conditions*: i) diethyl ethoxymethylene malonate, 100°C, 4-6 h; ii) Dowtherm A, 240°C.

Although employing the Gould-Jacobs's method was successful, particularly harsh conditions were required. Zewge *et al*[42] have reported a mild method for enamine cyclisations with excellent yields (over 90%). The cyclisation was carried out in Eaton's reagent (phosphorus pentoxide in methansulfonic acid) at significantly lower temperature (<90°C) for 2 hours.

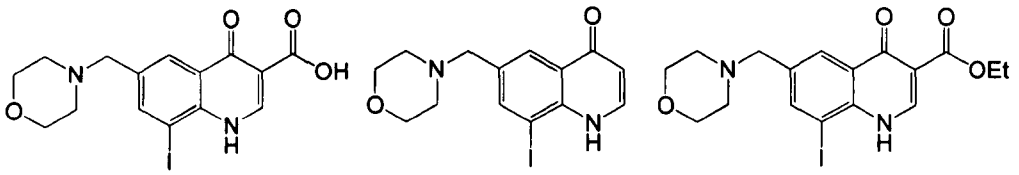
Cyclisation using Eaton's reagent was also examined on enamine **61** to generate quinolone **67**. Eaton's reagent was obtained from Alfa Asaer at 7.5% w/w of P_2O_5 in methanesulfonic acid (Scheme 12).



61 **67**

Scheme 12. *Reagents and conditions*: i) Eaton's reagent, 80-90°C.

The enamine cyclisation in Eaton's reagent was successful, although the relatively low yield was comparable to the Gould-Jacobs' method. In addition, the aqueous workup proved problematic, due to the formation of a highly viscous substance (polyphosphoric acid) when phosphorus pentoxide was treated with water. Consequently, conventional extraction and purification procedures were not possible, due to the poor solubility of the quinolone ester. Dorow *et al*,[43] whose work on the synthesis of pyrrolquinolone using Eaton's reagent also detected the formations of both the carboxylic acid (**73**) and decarboxylated analogue (**74**) in addition to the desired quinolone ester (**75**). (Figure 12)

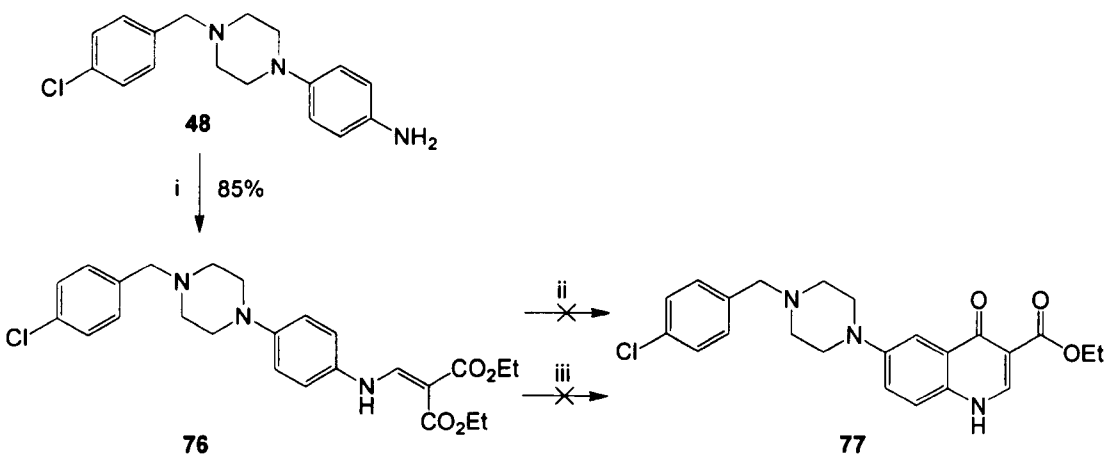


73 **74** **75**

Figure 12. The carboxylic acid (**73**) and decarboxylated analogue (**74**), and the desired quinolone ester (**75**) were observed by Dorow[43] in the cyclisation using Eaton's reagent.

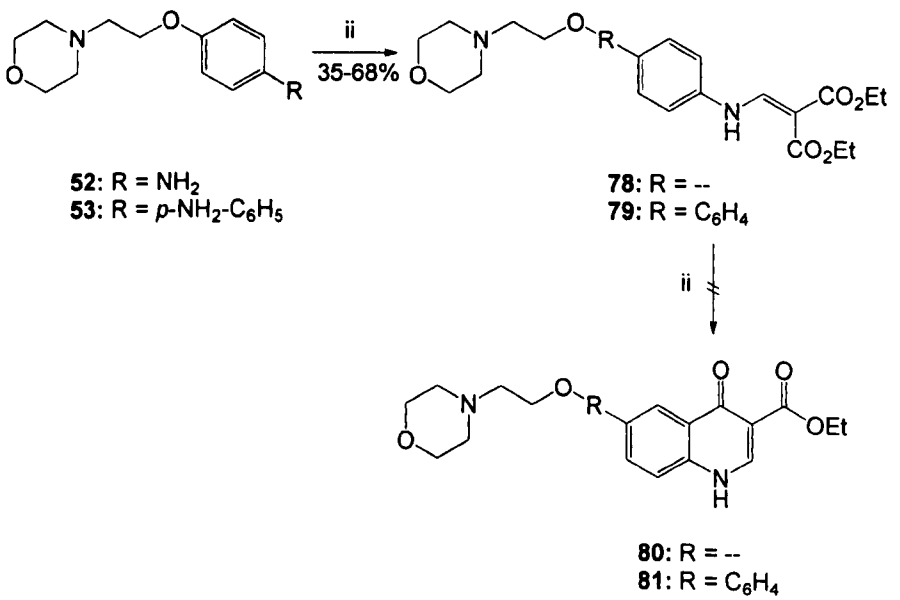
The coupling of aniline **48** with diethyl ethoxymethylene malonate successfully gave enamine **76**. However, the subsequent Gould-Jacobs reaction failed to achieve the quinolone **77**. It is likely that the product was unstable in these harsh conditions, as multiple components were observed by TLC. Repeating the reaction at a lower temperature (200°C) for a shortened period had no effect (no reaction by TLC).

Attempting the cyclisation using Eaton's reagent was also unsuccessful. Again, many side-products were formed, which could not be identified due to the difficulty in purification by column chromatography. Consequently, this target was abandoned (Scheme 13).



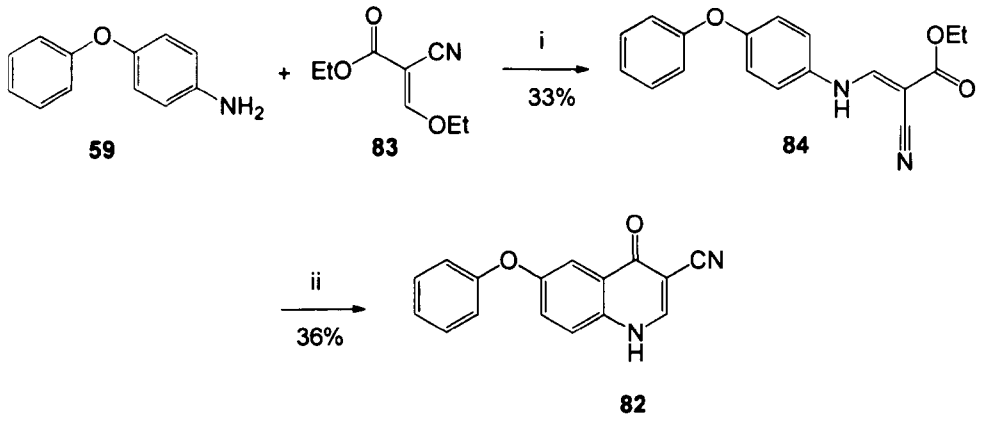
Scheme 13. *Reagents and conditions*: i) diethyl ethoxymethylene malonate, 100°C, 4-6 h; ii) Dowtherm A, 220 – 240°C; iii) Eaton's reagent, 90°C, 6 h.

The coupling of aniline **52-53** with diethyl ethoxymethylene malonate successfully formed the enamine **78-79**. Although the Gould-Jacobs reaction was attempted, subsequent purification and isolation of the product **80-81** failed due to the highly polar nature of the products (Scheme 14).



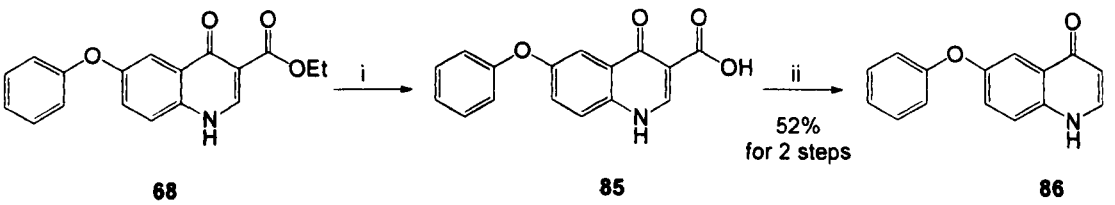
Scheme 14. *Reagents and conditions*: i) diethyl ethoxymethylene malonate, 100°C, 4-6 h; ii) Dowtherm A, 220 – 240°C.

To investigate the effect of substitution at the 3-position of the quinolone, we planned to synthesise a small range of analogues with different functionalities. A nitrile analogue of quinolone **68** (**82**) was prepared by similar methodology with the use of ethyl (ethoxymethylene)cyanoacetate in ethanol to form the enamine intermediate, which was then subjected to the thermal cyclisation conditions. (Scheme 15)



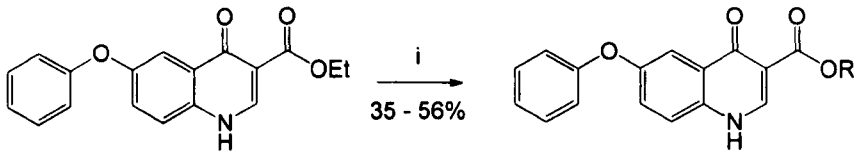
Scheme 15. *Reagents and conditions*: i) EtOH, 100°C; ii) Dowtherm A, 240°C.

Basic hydrolysis of the ethyl ester on quinolone **68** gave the carboxylic acid, followed by heating in Dowtherm A at 280°C to form the decarboxylated analogue **86** in 52% yield (Scheme 16).



Scheme 16. *Reagents and Conditions*: i) 10% aq. NaOH, MeOH, reflux, 2 h; (ii) dowtherm A, 240°C, 1 h.

Isopropyl ester **87** and cyclohexyl ester **88** analogues were also synthesised, employing a titanium catalysed transesterification[44] (Scheme 17). The quinolone **68** was heated with isopropanol or cyclohexanol in the presence of catalytic amounts of titanium (IV) isopropoxide.



68

87: R = ^{i}Pr
88: R = cyclohexyl

Scheme 17. *Reagents and conditions*: i) isopropanol or cyclohexanol, titanium (IV) isopropoxide (cat.), reflux, o/n.

3.3.1.2 Antimalarial activity and SAR studies

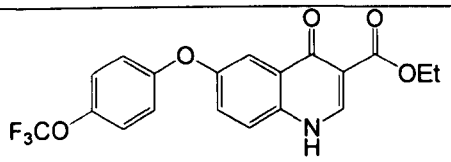
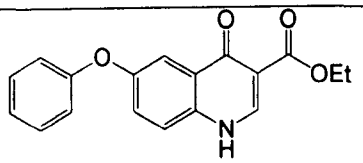
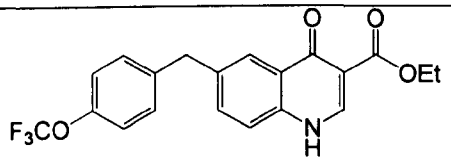
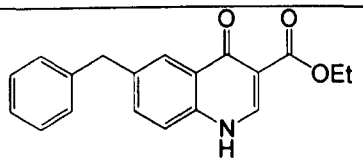
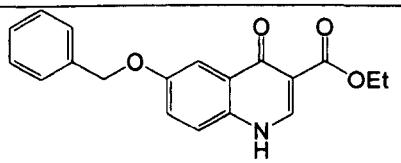
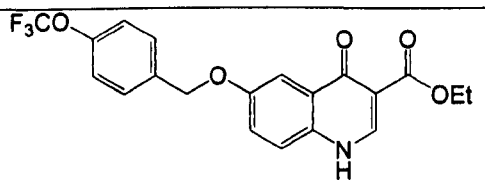
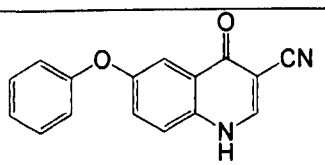
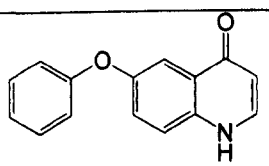
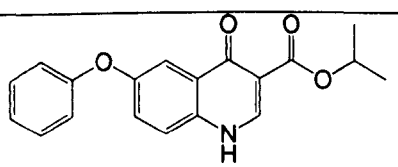
In vitro analysis of the quinolone compounds were performed on "wild-type" 3D7 chloroquine-sensitive parasite isolates of *Plasmodium falciparum* at the Liverpool School of Tropical Medicine. ClogP values were calculated using the ALogPS2.1 software programme.[45-46]

Table 1 shows the antimalarial activity for the 6-substituted quinolone esters. There was no IC_{50} data for quinolone **71** as it was found to be insoluble in the suspension vehicle for testing. The results show that the 6-benzyl analogue **70** displayed the best *in vitro* activity in the series.

Quinolone **68** displays superior activity (164nM) to the 3-nitrile **82** (>10 μ M) and decarboxylated analogues **86** (4698 nM), indicating that the 3-ester functionality is essential for activity. However, the size of the ester moiety is limited as exemplified by **87** and **88**. The activity drops when increasing the size of the ester indicating potential steric hindrance in the active binding site.

The activities of the quinolones **67** and **68** dropped when replacing a methylene linker with an oxygen linker. The incorporation of a *para*-trifluoromethoxy functionality on the phenyl ring generally decreases activity. Interestingly, this observation is contrary to the *in vitro* testing results of the corresponding 7-substituted series, which were prepared in the group[28] simultaneously. It was also found in general that the 7-series exhibited better activity than the corresponding 6-series (Table 2).

Table 1. *In vitro* growth inhibition of quinolone esters against 3D7 *Plasmodium falciparum*.

Compound	Structure	IC_{50} 3D7 (nM)	ClogP
67		229.8	4.1 ± 0.8
68		164.0	3.1 ± 0.4
69		141.5	4.4 ± 0.8
70		40.4	3.4 ± 0.4
71		No Data	3.0 ± 0.5
72		674.8	4.1 ± 0.8
82		>10 μM	2.8 ± 0.8
86		4698.0	2.7 ± 0.4
87		183.9	3.4 ± 0.5

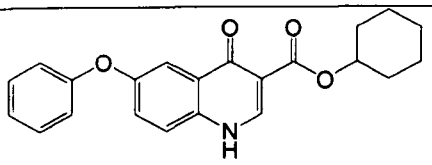
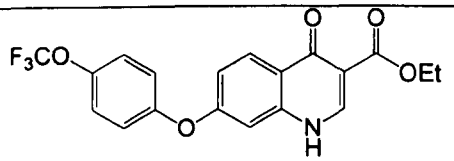
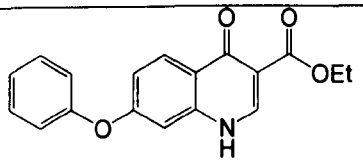
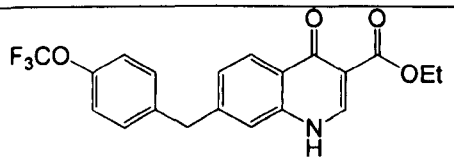
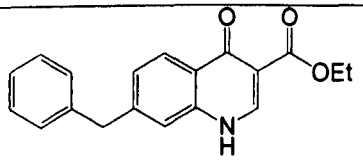
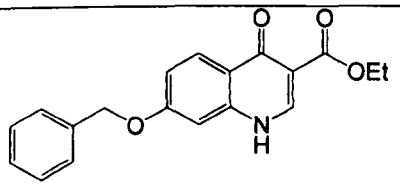
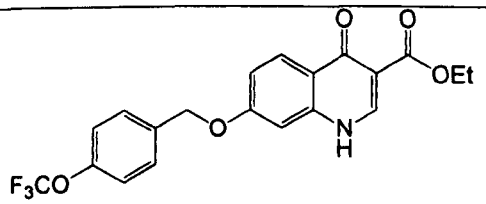
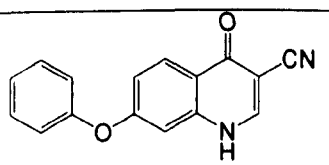
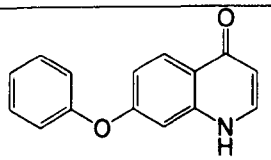
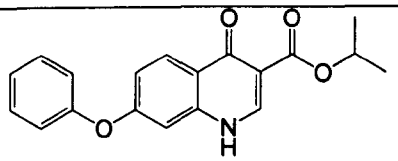
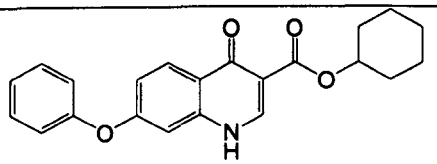
88		1820.4	4.4 ± 0.7

Table 2. *In Vitro* growth inhibition of the corresponding 7-substituted quinolone esters against 3D7 *Plasmodium falciparum*.[28]

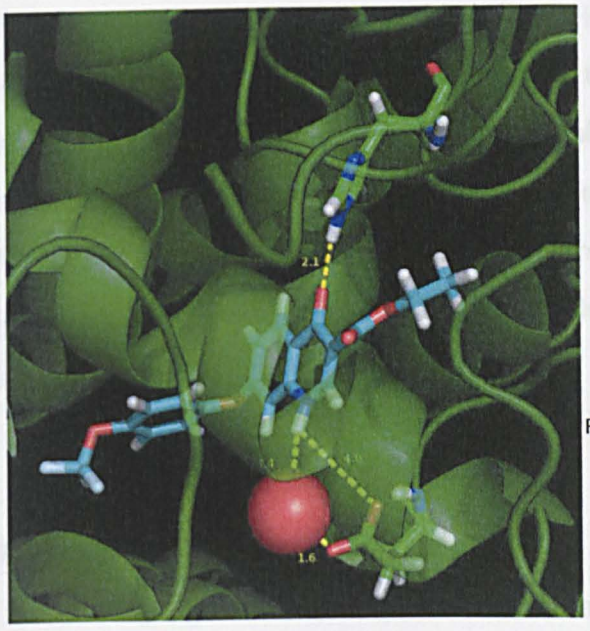
Compound	Structure	IC_{50} 3D7 (nM)	ClogP
89		5.30	4.1 ± 0.8
90		19.3	3.1 ± 0.4
91		0.46	4.4 ± 0.8
92		4.57	3.4 ± 0.4
93		No Data	3.0 ±0.5
94		5.00	4.1 ± 0.8
95		8102.5	2.8 ± 0.8

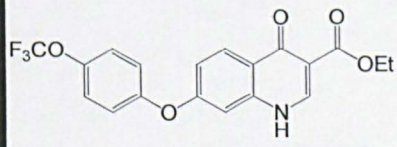
96		6725.0	2.7 ± 0.4
97		8.45	3.4 ± 0.5
98		29.6	4.4 ± 0.7

3.3.1.3 *In silico* studies

To explain the biological observations from 6- and 7-series, modeling studies were performed using the crystal structure yeast bc_1 model (Protein Data Bank accession code 3CX5) in collaboration with the Berry modelling group at the University of Liverpool. The yeast protein shares ~40% homology with the parasite, in particular, the Q_o binding site is well conserved.[47] The stigmatellin-bound yeast crystal structure (PDB code: 3CX5) is shown in Figure 11. Using GOLD (Genetic Optimisation for Ligand and Docking)[48], a reliable and well validated ligand docking programme, which predicts the binding modes of small molecule to macromolecules of known three-dimensional structure, stigmatellin can be removed and thereby other compounds docked in its place. This allows the prediction of binding modes of compounds that have not been crystallized in the protein of interest.

Docking studies of the active 7-series of quinolones, indicate that the quinolone head group adopts similar binding poses in the 7-series, whereby the quinolone carbonyl group interacts with the protonated imidazole *N* atom of ISP residue His181 (a residue of the [2Fe2S] cluster), and the quinolone N-H forms a water-mediated hydrogen bridge with the carboxyl group of cytochrome *b* residue Glu272 (Figure 13).

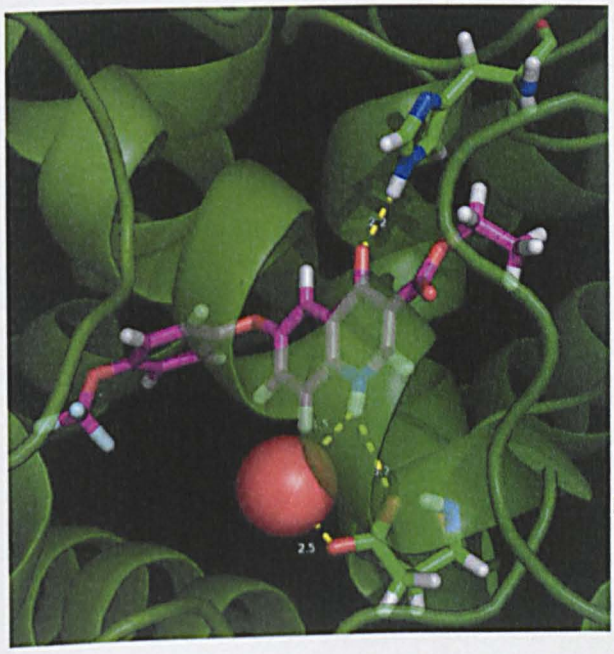


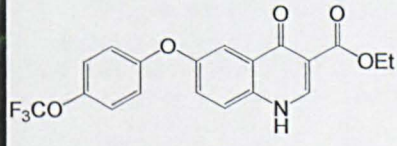


89
IC_{50} = 5.3 nM
GoldScore = 45.36

Figure 13. General docking pose for the 7-series of quinolones.

However, when docking the 6-series of quinolones under the same docking settings, it was found that their binding poses flipped. In contrast to the 7-series, only a partial Glu272 water-mediated interaction could be observed with no His181 interaction. Steric hindrance may be the main reason for the loss of His181 interaction since the active site appears to be less accommodating when the side chain is at the 6-position.





67
IC_{50} = 229.8 nM
GoldScore = 33.8765

Figure 14. Docking pose for 6-series of quinlones in the 7-series binding motif.

Further *in silico* studies by forcing the 6-series into the pose as the 7-series with His181 and Glu272 interactions have confirmed that it is not the preferred binding pose for the 6-series (Figure 14). An unfavourable interaction between the ester linker of **67** with the sulphur group of the methionine reside was observed. GoldScore (an indicator of binding affinity determined by GOLD) for **67** was found to be 33.8765 which was significantly lower than that for **89** (45.3582), suggesting **67** has a weaker binding with the target than **89**.

3.3.1.4 Summary

This section highlights the syntheses and antimalarial activities of a range of novel quinolone esters. Among the 6-substituted quinolones, the 6-benzyl quinolone ester **70** showed the best antimalarial activity (IC_{50} 40.4 nM). SAR studies for the 6- and corresponding 7-series of quinolones concluded that substitution at the 7-position is superior than the 6-position. Furthermore, *in silico* docking studies demonstrated that substitution at the 6-position is less favorable in the hydrophobic pocket of the active site due to steric hindrance preventing optimal positioning of the quinolone core with respect to key hydrogen bonding residues.

3.3.2 Pyrrolidine-fused quinolones

Previous studies in the group have identified quinolones SQ3 (**99**) and SQ4 (**100**), which possess good antimalarial activity. The tricyclic system structurally mimics the promising antimalarial floxacrine (**10**) with a rigid structure.

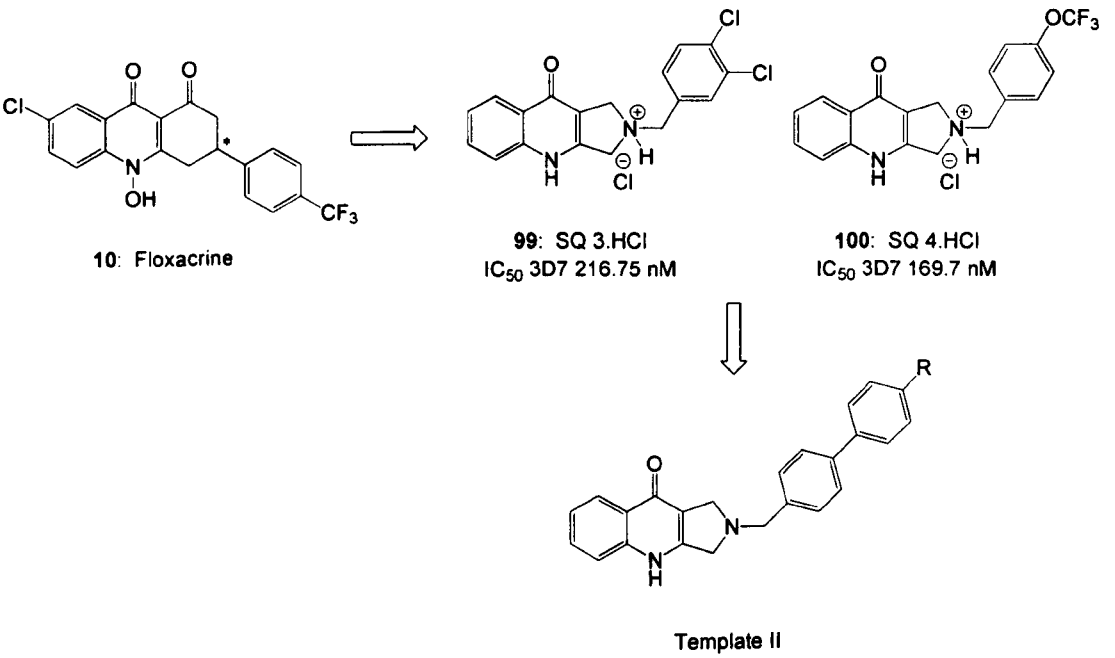


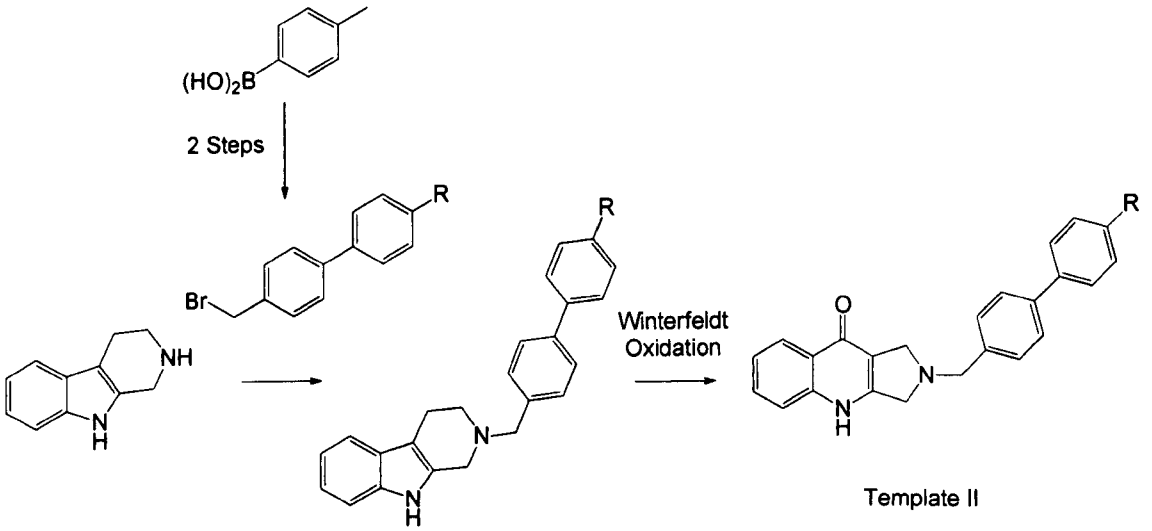
Figure 15. The structures of Floxacrine (**10**), SQ3 (**99**), SO4 (**100**) and Template II.

Encouraged by these results, the aim of this part of project was to synthesise and further explore the SAR of the quinolone template with a *N*-biaryl side chain in order to identify a potential template as drug lead. *N*-Biaryl side chain can allow the comparison to *N*-monoaryl and it was proposed that the longer aromatic side chains could better penetrate the hydrophobic pocket of the bc_1 Q_o active site. When comparing this target series with floxacrine, the advantage of the target structure (template II) is that it contains a basic nitrogen. The pKa of this nitrogen permits formation of salts which may help to increase the aqueous solubility of the poorly soluble quinolone template.

3.3.2.1 Synthesis

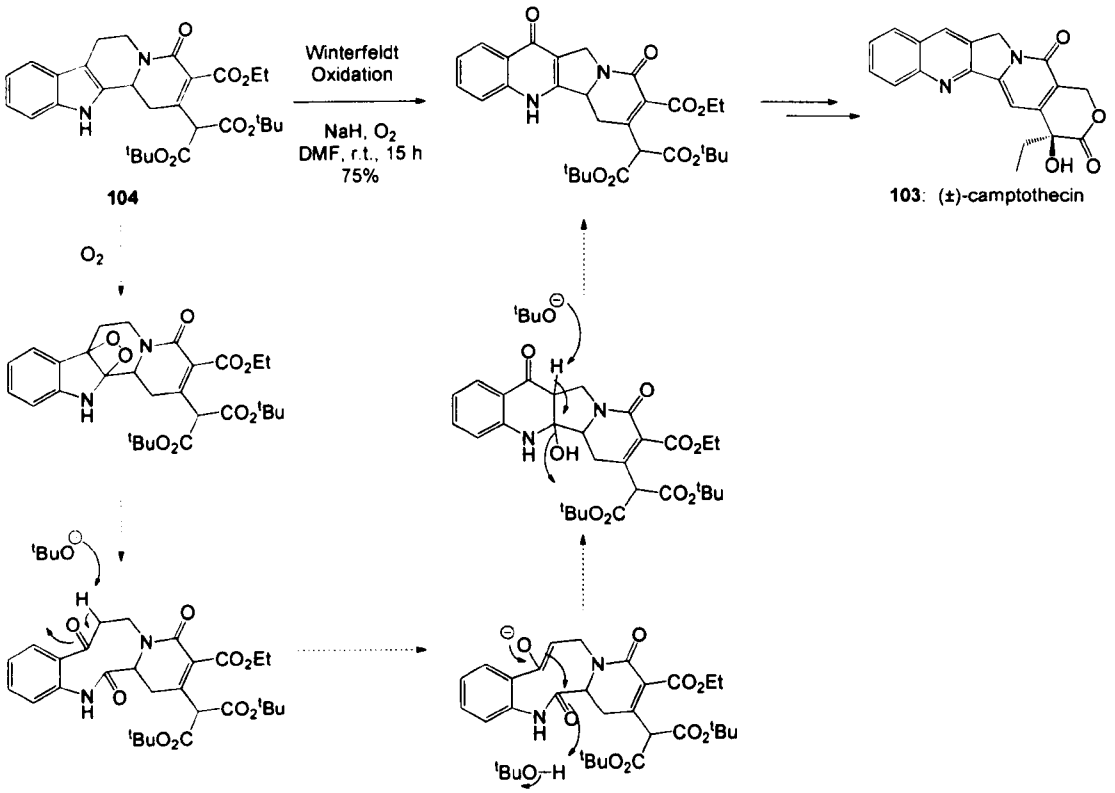
The good antimalarial activity of SQ4 (**100**) against whole cell *Plasmodium falciparum* prompted us to synthesise the corresponding biaryl derivative **101** and **102**.

In general, quinolones **101** and **102** were prepared via a four-step synthesis. A Winterfeldt oxidation was used to oxidize the fused tryptamine structure to the quinolone by a ring expansion reaction (Scheme 18).



Scheme 18. Synthetic route to template II.

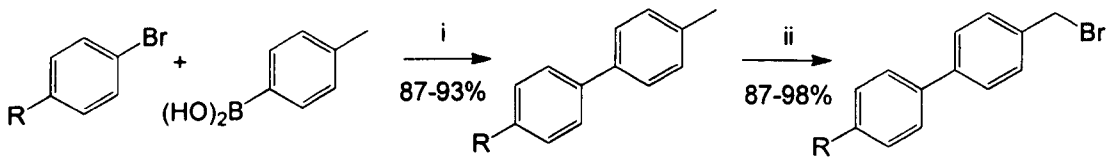
In the early 1950s, Witkop reported the synthesis of quinolones from indoles *via* an oxidation followed by Camps cyclisation.[32, 49] Thereafter, Winterfeldt had identified the conditions for the direct transformation of indoles into quinolones in the total synthesis of (±)-camptothecin (**103**).[50] The Winterfeldt oxidation comprises of a one pot Witkop-oxidation and Camps-cyclisation of the indole **104** by NaH/O_2 in DMF to form the pyrroloquinolone, without the necessity of isolating the dicarbonyl intermediates (Scheme 19).



Scheme 19. Winterfeldt's synthesis of camptothecin (**103**) + mechanism.[32]

Husson *et al*[51-53] have successfully used the Winterfeldt Oxidation with $^tBuOK/O_2$ to synthesise a series of quinolones in moderate to excellent yields (45-92%). As a result of these studies, Winterfeldt oxidation conditions were applied in the synthesis of pyrrolidine-fused quinolones studied herein.

The first step in the synthesis of the biaryl side chain **105-106** employed a Suzuki coupling of commercially available *p*-tolylboronic acid and the corresponding aryl bromide with a catalytic amount of $PdCl_2$(dppf) and potassium carbonate as the base in 87-93% yields. Wohl-Ziegler bromination conditions were then employed to synthesise the bromide species (**105-106**) in high yield under the condition (Scheme 20).



107: R= Cl
108: R = OCF_3

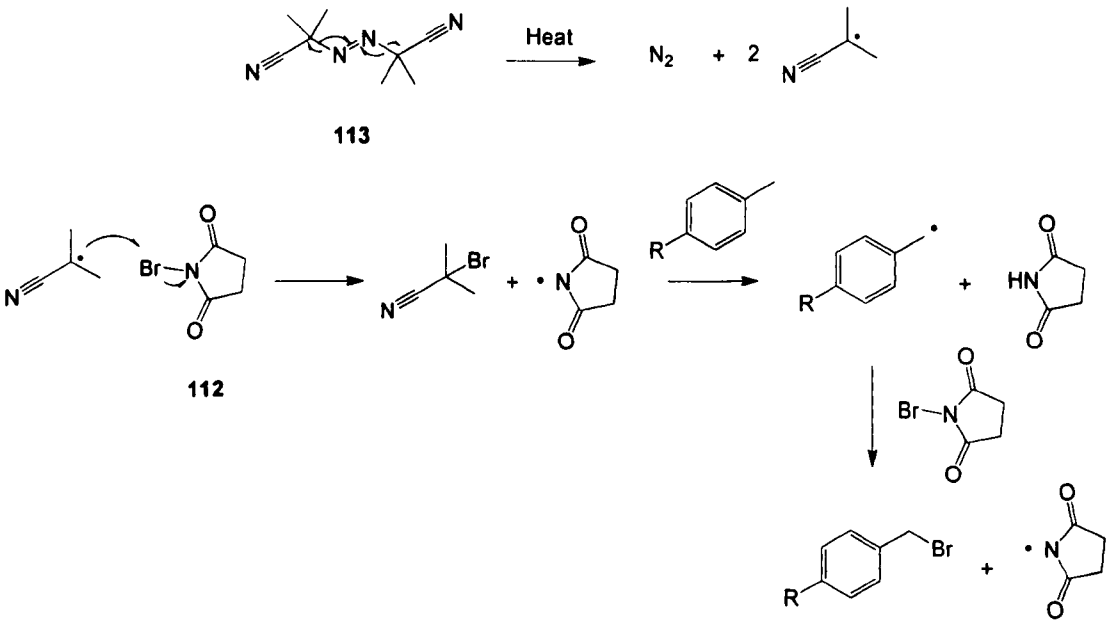
109

110: R= Cl
111: R = OCF_3

105: R= Cl
106: R = OCF_3

Scheme 20. *Reagents and conditions*: i) *p*-Tolylboronic acid (1.5 equiv), K_3PO_4 (4 equiv), $PdCl_2$(dppf) (0.05 equiv), 1,4-dioxane, 100°C, 4 h; ii) *N*-bromosucccinimide (2 equiv), AIBN (0.25 equiv), α,α,α-trifluorotoluene, reflux, 4 h.

Bromination conditions using *N*-bromosuccinimide (NBS, **112**) and a catalytic amount of 2,2'-azobis(isobutyronitrile) (AIBN, **113**) as initiator is a useful method for the preparation of substituted benzyl bromides. The mechanism involves the initiation of 2-cyanoprop-2-yl radical generated by homolysis reaction of AIBN. (Scheme 21).

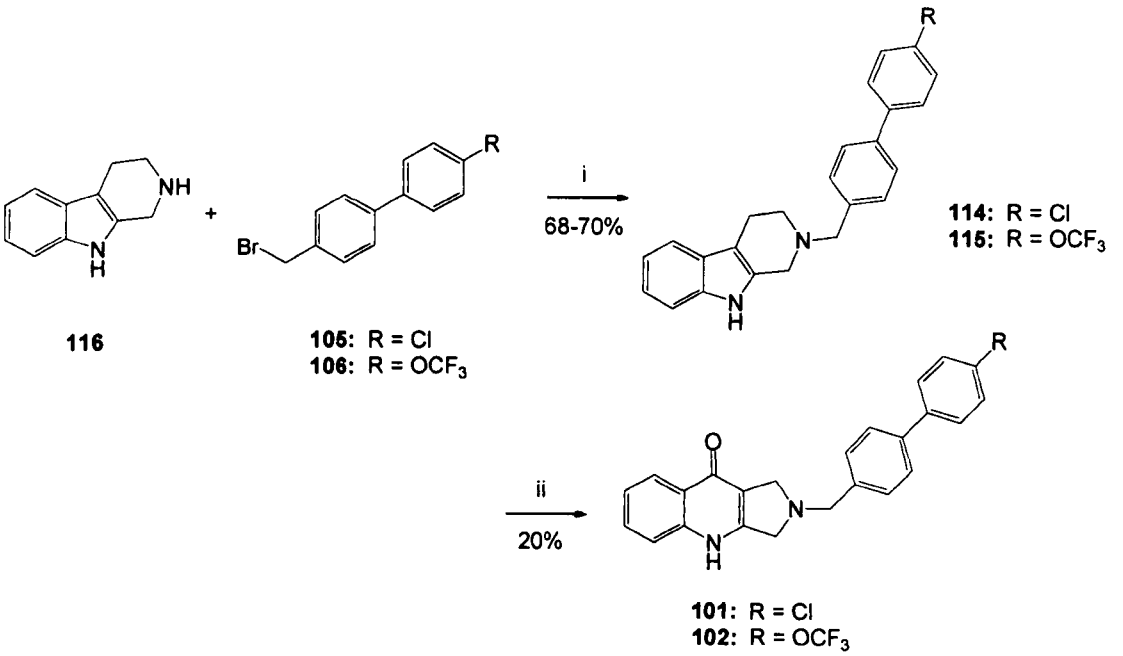


Scheme 21. The mechanism of the Wohl-Ziegler bromination.[54]

Traditionally, carbon tetrachloride or benzene is used as the solvent in the bromination. However, due to their high toxicities, the more environmentally-friendly α,α,α-trifluorotoluene isnow used to perform the reaction. The solvent is chosen due to its boiling point and its inert trifluoromethyl group.

Bromination under these conditions proceeded smoothly and gave excellent yields without any side reactions. However, care must be taken regarding both the quantity of NBS used and the temperature of the reaction in order to avoid dibromination.

The next step was to synthesise the *N*(*b*)-substituted indole intermediates **114-115**. This was achieved by alkylation of 1,2,3,4-tetrahydro-9*H*-pyrido[3,4-*b*]indole **116** with the substituted benzyl bromides **105-106** in the presence of triethylamine (68-70% yields). Finally, Winterfeldt's oxidation of the *N*(*b*)-substituted indoles to their respective quinolones **101-102** was achieved by passing compressed oxygen through a DMF solution of *N*(*b*)-substituted indole in the presence of *t*-BuOK at room temperature (Scheme 22).



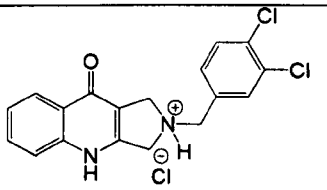
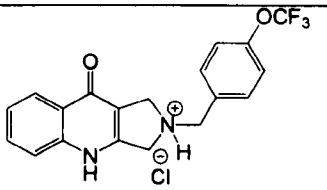
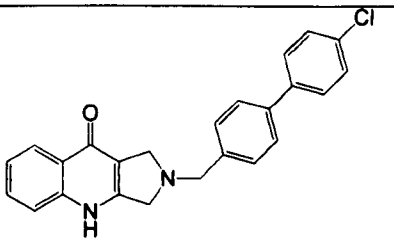
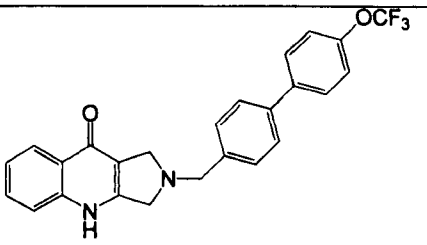
Scheme 22. *Reagents and conditions*: i) NEt_3, THF, 0°C – r.t., 1 h; ii) ^tBuOK, O_2, DMF, r.t., o/n.

Although the Winderfeldt oxidations were promising in forming the desired products, they were found to be low yielding. Another problem encountered was the high polarity of the quinolone products, which resulted in difficulties regarding purification on a small scale.

3.3.2.2 Antimalarial activity

The antimalarial activity of **101** and **102** was evaluated against chloroquine-sensitive 3D7 *Plasmodium falciparum* parasites (Table 3). Interestingly, **102** demonstrated a potent *in vitro* activity with an IC_{50} of 34 nM. *Para*-trifluoromethoxy substituent is favoured over the *para*-chloro group at the terminal side chain. In general, *N*-biary side chain is favored over N-monoaryl side chain as exemplified by **102** which is also more potent than the corresponding monoaryl analogue SQ4 (**100**).

Table 3. *In vitro* antimalarial activity of the tricyclic quinolones against 3D7 *Plasmodium falciparum*.

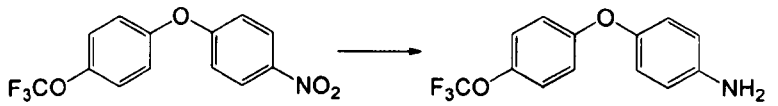
Compound	Structure	IC_{50} 3D7 (nM)
SQ3.HCl **(99)**		216.75
SQ4.HCl **(100)**		169.7
101		194nM*
102		34 ± 3
* Preliminary result based on 2 test-runs only.		

3.3.2.3 Summary

In summary, this short study highlights the syntheses and antimalarial activities of pyrrolidine-fused quinolones. Interestingly, **102** displayed potent antimalarial *in vitro* activity. More work is required to investigate the aqueous solubility profile of salts versus the free base. In addition, studies on the in vivo activity of this template are likely to follow should reasonable levels of solubility be achieved.

3.4 Experimental

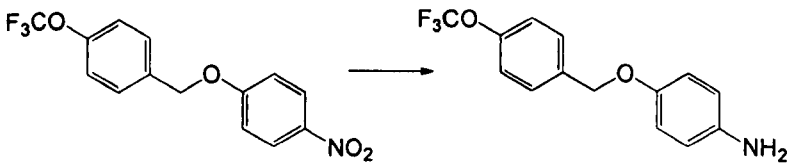
Preparation of **4-(4-(Trifluoromethoxy)phenoxy)aniline 31.**



35 **31**

To a solution of **35** (0.41 g, 1.37 mmol) in anhydrous methanol (8 mL) was added 10% palladium on carbon (40 mg). The mixture was allowed to stir under hydrogen environment for 5 ½ h (reaction followed by tlc). The mixture was diluted by dichloromethane and filtered through celite and the colourless filtrate was evaporated to give the title compound (0.33 g, 89 %) as a red liquid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 – 7.09 (m, 2H), 6.98 – 6.83 (m, 4H), 6.78 – 6.64 (m, 2H), 3.63 (s, 2H, NH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.91, 148.53, 143.50, 122.83, 121.65, 118.73, 118.21, 116.69; HRMS (CI) $C_{13}H_{11}NO_2F_3$ $[M+H]^+$ requires 270.07419, found 270.07406; Anal. $C_{13}H_{10}NO_2F_3$ requires C 58.00%, H 3.74%, N 5.20%, found C 57.93%, H3.77%, N 5.18%.

Preparation of **4-((4-(Trifluoromethoxy)benzyl)oxy)aniline 32.**

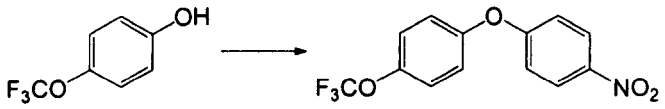


38 **32**

To a solution of **38** (1.22 g, 3.88 mmol) in anhydrous ethyl acetate (15 mL) was added tin(II) chloride dehydrate (4.38 g, 19.4 mmol, 5 equiv). The mixture was refluxed for 2 h, afterwhich the mixture was diluted with water and the pH was adjusted to pH 8 by addition of saturated $NaHCO_3$. It was then extracted with ethyl acetate (x 3) and washed with brine. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was then purified by column chromatography using 25% ethyl acetate in hexane gave **32** (0.72 g, 67 %) as a pale brown oil: R_f = 0.09, 25% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45

(d, *J* = 8.5 Hz, 2H, H-2'), 7.22 (d, *J* = 8.2 Hz, 2H, H-3'), 6.85 – 6.76 (m, 2H, H-3), 6.74 – 6.57 (m, 2H, H-2), 4.98 (s, 2H, OCH_2), 3.47 (s, 2H, NH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.83 (C-4), 146.85 (C-4'), 138.41 (C-1), 134.34 (C-1'), 126.93 (ArC x 2), 119.14 (ArC x 2), 118.56 (OCF_3), 114.53 (ArC x 2), 114.15 (ArC x 2), 67.98 (OCH_2); HRMS (ESI) $C_{14}H_{13}NO_2F_3$ $[M+H]^+$ requires 284.0898, found 284.0893; Anal. $C_{14}H_{12}NO_2F_3$ requires C 59.37%, H 4.27%, N 4.95%, found C 58.98%, H 4.37%, N 4.77%.

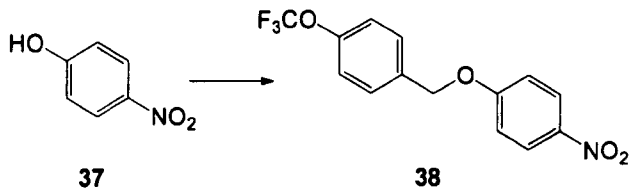
Preparation of **1-Nitro-4-(4-(trifluoromethoxy)phenoxy)benzene 35.**



33 **35**

To a solution of 4-fluoronitrobenzene (0.47 g, 3.30 mmol) in anhydrous DMF (10 mL) was added potassium carbonate (1.83 g, 13.20 mmol, 4 equiv), 4-(trifluoromethoxy)phenol (0.73 g, 4.13 mmol, 1.25 equiv) and 18-crown-6 (20 mg, 0.08 mmol). The mixture was allowed to stir at room temperature overnight. The reaction mixture was diluted with dichloromethane (25 mL), washed with water, 1N NaOH (3 x 10 mL), water (until pH 7.0), followed by brine, and the organic layer was dried over Na_2SO_4, filtered and concentrated to give a yellow oil. The crude product was then purified by column chromatography using pure hexane to give the title compound (0.86 g, 87 %) as a yellow oil: R_f = 0.17 in pure hexane; ^1H NMR (400 MHz, $CDCl_3$) δ 8.31 – 8.20 (m, 2H, H-2), 7.37 – 7.27 (m, 2H, H-2'), 7.22 – 7.09 (m, 2H, H-3'), 7.09 – 6.99 (m, 2H, H-3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.15 (C-4), 153.56, 146.49, 143.46, 126.46 (C-2), 123.48 (C-2'), 122.00 (C-3'), 119.55 (OCF_3), 117.74 (C-3); HRMS (CI) $C_{13}H_{12}N_2O_4F_3$ $[M+NH_4]^+$ requires 317.07492, found 317.07569; Anal. $C_{13}H_8NO_4F_3$ requires C 52.19%, H 2.70%, N 4.68%, found C 52.77%, H 2.86%, N 4.54%.

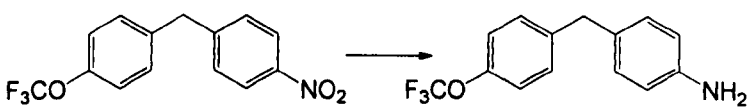
Preparation of **1-Nitro-4-((4-(trifluoromethoxy)benzyl)oxy)benzene 38.**



37 **38**

To a solution of 4-nitrophenol (3.30 g, 23.72 mmol) in anhydrous acetonitrile (22 mL) was added caesium carbonate (7.73 g, 23.72 mmol), followed by 4-(trifluoromethoxy)benzyl bromide (1.6 mL, 10 mmol) under N_2. The mixture was allowed to reflux for 4 h (followed by tlc). The mixture was then extracted with ethyl acetate, washed with brine and dired over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was then purified by column chromatography using 6% ethyl acetate in hexane to give the title compound (2.23 g, 69 %) as a white solid: mp 72 – 73°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37 – 8.18 (m, 2H, H-2), 7.61 – 7.44 (m, 2H, H-2'), 7.37 – 7.21 (m, 2H, H-3'), 7.14 – 6.97 (m, 2H, H-3), 5.16 (s, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.75, 149.63, 142.28, 134.56, 129.36 (C-2'), 126.41 (C-2), 122.10 (C-3'), 121.71, 115.20 (C-3), 70.14 (CH_2); IR ν_{max} (neat)/cm^{-1} 2977.6, 2888.8, 1592.9, 1508.1, 1494.6, 1340.3, 1245.8, 1201.4 and 1170.6; HRMS (ESI) $C_{14}H_{10}NO_4F_3{}^{23}Na$ $[M+Na]^+$ requires 336.0460, found 336.0456; Anal. $C_{14}N_{10}NO_4F_3$ requires C 53.68%, H 3.22%, N 4.47%, found C 53.10%, H 3.33%, N 4.34%.

Preparation of **4-(4-(Trifluoromethoxy)benzyl)aniline 39.**

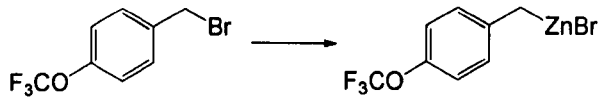


45 **39**

39 was prepared from **45** (0.36 g, 1.21 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 10% ethyl acetate in hexane gave **39** (0.27 g, 85 %) as an orange oil: R_f = 0.33, 30% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, *J* = 8.5 Hz, 2H, H-2'), 7.10 (d, *J* = 8.3 Hz, 2H, H-3'), 6.96 (d, *J* = 8.2 Hz, 2H, H-3), 6.63 (d, *J* = 8.3 Hz, 2H, H-2), 3.86 (s, 2H, CH_2), 3.61 (s, 2H, NH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 147.85(C-1), 145.13 (C-4'), 141.11

(C-1'), 130.86 (C-4), 130.34 (C-2'), 130.16 (C-3), 121.32 (C-3'), 119.64 (OCF_3), 115.76 (C-2), 40.74 (CH_2); HRMS (CI) $C_{14}H_{13}NOF_3$ $[M+H]^+$ requires 268.09492, found 268.09476; Anal. $C_{14}H_{12}NOF_3$ requires C 62.92%, H 4.53%, N 5.24%, found C 63.13%, H 4.56%, N 5.21%.

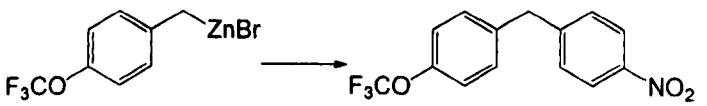
Preparation of **(4-(Trifluoromethoxy)benzyl)zinc(II) bromide 43.**



36 **43**

Activation of zinc dust – Zinc dust (1 g) was washed with 5% aqueous hydrochloric acid (3 x 10 mL), water (3 x 10 mL), ethanol (3 x 10 mL) and diethyl ether (3 x 10 mL), then dried *in vacuo* overnight. Zinc dust (0.77 g, 11.76 mmol) was suspended in anhydrous tetrahydrofuran (2.5 mL) in a dried-2-neck flask and heated to 60°C under N_2. 1,2-Dibromoethane (0.04 mL, 0.47 mmol) was added and the mixture was allowed to stir for 15 min at 60°C, then was cooled to room temperature. Trimethylsilyl chloride (0.05 mL, 0.39 mmol) was then added. The mixture was allowed to stir for 30 min at r.t., then a solution of 4-(trifluoromethoxy)benzyl bromide (2.5 g, 9.80 mmol) in anhydrous THF (10 mL) was added over 1 h and the flask was sealed and left in fridge to let the Zn dust settle. The resulting solution was used directly in the next step. (88 % coversion.)

Preparation of **1-Nitro-4-(4-(trifluoromethoxy)benzyl)benzene 45.**



43 **45**

Palladium acetate (0.03 g, 0.13 mmol, 0.05 equiv) and tri-(*o*-tolyl)phosphine (0.08 g, 0.26 mmol, 0.1 equiv) were dissolved in anhydrous tetrahydrofuran (4 mL) under N_2. The yellow mixture was allowed to stir for 5 min, the reaction was then cooled to 0°C and 1-iodo-4-nitrobenzene (0.59 g, 2.36 mmol) was added, followed by the

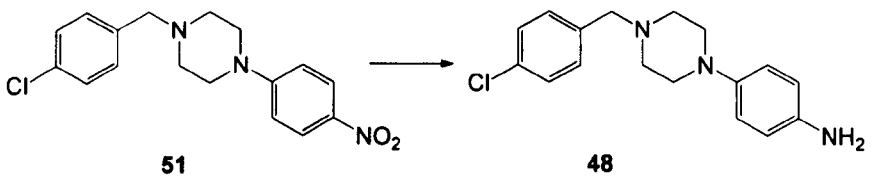
solution of **43** (1.13 g, 3.53 mmol, 1.5 equiv, in 3 mL THF) which was added over 2 min. The reaction was then left stirring at room temperature overnight. Saturated aqueous NH_4Cl was added and the reaction was stirred for another 10 min and then was extracted with diethyl ether (x 3) and washed with brine. The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give an oil. The crude product was then purified by column chromatography using 4% diethyl ether in hexane to give the title compound (0.45 g, 64 %) as a colourless oil: R_f = 0.29 in 10% diethyl ether in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 – 8.09 (m, 2H, H-2), 7.40 – 7.30 (m, 2H, H-3), 7.24 – 7.13 (m, 4H, ArH'), 4.09 (s, 2H, CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.48 (C-4), 148.45 (C-1), 147.08 (C-4'), 138.35 (C-1'), 130.66 (C-2'), 130.06 (C-3), 124.27 (C-2), 121.74 (C-3'), 120.86 (q, *J* = 257.1 Hz), 41.32 (CH_2); HRMS (ESI) $C_{14}H_9NO_3F_3$ $[M-H]^-$ requires 296.0535, found 296.0523; Anal. $C_{14}H_{10}NO_2F_3$ requires C 56.57%, H 3.39%, N 4.71%, found C 56.51%, H 3.42%, N 4.74%.

Preparation of **4-Benzylaniline 47.**

46 **47**

47 was prepared from 4-nitrodiphenylmethane (2.14 g, 10.03 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 8% ethyl acetate in hexane gave **47** (1.66 g, 90 %) as a light brown oil: R_f = 0.27, 20% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 – 7.24 (m, 3H, ArH'), 7.24 – 7.17 (m, 2H, ArH'), 7.14 – 6.98 (m, 2H, H-3), 6.85 – 6.53 (m, 2H, H-2), 3.87 (s, 2H, CH_2), 3.56 (s, 2H, NH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.88, 142.31, 131.61, 130.17 (C-3), 129.19, 128.76, 126.24 (C-4'), 115.70 (C-2), 41.47 (CH_2); HRMS (CI) $C_{13}H_{14}N$ $[M+H]^+$ requires 184.11262, found 184.11199; Anal. $C_{13}H_{13}N$ requires C 85.21%, H 7.15%, N 7.64%, found C 85.29%, H 7.16%, N 7.61%.

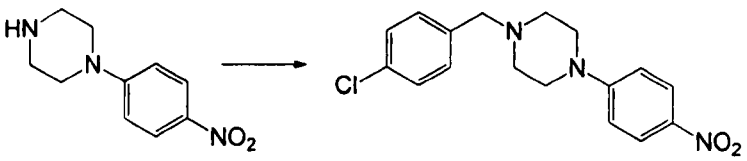
Preparation of **4-(4-(4-Chlorobenzyl)piperazin-1-yl)aniline 48.**



51 **48**

48 was prepared from **51** (0.27 g, 0.82 mmol) according to the procedure for the preparation of **32**. Purification by column chromatography using 1% methanol in dichloromethane to give **48** (0.21 g, 84 %) as a brown solid: R_f = 0.5 in 5% methanol in dichloromethane; mp 109°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.26 (m, 4H), 6.85 – 6.75 (m, 2H), 6.74 – 6.60 (m, 2H), 3.52 (s, 2H, CH_2), 3.13 – 2.98 (m, 4H, Ar-NCH_2), 2.68 – 2.55 (m, 2H, NCH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.91 (C-4), 140.52 (C-1), 136.96 (C-1'), 133.23 (C-4'), 130.90 (C-2'), 128.82 (C-3'), 119.02 (C-2), 116.61 (C-3), 62.65 (Ar<u>C</u>H_2), 53.63 (NCH_2), 51.28 (<u>C</u>H_2N-Ar); HRMS (CI) $C_{17}H_{21}N_3Cl$ $[M+H]^+$ requires 302.14240, found 302.14162; Anal. $C_{17}H_{20}N_3Cl$ requires C 67.65%, H 6.68%, N 13.92%, found C 67.60%, H 6.72%, N 13.90%.

Preparation of **1-(4-Chlorobenzyl)-4-(4-nitrophenyl)piperazine 51.**

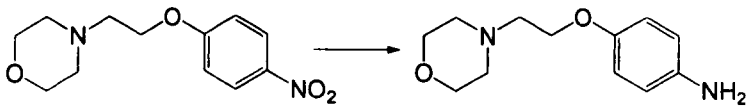


50 **51**

To a solution of 1-(4-nitrophenyl)piperazine **50** (2.14 g, 10.30 mmol) in acetonitrile (60 mL) was added potassium carbonate (8.26 g, 59.73 mmol, 5.8 equiv) and 4-chlorobenzyl bromide (2.33 g, 11.35 mmol, 1.1 equiv). The yellow mixture was heated to reflux for 5 h (followed by tlc). Afterwhich the reaction was allowed to cool to room temperature and diluted with dichloromethane (100 mL), washed with saturated NH_4Cl solution and brine. The organic layer was then dried over $MgSO_4$ and concentrated. The crude residue was purified by flash chromatography using dichloromethane to give the title compound (2.32 g, 68 %) as a yellow solid: R_f = 0.2 in dichloromethane; mp 118 – 119°C (lit.[55] 129 – 130°C); ^{1}H NMR (400 MHz, $CDCl_3$)

δ 8.17 – 8.05 (m, 2H, O_2N-C=CH), 7.39 – 7.26 (m, 4H, ArH), 6.90 – 6.76 (m, 2H, H_2CN-C=C<u>H</u>), 3.53 (s, 2H, CH_2), 3.45 – 3.38 (m, 4H, Ar-NCH_2), 2.65 – 2.53 (m, 4H, NCH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.26 (H_2CN-<u>C</u>), 138.84 (C-NO_2), 136.58 (<u>C</u>-CH_2N), 133.47 (C-Cl), 130.76 (ArC x 2), 128.95 (ArC x 2), 126.36 (O_2N-C=<u>C</u>H), 113.04 (H_2CN-C=<u>C</u>H), 62.49 (CH_2), 52.87 ($H_2CN\underline{C}H_2$), 47.44 (Ar-N<u>C</u>H_2); HRMS (CI) $C_{17}H_{19}N_3O_2Cl$ $[M+H]^+$ requires 332.11658, found 332.11707; Anal. $C_{17}H_{18}N_3O_2Cl$ requires C 61.54%, H 5.47%, N 12.66%, found C 61.48%, H 5.45%, N 12.71%.

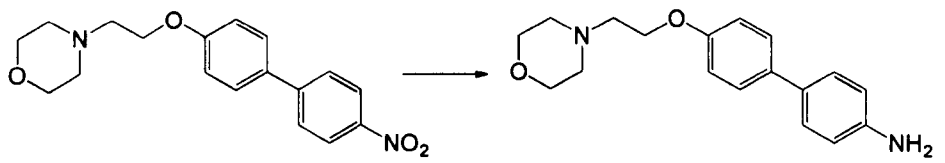
Preparation of **4-(2-Morpholinoethoxy)aniline 52.**



55 **52**

To a solution of **55** (0.63g, 2.50 mmol) in anhydrous ethanol (8 mL) was added 10% palladium on carbon (53 mg, 0.05 mmol, 0.02 equiv). The mixture was then allowed to stir under hydrogen environment overnight (reaction followed by tlc). The mixture was diluted by ethyl acetate and filtered through celite. The colourless filtrate was evaporated to give the title compound (0.46 g, 83 %) as a red liquid: R_f = 0.24, 10% methanol in ethyl acetate; ^1H NMR (400 MHz, $CDCl_3$) δ 6.95 – 6.71 (m, 2H, H-3), 6.71 – 6.53 (m, 2H, H-2), 4.05 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.86 – 3.69 (m, 4H, OCH_2), 3.45 (s, 2H, NH_2), 2.78 (t, *J* = 5.5 Hz, 2H, NCH_2), 2.71 – 2.51 (m, 4H, NCH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.24 (C-4), 140.62 (C-1), 116.77 (C-2), 116.23 (C-3), 67.28 (OCH_2), 66.74 (ArO<u>C</u>H_2CH_2), 58.21 (ArOCH_2<u>C</u>H_2), 54.46 (NCH_2); IR ν_{max} (neat)/cm^{-1} 3357.5, 3228.3 (N-H), 2940.9, 2879.2, 1513.9, 1234.2 and 1110.8; HRMS (ESI) $C_{12}H_{19}N_2O_2$ $[M+H]^+$ requires 223.1447, found 223.1439.

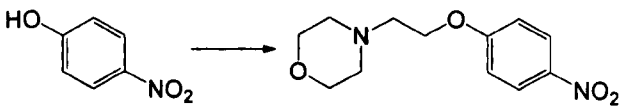
Preparation of **4'-(2-Morpholinoethoxy)-[1,1'-biphenyl]-4-amine 53.**



58 **53**

53 was prepared from **58** (0.28 g, 0.85 mmol) according to the procedure for the preparation of **52**. Filtration through celite and washed with ethyl acetate gave **53** (0.24 g, 95 %) as a yellow solid: R_f = 0.33, 4% methanol in dichloromethane; mp 106 – 107°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49 – 7.42 (m, 2H, H-6), 7.40 – 7.31 (m, 2H, H-3), 6.98 – 6.90 (m, 2H, H-7), 6.78 – 6.69 (m, 2H, H-2), 4.15 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.80 – 3.73 (m, 4H, OCH_2), 3.70 (s, 2H, NH_2), 2.83 (t, *J* = 5.5 Hz, 2H, NCH_2), 2.70 – 2.53 (m, 4H, NCH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.88 (C-8), 145.75 (C-1), 134.52 (C-4), 131.66 (C-5), 128.01 (C-6), 127.81 (C-3), 115.84 (C-2), 115.21 (C-7), 67.28 (OCH_2), 66.17 ($ArO\underline{C}H_2CH_2$), 58.09 ($ArOCH_2\underline{C}H_2$), 54.48 (NCH_2); HRMS (ESI) $C_{18}H_{23}N_2O_2$ $[M+H]^+$ requires 299.1760, found 299.1763; Anal. $C_{18}H_{22}N_2O_2$ requires C 72.46%, H 7.43%, N 9.39%, found C 72.30%, H 7.49%, N 9.44%.

Preparation of **4-(2-(4-Nitrophenoxy)ethyl)morpholine 55.**

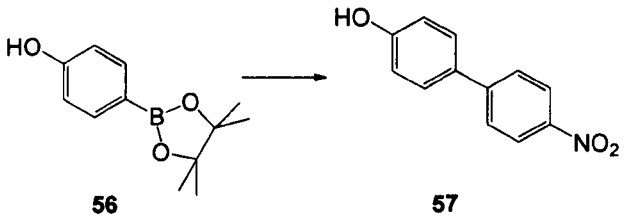


54 **55**

A mixture of 4-hydroxynitrobenzene (1.91 g, 13.71 mmol) and potassium carbonate (3.90 g, 28.25 mmol, 2 equiv) in anhydrous DMF (15 mL) were allowed to stir for 5 min under N_2. N-(2-Chloroethyl)morpholine (3.83 g, 2.06 mmol, 1.5 equiv) was added to the yellow mixture and the mixture was heated to 80°C for 3 h. 1 M Sodium hydroxide solution (15 mL) was added. The mixture was then extracted by ethyl acetate (3 x 30 mL), the organic layer was then washed with saturated $NaHCO_3$ (x 2), water (x 2), brine (x 2), dried over $MgSO_4$ filtered and concentrated *in vacuo* to a yellow oil. Upon cooling, a solid precipitated formed, which was collected by filtration and washed with hexane to give the title compound (2.83 g, 82 %) as a

pale yellow solid: mp 74 – 75°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 – 8.08 (m, 2H, H-2), 7.12 – 6.79 (m, 2H, H-3), 4.20 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.96 – 3.72 (m, 4H, OCH_2), 2.84 (t, *J* = 5.7 Hz, 2H, NCH_2), 2.72 – 2.47 (m, 4H, NCH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.12 (C-O), 142.04 (C-NO_2), 126.32 (C-2), 114.92 (C-3), 67.28 (OCH_2 x 2), 67.08 (OCH_2), 57.70 (NCH_2), 54.51 (NCH_2 x 2); HRMS (ESI) $C_{12}H_{17}N_2O_4$ $[M+H]^+$ requires 253.1188, found 253.1189; Anal. $C_{12}H_{16}N_2O_4$ requires C 57.13%, H 6.39%, N 11.10%, found C 56.98%, H 6.42%, N 11.01%.

Preparation of **4'-Nitro-[1,1'-biphenyl]-4-ol 57.**

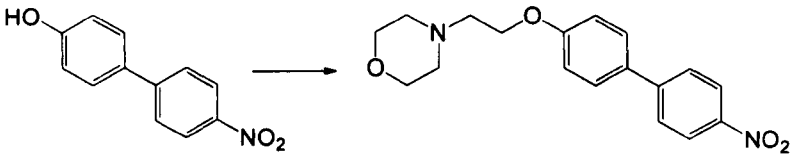


56 **57**

Nitrogen gas was bubbled through a solution of 50% ^{i}PrOH/water (12 mL) for 15 min. 4-Iodonitrobenzene (0.73 g, 2.93 mmol), 10% palladium on carbon (0.05 g, 0.04 mmol, 0.015 equiv) and sodium phosphate tribasic (1.26 g, 7.66 mmol, 2.6 equiv) were added to the solution. The mixture was allowed to stir at room temperature for 5 min. 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (0.74 g, 3.36 mmol, 1.15 equiv) was added. The mixture was heated to 50°C for 3 h (followed by tlc). The reaction was cooled and water (100 mL) and ethyl acetate (100 mL) were added. The mixture was then filtered and the filtrate was then extracted with ethyl acetate and the organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated to give a yellow solid. The crude product was purified by column chromatography using 5% ethyl acetate in hexane to give the title compound (0.46 g, 73%) yellow solid: R_f = 0.07, 10% ethyl acetate in hexane; mp 200°C (lit.[56] 211 – 213°C); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 – 8.06 (m, 2H, H-3'), 7.59 – 7.50 (m, 2H, H-2'), 7.41 – 7.33 (m, 2H, H-3), 6.84 – 6.75 (m, 2H, H-2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.45 (C-1), 151.99, 150.52, 134.20 (C-4), 132.91 (C-3), 131.16 (C-3'), 128.36 (C-2'), 120.33 (C-2); IR ν_{max} (neat)/cm^{-1} 3419.2 (O-H), 2979.5, 2927.4, 1591.0, 1504.2, 1444.4, 1332.6, 1270.9 and 1191.8; HRMS (ESI) $C_{12}H_9NO_3{}^{23}Na$ $[M+Na]^+$ requires

238.0480, found 238.0473; Anal. $C_{12}H_9NO_3$ requires C 66.97%, H 4.22%, N 6.51%, found C 66.91%, H 4.37%, N 6.29%.

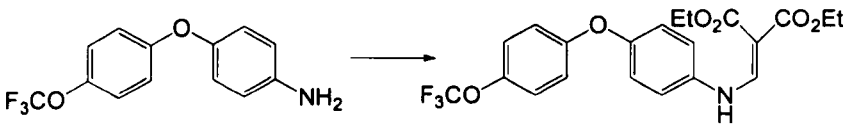
Preparation of **4-(2-((4'-Nitro-[1,1'-biphenyl]-4-yl)oxy)ethyl)morpholine 58.**



57 **58**

A mixture of **57** (0.31 g, 1.42 mmol) and potassium carbonate (0.51 g, 3.67 mmol, 2.6 equiv) in anhydrous DMF (5 mL) was allowed to stir at room temperature for 5 min under N_2. *N*-(2-Chloroethyl)morpholine (0.39 g, 2.13 mmol, 1.5 equiv) was added. The resulting yellow mixture was heated to 80°C for 5 h. The reaction mixture was cooled and 1M NaOH (aq) (5 mL) was added and the mixture was extracted by ethyl acetate (x 3). The combined organic extracts were washed with saturated $NaHCO_3$ (x 2), water (x 2) and brine (x 2), dried over $MgSO_4$, filtered and concentrated to yellow oil. The crude product was purified by column chromatography using 2% methanol in dichloromethane to give the title compound (0.41 g, 88 %) as a yellow oil: R_f = 0.22, 2% methanol in dichloromethane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (d, *J* = 8.8 Hz, 2H, H-2), 7.69 (d, *J* = 8.8 Hz, 2H, H-3), 7.58 (d, *J* = 8.8 Hz, 2H, H-6), 7.03 (d, *J* = 8.8 Hz, 2H, H-7), 4.18 (t, *J* = 5.7 Hz, 2H, $ArOC\underline{H}_2$), 3.91 – 3.69 (m, 4H, OCH_2), 2.85 (t, *J* = 5.7 Hz, 2H, NCH_2), 2.70 – 2.51 (m, 4H, NCH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.95 (C-8), 147.54, 146.98, 131.69 (C-5), 128.99 (C-6), 127.49 (C-3), 124.56 (C-2), 115.63 (C-7), 67.30 ($ArOCH_2$), 66.37 (OCH_2), 57.97 ($N\underline{C}H_2$), 54.52 ($N\underline{C}H_2$); HRMS (ESI) $C_{18}H_{21}N_2O_4$ $[M+H]^+$ requires 329.1501, found 329.1513.

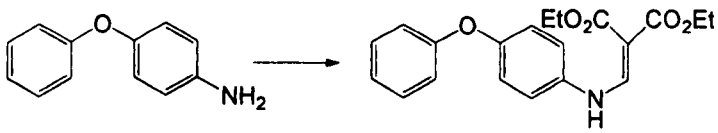
Preparation of **Diethyl 2-(((4-(4-(trifluoromethoxy)phenoxy)phenyl)amino) methylene)malonate 61.**



31 **61**

31 (1.24 g, 4.61 mmol) and diethylethoxymethylene malonate (0.99 g, 4.61 mmol) was heated at 100°C for 4 h. Afterwhich the reaction was allowed to cool to room temperature. The crude material was purified by column chromatography using 10% ethyl acetate in hexane to give the title compound (1.82 g, 90 %) as a brown oil: R_f = 0.41 in 20% ethyl acetate in hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.04 (d, *J* = 13.6 Hz, 1H, N-H), 8.47 (d, *J* = 13.7 Hz, 1H, C=CH), 7.23 – 7.11 (m, 4H, ArH), 7.10 – 6.92 (m, 4H, ArH), 4.31 (q, *J* = 7.1 Hz, 2H, CH_2), 4.25 (q, *J* = 7.1 Hz, 1H, CH_2), 1.39 (t, *J* = 7.1 Hz, 3H, CH_3), 1.33 (t, *J* = 7.1 Hz, 1H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.55 (C=O), 166.12 (C=O), 156.19, 154.25, 152.61 (C=CH), 144.99, 135.82, 123.11, 120.91, 119.75, 119.61, 119.23, 93.84 (C=CH), 60.84 (CH_2), 60.54 (CH_2), 14.83 (CH_3), 14.70 (CH_3); HRMS (ESI) $C_{21}H_{20}NO_6F_3{}^{23}Na$ $[M+Na]^+$ requires 462.1140, found 462.1129; Anal. $C_{21}H_{20}NO_6F_3$ requires C 57.40%, H 4.59%, N 3.19%, found C 57.33%, H 4.59%, N 3.15%.

Preparation of **Diethyl 2-(((4-phenoxyphenyl)amino)methylene)malonate 62.**

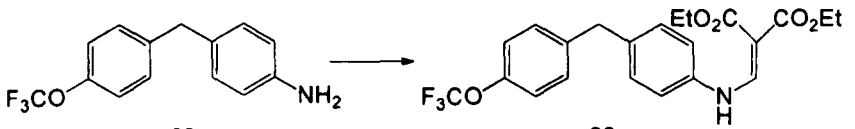


59 **62**

62 was prepared from 4-phenoxyaniline **59** (2. 9 g, 15.62 mmol) according to the procedure for the preparation of **61**. Recrystallisation from petroleum ether gave **62** (4.08 g, 74 %) as yellowish brown crystals: R_f = 0.38, 20% ethyl acetate in hexane; mp 70 – 71°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.03 (d, *J* = 13.6 Hz, 1H, N-H), 8.46 (d, *J* = 13.7 Hz, 1H, C=CH), 7.35 (t, *J* = 7.9 Hz, 2H, ArH), 7.20 – 6.88 (m, 7H, ArH), 4.31 (q, *J* = 7.1 Hz, 2H), 4.24 (q, *J* = 7.1 Hz, 2H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.32 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.56 (C=O), 166.14 (C=O), 157.56, 154.90, 152.72

(C=CH), 135.32, 130.25 (2 x C-H), 123.87, 120.62 (2 x C-H), 119.16 (2 x C-H), 119.09 (2 x C-H), 93.63 (C=CH), 60.76 (CH_2), 60.45(CH_2), 14.83 (CH_3), 14.71 (CH_3); HRMS (ESI) $C_{20}H_{21}NO_5{}^{23}Na$ $[M+Na]^+$ requires 378.1317, found 378.1307; Anal. $C_{20}H_{21}NO_5$ requires C 67.59%, H 5.96%, N 3.94%, found C 67.58%, H 5.97%, N 3.90%.

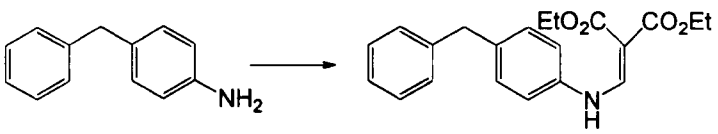
Preparation of **Diethyl 2-(((4-(4-(trifluoromethoxy)benzyl)phenyl)amino) methylene)malonate 63.**



39 **63**

63 was prepared from **39** (0.58 g, 2.17 mmol) according to the procedure for the preparation of **61**. Recrystallisation from petroleum ether gave **63** (0.84 g, 88 %) as a yellow solid: R_f = 0.4, 20% ethyl acetate in hexane; mp 98°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.00 (d, *J* = 13.6 Hz, 1H, N-H), 8.50 (d, *J* = 13.7 Hz, 1H, C=CH), 7.26 – 7.02 (m, 8H, ArH), 4.30 (q, *J* = 7.1 Hz, 2H, CH_2), 4.24 (q, *J* = 7.1 Hz, 2H, CH_2), 3.96 (s, 2H, CH_2), 1.38 (t, *J* = 7.1 Hz, 3H, CH_3), 1.32 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.49 (C=O), 166.14 (C=O), 152.37 (C=CH), 139.86 (C-N), 138.13, 137.66, 130.65 (ArC x 2), 130.45 (ArC x 2), 121.51 (ArC x 2), 117.85 (ArC x 2), 93.82 (C=CH), 60.77 (CH_2), 60.47 (CH_2), 40.87 (CH_2), 14.81 (CH_3), 14.70 (CH_3); IR ν_{max} (neat)/cm^{-1} 2985.3, 2904.3, 1681.6 (C=O), 1639.2, 1606.4, 1579.4, 1477.2, 1446.4, 1245.8 (C-O), 1213.0 and 1170.6; HRMS (ESI) $C_{22}H_{22}NO_5F_3{}^{23}Na$ $[M+Na]^+$ requires 460.1348, found 460.1352; Anal. $C_{22}H_{22}NO_5F_3$ requires C 60.41%, H 5.07%, N 3.20%, found C 60.38%, H 5.10%, N 3.16%.

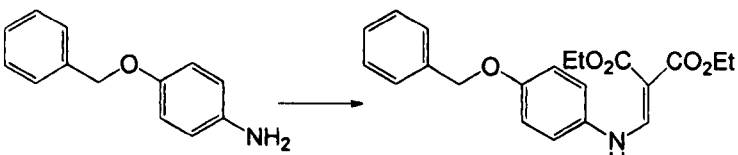
Preparation of **Diethyl 2-(((4-benzylphenyl)amino)methylene)malonate 64.**



49 **64**

64 was prepared from **49** (1.65 g, 8.99 mmol) according to the procedure for the preparation of **61**. Recrystallisation from petroleum ether gave **64** (2.14 g, 67 %) as white crystals: R_f = 0.47, 20% ethyl acetate in hexane; mp 59 – 60°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.99 (d, *J* = 13.7 Hz, 1H, N-H), 8.50 (d, *J* = 13.8 Hz, 1H, C=CH), 7.33 – 7.25 (m, 3H, ArH), 7.24 – 7.14 (m, 4H, ArH), 7.06 (d, *J* = 8.4 Hz, 2H, ArH), 4.30 (q, *J* = 7.1 Hz, 2H, CH_2), 4.24 (q, *J* = 7.1 Hz, 2H, CH_2), 3.96 (s, 2H, CH_2), 1.38 (t, *J* = 7.1 Hz, 3H, CH_3), 1.32 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.53 (C=O), 166.16 (C=O), 152.48 (C=CH), 141.12, 138.49, 137.86, 130.67 (ArC x 2), 129.25 (ArC x 2), 128.99 (ArC x 2), 126.68 (C-4'), 117.77 (ArC x 2), 93.57 (C=CH), 60.76 (CH_2CH_3), 60.45 (CH_2CH_3), 41.63 ($ArCH_2Ar$), 14.84 (CH_2CH_3), 14.73 (CH_2CH_3); IR ν_{max} (neat)/cm^{-1} 2985.3, 2925.5, 1683.5 (C=O), 1637.3, 1606.4, 1579.4, 1494.6, 1444.4, 1259.3 and 1240.0; HRMS (ESI) $C_{21}H_{23}NO_4{}^{23}Na$ $[M+Na]^+$ requires 376.1525, found 376.1509; Anal. $C_{21}H_{23}NO_4$ requires C 71.37%, H 6.56%, N 3.96%, found C 71.21%, H 6.55%, N 4.01%.

Preparation of **Ethyl 2-(((4-(benzyloxy)phenyl)amino)methylene)malonate 65.**

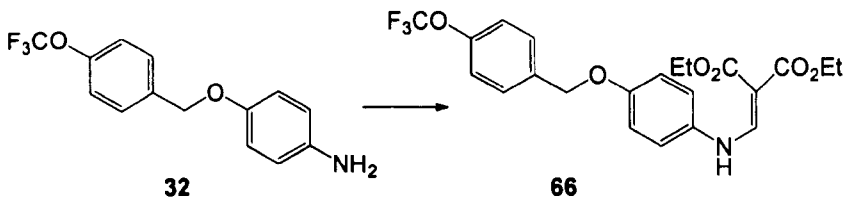


60 **65**

65 was prepared from 4-benoxyaniline (1.62g, 8.12 mmol) according to the procedure for the preparation of **61**. Recrystallisation from petroleum ether gave **65** (2.19 g, 73 %) as a brown powder: R_f = 0.44, 20% ethyl acetate in hexane; mp 106 – 107°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.99 (d, *J* = 13.2 Hz, 1H, N-H), 8.44 (d, *J* = 13.8 Hz, 1H, C=CH), 7.51 – 7.31 (m, 4H, ArH), 7.14 – 6.99 (m, 3H, ArH), 7.04 – 6.90 (m, 2H, ArH), 5.06 (s, 2H, CH_2), 4.30 (q, *J* = 7.1 Hz, 2H, CH_2), 4.24 (q, *J* = 7.1 Hz, 1H, CH_2), 1.38

(t, *J* = 7.1 Hz, 3H, CH_3), 1.32 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.65 (C=O), 166.24 (C=O), 156.71 (C-4), 153.00 (C=<u>C</u>H), 137.05 (C-1'), 133.40 (C-1), 129.06 (2 x C-H), 128.52 (C-5'), 127.87 (2 x C-H), 119.21 (2 x C-H), 116.45 (2 x C-H), 92.93 (<u>C</u>=CH), 70.80 (OCH_2), 60.70 (CH_2), 60.39 (CH_2), 14.87 (CH_3), 14.75 (CH_3); IR ν_{max} (neat)/cm^{-1} 3170.4, 3075.9, 1681.6 (C=O), 1631.5, 1612.2, 1583.3, 1475.3, 1442.5, 1253.5 and 1228.4; HRMS (ESI) $C_{21}H_{23}NO_5{}^{23}Na$ $[M+Na]^+$ requires 392.1474, found 392. 1461; Anal. C 68.28%, H 6.29%, N 3.79%, found C 68.36%, H 6.27%, N 3.77%.

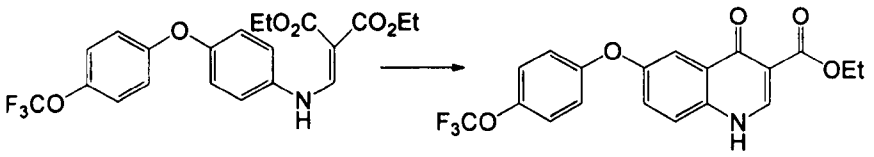
Preparation of **Diethyl 2-(((4-((4-(trifluoromethoxy)benzyl)oxy)phenyl)amino) methylene)malonate 66.**



32 **66**

66 was prepared from **32** (0.70 g, 2.48 mmol) according to the procedure for the preparation of **61**. Recrystallisation from petroleum ether gave **66** (0.77 g, 69 %) as a brown solid: R_f = 0.42, 6% methanol in dichloromethane; mp 72°C; ^1H NMR (400 MHz, $CDCl_3$) δ 11.00 (d, *J* = 13.7 Hz, 1H, N-H), 8.44 (d, *J* = 13.8 Hz, 1H, C=CH), 7.54 – 7.43 (m, 2H, ArH), 7.27 – 7.22 (m, 2H, ArH), 7.13 – 7.06 (m, 2H, ArH), 7.02 – 6.92 (m, 2H, ArH), 5.05 (s, 2H, OCH_2), 4.30 (q, *J* = 7.1 Hz, 2H, CH_2), 4.24 (q, *J* = 7.1 Hz, 2H, CH_2), 1.38 (t, *J* = 7.1 Hz, 3H, CH_3), 1.32 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.64 (C=O), 166.25 (C=O), 156.39, 152.95 (C=<u>C</u>H), 135.76, 133.66, 132.13, 130.75, 129.25 (ArC x 2), 121.57 (ArC x 2), 119.24 (ArC x 2), 116.42 (ArC x 2), 93.09 (<u>C</u>=CH), 69.91 (OCH_2), 60.72 (CH_2), 60.41 (CH_2), 14.85 (CH_3), 14.72 (CH_3); HRMS (ESI) $C_{22}H_{22}NO_6F_3{}^{23}Na$ $[M+Na]^+$ requires 476.1297, found 476.1291; Anal. $C_{22}H_{22}NO_6F_3$ requires C 58.28%, H 4.89%, N 3.09%, found C 58.14%, H 4.93%, N 3.06%.

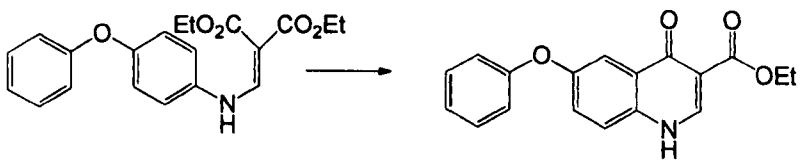
Preparation of **Ethyl 4-oxo-6-(4-(trifluoromethoxy)phenoxy)-1,4-dihydroquinoline -3-carboxylate 67.**



61 **67**

61 (3.2 g, 7.30 mmol) in Dowtherm A (8 mL) was heated at 240°C for 1 ½ h. The mixture was allowed to cool to room temperature and hexane was added. The resulting precipitate was filtered and the solid was then recrystallised from DMSO to give the title compound (1.69 g, 59 %) as a white solid: mp 274 – 275°C; ^{1}H NMR (400 MHz, DMSO + TFA) δ 8.57 (s, 1H, H-2), 7.72 (d, *J* = 8.9 Hz, 1H, H-8), 7.59 (d, *J* = 2.8 Hz, 1H, H-5), 7.52 (dd, *J* = 8.9, 2.9 Hz, 1H, H-6), 7.42 (dd, *J* = 9.0, 0.7 Hz, 2H, H-2'), 7.23 – 7.14 (m, 2H, H-3'), 4.21 (q, *J* = 7.1 Hz, 2H, CH_2), 1.27 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO + TFA) δ 173.02 (C=O), 165.13 ($\underline{C}O_2Et$), 155.58 (C-O), 154.04 (C-O), 144.93 (C-O), 144.48 (C-2), 135.70, 128.84, 123.45, 120.78, 119.68, 116.81 (C-2'), 113.95 (C-3'), 111.08 (C-5), 109.41 (C-3), 59.94 (CH_2), 14.58 (CH_3); IR ν_{max} (neat)/cm^{-1} 2989.1, 2954.4, 1699.0 (C=O), 1618.0, 1581.3, 1481.1, 1267.0, 1222.7, 1193.7 and 1164.8; HRMS (ESI) $C_{19}H_{15}NO_5F_3$ $[M+H]^+$ requires 394.0902, found 394.0876, $C_{19}H_{14}NO_5F_3{}^{23}Na$ $[M+Na]^+$ requires 416.0722, found 416.0712; Anal. $C_{19}H_{14}NO_5F_3$ requires C 58.02%, H 3.59%, N 3.56%, found C 58.23%, H 3.69%, N 3.48%.

Preparation of **Ethyl 4-oxo-6-phenoxy-1,4-dihydroquinoline-3-carboxylate 68.**



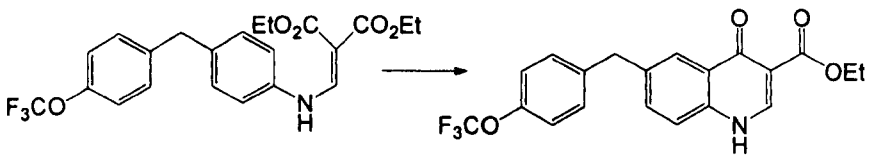
62 **68**

68 was prepared from **62** (1.00 g, 2.81 mmol) according to the procedure for the preparation of **67**. The solid formed was filtered and washed with ethyl acetate followed by dichloromethane. Further purification by column chromatography gave

68 (0.40 g, 46 %) as a white solid: R_f = 0.38, 4% methanol in dichloromethane; mp 261 – 262°C; ^{1}H NMR (400 MHz, DMSO) δ 12.43 (s, 1H, N-H), 8.55 (s, 1H, H-2), 7.70 (d, *J* = 8.8 Hz, 1H, H-8), 7.53 (d, *J* = 2.7 Hz, 1H, H-5), 7.50 (dd, *J* = 8.8, 2.9 Hz, 1H, H-7), 7.48 – 7.41 (m, 2H, H-3'), 7.27 – 7.19 (m, 1H, H-4'), 7.13 – 7.05 (m, 2H, H-2'), 4.20 (q, *J* = 7.1 Hz, 2H, CH_2), 1.26 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 173.04 (C=O), 165.20 ($\underline{C}O_2Et$), 156.47 (C-O), 154.68 (C-O), 144.79 (C-2), 135.29, 130.64 (C-3'), 128.87, 124.69 (C-7), 124.54 (C-4'), 121.59 (C-8), 119.67 (C-2'), 112.29 (C-5), 109.30 (C-3), 59.92 (CH_2), 14.67 (CH_3); IR ν_{max} (neat)/cm^{-1} 2981.4, 2904.3, 1699.0 (C=O), 1616.06, 1581.3, 1477.2, 1199.5 and 1166.7;

HRMS (ESI) $C_{18}H_{16}NO_4$ $[M+H]^+$ requires 310.1079, found 310.1071 (100%); $C_{18}H_{15}NO_4{}^{23}Na$ $[M+Na]^+$ requires 332.0899, found 332.0894; Anal. $C_{18}H_{15}NO_4$ requires C 69.89%, H 4.89%, N 4.53%, found C 69.69%, H 4.88%, N 4.55%.

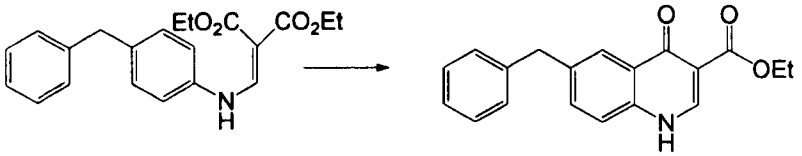
Preparation of **Ethyl 4-oxo-6-(4-(trifluoromethoxy)benzyl)-1,4-dihydroquinoline -3-carboxylate 69.**



63 **69**

69 was prepared from **63** (0.70 g, 1.61 mmol) according to the procedure for the preparation of **67**. The solid was filtered and washed with ethyl acetate. Recrystallisation from hot methanol/ethyl acetate gave **69** (0.45 g, 71 %) as a white solid: mp 282 – 284°C; ^{1}H NMR (400 MHz, DMSO) δ 12.29 (s, 1H, N-H), 8.51 (s, 1H, H-2), 7.99 (s, 1H, H-5), 7.77 – 7.50 (m, 2H, H-7 + H-8), 7.38 (d, *J* = 8.5 Hz, 2H, H-2'), 7.30 (d, *J* = 8.3 Hz, 2H, H-3'), 4.20 (q, *J* = 7.1 Hz, 2H, CH_2), 4.12 (s, 2H, CH_2), 1.27 (t, *J* = 7.1 Hz, 3H, CH_3); IR ν_{max} (neat)/cm^{-1} 2979.5, 2888.8, 1700.9 (C=O), 1616.1, 1581.3, 1488.8, 1456.0, 1267.0, 1209.2 and 1164.8; HRMS (ESI) $C_{20}H_{17}NO_4F_3$ $[M+H]^+$ requires 392.1110, found 392.1099 (61%); $C_{20}H_{16}NO_4F_3{}^{23}Na$ $[M+Na]^+$ requires 414.0929, found 414.0910; Anal. $C_{20}H_{16}NO_4F_3$ requires C 61.38%, H 4.12%, N 3.58%, found C 61.08%, H 4.14%, N 3.63%.

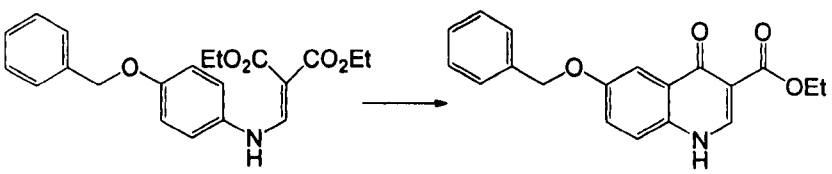
Preparation of **Ethyl 6-benzyl-4-oxo-1,4-dihydroquinoline-3-carboxylate 70.**



64 **70**

70 was prepared from **64** (1.00 g, 2.83 mmol) according to the procedure for the preparation of **67**. The solid was filtered and washed with ethyl acetate. Purification by column chromatography using 5% methanol in dichloromethane gave **70** (0.38 g, 44 %) as a white solid: mp 260°C; ^{1}H NMR (400 MHz, DMSO) δ 12.31 (s, 1H, N-H), 8.51 (d, *J* = 6.0 Hz, 1H, H-2), 7.98 (s, 1H, H-5), 7.59 (d, *J* = 8.4 Hz, 1H, H-7), 7.55 (d, *J* = 8.4 Hz, 1H, H-8), 7.39 – 7.12 (m, 5H, ArH'), 4.20 (q, *J* = 6.8 Hz, 2H, CH_2), 4.08 (s, 2H, CH_2), 1.27 (t, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 173.70 (C=O), 165.16 ($\underline{C}O_2Et$), 144.89 (C-2), 141.28, 138.43, 137.72, 133.65, 129.15 (C-3'), 128.89 (C-2'), 127.62, 126.49 (C-4'), 125.26, 119.36, 109.94 (C-3), 59.86 ($\underline{C}H_2CH_3$), 40.98 (CH_2), 14.68 (CH_3); IR ν_{max} (neat)/cm^{-1} 2979.5, 2898.5, 1700.9 (C=O), 1616.1, 1581.3, 1490.7, 1454.1, 1294 (C-O stretch), 1170.6 and 1093.44; HRMS (ESI) $C_{19}H_{18}NO_3$ $[M+H]^+$ requires 308.1287, found 308.1290; Anal. $C_{19}H_{17}NO_3$ requires C 74.25%, H 5.56%, N 4.56%, found C 74.39%, H 5.59%, N 4.55%.

Preparation of **Ethyl 6-(benzyloxy)-4-oxo-1,4-dihydroquinoline-3-carboxylate 71.**

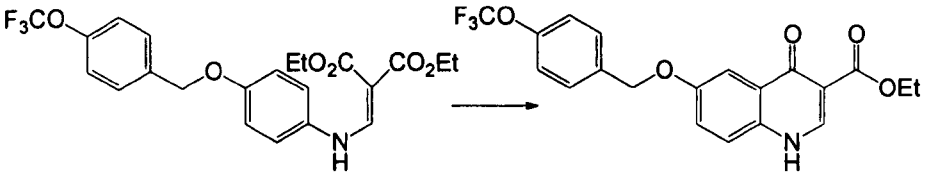


65 **71**

71 was prepared from **65** (0.97 g, 2.61 mmol) according to the procedure for the preparation of **67**. The solid was filtered and washed with ethyl acetate. Recrystallisation from hot methanol/ethyl acetate gave **71** (0.54 g, 64 %) as a white solid: mp 270 – 271°C; ^{1}H NMR (400 MHz, DMSO, 363K) δ 11.90 (s, 1H, N-H), 8.41 (s, 1H, H-2), 7.73 (d, *J* = 2.9 Hz, 1H), 7.58 (d, *J* = 8.9 Hz, 1H), 7.48 (d, *J* = 7.4 Hz, 2H), 7.44 – 7.37 (m, 3H), 7.34 (dd, *J* = 8.4, 6.0 Hz, 1H), 5.23 (s, 2H, $ArOC\underline{H}_2$), 4.25 (q, *J* =

7.1 Hz, 2H, CH_2), 1.31 (t, *J* = 7.1 Hz, 3H, CH_3); IR ν_{max} (neat)/cm^{-1} 2977.6, 2898.5, 1699.0 (C=O), 1618.0, 1583.3, 1490.7, 1454.1 and 1292.1 (C-O stretch); HRMS (ESI) $C_{19}H_{18}NO_4$ $[M+H]^+$ requires 324.1236, found 324.1223 (28%) $C_{19}H_{17}NO_4{}^{23}Na$ $[M+Na]^+$ requires 346.1055, found 346.1039 (100%); Anal. $C_{19}H_{17}NO_4$ requires C 70.58%, H 5.30%, N 4.33%, found C 70.63%, H 5.30%, N 4.31%.

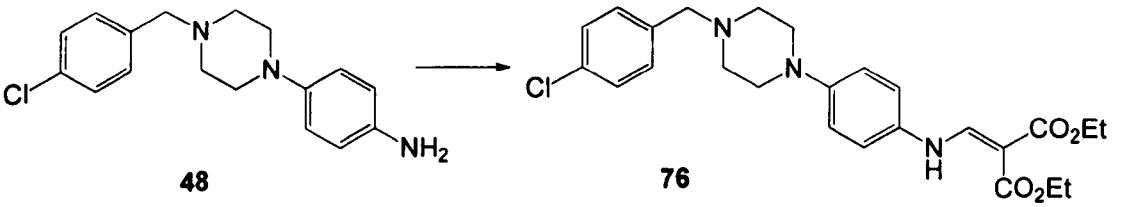
Preparation of **Ethyl 4-oxo-6-((4-(trifluoromethoxy)benzyl)oxy)-1,4-dihydroquinoline-3-carboxylate 72.**



66 **72**

72 was prepared from **66** (0.37 g, 0.82 mmol) according to the procedure for the preparation of **67**. The solid formed was filtered and washed with ethyl acetate and dichloromethane, followed by methanol gave **72** (0.14 g, 42 %) as a solid: mp 281 – 282°C; ^1H NMR (400 MHz, DMSO + TFA) δ 9.00 (s, 1H, H-2), 7.98 (d, *J* = 9.2 Hz, 1H, H-8), 7.71 (d, *J* = 2.7 Hz, 1H, H-5), 7.68 (dd, *J* = 9.2, 2.8 Hz, 1H, H-7), 7.45 (d, *J* = 8.7 Hz, 2H, H-2'), 7.16 (d, *J* = 8.0 Hz, 2H, H-3'), 5.17 (s, 2H, OCH_2), 4.38 (q, *J* = 7.1 Hz, 2H, CH_2), 1.27 (t, *J* = 7.1 Hz, 3H, CH_3); IR ν_{max} (neat)/cm^{-1} 3048.9, 2977.6, 2904.3, 1699.0 (C=O), 1619.9, 1581.3, 1490.7, 1276.7, 1192.2 and 1159.1; HRMS (ESI) $C_{20}H_{16}NO_5F_3{}^{23}Na$ $[M+Na]^+$ requires 430.0878, found 430.0862; Anal. $C_{20}H_{16}NO_5F_3$ requires C 58.97%, H 3.96%, N 3.44%, found C 58.83%, H 3.92%, N 3.41%.

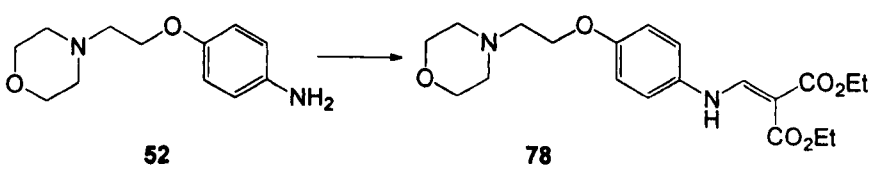
Preparation of **diethyl 2-(((4-(4-(4-chlorobenzyl)piperazin-1-yl)phenyl)amino) methylene)malonate 76.**



48 **76**

76 was prepared from **48** (0.92 g, 3.1 mmol) and diethylethoxymethylene malonate (0.66 g, 3.1 mmol) according to the procedure for the preparation of **61**. Purification by flash column chromatography using 20% ethyl acetate in hexane gave **76** (1.24 g, 85 %) as a yellow solid: R_f = 0.09, 20% ethyl acetate in hexane; mp 76 – 77°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.99 (d, *J* = 13.9 Hz, 1H, N-H), 8.44 (d, *J* = 13.9 Hz, 1H, HNC<u>H</u>=C), 7.35 – 7.25 (m, 4H, ArH'), 7.15 – 7.00 (m, 2H, H-2), 6.96 – 6.77 (m, 2H, H-3'), 4.29 (q, *J* = 7.1 Hz, 2H, CH_2), 4.23 (q, *J* = 7.1 Hz, 2H, CH_2), 3.50 (s, 2H, ArC<u>H</u>$_2$), 3.22 – 3.10 (m, 4H, ArNC<u>H</u>$_2$), 2.66 – 2.47 (m, 4H, NC<u>H</u>$_2$CH$_2$), 1.37 (t, *J* = 7.1 Hz, 3H, CH_3), 1.31 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.56 (C=O), 166.20 (C=O), 152.67, 149.33 (<u>C</u>H=C), 136.95, 133.18, 132.07, 130.72 (ArC' x 2), 128.77 (ArC' x 2), 118.78 (C-2), 117.36 (C-3), 92.53 (CH=<u>C</u>), 62.50 (CH_2), 60.49 (CH_2), 60.20 (CH_2), 53.26 (NCH_2), 49.59 (ArN<u>C</u>H$_2$), 14.82 (CH_3), 14.70 (CH_3); HRMS (ESI) $C_{25}H_{31}N_3O_4{}^{35}Cl$ $[M+H]^+$ requires, 472.2003, found 472.2005; $C_{25}H_{31}N_3O_4{}^{37}Cl$ $[M+H]^+$ requires 474.1974, found 474.1989; Anal. $C_{25}H_{30}N_3O_4Cl$ requires C 63.62 %, H 6.41%, N 8.90%, found C 63.50%, H 6.44%, N 8.87%.

Preparation of **Diethyl 2-(((4-(2-morpholinoethoxy)phenyl)amino)methylene) malonate 78.**

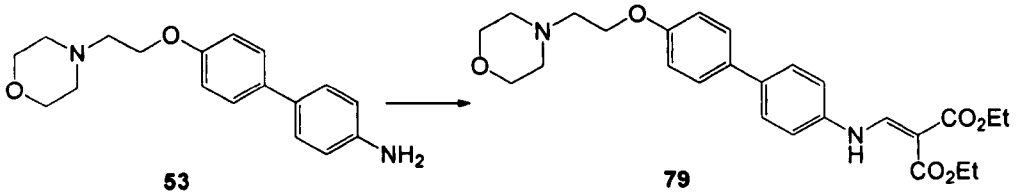


52 **78**

78 was prepared from SL89 (0.28 g, 1.25 mmol) according to the procedure for the preparation of **61**. Purification by column chromatography using 5% methanol in dichloromethane gave **78** (0.33 g, 68 %) as a yellow oil: R_f = 0.42, 6% methanol in

dichloromethane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.99 (d, *J* = 13.8 Hz, 1H, N-H), 8.44 (d, *J* = 13.8 Hz, 1H, C=CH), 7.17 – 7.02 (m, 2H, H-2), 6.97 – 6.68 (m, 2H, H-3), 4.30 (q, *J* = 7.1 Hz, 2H, CH_2), 4.24 (q, *J* = 7.1 Hz, 2H, CH_2), 4.10 (t, *J* = 5.7 Hz, 2H, $OC\underline{H}_2CH_2$), 3.84 – 3.66 (m, 4H, OCH_2), 2.80 (t, *J* = 5.7 Hz, 2H, NCH_2), 2.68 – 2.52 (m, 4H, NCH_2), 1.38 (t, *J* = 7.1 Hz, 3H, CH_3), 1.32 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.65 (C=O), 166.29 (C=O), 156.69, 153.02 (C=$\underline{C}$H), 133.36, 119.20 (C-2), 116.16 (C-3), 92.94 ($\underline{C}$=CH), 67.32 ($O\underline{C}H_2CH_2N$), 66.60 (OCH_2), 60.70 (CH_2), 60.40 (CH_2), 58.02 (NCH_2), 54.51 (NCH_2), 14.85 (CH_3), 14.73 (CH_3); HRMS (ESI) $C_{20}H_{29}N_2O_6$ $[M+H]^+$ requires 393.2026, found 393.3032; Anal. $C_{20}H_{28}N_2O_6$ requires C 61.21%, H 7.19%, N 7.14%, found C 60.99%, H 7.23%, N 7.17%.

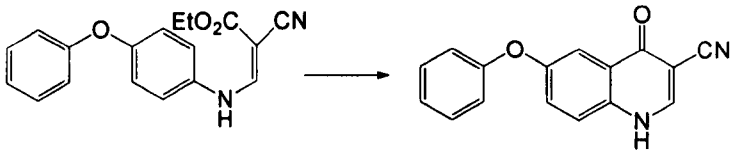
Preparation of **Diethyl 2-(((4'-(2-morpholinoethoxy)-[1,1'-biphenyl]-4-yl)amino) methylene)malonate 79.**



53 **79**

79 was prepared from **53** (0.23 g, 0.78 mmol) according to the procedure for the preparation of **61**. Purification by column chromatography using 5% methanol in dichloromethane gave **79** (0.13 g, 35 %) as a yellow oil: R_f = 0.78, 4% methanol in dichloromethane; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.07 (d, *J* = 13.7 Hz, 1H, N-H), 8.56 (d, *J* = 13.7 Hz, 1H, C=CH), 7.55 (d, *J* = 8.5 Hz, 2H, H-6), 7.53 – 7.44 (m, 2H, H-3), 7.19 (d, *J* = 8.6 Hz, 2H, H-7), 7.10 – 6.92 (m, 2H, H-2), 4.32 (q, *J* = 7.1 Hz, 2H, CH_2), 4.26 (q, *J* = 7.1 Hz, 2H, CH_2), 4.16 (t, *J* = 5.7 Hz, 2H, OCH_2), 3.82 – 3.68 (m, 4H, OCH_2), 2.83 (t, *J* = 5.7 Hz, 2H, NCH_2), 2.69 – 2.55 (m, 4H, NCH_2), 1.39 (t, *J* = 7.1 Hz, 3H, CH_3), 1.34 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.50 (C=O), 166.17 (C=O), 158.77 (C-8), 152.10 (C=$\underline{C}$H), 138.36, 137.89, 133.13, 128.34 (C-6), 128.23 (C-3), 117.89 (C-7), 115.40 (C-2), 93.96 ($\underline{C}$=CH), 67.33 (OCH_2), 66.31 (OCH_2), 60.81 (CH_2), 60.52 (CH_2), 58.05 (NCH_2), 54.51 (NCH_2), 14.84 (CH_3), 14.72 (CH_3); HRMS (ESI) $C_{26}H_{33}N_2O_6$ $[M+H]^+$ requires 469.2339, found 469.2358; Anal. $C_{26}H_{32}N_2O_6$ requires C 66.65%, H 6.88%, N 5.98%, found 66.67%, H 6.88%, N 6.02%.

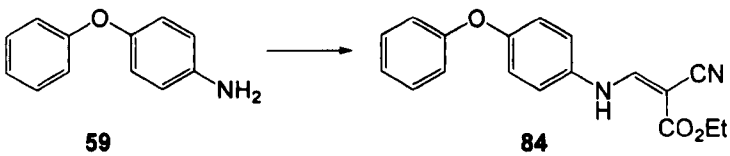
Preparation of **4-Oxo-6-phenoxy-1,4-dihydroquinoline-3-carbonitrile 82.**



84 **82**

82 was prepared from **84** (0.64 g, 2.09 mmol) according to the procedure for the preparation of **67**. The solid was filtered and washed with ethyl acetate. Recrystallisation from hot methanol/ethyl acetate gave **82** (0.20 g, 36 %) as a dark brown solid: mp 313 – 314°C; ^{1}H NMR (400 MHz, DMSO) δ 8.67 (s, 1H, H-2), 7.71 (d, *J* = 9.0 Hz, 1H, H-8), 7.56 (dd, *J* = 9.0, 2.9 Hz, 1H, H-7), 7.50 – 7.42 (m, 3H, H-5 + ArH), 7.24 (t, *J* = 7.4 Hz, 1H, ArH), 7.11 (d, *J* = 7.8 Hz, 2H, ArH); ^{13}C NMR (101 MHz, DMSO) δ 174.28 (C=O), 156.15 (C-O), 155.21 (C-O), 146.53 (C-2), 135.78, 130.73 (ArC x 2), 126.74, 125.40 (C-7), 124.82 (ArC), 122.47 (C-8), 119.85 (ArC), 117.35, 111.13 (C-5), 92.95 (C-CN); IR ν_{max} (neat)/cm^{-1} 3650.6 (N-H), 2979.5, 2887.0, 2217.7 (CN), 1702.8 (C=O), 1618.0, 1585.2, 1483.0, 1456.0 and 1380.8; HRMS (ESI) $C_{16}H_{10}N_2O_2{}^{23}Na$ $[M+Na]^+$ requires 285.0640, found 285.0636; Anal. $C_{16}H_{10}N_2O_2$ requires C 73.27%, H 3.84%, N 10.68%, found C 73.25%, H 3.86%, N 10.73%.

Preparation of **Ethyl 2-cyano-3-((4-phenoxyphenyl)amino)acrylate 84.**

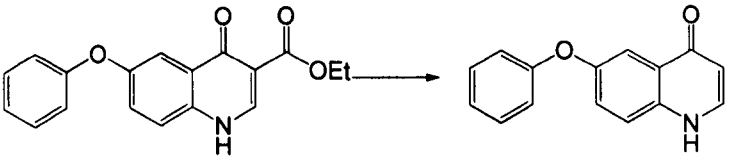


59 **84**

4-Phenoxyaniline (3.0 g, 16.22 mmol) and ethyl (ethoxymethylene) cyanoacetate (2.74 g, 16.22 mmol) were mixed and heated to 120°C for 3 ½ h. The mixture was cooled to room temperature, hexane was added and the mixture was further cooled in an ice bath. The precipitate was then filtered and washed with hexane to give the title compound (1.65 g, 33%) as a brown solid: mp 138 – 139°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.76 (d, *J* = 13.3 Hz, 1H, N-H), 7.78 (d, *J* = 13.5 Hz, 1H, C=CH), 7.45 – 7.29 (m, 2H, ArH), 7.23 – 6.97 (m, 7H, ArH), 4.30 (q, *J* = 7.1 Hz, 2H, CH_2), 1.37 (t, *J* = 7.1 Hz, 3H, CH_3); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.06 (C=O), 157.18 (C-O), 155.70 (C-O), 152.57 (C=CH), 134.16 (C-NH), 130.34 (ArC x 2), 124.18 (ArC), 120.53 (ArC x 2),

119.36 (ArC x 2), 119.25 (ArC x 2), 118.37 (C-CN), 75.44 (C-CN), 61.64 (CH_2), 14.70 (CH_3); HRMS (ESI) $C_{18}H_{16}N_2O_3{}^{23}Na$ $[M+Na]^+$ requires 331.1059, found 331.1047; Anal. $C_{18}H_{16}N_2O_3$ requires C 70.12%, H 5.23%, N 9.09%, found C 70.32%, H 5.26%, N 9.03%.

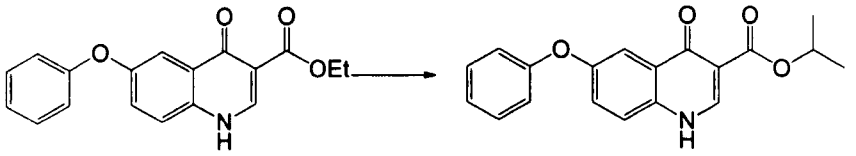
Preparation of **6-Phenoxyquinolin-4(1H)-one 86.**



68 **86**

To a solution of **68** (0.31 g, 0.99 mmol) in methanol (4 mL) was added 10% aqueous sodium hydroxide (4 mL). The mixture was then heated at reflux for 2 h. The reaction mixture was acidified with 2M hydrochloric acid (approx. 10 mL) and the resulting solid collected and washed with 15% methanol in dichloromethane to obtain the carboxylic acid (0.18 g) as a white solid. The solid was stirred at 280°C in Dowtherm A for 1 h. Afterwhich the mixture was allowed to cool in the fridge overnight. The solid was filtered and washed with petroleum ether followed by ethyl acetate to give the title compound (0.12 g, 52%) as a white solid: mp 218 – 219°C; ^{1}H NMR (400 MHz, DMSO) δ 11.87 (s, 1H, N-H), 7.90 (dd, *J* = 6.8, 4.2 Hz, 1H), 7.62 (d, *J* = 8.8 Hz, 1H, H-8), 7.53 – 7.38 (m, 4H, H-5 + H-6 + H-3'), 7.19 (t, *J* = 7.4 Hz, 1H, H-4'), 7.07 (d, *J* = 7.7 Hz, 2H, H-2'), 6.01 (d, *J* = 7.3 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 176.49 (C=O), 156.87 (C-1'), 153.32 (C-6), 139.48, 136.55, 130.56 (C-3'), 127.18, 124.50, 124.22 (C-4'), 121.04 (C-8), 119.42 (C-2'), 111.70, 108.16; IR ν_{max} (neat)/cm^{-1} 2921.6, 2854.1, 1591.0,, 1556.3, 1504.2 (C=C) and 1475.28; HRMS (ESI) $C_{15}H_{11}NO_2{}^{23}Na$ $[M+Na]^+$ requires 260.0687, found 260.0690; Anal. $C_{15}H_{11}NO_2$ requires C 75.94%, H 4.67%, N 5.90%, found C 75.66%, 4.84%, N 5.66%.

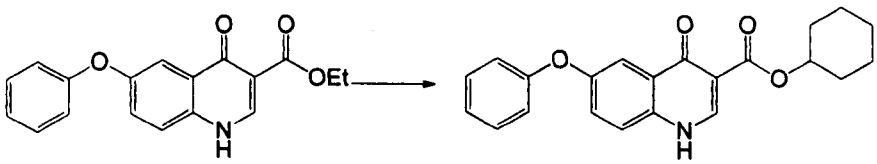
Preparation of **Isopropyl 4-oxo-6-phenoxy-1,4-dihydroquinoline-3-carboxylate 87.**



68 **87**

To a solution of **68** (0.21 g, 0.68 mmol) in propan-2-ol (10 mL) was added 1-2 drops of titanium(IV) isopropoxide. The mixture was heated at reflux overnight. Part of the solvent was removed *in vacuo*. The resulting solid was filtered and washed with hexane and ethyl acetate to give the title compound (0.12 g, 56 %) as a white solid: R_f = 0.51, 5% methanol in dichloromethane; mp 234 – 235°C; ^{1}H NMR (400 MHz, DMSO) δ 12.82 (s, 1H, N-H), 8.51 (s, 1H, H-2), 7.77 (d, *J* = 9.1 Hz, 1H, H-8), 7.59 – 7.37 (m, 4H, H-5 + H-7 + H-3'), 7.32 – 7.18 (m, 1H, H-4'), 7.11 (dt, *J* = 9.0, 1.8 Hz, 2H, H-2'), 5.21 – 4.92 (m, 1H, CH), 1.27 (d, *J* = 6.2 Hz, 6H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 173.32 (C=O), 164.55, 156.43, 154.73, 144.49, 135.15, 130.64 (C-3'), 128.76, 124.57 (C-2'), 121.60, 119.75, 119.15, 112.11, 109.36, 67.11 (CH), 22.14 (2 x CH_3); IR ν_{max} (neat)/cm^{-1} 3095.2, 3037.3, 1693.2 (C=O), 1616.1, 1581.3, 1477.2, 1203.4 (C-O) and 1170.6; HRMS (ESI) $C_{19}H_{17}NO_4{}^{23}Na$ $[M+Na]^+$ requires 346.1055, found 346.1070; Anal. $C_{19}H_{17}NO_4$ requires C 70.58%, H 5.30%, N 4.33%, found C 70.24%, H 5.33%, N 4.30%.

Preparation of **Cyclohexyl 4-oxo-6-phenoxy-1,4-dihydroquinoline-3-carboxylate 88.**

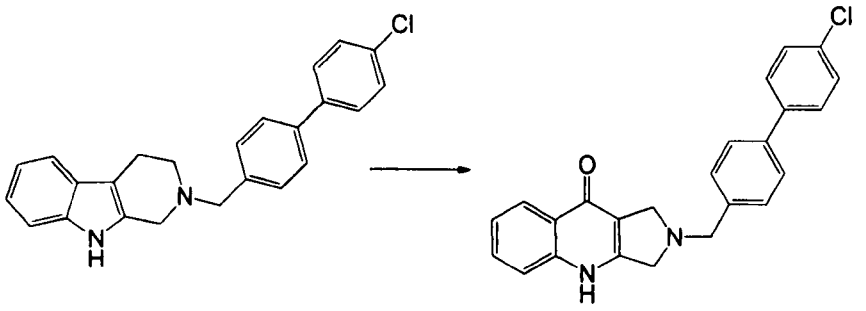


68 **88**

88 was prepared from **68** (0.30 g, 0.99 mmol) and cyclohexanol (50 mL) according to the procedure for the preparation of **87**. Purification by column chromatography using 5% methanol in dichloromethane gave **88** (0.12 g, 35 %) as a white solid: R_f = 0.56, 2% methanol in dichloromethane; mp 248 – 249°C; ^{1}H NMR (400 MHz, DMSO)

δ 12.40 (s, 1H, N-H), 8.52 (s, 1H, H-2), 7.70 (d, *J* = 8.8 Hz, 1H, H-8), 7.49 – 7.42 (m, 4H, H-5 + H-7 + H-3'), 7.26 – 7.20 (m, 1H, H-4'), 7.10 (dt, *J* = 9.0, 1.8 Hz, 2H, H-2'), 4.91 – 4.79 (m, 1H, CH), 1.87 – 1.79 (m, 2H, CH_2), 1.79 – 1.66 (m, 2H, CH_2), 1.60 – 1.25 (m, 6H, CH_2); ^{13}C NMR (101 MHz, DMSO) δ 171.14 (C=O), 162.67, 154.52, 152.71, 142.82, 133.51, 128.70 (C-3'), 126.95, 122.65, 122.60, 119.75, 117.77 (C-2'), 110.28, 107.66, 69.67 (CH), 29.59 (CH_2 x 2), 23.47 (CH_2), 21.49 (CH_2 x 2); IR ν_{max} (neat)/cm^{-1} 2935.1, 2852.2, 1693.2 (C=O), 1618.0, 1589.1, 1479.1, 1201.43 (C-O) and 1170.6; HRMS (ESI) $C_{22}H_{22}NO_4$ $[M+H]^+$ requires 364.1549, found 364.1537 (100%), $C_{22}H_{21}NO_4{}^{23}Na$ $[M+Na]^+$ requires 386.1368, found 386.1355 (79%); Anal. $C_{22}H_{21}NO_4$ requires C 72.71%, H 5.82%, N 3.85%, found C 72.62%, H 5.82%, N 3.80%.

Preparation of **2-((4'-Chlorobiphenyl-4-yl)methyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*] quinolin-9(4*H*)-one 101.**

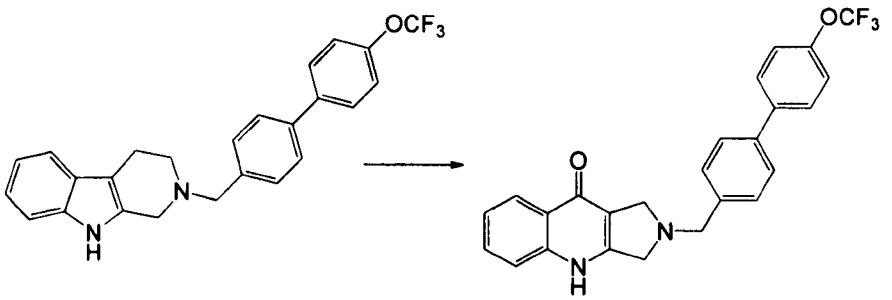


114 **101**

114 (0.12 g, 0.33 mmol) and potassium *tert*-butoxide (0.04 g, 0.37 mmol) were dissolved in DMF (8 mL). A steady stream of oxygen was bubbled through the solution. The reaction was allowed to stir at room temperature overnight (followed by tlc). Water (50 mL) was added and the solution was neutralized using 1M HCl and the yellow precipitate was filtered and the filtrate was extracted with ethyl acetate, washed with brine (x 2). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting solid was combined with the yellow precipitate, washed with dichloromethane and dried to give the title compound (25 mg, 20%) as a yellow solid: mp 238°C; ^1H NMR (400 MHz, MeOD) δ = 8.29 (1H, d, *J* 8.3 Hz, N-H), 7.76 – 7.81 (2H, m, Ar-H), 7.61 – 7.71 (8H, m, Ar'-H), 7.48 – 7.51 (2H, m, Ar-H), 4.90 (2H, s, N-CH_2), 4.76 (2H, s, 8-CH_2), 4.70 (2H, s, 6-CH_2); IR ν_{max} (neat)/cm^{-1} 2977.6,

2935.1, 1677.8 (C=O), 1589.1, 1515.8, 1473.4, 1376.9, 1253.5 and 756.0; HRMS (ESI) $C_{24}H_{20}N_2O^{35}Cl$ $[M+H]^+$ requires 387.1264, found 387.1276 (100).

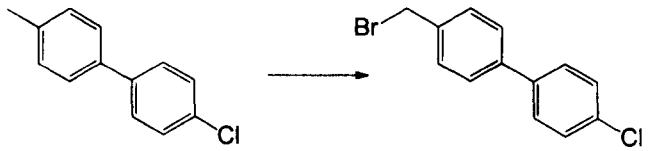
Preparation of **2-((4'-(Trifluoromethoxy)biphenyl-4-yl)methyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*b*]quinolin-9(4*H*)-one 102.**



115 **102**

102 was prepared from **115** (0.46 g, 1.09 mmol) according to the procedure for the preparation of **101**. Filtration of the mixture gave **102** (95 mg, 20 %) as a yellow solid: mp 266°C; ^{1}H NMR (400 MHz, DMSO) δ 12.06 (s, 1H, N-H), 8.11 (dd, *J* = 8.1, 1.2 Hz, 1H, H-5), 7.86 – 7.79 (m, 2H, H-6'), 7.69 (d, *J* = 8.2 Hz, 2H, H-3'), 7.62 (ddd, *J* = 8.4, 7.0, 1.5 Hz, 1H, H-7), 7.53 – 7.48 (m, 3H, H-8 + H-2'), 7.46 (d, *J* = 8.0 Hz, 2H, H-7'), 7.34 – 7.27 (m, 1H, H-6), 4.02 (s, 2H, NCH_2), 3.95 (s, 2H, $NC\underline{H}_2Ar$), 3.78 (s, 2H, NCH_2); ^{13}C NMR (101 MHz, DMSO) δ 172.86 (C=O), 150.80, 140.37, 139.69, 139.12, 137.82, 131.49, 129.54 (C-6'), 128.85 (C-2'), 127.18 (C-3'), 125.78, 125.26, 123.19, 121.84 (C-7'), 118.57, 116.64, 59.02 (CH_2), 57.43 (CH_2), 55.66 (CH_2); IR ν_{max} (neat)/cm^{-1} 2977.6, 2937.1, 1679.7 (C=O), 1591.0, 1492.6, 1473.4, 1376.9 and 1253.5; HRMS (ESI) $C_{25}H_{20}N_2O_2F_3$ $[M+H]^+$ requires 437.1477, found 437.1497 (100); Anal. $C_{25}H_{19}N_2O_2F_3$ requires C 68.80%, H 4.79%, N 6.42%, found C 68.48%, H 4.47%, N 6.25%.

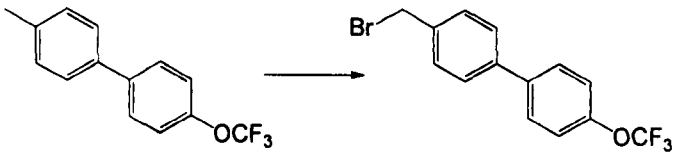
Preparation of **4-(Bromomethyl)-4'-chlorobiphenyl 105.**



110 **105**

110 (0.21 g, 1.01 mmol) was dissolved in anhydrous benzene (10 mL) and the system was flushed with nitrogen. 2,2'-Azobis(isobutyronitrile) (0.04 g, 0.26 mmol, 0.25 equiv) and *N*-bromosuccinimide (0.22 g, 1.23 mmol, 2 equiv) were added. The mixture was allowed to stir at reflux temperature overnight until all biphenyl was consumed (followed by tlc). The mixture was cooled to room temperature and the white solid was filtered. The filtrate was evaporated to give a crude product which was purified by flash column chromatography using pure hexane to give the title compound (0.28 g, 98 %) as a white solid: R_f = 0.38, hexane; mp 90°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.39 – 7.63 (8H, m, Ar-H, Ar'-H), 4.54 (2H, s, CH_2); ^{13}C NMR (100MHz, $CDCl_3$) δ = 140.5, 139.3, 137.6, 134.1, 130.0, 129.4, 128.7, 127.8, 33.6; MS (CI) $C_{13}H_{11}ClBr$ $[M+H]^+$ m/z 282; Anal. $C_{13}H_{10}ClBr$ requires C 55.45%, H 3.58%, found C 55.19%, H 3.44%.

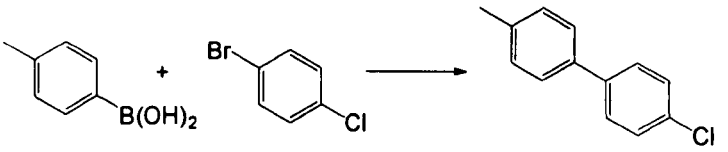
Preparation of **4-(bromomethyl)-4'-(trifluoromethoxy)biphenyl 106.**



111 **106**

106 was prepared from **111** (0.14 g, 0.57 mmol) according to the procedure for the preparation of **105**. Purification by flash column chromatography using 2% ethyl acetate in hexane gave **106** (0.16 g, 87 %) as a white solid: R_f = 0.44, 4% Ethyl acetate in hexane; mp 100°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.47 – 7.61 (6H, m, Ar-H, Ar'-H), 7.29 (2H, d, J 7.7 Hz, H-3', H-5'), 4.55 (2H, s, CH_2); ^{13}C NMR (100MHz, $CDCl_3$) δ = 149.4, 140.4, 139.6, 137.7, 130.0, 128.9, 127.9, 121.7, 33.5; HRMS (CI) $C_{14}H_{11}OF_3Br$ $[M]^+$ requires 329.98671, found 329.98690; Anal. $C_{14}H_{11}OF_3Br$ requires C 50.74%, H 3.04%, found C 50.59%, H 3.08%.

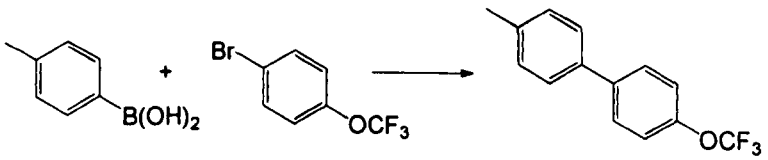
Preparation of **4-Chloro-4'-methylbiphenyl 110.**



109 **107** **110**

1-Bromo-4-chlorobenzene **107** (0.41 g, 2.12 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (0.08 g, 0.11 mmol, 0.05 equiv) and potassium phosphate tribasic (1.82 g, 8.56 mmol, 4 equiv) were placed in an oven-dried round-bottomed flask. Anhydrous 1,4-dioxane (20 mL) was added and the resulting yellow mixture was stirred. Vacuum and nitrogen cycles were applied three times. The mixture was allowed to stir under nitrogen environment for 10 min at room temperature. *p*-Tolylboronic acid (0.45 g, 3.27 mmol, 1.5 equiv) was added. Vacuum/N_2 cycles were applied again (x 10). The resulting mixture was heated at 100°C for 2 h (followed by tlc). Afterwhich it was cooled to room temperature and filtered through a pad of $MgSO_4$ and silica gel. The pad was further washed with 15% ethyl acetate in hexane (100 mL) and the filtrate was evaporated and the crude product was purified by flash column chromatography to give **110** (0.37 g, 87 %) as a white solid: R_f = 0.42, hexane; mp 121 – 122°C (Lit.[57] 122°C); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.24 – 7.51 (8H, m, ArH), 2.39 (3H, s, CH_3); ^{13}C NMR (100MHz, $CDCl_3$) δ = 140.0, 137.8, 137.5, 133.4, 130.0, 129.3, 128.6, 127.2, 21.5; HRMS (CI) $C_{13}H_{11}Cl$ $[M]^+$ requires 202.05493, found 202.05555; Anal. $C_{13}H_{11}Cl$ requires C 77.04%, H 5.47%, found C 77.03%, H 5.45%.

Preparation of **4-Methyl-4'-(trifluoromethoxy)biphenyl 111.**

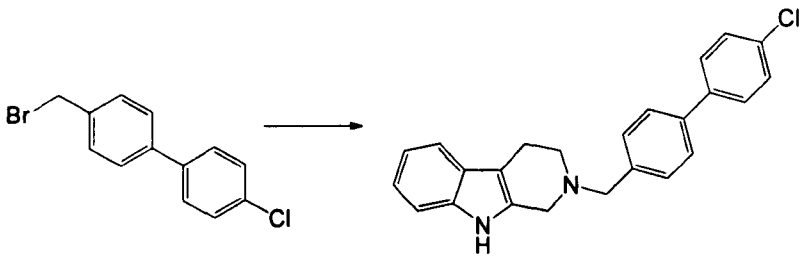


109 **108** **111**

111 was prepared from 1-bromo-4-(trifluoromethoxy)benzene (0.25 g, 1.05 mmol), and *p*-tolylboronic acid (0.21 g, 1.57 mmol, 1.5 equiv) according to the procedure for the preparation of **110**. Purification by flash column chromatography using

hexane gave **111** (0.25 g, 93%) as a white solid: R_f = 0.5, hexane; mp 100°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.25 – 7.59 (8H, m, ArH), 2.40 (3H, s, CH_3); ^{13}C NMR (100MHz, $CDCl_3$) δ = 137.9, 137.3, 130.0, 128.6, 127.3, 121.6, 21.2; MS (CI+) $[M]^+$ m/z 252(100), HRMS (CI) $C_{14}H_{11}OF_3$ $[M]^+$ requires 252.07620, found 252.07648; Anal. $C_{14}H_{11}OF_3$ requires C 66.67%, H 4.39%, found C 66.62%, H 4.38%.

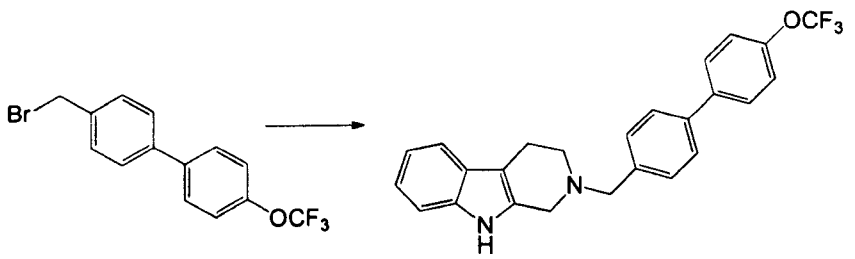
Preparation of **2-((4'-Chlorobiphenyl-4-yl)methyl)-1,2,3,4-tetrahydro-9*H*-pyrido [3,4-*b*]indole 114.**



105 **114**

1,2,3,4-Tetrahydro-9*H*-pyrido[3,4-*b*]indole (0.13 g, 0.78 mmol) was dissolved in anhydrous tetrahydrofuran (10 mL) at 0°C under nitrogen environment. Triethylamine (0.20 mL, 1.48 mmol, 2 equiv) was added dropwise, and the resulting solution was left to warm to room temperature over 1 h. **105** (0.18 g, 0.65 mmol) was then added and left to stir until all of the bromide was consumed (followed by tlc). The solvent was then evaporated, the crude product was purified by flash column chromatography using 15% ethyl acetate in hexane to give the title compound (0.16 g, 68%) as a yellow solid: R_f = 0.40, 40% ethyl acetate in hexane; mp 104 – 107°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.67 (1H, br. s, N-H), 7.49 – 7.63 (8H, m, Ar'-H), 7.28 – 7.45 (2H, m, Ar-H), 7.10 – 7.18 (2H, m, Ar-H), 3.84 (2H, s, N-CH_2), 3.72 (2H, s, 8-CH_2), 2.97 (2H, t, J 5.6 Hz, 5-CH_2), 2.87 (2H, t, J 5.4 Hz, 6-CH_2); ^{13}C NMR ($CDCl_3$, 100MHz) δ = 139.8, 139.3, 138.4, 136.4, 133.7, 132.2, 130.0, 129.3, 128.7, 127.7, 127.3, 121.8, 119.8, 118.4, 111.1, 108.9, 62.0, 51.4, 50.7, 21.6; HRMS (ESI) $C_{24}H_{22}N_2{}^{35}Cl$ $[M+H]^+$ requires 373.1472, found 373.1480 (100); Anal. $C_{24}H_{21}N_2Cl$ requires C 77.30%, H 5.68%, N 7.51%, found C 77.27%, H 5.67%, N 7.47%.

Preparation of **2-((4'-(Trifluoromethoxy)biphenyl-4-yl)methyl)-1,2,3,4-tetrahydro -9*H*-pyrido[3,4-*b*]indole 115.**



106 **115**

115 was prepared from **106** (0.38 g, 2.17 mmol) according to the procedure for the preparation of **114**. Purification by flash column chromatography using 30% ethyl acetate in hexane gave **115** (0.54 g, 70 %) as a yellow solid: R_f = 0.22, 30% ethyl acetate in hexane; mp 182°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.67 (1H, br. s, N-H), 7.47 – 7.64 (8H, m, Ar'-H), 7.26 – 7.30 (2H, m, Ar-H), 7.07 – 7.15 (2H, m, Ar-H), 3.83 (2H, s, N-CH_2), 3.72 (2H, s, 8-CH_2), 2.95 (2H, t, J 5.7 Hz, 5-CH_2), 2.85 (2H, t, J 5.4 Hz, 6-CH_2); ^{13}C NMR ($CDCl_3$, 100MHz) δ = 136.4, 132.2, 130.0, 128.8, 127.7, 127.5, 121.8, 121.7, 119.8, 118.4, 111.1, 62.0, 51.4, 50.7, 21.6; HRMS (ESI) $C_{25}H_{22}F_3N_2O$ $[M+H]^+$ requires 423.1684, found 423.1686 (100); Anal. $C_{25}H_{21}F_3N_2O$ requires C 71.08%, H 5.01%, N 6.63%, found C 71.21%, H 5.05%, N 6.59%.

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Chapter 4

Modular Synthesis and *In Vitro* and *In Vivo* Antimalarial Assessment of C-10 Pyrrole Mannich Base Derivatives of Artemisinin

Chapter 4

Modular Synthesis and in Vitro and in Vivo Antimalarial Assessment of C-10 Pyrrole Mannich Base Derivatives of Artemisinin[1]

[This chapter is represented by a joint-published paper in Journal of Medicinal Chemistry (2010, 53, 633-640). A series of semisynthetic C-10 pyrrole Mannich artemisinin derivatives (**7a-g**, **7j-m**, **7o**, **8** and **9**) were synthesised. Other analogues in the paper were prepared by Dr Bénédicte Pacorel while the biological work was performed by other authors.]

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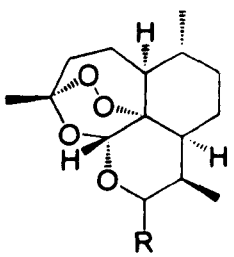
Abstract

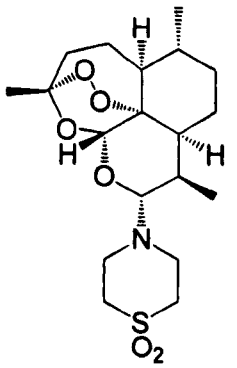
In two steps from dihydroartemisinin, a smally array of 16 semisynthetic C-10 pyrrole Mannich artemisinin derivatives (**7a – p**) have been prepared in moderate to excellent yield. *In vitro* analysis against both chloroquine sensitive and resistant strains has demonstrated that these analogues have nanomolar antimalarial activity, with several compounds being more than 3 times more potent than the natural product artemisinin. In addition to a potent antimalarial profile, these molecules also have very high *in vitro* therapeutic indices. Analysis of the optimal Mannich side chain substitution for *in vitro* and *in vivo* activity reveals that the morpholine and *N*-methylpiperazine Mannich side chains provide analogues with the best activity profiles, both *in vitro* and *in vivo* in the Peters' 4 day test.

Introduction

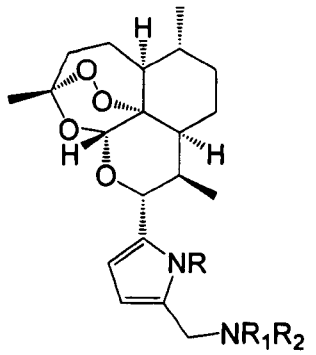
The therapeutic value of artemisinin (**1**), *qinghaosu*, is limited to a great extent by its low solubility in both oil and water. Therefore a number of more soluble derivatives have been developed, such as DHA (**2**), artemether (**3**),[2] arteether (**4**)[3] and sodium artesunate (**5**)[4-5] (**Figure 1**). Although artemisinins (**1-5**) are the most efficient and fast acting antimalarial drugs used in malaria chemotherapy the current first generation semi-synthetics (**2-5**) are cleared from blood within 2 hours and parasites that are not eliminated within this time can re-emerge resulting in parasite recrudescence. To prevent recrudescence,[6] the artemisinins are used in combination therapies with drugs that have longer half lives (eg amodiaquine, mefloquine, piperaquine and lumefantrine).[7-10]



Artemisinin **1**, R = =O
Dihydroartemisinin **2**, R = OH
Artemether **3**, R = β-OMe
Arteether **4**, R = β-OEt
Artesunate **5**, R = α-OC(O)$CH_2CH_2CO_2Na$



Artemisone **6**



C-10-α-Pyrrole Template **7**

Figure 1. Artemisinin and Semi-synthetic Analogues.

To treat advanced cases of *Plasmodium falciparum* malaria, a water-soluble derivative of artemisinin is required, which can be delivered quickly by intramuscular injection.[4] The water-soluble sodium artesunate is currently the drug of choice[11] and is administered in combination therapy most often with mefloquine.[12]

In terms of semi-synthetic analogues, the major challenge in this field is to prepare analogues from DHA in only one or two high yield steps to provide analogues with a log *P* of < 4 (ideally 3.25 – 3.75) and enhanced metabolic and chemical stability compared to artesunate (**5**) or artemether (**3**).[13] Artemisone (**6**) is a molecule that fulfils both of these criteria available in only three steps from DHA, an excellent *in vitro* and *in vivo* antimalarial activity profile with enhanced metabolic stability with acceptable exposure profiles and half-life following oral administration.[14]

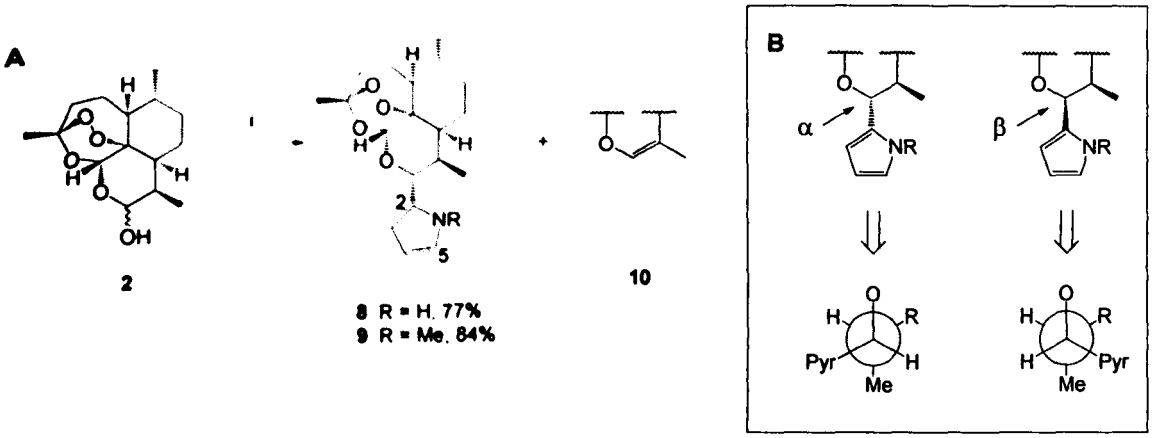
In this paper, we describe a modular approach to new, more water-soluble analogues of DHA based on the C-10 pyrrole template (**7**). Several "mechanism-based approaches" already have been investigated for improving the antimalarial activity of artemisinin-based trioxane derivatives; these include the incorporation of groups to enhance the stability of proposed "parasiticidal intermediates" [15] and the covalent attachment of "iron chelator functionality"[16] to enhance the interaction of the peroxide bridge with available "chelatable iron"[17] in the food vacuole of the parasite. From our earlier work, we proposed that the incorporation of protonatable amino functionality into an artemisinin derivative would enhance drug activity by increasing the level of cellular accumulation within the acidic (pH 4.7) parasite food vacuole by "ion trapping".[18] The higher concentration of drug available for interaction with heme or non-heme iron may have been responsible for the increased antimalarial activity observed in this work. The aim of this work was to prepare C-10 pyrrole analogues with alkylamine side chains. The C-10 pyrrole analogues were chosen for the following reasons: (i) C-10-aryl artemisinins or C-10 heteroaryl systems cannot generate DHA by hydrolysis or metabolism. (ii) The Mannich side chain provides molecules that have the potential to be formulated as salts, and as discussed, amine analogues should accumulate to higher concentrations than nonbasic derivatives such as artemether by an ion trapping mechanism.

Results and Discussion

Chemistry

Three different approaches were explored for the synthesis. First, C-10 analogues **8** and **9** were synthesized directly from **2** in the presence of a Lewis acid using the pyrrole and *N*-methylpyrrole (Scheme 1A). This particular heterocycle was chosen as the electron rich ring provides scope for the introduction of electrophiles at the C-5 position following incorporation into the artemisinin scaffold at the C-2 position.

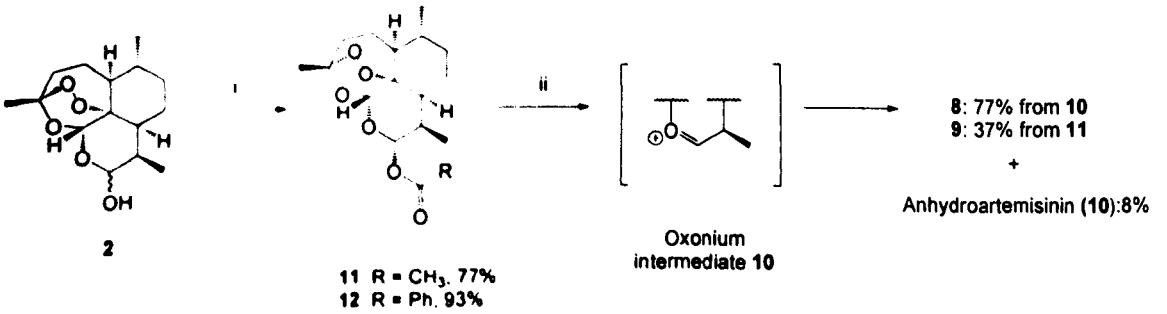
Scheme 1. (A) Synthesis of 8-10[a] and (B) Newan Projections of C-10α and C-10β Pyrrole Analogues.



[a] *Reagents and conditions*: (i) $BF_3{\cdot}Et_2O$, pyrrole or *N*-methylpyrrole, CH_2Cl_2, −50 °C, 30 min. In the synthesis of both **8** and **9**, 15 and 10% of the side product anydroartemisinin (**10**), respectively, was produced.

The stereochemistry at the C-10 position was determined by ^1H NMR spectroscopy. The signal due to H10 appears as a doublet at 4.49 ppm with a $^3J_{H10\text{-}H9}$ value of 10.8 Hz, which is indicative of a *trans-trans* diaxial relationship between H10 and H9 (**Scheme 1 B**).[19] This stereochemistry results from the bulky pyrrole nucleophile attacking the oxonium intermediate in an equatorial manner to minimise steric interaction with the C-9 β-methyl group. We also examined the synthesis of **8** via acetate **11** formed by treatment of DHA with acetic anhydride in DMAP.[20] $BF_3.Et_2O$-catalysed reaction of **11** with the pyrrole led to the formation of the product **8** (via oxonium **13**) in good yield

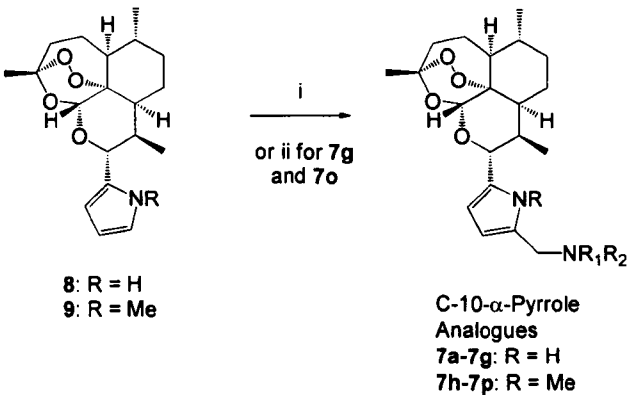
with minor amounts of side product **10** as shown (**Scheme 2**). Our observations are in accordance with the results obtained by the Posner group where a C-10β anomeric fluoride was employed in this Friedel-Crafts type chemistry.[21] In their approach *N*-methylpyrrole was produced in 84% yield. We have also previously used the C-10 anomeric benzoate as leaving group in the synthesis of C-10 carba analogues of artemisinin.[22] Thus, we also examined the reactivity of the benzoate **12** prepared from benzoyl chloride, dihydroartemisinin and pyridine as the nucleophilic catalyst.[20] In our hands, **12** proved to be a worse substrate for this reaction with a lower yield for the target molecule **8**. It can be concluded that there is no benefit from synthesis via C-10α acetate or C-10α benzoate derivatives as the overall yields were not as good as that for the direct route from **2** (77% yield).



Scheme 2. *Reagents and conditions*: i) DMAP, pyridine, acetic anhydride or benzoyl chloride, DCM; ii) pyrrole, $BF_3.Et_2O$, DCM, -50°C, 30 mins.

Having developed a high-yielding route to **8** and **9**, we decided to employ the Mannich reaction to incorporate the requisite amino alkyl side chain (**Scheme 3**). As noted the amino functionality in these analogues and expected enhanced water solubility, we anticipated improved *in vitro* and *in vivo* antimalarial activities based on the observations made with other semisynthetic amino alkyl artemisinin derivatives.[14, 20, 23] We chose to conduct this reaction on the pyrrole ring under acidic conditions using formaldehyde and several secondary amines. The reaction was allowed to proceed by sequentially dissolving **8** or **9** at room temperature in ethanol, followed by addition of secondary amine (3.2 equiv.), formaldehyde (3.2 equiv.) and acetic acid (1.0 mL in 5.0 mL EtOH). The reaction mixture was then left for 0.5 hour and the reaction quenched

with sodium hydroxide. The crude product was extracted with dichloromethane and the organic phase washed with brine.



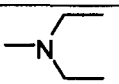
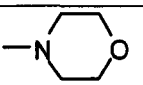
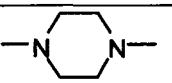
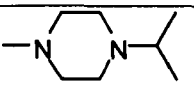
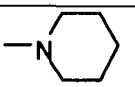
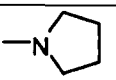
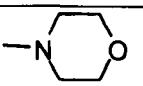
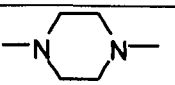
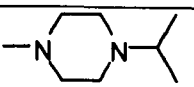
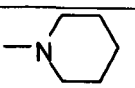
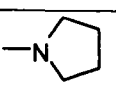
8: R = H
9: R = Me

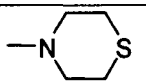
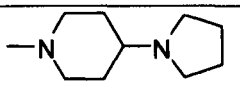
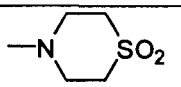
C-10-α-Pyrrole
Analogues
7a-7g: R = H
7h-7p: R = Me

Scheme 3. *Reagents and conditions*: i) CH_2O, secondary amine, AcOH, EtOH, r.t., 30 mins ii) $[CH_2N(CH_3)_2]^+I^-$, acetonitrile, r.t., 24 hrs.

The conditions described above were used to prepare **7a–g** (purified by flash column chromatography) from **8** in acceptable yields (Table 1). The Mannich reactions gave lower yields with amines diethylamine, piperidine, and pyrrolidine. Similarly, **7h–p** were synthesized from **9** in very good yields (Table 1). The higher yields are probably explained by the fact that *N*-methylpyrrole **9** is slightly more electron rich than its N-H counterpart **8**. Repetition of the Mannich reaction with commercial Eschenmoser's salt (Scheme 3) gave **7g** and **7o** in 70 and 86% yields, respectively. As one can see from Table 1, a range of ClogP values are encompassed within this array from 2.89 for **7p** to 4.53 for **7k**.

Table 1: Semi-synthetic artemisinins prepared

Compound	Structure -R	Structure –R'	Clog P[a]	Yield (%)
8	-H	-	3.53	77
9	-Me	-	3.77	84
7a	-H		4.13	24
7b	-H		3.01	70
7c	-H		3.00	60
7d	-H		3.80	70
7e	-H		4.24	35
7f	-H		3.83	24
7g	-H		3.40	70
7h	-Me		3.33	75
7i	-Me		3.38	83
7j	-Me		4.12	76
7k	-Me		4.53	88
7l	-Me		4.14	97

7m	-Me		4.22	90
7n	-Me		4.46	54
7o	-Me		3.98	86
7p	-Me		2.89	35

[a] ClogP values were calculated using the AlogPS 2.1 program. [24]

Biology

The antimalarial activity of the Mannich-based artemisinin derivatives was first evaluated *in vitro* against the chloroquine sensitive 3D7 strain of *P. falciparum*, and then selected compounds were examined against the chloroquine resistant K1 isolate with artemisinin and chloroquine as positive controls (Tables 2 and 3). For the Mannich analogues derived from **8**, in vitro testing versus the 3D7 strain revealed that the most potent analogue was morpholine derivative **7b** which was almost 5 times more potent than artemisinin. This molecule also had an excellent *in vitro* therapeutic index with an IC_{50} in the high micromolar region versus the control KB mammalian cell line. Two additional parent pyrrole analogues had slightly better potencies than artemisinin against 3D7, **7c** and **7d**. Surprisingly, compounds **7a**, **7e**, and **7g** had higher-than-anticipated IC_{50} values, and analysis of the testing ethanol stock solution by TLC revealed some decomposition of the samples with the appearance of several minor impurities. For this reason, these compounds were not selected further for biological evaluation; the remaining compounds (derived from *N*-methylpyrrole **9**) were shown to be stable in ethanol for 1 week at room temperature. For the *N*-methyl series, the compounds were all more potent than artemisinin and more stable apart from trioxane **7o** which was found to have poor stability in ethanol and other protic solvents.

Selected compounds from both series were then assessed against the K1 strain. The best compounds from each series were morpholine derivatives **7b** and **7h**; the latter compound has an IC_{50} of 1.9, making it nearly 4 times more potent than artemisinin and 2 times as active as artemether. As a series, it is clear that these molecules have a very high *in vitro* therapeutic window with values ranging from 2000 to 25000.

Table 2. *In vitro* antimalarial activity of Pyrrole Artemisinin Derivatives versus Chloroquine Sensitive 3D7 *P. falciparum.* [a]

Compound	IC_{50} (nM) for 3D7	IC_{50} (μM) for KB	TI (KB/3D7)	relative IC_{50} for artemisinin /analogue IC_{50}
Artemisinin	12.8	>354	>27656	1.00
Artemether	4.2	ND[c]	ND[c]	ND[c]
Chloroquine	29.6	112.5	3784	0.43
8	7.4	62.5	8446	1.73
9	5.2	60.3	11596	2.46
7a	75.8[b]	ND[c]	ND[c]	0.17
7b	2.5	56.5	22600	2.84
7c	6.5	85.2	13108	1.97
7d	8.5	45.3	5329	1.51
7e	59.4[b]	143.4	2414	0.22
7f	16.9	47.0	2781	0.76
7g	20.5[b]	43.6	2127	0.62
7h	3.5	86.2	24628	3.66
7i	6.5	96.5	14846	1.97
7j	5.1	ND[c]	ND[c]	2.51
7k	6.1	39.2	6426	2.10
7l	3.7	42.2	11405	3.46
7m	7.2	137.8	19139	1.78
7n	2.5	ND[c]	ND[c]	5.12
7o	31.6[b]	ND[c]	ND[c]	0.40
7p	2.2	68.32	31054	5.82

[a] Chloroquine was tested as the diphosphate salt. All other compounds were all tested as free bases. 3D7 is a chloroquine sensitive strains of *P. falciparum* [b] IC_{50} values may underestimate potency due to compound degradation. [c] Not determined.

Table 3. *In vitro* Antimalarial Activity of Pyrrole Mannich Analogues versus Chloroquine Resistant K1[a] *P. falciparum.*

Compound	IC_{50} (nM) for K1	relative IC_{50} for artemisinin/analogue IC_{50}
Artemisinin	8.2	1.00
Artemether	3.4	2.41
Chloroquine[b]	150.2	0.05
7b	3.2	2.56
7c	5.0	1.64
7d	3.5	2.34
7h	1.9	4.32
7i	3.8	2.16
7j	8.3	0.99
7n	7.3	1.12
7p	3.57	2.30

[a] K1 is a chloroquine resistant strain of *P. falciparum.*

[b] Chloroquine was tested as the diphosphate salt. All other compounds were tested as free bases.

On the basis of the chemical yield and *in vitro* performance, a selection of the compounds was screened for their *in vivo* activity against *Plasmodium berghei.* First, Peter's 4 day suppressive test was performed using 30 mg/kg over days 1–3 post-infection (Table 4). All the compounds tested displayed good activity, with **7b**, **7h**, and **7i** achieving 100% elimination of parasites according to this protocol.

Table 4. Results of Peters' 4 Day Suppressive Test at a dose of 3 x 30 mg/kg.

Compounds	% inhibition for 30 mg/kg
Artemether (**3**)	100
Artesunate (**5**)	100
7c	97.7
7d	90.5
7h	100
7i	100

Encouraged by the experiments, we conducted dose–response experiments with compounds **7b**, **7h**, and **7i** to determine ED_{50} and ED_{90} values which are definitive measurements of reduction of parasitemia by 50 and 90%, respectively, following oral dosing. All three compounds clearly outperformed the water-soluble sodium artesunate and the oil-soluble control trioxane artemether (Table 5). Data are also included for the activity of **9** from the previous work of Posner and co-workers.[21]

Table 5 *In Vivo* Antimalarial Activities versus *P. berghei* N Strain

Endoperoxide	ED_{50} mg/kg	ED_{90} mg/kg
7b	2.11	4.3
7h	1.77	5.20
7i	1.99	5.34
9	4.50	8.50
Artemether	5.88	10.57
Artesunate	3.23	>10

Finally, given the potential to formulate the piperazine analogues such as **7i**, some additional *in vivo* work was conducted at the Swiss Tropical Institute, and the data are compiled in Table 6. In these experiments, groups of three female NMRI mice (20–22 g) were intravenously infected with parasitized erythrocytes (2×10^7) on day 0 with GFP-transfected *P. berghei* strain ANKA.[25] The three Mannich derivatives were formulated in a standard suspending vehicle (SSV) and were administered orally on four consecutive days (4, 24, 48 and 72 h post-infection). Parasitemia was determined on day 4 post-infection (24h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean percent parasitaemia for the control (n = 5 mice) and treated groups expressed as a percent relative to the control group.

Table 6 *In Vivo* Antimalarial Activities versus the *P. berghei ANKA Strain.*

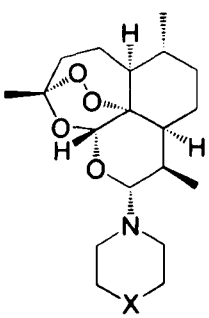
Endoperoxide	% Inhibition [a] (10 mg/kg x 4)	MSD [b]
7c	98	7
7d	90	6
7i	100	9
Artesunate	98	8

[a] Parasitemia was determined on day 4 post-infection (24h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean percent parasitaemia for the control (n = 5 mice) and treated groups expressed as a percent relative to the control group

[b] The mean survival time in days (MSD) is recorded up to a maximum of 30 days after infection. A compound was considered curative if the animal survived to day 30 after infection with no detectable parasites.

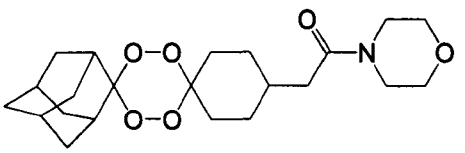
In this assay, **7i** was the most potent, achieving 100% clearance of parasitemia 24 h after the last treatment and extending mouse survival to 9 days compared with 8 days for the artesunate control.

In terms of SAR within this series of molecules, it is clear that the morpholine, *N*-methylpiperazine, and sulfonylmorpholine heterocyles provide molecules with improved efficacy both *in vitro* and *in vivo* (for morpholine and *N*-methylpiperazine). These heterocycles also appear in some of the most potent semisynthetic analogues prepared to data Figure 2 (see 6, 14 and 17) and in totally synthetic tetraoxanes [26] being developed at Liverpool Figure 2 (see 15 and 16).

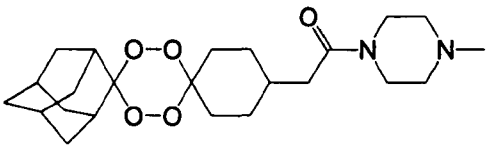


6: X = SO_2
ED_{90} = 1.3 mg/kg

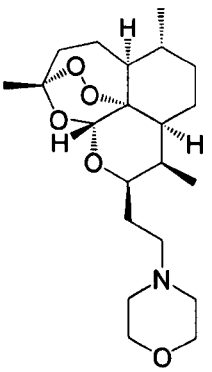
14: X = O
ED_{90} = 3.10 mg/kg



15 (RKA216)
IC_{50} (3D7 Strain) = 5.2 nM
ED_{50} = 3.18 mg/kg
ED_{90} = 3.88 mg/kg



16 (RKA071)
IC_{50} (3D7 Strain) = 0.5 nM
ED_{50} = 2.70 ± 0.1 mg/kg



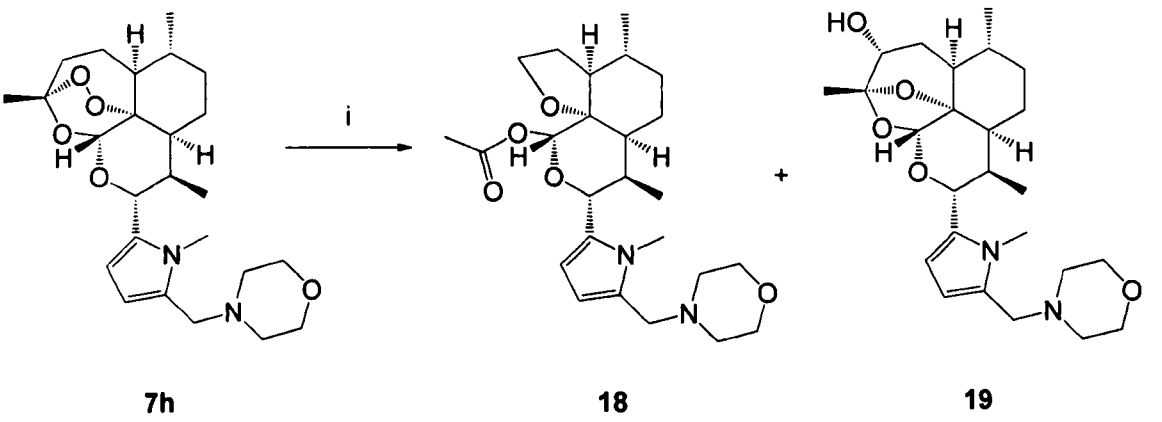
17
IC_{50} (K1 Strain) = 4.2 nM

Figure 2. Potent Semi-synthetic and synthetic endoperoxides containing the morpholine,[13, 23] sulfonylmorpholine [13] and piperazine side chains. [18, 23] Key data from original publications are included; ED_{50} and ED_{90} are for oral dosing in mice infected with *P. berghei*.

Artemisinins act via mechanisms that are distinct from those of other antimalarial classes. Antimalarial activity may arise from alkylation of vital intraparasitic biomolecules by free radicals generated within the malaria parasite through an

iron(II)-induced degradation process.[27-29] The parasite death that ensues in the presence artemisinin is more likely to involve specific radicals and targets rather than nonspecific cell damage caused by freely diffusing oxygen- and carbon-centered radical species.

To determine the impact of the polar Mannich side chain on the iron-mediated degradation process, we examined the ferrous iron-mediated degradation of **7h** with iron(II) sulfate and iron(II) chloride. As anticipated, iron(II)-mediated degradation of **7h** gave two main products as shown in Scheme 4. Isolation of **18** and **19**, surrogate markers of primary and secondary C radicals, indicates that carbon-centered radical intermediates can be readily produced during iron-dependent cleavage of morpholine **7h** and that these species may play a role in the mechanism of action of these semisynthetic artemisinins (Table 7).



7h **18** **19**

Scheme 4. *Reagents and conditions*: i) $FeSO_4$, 1/1 acetonitrile/water, 1 hr, r.t. or $FeCl_2.4H_2O$, acetonitrile, 30 min, r.t.

Table 7.

Iron (II)	Yield for **18** (%)	Yield for **19** (%)
$FeSO_4.6H_2O$	72	21
$FeCl_2\,4H_2O$	42	23

Summary

In summary, a small library of weak base and polar C-10 pyrrole analogues has been prepared with modular chemistry amenable to parallel synthesis methods. The in vitro antimalarial results have revealed that morpholine **7h** and *N*-methylpiperazine **7i** analogues have biological profiles superior to those of clinically used sodium artesunate. The *in vivo* profiles of **7h** and **7i** warrant further investigation, including combination and pharmacokinetic and preclinical toxicological evaluation to fully assess the potential of these compounds. Initial studies employing ferrous(II) salts indicate that members of this class of semisynthetic artemisinin generate both primary and secondary carbon-centered radicals in a manner similar to that of artemisinin. Further work is required to establish the role of these intermediates in the mechanism of action.

Experimental section

Chemistry

Air- and moisture-sensitive reactions were conducted in oven-dried glassware sealed with rubber septa under a positive pressure of dry nitrogen or argon from a manifold or balloon. Similarly sensitive liquids and solutions were transferred via syringe. Reaction mixtures were stirred using Teflon-coated magnetic stir bars. Organic solutions were concentrated using a Buchi rotary evaporator with a diaphragm vacuum pump.

(i) Purification of Reagents and Solvents. Anhydrous solvents were either obtained from commercial sources or dried and distilled immediately prior to use under a constant flow of dry nitrogen. DCM was distilled from CaH_2. All other reagents were used as received from commercial sources unless otherwise indicated.

(ii) Purification of Products. Analytical thin layer chromatography was performed with 0.25 mm silica gel 60F plates with 254 nm fluorescent indicator-coated aluminum sheets from Merck. Plates were visualized with ultraviolet light or by treatment with iodine, *p*-anisaldehyde, ninhydrin, or potassium permanganate followed by gentle heating. Chromatographic purification of products was accomplished by flash chromatography, as described by Still and co-workers.[30]

(iii) Analysis. Melting points were determined in open tubes in a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were recorded on Bruker AC 200 (^1H, 200 MHz) and Bruker AMX 400 (^1H, 400 MHz; ^{13}C, 100 MHz) spectrometers. Chemical shifts are described in parts per million downfield from an internal standard of trimethylsilane. Multiplicities are recorded as broad peaks (br), singlets (s), doublets (d), triplets (t), quartets (q), doublets of doublets (dd), doublets of triplets (dt), and multiplets (m). Coupling values are in hertz. Mass spectra were recorded on a VG analytical 7070E machine and Frisons TRIO spectrometers using electron ionization (EI), chemical ionization (CI), or electron spray (ES). Infrared spectra were recorded on a PerkinElmer RX1 FT-IR spectrometer and are reported in wavenumbers (cm^{-1}). Microanalyses (%C, %H, %N) were performed in the University of Liverpool Microanalysis laboratory. Reported atomic percentages are within error limits of

±0.4%. The artemisinin numbering scheme used by Oh was employed throughout this analysis.[31]

10α-(1*H*-Pyrrol-2-yl)artemisinin (8)

A solution of DHA (**2**) (250 mg, 0.88 mmol) in DCM (15 mL) was treated sequentially with pyrrole (295 mg, 4.40 mmol) and $BF_3{\cdot}Et_2O$ (187 mg, 1.32 mmol) and stirred at −50 °C for 1 h. The reaction was quenched with saturated $NaHCO_3$, and the mixture was extracted with DCM, washed with brine, dried with $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography (10% EtOAc/*n*-hexanes) to give a clear oil (125 mg, 77%): R_f = 0.36 (25% EtOAc/*n*-hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.64 (1H, s, NH), 6.76 (1H, dd, *J* = 2.5, 1.6, N-CH), 6.07 (1H, dd, *J* = 2.5, 5.5, pyr CH), 6.03 (1H, m, pyr CH), 5.40 (1H, s, H12), 4.49 (1H, d, *J* = 10.8, H10), 2.57 (1H, m, H9), 2.39 (1H, dt, *J* = 13.7, 4.1, H4α), 1.42 (3H, s, H14), 2.08–1.20 (10H, m), 0.97 (3H, d, *J* = 6.3, H15), 0.63 (3H, d, *J* = 7.1, H16); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.2, 117.7, 107.39, 106.8, 104.3, 92.1, 80.7, 72.1, 51.9, 45.9, 37.4, 36.4, 34.2, 33.1, 31.6, 26.1, 24.8, 22.6, 21.3, 20.3, 14.1, 14.0; HRMS calcd for $C_{19}H_{28}NO_4$ $[M + H]^+$ 334.2018, found 334.2012. Anal. ($C_{19}H_{27}NO_4$) C, H, N.

10α-(1-Methyl-pyrrol-2-yl)artemisinin (9)

A solution of dihydroartemisinin (300 mg, 1.05 mmol) in DCM (25 mL) at room temperature was treated sequentially with *N*-methylpyrrole (0.47 mL, 5.29 mmol) and $BF_3{\cdot}Et_2O$ (0.19 mL, 1.51 mmol), stirred for 10 min at rt, and then cooled at −50 °C for 20 min. The reaction was quenched with saturated $NaHCO_3$, and the mixture was extracted with DCM, washed with brine, dried with $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography (10% EtOAc/*n*-hexanes) to give a colorless crystal (378 mg, 84%): mp 95 °C; R_f = 0.42 in 25% EtOAc/*n*-hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.54 (1H, t, *J* = 2.2, N-CH), 5.90 (2H, m, pyr CH), 5.38 (1H, s, H12), 4.50 (1H, d, *J* = 11.3, H10), 3.84 (3H, s, N-CH_3), 2.83 (1H, m, H9), 2.39 (1H, dt, *J* = 14.0, 4.1, H4α), 1.39 (3H, s, H14), 2.08–1.20 (10H, m), 0.98 (3H, d, *J* = 6.3, H15), 0.61 (3H, d, *J* = 7.2 Hz, H16); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.2, 124.2, 109.9, 106.6, 104.6, 92.3, 81.1, 72.9, 52.4, 46.3, 37.8, 36.7, 35.4, 34.6, 31.3, 26.4, 25.2, 21.3, 20.7, 14.8; HRMS calcd for $C_{20}H_{30}NO_4$ $[M + H]^+$ 348.2175, found 348.2174. Anal. ($C_{20}H_{29}NO_4$) C, H, N.

General Procedure for 7a–o

Formaldehyde (0.1 mL, 3.2 equiv) and a secondary amine (3.2 equiv) solution were added to **8** or **9** (150 mg, 1 equiv) in anhydrous ethanol (5 mL). Then glacial acetic acid (1.0 mL) was added to the reaction mixture, which was left at rt for 30 min. The reaction mixture was basified (pH 8) with a 2 M sodium hydroxide solution (5 mL). The mixture was extracted with EtOAc (3 × 25 mL), and combined organic extracts were washed with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a crude product that was purified by flash chromatography using a 5% methanol/dichloromethane mixture.

10α-{5-[(Dimethylamino)methyl]-1*H*-pyrrol-2-yl}artemisinin (7g)

Eschenmoser's salt (124 mg) was dissolved in the minimum amount of anhydrous acetonitrile and added dropwise over a period of 30 min to a solution of **8** (140 mg) in anhydrous acetonitrile (10 mL). The mixture was left to stir at room temperature for 24 h. The mixture was then basified (pH 8) with a 2 M NaOH solution (3.0 mL). The organic layer was then separated and the aqueous layer extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with saturated H_2O and brine. The organic phase was then dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a crude product that was purified by silica gel chromatography using a 10–30% MeOH/DCM mixture as the eluent. This gave **7g** (70% yield): colorless sticky solid; mp 60 °C; R_f = 0.13 in 10% MeOH/DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.70 (1H, s, NH), 6.02 (2H, m, CH-CH [pyrrole]), 5.37 (1H, s, H12), 4.43 (1H, d, J = 10.8, H10), 3.78–3.69 (2H, AB quartet, J = 13.4, CH_2-N), 2.63 (1H, m, H9), 2.46 [6H, s, N-$(CH_3)_2$], 2.39 (1H, dt, J = 13.7, 4.1, H4α), 1.42 (3H, s, H14), 2.08–1.20 (10H, m), 0.97 (3H, d, J = 6.3, H15), 0.63 (3H, d, J = 7.1, H16); HRMS calcd for $C_{22}H_{35}N_2O_4$ $[M + H]^+$ 391.2597, found 391.2598. Anal. ($C_{22}H_{34}N_2O_4$) C, H, N.

10α-[1-Methyl-5-(sulfonylmorpholinomethyl)pyrrol-2-yl]artemisinin (7p)

To a solution of **7m** (100 mg, 0.22 mmol), prepared as previously described, in DCM at rt under nitrogen were added NMO (76 mg, 0.65 mmol), powered molecular sieves (500 mg), and TPAP (10 mg, catalytic). The mixture was stirred at rt overnight and then filtered through a pad of silica, and the residue was washed with EtOAc (3 × 15 mL). The filtrate was concentrated in vacuo. The residue was then purified by flash

chromatography (SiO_2; 35% EtOAc/*n*-hexanes) to give **7p** as a yellow solid (38 mg, 35% yield): mp 77 °C; R_f = 0.92 in 10% MeOH/DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.90 (1H, d, *J* = 3.5, pyr CH), 5.88 (1H, d, *J* = 3.5, pyr CH), 5.38 (1H, s, H12), 4.48 (1H, d, *J* = 11.3, H10), 3.82 (3H, s, N-CH_3), 3.59–3.49 (2H, AB quartet, *J* = 13.7, CH_2), 3.00 (4H, m, thiomorpholine CH_2), 2.95 (4H, m, thiomorpholine CH_2), 2.85 (1H, m, H9), 2.39 (1H, dt, *J* = 14.0, 4.1, H4α), 1.39 (3H, s, H14), 2.08–1.20 (10H, m), 0.98 (3H, d, *J* = 6.3, H15), 0.57 (3H, d, *J* = 7.2, H16); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 129.8, 109.2, 108.6, 104.6, 92.3, 81.0, 73.2, 55.5, 54.8, 52.4, 46.3, 37.8, 36.7, 34.6, 32.1, 31.2, 28.3, 26.4, 25.2, 21.3, 20.6, 14.8; HRMS calcd for $C_{25}H_{38}N_2O_6Na$ $[M + Na]^+$ 517.2348, found 517.2344.

$FeSO_4$-Mediated Degradation of 10α-[1-Methyl-5-(morpholinomethyl)-1*H*-pyrrol-2-yl]artemisinin with Iron(II) Sulfate

To a solution of **7h** (110 mg, 0.25 mmol) in acetonitrile (5 mL) and water (5 mL) was added $FeSO_4 \cdot 7H_2O$ (86 mg, 0.31 mmol). The reaction mixture was left stirring at rt for 1 h before being filtered through Celite and washed with acetonitrile. Concentration under reduced pressure and flash column chromatography using a 9/1 DCM/MeOH mixture as the eluent yielded the products **18** as a yellow oil (0.08 g, 72%) and **19** as a yellow oil (0.05 g, 21%).

(i) $FeCl_2$-Mediated Degradation of 10α-[1-Methyl-5-(morpholinomethyl)-1*H*-pyrrol-2-yl]artemisinin with Iron(II) Chloride. To a solution of **7h** (0.15 g, 0.34 mmol) in acetonitrile (13 mL) was added $FeCl_2 \cdot 4H_2O$ (74 mg, 0.34 mmol) under a nitrogen atmosphere. The reaction mixture was left stirring at room temperature for 30 min before being filtered through Celite and washed with acetonitrile. Concentration under reduced pressure and flash column chromatography using a 5/95 EtOAc/*n*-hexane mixture as the eluent yielded **18** (0.09 g, 42%) and **19** (0.03 g, 23%).

(ii) Furano acetate (18): yellow oil; R_f = 0.58 in 9/1 DCM/MeOH; ^{1}H NMR (400 MHz) δ_H 6.15 (1H, s, H12), 6.05 (1H, d, *J* = 3.6, pyr CH), 5.88 (1H, d, *J* = 3.6, pyr CH), 4.59 (1H, d, *J* = 11.1, H10), 4.27 (1H, t, *J* = 9.5, H4), 3.91 (1H, q, *J* = 8.0, H4), 3.68 (4H, m, morph CH_2), 3.61 (3H, s, N-CH_3), 3.36 (2H, AB quartet, *J* = 13.5, CH_2), 2.74 (1H, m, H9), 2.38 (4H, m, morph CH_2), 2.09 (3H, s, H14), 2.00–1.00 (9H, m), 0.95 (3H, d, *J* = 6.6, H15), 0.87 (3H, d, *J* = 7.0, H16); ^{13}C NMR (400 MHz) δ_C 169.8, 130.8, 108.8, 106.9, 92.9, 80.6, 71.8, 68.9,

67.5 (2C), 55.7, 55.5, 53.7 (2C), 48.5, 35.9, 32.4, 31.0, 30.9, 30.4, 28.1, 22.5, 22.0, 21.0, 15.0; HRMS calcd for $C_{25}H_{38}N_2O_5Na$ $[M + Na]^+$ 469.2678, found 469.2687.

(iii) 3α-Hydroxydeoxyartemisinin (19): yellow oil; R_f = 0.48 in 9/1 DCM/MeOH; ^1H NMR (400 MHz) δ_H 5.92 (1H, d, J = 3.5, pyr CH), 5.87 (1H, d, J = 3.5, pyr CH), 5.33 (1H, s, H12), 4.53 (1H, d, J = 10.8, H10), 3.72 (3H, s, N-CH_3), 3.66 (4H, m, morph CH_2), 3.57 (1H, brs, -OH), 3.38 (2H, AB quartet, J = 16.4, CH_2), 2.79 (1H, m, H9), 2.37 (4H, m, morph CH_2), 2.1–1.1 (9H, m), 1.55 (3H, s, H14), 0.90 (3H, d, J = 6.44, H15), 0.65 (3H, d, J = 7.2, H16); ^{13}C NMR (400 MHz) δ_C 131.8, 130.0, 108.8, 108.6, 107.7, 95.7, 84.5, 77.1, 71.8, 70.1, 67.5 (2C), 55.5, 53.7 (2C), 42.9, 35.3, 34.8, 31.8, 30.8, 30.3, 22.5, 21.4, 21.0, 14.6; HRMS calcd for $C_{25}H_{38}N_2O_5Na$ $[M + Na]^+$ 469.2678, found 469.2691.

Biology

(i) *P. falciparum* in Vitro Culture and Parasite Growth Inhibition Assays

All parasite clones, isolates, and strains were acquired from MR4 (Malaria Research and Reference Reagent Resource Center, Manassas, VA). Strains and isolates used in this study were the drug sensitive 3D7 clone of the NF54 isolate (unknown origin) and the chloroquine, pyrimethamine, and cycloguanyl resistant K1 strain (Thailand). In vitro culture of *P. falciparum* was conducted following standard methods[32] with modifications as described previously.[33] *In vitro* parasite growth inhibition was assessed by the incorporation of $[^3H]$ hypoxanthine based on the method used by Desjardins[34] and modified as described previously.[35]

Briefly, stock drug solutions were dissolved in 100% dimethyl sulfoxide (Sigma, Dorset, U.K.), and 50 μL of a 3-fold dilution series (10.0, 3.33, 0.111, 0.0370, 0.0123, and 0.0041 μg/mL) of the drugs prepared in assay medium [RPMI 1640 supplemented with 0.5% Albumax II (Invitrogen), 0.2% (w/v) glucose, 0.03% l-glutamine, and 5 μM hypoxanthine] was added to each well of 96-well plates in triplicate. Fifty microlitres of asynchronous (65–75% ring stage) *P. falciparum* culture (0.5% parasitemia) or uninfected erythrocytes (blank) were added to each well, reaching a final volume of 100 μL per well, a final hematocrit of 2.5%, and final dimethyl sulfoxide concentrations of ≤0.01%. Plates were incubated at 37 °C in a 5% CO_2/95% air mixture for 24 h, at

which point 10 µL (0.2 µCi/well) of [^{3}H]hypoxanthine (Perkin-Elmer, Hounslow, U.K.) was added to each well. After an additional 24 h incubation period, the experiment was terminated by placing the plates in a −80 °C freezer. Plates were thawed and harvested onto glass fiber filter mats using a 96-well cell harvester (Harvester 96, Tomtec, Oxon, U.K.) and left to dry. After the addition of MeltiLex solid scintillant (Perkin-Elmer), the incorporated radioactivity was counted using a Wallac 1450 Betalux scintillation counter (Wallac).

Data acquired by the Wallac BetaLux scintillation counter were exported into a Microsoft Excel spreadsheet, and the IC_{50} values of each drug were calculated by using XLFit line fitting software (ID Business Solution). Chloroquine diphosphate, as a standard drug, and control wells with untreated infected and uninfected erythrocytes were included in all assays.

(ii) ***In vitro* cytotoxicity assay**

The AlamarBlue (Accumed International, USA) method was used to assess cytotoxicity to KB cells as previously described. [33] BrieXy, microtiter plates were seeded at a density of 4 x 10^4 KB cells/mL in RPMI 1640 culture medium supplemented with 10% heat-inactivated foetal calf serum (complete medium) (Seralab). Plates were incubated at 37 °C in a 5% CO_2/95% air mixture for 24 h followed by addition of the compound to triplicate wells in a dilution series in complete medium. The positive control drug was podophyllotoxin (Sigma). Plates were incubated for a further 72 h followed by the addition of 10 µL of AlamarBlue (AccuMed International) to each well and incubation for 2–4 h at 37 °C in a 5% CO_2/95% air mixture. Fluorescence emission at 585 nm was measured in a SPECTRAMAX GEMINI plate reader (Molecular Devices) after excitation at 530 nm. IC_{50} values were calculated using XLFit (ID Business Solutions) line fitting software.

Peters' Fully Suppressive 4 Day Test. [36]

In vivo tests were performed under UK Home Office Animals (Scientific Procedures) Act 1986. The rodent malaria line used was the *P. berghei* ANKA (drug susceptible). Swiss outbred 20 g male CD-1 mice (Charles River) were kept in specific pathogen-free conditions and fed ad libitum. For oral administration, compounds were dissolved in standard suspending formula (SSV) [0.5% sodium carboxymethylcellulose, 0.5% benzyl alcohol, 0.4% Tween 80, and 0.9% NaCl (all from Sigma)]. Mice were infected intravenously with 4×10^6 infected red cells (day 0), randomized, and divided into groups of five mice for each dose. Oral treatment started after 3 h and continued for up to 3 days postinfection once a day. Artesunate doses were 30, 10, 3, and 1 mg/kg. Parasitemias were determined by microscopic examination of Giemsa-stained blood films taken on day 4.

Microscopic counts of blood films from each mouse were processed using Microsoft Excel and expressed as percentages of inhibition from the arithmetic mean parasitemias of each group in relation to the untreated group.

***In Vivo* Antimalarial Mean Survival Time Studies**

All efficacy studies were approved by the institutional animal experimentation ethics committee. *In vivo* antimalarial activity was assessed basically as previously described.[37] Groups of three female NMRI mice (20–22 g) were intravenously infected with 2×10^7 parasitized erythrocytes on day 0 with GFP-transfected *P. berghei* strain ANKA.[25] Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water, and administered intraperitoneally in a volume of 10 ml/kg on four consecutive days (4, 24, 48 and 72 h post-infection). Parasitemia was determined on day 4 post-infection (24h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean percent parasitaemia for the control (n = 5 mice) and treated groups expressed as a percent relative to the control group. The survival time in days was also recorded up to 30 days after infection. A compound was considered curative if the animal survived to day 30 after infection with no detectable parasites.

Acknowledgments

We thank Romark and the EPSRC for studentships to B.P. and S.C.L. and J. C. Janse (Leiden University, Leiden, The Netherlands) for providing the GFP-transfected *P. berghei* strain.

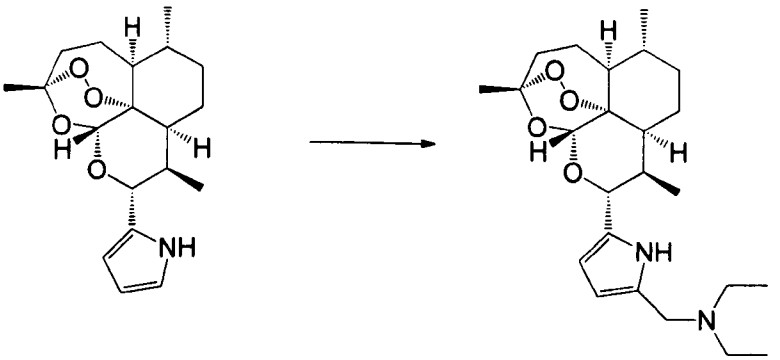
Supporting Information

Additional spectroscopic data for **7a–o**, **8**, and **9** and details of the synthesis of acetate **11** and benzoate **12** along with C,H,N analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

Further Information for Experimental

General procedure for 7a-7o. Formaldehyde (0.1 mL, 3.2 equiv.) and a secondary amine (3.2 equiv.) solution were added to **(8)** or **(9)** (150 mg, 1 equivalent) in anhydrous ethanol (5 mL). Then glacial acetic acid (1.0 mL) was added to the reaction mixture, which was left at r.t. for 30 min. The reaction was basified (pH 8) with 2M sodium hydroxide solution (5 mL). The mixture was extracted with EtOAc (3×25 mL) and combined organic extracts washed with brine. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a crude product that was purified by flash chromatography using 5% methanol/dichloromethane.

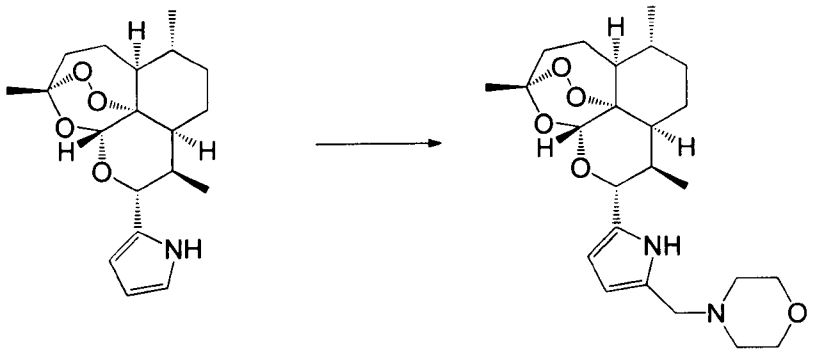
10α-(5-((Diethylamino)methyl)-1*H*-pyrrol-2-yl)artemisinin (7a).



8 **7a**

See general procedure for Mannich reaction. Colourless sticky solid (24%); R_f=0.04 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.50 (1H, s, NH), 5.99 (1H, t, *J*= 2.8 Hz, pyr CH), 5.93 (1H, t, *J*=2.8 Hz, pyr CH), 5.37 (1H, s, H12), 4.42 (1H, d, *J*=11.0 Hz, H10), 3.69 (2H, AB quartet, *J*= 13.5 Hz, CH_2-N), 2.62 (4H, m, N-CH_2), 2.57 (1H, m, H9), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 1.09 (6H, t, *J*= 7.2 Hz, dieth CH_3), 0.97 (3H, d, *J*=6.3 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 123.3, 106.8, 104.2, 92.1, 80.6, 72.0, 52.0, 46.1, 45.9, 37.4, 37.4, 36.3, 34.2, 33.0, 29.7, 29.7, 26.0, 24.8, 22.7, 21.4, 20.3, 14.1, 10.7 ppm; MS (ES+), $[M+H]^+$ (100) 419; HRMS calcd for $C_{24}H_{39}N_2O_4$ $[M+H]^+$ 419.2910, found 419.2927.

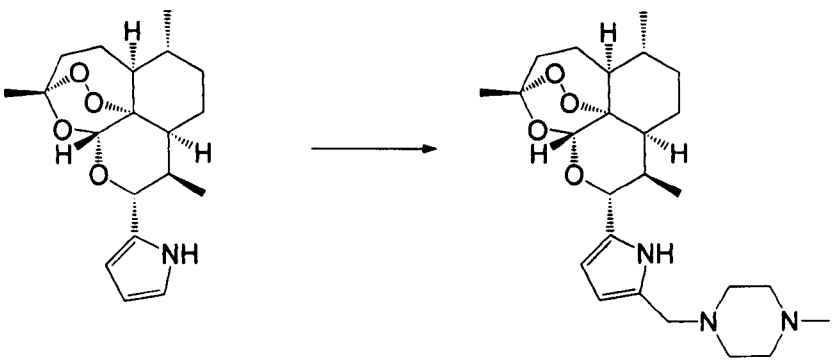
10α- (5-(Morpholinomethyl)-1*H*-pyrrol-2-yl)artemisinin (7b).



8 **7b**

See general procedure for Mannich reaction. Orange solid (60%): mp = 33 °C; R_f=0.75 in 10% MeOH/ DCM;^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.85 (1H, s, NH), 5.93 (1H, t, *J*=3.2 Hz, pyr CH), 5.89 (1H, t, *J*=3.2 Hz, pyr CH), 5.38 (1H, s, H12), 4.42 (1H, d, *J*=10.8 Hz, H10), 3.70 (4H, t, *J*=4.6 Hz, morph CH_2-O), 3.47 (2H, s, morph CH_2-N), 2.57 (1H, m, H9), 2.43 (4H, m, N-CH_2), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 0.97 (3H, d, *J*=6.3 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.3, 127.5, 107.6, 107.0, 104.2, 92.3, 80.7, 72.1, 67.0, 55.8, 53.3, 52.0, 45.9, 37.4, 36.3, 34.2, 33.0, 26.0, 24.8, 21.4, 20.3 and 14.1 ppm; IR, υ_{max}= 3372, 1650, 1456, 1376, 1303, 1152, 1120, 1057, 926, 880, 865, 849, 828, 770, 722 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 455; HRMS calcd for $C_{24}H_{36}N_2O_5Na$ $[M+Na]^+$ 455.2522, found 455.2536.

10α- (5-((4-Methylpiperazin-1-yl)methyl)-1*H*-pyrrol-2-yl)artemisinin (7c).

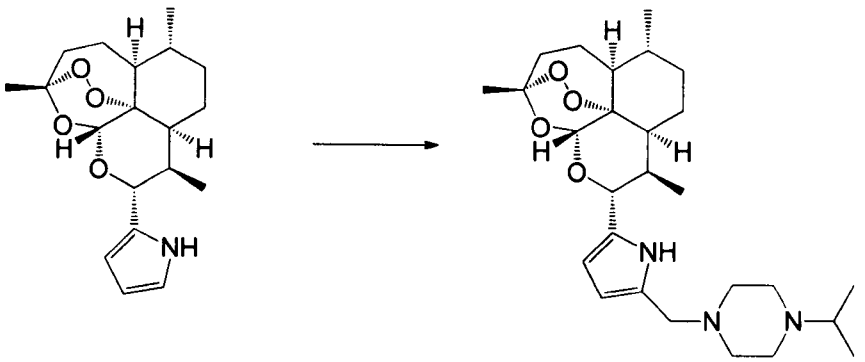


8 **7c**

See general procedure for Mannich reaction. Yellow crystal (60%): mp = 36 °C; R_f=0.32 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.07 (1H, s, NH), 5.94 (1H, d, *J*=3.2

Hz, pyr CH), 5.90 (1H, d, *J*=3.2 Hz, pyr CH), 5.38 (1H, s, H12), 4.42 (1H, d, *J*=10.8 Hz, H10), 3.51 (2H, s, CH_2), 2.57 (1H, m, H9), 2.55 (8H, m, pipz CH_2), 2.39 (1H, dt, *J*=13.7, 4.1 Hz), 2.31 (3H, s, N-CH_3), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 0.97 (3H, d, *J*=6.2 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.8, 127.8, 108.1, 107.4, 104.6, 92.5, 81.1, 72.5, 55.6, 55.0, 52.9, 52.4, 46.3, 37.8, 36.7, 34.5, 33.4, 30.0, 26.4, 25.1, 21.7, 20.7 and 14.4 ppm; MS (ES+), $[M+H]^+$ (100) 446; HRMS calcd for $C_{25}H_{40}N_3O_4$ $[M+H]^+$ 446.3019, found 446.3004; IR υ_{max}= 3268 ($\upsilon_{N\text{-}H,\ Pyrrole}$), 2926 ($\upsilon_{C\text{-}H}$), 1705 ($\upsilon_{C=N}$), 880, 826 ($\upsilon_{O\text{-}O}$) cm^{-1}.

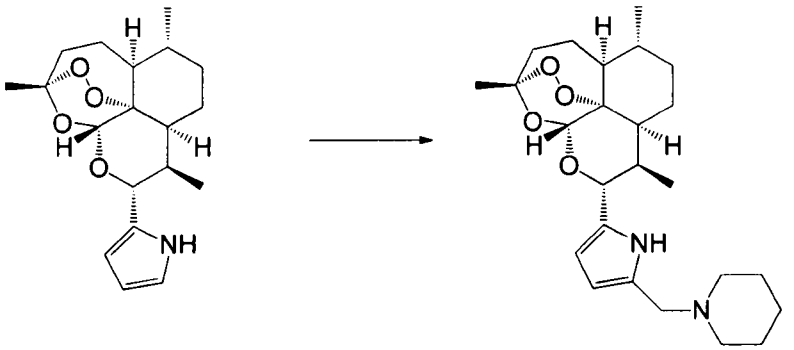
10α- (5-((4-Isopropylpiperazin-1-yl)methyl)-1*H*-pyrrol-2-yl)artemisinin (7d).



8 **7d**

See general procedure for Mannich reaction. Colourless crystal (70%): mp = 45 °C; Rf=0.17 in 10% MeOH/ DCM; ^1H NMR (400 MHz, $CDCl_3$) δ_H 9.26 (1H, s, NH), 5.96 (1H, t, *J*=2.7 Hz, pyr CH), 5.92 (1H, t, *J*=2.7 Hz, pyr CH), 5.38 (1H, s, H12), 4.42 (1H, d, *J*=10.8 Hz, H10), 3.57 (2H, s, CH_2), 2.88 (1H, m, ^{i}Pr CH), 2.72 (8H, m, pipz CH_2), 2.57 (1H, m, H9), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.42 (3H, s, H14), 1.2-2.1 (10H, m), 1.12 (6H, d, *J*=6.5 Hz, ^{i}Pr CH_3), 0.97 (3H, d, *J*=6.2 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.3, 130.1, 126.6, 108.7, 107.3, 104.6, 92.5, 81.1, 72.5, 55.4, 55.3, 52.3, 48.15, 46.3, 37.8, 36.7, 34.5, 33.4, 26.4, 25.1, 21.7, 20.7, 18.7, 18.5, 18.4 and 14.5 ppm; MS (ES+), $[M+H]^+$ (100) 474; HRMS calcd for $C_{27}H_{44}N_3O_4$ $[M+H]^+$ 474.3332, found 474.3318.

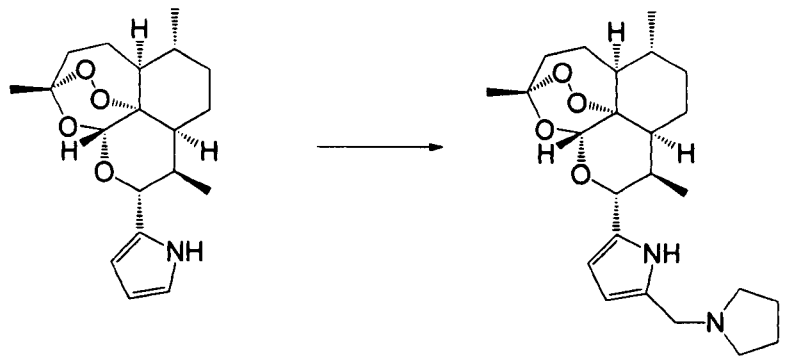
10α- (5-(Piperidin-1-ylmethyl)-1*H*-pyrrol-2-yl)artemisinin (7e).



8 **7e**

See general procedure for Mannich reaction. Colourless sticky solid (65%); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.30 (1H, s, NH), 5.99 (1H, d, *J*=2.8 Hz, pyr CH), 5.94 (1H, d, *J*=2.8 Hz, pyr CH), 5.37 (1H, s, H12), 4.42 (1H, d, *J*=10.8 Hz, H10), 3.64-3.58 (2H, AB quartet, *J*= 14.4 Hz, CH_2), 2.59 (1H, m, H9), 2.52 (4H, m, pipd CH_2), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.67 (4H, m, pipd CH_2), 1.48 (2H, d, *J*=3.9 Hz, pipd CH_2), 1.42 (3H, s, H14), 1.2-2.1 (10H, m), 0.97 (3H, d, *J*=6.3 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.7, 109.1, 107.1, 104.6, 92.5, 81.0, 72.4, 60.7, 56.0, 54.0, 52.4, 46.3, 37.8, 36.7, 34.6, 33.5, 26.4, 25.1, 24.1, 21.7, 21.4, 20.7 and 14.4 ppm; IR υ_{max}= 3250 ($\upsilon_{N\text{-}H,\ Pyrrole}$), 2933 ($\upsilon_{C\text{-}H}$), 1707 ($\upsilon_{C=N}$), 880, 827 ($\upsilon_{O\text{-}O}$) cm^{-1}.

10α-(5-(Pyrrolidin-1-ylmethyl)-1*H*-pyrrol-2-yl)artemisinin (7f).

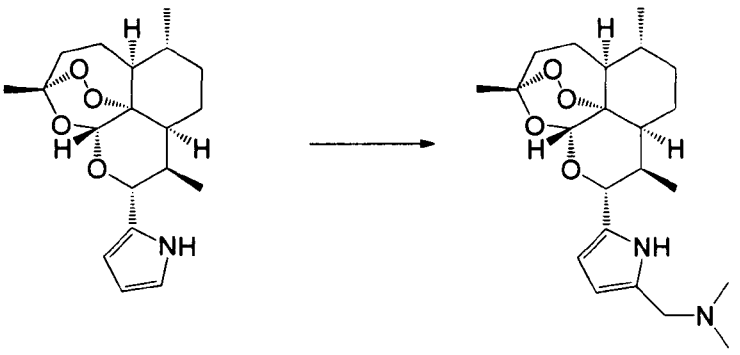


8 **7f**

See general procedure for Mannich reaction. Orange oil (70%); R_f=0.5 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 10.54 (1H, s, NH), 6.08 (1H, t, *J*=2.8 Hz, pyr CH), 6.04 (1H, t, *J*=2.8 Hz, pyr CH), 5.31 (1H, s, H12), 4.35 (1H, d, *J*=10.6 Hz, H10), 4.04-4.14

(2H, AB quartet, *J*= 14.9 Hz, CH_2), 3.10 (4H, m, pyro CH^iPr CH_2), 2.57 (1H, m, H9), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 2.00 (4H, m, pyro CH_2), 1.42 (3H, s, H14), 1.2-2.1 (10H, m), 0.97 (3H, d, *J*=6.3 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 134.1, 120.2, 111.4, 106.6, 104.2, 92.2, 80.6, 71.9, 52.2, 52.0, 51.4, 50.5, 45.9, 37.3, 36.3, 34.1, 33.3, 26.0, 24,7, 23.2, 23.0, 21.4, 20.3, 14.1 ppm; MS (ES+), $[M+H]^+$ (100) 417; HRMS calcd for $C_{24}H_{37}N_2O_4$ $[M+H]^+$ 417.2753, found 417.2739.

10α-(5-((Dimethylamino)methyl)-1*H*-pyrrol-2-yl)artemisinin (7g).



8 **7g**

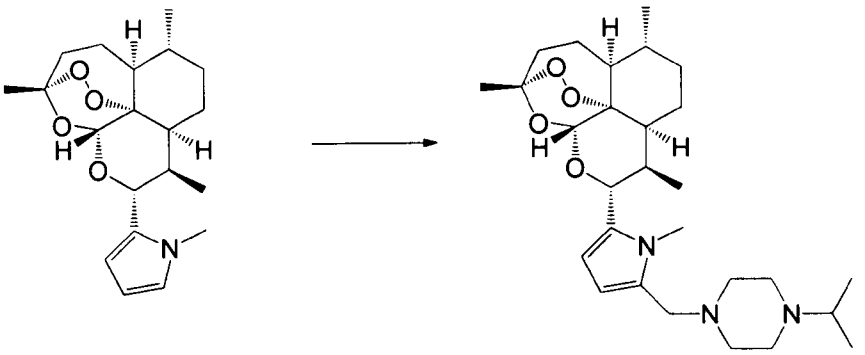
Eschenmoser's salt (124 mg) was dissolved in the minimum amount of anhydrous acetonitrile and added drop wise over a period of 30 mins to a solution of **(8)** (140 mg) in anhydrous acetonitrile (10 mL). The mixture was left to stir at room temperature for 24 h. The mixture was then basified (pH 8) with 2M NaOH solution (3.0 mL). The organic layer was then separated and the aqueous layer extracted with EtOAc (3×25 mL). The combined organic layers were washed with saturated H_2O and brine. The organic phase was then dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a crude product that was purified by silica gel chromatography using 10 to 30% MeOH/ DCM as eluent: This gave **7g** (70% yield); Colourless sticky solid, R_f= 0.01 in 10% MeOH/ DCM; ^1H NMR (400 MHz, $CDCl_3$) δ_H 9.70 (1H, s, NH), 6.02 (2H, m, CH-CH [pyrrole]), 5.37 (1H, s, H12), 4.43 (1H, d, *J*=10.8 Hz, H10), 3.78-3.69 (2H, AB quartet, *J*= 13.4 Hz, CH_2-N), 2.63 (1H, m, H9), 2.46 (6H, s, N-$(CH_3)_2$), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 0.97 (3H, d, *J*= 6.3 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; IR, υ_{max}= 3461, 2918, 2360, 1588, 1456, 1376, 1278, 1226, 1196, 1151, 1127, 1098, 1085, 1058, 1043, 940, 925, 894, 880, 848, 826,

772, 723 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 391; HRMS calcd for $C_{22}H_{35}N_2O_4$ $[M+H]^+$ 391.2597, found 391.2598.

10α-(1-Methyl-5-(morpholinomethyl)-pyrrol-2-yl)artemisinin (7h). See general procedure for Mannich reaction. Light yellow solid (89% yield); mp= 126 °C; R_f= 0.48 in 10% MeOH/ DCM; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.93 (2H, m, pyr CH), 5.43 (1H, s, H12), 4.53 (1H, d, *J*=11.1 Hz, H10), 3.88 (3H, s, N-CH_3), 3.72 (4H, t, *J*=4.4 Hz, morph CH_2), 3.52-3.45 (2H, AB quartet, *J*=12.8 Hz, CH_2), 2.90 (1H, m, H9), 2.47 (4H, m, morph CH_2), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.3 Hz, H15) and 0.61 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.1, 129.6, 108.9, 108.4, 104.6, 92.3, 81.1, 73.2, 67.3, 55.1, 53.4, 52.4, 46.3, 37.8, 36.7, 34.6, 32.1, 31.2, 26.4, 25.2, 21.9, 21.3 and 14.8 ppm; IR, υ= 1757, 1458, 1376, 1347, 1263, 1226, 1207, 1151, 1120, 1100, 1084, 1054, 1042, 1004, 979, 940, 926, 895, 880, 864, 852, 828, 800, 743 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 469; HRMS calcd for $C_{25}H_{38}N_2O_5Na$ $[M+Na]^+$ 469.2678, found 469.2664; Anal. ($C_{16}H_{26}N_2O_3$) C, H, N.

10α-(1-Methyl-5-((4-methylpiperazin-1-yl)methyl)-pyrrol-2-yl)artemisinin (7i). See general procedure for Mannich reaction. Yellow crystal (83%); mp = 115 °C; R_f= 0.25 in 10% MeOH/ DCM; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.89 (1H, d, *J*=3.5 Hz, pyr CH), 5.86 (1H, d, *J*=3.5 Hz, pyr CH), 5.39 (1H, s, H12), 4.47 (1H, d, *J*=11.1 Hz, H10), 3.82 (3H, s, pyr N-CH_3), 3.45-3.38 (2H, AB quartet, *J*= 13.5 Hz, CH_2), 2.85 (1H, m, H9), 2.35-2.70 (8H, m, pipz CH_2), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 2.34 (3H, s, pipz N-CH_3), 1.41 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.2 Hz, H15) and 0.56 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.9, 130.5, 108.5, 108.3, 104.5, 92.3, 81.1, 73.1, 55.4, 54.8, 52.5, 52.4, 46.3, 45.9, 37.8, 36.7, 34.5, 32.1, 31.2, 26.4, 25.1, 21.3, 20.7 and 14.8 ppm; IR, υ_{max}= 2670, 1456, 1376, 1302, 1159, 1100, 1057, 1041, 975, 890, 849, 828, 762, 722 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 460; HRMS calcd for $C_{26}H_{42}N_3O_4$ $[M+H]^+$ 460.3175, found 460.3176; Anal. ($C_{26}H_{41}N_3O_4$) C, H, N

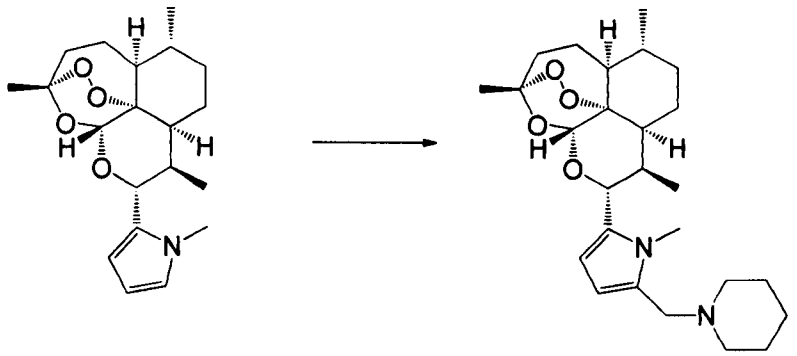
10α- (5-((4-Isopropylpiperazin-1-yl)methyl)-1-methyl-pyrrol-2-yl)artemisinin (7j).



8 **7j**

See general procedure for Mannich reaction. Colourless solid (76%); mp= 68 °C; Rf= 0.21 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.89 (1H, d, *J*= 3.5 Hz, pyr CH), 5.87 (1H, d, *J*= 3.5 Hz, pyr CH), 5.39 (1H, s, H12), 4.48 (1H, d, *J*=11.3 Hz, H10), 3.81 (3H, s, N-CH_3), 3.51-3.42 (2H, AB quartet, *J*=13.5, CH_2), 3.05 (4H, m, pipz CH_2), 2.85 (1H, m, H9), 2.73 (5H, m, pipz CH_2), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 1.39 (3H, s, H14), 1.25 (6H, d, *J*=6.5 Hz, iPr CH_3), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.3 Hz, H15) and 0.61 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.1, 130.0, 108.8, 108.5, 104.6, 92.3, 81.1, 73.2, 56.5, 54.4, 53.8, 48.7, 46.3, 37.8, 36.7, 34.5, 32.1, 31.2, 26.4, 25.1, 21.3, 20.7, 18.0 and 14.8 ppm; IR, υ_{max}= 3894, 3816, 3710, 3544, 3024, 2929, 2856, 2360, 1610, 1460, 1232, 1029 and 756 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 488; HRMS calcd for $C_{28}H_{46}N_3O_4$ $[M+H]^+$ 488.3488, found 488.3507; Anal. ($C_{28}H_{45}N_3O_4$) C, H, N

10α-(1-Methyl-5-(piperidin-1-ylmethyl)-pyrrol-2-yl)artemisinin (7k).

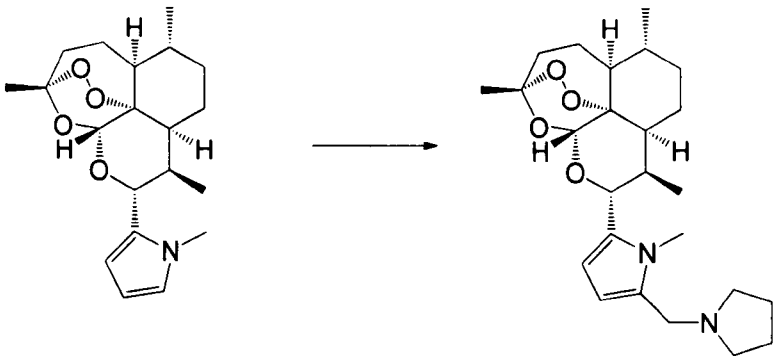


8 **7k**

See general procedure for Mannich reaction. Yellow solid (88%); R_f= 0.34 in 10% MeOH/ DCM; mp= 80 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.95 (1H, m, pyr CH), 5.38 (1H,

s, H12), 4.48 (1H, d, *J*=11.2 Hz, H10), 3.84 (3H, s, N-CH_3), 3.60 (2H, m, pipd CH_2), 2.85 (1H, m, H9), 2.52 (4H, m, pipd CH_2), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 1.67 (4H, m, pipd CH_2), 1.46 (2H, m, pipd CH_2), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.2 Hz, H15) and 0.58 (3H, d, *J*=7.1 Hz, H16) ppm; IR, υ_{max}= 3948, 3836, 3710, 3589, 3539, 2931, 2343, 1462, 1122, 1037, 827, 758 and 679 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 445; HRMS calcd for $C_{26}H_{41}N_2O_4$ $[M+H]^+$ 445.3066, found 445.3058; Anal. ($C_{26}H_{40}N_2O_4$) C, H, N.

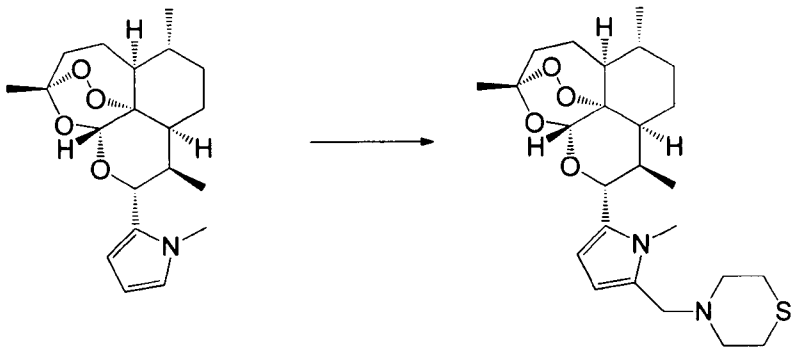
10α-(1-Methyl-5-(pyrrolidin-1-ylmethyl)-pyrrol-2-yl)artemisinin (7l).



8 **7l**

See general procedure for Mannich reaction. Pale yellow dry foam (97%); mp= 70 °C; R_f= 0.29 in 10% MeOH/ DCM; ^1H NMR (400 MHz, $CDCl_3$) δ_H 6.03 (1H, d, *J*=3.7 Hz, pyr CH), 5.97 (1H, d, *J*=3.6 Hz, pyr CH), 5.38 (1H, s, H12), 4.48 (1H, d, *J*=11.2 Hz, H10), 3.87 (3H, s, N-CH_3), 3.85 (2H, s, CH_2), 2.86 (1H, m, H9), 2.82 (4H, m, pyro CH_2), 2.39 (1H, dt, *J*= 14.0, 4.1 Hz, H4α), 1.89 (4H, m, pyro CH_2), 1.39 (3H, s, H14), 1.2-2.1 (10H, m), 0.98 (3H, d, *J*=6.4 Hz, H15) and 0.61 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 129.8, 109.0, 104.6, 92.3, 81.0, 72.9, 53.8, 53.2, 52.4, 50.3, 46.3, 37.8, 36.7, 34.5, 32.4, 31.2, 26.4, 25.2, 23.6, 21.3, 20.6 and 14.8 ppm; IR, υ_{max}= 3834, 3759, 3323, 2970, 2345, 1658, 1458, 1377, 1321, 1199, 1124, 1095, 1049, 881 (O-O), 825 (O-O), 762 and 681 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 431; HRMS calcd for $C_{25}H_{39}N_2O_4$ $[M+H]^+$ 431.2910, found 431.2907.

10α-(1-Methyl-5-(thiomorpholinomethyl)-pyrrol-2-yl)artemisinin (7m).



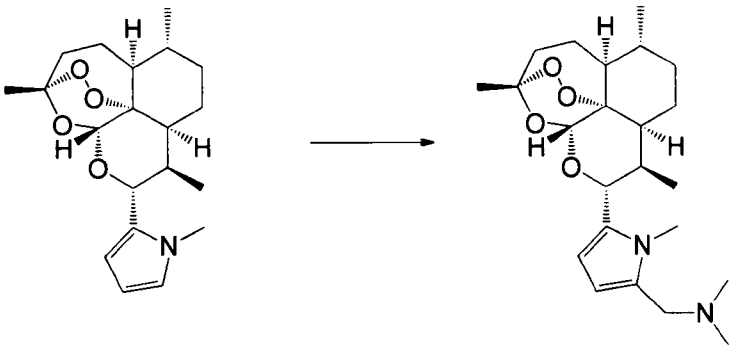
8 **7m**

See general procedure for Mannich reaction. Yellow foam (90%); R_f= 0.92 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.90 (1H, d, *J*=3.5Hz, pyr CH), 5.86 (1H, d, *J*=3.5 Hz, pyr CH), 5.38 (1H, s, H12), 4.46 (1H, d, *J*=11.1 Hz, H10), 3.81 (3H, s, N-CH_3), 3.46 and 3.41 (2H, AB quartet, *J*=12.8 Hz, CH_2), 2.85 (1H, m, H9), 2.69 (4H, m, thiomorph), 2.64 (4H, m, CH_2-S), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 1.39 (3H, s, H14), 1.2-2.1 (10H, m), 0.98 (3H, d, *J*=6.3 Hz, H15) and 0.61 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 129.8, 108.9, 108.4, 104.5, 92.3, 81.0, 73.1, 55.5, 54.8, 52.4, 46.3, 37.8, 36.7, 34.6, 32.1, 31.2, 28.3, 26.4, 25.2, 21.3, 20.6 and 14.8 ppm; IR, υ= 3892, 3759, 3712, 3356, 2972, 2814, 2370, 2331, 1658, 1460, 1414, 1371, 1333, 1279, 1203, 1124, 1099, 1051, 948, 880, 823 and 762 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 485; HRMS calcd for $C_{25}H_{38}N_2O_4SNa$ $[M+Na]^+$ 485.2450, found 485.2460; Anal. ($C_{24}H_{36}N_2O_4S$) C, H, N

10α-(1-Methyl-5-((4-(pyrrolidin-1-yl)piperidin-1-yl)methyl)-pyrrol-2-yl)artemisinin (7n). See general procedure for Mannich reaction. Colourless sticky solid (54%); R_f= 0.10 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.90 (1H, d, *J*= 3.5 Hz, pyr CH), 5.84 (1H, d, *J*= 3.5 Hz, pyr CH), 5.38 (1H, s, H12), 4.46 (1H, d, *J*=11.1 Hz, H10), 3.78 (3H, s, N-CH_3), 3.42-3.36 (2H, AB quartet, *J*=13.5, CH_2), 3.17 (4H, m, pipd CH_2), 2.99 (4H, m, pyro CH_2), 2.85 (1H, m, H9), 2.82 (1H, m, pipd CH), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 2.08 (4H, m, pyro CH_2), 1.92 (4H, m, pipd CH_2), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*= 6.3 Hz, H15) and 0.61 (3H, d, *J*= 7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 130.9, 108.1, 108.0, 104.5, 92.3, 81.0, 73.1, 62.6, 55.0, 52.6, 52.3,

51.7, 46.4, 37.8, 36.7, 34.6, 32.0, 31.6, 31.3, 26.4, 25.2, 23.6, 21.3, 20.6 and 14.8 ppm; IR, υ_{max}= 1703, 1458, 1376, 1263, 1151, 1041, 965, 880, 828, 743 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 514; HRMS calcd for $C_{30}H_{48}N_3O_4$ $[M+H]^+$ 514.3645, found 514.3657.

10α-(5-((Dimethylamino)methyl)-1-methyl-pyrrol-2-yl)artemisinin (7o).



8 **7o**

Eschenmoser's salt (124 mg) was dissolved in the minimum amount of anhydrous acetonitrile and added dropwise over a period of 30 mins to a solution of **(9)** (140 mg) in anhydrous acetonitrile (10 mL). The mixture was left to stir at r.t. for 24 h. The mixture was then basified (pH 8) with 2 M NaOH solution (3mL). The organic layer was then separated and the aqueous layer extracted with EtOAc (3×25 mL). The combined organic layers were washed with saturated H_2O and brine. The organic phase was then dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a crude product that was purified by silica gel chromatography using 10 to 30% MeOH/ DCM as eluent: This yielded **7o** (70%); yellow sticky solid, R_f= 0.01 in 10% MeOH/ DCM; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.93 (1H, d, *J*=3.5 Hz, pyr CH), 5.90 (1H, d, J=3.5 Hz, pyr CH), 5.38 (1H, s, H12), 4.48 (1H, d, *J*=11.2 Hz, H10), 3.81 (3H, s, N-CH_3), 3.4 (2H, s, CH_2), 2.7 (1H, m, H9), 2.37 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 2.22 (6H, s, N-$(CH_3)_2$), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.4 Hz, H15) and 0.61 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100MHz, $CDCl_3$) δ_C 131.2, 130.6, 108.5, 104.5, 92.3, 81.0, 72.9, 55.7, 52.4, 50.7, 46.3, 45.0, 37.8, 36.7, 34.5, 31.9, 31.1, 26.3,25.1, 21.3, 20.6 and 14.7ppm; IR υ= 3366 (N-H), 2924 (C-H), 2360, 1708 (C=N), 1498, 1458, 1376, 1320, 1297, 1278, 1248, 1227, 1196, 1151, 1128, 1100, 1085, 1055, 1043, 976, 940, 927, 880 (O-O), 851, 828 (O-O), 775, 721, 708 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 427; HRMS calcd for $C_{23}H_{36}N_2O_4Na$ $[M+Na]^+$ 427.2581, found 427.2573; Anal. ($C_{23}H_{36}N_2O_4$) C, H, N

10α-(1-Methyl-5-(sulfonylmorpholinomethyl)-pyrrol-2-yl)artemisinin (7p). To a solution of **(7m)** (100 mg, 0.22 mmol), prepared as previously described, in DCM at r.t. under nitrogen was added NMO (76 mg, 0.65 mmol), powered molecular sieves (500mg) and TPAP (10 mg, cat.). The mixture was stirred at r.t. over night after which it was filtered through a pad of silica and the residue was washed with EtOAc (3×15mL). The filtrate was concentrated in vacuo. The residue was then purified by flash chromatography (SiO_2; 35% EtOAc/ *n*-Hex) to give **7p** as a yellow solid (38 mg, 35%); mp= 77 °C; R_f= 0.92 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.90 (1H, d, *J*=3.5 Hz, pyr CH), 5.88 (1H, d, *J*=3.5 Hz, pyr CH), 5.38 (1H, s, H12), 4.48 (1H, d, *J*=11.3 Hz, H10), 3.82 (3H, s, N-CH_3), 3.59-3.49 (2H, AB quartet, *J*=13.7 Hz, CH_2), 3.00 (4H, m, thiomorph CH_2), 2.95 (4H, m, thiomorph CH_2), 2.85 (1H, m, H9), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.3 Hz, H15) and 0.57 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 129.8, 109.2, 108.6, 104.6, 92.3, 81.0, 73.2, 55.5, 54.8, 52.4, 46.3, 37.8, 36.7, 34.6, 32.1, 31.2, 28.3, 26.4, 25.2, 21.3, 20.6 and 14.8 ppm; MS (ES+), $[M+Na]^+$ (100) 517; HRMS calcd for $C_{25}H_{38}N_2O_6Na$ $[M+Na]^+$ 517.2348, found 517.2344.

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Chapter 5

Final Conclusions and Future work

5.1 Final conclusions and future work

The aims of this thesis were to synthesise novel antimalarial quinolones targeting *Pf*NDH2 (a new target) and cytochrome bc_1 of mitochondrial respiratory chain of *Plasmodium falciparum*, and investigate their antimalarial activities. An additional aim of this thesis was to synthesise a series of C-10 pyrrole mannich base derivatives of artemisinin with improved activity and stability. In this final chapter, a set of conclusions and possible future studies are suggested based on the results obtained.

4-Quinolones have been studied by many scientists extensively as they demonstrate interesting antibacterial and antitumor activities. The quinolone pharmacophore offers a unique scaffold for antimalarial drug development as this structure is not present in available antimalarial drugs, as such their introduction may prevent the development of resistance, particularly if the given quinolone template has the potential to target more than one essential parasite process.

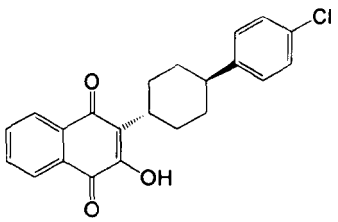
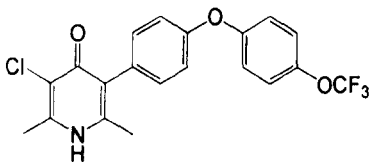
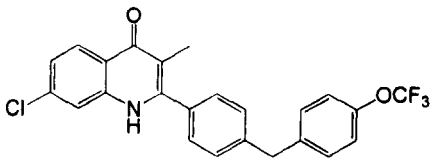
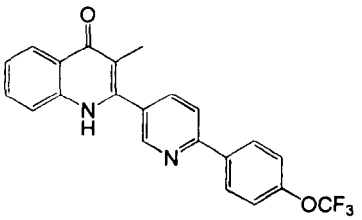
5.2 Quinolones targeting *Pf*NDH2

The first aim of this thesis was to optimise the CK-2-68 lead compound that was previously prepared in the group. A series of heterocyclic quinolones were successfully synthesised in 4-6 steps. The SAR of the pyridinyl series was studied in detail and conclusions drawn on the optimal substitution in the A, B, C, D ring system of the inhibitor.

Upon finishing this thesis, Boysen *et al* published a paper regarding the studies of the role of NDH2 in the murine malarial parasite *Plasmodium berghei* by targeted gene disruption. This study pointed out that NDH2 is dispensable as electron donor for the mitochondrial membrane potential, and thus NDH2 may not be a potential drug target.[1] This observation is obviously contradicted to earlier findings which suggested NDH2 is an attractive target.[2] Moreover, their studies were done in *P. berghei* model, which may be of limited applicability to human strains of the malaria parasite. Unpublished research studies in our group have been unable to knock out the NDH2 enzyme in *P. falciparum* genetically, and indicated that *P. berghei* and *P. falciparum* have several differences in mitochondria. Therefore, the knockout effect may be species specific.

Despite the controversy on the essentiality of the mitochondrial functions of the parasite, several compounds within the series prepared in this thesis have demonstrated potent antimalarial activity both *in vitro* and *in vivo*. They have been proved to be selectively active against the *Pf*NDH2 enzymatic target. The lead compounds SL-2-25 and SL-2-64 (quinolones **32** and **52** in chapter 2) also show good profiles with improvements in ClogP, solubility and in vivo activity compared to the previous lead CK-2-68. SL-2-25 is active against the atovaquone-resistant strain TM90C2B with IC_{50} 156 nM. Although SL-2-25 is also active against parasite bc_1 complex, it is less active in beef heart bc_1 (which is similar to human bc_1 complex) compared to CK-2-68 (Table 1). This might provide a greater selectivity and window to avoid toxicity in humans.

Table 1. Antimalarial activities of atovaquone, GW84420, CK-2-68 and SL-2-25.

Compound	**IC_{50} 3D7 (nM)**	**IC_{50} TM90C2B (nM)**	**IC_{50} *Pf*NDH2 (nM)**	**IC_{50} Parasite bc_1 (nM)**	**IC_{50} Bovine bc_1 (nM)**
Atovaquone 	0.9	12000	>10000	2	83
Pyridone (GW84420) 	15	-	14000	32	51
CK-2-68 (**10**) 	31	184	16	400	465
SL-2-25 (**32**) 	54	156	14	15	890

Recently available biological data showed that SL-2-34 (quinolone **14**, Chapter 2) and SL-2-64 (**52**) are also active against *P. falciparum* bc_1 complex (Table 2). This further suggests the cross-over of the activity against two targets NDH2 and bc_1 complex cannot be designed out completely. It is also noticed that the incorporation of a fluorine at 7-position increases selectivity for *Pf*NDH2 over bc_1, as demonstrated by **32** and **52.**

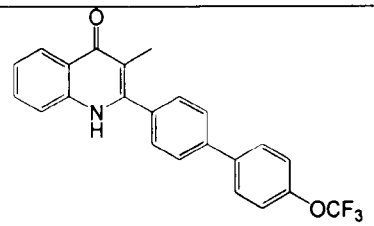
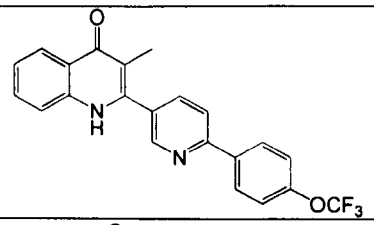
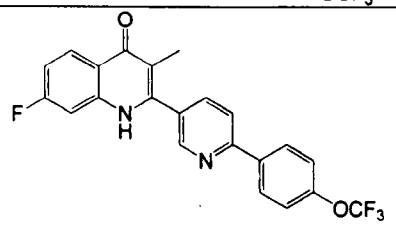
Compound		IC_{50} 3D7 (nM)	IC_{50} *Pf*NDH2 (nM)	IC_{50} *P. fal* bc_1[a] (nM)	Selectivity (*P. fal* bc_1/ *Pf*NDH2)
14		59±9	<1	38	>38
32		54±6	14.6	15.1	1.03
52		75±9	4.2	26.8	6.38

Table 2. Inhibitory profile of respiratory chain inhibitors showing differential selectivity for parasite mitochondrial bc_1 and *Pf*NDH2 enzymes.

[a] Cytochrome *c* reductase activity measurements were assayed in 50 mM potassium phosphate, pH 7.5, 2 mM EDTA, 10 mM KCN and 30 uM equine cytochrome *c* at room temperature. Cytochrome *c* reductase activity was initiated by the addition of decylubiquinol (50 uM). The cytochrome *b* content of membranes was determined from the dithionite-reduced minus ferricyanide-oxidized difference spectra. Inhibitors of bc_1 activity were added without prior incubation.

Further *in silico* studies in collaboration with the Berry computational drug design group at the University of Liverpool concluded that SL-2-25 (quinolone **32**) occupies a position within the Q_o site similar to that of stigmatellin, a potent inhibitor of bc_1 complex at the Q_o site. Key predicted interactions include a hydrogen bond between the quinolone headgroup and the imidazole ring of Rieske protein residue His181 (2.3Å). Another water-bridged hydrogen bond is predicted between the quinolone N-H and the side chain of cytochrome *b* residue Glu272 bond from the side chain of cytochrome *b* 'PEWY'(**ef**) loop (2.5Å and 2.3Å) (Figure 1a). Within cytochrome *b*, SL-2-25 binding site is formed from residues contained within the C-terminal region of transmembrane helix **C** (I122, I125), helix **cd1** (I147, I150), the **ef** loop (L269, P271, E272, L275) and the **F1-F2** linker region (F296, I299) (Figure 1b). The majority of interactions are hydrophobic and van der Waals associations. The side chain of I122 is predicted to form a 58Å^2 van der Waals interaction with the trifluoromethoxy moiety of SL-2-25, with significant hydrophobic interactions with the pyridinyl and quinolone groups of the inhibitor provided by the side chains of P271 and L275 (26- and 33Å^2 respectively).

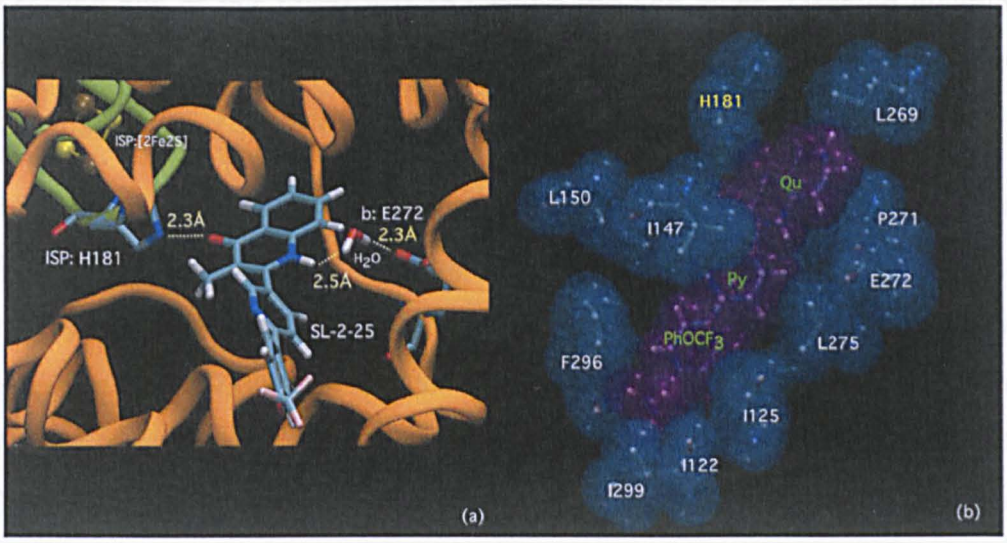


Figure 1. (a) SL-2-25 (quinolone **32**) docked within the Q_o site of yeast cytochrome bc_1 (3CX5.pdb). The a-carbon backbones of cytochrome *b* and the Rieske protein are represented in cartoon form in orange and green respectively. Hydrogen bonds between SL-2-25 and the sidechains of Glu-272(cytochrome *b*) and His-181(Rieske) are indicated by yellow dotted lines. (b) Predicted binding interactions between SL-2-25 (quinolone **32**) and the Q_o site of the yeast bc_1 complex. Cytochrome *b* residues are labeled in white, Rieske protein residues in orange. The quinolone, pyridinyl and trifluoromethoxyphenyl moieties of SL-2-25 are labeled 'Qu', 'Py' and '$PhOCF_3$' respectively.

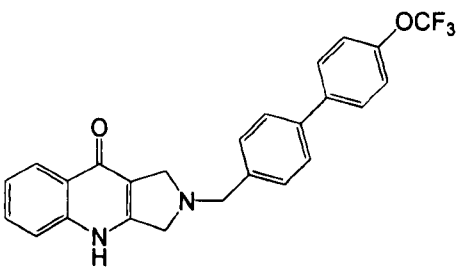
Therefore, based on the promising potency profile of the SL-2-25 lead (**32**), this class of quinolone compounds merit further development. Whilst the lead series has properties considered drug-like, the poor solubility of these analogues provide the final major hurdle for providing a significant and viable alternative to atovaquone.

5.3 Quinolones targeting *Pfbc$_1$* complex.

The second aim of this thesis was to explore the SAR of *bc$_1$* targeted quinolones based on two regioisomeric quinolone templates and to identify the possibility of identifying a lead for optimisation.

In the first part, a small library of 6-substituted quinolone esters were synthesised in 4 steps with good yields. Although SAR and *in silico* studies for the 6- and corresponding 7-series concluded that the antimalarial activity of quinolone with substitution at the 7-position is superior to the corresponding 6-position, 6-benzyl quinolone ester showed potent antimalarial activity in nanomolar range (IC_{50} 40.4 nM). Perhaps SAR of this class of quinolone esters should be further investigated with different side chains or substitutions on other positions of quinolone and effort should be made to improve the solubility of the quinolone.

In the second part, two pyrrolidine-fused quinolones with N-biary side chain were prepared in 4 steps using Winderfeldt Oxidation. While the solubility of the quinolone is the main issue, quinolone **102** (Chapter 3) showed a better activity against the 3D7 strain of *P. falciparum* with IC_{50} of 34 nM than the corresponding monoaryl analogue. The antimalarial activity of quinolone **102** makes this pyrrolidine-fused template attractive for future development. Future work should focus on the investigation of the SAR while attempt should be made to improve the yield of Winderfeldt Oxidation and improve the solubility of this class of quinolones.



102
IC_{50} 3D7 34 nM

5.4 Pyrrole mannich base Artermisinin derivatives

Artemisia annua has a long history in the treatment of fever even through its active ingredient, artemisinin, was only identified in 1971 in China.[3] Artemisinin has a high therapeutic value, however, it has a poor solubility in oil and water. Although many semi-synthetic artemisinin derivatives, such as artemether and artesunate, with improved aqueous solubility have been developed, most of them are easily hydrolysed back to dihydroartemisinin. Dihydroartemisinin is also a potent antimalarial, however, it has an elimination half life of 40 – 60 minutes when it is converted to inactive metabolites by hepatic cytochrome P-450 and other enzymes.[4-5]

The third aim of this thesis was to synthesise a series of derivatives of artemisinin with improved activity and stability. This aim also was successfully achieved as a series of C-10 pyrrole mannich base derivatives of artemisinin were designed and synthesised in 2 steps with excellent yields. The incorporation of pyrrole at the C-10 position of the artemisinin can prevent its hydrolysis to dihydroartemisin, therefore it is more metabolically stable. Also, several compounds among the series show a 3-fold more increase in potency in comparison to artemisinin. The lead compound **7b** (Chapter 4) which has a H-pyrrole and morpholine mannich side chain demonstrates the best activity both *in vitro* and *in vivo* (Figure 2). This molecule is undergoing additional evaluation at the time of writing this thesis.

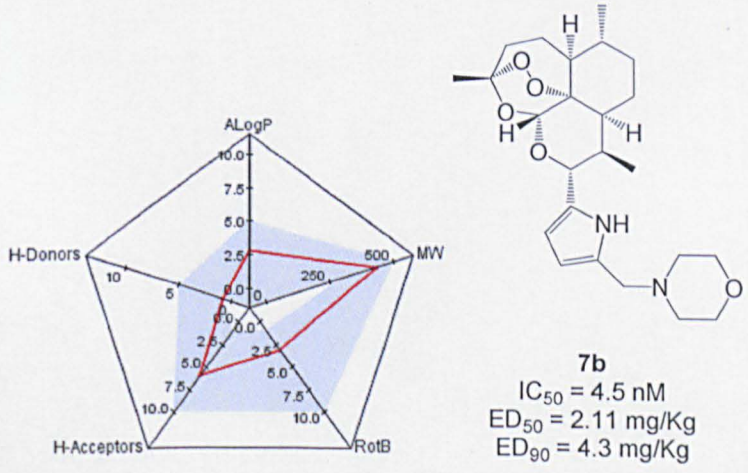


Figure 2. Radar plot of physicochemical properties and structure of the lead artemisinin derivative **7b**.

5.5 References

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