**Synthesis, Antiviral Activity, Preliminary Pharmacokinetics and Structural Parameters of Thiazolide Amine Salts**

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**Abstract**

**Background**

The thiazolides, typified by nitazoxanide (NTZ), are an important class of antiinfective agents. A significant problem with NTZ and its active circulating metabolite tizoxanide (TZ) is their poor solubility. **Results** We report the preparation and evaluation of a series of amine salts of tizoxanide (TZ) and the corresponding 5-Cl thiazolide. These salts demonstrated improved aqueous solubility and absorption as shown by physicochemical and *in vivo* measurements. They combine antiviral activity against influenza A virus with excellent cell safety indices. We also report the X-ray crystal structural data of the ethanolamine salt. **Conclusions** The ethanol salt of TZ retains the activity of the parent together with an improved cell safety index, making it a good candidate for further evaluation.

**Introduction**

**General**

Nitazoxanide [NTZ; 2-[(acetyloxy)-*N*-(5-nitro-2-thiazolyl)] benzamide] **1a**, Figure 1, was first reported in 1976 by Rossignol and Cavier [1] ; it resembled the known antiinfective agent niclosamide **2** [2], replacing the anilide by a nitrothiazolyl amide and showed promising antiparasitic activity *in vitro* and *in vivo*. Originally NTZ **1a** was developed for the treatment of protozoal and helminth parasitic infections [3-5], but later its most important application became the treatment of *Cryptosporidium* spp. infections [6,7]: to this day, it is the only FDA-approved treatment for *Cryptosporidium parvum*. It has been established from studies of its antiparasitic activity that one important mode of action of NTZ **1a** is inhibition of the folding chaperone protein disulfide isomerase [8]. NTZ **1a** also has valuable antibacterial activity against both aerobic and anaerobic species, operating by inhibition of pyruvate oxidoreductases in the case of anaerobes [9,10].



 **1a** R = COCH3 **2 3a** R = COCH3

 **1b** R = H **3b** R = H



 **4 5**

**Figure 1. Thiazolide Structures and Niclosamide**

**Thiazolides as Antiviral Agents: SAR and Breadth of Spectrum**

An exciting new chapter in the development of NTZ **1a** began with the discovery of its antiviral activity, during the course of treating cryptosporidiosis in patients with AIDS [11]. The first clinical trial with NTZ **1a** as an antiviral agent was against rotavirus-induced diahorrea [12], including young children as patients. Indeed, NTZ **1a** proved to be a broad- spectrum antiviral, also highly effective against hepatitis B, hepatitis C and influenza A virus, and has performed well in clinical trials [13-16] against all these viruses.

The nitro group is not essential for activity: the 5´-Cl analogue **3a** has an almost parallel spectrum of activity, at low micromolar values [17], and the 4´-ethanesulfonyl analogue **4** shows excellent *in vitro* activity against an H1N1 strain of influenza A virus, IC50 = 0.14 M. We have reported a number of 4´-/5´-substituted thiazolides, which show low micromolar activity against hepatitis B and C and influenza A [18-20].

In addition to the clinical trials noted above, NTZ **1a** and its active circulating metabolite tizoxanide **1b** exhibit broad-spectrum activity against many classes of viruses. Thus **1a** and **1b** were reported to be effective in cell culture assays against a broad range of ten classes of RNA and DNA viruses, namely the *Orthomyxoviridae* [(influenza A and B viruses) (IAV and IBV)], the *Paramyxoviridae* [parainfluenza Sendai virus (SeV) and respiratory syncytial virus A-2 (RSV)], the *Picornaviridae* (human rhinovirus type 2) and the *Coronaviridae* [canine coronavirus S-378 (CCoV)], the *Retroviridae* [(human immunodeficiency virus (HIV)], the *Flaviviridae* [Hepatitis C virus (HCV), Japanese Encephalitis Virus (JEV), the Yellow Fever virus 17DD (YFV), the Dengue fever virus -2 (DFV), the Zika virus (ZIKV)], the *Togaviridae* [chikungunya virus, Sindbis virus (SINV) and the Semliki Forest virus (SFV)], the *Reoviridae* [Simian A/SA11-G3P[2] and human Wa-G1P[8] rotaviruses], the *Caliciviridae* (norovirus), and the *Herpesviridae* (herpes simplex virus (HSV-1 and -2) [16].

More recently Wang et al. showed that nitazoxanide was effective against the emerging coronavirus SARS-CoV-2 (Covid-19) [21]. With or without safe vaccines, a chemoprophylactic treatment would be extremely useful. Two large clinical studies with nitazoxanide against SARS-CoV-2 in a total of 1,800 subjects have been recruited in the US among populations at risk testing the effect of the drug on post-exposure prophylaxis in health care workers and residents of the nursing homes [22, 23].

**Mode of antiviral action of NTZ**

Nitazoxanide and tizoxanide are modulators of mitochondrial activity by uncoupling oxidative phosphorylation. Studies indicate that tizoxanide decreases cellular ATP in a dose-dependent manner in MDCK cells infected with the influenza viruses. Maximum inhibition of ATP in influenza infected or uninfected MDCK cells reaches up to 45% after 24 hours of exposure to 100 µM tizoxanide. In these experiments a 10% reduction of ATP results from adding less than 10 µM of tizoxanide, sufficient to inhibit influenza virus replication by approximately 90%. The decrease in cellular ATP does not affect cell viability and is reversible after eliminating tizoxanide from the culture [24]. Studies of a number of different viruses have shown viral replication is ATP-dependent [25-29]. In the case of nitazoxanide, key viral proteins like hemagglutinin (influenza) and F-protein (RSV and parainfluenza) have been identified as the drug target [30, 31]. In addition, inhibition of ATP and its downstream effect on AMP-activated protein kinase activation has been shown to suppress secretion of pro-inflammatory cytokines [32-34]. We have very recently discovered that NTZ shares with other drugs the ability to inhibit viral syncytia formation induced by the SARS-CoV-2 spike protein [35]; furthermore, it also enhances RNA sensor activity [36, 37].

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 **6 7**

**Figure 2.** Examples of prodrug ester and salt formulations.

**Pharmacokinetic Characteristics of Nitazoxanide**

NTZ **1a** is administered orally but is only partially absorbed from the gastro-intestinal tract [38]. It is effectively a prodrug for the deacetyl derivative tizoxanide **1b**, which is formed immediately on absorption and subsequently excreted from the body largely as the *O*-glucuronide **5** [39]: **1a** has a plasma half-life of 1.3h. Such a biodisposition is perfectly acceptable for intestinal infections, but to achieve adequate systemic circulation of **1a/1b** for viruses such as influenza A is a major challenge. We have described the synthesis and evaluation of prodrug amino-acid esters such as **6**, Figure 2, which greatly improve the absolute oral bioavailability of **1a**, to about 20% in the case of **6** [40].

 Another approach to improve the pharmacokinetic parameters of **1a/1b** is to administer the active agent as an amine salt. Tizoxanide **1b** is relatively acidic, with *both* OH and NH protons being removed at pH 9 and above, and is reported to have a first pKa = 6.7 [41]. Ethanolamine proved to be a suitable amine for generation of a stable salt of **1b**: the beta-oxygen reduces its pKa compared with ethylamine (viz. 9.5 as opposed to 10.5), and crucially it is very well tolerated *in vivo*. The corresponding ethanolamine salt **7** [42], Figure 2, of niclosamide **2** is at least as well tolerated as the parent, with no adverse long-term effects seen after one year’s oral administration at high doses in rats and dogs [43, 44]. In parallel with NTZ **1a**, niclosamide **2** is excreted primarily as its *O*-aryl glucuronide but its pharmacokinetic profile is less favourable overall owing to oxidative metabolism, generating the 3-OH derivative [45].

**Materials & methods/experimental**

**Chemistry**

1H and 13C spectra were obtained on a Bruker 400MHz instrument (100MHz for 13C spectra) equipped with a multinuclear 5-mm BBFO probe. 1H spectra (at 400.13 MHz) and 13C(1H) spectra (at 100.61Mhz) were acquired at ambient temperature using standard parameters set; solvent resonances were used for referencing purpose.

Low- and high-resolution mass spectra were obtained by direct injection of sample solutions into a Micromass LCT mass spectrometer operated in the electrospray mode, by +ve or -ve ion as indicated (Micromass LCT Waters Micromass UK Ltd, Manchester, UK).

HPLC chromatograms were obtained on an Agilent 1200 instrument, equipped with UV (DAD) and MS (ESI +/-) detectors. Sample were eluted with a gradient water/acetonitrile (with formic acid buffer) through an Ascentis Express column (C18, 100 x 4.6mm, 2.7 µm, from Supelco) equipped with a column guard.

*2-Hydroxybenzoyl-N-[(5-nitro)thiazol-2-yl]amide, Ethanolamine Salt* ***8***

Tizoxanide **1b** (0.53 g, 2 mmol) was suspended in methanol (MeOH, 20 ml) containing ethanolamine (0.15 mL). The suspension was warmed to 50°C for a few minutes, giving a virtually clear yellow solution which was filtered and concentrated to 5 mL when crystallization readily began. Diethyl ether (Et2O, 5 mL) was added and the mixture was cooled to 0°C to complete crystallization. Filtration, washing with Et2O containing a little MeOH and drying afforded the title salt **8** (0.49 g, 75%) as a yellow crystalline solid, mp 158-160°C (decomposition); Found: C, 44.1; H, 4.2; N, 17.35; S, 9.8. C12H14N4O5S requires C, 44.2; H, 4.3; N, 17.2; S, 9.8%; 1H NMR [400 MHz, (CD3)2SO]  2.86 (2H, t, CH2CH2), 3.57 (2H, t, CH2CH2), 5.20 (1H, br s, OH), 6.81 (2H, m, ArH), 7.32 (1H, m, ArH), 7.67 (3H, br s, NH3+), 7.91 (1H, m, ArH), 8.51 (1H, s, 4´-H) and 14.71 (1H, br s, NH); 13C NMR [100 MHz, (CD3)2SO]  41.6, 57.9, 117.5, 118.2, 119.9, 130.1, 133.4, 137.9, 145.9, 161.3, 171.6 and 172.2; m/z (-ve ion electrospray mode) 264 [(M-H)]. Found: m/z, 264.0092. C10H6N3O4S requires m/z, 264.0085.

*2-Hydroxybenzoyl-N-[(5-chloro)thiazol-2-yl]amide, Ethanolamine Salt* ***9***

This salt was prepared similarly to **8** using RM4848 **3b** (0.51 g, 2 mmol), giving the product **9** (0.48 g, 76%); Found: C, 45.7; H, 4.5; N, 13.35; S, 10.15. C12H14ClN3O3S requires C, 45.6; H, 4.5; N, 13.3; S, 10.15%; 1H NMR [400 MHz, (CD3)2SO]  2.86 (2 H, t, CH2CH2), 3.58 (2H, t, CH2CH2), 5.20 (1 H, br s, OH), 6.67-6.73 (2 H, 2m, ArH), 7.20 (1 H, m, ArH), 7.23 (1 H, s, 4´-H) and 7.83 (1 H, dd, ArH); the NH3+ appears as a very broad signal centred at  7.65; 13C NMR [100 MHz, (CD3)2SO]  41.7, 58.0, 114.3, 116.8, 117.4, 120.4, 129.4, 132.1, 135.3, 162.4, 164.5 and 169.5; m/z (-ve ion electrospray mode) 253 [(M-H)]. Found: m/z, 252.9849. C10H635ClN2O2S requires m/z, 252.9844.

*2-Hydroxybenzoyl-N-[(5-nitro)thiazol-2-yl]amide, Morpholine Salt* ***10***

This salt was prepared similarly to **8** from tizoxanide **1b** (0.53 g, 2 mmol) and morpholine (0.24 mL), giving **10** as a yellow microcrystalline solid (0.66 g, 94%); Found: C, 47.7; H, 4.6; N, 16.0; S, 9.2. C14H16N4O5S requires C, 47.7; H, 4.6; N, 15.9; S, 9.1%; 1H NMR [400 MHz, (CD3)2SO]  3.11, 3.75 (8 H, 2m, 2xCH2CH2), 6.82 (2 H, m, ArH), 7.31 (1 H, t, ArH), 7.91 (1 H, m, ArH) and 8.51 (1 H, s, 4´-H); 13C NMR [100 MHz, (CD3)2SO]  43.4, 63.8, 117.5, 118.2, 119.8, 130.1, 133.4, 138.0, 145.9, 161.3, 171.5 and 172.1.

For the synthesis and characterisation of compounds **11-16** and details of the *in vivo* pharmacokinetic methods used, please see Supporting Information.

**Antiviral assay**

**Cell Culture and Treatments**

The following cell lines were used for antiviral assays: human A549 alveolar type II-like epithelial cells (American Type Culture Collection, ATCC), Madin-Darby canine kidney (MDCK) cells (ATCC) and African green monkey (*Cercopithecus aethyops sabeus*) kidney (AGMK) 37RC cells (a kind gift from Arrigo Benedetto, Centre of Virology, OO.RR., Rome, Italy) [46]. Cells were grown at 37oC in a 5% CO2 atmosphere in RPMI-1640 medium (LONZA-CAMBREX, Basel, Switzerland), supplemented with 10% fetal calf serum (FCS), 2 mM glutamine and antibiotics. Tizoxanide and RM-5071 **8** (Romark Laboratories, L.C.), dissolved in DMSO stock solution, were diluted in culture medium, added to infected cells after a one-hour virus adsorption period, and maintained in the medium for the duration of the experiment. Controls received equal amounts of DMSO vehicle, which did not affect cell viability or virus replication.

Cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to MTT formazan conversion assay (Sigma-Aldrich), as described [47]. The 50% cytotoxic dose (CC50) was calculated using Prism 5.0 software (Graph-Pad Software Inc.).

**Virus preparation, infection and titration**

Two influenza A virus (IAV) strains, A/PuertoRico/8/1934(H1N1) (PR8) a prototype strain of the H1N1 IAV subtype, and A/Wisconsin/1933 (H1N1) (WSN), and the parainfluenza Sendai virus (SeV) were utilized for this study. Viruses were grown in the allantoic cavity of 10-day-old embryonated eggs, and titers were determined by plaque-assay, as described previously [31, 48].

Confluent cell monolayers were mock-infected or infected with PR8, WSN and SeV viruses for 1h at 37°C at a multiplicity of infection (MOI) of 2.5 HAU (hemagglutinating units)/105cells, as described [31, 48].After the adsorption period, the viral inoculum was removed and cells were treated with different concentrations (0.01, 0.1, 1, 10, 50 and 100 mg/ml) of RM-5071***8,*** tizoxanide **1b** or vehicle and maintained at 37°C in RPMI-1640 medium containing 2% FCS for 24 h*;* in parallel, cell viability was determined in mock-infected cells by MTT-assay, as described above. Virus yield was determined 24h post infection (p.i.) by HA-titration, as described [48]. Compounds IC50 (50% inhibitory concentration) and IC90 (90% inhibitory concentration) were calculated using Prism 5.0 software.

**Results and Discussion**

**Synthesis and Solubility of Thiazolide Amine Salts**

 We now report the synthesis of a set of amine salts of thiazolides **1b** and RM4848 **3b**, their characterisation and selected pharmacokinetic data. In general alcohols were suitable salts for the salt formation, though individual solubilities varied significantly. Thus when tizoxanide **1b** was heated in methanol with a slight excess of ethanolamine for about 0.25 h, an almost clear solution was obtained. Filtration followed by concentration led to crystallisation of the desired salt **8**; after dilution with diethyl ether, cooling and filtration, **8** was obtained in good yield and excellent microanalytical purity. The 1H NMR showed a characteristic upfield shift of the aryl protons, consistent with the anionic nature of the thiazolide. Similarly, RM4848 **3b** afforded salt **9**. Figure 3 summarises a total of nine salts made similarly, employing hydroxyamines, morpholine and *N*-methyl piperazine.



**Figure 3**. Thiazolide amine salts prepared.

Some significant differences were noted with specific thiazolide/amine combinations. Thus the morpholine salt **10** was obtained in the normal manner from **1b**, but when RM4848 **3b** was used the first solid to precipitate was unreacted **3b**. Concentration of the filtrate led to the desired salt **11**, inevitably in rather low yield but still microanalytically pure. Salts **12** to **15** were similarly obtained using1-aminopropanol (**12**, **13**) and diethanolamine (**14**, **15**). Diamines proved more difficult to handle, and from piperazine we could not easily obtain pure salts; piperazine is a solid and difficult to remove by recrystallisation. However, from *N*-Me piperazine, a liquid, and **1b** we could obtain the salt **16** although in low yield; the site of protonation in this case was not determined. The amino-acid L-lysine did not give an isolable salt from either **1b** or **3b**. Naturally the scope of possible amines is very large and only a selection is shown; hydroxyamines and diamines were attractive because of the prospect of better water solubility, and in particular ethanolamine is very well tolerated *in vivo* as noted above.

The improved solubility of **8** over **1b** is illustrated for a variety of solvents in Table 1, where the data were obtained at a concentration of 1mg/mL in each case. Quantitatively, the aqueous solubility of **8** is about 100mg/L; that of **1b** is <10mg/L.

**Table 1**. The comparative qualitative solubilities of salt **8** and parent **1b** in various solvents, at 1mg/mL and 20°C.

|  |  |  |
| --- | --- | --- |
| Solvent | Compound **8** | Compound **1b**  |
| DMF | 1 |  |
| DMSO |  |  |
| MeCN | 4 | x |
| MeCN: H2O, 35:65 | 2 |  |
| MeOH | 3 |  |
| i-PrOH |  |  |
| EtOH |  |  |
| H2O |  | x |

 1 Readily soluble; 2 Soluble; 3 Almost soluble (complete dissolution requires a few hours without stirring); 4 Poorly soluble; x = Insoluble.

**Large-scale synthesis of ethanolamine salt 8**

The synthesis of ethanolamine salt **8** was successfully scaled up to an industrial process. It consists of a two-step synthesis (80 % yield) starting from FDA approved drug NTZ **1a**, following our published deacetylation procedure to convert **1a** into **1b** [39]. A pre-technical batch of **8** was prepared yielding 40 kg of pure material. Using production plant equipment, we demonstrated our process is reliable for large-scale manufacturing. Analytical development is ongoing following ICH guidelines to allow full characterization of **8** and definition of drug substance specification. Subsequently production of GMP validation batches is planned within the scope of a new drug application (NDA).

**Animal Pharmacokinetics of Thiazolide Amine Salts RM-5071 8 and RM-5072 10**

As we noted in 1.3, NTZ **1** is poorly absorbed following oral administration in animals and humans, behaving as a pro-drug for tizoxanide **1b** and glucuronide **5**. Absorption is significantly affected by food, and there is significant intra- and inter-subject variability in concentrations of **1b** and **5**. Two new salts of **1b**, synthesized as above, RM-5071 **8** and RM-5072 **10**, were tested to show a potential improvement in the bioavailability of **1b** and **5** following oral administration. The median concentrations of **1b** and **5** attained over a 12h period are shown in Figures 4 and 5 respectively. For details of the administration protocol please see Supporting Information.

Designation: RM-5071 = **8**, RM-5072 = **10**, NTZ = **1a**

**Figure 4.** Median concentrations of **1b** (µg/mL) in plasma over 12 hours.

Designation: RM-5071 = **8**, RM-5072 = **10**, NTZ = **1a**

**Figure 5.** Median concentrations of **5** (µg/mL) in plasma over 12 hours.

Because some animals convert **1b** into **5** faster than others, we evaluated the extent, rate and variability of absorption of these three compounds using the sum of free phenol **1b** and glucuronide **5** concentrations at each time point. To arrive at the concentrations of glucurono-conjugated T, we multiplied TG concentrations by 61% (molecular weight of T = 270 divided by molecular weight of TG = 441). The sums of median **1b** plus **5** concentrations in plasma over the 12 hours post-dose are presented in Figure 6.

**Figure 6.** Sum of median **1b** and **5** concentrations (µg/mL) in plasma over 12 hrs.

The mean AUC0-12h for **8** was almost double that of **10**, but it was roughly the same as that for **1a**. The comparison of AUC0-12h with **1a** is affected by the collection of only one plasma sample (the 6 h sample) between 2 and 12 hours and the fact that **1a** is absorbed more slowly than the other compounds. The actual AUC0-12h value for **1a** would likely have been much lower had additional samples been collected, particularly between the 6 and 12 h post-dose timepoints. Notably, the RSD associated with the mean AUC0-12h for **8** was only 16% compared to 36% for both **10** and **1a**. This indicates that the inter-subject variability of absorption associated with NTZ **1a** is significantly improved by **8**.

**Antiviral activity of Thiazolide Amine Salt RM-5071 8**

In order to determine whether the thiazolide amine salt RM-5071 retains the antiviral activity of tizoxanide **1b**, we utilized three different models of *in vitro* RNA virus infection: human A549 alveolar type II-like epithelial cells infected with the Influenza A virus (IAV) A/WSN/1933(H1N1) (WSN) strain; Madin-Darby canine kidney (MDCK) cells infected with the IAV A/Puerto Rico/8/1934(H1N1) (PR8) strain; and African green monkey kidney (AGMK) 37RC cells infected with the paramyxovirus Sendai (SeV). Confluent cell monolayers were infected with the different viruses under single-step growth conditions, and treated with different concentrations (0.01, 0.1, 1, 10, 50 and 100 mg/ml) of RM-5071**8*,*** tizoxanide **1b** or vehicle immediately after virus adsorption, and virus yield was determined at 24 hours post infection (p.i.), as described in *Experimental;* in parallel, cell viability was determined by MTT assay in mock-infected cells. Both RM*-*5071 andtizoxanide **1b** did not affect viability at the effective concentrations in all cell types. RM-5071 **8**was found to have improved solubility in culture medium as compared to the parent compound tizoxanide **1b*,*** which could not be tested at concentrations higher than 50 mg/ml.The results, shown in Table **2**, demonstrate that RM-5071 **8** is more effective than tizoxanide **1b**, presenting higher Selectivity Indexes (SI) in all models tested.

**Table 2**. The comparative antiviral activity of salt **8** and parent **1b** in models of influenza and parainfluenza virus infection *in vitro*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Influenza A (PR8)****MDCK cells** |  | **Influenza A (WSN)** **A549 cells** |  | **Sendai virus (SeV)** **AGMK cells** |  |
|  |  **TIZ RM5071** |  |  **TIZ RM5071** |  |  **TIZ RM5071** |  |
|  **IC50** |  **0.3 0.3** |  |  **0.9**  | **1.0** |  **0.4**  |  **0.4** |
|  **IC90** |  **9.0 8.0** |  |  **7.9**  | **8.6** |  **2.0** |  **4.0** |
|  **CC50** |  **>50 >100**  |  |  **>50** | **>100** |  **>50** |  **>100** |
|  **SI** | **>166.6 >333.3** |  |  **>55.5** | **>100** |  **>125** |  **>250** |

IC50 and IC90 values, in μg/ml, were determined in MDCK, A549 and AGMK cells following infection with H1N1 A/PR/8/34 (PR8) and A/WSN/33 (WSN) influenza A viruses, and Sendai (SeV) paramyxovirus respectively, as described in *Experimental*. All antiviral assays were determined at least in duplicate and a standard deviation of ±20% applies. Cell viability was determined using the MTT assay (see *Experimental*) and used to calculate the CC50 values, which are also given in μg/ml.

AGMK: African green monkey kidney; CC50: 50% cytotoxic concentration; IC50: 50% inhibitory concentration; IC90: 90% inhibitory concentration; MDCK: Madin-Darby canine kidney; SeV: Sendai virus; SI: selectivity index = ratio CC50:IC50.

**X-Ray Structure of amine salt 8**



**Figure 7.** The X-ray single-crystal structure of amine salt **8**.

Ethanolamine salt RM5071 **8** crystallised in a form suitable for single-crystal X-ray structure determination, with the result shown in Figure 7. The ammonium group is held in a strong ionic bond with the amide carbonyl oxygen, while the phenol donates an H bond to the amide nitrogen. The C-O bond length is 1.263(5) Å and the C-N amide bond is 1.34(5) Å, the latter value being fairly typical of a secondary amide C-N. A published X-ray single crystal structure of tizoxanide [49] shows the same *syn*-orientation of the OH group and amide nitrogen, but there the phenolic oxygen accepts an H bond donated by the amide NH; in this case the C-O bond length is shorter at 1.222Å and the C-N bond is longer, 1.383Å. Full structural details of **8** are given in the cif file (Supporting Information, q. v.).

**Conclusion**

By dissolution of thiazolides **1b** and **3b** with a range of amines in methanolic solution, stable amine salts of high purity and good crystalline form are obtained. We have studied the *in vivo* pharmacokinetic behaviour of the ethanolamine and morpholine salts RM-5071 **8** and RM-5072 **10**, derived from tizoxanide **1b**, in particular. Both **8** and **10** dramatically improve the speed of availability of tizoxanide **1b** in plasma compared to NTZ **1a**. These compounds are rapidly absorbed achieving Cmax within 5 minutes after an oral dose. RM-5071 **8** is associated with higher plasma concentrations of free and glucurono-conjugated **1b** and less variability of absorption than either RM-5072 **10** or NTZ **1a**. This study indicates that the rate, extent and variability of absorption is improved for RM-5071 **8** compared to RM-5072 **10** or NTZ **1a**. In addition RM-5071 **8** was found to be more effective than tizoxanide, presenting higher Selectivity Indexes in different models of *in vitro* RNA virus infection, including influenza A virus (PR8 and WSN strains) and the parainfluenza Sendai virus. The improved bioavailability offered by RM5071 compared to NTZ is promising for the wider use of thiazolides against non-gastrointestinal parasites and for antiviral indications.

**Future perspective**

The coronavirus pandemic has highlighted the need for small molecule drugs as antiviral agents. These will complement immunisation programmes and may be considered for both prophylactic and therapeutic roles. Nevertheless, poor solubility and absorption have restricted the use of many earlier generation antivirals. In the case of nitazoxanide (NTZ), where the absolute bioavailability is <5%, we have demonstrated that the ethanolamine salt, exploiting NTZ’s relatively high acidity, affords a much more soluble formulation. The good cellular tolerance of this salt, combined with maintenance of good antiviral activity and the broad SAR of the thiazolides, which we demonstrated previously, should make it a valuable antiviral agent which can be employed for many indications. We believe this derivative will maintain and enhance the value of thiazolides as antivirals.

**Summary points**

**Thiazolides as antiviral agents**

* The thiazolides, typified by nitazoxanide, are broad-spectrum antiviral agents.
* Tizoxanide is the active circulating metabolite of nitazoxanide in vivo.
* The SAR of thiazolides includes activity against respiratory diseases such as COVID-19.

**Amine salt derivatives**

* A number of amine salt derivatives of tizoxanide and its 5-chloro analogue have been prepared.
* The salts had greatly improved aqueous solubility compared to the parent drugs and good crystalline form.

**Biological data**

* The ethanolamine salt RM5071 of tizoxanide retained the activity of the parent and was slightly better tolerated in the MDCK cell line.
* RM5071 was rapidly absorbed, achieved higher plasma levels and showed less variability of absorption than the parent.

**Structural properties**

* RM5071 gave crystals suitable for X-ray structure determination.
* The structure shows the compound is a true salt with the thiazolide portion as an anion.

**Acknowledgements**

Separately recorded.

**Appendix A. Supplementary data**

Synthesis and characterization of compounds **11-16**; details of pharmacokinetic methods used; supplementary X-ray crystallographic data. The X-ray crystal structure has been deposited as CCDC-2056214.

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