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New *N*-Alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides as Antituberculous Agents with Improved Pharmacokinetics

Daniela Ubiali,[a] Giuseppe Pagani,[a] Massimo Pregnolato,[a] Claudio Piersimoni,[b] José L. Pedraz Muñoz,[c] Alicia Rodríguez Gascón[c] and Marco Terreni,[a,*]

[a]*Dipartimento di Chimica Farmaceutica, Via Taramelli 12, Università degli Studi, I-27100 Pavia, Italy*
[b]*Dipartimento di Microbiologia Clinica, Policlinico 'Umberto I-Torrette', I-60020 Ancona, Italy*
[c]*Laboratory of Pharmacy and Pharmaceutical Technology, University of the Basque Country, Vitoria, Spain*

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Abstract—Infections caused by multidrug-resistant *Mycobacterium tuberculosis* (MT) and non-tuberculous mycobacteria are difficult to treat and, indeed, new therapeutic agents are being sought. As a part of an ongoing research in our laboratories, novel *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides have been synthesized and evaluated against several strains of MT and *Mycobacterium avium* complex (MAC). The pharmacokinetics and relative bioavailability after intravenous administration of three derivatives have been investigated. Introduction of a hydroxyl or a tertiary amino group in the *N*-alkyl chain resulted in an improved pharmacokinetic profile without affecting sensitively the antituberculous potency.

Tuberculosis is a growing international health concern; it is the leading infectious cause of death in the world today.[1,2] Multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis* (MT) have emerged worldwide. Resistance has been described for all first-line drugs (isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin), and for several second-line and newer drugs (ethionamide, fluoroquinolones, macrolides, nitroimidazopyrans). Because MDR strains are the result of cumulative mutations, growth of MT can successfully be controlled in the host by concomitant treatment with more than one drug. Thus, treatment regimens that consist of three to four drugs are used routinely to treat patients with tuberculosis.[3]

Since the start of the acquired immunodeficiency syndrome (AIDS) epidemic, the role of *Mycobacterium avium* complex (MAC) as an opportunistic pathogen in advanced AIDS patients has become more and more clear. Treatment of MAC or disseminated MAC (DMAC) infections has historically been very difficult due to the inherent resistance of the MAC pathogen to most standard antimycobacterial agents. This has resulted in the development of new agents for the prevention of DMAC infection as well as combinations of both new and standard agents for its treatment.[4,5]

The search for more effective agents against MT and MAC is ongoing in an attempt to enhance survival and reduce morbidity, as proven by the high number of patents of new antituberculous drugs recently published.[6]

Preclinical data, such as in vitro measures of drug activity and pharmacokinetics, are used in the design of new treatment regimens.[7] Assessment of pharmacodynamic activity from standard in vitro minimum inhibitory concentrations (MICs) alone is insufficient to predict in vivo potency. Achievable serum and tissue concentrations as well as pharmacokinetic characteristics must be considered.[8]

Previous reports from our laboratories have identified the *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide structure (Fig. 1) as a novel class of potential antituberculous agents. Several derivatives have been recently synthesized and MICs for MT and MAC strains, either standard or isolated from infected patients, have been described.[9]

Particularly, among the more active substances, compounds **1** and **2** (ethyl and heptyl analogues, Fig. 1) were selected for a preliminary pharmacokinetic study.

*Corresponding author. Tel.: +39-0382-507265; fax: +39-0382-507589; e-mail: markt@pbl.unipv.it

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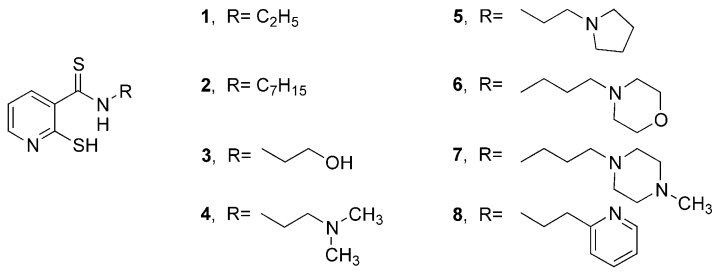


Figure 1. *N*-Alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides **1**–**8**.

The pharmacokinetic evaluation, performed after intravenous and oral administration to rats, resulted in a rapid elimination and a poor or absent absorption.[9] This means that neither **1** nor **2** have suitable properties for parenteral or oral administration. We have hypothesized that the very low levels detected following oral administration could be ascribed to a poor absorption but also to a significant first-pass metabolism. Several efforts were made in order to obtain new active compounds with a better pharmacokinetics; in this work we report the results of the evaluation of the pharmacokinetic profile after intravenous administration of more hydrophilic molecules structurally related to the reference compounds **1** and **2**. For this study, we have at first selected the *N*-hydroxyethyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide derivative **3**[9] (Fig. 1), which was only slightly less potent than compounds **1** and **2** (Table 1). Indeed, a good balance between in vitro activity and physical properties, particularly an increased water solubility, made compound **3** an interesting candidate to be studied. The pharmacokinetic evaluation of **3** was performed after iv administration to rats at a single dose of 20 mg/kg. The analysis was determined from plasma samples by HPLC according to a non compartmental method using the WINNONLIN program.[10,11] The data obtained, summarized in Table 2, show no sensitive changes in pharmacokinetic parameters for the new compound. Although clearance (5.59 ± 1.31 L/h kg) and distribution volume (4.17 ± 2.23 L/kg) are higher, mean residence time (0.72 ± 0.23 h) and half-life (0.67 ± 0.35 h) are similar to the reference molecules.

The improved hydrophilicity of **3** seems still to be insufficient to affect the pharmacokinetic profile. According to these results, attempts to improve the pharmacokinetics by manipulating the lipophilicity and polarity of the target compounds resulted in the design of novel derivatives bearing a tertiary amino group in the *N*-alkyl chain instead of an hydroxyl group (Fig. 1, compounds **4**–**8**).

These compounds are a good starting point in order to have more water soluble substances. Indeed, the corresponding hydrochloride salts (i.e., **4s**–**8s**) were easily prepared (Scheme 1) and then tested in vitro for their antimycobacterial activities. All compounds were generally more active against MT rather than against MAC as resumed in Table 1.

The first compound of this series (**4s**), which represents the *N,N*-(dimethylamino)ethyl derivative of the reference molecule **1**, has been selected for the pharmacokinetic study. This compound has been evaluated according to the same protocol described above for **3**. After intravenous administration of **4s**, the elimination half-life ($t_{1/2}$) was 2.72 ± 0.60 h (Table 2), about 4- and 6-fold higher than the values obtained for the reference molecules **1** and **2** previously investigated, and 4-fold higher in comparison with **3**. Thus, we concluded that, as far as the hydrophilicity increases (**2** < **1** < **3** < **4s**), the pharmacokinetic profile sensitively improves.

In order to have new hydrophilic compounds closely related to **4s** but endowed with a better in vitro activity, *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides **5s**–**8s** were synthesized. Compound **5s** can be considered as the result of the ring closure built upon the dimethylamino moiety of **4s**. The structures **6s**–**8s** were prepared to evaluate the effect due to the lenghtening of the

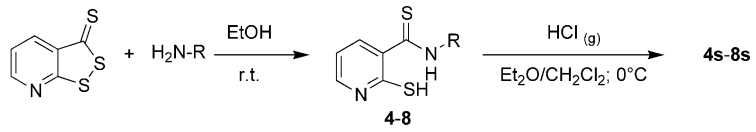
Table 1. In vitro anti-*M. tuberculosis* and anti-*M. avium* activity of compounds **1**–**3**[c] and **4s**–**8s** (MIC μg/mL)

Compd	MT ATCC H37-Rv	MT-1[b]	MT-2[b]	MAC ATCC[a] 15769	MAC ISS 486[b]	MAC AN1[b]	MAC AN2[b]	MAC AN3[b]
1[c]	0.5	1	1	4	4	4	8	8
2[c]	0.5	2	2	2	4	4	4	4
3[c]	2	4	8	8	8	4	8	4
4s	8	16	>16	>16	>16	>16	>16	>16
5s	8	16	>16	>16	>16	>16	>16	>16
6s	4	8	8	>16	>16	>16	>16	>16
7s	8	16	>16	>16	>16	>16	>16	>16
8s	4	4	1	4	8	8	8	8
Ethambutol	≤2	≤2	≤2	ND	2	1	0.5	2

ND, not done.
[a]std. strains.
[b]*Wild-type* strains (isolated from infected patients).
[c]MICs as reported in ref 9.

Table 2. Pharmacokinetic values of compounds **1**–**3**, **4s** and **8s** after iv administration to rats

Compd	$t_{1/2}$ (h)	Cl (L/h kg)	V_{ss} (L/kg)	MRT (h)	AUC_{0-8} (mg h/L)
1[a]	0.62±0.33	1.2±0.42	0.93±0.34	0.79±0.24	15.8±6.4
2[a]	0.47±0.26	3.0±1.8	1.28±0.51	0.48±0.24	8.25±6.4
3	0.67±0.35	5.59±1.31	4.17±2.23	0.72±0.23	3.73±0.79
4s	2.72±0.60	3.65±1.08	11.19±2.33	2.83±0.01	6.91±0.47
8s	2.22±0.54	8.66±1.25	14.51±5.46	1.77±0.97	4.70±0.78



Scheme 1.

N-alkyl chain and the replacement of the dimethylamino moiety with different heterocycles. Amongst the considered salts **5s**–**8s**, the most active compound **8s** was then selected for the pharmacokinetic evaluation. Data obtained for **8s** (Table 2) support again the importance of a polar moiety in the molecule to have an improvement of the bioavailability. In fact, the half-life for **8s** (2.22±0.54 h) was comparable with the value obtained for compound **4s** (2.72±0.60 h). Simultaneously, the compounds **3**, **4s** and **8s** have been tested for toxicity according to the protocol previously described[9] affording LD_{50} values >2000 mg/kg.

Compounds **4s**–**8s** were synthesized according to the following general procedure (Scheme 1). A mixture of 3*H*-[1,2]-dithiolo[3,4-*b*]pyridine-3-thione[12] and the appropriate amine (ratio 1:1.2) was stirred in absolute ethanol at room temperature for 1–2 h, the optimum reaction time being determined by TLC monitoring. After cooling, the resulting precipitate was filtered and washed with hot petroleum ether. The residue was recrystallized from the appropriate solvent and used for the next step. To the solution of the free base obtained (**4**–**8**) in dichloromethane, a solution of anhydrous diethyl ether saturated with HCl was added dropwise at 0 °C under magnetic stirring. The resulting precipitate (**4s**–**8s**) was filtered and recrystallized.

***N*-(2-Dimethylamino-ethyl)-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide hydrochloride (4s).** Yellow crystals (chloroform), mp 185–190 °C. ^{1}H NMR (DMSO-d_6): δ 3.00 (m, 2H, NH–CH_2–CH_2–), 3.50 (d, 6H, 2×CH_3), 4.24 (m, 2H, NH–CH_2–CH_2–), 7.00 (t, 1H, H-5), 7.99 (d, 1H, H-4), 8.24 (d, 1H, H-6), 10.60 (bs, 1H, NH), 11.60 (s, 1H, $^+$N–H), 14.10 (bs, 1H, SH). Elemental analysis ($C_{10}H_{16}N_3S_2Cl$): calcd C, 43.23; H, 5.80; N, 15.12. Found C, 43.02; H, 5.93; N, 15.44.

***N*-(2-Pyrrolidin-1-yl-ethyl)-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide hydrochloride (5s).** Yellow crystals (diethyl ether), mp 200–204 °C. ^{1}H NMR (DMSO-d_6): δ 1.88 (m, 4H, –CH_2–CH_2–), 3.26 (m, 4H, –H_2C–N–CH_2–), 3.65 (t, 2H, –NH–CH_2–CH_2–), 4.03 (t, 2H, –NH–CH_2–), 6.93 (t, 1H, H-5), 7.85 (d, 1H, H-4), 8.07 (d, 1H, H-6), 9.99 (bs, 1H, NH), 10.18 (s, 1H, $^+$N-H), 11.42 (s, 1H, SH). Elemental analysis ($C_{12}H_{18}N_3S_2Cl$): calcd C, 47.43; H, 5.97; N, 13.83. Found C, 47.50; H, 6.00; N, 13.64.

***N*-(3-Morpholin-4-yl-propyl)-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide hydrochloride (6s).** Yellow crystals (diethyl ether), mp 210–214 °C. ^{1}H NMR (DMSO-d_6): δ 2.02 (q, 2H, –CH_2–CH_2–CH_2–), 2.50 (t, 4H, –H_2C–N–CH_2–), 2.60 (t, 2H, –CH_2–CH_2–CH_2–N), 3.67 (t, 4H, –H_2C–O–CH_2–), 3.86 (t, 2H, –NH–CH_2–), 6.95 (t, 1H, H-5), 7.77 (dd, 1H, H-4), 8.78 (dd, 1H, H-6), 11.50 (bs, 1H, NH), 11.81 (s, 1H, $^+$N-H), 13.72 (s, 1H, SH). Elemental analysis ($C_{13}H_{20}N_3S_2OCl$): calcd C, 46.76; H, 6.04; N, 12.58. Found C, 46.89; H, 6.01; N, 12.56.

***N*-[3-(4-Methyl-piperazin-1-yl)-propyl]-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide hydrochloride (7s).** Yellow crystals (ethyl acetate), mp 215–220 °C. ^{1}H NMR (DMSO-d_6): δ 1.99 (q, 2H, CH_2–CH_2–CH_2–N), 2.27 (s, 3H, CH_3), 2.51 (m, 8H, CH_2×4), 2.62 (t, 2H, CH_2–CH_2–CH_2–N), 3.85 (t, 2H, NH–CH_2–CH_2–CH_2–), 6.95 (t, 1H, H-5), 7.79 (dd, 1H, H-4), 8.78 (dd, 1H, H-6), 11.62 (bs, 1H, NH), 11.90 (d, 2H, 2×$^+$N-H), 13.98 (s, 1H, SH). Elemental analysis ($C_{14}H_{24}N_4S_2Cl_2$): calcd C, 43.85; H, 6.31; N, 14.61. Found C, 43.92; H, 6.57; N, 14.60.

***N*-(2-Pyridin-2-yl-ethyl)-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide hydrochloride (8s).** Yellow crystals (chloroform), mp 190–193 °C. ^{1}H NMR (DMSO-d_6): δ 3.54 (t, 2H, NH–CH_2–CH_2–), 4.28 (t, 2H, NH–CH_2–CH_2–), 7.06 (m, 1H, H-5′), 7.95 (m, 2H, H-5′ and H-4′), 8.10 (d, 1H, H-3′), 8.31 (d, 1H, H-4), 8.50 (d, 1H, H-6′), 8.93 (d, 1H, H-6), 11.80 (s, 1H, NH), 12.12 (s, 1H, $^+$N-H), 13.99 (s, 1H, SH). Elemental analysis ($C_{13}H_{14}N_3S_2Cl$): calcd C, 50.07; H, 4.52; N, 13.47. Found C, 50.02; H, 4.67; N, 13.37.

Conclusions

This study was conducted to evaluate the in vitro antimycobacterial activity of novel *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides and particularly, to characterize the single-dose pharmacokinetics after iv administration of three derivatives of this series (**3**, **4s** and **8s**), in order to assess the effect of the introduction of a more hydrophilic functionality on the absorption in a animal model. Modulation of the physical properties of these compounds was achieved without affecting sensitively the antituberculous potency.

Pharmacokinetic properties have changed especially for **4s** and **8s**, being their half-lives about 4- and 6-fold higher than the values obtained for the reference molecules **1** and **2** previously investigated.[9] According to the experimental data, as far as the hydrophilicity increases (**2** < **1** < **3** < **8s** ≤ **4s**), the pharmacokinetic profile sensitively improves ($t_{1/2} = 0.47$ h < 0.62 h < 0.67 h < 2.22 h < 2.72 h).

Amongst the considered compounds, **8s** appears to possess desirable attributes for a promising candidate (good activity in vitro, absence of toxicity, reasonable pharmacokinetics). Consequently, further studies are now in progress in order to highlight also the metabolic pathways this compound undergoes after oral administration.

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