Synthesis and in Vitro Anti-*Mycobacterium* Activity of *N*-Alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides. Preliminary Toxicity and Pharmacokinetic Evaluation

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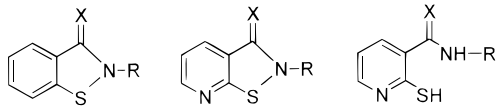
Disseminated infections with *Mycobacterium tuberculosis* (MT) and *Mycobacterium avium* complex (MAC) are increasingly opportunistic diseases in patients with advanced acquired human immunodeficiency syndrome (AIDS). A series of *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides has been synthesized, and MICs for MT and MAC strains, either standard or isolated from infected patients, have been determined. Preliminary tests show a good activity and a very low toxicity for some derivatives. Pharmacokinetic studies in the rat show a very rapid elimination from the body after intravenous administration and a poor absorption after oral administration.

Introduction

Among the several pathogens afflicting AIDS-infected patients, two groups of mycobacteria pose a significant problem to the clinical management: the *Mycobacterium tuberculosis* (MT) and the *Mycobacterium avium* complex (MAC).[1−3] Disseminated MAC (dMAC) infection is the most frequent complication[4,5] and occurs late in the course of AIDS when patients are severely immunocompromised.[6,7] Although several reports suggest that chemotherapy of dMAC infections may reduce symptoms and prolong survival, attempts to eradicate the infections have been unsuccessful.[3,8] Conventional antimycobacterial agents such as isoniazid, rifampicin, and pyrazinamide are inactive against almost all strains of MAC,[9] and single drugs such as rifabutin, ethambutol, and ciprofloxacin are inadequate for therapy.[10]

The most valuable group of antimicrobial agents availaible for the treatment of dMAC disease is represented by the new macrolides such as azithromycin, clarithromycin, and roxithromycin.[3] However, the selection of resistant mutants occurs at such a rate with macrolide therapy that at least two agents have to be used. Combinations of agents for dMAC treatment are highly variable in content, but all associations should contain one of the advanced generation macrolides.

The control of MT infections is still considered of relevance, not only for the survival of AIDS patients. The recent emergence of drug-resistant MT, in fact, also has become a serious concern. *Mycobacterium tuberculosis* becomes drug resistant through random, spontaneous genetic mutation, and administration of a single drug often leads to the development of a bacterial population resistant to that drug. Consequently, effective regimens for the treatment of MT also must contain multiple drugs. Generally, a four-drug regimen with isoniazid, rifampin, pyrazinamide, and ethambutol is preferred for the initial, empiric treatment of MT. This four-drug regimen is highly effective even for isoniazid-resistant organisms.[11]



I: X=O II: X=S III: X=O IV: X=S V: X=O VI: X=S

Figure 1. Structures of the considered compounds.

Because of the concern of the resistance to most of the commonly used drugs displayed by the considered mycobacteria, our studies have been focused on the development of new potential therapeutic agents. The 1,2-benzisothiazol-3(2*H*)-ones **I** (BITs) (Figure 1) are known to possess several biological activities, and the antimicrobial properties have been largely described.[12−14] Some *N*-hydroxyalkyl derivatives of BIT have been claimed to possess in vitro antibacterial activity against MT and other mycobacteria.[15] Recently, our interest in 1,2-benzisothiazol-3(2*H*)-ones and their corresponding thiono derivatives **II** has increased,[16] and the reported antibacterial activities have enhanced the development of new derivatives. A series of *N*-(2-hydroxyethyl)-1,2-benzisothiazol-3(2*H*)-one and -thione carbamic esters have been recently synthesized and tested against *Mycobacterium avium* strains.[17]

In a preliminary approach, our attention has been directed to the synthesis of bioisosteric derivatives of

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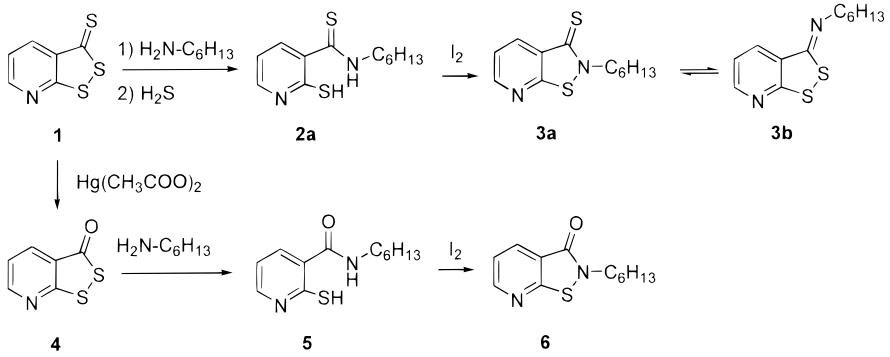
Scheme 1



BIT in which the benzene ring is replaced by a heterocycle such as pyridine. Thus, *N*-alkylisothiazole[5,4-*b*]-pyridin-3(2*H*)-ones **III** and thiones **IV**, reported in Figure 1, have been synthesized. Compounds **III** and **IV** and their intermediates of synthesis (**V**, **VI**) have been evaluated in vitro against MAC (ATCC 15769) and MT, either standard (H37-Rv ATCC) or isolated from blood of infected patients (MT-1, MT-2). For the most active structure **VI**, the study has been enlarged to several *N*-alkyl derivatives in order to evaluate the effect of the *N*-alkyl side chain on anti-MAC and anti-MT activities toward both standard and wild strains. The most interesting substances have been preliminarily evaluated for the in vivo toxicity and pharmacokinetic studies in the rat.

Results and Discussion

The first study on the bioisosteric isothiazole[5,4-*b*]-pyridin-3(2*H*)-ones and related compounds (Figure 1: **III**−**VI**) has been conducted choosing the *n*-hexyl group for the *N*-substitution in order to prepare model derivatives for anti-mycobacteria tests. The synthesis has been performed by reacting the 3*H*-1,2-dithiolo[3,4-*b*]pyridine-3-thione **1** and the 3*H*-1,2-dithiole[3,4-*b*]pyridin-3-one **4** with *n*-hexylamine. The obtained *N*-hexyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide **2a** and *N*-hexyl-1,2-dihydro-2-thioxo-3-pyridinecarbamide **5** have been cyclized by iodine treatment to give the *N*-hexylisothiazolo[5,4-*b*]pyridine-3(2*H*)-thione **3a** in equilibrium with its isomer *N*-hexyl-3-imino-3*H*-1,2-dithiolo[3,4-*b*]pyridine **3b**[18,19] and the *N*-hexylisothiazolo[5,4-*b*]-pyridine-3(2*H*)-one **6**, respectively (Scheme 1).

The in vitro activity of all synthesized compounds **1**−**6** on mycobacteria strains has been determined by the radiometric broth dilution method, and results are reported in Table 1. Although it is not difficult to isolate the two isomeric forms **3a** and **3b**, an equilibrium between the two structures easily occurs upon being dissolved in polar solvent (DMSO, DMF, acetone, water).[18,19] As a consequence, the biological tests for **3a** and **3b** have been performed on the isomeric mixture.

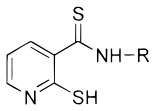
The results show that the pyridinecarbothioamide **2a** displays the best activity (MICs: 1−2 μg/mL). Compounds **1** and **4** (MICs: 8−16 μg/mL and 2−8 μg/mL, respectively), the oxo-analogue pyridinecarbamide **5** (MICs: 8−16 μg/mL), the cyclic compounds **3a**−**3b** (MICs: 4−8 μg/mL), and **6** (MICs: 8−16 μg/mL) are, in fact, less active.

As reported in a previous study,[17] the substitution of sulfur for oxygen in the ketobenzisothiazole system

Table 1. In Vitro Anti-*M. Avium* and Anti-*M. Tuberculosis* Activities of Compounds **1**−**6** (MIC μg/mL)

compd	MAC ATCC 15769[a]	MT H37-Rv ATCC[a]	MT-1[b]	MT-2[b]
1	8	16	8	8
2a	1	1	1	2
3a,b[c]	8	4	8	8
4	2	4	8	8
5	8	8	8	16
6	16	16	8	8
ethambutol	ND[d]	≤2	≤2	≤2

[a] Standard strains. [b] Wild-type strains (isolated from infected patients). [c] The biological test has been conducted on the isomeric mixture because of the rapid equilibrium of the single isomers in polar solvents.[17,18] [d] ND: not determined.



2a, R=C_6H_{13} **2h**, R=cyclohexyl
2b, R=CH_3 **2i**, R=C_7H_{15}
2c, R=C_2H_5 **2l**, R=C_8H_{17}
2d, R=$(CH_2)_2OH$ **2m**, R=C_9H_{19}
2e, R=C_3H_7 **2n**, R=$C_{10}H_{21}$
2f, R=C_4H_9 **2o**, R=$C_{16}H_{33}$
2g, R=C_5H_{11} **2p**, R=$C_{18}H_{37}$

Figure 2. *N*-Alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides **2a**−**2p**.

increases the lypophilicity and affects a modification on the electronic structure, suggesting a possible relationship with the improved biological activity. Comparing the MICs for compounds **5** and **6** with **2a** and **3a**−**b**, it is evident that the thioxo-analogues are generally more active against MAC and MT strains, in accordance with results previously reported.[18]

The preliminary microbiological data indicate that the *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide structure is the most active. Thus, the research has been focused on the study of the carbothioamidic moiety, and the effect of the *N*-alkyl substitution has been evaluated. Through the same procedure employed for the synthesis of the *N*-hexyl-2-mercapto-3-pyridinecarbothioamide **2a**, a series of primary alkylamines have been reacted with 3*H*-1,2-dithiole[3,4-*b*]pyridin-3-thione **1**. The reaction gave the 1,2-dihydro-2-thioxo-3-pyridinecarbothioamides **2a**−**2p** in 75−83% yields (Figure 2). ^{1}H NMR spectrum of compound **2a** shows the typical pyridinic signals at δ 6.95 (q, 1H, 5-H), 7.67 (q, 1H, 4-H), 9.42 (q, 1H, 6-H) and the multiplet of the

Table 2. In Vitro Anti-*M. Avium* and Anti-*M. Tuberculosis* Activities of Compounds **2a**−**2p** (MIC μg/mL)

compd	R	cLogP	MAC ATCC 15769[a]	MAC ISS 486[b]	MAC AN1[b]	MAC AN2[b]	MAC AN3[b]	MT H37-Rv ATCC[a]	MT-1[b]	MT-2[b]
2a	C_6H_{13}	3.66	1	0.5	1	1	1	1	1	2
2b	CH_3	1.58	4	4	8	4	4	2	1	1
2c	C_2H_5	1.92	4	4	4	8	8	0.5	1	1
2d	$(CH_2)_2$-OH	ND	8	8	4	8	4	2	4	8
2e	C_3H_7	2.41	2	1	2	4	2	2	4	4
2f	C_4H_9	2.83	1	2	4	4	4	4	4	4
2g	C_5H_{11}	3.24	1	1	1	2	1	2	4	4
2h	cyclohexyl	ND	4	4	4	4	4	0.5	4	1
2i	C_7H_{15}	4.08	2	4	4	4	4	0.5	2	2
2l	C_8H_{17}	4.50	4	8	8	4	4	1	4	4
2m	C_9H_{19}	4.91	8	8	8	8	8	4	16	16
2n	$C_{10}H_{21}$	5.33	16	32	32	32	32	16	64	64
2o	$C_{16}H_{33}$	7.83	8	8	8	8	8	16	16	32
2p	$C_{18}H_{37}$	8.67	64	32	32	32	32	32	64	64
ethambutol		ND	ND	2	1	0.5	2	≤2	≤2	≤2

[a] Standard strains. [b] Wild-type strains (isolated from infected patients). ND: not determined.

CH_2−NH group at δ 3,85 ($J_{NH-CH_2} = 5.0$ Hz) as well as in all prepared compounds.[18]

In vitro activities of the synthesized compounds versus *M. tuberculosis* (H37-Rv ATCC, MT-1, 2) and *M. avium* (MAC ATCC 15769, MAC ISS 486, MAC AN1, AN2, AN3) expressed as MIC (μg/mL) have been reported in Table 2. The biological results show a remarkable activity especially for compounds **2c** and **2i** against *M. tuberculosis* H37-Rv ATCC (MIC: 0.5 μg/mL); these substances result, however, as less active on the MAC strains (MICs: 4−8 μg/mL for **2c** and 2−4 μg/mL for **2i**). Compounds **2a** and **2b** exhibit a positive activity against *M. tuberculosis* (MICs: 1−2 μg/mL). Besides, compound **2a** has a good activity versus MAC wild strains (MICs: 0.5−2 μg/mL), even higher than that of ethambutol for MAC ISS 486 and MAC AN3. It is of relevance that **2a** also displays a comparable activity with the reference drug against all MT strains, resulting, indeed, in the most active compound both against MAC and MT strains.

To verify the existence of a correlation between the lipophilicity apported to the moiety by the different *N*-alkyl side chains and the biological activity, the relationship between hydrophobicity of the derivatives and anti-*Mycobacterium* activity has been evaluated. An attempt to find a relationship between the calculated LogP[20] (Table 2) and the −log MMIC (expressed in mmol/mL) of compounds **2a**−**2n** (chain length: C_1−C_{10}) for all strains considered resulted in a quadratic fit equation with *r* values higher than 0.92. The best correlation has been found for MAC ATCC 15769. The equation is reported below, and the curve fitting is depicted in Figure 3.

$$-\log \text{MMIC} = -0.2224(\text{LogP})^2 + 1.4341\ \text{LogP} - 0.03439$$
$$(r = 0.992;\ S = 0.113;\ n = 10)$$

The curves obtained present a maximum of antitubercular activity for clogP values between 2.4 and 4.1 (MICs: 0.5−4 μg/mL). Molecules having higher clogP values (chain length C_8−C_{18}) have lower activities, probably because of the interference of long alkyl chains with cellular lipophilic materials.

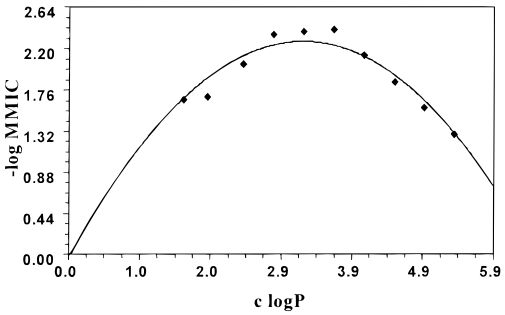


Figure 3. Plot of clogP vs −log MMIC for compounds **2a**−**2n**.

Preliminary Toxicity and Pharmacokinetic Evaluation

Among the more active compounds (**2a**−**l**), two derivatives have been selected for a preliminary toxicity and pharmacokinetic study. To evaluate the influence of the hydrophobicity properties on the pharmacokinetic profile, *N*-ethyl and *N*-heptyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides (**2c** and **2i** respectively) have been considered. These compounds have, in fact, different cLogP values (namely 1.92 for **2c** and 4.08 for **2i**) but similar and positive biological activity.

Toxicity. A single dose of compounds **2c** and **2i** was orally administered to rats and mice (5 males and 5 females/species/group/dose) at a dose ranging from 200 to 2000 mg/kg. Intraperitoneal administration was only performed at the dose of 200 mg/kg, being that a higher dose would not be applicable because of the low solubility of the compounds. Observations of mortality and anomalous clinical signs were made during 14 days but no clinical effects and mortality were observed.

According to these experimental conditions, LD_{50} after intraperitoneal administration of compounds **2c** and **2i** is higher than 200 mg/kg, for both mice and rats. No toxic effects were in fact observed at the dose of 200 mg/kg i.p. The lack of toxicity of the compounds following oral administration is probably due to their poor bioavailability by this route (see Pharmacokinetic Studies).

Pharmacokinetic Studies. The pharmacokinetic evaluation of **2c** and **2i** has been performed after intravenous and oral administration to rats (20 mg/kg). The analysis of **2c** and **2i** was performed from plasma samples, by gas chromatography−mass spectrometry. Further procedure details are reported in the Experi-

Table 3. Pharmacokinetic Values of **2c** and **2i** after i.v. Administration to Rats

compd	$t_{1/2\lambda}$ (min)	Cl (L/min)	V_{ss} (L)	MRT (min)	$AUC_{0-\infty}$ (mg min/L)
2c	37.18 ± 19.71	0.02 ± 0.007	0.93 ± 0.34	47.65 ± 14.52	1110 ± 384
2i	28.0 ± 15.6	0.05 ± 0.03	1.28 ± 0.51	28.9 ± 14.4	494.8 ± 193.7

mental Section, while the pharmacokinetic parameters are reported in Table 3. After intravenous administration of **2c**, the elimination half-life ($t_{1/2\lambda}$) was 37.18 min and the clearance (Cl) and steady-state volume of distribution (V_{ss}) were 0.02 L/min and 0.93 L/kg, respectively. The area under the plasma concentration−time curve from zero to infinity ($AUC_{0-\infty}$) was 1110 mg min/L, and the mean residence time (MRT) was 47.65 min. Concerning **2i** intravenous administration, the elimination half-life ($t_{1/2\lambda}$) was 28.0 min and the clearance (Cl) and steady-state volume of distribution (V_{ss}) were 0.05 L/min and 1.28 L/kg, respectively. The area under the plasma concentration−time curve from zero to infinity ($AUC_{0-\lambda}$) was 494.8 mg min/L, and the mean residence time (MRT) was 28.9 min. These data show that both compounds are rapidly eliminated from the body; however, the results obtained indicate that the less lipophilic compound **2c** shows a higher bioavailability, compared with the *N*-heptyl analogue **2i**, after intravenous administration.

After oral administration of **2c** and **2i** to the rats, drug levels in the plasma samples were under detection level. In some samples, the presence of the compounds was detectable under quantification levels.

Conclusions

All synthesized compounds generally display a positive activity against *Mycobacterium tuberculosis* and *Mycobacterium avium* complex strains, both standard (MAC ATCC 15769, MT H37-Rv ATCC) and isolated from infected patients (MAC ISS 486, MAC AN1, 2, 3; MT1, 2). The significant anti-mycobacterial activity observed indicate that these substances could be considered as important leads for new potential anti-*Mycobacterium* drugs development.

In vitro antibacterial activity of compounds **2a** and **2g** against MAC strains appears to be promising: MICs are considerably low, and these compounds deserve further attention. Activity against MT of compounds **2c** and **2i** is interesting. It is, indeed, very similar to the activity shown by ethambutol. However, in vitro drug susceptibility testing against first-line drug-resistant strains is mandatory to confirm good activity shown on drug-susceptible ones. Compound **2a** exhibits a broad anti-mycobacterial activity covering both MAC and MT. Furthermore, the preliminary studies of toxicity after intraperitoneal administration carried out for two derivatives, the *N*-ethyl and the *N*-heptyl compounds (**2c** and **2i**), are very favorable. However, the pharmacokinetic data obtained following intravenous administration of the two analogues to rats indicate that they are rapidly cleared (either hepatically or renally or both), while the data obtained after oral administration, appear to be not significative because of the very low levels detected. This means that neither the ethyl nor the heptyl derivatives (**2c** and **2i**, respectively) have suitable properties for oral or parenteral administration.

Since no urinary or fecal analyses or analyses for potential metabolites have been performed, it could be hypothesized that the compounds are well orally absorbed, but undergo significant first-pass metabolism, leading to poor or undetectable oral bioavailability. Oxidative metabolism of both sulfur substituents (as in thiobarbiturates and ethionamide) or oxidative *N*-dealkylation is highly likely. The higher bioavailability after intravenous administration of the less lipophilic *N*-ethyl derivative **2c**, in comparison with the *N*-heptyl analogue **2i**, indicates that an increased water solubility (or a decreased lipophilicity) is a likely desirable property in order to improve the pharmacokinetic profile. Further studies are in progress and will be the matter of forthcoming papers.

Experimental Section

Chemistry. All reagents are commercial reagent grade and were purchased from their suppliers and used without further purification. Melting points were measured using a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR were recorded at 300 MHz using a Bruker ACE-300 spectrometer in $CDCl_3$ or DMSO-d_6. ^{1}H chemical shift (δ) were reported with Me_4Si (δ = 0.00 ppm) as internal standard. The following abbreviations are used: br = broad, s = singlet, d = doublet, dd = double doublet, t = triplet, and m = multiplet. Mass spectra were obtained on a Finnigan MAT 8222 spectrometer via the direct inlet. Electron ionization was performed at 70 eV and 0.5 mA with a source temperature of 250 °C. Elemental analyses were within ±0.4% of the theoretical values and were performed on a Carlo Erba 1106 elemental analyzer. All reactions were monitored by thin-layer chromatography carried out on 0.25 mm Merck silica gel (60 F_{254}) and visualized by UV light (λ = 264 or 365 nm); flash chromatography was performed using silica gel 60 (60−200 μm, Merck). Compound **3a,b** was prepared as previously reported.[18]

General Procedure for 1,2-Dihydro-2-thioxo-3-pyridinecarbothioamide Derivatives, 2a−2p. A mixture of 3*H*-1,2-dithiolo[3,4-*b*]pyridine-3-thione **1** (16 mmol) and the appropriate amine (19 mmol) was refluxed in 150 mL of ethanol for 30−60 min, the optimum reaction time being determined by TLC monitoring (eluent: hexane/ethyl acetate). After cooling, the solvent was removed under reduced pressure and the residue was recrystallized from the appropriate solvent. Analytical data of compounds **2a**,**e**,**f** are consistent with those reported in the previous paper.[18]

***N*-Methyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2b.** This compound was obtained as yellow crystals (ethyl acetate/hexane), mp 130−133 °C: ^{1}H NMR ($CDCl_3$) δ 3.40 (d, 3H, CH_3, J = 5.0 Hz), 6.99 (dd, 1H, 5-H, $J_{4,5}$ = 6.0 Hz, $J_{6,5}$ = 7.5 Hz), 7.72 (dd, 1H, 4-H, $J_{4,5}$ = 6.0 Hz, $J_{6,4}$ = 1.5 Hz), 9.41 (dd, 1H, 6-H, $J_{6,5}$ = 7.5 Hz, $J_{6,4}$ = 1.5 Hz), 10.6 and 12.5 (br s, 2H, NH and SH); MS *m*/*z* (% ra) 184 (14), 182 (100), 118 (62), 103 (7), 91 (14), 78 (15). Anal. ($C_7H_8N_2S_2$) C, H, N.

***N*-Ethyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2c.** This compound was obtained as yellow crystals (ethyl acetate/petroleum ether), mp 150−152 °C: ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H, CH_3, J = 7.5 Hz), 3.90 (m, 2H, N−CH_2, J = 5.0 Hz), 6.98 (dd, 1H, 5-H, $J_{6,5}$ = 7.5 Hz, $J_{4,5}$ = 6.0 Hz), 7.70 (dd, 1H, H-4, $J_{4,5}$ = 6.0 Hz, $J_{6,4}$ = 1.5 Hz), 9.39 (dd, 1H, 6-H, $J_{6,5}$ = 7.5 Hz, $J_{6,4}$ = 1.5 Hz), 12.4 (br s, 2H, NH and SH); MS *m*/*z* (% ra) 198 (38), 196 (100), 181 (31), 168 (46), 155 (23), 137 (22), 104 (70), 77 (12). Anal. ($C_8H_{10}N_2S_2$) C, H, N.

***N*-Hydroxyethyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2d.** This compound was obtained as yellow crystals (2-propanol/ethanol/water), mp 151−153 °C: ^{1}H NMR (DMSO-d_6) δ 3.70 (m, 2H, N−CH_2, J = 5.0 Hz), 3.80 (m, 2H,

CH_2OH, $J = 5.0$ Hz), 4.85 (t, 1H, OH), 7.00 (m, 1H, 5-H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 7.90 (dd, 1H, H-4, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.5$ Hz), 8.55 (dd, 1H, 6-H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.5$ Hz), 12.01 (s, 1H, NH), 13.97 (s, 1H, SH); MS *m*/*z* (% ra) 214 (26), 196 (21), 181 (100), 168 (55), 152 (44), 137 (59), 104 (75), 92 (17), 77 (16). Anal. ($C_8H_{10}N_2S_2$) C, H, N.

***N*-Pentyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2g.** This compound was obtained as yellow crystals (ethyl acetate/hexane), mp 123−126 °C: ^{1}H NMR ($CDCl_3$) δ 3.90 (m, 2H, N−CH_2, $J = 5.0$ Hz), 6.95 (dd, 1H, 5-H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 7.67 (dd, 1H, 4H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.5$ Hz), 9.42 (dd, 1H, 6-H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.5$ Hz), 12.3 and 12.45 (br s, 2H, NH and SH); MS *m*/*z* (% ra) 240 (30), 205 (70), 181 (24), 168 (100), 155 (20), 137 (34), 104 (48), 77 (14), 55 (16). Anal. ($C_{11}H_{16}N_2S_2$) C, H, N.

***N*-Cyclohexyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2h.** This compound was obtained as yellow crystals (water), mp 151−155 °C: ^{1}H NMR (DMSO-d_6) δ 1.20−2.10 (m, 11H, cyclohexyl), 4.40 (m, 2H, N−CH_2, $J = 5.0$ Hz), 6.95 (dd, 1H, 5-H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 7.85 (dd, 1H, 4H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.5$ Hz), 8.50 (dd, 1H, 6-H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.5$ Hz), 12.10 (s, 1H, NH), 13.92 (s, 1H, SH); MS *m*/*z* (% ra) 250 (22), 168 (100), 137 (7), 104 (15), 83 (26), 77 (7), 55 (50), 41 (29). Anal. ($C_{12}H_{16}N_2S_2$) C, H, N.

***N*-Heptyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2i.** This compound was obtained as yellow crystals (ethanol/water), mp 106−108 °C: ^{1}H NMR ($CDCl_3$) δ 3.82 (m, 2H, N−CH_2, $J = 5.0$ Hz), 6.92 (br t, 1H, 5-H), 7.82 (br s, 1H, 4-H), 9.30 (d, 1H, 6-H), 9.50 and 12.38 (br s, 2H, NH and SH); MS *m*/*z* (% ra) 268 (23), 233 (100), 203 (38), 181 (47), 168 (80), 137 (36), 104 (50), 57 (75). Anal. ($C_{13}H_{20}N_2S_2$) C, H, N.

***N*-Octyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2l.** This compound was obtained as yellow crystals (ethanol/water), mp 101−103 °C: ^{1}H NMR ($CDCl_3$) δ 3.85 (m, 2H, N−CH_2, $J = 5.0$ Hz), 6.99 (br t, 1H, 5-H), 7.70 (br s, 1H, 4-H), 9.38 (d, 1H, 6-H), 12.28 (br s, 1H, SH); MS *m*/*z* (% ra) 282 (4), 280 (16), 247 (100), 203 (26), 168 (91), 154 (33), 104 (24), 57 (35). Anal. ($C_{14}H_{22}N_2S_2$) C, H, N.

***N*-Nonyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2m.** This compound was obtained as yellow crystals (ethanol/water), mp 105−108 °C: ^{1}H NMR (DMSO-d_6) δ 3.90 (m, 2H, N−CH_2, $J = 5.0$ Hz), 7.00 (t, 1H, 5-H), 7.90 (d, 1H, 4-H), 9.40 (d, 1H, 6-H), 8.70 and 12.50 (br s, 2H, NH and SH); MS *m*/*z* (% ra) 296 (4), 261 (100), 203 (36), 168 (83), 154 (9), 137 (16), 104 (24), 57 (36). Anal. ($C_{15}H_{24}N_2S_2$) C, H, N.

***N*-Decyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2n.** This compound was obtained as yellow crystals (ethanol/water), mp 111−114 °C: ^{1}H NMR ($CDCl_3$) δ 3.86 (m, 2H, N−CH_2, $J = 5.0$ Hz), 6.98 (dd, 1H, 5-H, $J_{4,5} = 6.0$ Hz, $J_{6,5} = 7.5$ Hz), 7.67 (dd, 1H, 4-H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.5$ Hz), 9.40 (d, 1H, 6-H), 12.19 and 12.41 (br s, 2H, NH and SH); MS *m*/*z* (% ra) 310 (51), 275 (100), 203 (23), 168 (81), 137 (78), 104 (22), 57 (58). Anal. ($C_{16}H_{26}N_2S_2$) C, H, N.

***N*-Hexadecyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2o.** This compound was obtained as yellow crystals (2-propanol), mp 94−97 °C: ^{1}H NMR (DMSO-d_6) δ 3.65 (m, 2H, N−CH_2, $J = 5.0$ Hz), 6.97 (t, 1H, 5-H), 7.88 (d, 1H, 4-H), 8.45 (d, 1H, 6-H), 11.96 and 13.93 (br s, 2H, NH and SH); MS *m*/*z* (% ra) 394 (17), 359 (100), 240 (13), 203 (30), 160 (40), 137 (18), 104 (8), 57 (23). Anal. ($C_{22}H_{38}N_2S_2$) C, H, N.

***N*-Octadecyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamide, 2p.** This compound was obtained as yellow crystals (ethanol/water), mp 103−105 °C: ^{1}H NMR (DMSO-d_6) δ 3.65 (m, 2H, N−CH_2, $J = 5.0$ Hz), 6.94 (dd, 1H, 5-H), 7.87 (dd, 1H, 4-H), 8.45 (dd, 1H, 6-H), 12.80 (br s, 1H, SH); MS *m*/*z* (% ra) 422 (2), 387 (100), 240 (13), 203 (30), 160 (40), 137 (18), 104 (8), 57 (23). Anal. ($C_{24}H_{42}N_2S_2$) C, H, N.

3*H*-1,2-Pyridindithiol-3-one, 4. A suspension of pyridin-1,2-dithiol-3-thione (0.84 mol) in 300 mL of chloroform was added, at room temperature, to a suspension of mercuric acetate (0.18 mol) in 700 mL of glacial acetic acid. The reaction was monitored by TLC (hexane/ethyl acetate 80:20) until disappearance of the starting material. The raw material was filtered through Celite and the clear solution obtained was evaporated under vacuum to yield the product as a pure white solid (yield: 98%), mp 94−96 °C: ^{1}H NMR δ ($CDCl_3$) 7.40 (dd, 1H, H-5, $J_{5,4} = 7.5$ Hz, $J_{5,6} = 5.0$ Hz), 8.23 (dd, 1H, H-4, $J_{4,5} = 7.5$ Hz, $J_{4,6} = 1.5$ Hz), 8.85 (dd, 1H, H-6 $J_{6,5} = 5.0$ Hz, $J_{6,4} = 1.5$ Hz); MS *m*/*z* (% ra) 169 (100) 141 (30), 105 (18), 77 (14). Anal. ($C_6H_3S_2NO$) C, H, N.

***N*-Hexyl-1,2-dihydro-2-thioxo-3-pyridinecarboamide, 5.** A solution of 3*H*-1,2-dithiole[3,4-*b*]pyridin-3-one **4** (3.5 mmol) and *n*-hexylamine (4.2 mmol) in 20 mL of ethanol was refluxed for 1 h, then the solvent was removed under reduced pressure, and the residue was purified through flash chromatography ($CHCl_3$/acetone 80:20). This compound was obtained as yellow crystals (ethanol/water), mp 108−112 °C: ^{1}H NMR ($CDCl_3$) δ 3.48 (m, 2H, N−CH_2, $J = 5.0$ Hz), 6.91 (dd, 1H, 5-H, $J_{6,5} = 7.5$ Hz, $J_{4,5} = 6.0$ Hz), 7.69 (dd, 1H, 4-H, $J_{4,5} = 6.0$ Hz, $J_{6,4} = 1.7$ Hz), 8.82 (dd, 1H, 6-H, $J_{6,5} = 7.5$ Hz, $J_{6,4} = 1.7$ Hz), 10.78 (bs, 1H, NH o SH); MS *m*/*z* (% ra) 238 (68), 205 (30), 165 (14), 154 (12), 138 (45),111 (39), 100 (100), 67 (10). Anal. ($C_{12}H_{18}N_2OS$) C, H, N.

***N*-Hexylisothiazolo[5,4-*b*]pyridin-3(2*H*)-one, 6.** An ethanolic solution of iodine (6%) was added dropwise to a stirred mixture of *N*-hexyl-1,2-dihydro-2-thioxo-3-pyridinecarboamide **5** (6 mmol) and sodium hydrogen carbonate (10 mmol) in 100 mL of ethanol, until a persistent brown color is noted. A precipitate was removed by filtration and washed with dichloromethane. Collected organic phases were evaporated under reduced pressure, and the residue was purified through flash chromatography (hexane/ethyl acetate 70:30) to give pure product (36% yield). Analytical data are in agreement with those previously reported.[18]

Microbiology. Stock solutions were made in dimethyl sulfoxide (DMSO). Working solutions, whose concentrations were 40-fold greater than the desired concentrations, were prepared from stock solutions in sterile distilled water. It has been verified that DMSO did not suppress or delay *M. avium* or *M. tuberculosis* strains' growth when added undiluted (producing 5% concentration in the medium). Ethambutol was employed as reference drug.

Radiometric Method. The growth of bacteria was recorded radiometrically by using the BACTEC 460-TB system (Becton Dickinson, Sparks, USA). Growth in 7H12 liquid medium (Becton Dickinson) containing ^{14}C-labeled palmitic acid leads to the consumption of this substrate, with subsequent release of $^{14}CO_2$ in the confined atmosphere above the medium.[21] The BACTEC instrument detects the amount of $^{14}CO_2$ and records it as a growth index (GI) on a scale from 0 to 999.

MIC Determination. Determination of MIC for MAC strains was performed as previously reported.[17] Determination of MIC for MT was performed according to the protocol reported.[22]

Toxicity. Compounds **2c** and **2i** were suspended in carboximethylcellulose and orally administered (by gastric gavage) as a single dose to Sprague−Dawley rats and CD1 mice (5 males and 5 females/species/group/dose) at a dose ranging from 200 to 2000 mg/kg and by intraperitoneal administration at the single dose of 200 mg/kg diluted in saline (higher doses were not applicable because of the low solubility of the compounds). The control group of mice and rats (5 males and 5 females/species/group) was treated with carboximethylcellulose or saline by oral and i.p. routes, respectively. Observations of mortality and of anomalous clinical signs were made 15 min and 1, 2, and 4 h after treatment and every day during the study (14 days).

Pharmacokinetic Studies. Compounds (**2c** and **2i**) were dissolved in 1 mL of poly(ethylene glycol) 400. The animals (rats Wistar, male, 250−300 g body weight) received a single dose (20 mg/kg) of both compounds by intravenous or oral route through a gastric tube. Intravenous drug administration and blood samples were collected through a permanent cannula inserted into the jugular vein. Plasma was obtained by centrifugation and stored until analysis.

The analysis of **2c** and **2i** was performed from plasma samples by gas chromatography−mass spectrometry. Col-

umn: methyl silicone (12 m × 0.2 mm; 0.33 μm film thickness). Carrier gas: highly purified helium (0.5 mL/min). Column temperatures: initial temperature, 100 °C for 3 min and then 10 °C/min to the final temperature of 300 °C. Injector temperature: 220 °C. Detector temperature: 280 °C. Electron impact: energy: 70 eV.

Compound levels in the plasma samples were determined by monitoring the following ions: 233, 181, 168, 137, 104. Pharmacokinetic parameters were determined according to a noncompartmental analysis by using the WINNONLIN program.[23] The parameters were obtained from the usual relationship.[24]

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Supporting Information Available: Elemental analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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