dry DMF (10 mL) was added dropwise a solution of imidazole (0.35 g, 5.14 mmol) in dry DMF (10 mL) at room temperature under dry N_2 and the mixture stirred for 30 min. Then, a solution of **40** (0.78 g, 2.56 mmol) in dry DMF (20 mL) was added dropwise at room temperature and the mixture then heated at 50 °C for 2 h. The reaction mixture was poured into water and extracted with EtOAc. The extract was washed with brine for several times, dried over Na_2SO_4, and evaporated. The residue was chromatographed on silica gel. Elution with EtOAc/TEA/EtOH (20/1/1 v/v) gave the product as an oil: yield 0.52 g (73%); ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3 H, $COOCH_3$), 3.95 (d, J = 6.0 Hz, 2 H, CH_2N), 6.85–7.6 (4 H, 3-H and imidazole). Anal. ($C_{14}H_{16}N_2O_2S$) C, H, N, S.

5-(1-Imidazolylmethyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-carboxylic Acid Hydrochloride (42). The title compound **42** was prepared similarly as described above for the preparation of **13a** from **41** as colorless needles from IPA/ether: yield 92%; mp 138.0–140.0 °C. Anal. ($C_{13}H_{14}N_2O_2S$·HCl) C, H, N, S, Cl.

6-[[(Methylsulfonyl)oxy]methyl]-4,5,6,7-tetrahydrobenzo[*b*]thiophene (43). The title compound **43** was prepared similarly as described above for the preparation of **37** from **6** as an oil: yield 78%; ^{1}H NMR ($CDCl_3$) δ 2.98 (s, 3 H, OSO_2CH_3), 4.17 (d, J = 6.0 Hz, 2 H, CH_2O), 6.88 (AB q, 2 H, H-2 and H-3).

2-Formyl-6-[[(methylsulfonyl)oxy]methyl]-4,5,6,7-tetrahydrobenzo[*b*]thiophene (44). The title compound **44** was prepared similarly as described above for the preparation of **38** from **43** as an oil: yield 64%; ^{1}H NMR ($CDCl_3$) δ 3.05 (s, 3 H, OSO_2CH_3), 4.24 (d, J = 5.0 Hz, 2 H, CH_2O), 7.46 s, 1 H, H-3), 9.85 (s, 1 H, CHO). Anal. ($C_{11}H_{14}O_4S_2$) C, H, S.

Methyl 6-[[(Methylsulfonyl)oxy]methyl]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-carboxylate (46). The title compound was prepared similarly as described above for the preparation of **40** from **44** as colorless needles from EtOAc/hexane: yield 85%; mp 188.0–191.0 °C; ^{1}H NMR ($CDCl_3$) δ 3.05 (s, 3 H, OSO_2CH_3), 3.86 (s, 3 H, $COOCH_3$), 4.20 (d, J = 5.0 Hz, 2 H, CH_2O), 7.48 (s, 1 H, H-3). Anal. ($C_{12}H_{16}O_5S_2$) C, H, S.

Methyl 4-(1-Imidazolylmethyl)-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-2-carboxylate (47). The title compound **47** was prepared similarly as described above for the preparation of **41** from **46** as a colorless amorphous powder: yield 96%; ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 3 H, $COOCH_3$), 3.96 (d, J = 6.0 Hz, 2 H, CH_2N), 6.9–7.6 (4 H, H-3 and imidazole). Anal. ($C_{14}H_{16}N_2O_2S$) C, H, N, S.

6-(1-Imidazolylmethyl)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-2-carboxylic Acid Hydrochloride (48). The title compound **48** was prepared similarly as described above for the preparation of **13a** from **47** as a colorless amorphous powder from IPA/ether: yield 64%. Anal. ($C_{13}H_{14}N_2O_2S$·HCl) C, H, N, S, Cl.

Registry No. **1**, 13414-95-4; **2**, 112101-60-7; **3**, 112101-59-4; **3** (4,5,6,7-tetrahydro), 112101-66-3; **4**, 1468-84-4; **5**, 114686-05-4; **6**, 114686-04-3; **7a**, 112101-55-0; **7b**, 112101-62-9; **7c**, 112101-70-9; **7d**, 112135-62-3; **8a**, 112101-53-8; **8b**, 112101-54-9; **8c**, 112101-72-1; **8d**, 114685-81-3; **10a**, 112101-36-7; **10b**, 112101-40-3; **10c**, 112101-49-2; **10d**, 112101-47-0; **11**, 3005-50-3; **11a**, 114686-23-6; **11a**·Na, 112101-37-8; **11b**, 114686-01-0; **11b**·Na, 112101-41-4; **11c**, 119971-54-9; **11c**·Na, 112101-50-5; **11d**, 119971-55-0; **11d**·Na, 112101-48-1; **12a**, 112101-38-9; **12b**, 119971-61-8; **12c**, 112101-51-6; **12d**, 119971-62-9; **13a**, 114686-21-4; **13a**·HCl, 112101-39-0; **13b**·HCl, 119971-59-4; **13c**·HCl, 112101-52-7; **13d**·HCl, 119971-60-7; **14**, 119971-43-6; **15**, 119971-44-7; **16**, 119971-45-8; **17**, 119971-46-9; **18**, 112135-60-1; **19**, 114686-02-1; **19**·Na, 112101-44-7; **20**, 119971-47-0; **21**, 119971-48-1; **21**·HCl, 119971-56-1; **22**, 114685-92-6; **23**, 114707-26-5; **24**, 119971-49-2; **25**, 114685-83-5; **26**, 114685-12-3; **26**·Na, 113817-57-5; **27**, 114685-91-5; **28**, 114686-11-2; **28**·HCl, 114685-78-8; **29**, 114686-86-8; **30**, 114685-85-7; **31**, 114685-88-0; **32**, 114685-87-9; **33**, 114685-89-1; **34**, 114686-19-0; **34**·Na, 114685-90-4; **35**, 114685-69-7; **36**, 114686-14-5; **36**·HCl, 114685-70-0; **37**, 114685-57-3; **37** alcohol, 114685-56-2; **38**, 114685-58-4; **39**, 119971-50-5; **40**, 114685-59-5; **41**, 114685-45-9; **42**, 114686-03-2; **42**·HCl, 114685-46-0; **43**, 114685-34-6; **44**, 114685-35-7; **45**, 119971-51-6; **46**, 114685-36-8; **47**, 114685-49-3; **48**, 119971-53-8; **48**·HCl, 114685-50-6; $CHCl_2OCH_3$, 4885-02-3; $ClCOCH_2Br$, 22118-09-8; cyclohexanecarbonyl chloride, 2719-27-9; pivaloyl chloride, 3282-30-2; 1*H*-imidazole, 288-32-4; thromboxane synthetase, 61276-89-9.

Hybrid Molecules: Growth Inhibition of *Leishmania donovani* Promastigotes by Thiosemicarbazones of 3-Carboxy-β-carbolines

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The thiosemicarbazones of β-carboline-3-carboxaldehyde (compound **2**) and 3-acetyl-β-carboline (compound **3**) were found to effectively inhibit the in vitro growth of the promastigote form of *Leishmania donovani*, 50% inhibition being obtained at concentrations of 5.0 and 2.5 μM, respectively, while irreversible growth inhibition was achieved at 40 (compound **2**) and 17.5 μM (compound **3**). The thiosemicarbazone of pyridine-2-carboxaldehyde (compound **4**) was considerably less active while both methyl β-carboline-3-carboxylate (compound **1**) and the thiosemicarbazone of ethyl 5-formyl-6-azaindole-2-carboxylate (compound **5**) were inactive at the highest concentrations tested. At concentrations provoking approximately 50% growth inhibition of promastigotes, compound **2** was observed to preferentially block DNA rather than RNA synthesis, but for compound **3**, the reverse was true. Compound **3**, the most active analogue studied, may thus act, at least partly, via a novel, though as yet unelucidated, mechanism.

Human leishmaniasis is a major and often fatal tropical parasitic disease for which few efficacious and easily administered treatments are known at present.[1] The causative agents of leishmaniasis are various species of the protozoa *Leishmania* belonging to the family Trypanosomatidae. One of these, *Leishmania donovani*, infects macrophages of reticuloendothelial organs in its amastogote form, giving rise to visceral leishmaniasis. Recently, it has been shown that certain compounds known to interact with the mammalian central nervous system also exhibit leishmanicidal properties. Thus, both tricyclic antidepressants such as clomipramine[2] as well as the structurally related neuroleptic phenothiazines[3] demonstrate potent cytotoxic effects against both the extracellular

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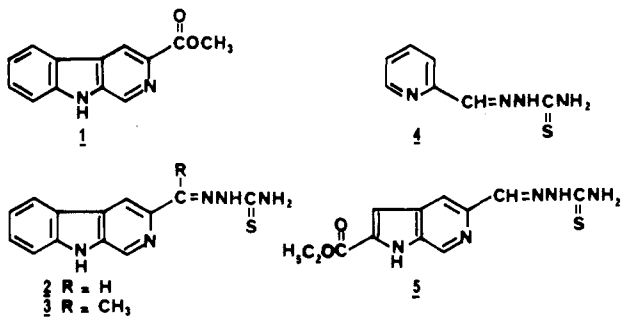
promastigote form of *L. donovani* and the macrophagic amastigotes. These compounds apparently disrupt the membrane function of the protozoa, inhibiting L-proline transport in the parasite.

Another class of compounds known to exhibit antiparasitic activity (*Trypanosoma cruzi*,[4] *Plasmodium berghei*,[5] *Plasmodium falciparum*,[6] and *Trypanosoma rhodesiense*[7]) are α-N-heterocyclic carboxaldehyde thiosemicarbazones (e.g. **4**). These compounds act by selectively inhibiting the enzyme ribonucleoside diphosphate reductase, thus interfering with DNA synthesis in the infecting protozoa.[8] The activity of these thiosemicarbazones against the various causative agents of leishmaniasis has not, to our knowledge, been reported however.

Among other centrally acting agents, it has been observed that some (e.g. the benzodiazepine Ro 11-3128[9] and avermectins[10]) known to interact with the benzodiazepine receptor and its associated γ-aminobutyric acid (GABA) receptor exhibit antiparasitic activity, though mainly against helminths and schistosomes. Since 3-carboxy-β-carbolines (e.g. β-CCM, compound **1**) also possess a high affinity for these benzodiazepine receptors[11] and are, moreover, amenable to derivitization to α-*N*-carboxaldehyde-type thiosemicarbazones, we reasoned that such hybrid compounds would perhaps demonstrate interesting antiparasitic properties. Here we report that the thiosemicarbazones of β-carboline-3-carboxaldehyde (compound **2**) and of 3-acetyl-β-carboline (compound **3**) are highly toxic in vitro to extracellular promastigotes of *L. donovani*. A preliminary investigation suggests that thiosemicarbazone **3** may act through a novel mechanism.



Chemistry

Compound **1** was synthesized in our laboratory according to published procedures.[12] Thiosemicarbazones **2** and **3** were formed by using standard procedures from β-carboline-3-carboxaldehyde and 3-acetyl-β-carboline,[12] respectively. Compound **4** was synthesized from commercial-grade pyridine-2-carboxaldehyde.[13] Thiosemicarbazone **5** was formed from the 5-formyl-6-azaindole precursor **11** whose synthesis is shown in Scheme I. Thus, the 2-methyl group of 2,4-dimethyl-5-nitropyridine **6**[14] was selectively oxidized to the aldehyde **7** by use of selenium dioxide in refluxing dioxane. The aldehyde function of **7** was protected as its acetal **8**. Compound **8** was then converted to the 6-azaindole derivative **10** by the method of Frydman and co-workers:[15] treatment of the potassium salt of **8** with diethyl oxalate in ethanol led to formation of the pyruvate **9**, which upon reductive cyclization gave **10**. Acid hydrolysis of the acetal blocking group of **10** yielded the desired aldehyde **11**.

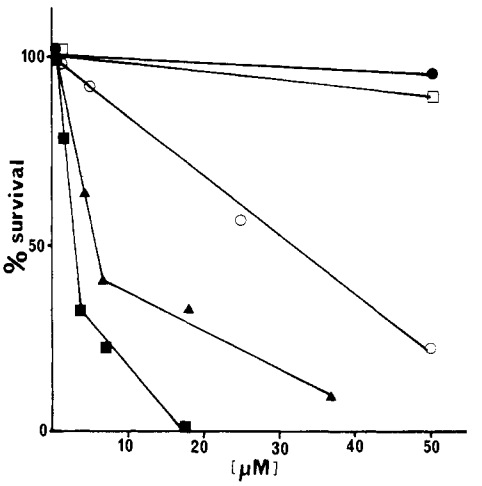


Figure 1. Growth inhibition of *L. donovani* promastigotes in the presence of various concentrations of test compounds. Symbols: ●, compound 1; ▲, compound 2; ■, compound 3; ○, compound 4; □, compound 5.

Table I. In Vitro Growth Inhibitory Effects of β-Carboline and Thiosemicarbazone Analogues on *Leishmania donovani* Promastigote Multiplication

compound	I_{50},[a] μM	I_{max},[b] μM
pentamidine	0.56	1.1
1	>50	ND
2	5	40
3	2.5	17.5
4	29	67
5	>50	>75

[a] Concentration of compound provoking 50% inhibition of growth. The number of promastigotes was determined 3 days after the drugs were added. Each value is the mean of five independent experiments. [b] Concentration of compound causing >99% irreversible inhibition of growth of promastigotes as determined 3 days after removal of drugs from the culture medium. Each value is the mean of three experiments.

Results and Discussion

Although the *Leishmania* parasite exists in the human host in its amastigote form, infecting mainly macrophages, the activity of the thiosemicarbazones was verified against the extracellular promastigote form for several reasons. Firstly, the promastigote form of *Leishmania* is biochemically very similar to the amastigote form. The antileishmanial activity measured is thus independent of such factors as cell penetration, metabolism, and replication.[16]

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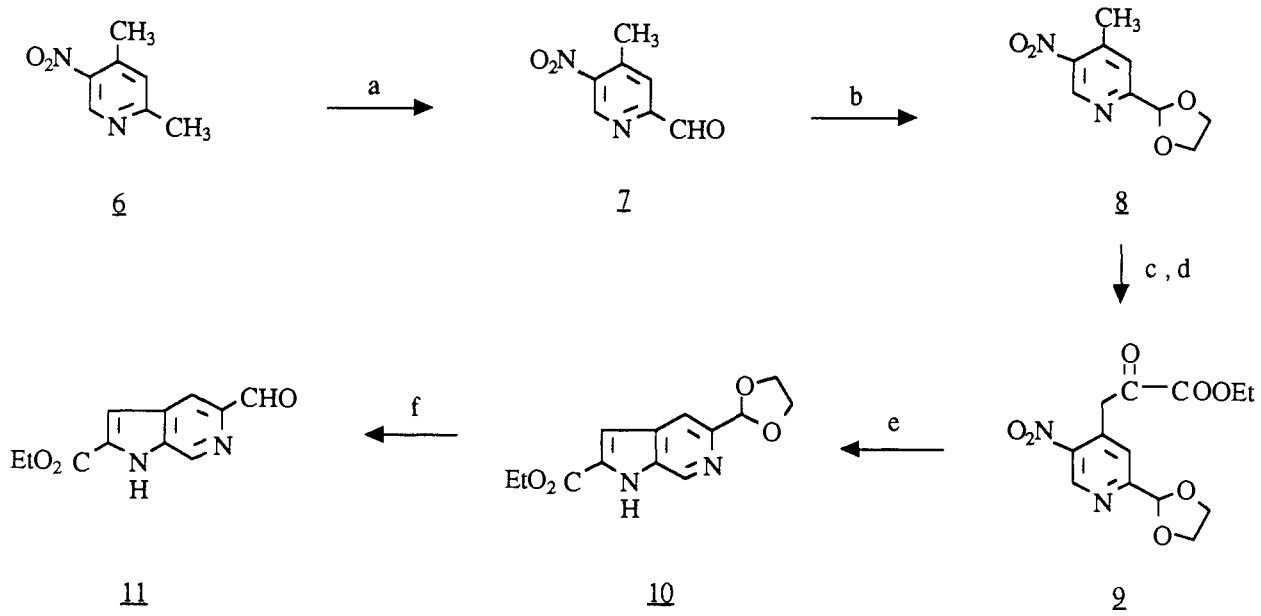
Scheme I[a]



[a] (a) SeO_2, dioxane reflux; (b) $HOCH_2CH_2OH$, pTSA, toluene reflux; (c) KOEt, EtOH; (d) EtOOCCOOEt; (e) H_2, Pd–C, CH_2Cl_2; (f) pTSA, CH_3CN, H_2O, reflux.

Secondly, with the notable exception of sodium stibogluconate, practically all compounds active against amastigotes are also active against promastigotes. Furthermore, pentamidine, used clinically, shows only moderate activity against amastigotes but good activity against promastigotes.[16]

The effects of the four thiosemicarbazones (**2–5**) and of β-CCM (**1**) on the growth of promastigotes of *Leishmania d. donovani* in vitro are shown in Figure 1. The best results were obtained with compounds **3** and **2**, whose I_{50} values were 2.5 and 5 μM, respectively. As can be seen in Table I, the I_{50} of the model compound **4** was much higher (29 μM), while the analogue **5** and β-CCM (**1**) were practically inactive up to 50 μM. The in vitro antipromastigote activities of the β-carboline derivatives **3** and **2** compare favorably with those reported for several other classes of antileishmanial drugs currently under investigation such as allopurinol,[17] formycin B (and related derivatives),[18] and tricyclic neuroleptics and antidepressants (chlorpromazine, clomipramine).[2,3]

In order to see whether the effect of these molecules was leishmanicidal or only leishmanistatic, the drugs were withdrawn from the culture media after 3 days of treatment and the number of living cells was then determined after 3 days incubation in the drug-free medium. As shown in Figure 2 and Table I, the best results were again obtained with compound **3**, which was leishmanicidal at 17.5 μM, whereas the leishmanicidal concentrations of compounds **2** and **4** were 40 and 67 μM, respectively. In contrast, compound **5** had no irreversible toxicity on promastigotes of *Leishmania d. donovani*.

Thus, the ability of compounds **2–4** to irreversibly inhibit the growth of *L. donovani* promastigotes correlates with their respective I_{50} values. Again, this activity of **3** is comparable to those of other known leishmanicidal compounds.

The azaindole derivative tested, compound **5**, represents compound **2** in which the electron-rich phenyl ring of the latter has been replaced by another electron-rich group,

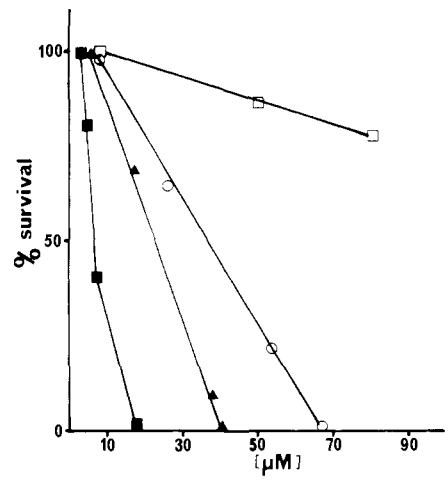


Figure 2. Survival of *L. donovani* promastigotes measured 3 days after removal of test compounds: ▲, compound **2**; ■, compound **3**; ○, compound **4**; □, compound **5**.

ethoxycarbonyl. That compound **5** exhibits no antileishmanial activity suggests that the nature of the heterocycle to which the α-*N*-formyl thiosemicarbazone side chain is attached is of considerable importance in conferring activity. Moreover, it appears from our study that the β-carboline ring system imparts significantly higher antileishmanial activities to the thiosemicarbazones than the analogous pyridine derivative. This is coherent with our stated working hypothesis regarding the potential antiparasitic activity of compounds affecting the central nervous system. In this connection, Evans and Croft[19] reported while this work was in progress than harmaline (3,4-dihydro-7-methoxy-β-carboline), a naturally occurring β-carboline having well-documented effects on the mammalian central nervous system, also exhibits significant antiparasitic activity against *Leishmania mexicana amazonensis* both in vitro and in vivo in mice. However, the mere presence of a β-carboline moiety (in our case, of the completely aromatic species) is apparently not by itself always sufficient to impart leishmanicidal activity, as the

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Table II. Effect of the Compounds on the Growth and Macromolecular Biosynthesis of Promastigotes of *L. donovani* as a Function of Drug Concentration

compd	concn, (μM)	% inhibition[a] growth	synthesis protein	synthesis RNA	synthesis DNA
2	3.7	37	7	34	89
	37	90	81	96	99.7
3	3.5	68	25	36	0
	5.25	ND	36	52	0.5
	17.5	100	95	98.7	99.6
4	26.6	56	20	50	90
	53	78	17	53	95

[a] Each value is the mean of three independent experiments.

total inactivity of β-CCM (1) attests.

This, and the fact that the pyrido thiosemicarbazone **4** shows considerable activity, argues in favor of the primordial importance of the side chain rather than of the nature of the heterocycle. Though thiosemicarbazones of pyridine and isoquinoline derivatives have been shown to be active against several types of parasites (*T. cruzi*,[4] *P. falciparum*,[6] *P. Berghei*[5]), their leishmanicidal activities have never, to our knowledge, been reported. These derivatives would perhaps merit closer attention in view of our findings. It is also interesting to note that compound **4** apparently demonstrates no antimalarial activity in vitro.[20]

Since α-N-heterocyclic carboxaldehyde thiosemicarbazones are considered to be, in vitro, inhibitors of ribonucleoside diphosphate reductase and, consequently, of DNA biosynthesis,[8] a preliminary study was performed in order to verify whether compounds **2–4** have similar actions on *Leishmania d. donovani* promastigotes. The effects of these compounds on macromolecular biosynthesis after a 4-h treatment is shown in Table II. The compounds were tested at concentrations provoking about 50% inhibition of growth.

As expected, compounds **2** and **4** strongly inhibited the incorporation of thymidine into DNA while the effect on RNA and protein synthesis was much less pronounced. This would most probably be the result of an inhibition of ribonucleoside diphosphate reductase, as has been amply demonstrated for other α-N-heterocyclic carboxaldehyde thiosemicarbazones.

Of particular interest, however, is the behavior of the most active thiosemicarbazone, compound **3**. At a concentration that inhibits 50% growth of the parasite (3.5 μM) as well as at a higher concentration (5.25 μM), this compound, in contrast to **2** and **4**, has little or no effect on DNA synthesis. Instead, it would appear that the antiparasitic action of **3** at these concentrations is limited to an inhibition of only RNA and protein synthesis. This suggests that compound **3** acts, at least partially, via a different mechanism than **2** and **4**. A direct investigation of the inhibition of ribonucleoside diphosphate reductase by these β-carboline derivatives will help to resolve this question.

In order to evaluate the possibility of clinical development of these compounds, the acute toxicities of **2** and **3** were determined in mice. These compounds, as well as their hydrochloride salts are, unfortunately, poorly soluble in aqueous media. However intravenous administration of compound **3** in DMSO was tolerated up to 50 mg/kg and intraperitoneal injection up to 100 mg/kg. The LD_{50} of **3** administered iv in DMSO was estimated to be 50–100 mg/kg while the LD_{50} ip was >100 mg/kg. Compound **2** was somewhat more toxic than **3** when administered iv in DMSO, the maximal dose tolerated being 10 mg/kg. However, when **2** was injected ip in (carboxymethyl)cellulose, the LD_{50} was found to be greater than 100 mg/kg.

In conclusion, we have shown that, as hypothesized, prototype hybrid molecules incorporating both a centrally active pharmacophore (a β-carboline) and a pharmacophore (α-N-pyrido carboxaldehyde thiosemicarbazones) known for antitrypanosomal (though not antileishmanial) activity are effectively lethal to promastigotes of *Leishmania donovani*. In particular, the high in vitro antileishmanial activity of 3-acetyl-β-carboline thiosemicarbazone (**3**) makes this compound a promising lead for the development of an effective therapeutic agent. This class of compound also benefits from structural and synthetic simplicity, not unimportant criteria in designing therapeutic agents destined in large part for Third World countries. We are currently synthesizing various analogues of **3** in an effort to optimize its activity and its solubility as well as investigating more closely the mechanism of action of these compounds.

Experimental Section

Chemistry. Melting points were determined on a Büchi apparatus and are uncorrected. Proton NMR spectra were determined on Varian T-60 or Bruker 80 or 200-MHz instruments. Chemical shifts are given as δ values with reference to Me_4Si as internal standard. Thin-layer chromatography was performed on Merck silica gel 60 plates with fluorescent indicator generally with use of 9:1 toluene–ethanol as developer. The plates were visualized with UV light (254 and 366 nm). Mass spectral measurements were done on an AEI MS-9 or an AEI MS-50 spectrometer. Elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette, France.

General Procedure for Synthesis of Thiosemicarbazones. A solution of the heterocyclic aldehyde in ethanol containing 1 equiv of thiosemicarbazide (and a trace of acetic acid for compound **3**) was refluxed for 1–6 h. After completion of the reaction, as indicated by TLC, the solution was cooled and the solid that precipitated was collected by filtration. Recrystallization of this material from ethanol gave pure thiosemicarbazone in 60–80% yield.

β-Carboline-3-carboxaldehyde thiosemicarbazone (2): mp 188–190 °C; EIMS *m/z* 269 (M^+); NMR (Me_2SO-d_6) δ 7.58 (1 H, t, H-6), 7.94 (2 H, m, H-5,7), 8.42 (1 H, s, C*H*=N), 8.58 (1 H, d, H-8), 8.74 (1 H, s, NH, D_2O exchangeable), 8.98 (1 H, s, NH, D_2O exchangeable), 9.22 (1 H, s, H-4), 9.30 (1 H, s, H-1), 12.22 (1 H, s, NH, D_2O exchangeable), 13.02 (1 H, s, NH, D_2O exchangeable); HRMS *m/z* calcd for $C_{13}H_{11}N_5S$ 269.0711, found 269.0723. Anal. ($C_{13}H_{11}N_5S$) C, H, N.

3-Acetyl-β-carboline thiosemicarbazone (3): mp 215–217 °C; EIMS *m/z* 283 (M^+); NMR (Me_2SO-d_6) δ 2.64 (3 H, s, CH_3, partially masked by Me_2SO), 7.42 (1 H, t, H-6), 7.71 (2 H, m, H-5,7), 8.31 (1 H, s, NH, D_2O exchangeable), 8.45 (1 H, d, H-8), 8.58 (1 H, s, NH, D_2O exchangeable), 8.98 (1 H, s, H-4), 9.31 (1 H, s, H-1), 10.35 (1 H, s, NH, D_2O exchangeable), 11.81 (1 H, s, NH, D_2O exchangeable); HRMS *m/z* calcd for $C_{14}H_{13}N_5S$ 283.0895, found 283.0893. Anal. ($C_{14}H_{13}N_5S$) C, H, N.

2-(Ethoxycarbonyl)-1*H*-pyrrolo[2,3-*c*]pyridine-5-carboxaldehyde thiosemicarbazone (5): mp 278–280 °C; EIMS *m/z* 291 (M^+); NMR (Me_2SO-d_6) δ 1.49 (3 H, t, CH_2CH_3), 4.48 (2 H, q, CH_2CH_3), 7.28 (1 H, s, H-3), 8.21 (1 H, s, NH, D_2O exchangeable), 8.34 (1 H, s, CH=N), 8.38 (1 H, s, NH, D_2O exchangeable), 8.65 (1 H, s, H-4), 8.95 (1 H, s, H-7), 11.64 (1 H, s, NH, D_2O exchangeable), 12.68 (1 H, br s, NH, D_2O exchangeable); HRMS *m/z* calcd for $C_{12}H_{13}N_{15}O_2S$ 291.0775, found 291.0782. Anal. ($C_{12}H_{13}N_5O_2S$) C, H, N, S.

4-Methyl-5-nitropyridine-2-carboxaldehyde (7). A mixture of 2,4-dimethyl-5-nitropyridine (**6**)[14] (1 g, 6.6 mmol) and selenium dioxide (900 mg, 8.4 mmol) in anhydrous 1,4-dioxane (25 mL) was refluxed under an atmosphere of nitrogen for 3 h. The reaction mixture was cooled to room temperature, the black selenium that precipitated was removed by filtration, and the filtrate was

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concentrated under vacuum. The gummy residue was suspended in diethyl ether and washed successively with saturated, aqueous sodium hydrogen carbonate and water. The organic layer was dried over sodium sulfate, the solvent was removed under vacuum, and the residue was purified by filtration on a short column of silica gel with toluene as developer. The resulting solid was crystallized from dichloromethane–hexane, yielding almost colorless crystals (433 mg, 40%): mp 81–82 °C; IR (ν_{max} cm^{-1}, KBr) 1720 (C=O), 1605 (N=C), 1525 (NO_2); EIMS m/z 166 (M^+), 138, 92, 77; ^{1}H NMR (80 MHz, $CDCl_3$) δ 2.70 (3 H, s, CH_3), 7.87 (1 H, s), 9.15 (1 H, s), 10.02 (1 H, s). Anal. ($C_7H_6O_3N_2$) C, H, N.

2-(1,3-Dioxolanyl)-4-methyl-5-nitropyridine (8). A solution of aldehyde **7** (131 mg, 0.79 mmol) in toluene (20 mL) containing ethylene glycol (287 mg, 4.63 mmol) and *p*-toluenesulfonic acid monohydrate (5 mg, 0.02 mmol) was refluxed until complete disappearance of starting material was observed by TLC (toluene–ethyl acetate 4:1). The solution was cooled, diluted with toluene, and washed with saturated aqueous $NaHCO_3$ solution, water, and brine. The organic layer was dried over $MgSO_4$, the solvent was removed under vacuum, and the residue was crystallized in dichloromethane–*n*-hexane, affording **8** (148 mg; 89%): mp 77 °C; IR (ν_{max} cm^{-1}, KBr) 1620 (N=C), 1535 and 1365 (C—NO_2), 1190, 1120, 1085, and 1035 (C—O); EIMS m/z 210 (M^+), 209, 167, 164, 137; ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.66 (3 H, s, CH_3), 4.13 (4 H, s, CH_2CH_2), 5.86 (1 H, s), 7.50 (1 H, s), 9.10 (1 H, s). Anal. ($C_9H_{10}O_4N_2$) C, H, N.

Ethyl 2-(1,3-Dioxolanyl)-5-nitro-4-pyridinepyruvate (9). To a solution of anhydrous ethanol (500 mL), potassium (3.6 g, 92.1 mequiv), and freshly distilled diethyl oxalate (12.9 mL, 95 mmol) was added over 15 min at room temperature and under nitrogen a solution of compound **8** (17.5 g, 83 mmol) in dry toluene (200 mL). The reaction mixture immediately turned red, and after 2 h of stirring, the red precipitate that had formed was collected by filtration and washed copiously with dry diethyl ether. The solid was then dissolved in water and acetic acid was added until disappearance of the intense red color was observed. This aqueous solution was extracted with ethyl acetate (3×), and the combined organic extracts were washed once with water, dried over sodium sulfate, and evaporated to dryness under vacuum. The resulting orange syrup crystallized on standing, affording 88% of **9**, which could be recrystallized from ethanol: mp 86–87 °C; EIMS m/z 310 (M^+), 267, 237 ($M^+ - CO_2Et$); ^{1}H NMR (200 MHz, $CDCl_3$) (as 1:1 mixture of enol and keto ester tautomers) δ 1.43 (6 H, 2 t, CH_2CH_3), 4.17 (m, 8 H, CH_2CH_2), 4.44 (4 H, 2 q, CH_2CH_3), 4.66 (2 H, s, CH_2 of keto form), 4.82 (4 H, s, exchangeable with D_2O, OH of enol form + H_2O), 5.92 (1 H, s, CHO), 5.95 (1 H, s, CHO), 7.00 (s, 1 H, CH=C), 7.53 (1 H, s, H-3), 8.39 (1 H, s, H-3), 9.13 (1 H, s, H-6), 9.33 (1 H, s, H-6). Anal. ($C_{13}H_{14}O_7N_2 \cdot {}^3/_4H_2O$) C, H, N.

Ethyl 5-(1,3-Dioxolanyl)-1*H*-pyrrolo[2,3-*c*]pyridine-2-carboxylate (10). A solution of compound **9** (1 g) in dichloromethane (60 mL) was hydrogenated in a Parr apparatus at 30 psi for 2 h in the presence of 10% palladium on carbon as catalyst. The reaction mixture was filtered on Celite, the catalyst was washed copiously with a mixture of dichloromethane–ethanol (1:1), and the combined filtrate and washings were evaporated to dryness under vacuum. The resulting solid was crystallized from dichloromethane–*n*-hexane, affording 524 mg (62%) of pure **10**: mp 190–191 °C; IR (ν_{max} cm^{-1}, KBr) 3050 (NH), 1705 (C=O), 1615 (C=C); EIMS m/z 262 (M^+), 218 ($M^+ - OCH_2CH_2$); ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.43 (3 H, t, CH_2CH_3), 4.16 (4 H, m, OCH_2CH_2), 4.46 (2 H, q, CH_2CH_3), 6.03 (1 H, s, CHO), 7.26 (1 H, s, H-3), 7.85 (1 H, s, H-4), 9.53 (1 H, s, H-7). Anal. ($C_{13}H_{14}O_4N_2$) C, H, N.

2-(Ethoxycarbonyl)-1*H*-pyrrolo[2,3-*c*]pyridine-5-carboxaldehyde (11). A solution of the acetal **10** (140 mg) in 10% aqueous acetonitrile (10 mL) was refluxed for 5 h in the presence of *p*-toluenesulfonic acid monohydrate (30 mg). The reaction mixture was cooled and concentrated in vacuo to remove excess acetonitrile. The residue was diluted with chloroform (50 mL) and washed successively with saturated aqueous sodium hydrogen carbonate (2 × 20 mL) and water (20 mL). The organic phase was dried over sodium sulfate and the solvent removed under vacuum, leaving crude solid **11**, which was crystallized from dichloromethane–*n*-hexane (90 mg, 77%): mp 173–174 °C; IR (ν_{max} cm^{-1}, KBr) 1740 (C=O), 1700 (C=O); EIMS m/z 218 (M^+), 190 ($M^+ - CO$); ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.40 (3 H, t, CH_2CH_3), 4.53 (2 H, q, CH_2CH_3), 7.36 (1 H, s, H-3), 8.36 (1 H, s, H-4), 9.00 (1 H, s, H-7), 10.09 (2 H, s + br s, CHO, NH). Anal. ($C_{11}H_{10}N_2O_3$) C, H, N.

Biological Methods. Cells. *Leishmania d. donovani* (strain LRC L-52) originated from the strain collection of the World Health Organization's International Reference Center for leishmaniasis (WHO-LRC) and were kindly provided by Dr. L. F. Schnur (Hebrew University, Hadassah Medical School, Jerusalem, Israël).

Culture Conditions. The medium was composed of 45% Dulbecco modified Eagle medium, 45% RPMI 1640 medium containing 25 mM HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid) (pH 7.4), and 10% heat-inactivated fetal calf serum. Streptomycin at 5 μg/mL, penicillin at 5 units/mL, and kanamycin at 5 μg/mL were also added.

Effect of the Compounds on Cell Multiplication. Promastigotes (5×10^5/mL) were inoculated into 25-mL Nuclon flasks containing 5 mL of the above-described medium and incubated at 26 °C. One day after seeding, the cells were counted in each flask with a hemacytometer and the compounds to be tested were added at the desired concentration. Each test was performed in duplicate, and the number of promastigotes was counted from two dilutions. Untreated cultures were run in parallel. On day 3 of treatment, the cultures were centrifuged, the compounds washed out, and the promastigotes suspended in new medium. The number of cells was counted again 3 days later. Irreversible growth arrest and decrease in cell number was attributed to leishmanicidal effect. Pentamidine (1 μM) was used as control in separate experiments.

Effect of the Compounds on Macromolecular Synthesis. Promastigotes (5×10^6 cell/mL) were cultured with and without the compounds to be tested, for 4 h, and then the appropriate radioactive precursor was added for 1 h to the medium: [4,5-^{3}H]leucine, 5 μCi/mL, or [5-^{3}H]uridine, 2.5 μCi/mL, or [*methyl*-^{3}H]thymidine, 2.5 μCi/mL. After 1 h of labeling, the cells were centrifuged and rinsed twice in cold phosphate-buffered saline (PBS). The uptake into the soluble pool and the incorporation into nucleic acids and proteins were obtained from the cold TCA-soluble, hot TCA-soluble, and insoluble materials, respectively.[21] Protein concentration was determined by the Lowry procedure[22] with bovine serum albumin as the standard.

In Vivo Toxicity Studies. The toxicities of compounds **2** and **3** were evaluated in female Swiss mice weighing approximately 20 g. Solutions of the test compounds were prepared in dimethyl sulfoxide at a concentration not exceeding 10% or in (carboxymethyl)cellulose (1%). The solutions were injected iv or ip in a volume of 0.5 mL/20 g (body weight). Single injections of 10, 50, or 100 mg/kg were administered to groups of 5 or 10 mice and the animals were observed for 14 days afterward.

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