Antileishmanial Pyrazolopyridine Derivatives: Synthesis and Structure−Activity Relationship Analysis

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Three series of 4-anilino-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic esters were synthesized as part of a program to study potential anti-*Leishmania* drugs. These compounds were obtained by a condensation reaction of 4-chloro-1*H*-pyrazolo[3,4-*b*]pyridine with several aniline derivatives. Some of them were also obtained by an alternative pathway involving a Mannich-type reaction. The hydrophobic parameter, log *P*, was determined by shake-flask methodology, and using the Hansch−Fujita addictive hydrophobic fragmental constants. These compounds were tested against promastigote forms of *Leishmania amazonensis*. The very promising results showed the 3′-diethylaminomethyl-substituted compounds as the most active [$IC_{50} = 0.39$ (**21**) and 0.12 μM (**22**)]. Molecular modeling, using semiempirical AM1 method, predicted the most active compounds through the low-energy conformers superimposition on amodiaquine structure. QSAR equations, derived from the IC_{50} values against *L. amazonensis*, showed the hydrophobic (log *P*) and Sterimol steric (L and B2) parameters as most significant contributions on biological activity.

Introduction

Parasitic diseases cause enormous suffering in many parts of the world. Leishmaniasis is widespread in many tropical and subtropical regions of the world where they constitute a serious health problem.[1] *Leishmania* is a parasitic protozoa (Kinetoplastida: Trypanosomatideae) which causes a disease that has been associated with different clinical forms, including cutaneous, mucocutaneous, and the visceral leishmaniasis.[1,2] The phlebotominae vector transmits by inoculation the flagellate promastigotes into the mammalian host, where they enter macrophages differentiating and multiplying into no motile amastigotes. *Leishmania amazonensis* has been associated with different forms of the disease, including cutaneous, hyperergic mucocutaneous, and the anergic diffuse cutaneous leishmaniasis.[3] Besides, this species has been isolated from patients with visceral disease or with postkalazar dermal leishmaniasis.[3,4] Therapy of patients with leishmaniasis is still a serious problem. The drugs for leishmaniasis's treatment of all their clinical forms are sodium stibogluconate (Pentostam) and meglumine antimonate (Glucantime), despite the fact that they exhibit renal and cardiac toxicity.[5] Alternative drugs, such as pentamidine, amphotericin B, and some azo-derivatives are also very toxic with serious side effects.[6] Miltefosine, a phosphocholine analogue originally developed as an anticancer agent, has been found to be highly effective against leishmaniasis in vitro and in vivo. Now, this compound is the only oral agent against both cutaneous[7] and visceral[8] leishmaniasis, although presenting severe gastrointestinal problems.[9] Since the chemotherapy against leishmaniasis is still inefficient, there is an urgent need for the development of new, efficient, and safe drugs for the treatment of this disease.[10]

Fused heterocyclic systems containing pyrazole ring are ranked among the most versatile bioactive compounds, and a variety of procedures have been developed for their synthesis.[11,12] 1*H*-Pyrazolo[3,4-*b*]pyridine is an example of such fused system, which is known to possess remarkable and significant biological and medicinal importance.[12−17] It has been reported that pyrazolo[3,4-*b*]pyridines were potential specific antagonists of nucleic acid metabolism. Derivatives of this heterocyclic ring system have been shown to be substrate inhibitors of purine-requiring enzymes and also exhibit potential nonsedative anxiolytic activity.[18] The pyrazolopyridine system can be viewed as an aminoquinoline analogue, and the aminoquinoline derivatives such as chloroquine and amodiaquine have presented a high antimalarial activity. They are still in use, although their use is limited due to development of resistant parasites.[19−21] However, concerning leishmaniasis, amodiaquine was never been used.

Considering the similarity of amodiaquine and pyrazolopyridines structures, we synthesized thirteen 4-(3′- or 4′-X-phenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (series **I**), four 4-(4′-hydroxyphenyl)amino- and 4-(4′-hydroxy-3′-diethylaminomethylphenyl)amino-5-carbethoxy-1,3-X,Y-1*H*-pyrazolo[3,4-*b*]pyridine derivatives, series **II** and **III**, respectively. Those compounds together with amodiaquine were assayed against *L. amazonensis* promastigotes. The lipophilic parameter, log *P*, was experimentally determined, and electronic parameters, including dipolar moment, HOMO and LUMO frontier orbital energies, and heat of formation, were calculated for structure−

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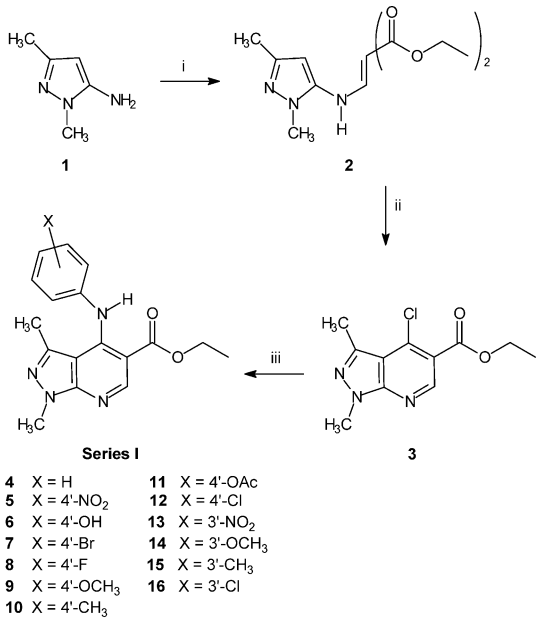
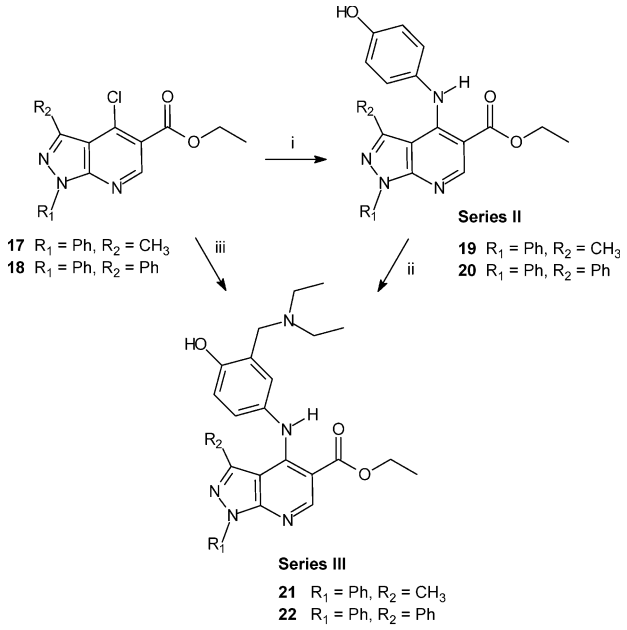
Scheme 1[a]



[a] Reaction conditions: (i) Diethyl ethoxymethylenemalonate, ethanol; (ii) $POCl_3$, △ (iii) substituted aniline, △.

Scheme 2[a]



[a] Reaction conditions: (i) 4-hydroxyaniline, △; (ii) $(CH_3CH_2)_2NH$, HCOH, 2-propanol; (iii) diethylaminomethyl-4-acetylaminophenol.

activity relationship studies. Furthermore, a molecular modeling study involving the comparison of bioactive molecular structures by superimposition of the most stable conformations on the amodiaquine structure was also performed.

Results and Discussion

Chemistry. The pyrazolopyridine derivatives of series **I**, 4-(3′- or 4′-X-phenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine, where X = H (**4**), 4′-NO_2 (**5**), 4′-OH (**6**), 4′-Br (**7**), 4′−F (**8**), 4′-OCH_3 (**9**), 4′-CH_3 (**10**), 4′-OAc (**11**), 4′-Cl (**12**), 3′-NO_2 (**13**), 3′-OCH_3 (**14**), 3′-CH_3 (**15**), and 3′-Cl (**16**), were prepared[22−24] by nucleophilic substitution reactions between the intermediate 4-chloro-1*H*-pyrazolo[3,4-*b*]pyridine (**3**) and the appropriate 3- or 4-substituted anilines (Scheme 1).

The procedure developed for the synthesis of **3** started from the previously prepared 5-amino-1,3-dimethylpyrazole (**1**), obtained by the condensation of methylhydrazine and β-cyanocrotonitrile, and diethyl ethoxymethylenomalonate to yield the intermediate diethyl α-carboethoxy-β-(1,3-dimethyl-5-aminopyrazolyl)acrylate (**2**).[23] Later, the intermediate **2** was treated with $POCl_3$ following the Lynch modified procedure[25] to afford **3** (Scheme 1). The target compounds **4**−**16** were obtained with 30−88% of yield, purified by ethanol recrystallization and fully characterized by ^1H and ^{13}C NMR, IR, and UV spectroscopies. The compounds of series **II** and **III** were obtained in a similar approach with appropriate intermediates starting from the previously prepared 4-chloropyrazolopyridines **17** and **18** as described in the literature.[24,25] The treatment of these compounds with 4-hydroxyaniline hydrochloride led, respectively, to **19** and **20** (Scheme 2). The compounds **21** and **22** were obtained by Mannich-type reaction or by a nucleophilic displacement with diethylaminomethyl-4-acetylaminophenol.[26−28] The derivatives of series **II** and **III** were obtained in 30−80% yield.

The theoretical calculations based on molecular mechanics and semiempirical methods using the AM1 Hamiltonian were performed for all compounds of series **I**, **II**, **III** and amodiaquine. Then, the most stable conformations were obtained, and the resulting geometry was utilized for the determination of theoretical parameters such as HOMO and LUMO (energies of the highest occupied and lowest unoccupied molecular frontier orbital, respectively), charges, and dipole moments for each molecule, using the MOPAC 6.0 software package.[29,30] In addition, the Hammett σ as well as the Swain−Lupton F and R substituent constants for field and resonance effects, respectively, were used as electronic parameters[31] for series **I** derivatives. The steric parameters, Es, and the polarizibility-related MR, L, B1, and B2, were taken from the literature[32] and used for QSAR analysis. Furthermore, a theoretical study involving the comparison of the molecular structures of bioactive derivatives of series **II** and **III** was realized by superimposition of the most stable conformations on the amodiaquine modeled structure. Their stereoviews showed a correlation of the best overlay of the pyridinic substituent moieties on amodiaquine, despite the out of plane pyrazolic ring, and the best results were observed for **21** and **22** (Figure 1).

The overall lipophilicity of each pyrazolopyridine derivative was assessed by the logarithm of its octanol/water partition coefficient (log *P*). The log *P* values were measured for **4**, **6**, **8**, **12**, **15**, and **16**, using the shake-flask method[33,34] under optimized conditions as described in the Experimental Section. The remaining compounds, **5**, **7**, **9**−**11**, **13**, **14**, and **19**−**22**, had the log *P* values calculated from log *P* of **4**, unsubstituted derivative, by hydrophobic fragmental constant using the addictive-constitutive property:[32,35] $\log P_X = \log P_H + \pi_X$. The Hansch−Fujita hydrophobicity constant, π, were taken from the literature.[32] Data for the octanol/water partition coefficients of pyrazolopyridine derivatives, log *P* and pK_a, are summarized in Table 1 for series **I**, and Table 2 for series **II** and **III**. Differences between the experimental and calculated log *P* values

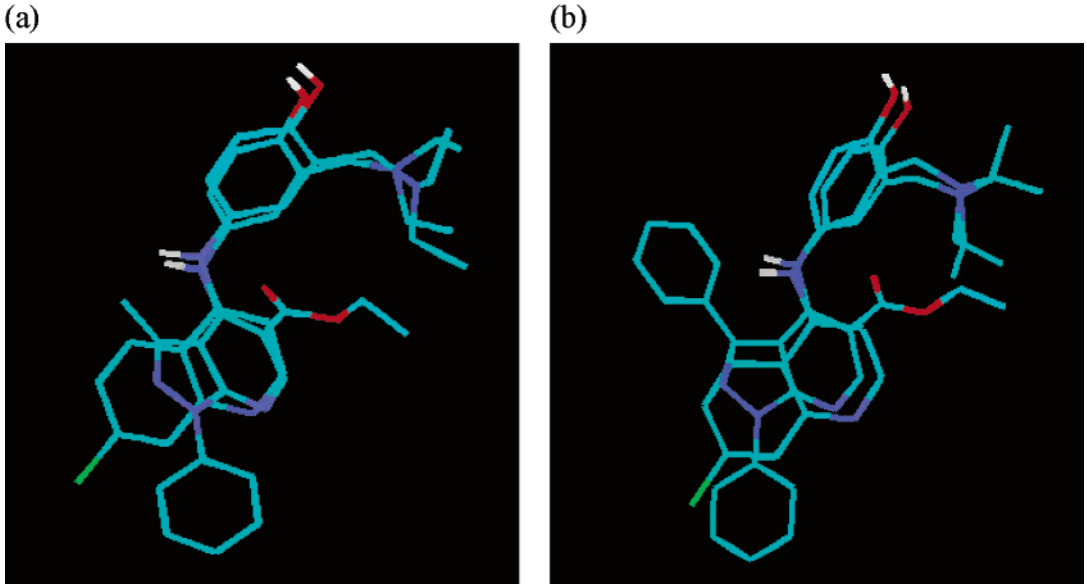


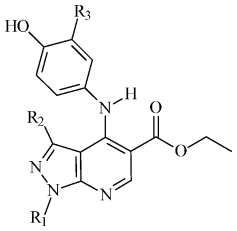
Figure 1. (a) Superposition of the **21** on amodiaquine. (b) Superposition of the **22** on amodiaquine.

Table 1. Experimental and Calculated log *P*, pK_a, and IC_{50} Values for Series **I** of Pyrazolopyridine Derivatives Assayed against *L. amazonensis* Promastigotes

compound	X	IC_{50} (μg/mL)	IC_{50} (μM)	log *P*	pK_a
4	H	30.69	98.89	0.96	8.23
5	4′-NO_2	114.15	321.23	0.68[a]	8.80
6	4′-OH	1.37	4.20	1.14	9.98
7	4′-Br	4.57	11.74	1.82[a]	8.39
8	4′−F	12.82	39.04	0.70	8.14
9	4′-OCH_3	10.91	32.05	0.94[a]	8.07
10	4′-CH_3	10.57	32.59	1.52[a]	8.61
11	3′-Cl	4.79	13.89	1.67	9.05
12	4′-Oac	0.44	1.23	0.32[a]	9.73
13	4′-Cl	1.34	3.89	1.32	7.98
14	3′-NO_2	6.11	17.19	0.68[a]	8.38
15	3′-OCH_3	7.45	21.89	0.94[a]	7.64
16	3′-CH_3	4.82	14.86	2.27	7.06
pentamidine isethionate[b]	-	0.27	0.46	-	-
amodiaquine	-	0.34	0.95	3.01	-

[a] Calculated by Hansch hydrophobicity constant[32,33] (π) and log P_H. [b] Reference drug.

Table 2. Experimental and Calculated Values of IC_{50} and log *P*, Respectively, for Series **II** and **III** of Pyrazolopyridine Derivatives Assayed against *L. amazonensis* Promastigotes



compound	R	IC_{50} (μg/mL)	IC_{50} (μM)	log *P*
19	R_1 = phenyl; R_2 = methyl R_3 = H	0.21	0.55	1.69
20	$R_1 = R_2$ = phenyl R_3 = H	0.34	0.75	3.09
21	R_1 = phenyl; R_2 = methyl R_3 = diethylaminomethyl	0.18	0.39	2.05
22	$R_1 = R_2$ = phenyl R_3 = diethylaminomethyl	0.064	0.12	3.45
amodiaquine	-	0.34	0.95	3.01
pentamidine isethionate[a]	-	0.27	0.46	-

[a] Reference drug.

for series **I** derivatives were within a range of ±0.17 units with **15** as the only exception (0.75). Determination of log *P* values reveals a range from 1.82 for **7** (X = 4′-Br) to 0.67 for **11** (X = 3′- and 4′-NO_2), exhibiting a lipophilicity spectrum of 1.15 log units.

Antileishmanial Assays. The 50% growth inhibitory activity value, IC_{50}, of each compound was determined using *L. amazonensis*, in the evolutive form of promastigotes. The dead and alive parasites were counted in a Neubauer chamber, and the IC_{50} values were determined by linear regression, relating the inhibition percentage and log of drug concentration in μg/mL and μM, as shown in Tables 1 and 2. The most active compounds of series **I** were the 4′-OAc (**11**, IC_{50} = 1.23 μM), 4′-Cl (**12**, IC_{50} = 3.9 μM), and 4′-OH (**6**, IC_{50} = 4.2 μM) derivatives when compared with the nonsubstituted derivative (**4**, IC_{50} = 98.9 μM).

An increase of antileishmanial activity was observed for series **II** and **III** derivatives (Table 2). Comparison of IC_{50} values indicated that series **III** derivatives are the most active compounds with values of 0.39 and 0.12 μM for **21** and **22**, respectively, while amodiaquine showed IC_{50} = 0.89 μM.

QSAR Analysis. The preliminary analysis correlating a single physicochemical property of pyrazolopyridine derivatives with biological activity against *L. amazonensis* promastigotes have not showed a linear significant correlation ($r \leq 0.50$). Thus, the multiple regressions were performed, fixing log *P* or π, the hydrophobic parameter, and changing the other electronic or steric parameter. Partitioning processes are usually studied through solvent models, e.g., octanol/water or through more structured systems such as micelles[36] and liposomes.[37] The π_{oct} hydrophobic constant for the octanol/water system can be used to determine the importance of substructural contributions to the biopotence of drugs. Thus, we have explored the behavior of the pyrazolopyridine derivatives through log *P* and π_{oct} physicochemical descriptors, and eq 1 shows a parabolic relationship with log *P*.

$$\log 1/IC_{50} = 2.11\ (\pm\ 0.45) \log P^2 - 4.88\ (\pm\ 1.02) \log P + 1.18\ (\pm\ 0.53) \quad (1)$$

$$n = 11;\ r^2 = 0.74;\ F = 11.44;\ \mathrm{sd} = 0.29$$

The most important physicochemical properties that explain the variance in the leishmanicidal activity were hydrophobic (log *P*) and Sterimol steric parameters (L and B2). Equations 2 and 3 indicated the dependence of log $1/IC_{50}$ for *L. amazonensis* promastigotes, with log *P*, log P^2, and Sterimol parameters. Nevertheless, the positive contribution of the steric parameter indicates that the more bulky the substituent on the aromatic moiety attached to the pyridine ring the more potent is the drug.

$$\log 1/IC_{50} = 1.52\ (\pm\ 0.35) \log P^2 - 3.44\ (\pm\ 0.76) \log P + 0.25\ (\pm\ 0.11)\ \mathrm{L} - 0.43\ (\pm\ 0.59)$$

$$n = 12;\ r^2 = 0.83;\ F = 12.67;\ \mathrm{sd} = 0.25 \quad (2)$$

$$\log 1/IC_{50} = 1.60\ (\pm\ 0.36) \log P^2 - 3.77\ (\pm\ 0.76) \log P + 0.58\ (\pm\ 0.29)\ \mathrm{B2} - 0.34\ (\pm 0.62)$$

$$n = 12;\ r^2 = 0.81;\ F = 11.33;\ \mathrm{sd} = 0.26 \quad (3)$$

Equation 2 showed a good correlation between experimental and predicted IC_{50} values ($r = 0.9408$), indicating the importance of hydrophobic and steric contribution of substituents on antileishmanial activity.

Furthermore, in an attempt to compare the antileishmanial activity of series **II** and **III** derivatives with amodiaquine, based on the similarity of the structural features, the low-energy conformers were superimposed on the amodiaquine modeled structure. The results indicated that the most superimposed structures, considering three distinct pharmacophoric site points: pyridinic ring, aniline ring, and diethylamino moiety, corresponded with the most active drugs, **21** and **22** (IC_{50} = 0.39 and 0.12 μM, respectively), despite the presence of carbethoxy group (Figure 1). Moreover, these results suggested that the diethylamino side group in **21** and **22** compared with **19** and **20** were significant for bioactivity, and the pyrazolopyridine moiety was a useful bioisostere of the quinoline group.

Conclusions

QSAR analysis performed for compounds of set **I** demonstrated the importance of the hydrophobic and steric properties of the substituent of dimethylpyrazolopyridine derivatives for growth inhibition activity against *L. amazonensis* promastigotes. Also, the results of antileishmanial activity observed for series **I**, **II**, and **III** may reflect some differences, especially of the hydrophobic character of C-3- and N-moieties on the pyrazolo ring, which is corroborated by log *P* values. No significant effect of the 5-carbethoxy moiety present on the pyridine ring was observed compared to amodiaquine activity. The antileishmanial activity presented by pyrazolopyridine derivatives indicated a promising new class of leishmanicidal drugs.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot plate apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer using potassium bromide tablets. ^{1}H and ^{13}C NMR spectra were obtained in a Bruker AC-200 spectrometer, with tetramethylsilane as the internal reference, in DMSO-d_6 as solvent; the chemical shifts were reported in ppm. The UV spectra and absorbance measurements for log *P* determinations were performed on Varian DMS-80 spectrophotometer in 1,4-dioxane:buffer as solvent.

The 5-aminopyrazole compounds 1,α-carbethoxy-β-(1,3-dimethyl-5-aminopyrazolylamino)acrylate **2** and 5-carbethoxy-4-chloro-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine **3** were prepared according to the literature.[24,27]

General Procedure for the Preparation of the 4-(3′- and 4′-X-phenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridines 4 and 5; 7−10; 12−16. A mixture of **3** (1.1 mmol) and the corresponding anilines (1 mmol) was heated for 1 h. After cooling, the mixture was neutralized with NaOH to afford the target compounds, purified by ethanol recrystallization.

4-(Phenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (4). Yield 80%; mp 137−140 °C; IR (KBr): ν 3235, 2982, 2931, 1590, 1565, 1669, 1266 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.4 (t, J = 7.0, 3H, CH_2CH_3), 1.6 (s, C3-CH_3), 4.0 (s, N1-CH_3), 4.3 (q, J = 7.0, CH_2), 7.1 ((m, H2′, 4′, and 6′), 7.3 (m, 2H, H3′ and 5′), 8.9 (s, H6), 10.4 (s, NH); ^{13}C NMR (DMSO-d_6): δ 14.2, 15.5, 33.5, 60.7, 103.4, 104.7, 122.9, 125.0, 129.5, 142.1, 142.9, 151.3, 152.4, 154.0, 168.8. Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

4-(4′-Nitrophenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (5). Yield 88%; mp 168−170 °C; IR (KBr): ν 3252, 2983, 2936, 1559,1601, 1681, 1269, 1501, 1334 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.4 (t, J = 7.1, 3H, CH_2-CH_3), 1.9 (s, C3-CH_3), 4.0 (s, N1-CH_3), 4.5 (q, J = 7.1, CH_2), 7.1 (d, J = 8.9, H2′ and 6′), 8.2 (d, J = 8.9, H3′ and 5′), 9,0 (s, H6), 10.3 (s, NH); ^{13}C NMR (DMSO-d_6): δ 14.1, 15.7, 33.6, 61.3, 105.9, 106.5, 119.5, 125.5, 141.2, 143.0, 148.1, 149.2, 151.9, 152.2, 168,1. Anal. ($C_{17}H_{17}N_5O_4$) C, H, N.

4-(4′-Bromophenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (7). Yield 50%; mp 110−112 °C; IR (KBr): ν 3182, 2989, 2934, 1591, 1564, 1673, 1264 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.4 (t, J = 7.1, 3H, CH_2CH_3), 1.7 (s, C3-CH_3), 4.0 (s, N1-CH_3), 4.4 (q, J = 7.1, CH_2), 7.0 (d, J = 8.7, H2′ and 6′), 7.4 (d, J = 8,9, H3′ and 5′), 9.0 (s, H6), 10.3 (s, NH); ^{13}C NMR (DMSO-d_6): δ 14.2, 15.9, 33.6, 61.0, 117.5, 104.0, 105.0, 124.1, 132.6, 141.8, 142.3, 150.7, 152.4, 152.5, 168.8. Anal. ($C_{17}H_{17}N_4O_2Br$) C, H, N.

4-(4′-Fluorophenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (8). Yield 60%; mp 104−106 °C; IR (KBr): ν 3222, 2978, 2927, 1595, 1569, 1669, 1269 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.4 (t, J = 7.0, 3H, CH_2CH_3), 1.6 (s, C3-CH_3), 3.9 (s, N1-CH_3), 4.4 (q, J = 7.0, CH_2), 7.0 (d, H3′ and 5′), 7.1 (d, H2′ and 6′), 8.9 (s, H6), 10.4 (s, NH); ^{13}C NMR (DMSO-d_6): δ 14.3, 15.7, 33.6, 60.9, 103.3, 104.0, 139.1 141.9, 151.6, 152.4, 152.6, 162.6, 168.9, d 124.9 and 116.4 (d, $^1J_{CF}$=245,2; $^2J_{CF}$ 22,6; $^3J_{CF}$ 7,0 Hz). Anal. ($C_{17}H_{17}N_4O_2F$) C, H, N.

4-(4′-Methoxyphenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (9). Yield 60%; mp 135−137 °C; IR (KBr): ν 3242, 2926, 2851, 1592, 1565, 1669, 1265 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.4 (t, J = 7.0, 3H, CH_2CH_3), 1.6 (s, C3-CH_3), 3.8 (s, C4′-OCH_3), 3.9 (s, N1-CH_3), 4.3 (q, J = 7.0, CH_2), 7.1 (d, J = 8.4, H2′ and 6′), 6.8 (d, J = 8.4, H3′ and 5′), 8.9 (s, H6), 10.4 (s, N−H); ^{13}C NMR (DMSO-d_6): δ 14.3, 15.7, 33.5, 55.5, 60.7, 102.6, 104.1, 114.8, 125.2, 135.9, 142.0, 152.3, 152.5, 152.7, 169.0, 157.4. Anal. ($C_{18}H_{20}N_4O_3$) C, H, N.

4-(4′-Methylphenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (10). Yield 30%; mp 175−177 °C; IR (KBr): ν 3201, 2984, 2933, 1567, 1666, 1266 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.4 (t, J = 7.1, 3H, CH_2CH_3), 1.5 (s, C3-CH_3), 2.1 (s, C4′-CH_3), 3,9 (s, N1-CH_3), 4.3 (q, J = 7.1, CH_2), 7.1 (d, J = 8.1, H2′ and 6′), 7.9 (d, J = 8.1, H3′ and 5′), 8.8 (s, H6), 10.1 (s, NH); ^{13}C NMR (DMSO-d_6): δ 14.1, 15.2, 33.3, 60.7, 103.1, 104.2, 122.8, 130.1, 134.4, 140.2, 140.9, 150.6, 151.4, 152.2, 168.1. Anal. ($C_{18}H_{20}N_4O_2$) C, H, N.

4-(4′-Chlorophenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (12). Yield 30%; mp 100−101 °C; IR (KBr): ν 3216, 2985, 2933, 1593,1580, 1673, 1267 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.4 (t, J = 7.1, 3H, CH_2CH_3), 1.7 (s, C3-CH_3), 4.0 (s, N1-CH_3), 4.4 (q, J = 7.1, CH_2), 7.1 (d, J = 8,5, H2′ and 6′), 7.3 (d, J = 8.5, H3′ and 5′), 9.0 (s, H6), 10.3 (s, NH); ^{13}C NMR (DMSO-d_6): δ 14.2, 15.8, 33.6, 60.9, 103.8, 104.9, 123.8, 129.6, 129.9, 141.6, 141.8, 150.9, 152.3, 152.4, 168.7. Anal. ($C_{17}H_{17}N_4O_2Cl$) C, H, N.

4-(3′-Nitrophenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (13). Yield 65%; mp 168−170 °C; IR (KBr): ν 3208, 2982, 1673, 1597, 1562, 1526, 1348, 1271 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.4 (t, J = 7.0, 3H, CH_2CH_3), 1.7 (s, C3-CH_3), 4.0 (s, N1-CH_3), 4.4 (q, J = 7.0, CH_2), 7.4 (m, 2H, H5′ and 6′), 7.9 (sl, 2H, H2′ and 4′), 9.0 (s, H6), 10.4 (s, NH); ^{13}C NMR (DMSO-d_6): δ 14.2, 15.9, 33.6, 61.2, 116.2, 118.9, 104.9, 105.5, 127.1, 130.3, 141.1, 149.2, 144.6, 149.6, 152.3, 152.4, 168.5. Anal. ($C_{17}H_{17}N_5O_4$) C, H, N.

4-(3′-Methoxyphenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (14). Yield 30%; mp 123−124 °C; IR (KBr): ν 3242, 2926, 1669, 1592, 1565, 1265 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.4 (t, J = 6.8, 3H, CH_2CH_3), 1.7 (s, C3-CH_3), 3.7 (s, C3′-OCH_3), 4.0 (s, N1-CH_3), 4.4 (q, J = 6.8, CH_2), 6.7 (sl, 3H, H2′,4′ and 6′), 7.2 (m, H5′), 9.0 (s, H6), 10.4 (s, NH). ^{13}C NMR (DMSO-d_6): δ 14.2, 15.5, 33.5, 55.2, 60.8, 103.6, 105.0, 110.5, 115.0, 118.6, 130.2, 142.3, 151.1, 152.2, 152.4, 144.0, 160.7, 168.7. Anal. ($C_{18}H_{20}N_4O_3$) C, H, N.

4-(3′-Methylphenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (15). Yield 40%; mp 130−133 °C; IR (KBr): ν 3237, 2983, 2931, 1670, 1564, 1591, 1265 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.3 (t. J = 7.1, 3H, CH_2CH_3), 1.6 (s, C3-CH_3), 2.5 (s, C3′-CH_3), 3.9 (s, N1-CH_3), 4.3 (q, J = 7.1, CH_2), 7.21 (m, 3H, H2′ and 4′-6′), 8.8 (s, H6), 10.1 (s, NH); ^{13}C NMR (DMSO-d_6): δ 14.1, 15.2, 20.7, 33.3, 60.8, 104.0, 105.0, 119.4, 122.9, 125.5, 129.5, 139.2, 140.9, 142.6, 147.3, 151.5, 152.5, 168.0. Anal. ($C_{18}H_{20}N_4O_2$) C, H, N.

4-(3′-Chlorophenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (16). Yield 34%; mp 155−157 °C; IR (KBr): ν 3220, 2982, 2928, 1673, 1594, 1565, 1268 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.0 (t, J = 8.0, 3H, CH_2CH_3), 1.8 (s, C3-CH_3), 4.0 (s, N1-CH_3), 4.4 (q, J = 8.0, CH_2), 9.0 (s, H6), 7.22−7.53 (m, 4H, H-2′ and 4′ to 6′), 10.1 (s, NH); ^{13}C NMR (DMSO-d_6): δ 13.8, 15.0, 33.1, 60.8, 104.5, 105.1, 119.9, 121.3, 123.6, 129.5, 131.1, 140.7, 147.0, 149.0, 151.4, 152.2, 167.8. Anal. ($C_{17}H_{17}N_4O_2Cl$) C, H, N.

4-(4′-Hydroxyphenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (6). An ethanolic solution of 4-aminophenol (2.2 mmol) and **3** (1.1 mmol) was heated at reflux for 18 h. The target compound was obtained by precipitation and recrystrallized from ethanol. Yield 55%; mp 274−276 °C; IR (KBr): ν 3300, 3130, 2999, 2934, 1574, 1590, 1681, 1269, cm^{-1}; ^1H NMR (DMSO-d_6): δ 8.9 (s, H-6), 6.9 (d, J = 8.6, H2′ and 6′), 7.2 (d, J = 8,6, H3′ and 5′), 1.6 (s, C3-CH_3), 4.0 (s, N1-CH_3), 4.4 (q, J = 7,0, CH_2), 1.4 (t, J = 7.0, 3H, CH_2CH_3), 10.3 (s, NH), 8.7 (s, OH); ^{13}C NMR (DMSO-d_6): δ 14.1, 15.2, 33.3, 60.6, 101.9, 103.3, 116.2, 125.5, 133.8, 141.0, 151.7, 151.9, 153.7, 155.5, 168.4. Anal. ($C_{17}H_{18}N_4O_3$) C, H, N.

4-(4′-Acetoxyphenylamino)-5-carbethoxy-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridine (11). A mixture of **6** and acetic anhydride (1.5 mmol) was heated at reflux for 30 min. After cooling, the crude product **11** was filtered and purified by recrystallization from ethanol. Yield 57%; mp 127−130 °C; IR (KBr): ν 3051, 2981, 2933, 1596, 1581, 1759, 1662, 1266, 1224 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.4 (t, J = 6,9, 3H, CH_2CH_3), 1.7 (s, C3-CH_3), 2.3 (s, CH_3CO), 4.0 (s, N1-CH_3), 4.4 (q, J = 6.9, CH_2), 7.1(d, J = 8.2, H-2′ and 6′), 7.0 (d, J = 8.2, H3′ and 5′), 8.9 (s, H6), 10.4 (s, NH); ^{13}C NMR (DMSO-d_6): δ 14.2, 15.7, 21.0, 33.5, 60.8, 103.5, 104.7, 122.8, 123.5, 140.6, 142.0, 147.7, 151.3, 152.3, 152.5, 168.7, 169.3. Anal. ($C_{19}H_{20}N_4O_4$) C, H, N.

Partition Coefficient and pK_a Determinations. The octanol/water partition coefficient of pyrazolopyridine derivatives was determined by the shake-flask method.[33,34] Octanol and aqueous buffer (0.2 M $Na_2HPO_4 \cdot 7H_2O$ and 0.1 M citric acid) were mutually saturated for 4 h, and the phases were separated. A stock solution of each pyrazolopyridine derivative (4×10^{-3} M) was prepared using 1,4-dioxane solution. Samples were partitioned between appropriately saturated octanol and aqueous buffer at pH = 7.4. Then the phase mixtures were shaken for 1 h in thermostated double-walled glass cells at constant temperature (25.0 ± 0.1 °C). After separation, the two layers were centrifuged for 15 min (300 rpm), and the absorbance of the aqueous and octanol solutions were measured by UV spectrophotometry in λ_{max} 320−354 nm range. Three replicates were performed for each compound. The P value corresponded to the quotient between buffer and octanol concentrations of the drug. Log P values were an average of three independent experiments.

The pK_a determination was realized using a classical potentiometric method.[38] 1,4-Dioxane and 1-octanol analytical grades were obtained after usual treatment of commercial purchase. Water was obtained from Millipore Milli-Q Water Purification System.

Biology. Parasite Culture. *L. amazonensis* promastigotes, MHOM/BR/77/LTB0016 strain, were grown at 25 °C in LIT medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS). Cells were harvested in the late log phase, resuspended in fresh medium, counted in Neubauer's chamber, and adjusted to a final concentration of 4×10^6/mL. This strain has been characterized by molecular and immunological techniques.[39]

Antileishmanial Assays.[40] The assays were carried out in 96-well plates in a volume of 180 μL/well. The drugs were added to a parasite culture in a concentration range from 160 to 5 μg/mL, solubilized in DMSO (the highest percentage used was 1.6%, v/v, which was not hazardous to the parasites). After 24 h incubation, the remaining parasites were counted and the percentage of inhibition was calculated, comparing to the controls (DMSO without the drugs and with the parasites alone). The IC_{50} values were determined by linear regression from these percentages of inhibition using statistical error limits up to 10%. All tests were done in triplicate and pentamidine isethionate (May & Baker Lab., England) was used as reference drug.

Molecular Modeling. The modeling was performed by applying the standard tools in MOPAC 6.0 package.[29,30] Geometry optimization were performed initially by molecular mechanics force field from PCModel program. The SCF convergence criteria for MOPAC optimization was realized using the keywords SCFRT = AM1 PRECISE, EF HESS = 1, VECTORS, BONDS, XYZ, DUMP = 1800 and GRAD. The geometry termination was obtained with a 0.00592−0.00989 range norm gradient.

QSAR Analysis. QSAR models were derived by multiple regression analyses that were performed using the BILIN program[41] to determine the coefficients of the correlation equations. In all equations in this paper, the numbers in parentheses represent the 95% confidence intervals of the coefficients, n is the number of points on which the equation is based, r is the correlation coefficient, and sd is the standard deviation, respectively.

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