Journal of Enzyme Inhibition and Medicinal Chemistry, 2002 Vol. 17 (6), pp. 353–358

Synthesis and Antileishmanial Activity of 3-(α-Azolylbenzyl)indoles

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(Received 20 March 2002)

The goal of the present study was to evaluate several azolyl-substituted indoles as new antileishmanial agents. Ten 3-(α-azolylbenzyl)indoles have been synthesized using Friedel-Crafts acylation as a key-step. All the target compounds were found to display high levels of activity when tested against *Leishmania mexicana* promastigotes *in vitro*. The most active compounds, showing an $IC_{50} < 1\ \mu M$, were 5-bromo-1-ethyl-3-[(2,4-dichlorophenyl)(1*H*-imidazol-1-yl)methyl]-1*H*-indole 15 and its triazole analogue 17. Four representative compounds 15, 17, 22 and, 23 were also tested against intracellular amastigotes of *L. mexicana* using ketoconazole and meglumine antimoniate as reference compounds, the results of which are discussed.

Keywords: Indoles; Azoles; Antileishmanial agents; Promastigotes; Amastigotes

INTRODUCTION

Transmitted by the bite of the infected female phlebotomus sandfly, the leishmaniases are major third-world diseases. After a bite, flagellated extracellular promastigotes are rapidly transformed into nonflagellated amastigotes, which actively divide within the mononuclear phagocytes of the vertebrate host. It has been estimated that 12 million people are infected with leishmaniasis and that 350 million people are at risk in about 88 countries on four continents (Africa, Asia, Europe, America). There are approximately 2 million new cases of leishmaniasis per year, of which only 600,000 are officially declared. In humans, the disease occurs in at least four major forms, depending on the parasite species and the cellular immune system of the patient. CL can produce serious and permanent skin lesions mainly on the face, arms and legs. DCL is especially related to a defective immune system. ML, also called "espundia" in South America, causes disfiguring lesions to the face. VL, also known as "kala azar", is characterized by fever, hepatosplenomegaly, pancytopenia and is the most severe form of the disease (a 100% mortality rate if not treated). VL has also emerged as an opportunistic infection in immunosuppressed patients (e.g. as a result of advanced HIV infections, immunosuppressive treatment for organ transplants). *Leishmania*/HIV coinfections are considered to be an extremely serious, new disease, particularly in southern Europe.[1–3]

Current drugs for the leishmaniasis therapy remain the parenteral pentavalent antimonials, sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®).[4] They have severe side effects,[5] require long-term treatment and many cases of clinical resistance have been reported.[6,7] Alternative parenteral treatment agents include pentamidine and AmB, which may cause serious side effects, such as renal toxicity and pancreatitis.[5] Diverse lipid formulations of AmB have been evaluated against leishmaniasis and are now competitive with antimonials as primary therapy for CL or VL.[8,9] In the case of VL, only a few drugs are quite effective by oral administration. Among these,

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Abbreviations: AmB, amphotericin B; CDI, 1,1′-carbonyldiimidazole; CC, column chromatography; CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; ML, mucocutaneous leishmaniasis; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SDT, sulfinylditriazole; TLC, thin-layer chromatography; VL, visceral leishmaniasis

ISSN 1475-6366 print/ISSN 1475-6374 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1475636021000005613

the membrane-active phospholipid derivative hexadecylphosphocholine (miltefosine) was reported with an interesting therapeutic profile.[10] Nevertheless, the search for new, effective, and non toxic drugs has become urgent.

In *Leishmania* species, ergosterol is an important component of their membrane structure.[11] Ergosterol biosynthesis, as in fungal cells, requires the C-14 demethylation of lanosterol. This biotransformation involves a cytochrome P450 enzyme called 14-α demethylase. Azole antifungals, known to interfere with the cytochrome P450 part of the 14-α demethylase, are potent candidates to treat leishmaniasis by inhibiting the sterol synthesis pathway of *Leishmania* spp. Few conazoles such as ketoconazole, itraconazole and SCH 56592 have been used to treat CL or VL with variable success.[12–17]

In the present work, a three-step synthesis of some novel azolyl-substituted indoles has been developed using indole as the substrate under Friedel-Crafts acylation reaction conditions.[18,19] N^1-Protection of the indole nitrogen by an alkyl or benzyl chain and introduction of an azole moiety (imidazole or triazole) were performed to obtain azolyl-substituted indoles, potential candidates for antileishmanial agents.[20] For all target compounds, *in vitro* antileishmanial activity was performed against cultured extracellular promastigotes of *L. mexicana*. The inhibitory effect of four compounds (**15**, **17**, **22**, **23**) against the intracellular form of the parasite (amastigote) was evaluated in a Balb/c mice peritoneal macrophage model. In this paper, we describe the general procedures to obtain 3-(α-azolylbenzyl)indoles and present the results of *in vitro* activities.

MATERIALS AND METHODS

Chemistry

All common chemicals and solvents utilized were reagent grade and purchased from Sigma-Aldrich (Saint Quentin, France). Melting points were determined on a Electrothermal IA9300 melting point digital apparatus and are reported uncorrected. Infrared (IR) spectra were obtained in KBr pellets or neat liquid films with a Perkin-Elmer Paragon FTIR 1000 PC spectrometer. ^{1}H-NMR spectra were obtained using a Bruker AC 250 apparatus operating at 250 MHz in d_6-DMSO as solvent. Chemical shifts are expressed as δ values (ppm) relative to Me_4Si as internal standard. All reactions were monitored by TLC, using 0.25 mm-thick precoated silica gel plates (E. Merck) eluted with CH_2Cl_2/EtOH gradients. Compounds were purified by CC using silica gel 60 as stationary phase and eluted with CH_2Cl_2/EtOH gradients.

General Procedure for the Preparation of Compounds (3, 4)

To anhydrous DMF (30 ml) at 25°C was added 1.20 g (30 mmoles) of sodium hydride as a suspension (60%) in mineral oil. The mixture was gently heated at 40°C and 10 mmoles of 1*H*-indole (**1**,**2**) were gradually added. When evolution of H_2 ceased, the mixture was cooled to 25°C, and 1.21 ml of ethyl iodide (15 mmoles) was added. The reaction mixture was heated at reflux for 3 h (TLC control). Most of the DMF was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated. The residue was purified by CC using CH_2Cl_2 as eluent and appropriate fractions gave the desired compound.

5-Bromo-1-ethyl-1*H*-indole (**4**). As yellow liquid (yield: 93%). IR (neat) cm^{-1}: 3099, 3074, 3060 (νCH arom.); 2974, 2934, 2884 (νCH alkane); 1610, 1566, 1509 (νC = C). ^{1}H NMR (DMSO d_6), δ ppm: 1.36 (t, 3H, $^3J = 7.20$ Hz, CH_3), 4.20 (q, 2H, $^3J = 7.20$ Hz, CH_2), 6.45 (d, 1H, $^3J = 3.15$ Hz, H^3), 7.27 (dd, 1H, $^4J_{H^6H^4} = 1.90$ Hz, $^3J_{H^6H^7} = 8.85$ Hz, H^6), 7.46 (d, 1H, $^3J = 3.15$ Hz, H^2), 7.47 (d, 1H, $^3J = 8.85$ Hz, H^7), 7.76 (d, 1H, $^4J = 1.90$ Hz, H^4).

General Procedure for the Preparation of Compounds (5–10)

To a magnetically stirred suspension of $AlCl_3$ (0.71 g, 5.4 mmoles) in CH_2Cl_2 (25 ml) at 25°C was added benzoyl chloride (5.4 mmoles) and the mixture was cooled at 0°C and stirred for 1 h. A solution of 4.5 mmoles of *N*-substituted 1*H*-indole (**3**, **4**) in CH_2Cl_2 (10 ml) was added dropwise. The reaction mixture was heated at reflux for 7 h. The solution was poured onto crushed ice and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 ml), and the combined organic layers were washed with brine, saturated aqueous $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated in vacuum. The obtained product was recrystallized from the solvent specified below.

5-Bromo-1-ethyl-3-(4-fluorobenzoyl)-1*H*-indole (**10**). As a white powder (yield: 80%), m.p. 155–157°C (diisopropyl ether/methanol). IR (KBr) cm^{-1}: 3066 (νCH arom.); 2975, 2942 (νCH alkane); 1616 (νC = O); 1526, 1460, 1400 (νC = C); 1044 (νC-F). ^{1}H NMR (DMSO d_6), δ ppm: 1.42 (t, 3H, $^3J = 7.20$ Hz, CH_3), 4.36 (q, 2H, $^3J = 7.20$ Hz, CH_2), 7.42 (dd, 2H, $^3J_{HH} = {}^3J_{HF} = 8.80$ Hz, $H^{3'}$ and $H^{5'}$), 7.50 (dd, 1H, $^4J = 2.0$ Hz, $^3J = 8.70$ Hz, H^6), 7.69 (d, 1H, $^3J = 8.70$ Hz, H^7), 7.93 (dd, 2H, $^4J_{HF} = 5.60$ Hz, $^3J_{HH} = 8.80$ Hz, $H^{2'}$, $H^{6'}$), 8.19 (s, 1H, H^2), 8.45 (d, 1H, $^4J = 2.0$ Hz, H^4).

General Procedure for the Preparation of Compounds (11–16, 21, 22)

A solution of sodium borohydride (21 mmoles) in methanol (10 ml) was added dropwise to a solution of 7 mmoles of 3-benzoyl-1*H*-indole (**5**–**10**, **19**, **20**) in methanol (10 ml). The reaction mixture was stirred at room temperature for 1 h. Water (30 ml) was added and the solution was extracted three times with diethyl ether. The combined organic layers were dried (Na_2SO_4) and the solvent was carefully evaporated leaving a light yellow oil. Corresponding carbinol (6.1 mmoles) and CDI (6.1 mmoles) in dry THF (20 ml) were stirred at room temperature for 3 h. The reaction mixture was partitioned between H_2O and diethyl ether and extracted three times with diethyl ether. The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by CC (CH_2Cl_2:absolute ethanol = 19:1) and recrystallized from the solvent specified below.

5-Bromo-1-ethyl-3-[(4-fluorophenyl)(1*H*-imidazol-1-yl)methyl]-1*H*-indole (**16**). As fine white crystals (yield: 71%), m.p. 143–145°C (isopropyl ether). IR (KBr) cm^{-1}: 3170, 3120, 3068, 3038 (νCH arom.); 2974, 2942 (νCH alkane); 1602, 1506, 1476 (νC = C and νC = N); 1071 (νC-F). ^{1}H NMR (DMSO d_6), δ ppm: 1.33 (t, 3H, $^3J = 7.20$ Hz, CH_3), 4.22 (q, 2H, $^3J = 7.20$ Hz, CH_2), 6.97 (s, 1H, H imidazole), 7.12 (s, 1H, CH), 7.14 (s, 1H, H^2), 7.20 (s, 1H, H imidazole), 7.24–7.34 (m, 4H, $H^{2'}$, $H^{3'}$, $H^{5'}$ and $H^{6'}$), 7.30–7.34 (m, 2H, H^4 and H^6), 7.54 (d, 1H, $^3J = 8.60$ Hz, H^7), 7.75 (s, 1H, H imidazole).

General Procedure for the Preparation of Compounds (17, 23)

Thionyl chloride (0.61 ml, 8.4 mmoles) was dropped onto an ice-cooled solution of 1*H*-1,2,4-triazole (2.32 g, 33.6 mmoles) in dry acetonitrile (30 ml). The mixture was stirred at room temperature for 1 h, then filtered. This solution was added dropwise to a solution of carbinol (0.5 g, 2.1 mmoles) in dry acetonitrile (10 ml). After addition, the mixture was stirred at room temperature for 2–4 h, then filtered and concentrated. The residue was dissolved in CH_2Cl_2 and the organic solution was washed with brine, dried over Na_2SO_4, filtered, and evaporated to provide the crude product mixture, which was purified by CC (CH_2Cl_2:absolute ethanol = 19:1) and recrystallized from the solvent specified below.

5-Bromo-1-ethyl-3-[(2,4-dichlorophenyl)(1*H*-1,2,4-triazol-1-yl)methyl]-1*H*-indole (**17**). As white crystals (yield: 30%), m.p. 124–126°C (acetonitrile). IR (KBr) cm^{-1}: 3156, 3080, 3018 (νCH arom.); 2982, 2942 (νCH alkane); 1538, 1498, 1464 (νC = C and νC = N); 791 (νC-Cl). ^{1}H NMR (DMSO d_6), δ ppm: 1.33 (t, 3H, $^3J = 7.20$ Hz, CH_3), 4.22 (q, 2H, $^3J = 7.20$ Hz, CH_2), 7.18 (s, 1H, H^2), 7.22 (d, 1H, $^3J = 8.50$ Hz, $H^{6'}$), 7.33 (dd, 1H, $^4J = 1.80$ Hz, $^3J = 8.70$ Hz, H^6), 7.48 (s, 1H, CH), 7.49–7.57 (m, 3H, H^4, H^7 and $H^{5'}$), 7.76 (d, 1H, $^4J = 2.0$ Hz, $H^{3'}$), 8.11 (s, 1H, H triazole), 8.69 (s, 1H, H triazole).

General Procedure for the Preparation of Compound (18)

To a magnetically stirred suspension of $AlCl_3$ (1.33 g, 10 mmoles) in CH_2Cl_2 (25 ml) at 25°C was added 4-fluorobenzoyl chloride (1.18 ml, 10 mmoles) and the mixture was stirred for 1 h. A solution of 4.5 mmoles of 5-bromo-1*H*-indole (**4**) in CH_2Cl_2 (10 ml) was added dropwise. The reaction mixture was stirred at room temperature for 48 h. The solution was poured onto crushed ice and ethyl acetate. After extraction with ethyl acetate (2 × 50 ml), the combined organic layers were washed with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and concentrated in vacuum. Crystallization of the solid from ethyl acetate gave (**18**).

5-Bromo-3-(4-fluorobenzoyl)-1*H*-indole (**18**). As fine white crystals (yield: 45%), m.p. >250°C (ethyl acetate). IR (KBr) cm^{-1}: 3157 (νNH), 3028 (νCH arom.); 2938, 2906 (νCH alkane); 1596 (νC = O); 1515, 1438 (νC = C); 1086 (νC-F). ^{1}H NMR (DMSO d_6): δ ppm: 7.40 (dd, 2H, $^3J_{HH} = {}^3J_{HF} = 8.70$ Hz, $H^{3'}$ and $H^{5'}$), 7.44 (dd, 1H, $^4J_{H^6H^4} = 1.70$ Hz, $^3J_{H^6H^7} = 8.60$ Hz, H^6), 7.54 (d, 1H, $^3J = 8.60$ Hz, H^7), 7.92 (dd, 1H, $^4J_{HF} = 5.70$ Hz, $^3J_{HH} = 8.70$ Hz, $H^{2'}$ and $H^{6'}$), 8.09 (d, 1H, $^3J = 3.10$ Hz, H^2), 8.43 (d, 1H, $^4J = 1.70$ Hz, H^4), 12.35 (s, 1H, NH).

General Procedure for the Preparation of Compounds (19, 20)

To anhydrous acetonitrile (15 ml) at 25°C were added 0.80 g (2.5 mmoles) of 5-bromo-3-(4-fluorobenzoyl)-1*H*-indole (**18**) and 1.63 g (5 mmoles) of cesium carbonate. The mixture was heated at reflux for 2 h and then 2.8 mmoles of halogenobenzyl chloride was added. The reaction mixture was heated at reflux for 1 h (TLC control). After filtration, the solvent was removed under reduced pressure, and the residue was dissolved in water and CH_2Cl_2. The organic layer was separated, washed with brine, dried over Na_2SO_4, filtered and evaporated. The obtained product was recrystallized from the solvent specified below.

5-Bromo-3-(4-fluorobenzoyl)-1-(4-fluorobenzyl)-1*H*-indole (**20**). As white crystals (yield: 93%) m.p. 150–152°C (diisopropyl ether). IR (KBr) cm^{-1}: 1629 (νC = O); 1602, 1526 (νC = C); 1153 (νC-F). ^{1}H NMR (DMSO d_6): δ ppm: 5.58 (s, 2H, CH_2), 7.19 (dd, 2H, $^3J_{HH} = {}^3J_{HF} = 8.80$ Hz, H^3 and H^5 benzyl), 7.38–7.43 (m, 2H, H^2 and H^6 benzyl), 7.43 (dd, 2H, $^3J_{HH} = {}^3J_{HF} = 8.80$ Hz, $H^{3'}$ and $H^{5'}$), 7.45 (dd, 1H, $^4J_{H^6H^4} = 1.90$ Hz, $^3J_{H^6H^7} = 8.80$ Hz, H^6), 7.59

(d, 1H, $^3J = 8.80$ Hz, H^7), 7.95 (dd, 1H, $^4J_{HF} = 5.60$ Hz, $^3J_{HH} = 8.80$ Hz, $H^{2'}$ and $H^{6'}$), 8.42 (s, 1H, H^2), 8.45 (d, 1H, $^4J = 1.90$ Hz, H^4) (Scheme 1).

Biological Experiments

Leishmania (*L.*) *mexicana* promastigotes (MHOM/-MEX/96/UPN5) were maintained in Schneider's insect medium (Sigma Chemical Co., St. Louis, MO, USA), plus 10% heat-inactivated foetal calf serum (FCS) (Sigma), penicillin and streptomycin, at 26°C, by passage every 7 days.

Studies In Vitro

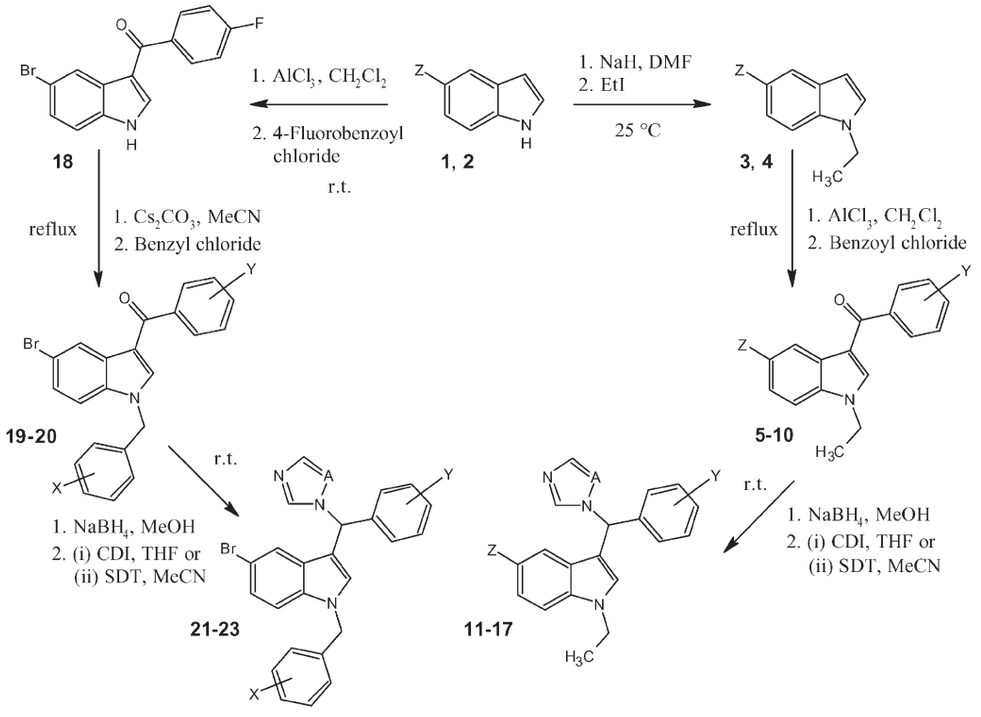
L. mexicana promastigotes were inoculated into 96-well plates (Nunc Inc., Naperville, IL, USA). The cultures were exposed for 96 h at 26°C to test 3-(α-azolylbenzyl)indoles **11**–**17** and **21**–**23** with a triplicate culture for each concentration (100, 10 and 1 μmol). The antiproliferative effect was determined by a colorimetric method based on the conversion of MTT into a blue formazan product, by mitochondrial dehydrogenases. Absorbance was monitored at 570 nm.[21,22]

The activity against the intracellular stage of the parasite (amastigote) was determined in a Balb/c mouse peritoneal macrophage model (Centre d'élevage R. Janvier, Le Genest, France) as previously described.[23] Briefly, peritoneal cells were placed into a 24-well plate (Nunc Inc.) and infected with stationary phase promastigotes. After a 24-hour incubation time, the culture was washed and exposed to the test compounds. Cytotoxicity was determined after exposure to the target azolylindoles during 96 h. IC_{50s} were calculated using the values of number of amastigotes per macrophage (Figure 1).

RESULTS AND DISCUSSION

The 10 potential chemotherapeutics were evaluated *in vitro* against promastigote *Leishmania mexicana* parasites (see. Table I), and four (**15**, **17**, **22**, **23**) were further evaluated *in vitro* against *L. mexicana* amastigotes using a Balb/c mouse peritoneal macrophage model (see Table II).

All synthesized compounds displayed *in vitro* activity against *L. mexicana* promastigotes ($IC_{50} < 35$ μM), while Glucantime®, the drug of first choice in leishmaniasis treatment, had an IC_{50} value of 41,500 μM. Among the N^1-ethyl derivatives (**11**–**17**), 5-bromo-1-ethyl-3-[(2,4-dichlorophenyl)(1*H*-imidazol-1-yl)methyl]-1*H*-indole (**15**) displayed the lowest IC_{50} value (0.23 ± 0.04 μM). The data indicated that the introduction of a 5-bromo group enhanced activity; compounds (**14** and **15**) were four- and eleven-fold more active, respectively, than the non-bromo compounds (**11** and **12**), with only one



SCHEME 1 Synthesis of 3-(α-azolylbenzyl)indoles.

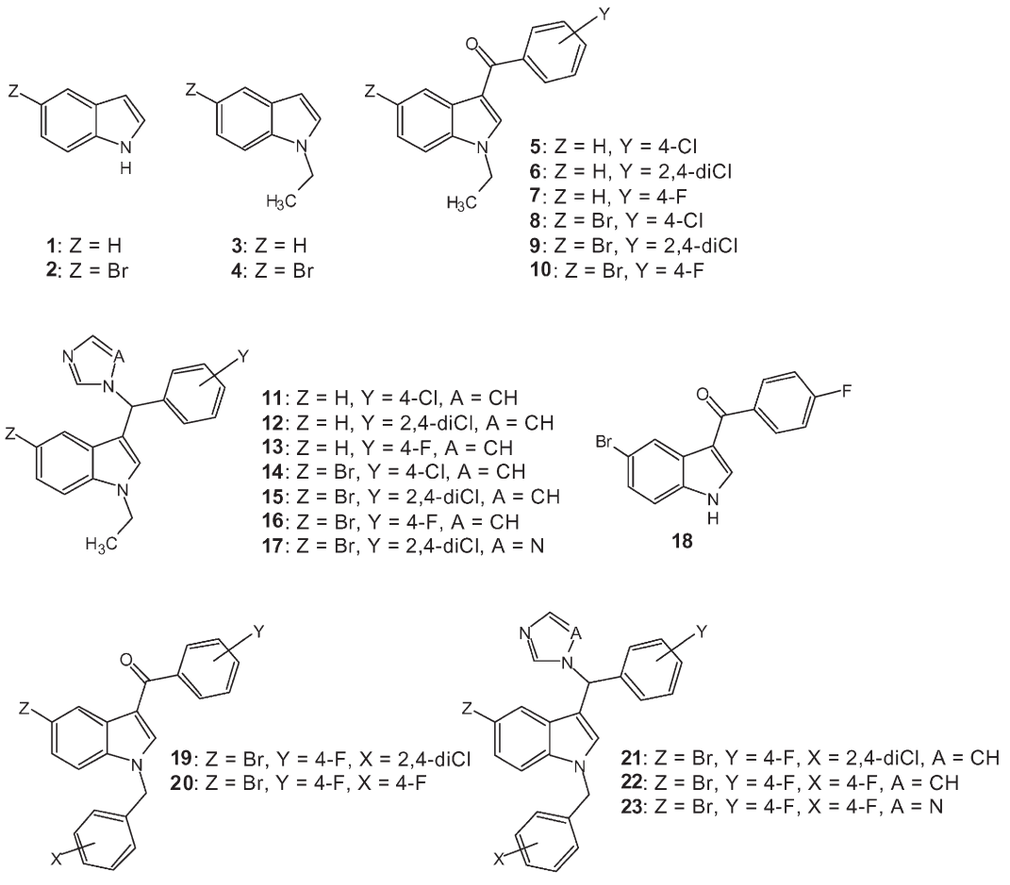


FIGURE 1 Chemical structures of 3-(α-azolylbenzyl)indoles (**11**–**17, 21**–**23**) and their precursors (**1**–**10**, **18**–**20**).

exception, compound (**16**) with a 3-(4-fluorobenzyl) moiety. The influence of the halogeno group on the (α-azolylbenzyl) moiety seemed to indicate a better activity for 4-chloro and 2,4-dichloro groups than for a 4-fluoro group. 5-Bromo-1-ethyl-3-[(4-chlorophenyl)(1*H*-imidazol-1-yl)methyl]-1*H*-indole (**14**) and 5-bromo-1-ethyl-3-[(2,4-dichlorophenyl)(1*H*-imidazol-1-yl)methyl]-1*H*-indole (**15**) had IC_{50} values less than 1 μM, while compound (**16**), characterized by a 4-fluorophenyl chain, displayed an IC_{50} value up to 30 μM. Only one triazole analogue has been synthesized, compound (**17**); its IC_{50} value was similar to one of the imidazole derivative (**15**) (0.37 and 0.23 μM, respectively).

With the N^1-benzyl derivatives (**21**–**23**), the IC_{50} values were quite similar for the imidazole compounds (3 and 5.4 μM, respectively), while the triazole analogue had a lower IC_{50} (1.2 ± 0.6 μM). The N^1-benzyl derivatives (**21** and **22**) demonstrated higher activities than the N^1-ethyl derivative (**16**).

TABLE I 3-(α-Azolylbenzyl)indoles (**11**–**17**, **21**–**23**) and their *in vitro* activities against promastigotes of a strain of *L. mexicana*

Compound	IC_{50} (μM)* promastigotes
11	2.6 ± 1.00
12	2.7 ± 1.50
13	5.9 ± 0.14
14	0.57 ± 0.01
15	0.23 ± 0.04
16	35 ± 3.00
17	0.37 ± 0.10
21	3 ± 1.00
22	5.4 ± 0.10
23	1.2 ± 0.60
Glucantime®	>100 (41,500)

*Mean from at least three determinations.

TABLE II 3-(α-Azolylbenzyl)indoles (**15**, **17**, **22**, **23**) and their *in vitro* activities against intracellular amastigotes of a strain of *L. mexicana*

Compound	IC_{50} (μM) Intracellular amastigotes
15	<1
17	7.3 ± 0.1
22	2.68 ± 0.1
23	6.47 ± 0.1
meglumine antimoniate*	48.7 ± 8.4
ketoconazole	1.3 ± 0.2

*Expressed as antimony.

The protozoan parasite *L. mexicana* survives and multiplies within mammalian macrophages as the amastigote form. It was therefore of interest to evaluate the efficacy of active 3-(α-azolylbenzyl)indoles on the clinical relevant stage. Four compounds (**15**, **17**, **22**, **23**) were selected for further evaluation against *L. mexicana* amastigotes using ketoconazole and meglumine antimoniate (based on antimony content) as reference compounds. All tested compounds were more active against the intracellular form of the parasite than meglumine antimoniate ($IC_{50} = 48.7 \pm 8.4\,\mu M$). Triazole compounds (**17** and **23**) were almost equally effective with IC_{50} values in the range 6.5–7.3 μM. They were less active than their imidazole analogues. Compound (**15**), the most active imidazole against *L.* promastigotes was, as well, the most active against *L.* amastigotes. It exhibited an IC_{50} value less than 1 μM. This result was an order of magnitude lower than the IC_{50} value obtained for the reference conazole, ketoconazole ($IC_{50} = 1.3 \pm 0.2\,\mu M$).

In conclusion, careful analysis of the antileishmanial activity of the target compounds revealed encouraging results obtained with all 3-(α-azolylbenzyl)indoles and especially the 3-(2,4-dichlorobenzyl) derivative (**15**). Further pharmacomodulation in that promising series will be conducted in order to lead to a drug with a high intrinsic activity and interesting pharmacokinetic profile. This most potent compound (**15**) was selected for an *in vivo* evaluation to confirm its chemotherapeutic potency. The results of this study and first approaches to explain possible mechanisms of action will be presented in due course.

Acknowledgements

The authors would like to thank YANG JI CHEMICAL COMPAGNY for financial support.

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