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Synthesis and Structure-Activity Relationships of Lansine Analogs as Antileishmanial Agents

Marco Pieroni^{[a],[c]}, Sisay Girmay^[a], Dianqing Sun^[a], Rajnish Sahu^[b], Babu L. Tekwani^[b], and Ghee T. Tan^[a]

^[a]Tan College of Pharmacy, University of Hawai'i at Hilo Hilo, HI 96720-4029 (USA), Fax: (+1) 808-933-2974 gheetan@hawaii.edu

^[b]National Center for Natural Products Research & Department of Pharmacology, School of Pharmacy, University of Mississippi University, MS 38677-1848 (USA), Fax: (+1) 662-915-7062 btekwani@olemiss.edu

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Among all the parasitic infections, leishmaniasis and malaria are considered the most significant from both a pathological and socioeconomic point of view. According to a recent report from WHO,^[1] leishmaniasis [visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and diffuse cutaneous leishmaniasis (DCL)] collectively affect 12 million people in 88 countries (especially in the world's inter-tropical and temperate regions), and 300 million more are at risk of infection. Moreover, there are 1.5-2 million new cases and 70,000 deaths attributed to this disease each year.^[1] These figures are especially alarming in developing countries, where co-infection with HIV is drastically changing the disease burden of leishmaniasis by way of its rapid expansion into new geographical areas; for instance, it has been reported that, in south Europe, up to 70% of adult cases of VL are associated with HIV infection.^[2] Since a vaccine is, as yet, unavailable, chemotherapy remains the only means to control the spread of the disease. Miltefosine, paromomycin and liposomal amphotericin B are gradually replacing pentavalent antimonials (meglumine antimoniate and sodium stibogluconate) and conventional amphotericin B as the preferred treatments in some regions of the world.^[3] While these agents continue to play a major role in mono- and combination therapies, it is evident that new drugs or treatment strategies must circumvent current chemotherapeutic limitations that include the high cost of drugs or their formulations,^[4] severe adverse effects^[5] or teratogenicity,^[6] and the need for daily parenteral administration due to the lack of oral efficacy.^[7] In addition, the emergence of drug resistance threatens to reduce the efficacy of these treatments.^[8] In view of the foregoing facts, there is an urgent need for the development of innovative antileishmanial agents based on new molecular scaffolds that are associated with improved efficacy and pharmacokinetic properties, in addition to the lack of toxicity.

Nature has been an inexhaustible source of lead molecules for antiparasitic, antibacterial and anticancer drug discovery since millennia. Indeed, before the advent of high-throughput screening and the post-genomic era, more than 80% of drug substances were natural

^[c]Current address: Interdepartmental Center Biopharmaceutics, Università degli Studi di Parma, Via G. P. Usberti 27, Parma Italy. Supporting information for this article is available on the WWW under <http://www.chemmedchem.org> or from the author.

products or inspired by naturally-occurring compounds.^[9] This still holds true for many anti-infectives. In recent years, a considerable number of carbazoles have been isolated from terrestrial plants, and evaluated primarily for their anticancer activity and/or as anti-infectives.^[10] The interest surrounding these molecules has stimulated efforts to develop straightforward and efficient synthetic pathways to afford the carbazole nucleus in good yields and in a relatively short amount of time.^[11] Although novel carbazole analogs have continuously been discovered or synthesized, no attempt has been made to systematically investigate the structure-activity relationships (SAR) for these tricyclic planar molecules with regard to antileishmanial/antiparasitic activity.

This prompted us to evaluate synthetic carbazoles against *Leishmania donovani*, the causative agent of VL, as a rational expansion of our work on the antitubercular activity of the carbazole lansine (Figure 1),^[12] an alkaloid first isolated from *Clausena lansium*.^[13] Lansine was deemed a hit compound worthy of further investigation by virtue of its potency in relation to the notable lack of cytotoxicity to VERO cells.^[12] Its relative ease of synthesis and favorable predicted physicochemical characteristics provided additional justification for the study. We synthesized a series of lansine analogs, and the rational modifications made at positions C-2, C-3, C-6 and N-9 allowed us to construct a plausible SAR for these compounds with respect to antileishmanial activity. Six derivatives (**5a**, **6c**, **6d**, **6e**, **8**, **13**) demonstrated improved potency against *L. donovani* promastigotes in comparison to the parent hit compound lansine (Figure 1, Table 1, **6a**). Four of these derivatives (**5a**, **6c**, **6d**, **6e**) also demonstrated significantly enhanced activity against axenic amastigotes with IC₅₀ values in the range of 7 - 23 μ M. The reference compound pentamidine yielded IC₅₀ values of 3.5 and 2.9 μ M against axenic amastigotes and promastigotes, respectively. Nevertheless, the insignificant cytotoxicity (CC₅₀ >20 μ g/mL) of almost all carbazole analogs examined (Table 1) warrants a more comprehensive and detailed investigation of these compounds in the quest for new antileishmanial agents.

The target compounds **5a**, **5g**, **6a-f**, and **13** were synthesized by employing an efficient protocol previously reported by Schmidt *et al* (Scheme 1).^[14] Briefly, the 4-substituted bromobenzene **1a-f** underwent a Buchwald-Hartwig cross coupling reaction with 3-(*tert*-butyldiphenylsilyloxy)-4-methylaniline **2** that was easily prepared from commercially available 2-methyl-nitrophenol in two steps. Subsequent palladium-catalyzed cyclization of **3a-g** afforded the carbazole derivatives **4a-g** in its less hindered conformation. The oxidation of the methyl group with DDQ, followed by the deprotection of the hydroxyl moiety, led to the synthesis of the title compounds **6a-f** in good overall yields. Reduction of the aldehyde with NaBH₄, carried out before the deprotection of the phenol moiety, afforded compound **11** in high yield from **5a**. Methylation of lansine (**6a**) with MeI using NaH as a base, resulted in the formation of compounds **8**^[14] and **9**, while *O*-demethylation of lansine (**6a**) with BBr₃ led to the first synthesis of the known natural product **7**.^[15]

In this work, a total of 17 compounds were evaluated, for the first time, for their ability to inhibit the growth of both the extracellular and intracellular forms of *Leishmania donovani*, the etiological agent responsible for visceral leishmaniasis (VL). The amastigote stage is the second evolutive form of the parasite that is responsible for systemic infections in a clinical setting. Results were expressed as IC₅₀ (median inhibitory concentration). Of all the compounds synthesized, seven are new chemical entities (**6c-f**, **9**, **11**, **12**), while ten are known compounds. Two of the known compounds (**6b**, **8**) were synthesized with a procedure different from that previously reported,^[14,16] while four (**5a**, **5g**, **6a**, **13**) were synthesized according to an established procedure.^[14,17] One known compound (**7**) was synthesized for the first time, and three (**14-16**) were purchased from Sigma-Aldrich. The *in vitro* toxicity profile of all compounds synthesized was obtained in three different cell lines: African Green Monkey kidney (VERO) cells, murine macrophages (J-774) and human lung

carcinoma (LU-1). Selectivity index (SI) is defined as CC_{50}/IC_{50} , where CC_{50} is the median cytotoxic concentration. Antiparasitic IC_{50} values, with associated selectivity indices in LU-1 and J-774, are reported in Table 1. All compounds are nontoxic to VERO cells at 20 $\mu\text{g/mL}$ ($CC_{50} > 20 \mu\text{g/mL}$). In addition, a previous report has indicated that lansine (**6a**) and **5g** are associated with CC values of $>102 \mu\text{g/mL}$ in VERO cells,^[15] which further support the favorable safety profile of this series of derivatives.

In order to build a reliable, albeit preliminary, body of SAR centered on the substitution pattern of the hit compound lansine (**6a**), we investigated the contribution of rationally selected substituents at positions N-9, C-2, C-3, and C-6 (Figure 1) to the overall antileishmanial activity of these molecules. Basic medicinal chemistry rules led us to generalize that more hydrophilic moieties such as $-\text{NH}$, the hydroxyl at C-2, and the aldehyde at C-3, were all responsible for critical interactions with the target, whereas substituents at C-6 might play a role in the modulation of pharmacological potency. In particular, since the mechanism of action reported for many carbazoles involves intercalation of the planar tricyclic scaffold into DNA,^[18-20] we can hypothesize that the substituent at C-6 may affect the H-bond donor/acceptor characteristics of the $-\text{OH}$ and $-\text{NH}$ groups, thus modulating the effectiveness of binding of the molecules to the bases of DNA.

The critical presence of a substituent at C-6 is confirmed by the fact that compound **6b** (IC_{50} 167.6 μM),^[16] unsubstituted at the C-6 position, was found to have much higher IC_{50} and CC_{50} values than lansine (**6a**, IC_{50} 89.1 μM) in the promastigote assay. Similarly, **6b** was less effective in inhibiting the growth of axenic amastigotes than lansine (**6a**) (Table 1). Given this precedence, and capitalizing on the feasible and straightforward synthesis of analogs variously substituted at C-6, a number of lansine analogs were synthesized with an array of C-6 substituents strategically adopted based on their size (bulky or small), electronic (EDGs and EWGs) and physicochemical (lipophilic and hydrophilic) characteristics.

From this first set of compounds, lipophilic groups such as $-\text{CF}_3$ (**6c**), $-\text{OCF}_3$ (**6d**) and $-\text{tBu}$ (**6e**) were found to significantly enhance the antileishmanial activity of the molecule, with **6d** and **6e** showing the greatest efficacy against both promastigotes and axenic amastigotes. The potency of these compounds is about 2-6-fold greater than that of lansine. It is also noteworthy that the most active compound **6d** also demonstrated the highest selectivity in the carbazole series examined. The focused body of data collected suggests that lipophilicity, rather than the electron donor/acceptor characteristics or the bulkiness of the substituent at C-6, plays the most significant role in ensuring antileishmanial potency. In confirmation of this, when the carbazole bears a more hydrophilic substituent at C-6, the activity is drastically compromised, regardless of whether the group is a bulky EWG such as $-\text{NO}_2$ (**6f**) or a small EDG such as $-\text{OH}$ (**7**) (Table 1). The enhanced activity conferred by a more lipophilic C-6 substituent may be explained either by the presence of a hydrophobic pocket in the target binding site or, more likely, by improved parasite membrane penetration.

In order to assess the importance of the $-\text{OH}$ at the C-2 position, this substituent was either abolished (**5g**),^[17] or methylated (**8**). In addition, compound **5a**,^[14] a key intermediate for the synthesis of lansine (**6a**), was also tested for comparison, since we presumed that the lipophilic protecting group might enhance membrane permeability, and, once in the cell, the labile $-\text{OTBDPS}$ ether would be cleaved to yield the active molecule. This supposition proved to be correct since compound **5a** yielded an IC_{50} (22.9 μM) value that is 2-fold lower than that of lansine (**6a**, IC_{50} 45.6 μM) in the axenic amastigote assay. A similar effect was observed against the promastigotes, albeit to a lesser extent. Compound **5a** demonstrated an IC_{50} of 71.9 μM which represents a discernible improvement over that of lansine (**6a**, IC_{50}

89.1 μM). Indeed, the prodrug strategy of employing a silyl ether to enhance the cellular penetration and pharmacological potency of a potential drug molecule has already been reported,^[21] although the hypothesized intracellular cleavage of silyl ethers by hydrolytic enzymes or otherwise, has yet to be demonstrated. This approach is directly applicable to amastigotes resident within human macrophages; greater penetration and accumulation of the compound will invoke sterilizing activity within the infected host cell. It is also reasonable to assume that the intact TBDPS ether itself may also be pharmacologically active, since planar chromophores with bulky peripheral substituents have been known to retain their ability to interact with DNA.^[22] While removal of the C-2-OH moiety (**5g**) reduced overall antileishmanial activity compared to that of lansine (**6a**), the methylation of this moiety led to an appreciable improvement in potency against promastigotes, since the IC_{50} value of **8** (34.9 μM) is more than 2-fold lower than that of lansine (IC_{50} 89.1 μM). Interestingly, the opposite trend was observed for the amastigotes, where the methylation of C-2-OH completely abolished activity. Although further investigation into this observation is pending, we may tentatively conclude that the dual H-bond donor/acceptor nature of the C-2-OH is not required at this position, whereas only the H-bond acceptor characteristic is needed to afford the observed anti-promastigote activity. Coincidentally, the removal or methylation of the -OH at C-2 also abolished cytotoxicity in both the LU-1 and J-774 cell lines, resulting in an appreciable increase in the SI values of compounds **5g** and **8** compared to that of lansine.

In contrast to the 2-fold improvement in antipromastigote activity observed with methylation of the C-2-OH, methylation of N-9 led to the complete loss of activity (**9**, IC_{50} >148.5 μM vs **8**, IC_{50} 34.9 μM). Along similar lines, it was observed that alkylation of N-9 (compounds **9**, **14** and **16**) resulted in the complete loss of activity against both promastigotes and amastigotes, regardless of the nature of the other substituents on the molecule. These observations reveal a crucial role of the N-9 hydrogen in target site interactions. Reduction of the -CHO moiety at C-3 (**11**) resulted in the complete loss of antipromastigote activity, whereas, surprisingly, its replacement with a -CH₃ (**13**,^[14] IC_{50} 78.0 μM) did not result in an appreciable change in the activity of the molecule. In contrast, compounds **11** and **13** demonstrated antiamastigote activity that was comparable to that of lansine. Finally, the complete removal of the substituent at C-3 did not lead to any appreciable change in overall antileishmanial potency (**6b** vs **15**), with activity against promastigotes and amastigotes remaining at ~160 and ~53 μM , respectively. Collectively, these observations are consistent with the dispensable nature of the substituent at C-3.

In general, similar SAR trends apply to both promastigotes and axenic amastigotes. In addition, the most active carbazole derivatives **5a**, **6c**, **6d**, **6e** retained potency in both these life stages of the parasite. Although the molecular target(s) and mode(s) of parasite growth inhibition are, as yet, uncharacterized for these planar tricyclic compounds, it is reasonable to assume that DNA interaction (via intercalation or groove binding), with downstream disruption of vital DNA-dependent cellular processes, might constitute a highly plausible mechanism.^[19-20] This hypothesis is supported by the fact that cleavage of ring B abolishes both antileishmanial and cytotoxic activity (**12**).

In conclusion, this work represents the first attempt to establish a reliable SAR for lansine analogs as antileishmanial agents. Overall, this work highlights the fact that lansine derivatives and, in general, compounds bearing a tricyclic carbazole nucleus, are amenable to modifications that enhance physicochemical and toxicological properties, while retaining antileishmanial potency. Significant improvements in terms of activity and selectivity with respect to the parent lansine have been achieved in this study. Most notably, compound **6d** demonstrated a 6-fold improvement in activity against amastigotes. Concomitantly, SI values improved 10-fold in LU-1 cells and 2-fold in J-774 macrophages. These encouraging

observations provide a firm foundation for the further development of this versatile pharmacophore.

Experimental Section

Chemistry

All reagents and solvents obtained from commercial sources were used without further purification. Reactions were monitored either by thin-layer chromatography (TLC) or HPLC with a Shimadzu LC-20A series high performance liquid chromatography (HPLC) system. TLC was performed using glass plates pre-coated with silica gel (0.25 mm, 60 Å pore size, 230-400 mesh, Sorbent Technologies, GA) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV). Hydrogenation reactions were done using domnick hunter NITROX UHP-60H hydrogen generator, USA. Reactions in sealed tube were carried out using Q-Tube™ pressure tube reactors from Q Labtech, USA. Flash column chromatography was performed using a Biotage Isolera One system with a Biotage SNAP cartridge. Proton and carbon nuclear magnetic resonance (^1H and ^{13}C NMR) spectra were recorded employing a Bruker Avance DRX-400 spectrometer. Chemical shifts and J values were expressed in parts per million (ppm) and Hertz, respectively. Mass spectra were recorded on a Varian 500-MS IT mass spectrometer using electron spray ionization (ESI). The purity of compounds was determined by analytical HPLC using a Gemini, 3 μm , C18, 110 Å column (50 mm $\times$ 4.6 mm, Phenomenex) and a flow rate of 1.0 mL/min. Gradient conditions: solvent A (0.1% trifluoroacetic acid in water) and solvent B (acetonitrile): 0-2.00 min 100% A, 2.00-7.00 min 0-100% B (linear gradient), 7.00-8.00 min 100% B, UV detection at 254 and 220 nm. The purity of the compounds tested was determined to be 97.8 % by analytical HPLC.

General procedure for the synthesis of compounds 3a-g (Buchwald-Hartwig cross coupling)

The appropriate substituted bromobenzene **1a-f** (1 eq) and the aniline **2** (1.1 eq) were solubilized in dry toluene (7 mL/mmol of bromobenzene), after which Cs_2CO_3 (1.2 eq), BINAP (6 mol%) and $\text{Pd}(\text{OAc})_2$ (6 mol%) were added and the reaction mixture was stirred vigorously at 115°C until complete consumption of the starting material as indicated by TLC. The solvent was evaporated and the residue was washed with water. The aqueous layer was then extracted with ethyl acetate (3 $\times$ 20 mL), and the combined organic extracts were washed with brine, dried over MgSO_4 , and concentrated under reduced pressure. The residue was purified by flash chromatography to obtain the desired product as a brown oil. Analytical data for compounds **3a**,^[14] **3g**^[17] matched data previously published.

General procedure for the synthesis of compounds 4a-g

In a sealed tube, $\text{Pd}(\text{OAc})_2$ (10 mol%), and $\text{Cu}(\text{OAc})_2$ (2.5 eq) were added to a solution of the diarylamines **3a-g** (1 eq) in acetic acid, and the mixture was heated at 135°C for 4 h. After cooling, the solution was carefully neutralized with Na_2CO_3 . The aqueous layer was extracted with ethyl acetate (3 $\times$ 10 mL) and the combined organic extracts were washed with brine, dried over MgSO_4 , and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to obtain the desired products in good overall yields (see supporting information). Analytical data for compounds **4a**,^[14] **4g**^[17] matched data previously reported.

General procedure for the synthesis of compounds 5a-g

DDQ (2.2 eq) was added to a solution of the carbazoles **4a-g** (1 eq) in a mixture of MeOH/THF (10:3, v/v), and the resulting dark solution was allowed to react at rt for 1 h. The

solvent was evaporated and the slurry residue was washed with water. The aqueous layer was extracted with ethyl acetate (3×10 mL), and the combined organic extracts were washed with brine, dried over MgSO_4 , and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to obtain the desired products. Interestingly, the synthetic reactions of compounds **5a** and **5c** afforded, among the other side products, a negligible amount of the corresponding deprotected carbazoles **6a** and **6c**. Analytical data for compounds **5a**,^[14] **5g**^[17] matched data previously reported.

General procedure for the synthesis of compounds **6a-f**, **11-13**

To a stirred solution of the *tert*-butyldiphenylsilyl ether (1 eq) in DMF (10 mL/mmol), TBAF·H₂O (1.6 eq) was added portion wise. After 30 min at rt, the mixture was poured in ice-water, the aqueous layer was extracted with ethyl acetate (3×5 mL), and the combined organic extracts were washed with brine, dried over MgSO_4 , and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel to obtain the title compounds **6a-f**, **11-13** in good overall yields (see supporting information). Analytical data for compounds **6a**,^[14] **6b**,^[16] **13**^[14] matched data previously reported.

2,6-Dihydroxy-9*H*-carbazole-3-carbaldehyde (**7**)

^[15] To a stirred solution of **6a** (70 mg, 0.29 mmol) in dry DCM (2 mL), BBr₃ (0.14 mL, 1.45 mmol) was added at 0 °C, and the dark red solution was stirred for 30 minutes. The mixture was then washed with water. The organic phase was washed with brine, dried over MgSO_4 , and concentrated under reduced pressure. The residue was purified by flash chromatography (from EtOAc-hexane 20–70%) to obtain the title compound as a yellow solid in 87% yield. Analytical data for compound **7** matched data previously reported.^[15]

2,6-Dimethoxy-9*H*-carbazole-3-carbaldehyde (**8**)¹⁴ and 2,6-dimethoxy-9-methyl-9*H*-carbazole-3-carbaldehyde (**9**)

A suspension of **6a** (40 mg, 0.16 mmol), K₂CO₃ (91 mg, 0.66 mmol) and MeI (0.015 mL, 0.248 mmol) in dry acetone (2 mL), was refluxed for 3 h. The mixture was then poured in ice-water, after which the aqueous layer was extracted with ethyl acetate (3×10 mL). The combined organic extracts were washed with brine, dried over MgSO_4 , and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (from EtOAc-hexane 20–70%) to obtain the title compounds **8** and **9** in 3:1 proportion, respectively. Analytical data for compound **8** matched data previously reported.¹⁴ **9** (light brown solid). ¹H NMR (400 Mz, CDCl₃) = 3.81 (s, 3H), 3.93 (s, 3H), 4.06 (s, 3H), 6.73 (s, 1H), 7.08 (dd, $J_1 = 2.4$ Hz, $J_2 = 8.4$ Hz 1H), 7.28 (d, $J = 8.4$ Hz, 1H), 7.55 (d, $J = 2.4$ Hz, 1H), 8.55 (s, 1H), 10.50 (s, 1H). ¹³C NMR (100 Mz, CDCl₃) = 191.5, 161.5, 153.6, 135.7, 125.9, 125.1, 123.3, 122.9, 117.0, 112.0, 96.7, 55.8, 55.6, 28.9. LRMS (ESI) calculated for C₁₆H₁₅NO₃ [M + H]⁺ 270.1052, found 270.2. HPLC purity 99.0%, peak 6.29 min.

2-(*Tert*-butyldiphenylsilyloxy)-6-methoxy-3-hydroxymethyl-9*H*-carbazole (**10**)

To a solution of **5a** (100 mg, 0.20 mmol) in dry methanol (3 mL), NaBH₄ (9.2 mg, 0.24 mmol) was added at 0 °C, and the solution was stirred at rt for 5 h. The solvent was evaporated, and the residue solubilized with ethyl acetate and washed with water (3×10 mL). The organic layers were washed with brine, dried over MgSO_4 , and concentrated under reduced pressure to give derivative **10** (85 mg, 83%) as a pale yellow powder, used in the next reaction step without further purification (HPLC purity 93%). ¹H NMR (400 Mz, CDCl₃) = 1.17 (s, 9H), 3.90 (s, 3H), 5.04 (s, 2H), 6.48 (s, 1H), 6.94 (dd, $J_1 = 2.4$ Hz, $J_2 = 8.8$ Hz 1H), 7.13 (d, $J = 8.8$ Hz, 1H), 7.37-7.48 (m, 8H), 7.77-7.81 (m, 4H), 7.97 (s, 1H). LRMS (ESI) calculated for C₃₀H₃₁NO₃Si [M + Na]⁺ 504.1971, found 504.5.

Biology

In vitro inhibition of *L. donovani*—The effect of carbazoles on the viability of *L. donovani* promastigotes and axenic amastigotes (2×10^6 cells/mL) was determined using a 72 hour Alamar blue assay based on the intracellular metabolic reduction of the redox indicator.^[23] All compounds were tested in duplicates and IC₅₀ values were computed from the mean dose response curves by Excel-Fit. The assay shows overall variation of +10%. The axenic amastigotes demonstrate a drug sensitivity profile similar to that of intracellular amastigotes.^[24] Pentamidine (IC₅₀ 1.2 µg/mL [3.5 µM]) and amphotericin B (IC₅₀ 0.14 µg/mL [0.2 µM]) were tested as the standard antipromastigote agents. The antiproliferative activity of compounds against axenic amastigotes was assessed as described for promastigotes except that the pH of the medium was acidified to pH 5.5 and incubations were performed at 37°C instead of 26°C.^[25] Reference compounds, pentamidine and amphotericin B demonstrated IC₅₀ values of 1.0 µg/mL [2.9 µM] and 0.15 µg/mL [0.2 µM], respectively. All carbazoles were simultaneously tested for cytotoxicity against African Green Monkey kidney epithelial (VERO) cells using Neutral Red as the metabolic indicator,^[26] and against murine macrophage cells (J774) using the Alamar blue assay.^[27]

Sulforhodamine (SRB) assay for cytotoxicity—A standard protocol for the assessment of cellular toxicity monitors the ability of cultured human lung carcinoma (LU-1) cells to proliferate in the presence of a test compound, and subsequently quantitates total protein content with sulforhodamine B (SRB) dye as a measure of the percentage of surviving cells.^[28] Data is expressed as a percentage relative to the DMSO-treated control incubations. IC₅₀ values are then calculated using non-linear regression analysis of plots of % survival versus concentration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- [1]. [last access, August 2012] <http://www.who.int/leishmaniasis/burden/en/>
- [2]. Alvar J, Aparicio P, Aseffa A, Den Boer M, Cañavate C, Dedet JP, Gradoni L, Ter Horst R, López-Vélez R, Moreno J. Clin. Microbiol. Rev. 2008; 21:334–359. [PubMed: 18400800]
- [3]. a) Kedzierski L, Sakthianandeswaren A, Curtis JM, Andrews PC, Junk PC, Kedzierska K. Curr. Med. Chem. 2009; 16(5):599–614. [PubMed: 19199925] b) Singh N, Kumar M, Singh RK. Asian Pac. J. Trop. Med. 2012; 5:485–497. [PubMed: 22575984]
- [4]. Meheus F, Balasegaram M, Oliaro P, Sundar S, Rijal S, Faiz MA, Boelaert M. PLoS Negl. Trop. Dis. 2010; 4(9):e818. [PubMed: 20838649]
- [5]. a) Oliveira LF, Schubach AO, Martins MM, Passos SL, Oliveira RV, Marzochi MC, Andrade CA. Acta Trop. 2011; 118(2):87–96. [PubMed: 21420925] b) Maltezou HC. Recent Pat. Antiinfect. Drug Discov. 2008; 3(3):192–198. [PubMed: 18991801]
- [6]. a) Sindermann H, Engel J, Trans J. R. Soc. Trop. Med. Hyg. 2006; 100(Suppl. 1):S17–20. b) Dorlo TP, Balasegaram M, Beijnen JH, de Vries PJ. J. Antimicrob. Chemother. Jul 24.2012 Epub ahead of print.

- [7]. Frézard F, Demicheli C, Ribeiro RR. *Molecules*. 2009; 30(147):2317–2336. [PubMed: 19633606]
 b) Rybníček J, Goede V, Mertens J, Ortmann M, Kulas W, Kochanek M, Benzing T, Arribas JR, Fätkenheuer G. *Int. J. Infect. Dis.* 2010; 14(6):e522–525. [PubMed: 19726213]
- [8]. a) Croft SL, Sundar S, Fairlamb AH. *Clin. Microbiol. Rev.* 2006; 19(1):111–126. [PubMed: 16418526] b) Maltezou HC. *J. Biomed. Biotechnol.* 2010; 2010:617521. [PubMed: 19888437] c) Aït-Oudhia K, Gazanion E, Vergnes B, Oury B, Sereno D. *Parasitol. Res.* 2011; 109(5):1225–1232. [PubMed: 21800124]
- [9]. Sneader, W. *Drug Prototypes and Their Exploitation*. Wiley; UK: 1996.
- [10]. a) Yan J, Zhou C, Ji Q, Geng R. *Guoji Yaoxue Yanjiu Zazhi*. 2011; 38(2):118–122. b) Zhang F-F, Gan L-L, Zhou CH. *Bioorg. Med. Chem. Lett.* 2010; 20(6):1881–1884. [PubMed: 20176480] c) Guo S, Tipparaju SK, Pegan SD, Wan B, Mo S, Orjala J, Mesecar AD, Franzblau SG, Kozikowski AP. *Bioorg. Med. Chem.* 2009; 17(20):7126–7130. [PubMed: 19783449] d) Thevissen K, Marchand A, Chaltin P, Meert EMK, Cammue BPA. *Curr. Med. Chem.* 2009; 16(17):2205–2211. [PubMed: 19519387]
- [11]. Kataeva O, Krahel MP, Knölker HJ. *Org. Biomol. Chem.* 2005; 3(17):3099–3101. [PubMed: 16106290] b) Knölker HJ, Reddy KR. *Alkaloids Chem. Biol.* 2008; 65:1–410. [PubMed: 18499945]
- [12]. Ma CY, Case RJ, Wang Y, Zhang HJ, Tan GT, Van Hung N, Cuong NM, Franzblau SG, Soejarto DD, Fong HH, Pauli GF. *Planta Med.* 2005; 17:261–267. [PubMed: 15770548]
- [13]. Prakash D, Raj K, Kapil RS, Indian SP. *J. Chem.* 1980; 19B:1075–1076.
- [14]. Schmidt M, Knölker HJ. *Synlett*. 2009:2421–2424.
- [15]. Shi XJ, Ye G, Tang WJ, Zhao WM. *Helv. Chim. Acta*. 2010; 93(5):985–990.
- [16]. Oliveira AMAG, Oliveira-Campos AMF, Rodrigues LM, Raposo MM, Machado AEH, Nascimento MSJ, Nazareth N, Pinto M. *Chem Biodivers.* 2007; 4(5):980–990. [PubMed: 17510993]
- [17]. Forke R, Krahel M, Krause T, Schlechtingen G, Knölker HJ. *Synlett*. 2007; 2:268–272.
- [18]. Saeed A, Kazmi M, Ameen Samra S, Irfan M, Bolte M. *Acta Crystallogr. E*. 2010; 66(Pt 8):o2118.
- [19]. Asche C, Demeunynck M. *Anticancer Agents Med. Chem.* 2007; 7:247–267. [PubMed: 17348830]
- [20]. Knölker, HJ.; Reddy, KR. *The Alkaloids*. Cordell, GA., editor. Vol. 65. Academic press; 2008. p. 181–191.
- [21]. Pungitore CR, Leon LG, Garcia C, Martin VS, Tonn CE, Padron JM. *Bioorg. Med. Chem. Lett.* 2007; 17:1332–1335. [PubMed: 17178223]
- [22]. Wheate NJ, Brodie CR, Collins JG, Kemp S, Aldrich-Wright JR. *Mini Rev. Med. Chem.* 2007; 7(6):627–648. [PubMed: 17584161]
- [23]. Mikus J, Steverding D. *Parasitol. Int.* 2009; 48(3):265–269. [PubMed: 11227767]
- [24]. Le Pape P, Pagniez F, Abdala-Valencia H. *Acta Parasitol.* 2003; 48(4):301–305.
- [25]. Luque-Ortega JR, Martinez S, Saugar JM, Izquierdo LR, Abad T, Luis JG, Piñero J, Valladares B, Rivas L. *Antimicrob. Agents Chemother.* 2004; 48(5):1534–1540. [PubMed: 15105102]
- [26]. Babich H, Borenfreund E. *Appl. Environ. Microbiol.* 1991; 57(7):2101–2103. [PubMed: 1892400]
- [27]. Ahmed SA, Gogal RM Jr, Walsh JE. *J. Immunol. Methods*. 1994; 170(2):211–224. [PubMed: 8157999]
- [28]. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. *J. Natl. Cancer Inst.* 1990; 82:1107–1112. [PubMed: 2359136]

Figure 1.
Chemical structure of lansine

Scheme 1.

a,ba Reagents and conditions: (a) $\text{Pd}(\text{OAc})_2$, BINAP, Cs_2CO_3 , Toluene, 115°C , 66-90%; (b) $\text{Pd}(\text{OAc})_2$, $\text{Cu}(\text{OAc})_2$, AcOH, 140°C in a sealed tube, 48-83%; (c) DDQ, MeOH/THF 3:1, rt, 45-76%; (d) TBAF, DMF, 0°C to rt, 78-92%; (e) BBR_3 , CH_2Cl_2 , 0°C to rt, 87%; (f) NaBH_4 , MeOH, 0°C to rt, 93%; (g) MeI, K_2CO_3 , acetone, 65°C ; ^bFor complete structures see Table 1.

Table 1

Activity of compounds **5a**, **5g**, **6a–f**, **7–9**, **11–16** against promastigotes and amastigotes of *Leishmania donovani*

<i>L. donovani</i>						Cytotoxicity ^[a]			
Compd	R ²	R ³	R ⁶	R ⁹	Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	LU-1 ^[c]	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]				
5a	OTBDPS	CHO	OCH ₃	H	34.5 (71.9)	11.0 (22.9)	55 (114.7)		5	19 (39.6)	2

<i>L. donovani</i>						Cytotoxicity ^[a]			
Compd	R ²	R ³	R ⁶	R ⁹	Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	LU-1 ^[c]	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]				
5g	H	CHO	OCH ₃	H	27 (119.9)	NA ^[g]	NC ^[h]	-	NC	NC	-

<i>L. donovani</i>									
		Promastigotes	Axenic amastigotes	LU-1 ^[c]	Cytotoxicity ^[a]						
Compd	R ²	R ³	R ⁶	R ⁹	IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	LU-1 ^[c]	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]
6a (lansine)	OH	CHO	OCH ₃	H	21.5 (89.1)	11.0 (45.6)	16.5 (68.4)	2	2	20 (82.90)	2

<i>L. donovani</i>							Cytotoxicity ^[a]			
Compd	R ²	R ³	R ⁶	R ⁹	Promastigotes		Axenic amastigotes		LU-1 ^[c]		J-774 murine macrophages				
					IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]			
6b	OH	CHO	H	H	35.4 (167.6)	11.0 (52.0)	45 (213.0)	21 (99.4)	4	21 (99.4)	2

<i>L. donovani</i>						Cytotoxicity ^[a]							
Compd	R ²	R ³	R ⁶	R ⁹	Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	LU-1 ^[c] CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e] CC ₅₀ ^[d] μg/mL (μM)	J-774 murine macrophages CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]									
6c	OH	CHO	CF ₃	H	7.3 (26.2)	6.0 (21.5)	21 (75.2)	4	9.8 (35.1)	2

Cytotoxicity ^[a]						
Compd	R ²	R ³	R ⁶	R ⁹	<i>L. donovani</i>					
					Promastigotes	Axenic amastigotes	LU-1 ^[c]	J-774 murine macrophages		
					IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]
6d	OH	CHO	OCF ₃	H	3.7 (12.6)	2.1 (7.1)	45 (152.4)	21	8.0 (27.1)	4

Cytotoxicity ^[a]						
Compd	R ²	R ³	R ⁶	R ⁹	<i>L. donovani</i>					
					Promastigotes	Axenic amastigotes	LU-1 ^[c]	J-774 murine macrophages		
					IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e]	SI-2 ^[f]
6e	OH	CHO	<i>t</i> -Bu	H	3.9 (14.7)	3.0 (11.2)	4.4 (16.5)	10.0 (37.4)	2	3

Compd	R ²	R ³	R ⁶	R ⁹	<i>L. donovani</i>					
					Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	LU-1 ^[c] CC ₅₀ ^[d] μg/mL (μM)	Cytotoxicity ^[a]		
								SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]
6f	OH	CHO	NO ₂	H	NA	NA	NC	-	20.0 (78.0)	-

<i>L. donovani</i>							Cytotoxicity ^[a]		
Compd	R ²	R ³	R ⁶	R ⁹	Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	LU-1 ^[c] CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e] CC ₅₀ ^[d] μg/mL (μM)	J-774 murine macrophages CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]			
7	OH	CHO	OH	H	34.2 (150.5)	NA	NC	-	32 (140.8)	-

Compd	R ²	R ³	R ⁶	R ⁹	<i>L. donovani</i>					
					Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	LU-1 ^[c]	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	J-774 murine macrophages CC ₅₀ ^[d] μg/mL (μM)
8	OCH ₃	CHO	OCH ₃	H	8.9 (34.9)	NA	NC	-	NC	-

Compd	R ²	R ³	R ⁶	R ⁹	<i>L. donovani</i>					
					Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	LU-1 ^[c] CC ₅₀ ^[d] μg/mL (μM)	Cytotoxicity ^[a]		
								SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]
9	OCH ₃	CHO	OCH ₃	CH ₃	NA	NA	NC	-	NC	-

<i>L. donovani</i>							Cytotoxicity ^[a]		
Compd	R ²	R ³	R ⁶	R ⁹	Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	LU-1 ^[c] CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e] CC ₅₀ ^[d] μg/mL (μM)	J-774 murine macrophages CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]			
11	OH	CH ₂ OH	OCH ₃	H	NA	10.0 (41.1)	>60 (>246.6)	>6	NC		>4

<i>L. donovani</i>					Cytotoxicity ^[a]			
Compd	R ²	R ³	R ⁶	R ⁹	Promastigotes		Axenic amastigotes		LU-1 ^[c]		J-774 murine macrophages		
					IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]	
13	OH	CH ₃	OCH ₃	H	17.7 (78.0)	11.5 (50.6)	11.5 (50.6)	30 (132.1)	3	20 (88.1)	2

Compd	R ²	R ³	R ⁶	R ⁹	<i>L. donovani</i>					
					Promastigotes	Axenic amastigotes	LU-1 ^[c]		Cytotoxicity ^[a]	
					IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]
14	H	CHO	H	CH ₂ CH ₃	38.6 (172.9)	NA	25 (112.0)	3	21 (94.1)	2

<i>L. donovani</i>					Cytotoxicity ^[a]			
Compd	R ²	R ³	R ⁶	R ⁹	Promastigotes		Axenic amastigotes		LU-1 ^[c]		J-774 murine macrophages		
					IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]		
15	OH	H	H	H	28.5 (155.6)	10.0 (54.6)	40 (218.3)	4	NC	>4			

Compd	R ²	R ³	R ⁶	R ⁹	<i>L. donovani</i>						
					Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	Cytotoxicity ^[a]			J-774 murine macrophages CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]
16	H	H	H	CH ₃	37.0 (204.2)	NA	NC	LU-1 ^[c]	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]

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Compd	R ²	R ³	R ⁶	R ⁹	<i>L. donovani</i>					Cytotoxicity ^[a]		
					Promastigotes IC ₅₀ ^[b] μg/mL (μM)	Axenic amastigotes IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	LU-1 ^[c]	SI-1 ^[e]	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]	
12	H ₃ CO				NA	NA	NC	-	NC	NC	-	-

					<i>L. donovani</i>							Cytotoxicity ^[a]		
Compd	R ²	R ³	R ⁶	R ⁹	Promastigotes		Axenic amastigotes		LU-1 ^[c]		J-774 murine macrophages			
					IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	LU-1 ^[c] μg/mL (μM)	SI-1 ^[e] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	SI-2 ^[f]		
Pentamidine					1.2 (3.5)	1.0 (2.9)	1.0 (2.9)	0.5 (1.5)	2	NT	-	-

Compd	R ²	R ³	R ⁶	R ⁹	<i>L. donovani</i>				Cytotoxicity ^[a]
					Promastigotes	Axenic amastigotes	LU-1 ^[c]	J-774 murine macrophages	
					IC ₅₀ ^[b] μg/mL (μM)	IC ₅₀ ^[b] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	CC ₅₀ ^[d] μg/mL (μM)	SI-1 ^[e]
Amphotericin B					0.14 (0.2)	0.15 (0.2)	NC	-	NT

- ^[a] All compounds were nontoxic to VERO cells at 20 µg/mL (i.e., CC₅₀>20 µg/mL).
- ^[b] IC₅₀: median *Leishmania* inhibitory concentration
- ^[c] LU-1: human lung carcinoma cell line
- ^[d] CC₅₀: median cytotoxic concentration
- ^[e] SI-1 (Selectivity Index-1) = CC₅₀ (LU-1)/IC₅₀ (amastigotes)
- ^[f] SI-2 (Selectivity Index-2) = CC₅₀ (J-774)/IC₅₀ (amastigotes)
- ^[g] NA (Not active); IC₅₀ > 40 µg/mL
- ^[h] NC (Noncytotoxic); CC₅₀ > 40 µg/mL
- ^[i] NT (Not tested)