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COMMUNICATION

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Design, Synthesis of Biaryl Piperidine Derivatives and Their Evaluation as Potential Antileishmanial Agents against *Leishmania donovani* Strain Ag83

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We have developed a new series of simple biaryl piperidine derivatives (**11**–**19**) based on biaryl naphthylisoquinoline alkaloid Ealamine-A. The target compounds were synthesized, analyzed by spectral data, and evaluated for antileishmanial activity against *Leishmania donovani* strain Ag83 by MTT assay. The compounds have shown the best to moderate antileishmanial activity. The 5′-fluoro-2′-methoxyphenyl derivative **14** and 3′,5′-difluorophenyl derivative **16** have inhibited the promastigotes by 86% and 85% after 24 h and 92% and 91% after 48 h incubation, respectively, at 400 μM concentration. The % inhibition was lower with the lowering of the concentration and increased with the incubation time. Compounds **12**, **15**, and **18** have solubility issues and proved to be less active than the rest of the compounds. Molecular docking studies were performed on selective active compounds and the results indicate that these compounds may act by binding to the Leishmanolysin and the docking scores are in good correlation with the antileishmanial activity. These results provide an initial insight into the design of new therapeutics for neglected tropical diseases.

Keywords: antileishmanial activity, promastigotes, biaryl piperidine derivatives, amphotericin B, neglected tropical diseases.

Introduction

Leishmaniasis is one of the neglected tropical diseases and is caused by several species of the genus *Leishmania* and transmitted by the bite of sandflies. Sandflies are primarily infected by the animal reservoir including humans. Leishmaniases are currently endemic in 98 countries and threaten approximately 1 million people around the world. Risk factors for transmission and disease development include poor housing, malnutrition, population mobility, and a weak immune system. Three main forms of Leishmaniases comprise visceral leishmaniasis (VL) also known as kala-azar is the most serious form of the disease followed by cutaneous and mucocutaneous leishmaniasis. Visceral leishmaniasis is the second-largest parasitic killer disease caused by *Leishmania donovani* and affects the most vital internal organs, causing black skin coloration. VL is a systemic disease characterized by irregular bouts of fever, weight loss, anemia, and substantial swelling of the spleen and liver.[1–4] The advancement of antileishmanial chemotherapeutic agents has been widely neglected in the past decades, despite variable efficacies, significantly associated toxicity, and severe side effects of the existing drugs. The traditional treatment was initiated with the application of pentavalent antimonial sodium stibogluconate (SSG, Pentostam) and other related compounds. Pentami-

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dine, miltefosine, amphotericin B, and paromomycin (*Figure 1*) are used as the second line of therapeutic agents and various combination medications are also available.[5–7] Resistance is a common problem in chemotherapy and the onset of resistance for the available medications is a well-recognized fact.[8,9] The chemotherapeutic agents currently in use are often inadequate, requiring long courses of parenteral administration, elevated costs, and are becoming less effective due to the emergence of resistant strains. The present situation demands the discovery of effective, more potent, and inexpensive drugs. The development and introduction of new antileishmanial drugs should be less toxic, easily available, and within the reach of poor people who are the most affected by the disease.

The seriousness of the disease prompted several research groups to work on the development of new antileishmanial agents and the class of scaffolds explored includes various quinolines,[10–13] chalcones,[14,15] nitroimidazole,[16] triazolopyrimidine,[17] pyrazolopyrimidine,[18,19] aminopyrazole,[20] triazine,[21,22] quinazoline,[23] thymidine,[24] triterpenoids,[25] and quinazolinone.[26] The isolation and synthesis of a new class of biaryl naphthylisoquinoline alkaloid ancistroealaine A (**1a**) from *Ancistrocladus ealaensis* and other alkaloids (**1b**, **1c**), and their antileishmanial activity has generated interest in the development of new antileishmanial agents.[27–29] The modified analog of **1a** & **1b** (C–C axis biaryls), **1d** (N–C axis biaryls, biaryl isoquinolinium salt) has shown much better antileishmanial activity.[30] Similar to these biaryls, the substituted diindolylmethanes (**1f**),[31] quinone based amino acid conjugates (**1g**),[32] as the modulators of amino acid transporters of Leishmania, and the biaryl based imidazooxazines (**1e**),[33] developed as antileishmanial agents. Compound **1e** has improved safety profile and shown efficacy in two animal models of visceral leishmaniasis. The biaryl group in both **1c** and **1e** provided much-needed insights in designing the proposed target compounds. The simple biphenyl unit from **1e** was kept intact and the piperidine part of the tetrahydroisoquinoline unit of **1c** was modified in the designing of a new class of antileishmanial agents (*Figure 2*). This led to the development and synthesis of simple piperidine based biaryls **11**–**19**.

Results and Discussion

Chemistry

The synthesis of biaryl incorporated piperidine derivatives was achieved as outlined in *Scheme 1*. The 5-amino-2-bromobenzoic acid **2** was esterified with methanol in the presence of catalytic H_2SO_4 to yield compound **3**, which was subjected to Suzuki coupling with [1-(*tert*-butoxycarbonyl)-1,2,5,6-tetrahydropyridin-

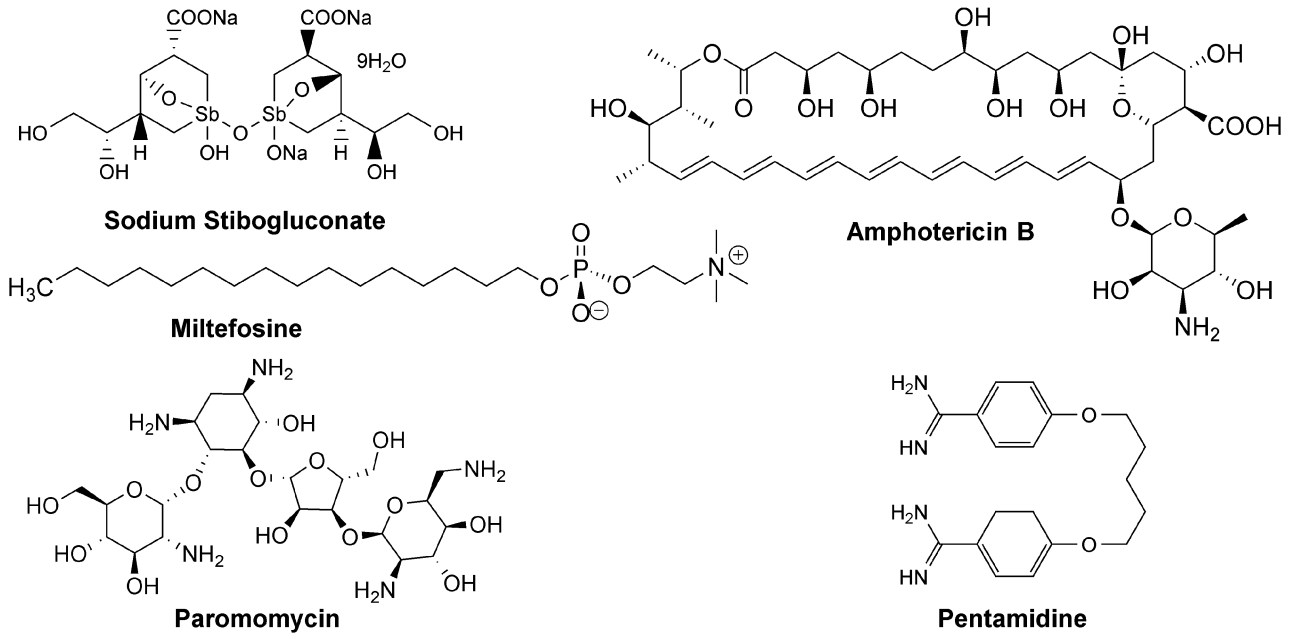


Figure 1. Antileishmanial drugs.

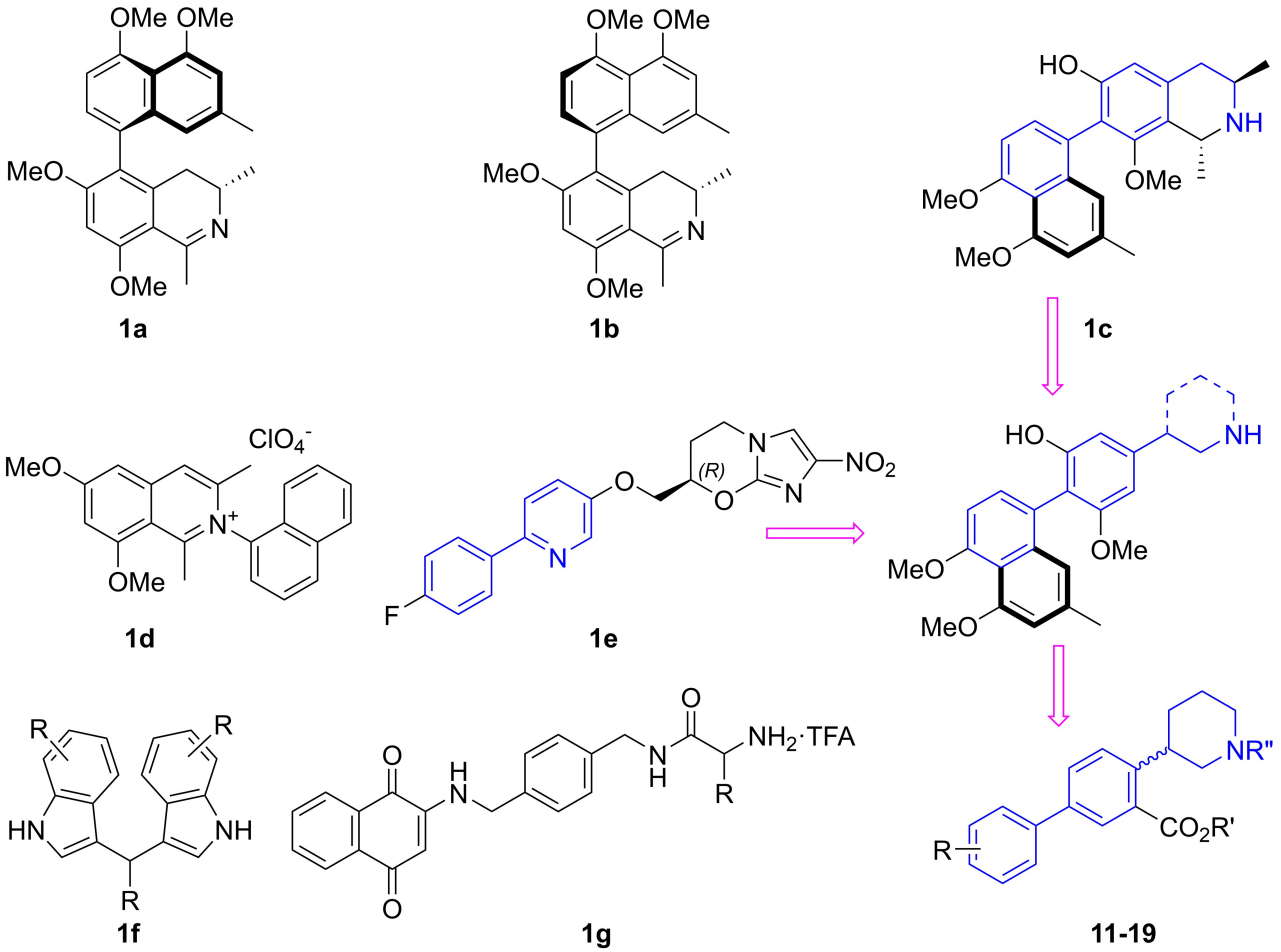
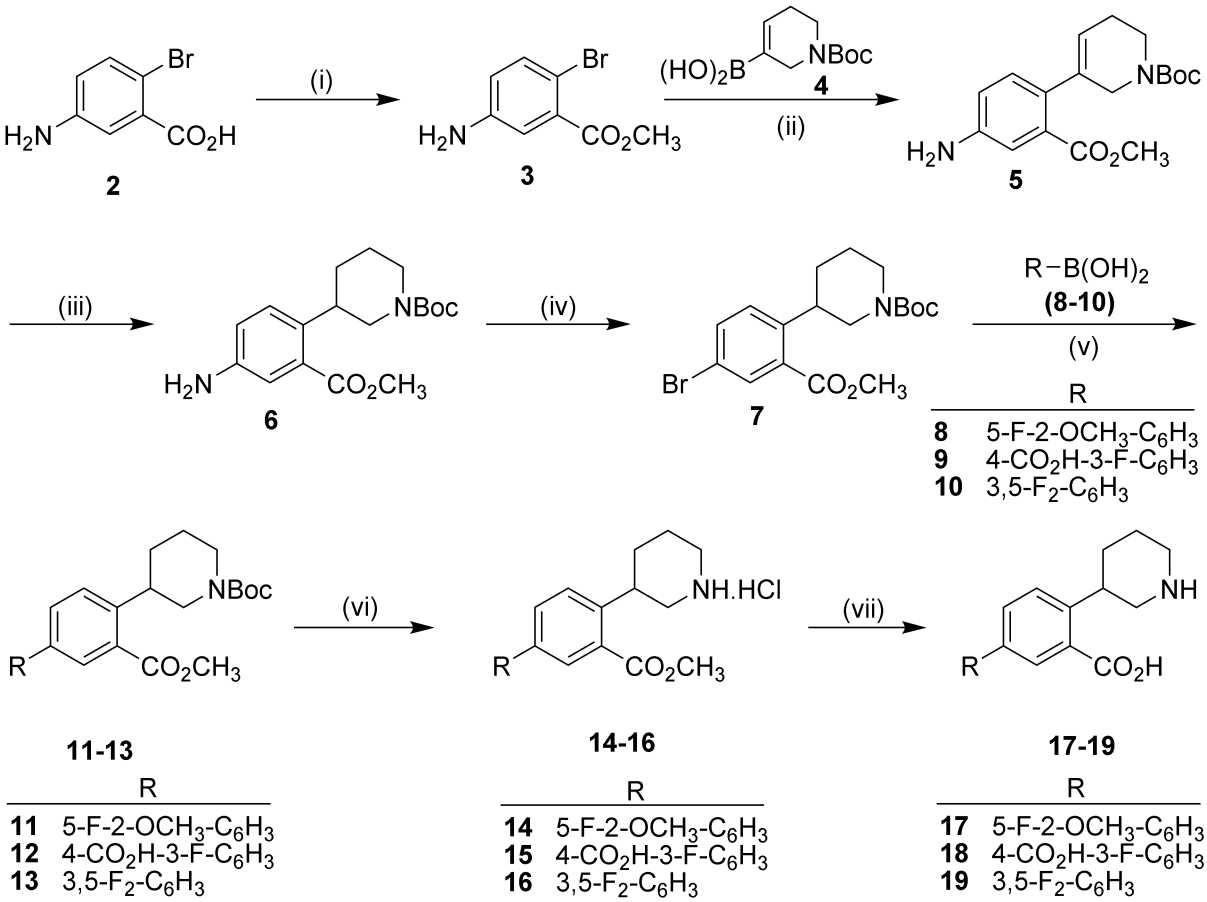


Figure 2. Biaryl antileishmanial compounds and designing of proposed target compounds. **1a:** Ancistroealaine A, **1b:** Ancistrotanzaanine B, **1c:** Ealamine A, **1d:** Isoquinolinium salt, **1e:** Biaryl based imidazooxazines, **1f:** Diindolyl methanes **1g:** Quinone based amino acid conjugates, **11**–**19:** Proposed target compounds.

3-yl]boronic acid (**4**) in the presence of $Pd(dppf)Cl_2$ and K_2CO_3 in 1,4-dioxane to afford *tert*-butyl 5-[4-amino-2-(methoxycarbonyl)phenyl]-3,6-dihydropyridine-1(2*H*)-carboxylate (**5**) in excellent yield. It was reduced with 10% Pd/C-H_2 (35–40 psi) to yield saturated piperidine derivative **6**. Sand Mayer reaction of compound **6** with ^{t}BuONO and CuBr afforded the key intermediate *tert*-butyl 3-[4-bromo-2-(methoxycarbonyl)phenyl]piperidine-1-carboxylate (**7**). Compound **7** was reacted with different types of aryl boronic acids (**8**–**10**) under Suzuki-coupling conditions using $Pd(dppf)Cl_2$, K_2CO_3 to give biaryl compounds **11**–**13**. The Boc-deprotection of compounds **11**–**13** was achieved with dil. HCl in 1,4-dioxane to afford piperidine derivatives as hydrochloride salts (**14**–**16**), which underwent basic hydrolysis with LiOH in methanol/water to afford pure amino-acid biaryl piperidine derivatives **17**–**19**. All the synthesized compounds were characterized by spectroscopic analysis (^{1}H-NMR and LCMS) and are racemic. The purity and mass spectral data of the target compounds were achieved by LCMS. The spectral data recording was challenging for the compounds **12**, **15**, and **18** as they were highly insoluble in regular solvents like $CDCl_3$ and CD_3OD. The extreme insolubility of compound **18**, provided D_2O exchange spectra in (D_6)DMSO with unwieldy peaks.

All the target compounds have fluorine substitution and have shown well-resolved peaks in both ^{1}H- and ^{13}C-NMR spectrum. Compound **11** has fluorine at 5′ position and has shown coupling with 4′, 5′, and 6′ carbons, and 3′, 4′, and 6′ protons. The fluorine coupled 4′ and 6′ protons appeared as a multiplet in the ^{1}H-NMR spectrum and the 3′ proton as a double doublet at δ 6.91 ppm with coupling constants 10.0 ($J_{H\text{-}H}$) and 5.0 ($J_{H\text{-}F}$). The 5′ carbon in the ^{13}C-NMR



Scheme 1. Synthesis of biaryl piperidine derivatives (**11**–**19**). Reaction conditions: i) MeOH, conc. H_2SO_4 (cat), 70 °C, 8 h. ii) Pd(dppf)Cl_2, K_2CO_3, 1,4-dioxane, 100 °C, 4 h. iii) Pd/C, H_2, r.t., 6 h. iv) ^{t}BuONO, CuBr, MeCN, 0 °C to r.t., 18 h. v) Pd(dppf)Cl_2, K_2CO_3, 1,4-dioxane, 100 °C, 8 h. vi) 4 N HCl, 1,4-dioxane, 30 °C, 6 h. vii) LiOH·H_2O, MeOH, H_2O, r.t., 6 h.

spectrum appeared as a doublet at δ 153.6 ppm with a $^1J_{C\text{-}F}$ coupling constant of 242. The fluoro coupled 4′ and 6′ carbons appeared as doublets upfield from C5′ at δ 113.71 ($J_{C\text{-}F}$ = 22.5), and δ 111.18 ppm ($J_{C\text{-}F}$ = 28.7), respectively. The carbonyl carbon of the methyl ester appeared at δ 164.88 ppm and the t-butyl ester at δ 151.44 ppm. The remaining protons and carbons are in line with the proposed structure.

Antileishmanial Activity

All the synthesized compounds were screened for their antileishmanial activity by MTT assay and most of the compounds inhibited the Leishmania promastigotes proliferation in a dose-dependent manner. Promastigotes were incubated for 24 h and 48 h with different concentrations of the compounds and DMSO as the control. The viability of the promastigotes is expressed as the percentage and is relative to DMSO (*Figures 3* and *4*). At a higher concentration of 400 μM, compounds **11**–**17** inhibited the promastigotes pro-

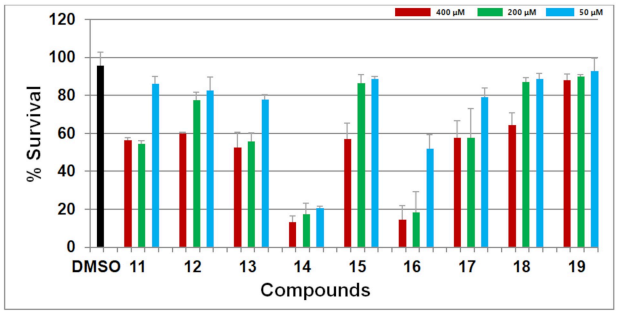


Figure 3. Effect of compounds (**11**–**19**) on *L. donovani* promastigotes survival. *L. donovani* promastigotes were treated with different concentrations of target compounds and survival was assessed 24 h later. Data represent the mean ± standard deviation of the experiments done in triplicates.

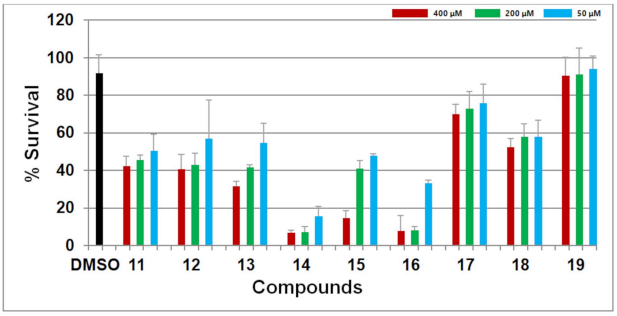


Figure 4. Effect of compounds (**11**–**19**) on *L. donovani* promastigotes survival. *L. donovani* promastigotes were treated with different concentrations of target compounds and survival was assessed 48 h later. Data represent the mean ± standard deviation of the experiments done in triplicates.

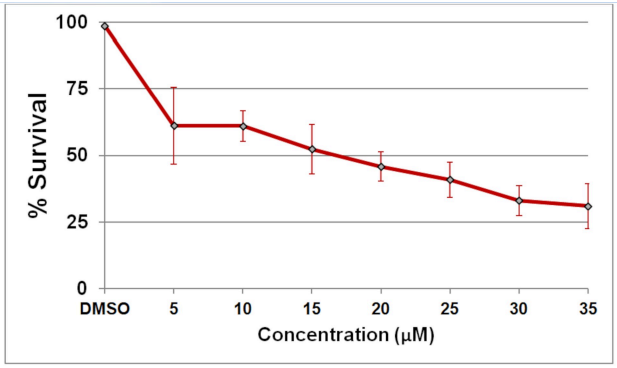


Figure 5. Dose-response curve of compound **14** against *L. donovani* promastigotes. *L. donovani* promastigotes were treated with different concentrations of compound-14 and survival was assessed 24 h later. Data represent the mean ± standard deviation of the experiments done in triplicates.

liferation by ~40% or higher after 24 h of incubation and compounds **11**–**16** by 50% or higher after 48 h of incubation at a concentration of ≥200 µM. The results indicate that compounds **14** and **16** are showing the best activity as compared to other derivatives in the series. Compound **14** inhibited the proliferation by 86%, 81%, and 78% after 24 h, and 92%, 92%, and 82% inhibition after 48 h of incubation at the concentration of 400 µM, 200 µM, and 50 µM, respectively. Similarly, compound **16** also inhibited the proliferation of promastigotes by 84%, 80%, and 45% after 24 h, and 91%, 91%, and 63% inhibition after 48 h of incubation at the concentration of 400 µM, 200 µM, and 50 µM, respectively. Further at 50 µM concentration, compound **14** has shown better activity as compared to compound **16**, which is 78% *vs.* 45% after 24 h and 82% *vs.* 63% after 48 h incubation, respectively. The other compounds (**11**, **13**, **17**) have moderate activity whereas compounds **12**, **15**, **18**, **19** demonstrated very little or negligible antileishmanial activity. The dose-response of the active compound **14** was studied by MTT assay with various concentrations ranging from 5 µM–35 µM (*Figure 5*). The IC_{50} was calculated from the dose response curve using the linear interpolation equation and was found to be 17 µM. The SAR analysis of the test compounds indicates that the compounds with fluoromethoxy or difluoro substitution are showing the best activity. The introduction of carboxylic acid substitution (**12**, **15**, and **18**) resulted in less solubility and lower activity, and further the esters (**14**–**16**) on conversion to carboxylic acids (**17**–**19**) also showed the same pattern of lower activity and much less solubility. From the results, it is clear that the substitution pattern and solubility are playing a significant role in the antileishmanial activity and gives direction in designing the newer compounds.

Molecular Docking Studies

Molecular docking studies of the selected compounds **11**, **14**, **16**, and **17** were performed with Leishmanolysin (gp63) protein (PDB_ID: 1LML), which is the Leishmania major surface metalloprotease. Amphotericin B was used in the docking studies to compare the binding affinities. The study was conducted employing the active site residues comprised of His264, Glu265, His268, His334, and Met345 and a Zn atom.[34,35] All the four compounds have the same common core and differentiate at the phenyl ring substitutions, and showed similar binding conformations toward 1LML protein. The binding orientations of the docked compounds and amphotericin B are shown in *Figure 6(A–E)*.

The core biphenyl piperidine ring of the compounds considered in the present study has been stabilized by hydrogen bonding and hydrophobic interactions. The binding energies of the compounds **11**, **14**, **16**, and **17**, and the standard reference compound are presented in *Table 1*. One of the aromatic rings of compound **11** is involved in the π-π interaction with His268 residue and the other aromatic ring is involved in cation-π interaction with Zn metal ion. The Glu265 is forming anion-π interaction with a phenyl ring of the compound. Further methyl groups from Boc formed the hydrophobic interactions with Ala349, Val261, and Leu224 residues. The phenyl ring of the de-Boc compound **14** is involved in π-π interaction with His268 residue and hydrophobic

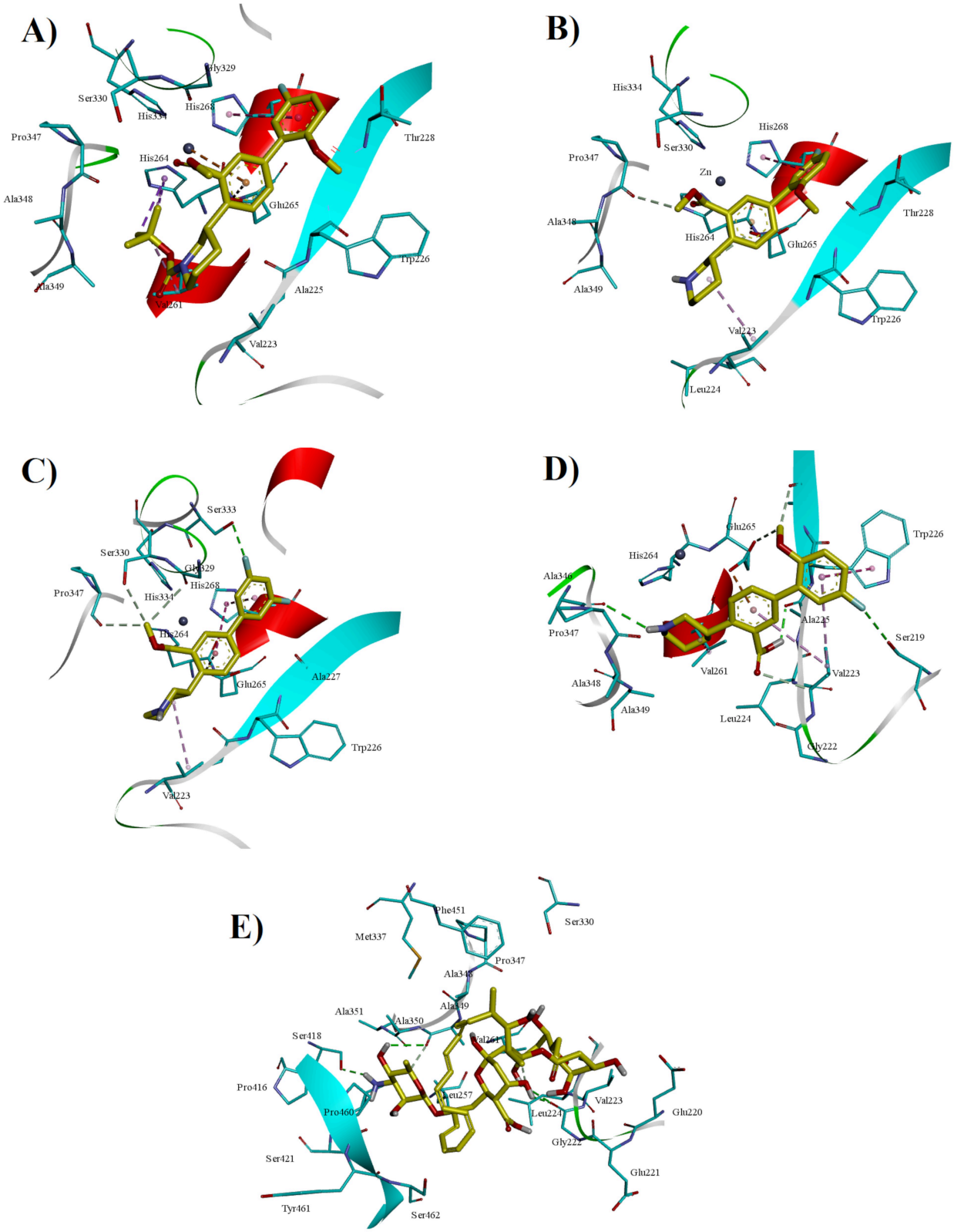


Figure 6. Molecular docking of compound **11** (*Figure 6A*), **14** (*Figure 6B*), **16** (*Figure 6C*), **17** (*Figure 6D*), and amphotericin B (*Figure 6E*) into the active site of Leishmanolysin. Protein amino acids are shown in blue lines and the compounds are shown in stick style with yellow color. Zn atom is shown in purple ball style and lined to His268. Hydrogen bonds are shown in broken green lines.

Table 1. The binding energies of docked compounds in the active site of Leishmanolysin.

Compounds	Binding affinity (in kcal/mol)	Key interacting residues
11	−6.2	Glu265, His268, His264, Ala349, and Val261
14	−6.5	His268, Glu265, His334, His264, Val223, and Thr228
16	−6.4	His268, Glu265, His264, Val223, Val261, and Thr228
17	−6.3	Glu265, His264, Ala349, and Val261
Amphotericin B	−7.6	Gly222, Ala349, and Ser418, Leu224, Leu257, Phe451, and Leu420

interactions with the Val261, Leu224, and Ala349 residues. The Glu265 is forming anion-π interaction with a phenyl ring of the compound. The difluoro amino-acid derivative **16**, the difluoro phenyl ring of ligand involved in π-π interaction with His268 residue, and the amino acid Glu265 forming a bifurcated anion-π interaction with two aromatic rings of ligand molecule. Compound **16** has hydrophobic interactions with Val223, Thr228, and His334 residues. The carboxylic group of compound **17** showed a hydrogen-bonding interaction with His264 residue. The aromatic ring of ligand involved in anion-π interaction with Glu265 residue and hydrophobic interactions with Val261 and Ala349 residues. Amphotericin B is also docked at the same site as compounds and is forming hydrogen bonding interactions with Gly222, Ala349, and Ser418 residues. Amphotericin B is also stabilized by key hydrophobic interactions from Leu224, Leu257, Phe451, and Leu420 residues in the active site of the protein. In conclusion, the four compounds bound in the active site properly with good binding affinities in comparison to the amphotericin B and also stabilized mostly with hydrophobic interactions in the active site. The results reveal that the compounds considered in the study can be new lead molecules for the development of new antileishmanial agents.

Conclusions

We have developed a new class of biaryl piperidine derivatives (**11**–**19**) and evaluated their antileishmanial activity. These compounds are showing the best to moderate antileishmanial activity against *Leishmania donovani* strain Ag83. 2′-Methoxy-5′-fluoro phenyl derivatives (**11**, **14**, and **17**) and compound **16** have shown the best antileishmanial activity. Compounds **12**, **15**, and **18** have less solubility and are less active compared to the other compounds in the series. The molecular docking scores of the compounds are in line with the biological results. These results will provide a way for designing newer compounds for Leishmaniasis. The plan of action for further development of these derivatives is to improve solubility as one of the key criteria.

Experimental Section

The synthetic procedures, characterization data of target compounds, and the biological assay methods are presented in the *Supporting Information.*

Acknowledgment

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Author Contribution Statement

M. Satyanarayana designed the experiments and wrote the article. B. Rathnakar has completed the synthesis of the target compounds and N. Rameshwar and Ch. Narsaiah assisted in the synthesis and data collection. K. K. Sinha, S. R. Prasad, and Mohd Imran Khan at NIPER performed the antileishmanial assay and analyzed the data.

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