Original Article

Synthesis and antileishmanial effect of a few cyclic and non-cyclic *n*-aryl enamino amide derivatives

Behnam Mohammadi-ghalehbin[1,2], Sima Najafi[3,4], and Nima Razzaghi-Asl[3,*]

[1]*Department of Microbiology and Parasitology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, I.R. Iran.*
[2]*Research Center for Zoonoses, Parasitic and Microbial Diseases, Ardabil University of Medical Sciences, Ardabil, I.R. Iran.*
[3]*Department of Medicinal Chemistry, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, I.R. Iran.*
[4]*Students Research Committee, School of Pharmacy, Ardabil University of Medical Sciences, Ardabil, I.R. Iran.*

Abstract

Background and purpose: The prevalence of leishmaniasis is reported in more than 98 countries and Iran is one of the endemic areas. There is no vaccine for this disease and few effective drugs are available to treat it. Moreover, drug resistance to the disease is increasing. During the past decade, several *in vitro* and *in vivo* studies have been performed on dihydropyrimidine derivatives as antileishmanial agents.
Experimental approach: In the present project, a few 6-methyl-4-aryl-*N*-aryl dihydropyrimidinone/thiones (**A7-A11**) and *N*-heteroaryl-3-(*para*-methoxy benzyl) amino but-enamides (**A1-A6**) were synthesized, structurally characterized, and finally subjected to *in vitro* anti-leishmanial effect against *Leishmania major* promastigotes.
Findings / Results: Results of the study showed that compound **A10**, 4-(3-chlorophenyl)-6-methyl-*N*-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide, exhibited superior anti-leishmanial effect with IC_{50} value of 52.67 µg/mL (more active than standard drug Glucantim® with IC_{50} 71000 ± 390 µg/mL).
Conclusion and implications: It was demonstrated that some dihydropyrimidine thiones were able to inhibit *Leishmania major* promastigotes. Structure-activity relationship evaluations indicated that more electron-poor rings such as isoxazole afforded higher activity within **A1**-**A6** series and in these derivatives, *N*-benzothiazole rings reinforced anti-leishmanial activity concerning thiazole. It was also observed that higher anti-parasite activities of **A10** and **A11** concerning **A7**-**A9** might be related to the incorporation of the sulfur atom into C2 position, replacement of N-thiazole carboxamide by N-phenyl carboxamide on C5 position of dihydropyrimidine ring, and also replacement of *para* with *meta*-substituted phenyls within C4 of dihydropyrimidine ring. The results may help unveil new 4-aryl-5-carboxamide dihydropyrimidines as potential anti-leishmanial agents and their further structural modification toward more potent derivatives.

Keywords: Dihydropyrimidinone; Leishmaniasis; MTT; Synthesis enamino amide.

INTRODUCTION

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania* and transferred into the host by the bite of infected female phlebotomine sandflies. Based on WHO reports, the pathogen is endemic over 90 countries (1) and Iran is one of the endemic areas of this pathogen (2,3). Leishmaniasis is estimated to involve about 2 million new cases per year (4) and despite such prevalence, leishmaniasis is categorized as a neglected tropical disease due to its strong relationship with poverty and also considering the limited resources invested in its diagnosis, treatment, and control (5). *Leishmania* is characterized by diverse clinical manifestations that range from self-limiting cutaneous lesions to highly fatal visceral leishmaniasis (6).

*Corresponding author: N. Razzaghi-Asl
Tel: +98-4533523833, Fax: +98-4533522197
Email: n.razzaghi@pharmacy.arums.ac.ir

Owing to the lack of effective antileishmanial vaccines up to now (4), existing *leishmania* therapy relies entirely on a limited number of chemotherapeutic agents such as sodium stibogluconate, meglumine antimoniate, amphotericin B, pentamidine, paromomycin, and miltefosine (7,8). In spite of chemotherapeutic advantages, current drugs suffer from severe adverse effects and increased incidence of treatment failures (9). Although current chemotherapy in some regions relies on liposomal amphotericin B which is effective and less toxic than non-liposomal formulation, problems such as treatment failure and post-kala-azar dermal leishmaniasis are major concerns (10).

In the light of above explanations, continued use of present antileishmanial drugs for the treatment of leishmaniasis is restricted, and potent and selective antileishmanial agents based on new molecular scaffolds are urgently required. Nitrogen-based heterocycles are privileged medicinal structures with valuable pharmacological properties. Besides previous reports on the antileishmanial activity of similar nitrogen-containing compounds such as quinazolines (11), a few dihydropyrimidinone (DHPM) derivatives were also reported to exhibit antileishmanial activities (12-14). To our best knowledge, previous reports mostly included DHPMs with 5-carboxylate (12), 5-alkanoyl (14), or 5-hyetroatyl (15) moieties. Fewer studies were dedicated to the antileishmanial activities of 5-carboxamide substituents and within this category, some aliphatic carboxamide derivatives were demonstrated to possess antileishmanial effects (13).

Based on previous reports and also intending to achieve more diverse structural patterns within *N*-heteroaryl 5-carboxamide DHPM and dihydropyrimidine thiones (DHPMT)-based antileishmanials, current work was dedicated to the synthesis, structural characterization and *in vitro* antileishmanial assessment of a few 6-methyl-4-aryl-*N*-aryl DHPM/DHPMTs (**A7**-**A11**) and some acyclic analogues with *N*-heteroaryl-3-(*para*-methoxy benzyl) amino but-enamide structure (**A1**-**A6**) against *Leishmania major* (*L. major)* promastigotes.

MATERIALS AND METHODS

Chemistry

All chemical reagents were purchased from Merck (Germany) and Aldrich (India) Companies and applied without further purification. Melting points were determined on an Electrothermal type 9200 melting point apparatus (England) and uncorrected. Infrared (IR) spectra were recorded on a Perkin Elmer-400 FT-IR spectrophotometer (England) while proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained on a Bruker DRX400 spectrometer (400 MGHz). Mass (MS) spectra were recorded on an Agilent 7890A spectrometer.

General procedure for the synthesis of N-heteroaryl-3-(para-methoxy benzyl) amino but-enamides (A1-A6)

The initial reaction of primary aromatic amines with dioxin and xylene under reflux afforded corresponding beta-keto amides (**B1**-**B3**) (16,17). To explain more; 1 mmol of **B1**-**B3** and 1.5 mmol of 4-methoxy benzylamine was dissolved in an appropriate amount of isopropyl alcohol and refluxed for 10 min to 3 h. The progress of the reaction was monitored by thin-layer chromatography (TLC) and after completion of the reaction, the precipitate was washed with cold ethanol (2 × 3 mL). The precipitate was dried in a desiccator to afford the final product (**A1**-**A6**) (Table 1).

General procedure for the synthesis of 6-methyl-4-aryl-N-aryl dihydropyrimidinones (A7-A9)

B1-**B6** (3.9 mmol), 3 mmol 4-fluoro benzaldehyde, and 3.6 mmol urea were dissolved in an appropriate amount of ethanol. After adding 5 drops of hydrochloric acid, the whole mixture was refluxed for 24 h. The progress of the reaction was monitored by TLC and after completion of the reaction, ice and water were added to the contents of the reflux flask. After 15 min, the formed precipitate was washed with cold water and recrystallized in ethanol. Final products were achieved after drying (**A7**-**A9**) (Table 2).

Table 1. Synthetized derivatives of *N*-heteroaryl-3-(*para*-methoxy benzyl) amino but-enamides (**A1-A6**).
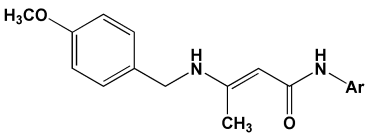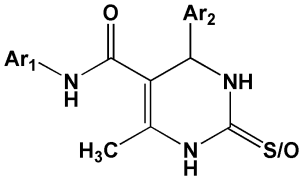

Compounds	Aryl	Molecular weight	Yield (%)
A1	6-methoxybenzothiazol-2-yl	383.1	56.9
A2	6-methylbenzothiazol-2-yl	367.1	77.1
A3	Benzothiazol-2-yl	353.1	99.7
A4	Thiazol-2-yl	303.1	99.3
A5	4-methylbenzothiazol-2-yl	367.1	61.6
A6	5-methylisoxazol-3-yl	301.1	60.9

Table 2. Synthetized derivatives of 6-methyl-4-aryl-*N*-aryl dihydropyrimidinone/thiones (**A7-A11**).

Compounds	Aryl₁	Aryl₂	X	Molecular weight	Yield (%)
A7	6-methoxybenzothiazol-2-yl	4-fluoro phenyl	O	412.1	48.3
A8	6-methylbenzothiazol-2-yl	4-fluoro phenyl	O	396.1	31.3
A9	Benzothiazol-2-yl	4-fluoro phenyl	O	382.1	41.2
A10	phenyl	3-chloro phenyl	S	357.1	8.7
A11	phenyl	3-fluoro phenyl	S	341.4	60.8

General procedure for the synthesis of 6-methyl-4-aryl-N-aryl dihydropyrimidine thiones (A10 and A11)

N-phenyl acetoacetamide (1.2 mmol), 1.3 mmol thiourea, 0.5 mmol $CO(HSO_4)_2$ in 5 mL ethanol was added to 1 mmol corresponding aldehyde and the solution was refluxed for 24 h (18,19). The progress of the reaction was monitored by TLC and after completion of the reaction, ice and water were added to the contents of the reflux flask. After 15 min, the formed precipitate was washed with cold water and recrystallized in ethanol and methanol mixture. Final products were achieved after drying (**A10** and **A11**) (Table 2).

Biological assessment

Leishmania major (MRHO/IR/75/ER) promastigotes were provided for this experiment. The parasites were cultivated in RPMI1640 supported with 10% inactivated fetal bovine serum (FBS) and 1% penicillin-streptomycin. All the parasite cultivating and cytotoxicity evaluation procedures were conducted due to the previous reports (20,21). Briefly, 100 µL of medium containing 2.5×10^6 promastigotes of *L. major* was added to each well of 96 well plates. The promastigotes were treated with 10 µL of different concentrations of all derivatives at 25 °C for 48 h. To evaluate the cytotoxic effects of the dihydropyrimidinones (DHPMs) and promastigote viability, colorimetric MTT assay was used and IC_{50} for each derivative was calculated (18,19). Glucantime was used as control. All experiments were performed triplicate and repeated three times. The IC_{50} values for *in vitro* antileishmanial activity of different concentrations were calculated using sigmoid dose-response curves generated by the software SigmaPlot version 14.

Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistically significant differences among groups were determined by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. $P \leq 0.05$ were considered significant.

RESULTS

Chemistry

Chemical structures of all synthesized derivatives were confirmed by IR, MS, and ^{1}H NMR methods. Spectroscopic results of synthesized compounds are illustrated below.

N-3- (4-methoxybenzylamino)-N- (6-methoxy-benzothiazol-2-yl) but-2-enamide (A1)

Yield: 56.91%, R_f = 0.695 (petroleum ether (PE) / EtOAc, 1:1), R_f = 0.839/ethanol (16 drop)/chloroform (4 mL)), mp: 225 °C; ^{1}H NMR (DMSO-d$_6$) δ (ppm) 11.38 (1H, brs, NH-amide), 9.36 (1H, t, J = 5.6 Hz, NH-enamine), 7.50 (1H, d, J = 8.8 Hz, C4′H-benzothiazole), 7.43 (1H, s, C7′H-benzothiazole), 7.24 (2H, d, J = 8.4 Hz, CH-phenyl), 6.94 (3H, d, J = 8.8 Hz, C5′H-benzothiazole and CH-phenyl), 4.74 (1H, s, CH-alkene), 4.41 (2H, d, J = 6 Hz, CH2-benzyl), 3.77 (3H, s, 6′-OCH$_3$-benzothiazole), 3.74 (3H, s, Ph-OCH$_3$), 1.95 (3H, s, CH$_3$), IR (KBr) v (cm$^-$): 3204.68 (N-H, amide), 3140.62 (NH, amine) 2944.91 (CH, aromatic), 2898.43 and 2842.18 (CH, aliphatic), 1638.10 (C=O, amide), 1577.05 (C=C); 833.47 (para-disubstituted benzene), 1166.39 and 1255.24 and 1270.59 (CN), MS m/z (%): 383.1 (6) [M$^+$], 121.1 (100), 180.1 (30),206(23), 77.1 (8).

3-(4- methoxybenzylamino)- N- (6-methyl-benzo[d]thiazol-2-yl) but-2-enamide (A2)

Yield: 77.11%, R_f = 0.575 (PE / EtOAc, 1:1), R_f = 0.818 (ethanol (16 drop) / chloroform (4 mL)), mp: 223 °C; ^{1}H NMR (DMSO-d$_6$) δ (ppm) 11.43 (1H, brs, NH-amide), 9.38 (1H, t, J = 6 Hz, NH-enamine), 7.63 (1H, s, C7′H-benzothiazole), 7.49 (1H, d, J = 8 Hz, C4′H-benzothiazole), 7.25 (2H, d, J = 8.8 Hz, CH-phenyl), 7.16 (1H, d, J = 8 Hz, C5′H-benzothiazole), 6.94 (2H, d, J = 8.4 Hz, CH-phenyl), 4.75 (1H, s, CH-alkene), 4.41 (2H, d, J = 6 Hz, CH$_2$-benzyl), 3.74 (3H, s, Ph-OCH$_3$),

2.37 (3H, s, Ph-CH$_3$), 1.96 (3H, s, CH$_3$), IR (KBr) v (cm^{-1}): 3187.5 (NH, amide), 3118.38 (NH, amine), 3004.35 (CH, aromatic), 2951.37 and 2921.87 (CH, aliphatic), 1641.52 (C=O, amide), 1581.76 (C=C); 818.06 (para-disubstituted benzene), 1167.33, 1248.99, and 1272.28 (CN), MS m/z (%): 367.1 (15) [M$^+$], 121.2 (100), 164.1 (44),204.1 (44), 77.1 (19).

3-(4- methoxybenzylamino)- N- (2,3-dihydro-benzo[d]thiazol-2-yl) but-2-enamide (A3)

Yield: 99.7%, R_f = 0.609 (PE / EtOAc, 1:1), R_f = 0.826 (ethanol (16 drop) / chloroform (4 mL), mp: 222-225 °C; ^{1}H NMR (DMSO-d$_6$) δ (ppm) 11.51 (1H, brs, NH-amide), 9.40 (1H, t, J = 5.6 Hz, NH-enamine), 7.83 (1H, d, J = 8 Hz, C4′H-benzothiazole), 7.61 (1H, d, J = 8 Hz, C7′H-benzothiazole), 7.35 (1H, t, J = 7.2 Hz, C5′H-benzothiazole), 7.25 (2H, d, J = 8.4 Hz, CH-phenyl), 7.19 (1H, t, J = 7.6 Hz, C6′H-benzothiazole), 6.94 (2H, d, J = 8.4 Hz, CH-phenyl), 4.77 (1H, s, CH-alkene), 4.42 (2H, d, J = 5.6 Hz, CH$_2$-benzyl), 3.74 (3H, s, OCH$_3$-phenyl), 1.96 (3H, s, CH$_3$), IR (KBr) ν (cm-1): 3294.01 (NH, amide), 3184.62 (NH, amine), 3128.93 (CH, aromatic), 3014.74 and 2937.41 (CH, aliphatic), 1644.65 (C=O, amide), 1578.79 (C=C); 812.51(para-disubstituted benzene), 1167.08, 1249.37, and 1278.71 (C-N), MS m/z (%): 353.1 (5) [M$^+$], 121.1 (100), 150.1 (60),176 (21), 78.1 (19).

E-3-(4-methoxybenzylamino)-N-(thiazol-2-yl) but-2-enamide (A4)

Yield: 99.31%, R_f = 0.5 (PE / EtOAc, 1:1), R_f = 0.857 (ethanol (16 drop) / chloroform (4 mL)), mp: 218 °C; ^{1}H NMR (DMSO-d$_6$) δ (ppm) 11.27 (1H, brs, NH-amide), 9.30 (1H, t, J = 6 Hz, NH-enamine), 7.35 (1H, d, J = 3.6 Hz, C4′H-thiazole), 7.23 (1H, d, J = 8.8 Hz, C5′H-thiazole), 7.00 (2H, d, J = 3.6 Hz, CH-phenyl), 6.92 (2H, d, J = 6.8 Hz, CH-phenyl), 4.73 (1H, s, CH-alkene), 4.38 (2H, d, J = 6 Hz, CH$_2$-benzyl), 3.73 (3H, s, OCH$_3$-phenyl), 1.92 (3H, s, CH$_3$), IR (KBr) v (cm^{-1}): 3274.34 (NH, amide), 3196.25 (NH, amine), 3108.96 and 3006.45 (CH, aromatic), 2937.27 and 2835.12 (CH, aliphatic), 1643.22 (C=O, amide), 1536.76 (C=C); 812.51(815.99-disubstituted benzene), 1099.16, 1170.95, and 1161.90 (CN), MS m/z (%): 303.1 (34) [M$^+$], 121.2 (100), 204.2 (84), 77.1 (18).

3-(4- methoxybenzylamino) -N- (4-methyl-benzo[d]thiazol-2-yl) but-2-enamide (A5)

Yield: 61.58%, R_f = 0.64 (PE / EtOAc, 1:1), R_f=0.878 (ethanol (16 drop) / chloroform (4 mL)), mp: 180 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 11.59 (1H, brs, NH-amide), 9.39 (1H, t, J = 6 Hz, NH-enamine), 7.64 (1H, d, J = 7.6 Hz, C7′H-benzothiazole), 7.25 (2H, d, J = 8.8 Hz, CH-phenyl), 7.17 (1H, d, J = 7.2 Hz, C5′H-benzothiazole), 7.09 (1H, t, J = 7.6 Hz, C6′H-benzothiazole), 6.94 (2H, d, J = 8.4 Hz, CH-phenyl), 4.78 (1H, s, CH-alkene), 4.42 (2H, d, J = 6 Hz, CH2-benzyl), 3.74 (3H, s, OCH3-phenyl), 2.49 (3H, s, 4′-CH3-benzothiazole), 1.95 (3H, s, CH3), IR (KBr) ν (cm⁻¹): 3241.87 (NH, amide), 3204.85 (NH, amine), 3046.87 (CH, aromatic), 2981.25 and 2930.97 (CH, aliphatic), 1645.01 (C=O, amide), 1582.35 (C=C); 818.37(*ortho*-disubstituted benzene), 1299.71, 1253.54, and 1172.19 (CN), MS m/z (%): 367.1 (15) [M⁺], 121.1 (100), 204.1 (20), 164.1 (38), 77.1 (18).

3-(4-methoxybenzylamino)-N-(5-methyloxazol-2-yl) but-2-enamide (A6)

Yield: 60.87%, R_f = 0.6 (PE / EtOAc, 1:1), R_f = 0.807 (ethanol (16 drop) / chloroform(4 mL)), mp: 187-192 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 10.01 (1H, brs, NH-amide), 9.37 (1H, t, J = 6 Hz, NH-enamine), 7.21 (2H, d, J = 8.8 Hz, CH-phenyl), 6.92 (2H, d, J = 8.4 Hz, CH-phenyl), 6.60 (1H, s, C4′H-isoxazole), 4.65 (1H, s, CH-alkene), 4.35 (2H, d, J = 6 Hz, CH2-benzyl), 3.73 (3H, s, OCH3-phenyl), 2.30 (3H, s, 5′-CH3-isoxazole), 1.89 (3H, s, CH3); IR (KBr) ν (cm⁻¹): 3234.28 (NH, amide), 3179.68 (NH, amine), 3173.99 and 3097.54 (CH, aromatic), 2964.14 and 2872.22 (CH, aliphatic), 1653.11 (C=O, amide), 1587.72 (C=C); 810.26 (para-disubstituted benzene),1176.59, 1253.30, and 1280.67 (CN), MS m/z (%): 301.1 (6) [M⁺], 121.2 (100), 204.1 (14), 77.1 (14).

4-(4-fluorophenyl) N-(6-methoxybenzothiazol-2-yl)-6-methyl -2-oxo-1,2,3,4 –tetrahydro-pyrimidine-5-carboxamide (A7)

Yield: 48.35 %, R_f = 0.78 (PE / EtOAc, 1:1), R_f = 0.75 (ethanol (16 drop) / chloroform (4 mL)), mp: 245-247 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 11.39 (1H, brs, NH-amide), 9.11 (1H,

brs, N1H), 7.80 (1H, brs, N3H), 7.57 (1H, d, J = 8.8 Hz, C4′H-benzothiazole), 7.50 (1H, s, C7′H-benzothiazole), 7.32 (2H, d, J = 8.8 Hz, CH-phenyl), 7.15 (2H, d, J = 8.8 Hz, CH-phenyl), 7.00 (1H, d, J = 8.8 Hz, C5′H-benzothiazole), 5.59 (1H, brs, C4H-DHPM), 3.78 (3H, s, OCH3-benzothiazole), 2.19 (3H, s, CH3-DHPM), IR (KBr) ν (cm⁻¹): 3399.8 (brs, NH-amide and N1H urea), 3249.10 (N3H, urea), 3099.66 and 2943.56 (CH, aromatic), 1700.82 and 1593.69 (C=O, amide and urea) 1552.08 and 1477.19 (C=C, aromatic);1262.33 (CF), MS m/z (%): 412.1 (4) [M⁺], 191.1 (100), 165.1 (56), 231.1(16), 69.1 (11).

4-(4- fluorophenyl)-6- methyl N-(6-methyl-benzothiazol -2-yl)-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxamide (A8)

Yield: 31.27%, R_f = 0.717 (PE / EtOAc 1:1), R_f = 0.767 (ethanol (16 drop) / chloroform (4 mL)), mp: 217-219 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 11.83 (1H, brs, NH-amide), 9.12 (1H, brs, N1H), 7.80 (1H, brs, N3H), 7.70 (1H, brs, C7′H-benzothiazole), 7.63 (1H, d, J = 8.4 Hz, C4′H-benzothiazole), 7.58 (1H, d, J = 8.0 Hz, C5′H-benzothiazole), 7.32 (2H, d, J = 8.4 Hz, CH-phenyl), 7.17 (2H, d, J = 8.8 Hz, CH-phenyl), 5.60 (1H, brs, C4H-DHPM), 2.38 (3H, s, CH3-benothiazole), 2.19 (3H, s, CH3-DHPM), IR (KBr) ν (cm⁻¹): 3172.68 (brs, NH amide and N1H urea), 3072.82 (N3H, urea), 3003.72 (CH, aromatic), 1710.68 and 1658.28 (C=O, amide and urea) 1560.25 (C=C, aromatic);1314.26 (CF), MS m/z (%): 396.1 (7) [M⁺], 164.1 (100), 248.1 (48), 1901.1 (19), 77.1 (9).

N-(benzothiazol-2-yl)-4- (4-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide (A9)

Yield: 41.25%, R_f = 0.38 (PE / EtOAc, 1:1), R_f = 0.5 (ethanol (16 drop) / chloroform (4 mL)), mp: 288 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 11.91 (1H, brs, NH-amide), 9.14 (1H, brs, N1H-amide), 7.91 (1H, d, J = 7.2 Hz, C4′H-benzothiazole), 7.82 (1H, brs, N3H), 7.69 (1H, d, J = 7.2 Hz, C7′H-benzothiazole), 7.41 (1H, t, J = 7.2 Hz, C5′H-benzothiazole), 7.27 (1H, t, J = 7.6 Hz, C6′H-benzothiazole), 7.33 (2H, d, J = 8.4 Hz, CH-phenyl), 7.17 (2H, d, J = 8.8 Hz, CH-phenyl), 5.61 (1H, brs, C4H-DHPM), 2.20

(3H, s, CH$_3$-DHPM), IR (KBr) ν (cm^{-1}): 3436.61 (brs, NH-amide and N1H urea), 3242.36 (N3H, urea), 3072.27 (CH, aromatic), 1683.16 and 1628.95 (C=O, amide and urea) 1540.50 and 1447.46 (C=C, aromatic);1243.75 (CF), MS m/z (%): 382.1 (47) [M$^+$], 150.1 (100), 190.1 (47), 233.1(54), 69.1 (9).

4-(3-chlorophenyl) -6-methyl-N- phenyl-2-thioxo -1,2,3,4- tetrahydropyrimidine-5-carboxamide (A10)

Yield: 8.73%, R$_f$ = 0.48 (PE / EtOAc, 1:1), R$_f$ = 0.61 (ethanol (16 drop) / chloroform (4 mL)), mp: 170 °C; ^{1}H NMR (DMSO-d$_6$) δ (ppm) 10.24 (1H, brs, NH-amide), 9.90 (1H, brs, N1H), 9.63 (1H, brs, N3H), 7.66 (2H, d, *J* = 7.6 Hz, CH-phenyl), 7.33-7.55 (7H, m, CH-phenyl), 5.51 (1H, brs, C4H-DHPM), 2.20 (3H, s, CH$_3$-DHPM); IR (KBr) ν$_{max}$ (cm^{-1}): 3413.9 (NH, amide), 3267.4 and 3185.3 (N-H, DHPM), 3066.5 and 3015.8 (CH, aromatic), 1678.5 (C=O), 1629.6 and 1584.1 (C=C, CN); MS m/z (%): 357 (63) [M$^+$], 281 (24), 265 (100), 246 (86), 77 (25).

4-(3- fluorophenyl)-6- methyl-N- phenyl-2-thioxo -1,2,3,4- tetrahydropyrimidine-5-carboxamide (A11)

Yield: 60.78%, R$_f$ = 0.48 (PE/EtOAc, 1:1), R$_f$ = 0.5 (ethanol (16 drop) / chloroform (4 mL)), mp: 178 °C; ^{1}H NMR (DMSO-d$_6$) δ (ppm) 10.23 (1H, brs, NH-amide), 9.90 (1H, brs, N1H), 9.64 (1H, brs, N3H), 7.67 (2H, d, *J* = 7.6 Hz, CH-phenyl), 7.22-7.57 (7H, m, CH-phenyl), 5.53 (1H, brs, C4H-DHPM), 2.20 (3H, s, CH$_3$-DHPM); IR (KBr) ν$_{max}$ (cm^{-1}): 3406.4 (NH, amide), 3174.2 and 3108.9 (NH, DHPM), 3064.3 and 3016.9 (CH, aromatic), 1678.7 (C=O), 1629.8 and 1571.4 (C=C, CN); MS m/z (%): 341 (66) [M$^+$], 249 (100), 190 (69), 77 (23).

in vitro antileishmanial effect

Prepared *N*-heteroaryl enamino amides and DHPMs/DHPMTs were assessed for their antileishmanial effect against *L. major* promastigotes through colorimetric MTT assay within RPMI culture medium. Results were reported in terms of IC$_{50}$ values with glucantime as the reference drug (Table 3). Biological results showed that **A10** and **A7**

were the best (IC$_{50}$ 52.67 μg/mL) and the worst (IC$_{50}$ 36566.71 μg/mL) antileishmanial derivatives. Moreover, relatively all the compounds exhibited higher antileishmanial effect than glucantime (IC$_{50}$ 71000 ± 390 μg/mL). For more information, the concentration-response curve for **A10** is depicted in Fig. 1. As could be seen in Fig. 1, after an initial concentration of 6.25 μg/mL, a steep decreasing slope of the curve occurred in a way that in 200 μg/mL, 0.27% of the promastigote cells were viable.

Table 3. Antileishmanial activity of dihydropyrimidine derivatives against *Leishmania major* promastigotes. Data are presented as mean ± SD, n = 3.

Compounds	IC$_{50}$ (μg/mL)
A1	302.61 ± 0.92
A2	876.34 ± 0.72
A3	2546.40 ± 0.12
A4	17250.84[a]
A5	2126.87 ± 0.53
A6	2146.99 ± 0.36
A7	36566.71[a]
A8	15602.35[a]
A9	9970.96[a]
A10	52.67 ± 0.28[b]
A11	182.55 ± 0.41
Glucantim	71000 ± 390

[a] Corresponding points were located out of the curve;
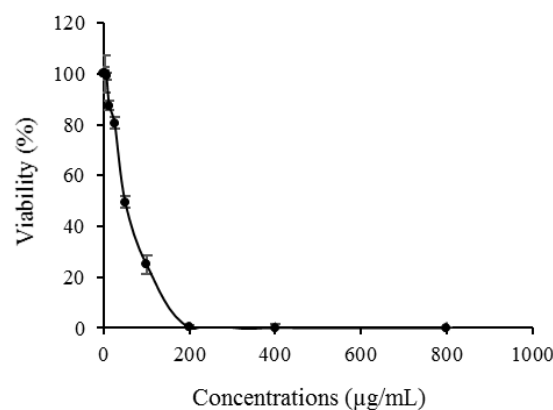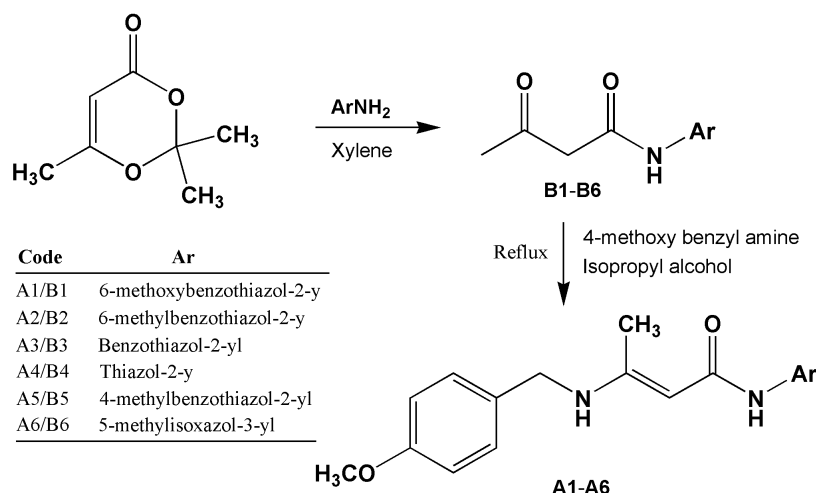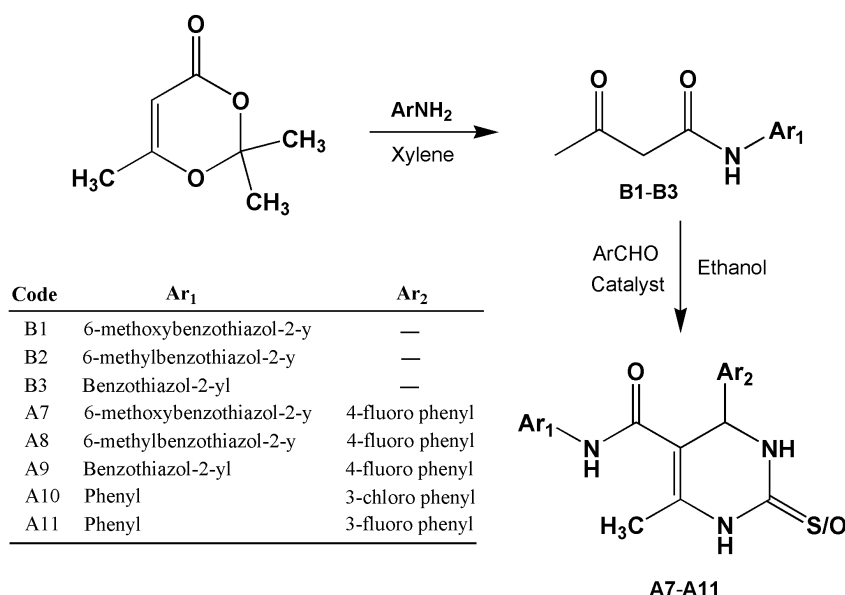[b] The best IC$_{50}$ among antileishmanial derivatives.

Fig. 1. Concentration-response curve for the antileishmanial effect of compound **A10** against *Leishmania major* promastigotes. Data represented as mean ± SD, n = 3.

Scheme 1. The synthetic route toward beta-keto amides (**B1-B6**) and *N*-heteroaryl-3-(*para*-methoxy benzyl) amino but-enamides (**A1-A6**).

Scheme 2. The synthetic route toward 6-methyl-4-aryl-*N*-aryl dihydropyrimidinones (**A7-A11**), compounds **A10** and **A11** were synthesized from commercially available *N*-phenyl acetoacetamide.

DISCUSSION

Synthetic routes to compounds **A1** to **A11** are depicted in Schemes 1 and 2. From the mechanistic aspect of view, synthesis of cyclic derivatives is initiated by the nucleophilic attack of the nitrogen atom in thiourea or urea into aldehyde carbonyl. After tautomerism, intramolecular nucleophilic attack and water elimination generates imine intermediate. 3-oxo butanamide carbonyl captures proton to produce enol which reacts with imine intermediate to give the second intermediate. The new intermediates are converted to target compounds *via* cyclization and water removal.

Synthesized derivatives were evaluated for their *in vitro* antileishmanial activity against *L. major* promastigotes. Based on obtained biological data, following structure activity relationship (SAR) guidelines may be ruled out based on two main scaffolds:

(a) *N*-heteroaryl amino but-enamides

(1) Among structures, different IC_{50}s of **A3**, **A4**, and **A6** could be attributed to the type of ring attached to the amide nitrogen. It was concluded that isoxazole was an electron-poor ring about thiazole and proposed a possible chemical interaction with an electron-rich site in the pathogen receptor. Such results were also reported previously in a way that neolignanes

possessing isoxazole and triazole rings exhibited better antileishmanial activities than furan containing derivatives and it was also revealed that isoxazole bearing compounds were better than triazole based ones (22). Other studies could also exhibit the effect of electron-poor or rich rings on the antileishmanial activities (23).

(2) Regarding the obtained IC_{50} values for compounds **A1**-**A3,** and **A5,** *N*-benzothiazolyl derivatives were better antileishmanial agents than *N*-thiazole one (**A4**) suggesting a possible hydrophobic interaction *via* the relevant site of the pathogen receptor.

(3) Results exhibited that in addition to the heterocycle type, the location of the substituent on the benzothiazole ring had also a significant effect on biological activity since 6-methoxy group (**A1**) led to the 7-fold increase in activity regarding the benzothiazole without any substituent (**A3**).

(4) Superior effect of **A1** concerning other *N*-benzothiazolyl based derivatives proposed a possible H-bonding as an H-bond acceptor with the site of interaction in pathogen receptor. However, this molecular site needs further attention *via* the incorporation of different H-bond acceptor groups. Similar results on a few 9-methyl-1-phenyl-9H-pyrido [3,4-b] indoles indicated the importance of methoxy

substituents on antileishmanial effects.

(b) Dihydropyrimidinones/thiones

(1) Within cyclic derivatives, it seemed that the replacement of 2-carbonyl with 2-thiocarbonyl moiety had a significant effect on antileishmanial inhibitory activity (24).

(2) Compound **A10** (IC_{50}: 52.67 µg/mL) and **A11** (IC_{50}: 182.55 µg/mL) were only structurally different concerning their C4 position in DHPM ring. 4-(*meta*-chloro phenyl) substituent led to an about 3.5-folds increase in antileishmanial effect when compared to 4-(*meta*-fluoro phenyl) and such observation prompted us to go through different *meta*-substituted corresponding derivatives in our future studies.

(3) In the case of **A7**-**A9** no considerable antileishmanial effect could be detected and in overall it could be concluded that higher antiparasitic activities of **A10** and **A11** relative to **A7**-**A9** might be related to the incorporation of sulfur atom into C2 position, replacement of 5-(*N*-thiazole carboxamide) by 5-(*N*-phenyl carboxamide) on C5 position of DHPM ring, and also replacement of *para* with *meta* substituted phenyls within C4 of DHPM ring.

In light of the above explanations, schematic SARs might be plausible for further molecular modifications into more potent antileishmanial agents (Figs. 2 and 3).
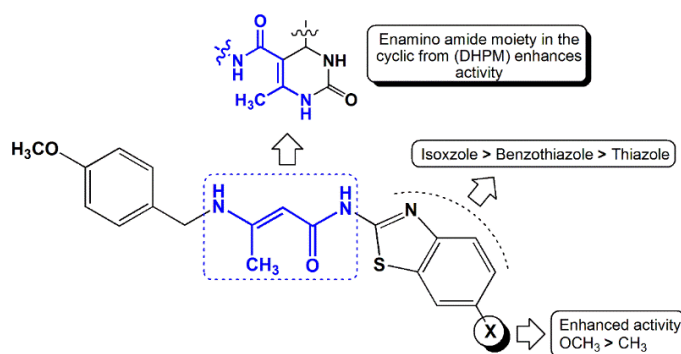

Fig. 2. Proposed structure-activity relationship for assessed *N*-heteroaryl-3-(*para*-methoxy benzyl) amino but-enamides (**A1-A6**) antileishmanial agents against *Leishmania majo*r.
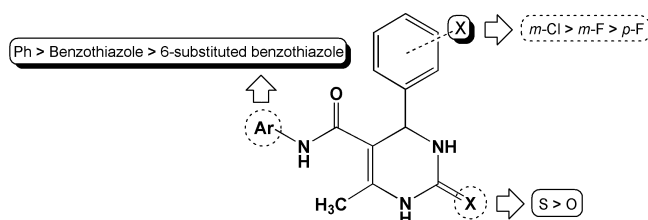

Fig. 3. Proposed structure-activity relationship for assessed 6-methyl-4-aryl-*N*-aryl dihydropyrimidinones/thiones (**A7-A11**) antileishmanial agents against *Leishmania major*.

CONCLUSION

SAR evaluations indicated that DHPMTs with halogen on *meta* position of 4-phenyl ring had superior antileishmanial effect than DHPMs bearing halogen on *para* position of 4-phenyl ring. Moreover; the replacement of 5-(*N*-thiazole carboxamide) by 5-(*N*-phenyl carboxamide) reinforced the activity. Based on obtained results, future efforts might be directed toward synthesis and antileishmanial assessment of diverse 5-(*N*-phenyl carboxamide)-4-(meta-substituted phenyl)-2-thiocarbonyl DHPMs with possible hydrophobic substituents on the *N*-phenyl moiety to span more extended chemical space to find potent antileishmanial small molecules.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest in this study.

AUTHORS' CONTRIBUTION

All authors contributed equally to this work.

REFERENCES

1. World Health Organization, WHO report on global surveillance of epidemic-prone infectious diseases-leishmaniasis.
https://www.who.int/csr/resources/publications/CSR_ISR_2000_1leish/en/, visited 20 December 2019.

2. Shirzadi MR, Esfahania SB, Mohebalia M, Ershadia MR, Gharachorlo F, Razavia MR, *et al*. Epidemiological status of leishmaniasis in the Islamic Republic of Iran, 1983-2012. East Mediterr Health J. 2015;21(10):736-742.
DOI: 10.26719/2015.21.10.736.

3. Sajjadi SE, Eskandarian AA, Shokoohinia Y, Yousefi HA, Mansourian M, Asgarian-Nasab H, *et al*. Antileishmanial activity of prenylated coumarins isolated from *Ferulago angulata* and *Prangos asperula*. Res Pharm Sci. 2016;11(4):324-331.
DOI: 10.4103/1735-5362.189314.

4. Savoia D. Recent updates and perspectives on leishmaniasis. J Infect Dev Ctries. 2015;9(6):588-596.
DOI: 10.3855/jidc.6833.

5. Ortalli M, Ilari A, Colotti G, De Ionna I, Battista T, Bisi A, *et al*. Identification of chalcone-based antileishmanial agents targeting trypanothione reductase. Eur J Med Chem. 2018;152:527-541.
DOI: 10.1016/j.ejmech.2018.04.057.

6. Mendoza-Martínez C, Correa-Basurto J, Nieto-Meneses R, Marquez-Navarro A, Aguilar-Suarez R, Montero-Cortes MD, *et al*. Design, synthesis and biological evaluation of quinazoline derivatives as anti-trypanosomatid and anti-plasmodial agents. Eur J Med Chem. 2015;96:296-307.
DOI: 10.1016/j.ejmech.2015.04.028.

7. Vijayakumar S, Das P. Recent progress in drug targets and inhibitors towards combating leishmaniasis. Acta Trop. 2018;181:95-104.
DOI: 10.1016/j.actatropica.2018.02.010.

8. Sheikhmoradi V, Saberi S, Saghaei L, Pestehchian N, Fassihi A. Synthesis and antileishmanial activity of antimony (V) complexes of hydroxypyranone and hydroxypyridinone ligands. Res Pharm Sci. 2018;13(2):111-120.
DOI: 10.4103/1735-5362.223793.

9. Ponte-Sucre A, Gamarro F, Dujardin J, Barret MP, Lopez-Velez R, García-Hernandez R, *et al*. Drug resistance and treatment failure in leishmaniasis: a 21st century challenge. PLoS Negl Trop Dis. 2017;11(12):e0006052,1-24.
DOI: 10.1371/journal.pntd.0006052.

10. Bhattacharya SK, Dash AP. Elimination of Kala-Azar from the southeast Asia region. Am J Trop Med Hyg. 2017;96(4):802-804.
DOI: 10.4269/ajtmh.16-0279.

11. Rezaee Nasab R, Hassanzadeh F, Mansourian M. Synthesis, antimicrobial evaluation and docking studies of some novel quinazolinone Schiff base derivatives. Res Pharm Sci. 2018;13(3):213-221.
DOI: 10.4103/1735-5362.228942.

12. Singh N, Kaur J, Kumar P, Gupta S, Singh N, Ghosal A, *et al*. An orally effective dihydropyrimidone (DHPM) analogue induces apoptosis-like cell death in clinical isolates of *Leishmania donovani* overexpressing pteridine reductase 1. Parasitol Res. 2009;105(5):1317-1325.
DOI: 10.1007/s00436-009-1557-z.

13. Saudi MNS, El-Semary MMA, Elbayaa RY, Jaeda MI, Eissa MM, Amer EI, *et al*. Synthesis and biological evaluation of a novel class as antileishmanial agent. Med Chem Res. 2012;21:257-267.
DOI: 10.1007/s00044-010-9532-x.

14. Rashid U, Sultana R, Shaheen N, Hassan SF, Yaqoob F, Ahmad MJ, *et al*. Structure based medicinal chemistry-driven strategy to design substituted dihydropyrimidines as potential antileishmanial agents. Eur J Med Chem. 2016;115:230-244.
DOI: 10.1016/j.ejmech.2016.03.022.

15. Mishra R, Mishra B, Hari Narayana Moorthy NS. Synthesis and anti-microbial evaluation of some 3,4-dihydro pyrimidine-2-one derivatives. Trends Appl Sci Res. 2008;3(20):203-208.
    DOI: 10.3923/tasr.2008.203.208.

16. Razzaghi-Asl N,Omidreza F, Hemmateenejad B, Javidnia K, Edraki N, Miri R. Design and synthesis of novel 3,5-bis-*N*-(aryl/heteroaryl) carbamoyl-4-aryl-1,4-dihydropyridines as small molecule BACE-1 inhibitors. Bioorg Med Chem. 2013;21(22): 6893-6909.
    DOI: 10.1016/j.bmc.2013.09.033.

17. Tavangar S, Bohlooli S, Razzaghi-Asl N. Synthesis and cytotoxic effect of a few *N*-heteroaryl enamino amides and dihydropyrimidinethiones on AGS & MCF-7 human cancer cell lines. Res Pharm Sci. 2020;15(2):154-163.
    DOI: 10.4103/1735-5362.283815.

18. Memarian HR, Ranjbar M. Synthesis of Biginelli compounds using Cobalt Hydrogen sulfate. J Chin Chem Soc. 2011;58(4):522-527.
    DOI: 10.1002/jccs.201190016.

19. Jalali M, Mahdavi M, Memarian HR, Ranjbar M, Soleymani M, Fassihi A, *et al*. Antimicrobial evaluation of some novel derivatives of 3,4-dihydropyrimidine-2(1*H*)-one. Res Pharm Sci. 2012;7(4):243-247.

20. Niapour A, Bohlooli S, Sharifi Pasandi M, Mohammadi-ghalehbin B. *In vitro* anti leishmanial effect of *Agrostemma githago* extract on *Leishmania major* promastigotes by cell count and MTT assay. J Mazandaran Univ Med Sci. 2018;28(165):13-23.

21. Heidari-Kharaji M, Fallah-Omrani V, Badirzadeh A, Mohammadi-Ghalehbin B, Nilforoushzadeh MA, Masoori L, *et al*. *Sambucus ebulus* extract stimulates cellular responses in cutaneous leishmaniasis. Parasite Immunol. 2019;41(1):e12605,1-37.
    DOI: 10.1111/pim.12605.

22. das Neves AR, Trefzger OS, Barbosa NV, Honorato AM, Carvalho DB, Moslaves IS, *et al*. Effect of isoxazole derivatives of tetrahydrofuran neolignans on intracellular amastigotes of *Leishmania (Leishmania) amazonensis*: a structure-activity relationship comparative study with triazole-neolignan-based compounds. Chem Biol Drug Des. 2019;94(6):2004-2012.
    DOI: 10.1111/cbdd.13609.

23. Liu M, Wilairat P, Croft SL, Tan AL, Go ML. Structure-activity relationships of antileishmanial and antimalarial chalcones. Bioorg Med Chem. 2003;11(13):2729-2738.
    DOI: 10.1016/s0968-0896(03)00233-5.

24. Ashok P, Chander S, Smith TK, Prakash Singh R, Jha PN, Sankaranarayanan M. Biological evaluation and structure activity relationship of 9-methyl-1-phenyl-9*H*-pyrido [3, 4-b] indole derivatives as anti-leishmanial agents. Bioorg Chem. 2019;84: 98-105.
    DOI: 10.1016/j.bioorg.2018.11.037.