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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of 2,4,6-trisubstituted pyrimidine derivatives as novel antileishmanial agents

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Abstract A series of new 2,4,6-trisubstituted pyrimidine derivatives **8(a–j)** were synthesized by reacting substituted chalcones containing imidazole **6(a–d)** and benzimidazole **7(a–f)** with guanidine hydrochloride in the presence of strong base. Substituted chalcones were synthesized by reacting 4-(1H-imidazol-1-yl)benzaldehyde or 4-(1H-benzo [d]imidazol-1-yl)benzaldehyde with different substituted acetophenones in the presence of 40 % NaOH in methanol. The synthesized compounds were confirmed by IR, ^{1}HNMR, and mass spectral data and screened for antileishmanial activity. Antileishmanial activity was performed against *Leishmania donovani* parasite, and percentage lysis inhibition were calculated by meglumine antimoliate taking a positive control and chloroform (0.1 % $CHCl_3$) treatment served as control. Among all the compounds, **8h** and **8j** exhibited 50–57 % inhibition against promastigotes, thus providing new structural lead for antileishmanials.

Keywords Synthesis · Chalcones · Pyrimidines · Antileishmanial activity

Introduction

Leishmaniasis is a parasitic disease caused by 17 species of the protozoan parasite *Leishmania* (Chandra *et al.*, 2005) and transmitted by the bite of a sand-fly genus *Phlebotomus*. Three different forms of the diseases: visceral leishmaniasis, cutaneous leishmaniasis, and mucocutaneous leishmaniasis are present in the world (Bouhlel *et al.*, 2010). This disease is mostly endemic in 88 countries in the world, and visceral form of leishmaniasis is the most severe type among all types of leishmaniasis, known as kala-azar, which is caused by *Leishmania donovani* and is nearly always fatal, if untreated (Sunduru *et al.*, 2009). More than 12 million people were infected by this disease around the world (Bhandari *et al.*, 2010). Five lakh new cases of visceral leishmaniasis or kala-azar were found every year (Perez-Victoria *et al.*, 2006). Currently available drugs are ineffective due to emergence of resistance among parasites; also, chemotherapy of leishmaniasis is quite difficult due to significant toxicity, variable efficacy, lack of oral bioavailability, and high cost of the therapeutic agents. So novel antileishmanial agents are urgently needed (Srinivas *et al.*, 2009).

Mostly the nitrogen heterocycles such as quinolines, acridines, phenothiazines, pyrimidines, purines, bis-benzamidines, pyrazolo[3,4b]pyridine, benzothiazoles, and imidazolidine were reported as antileishmanial agents (Agarwal *et al.*, 2009).

Most of the clinically used DHFR inhibitor shows less selectivity for leishmanial enzymes because the gene for pteridine reductase (PTR1) is amplified in some leishmanial mutants. Antifolate drugs simultaneously target both DHFR and PTR1 to be successful antileishmanials because PTR1 can reduce pterins and folates, and therefore, act as a bypass for DHFR inhibition. A number of compounds having pyrimidine moiety is reported to be potent inhibitor of PTR1 in *Leishmania* (Sunduru *et al.*, 2006).

Based on the above observations, we have synthesized hybrid derivatives of pyrimidine containing imidazole or benzimidazole. The synthesized compounds act as an

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Scheme 1 Synthesis of 2,4,6-trisubstituted pyrimidine derivatives. *Reagent and conditions*: (*I*) K_2CO_3/$C_{16}H_{33}(CH_3)_3N^+Br^-$, 100 °C, stirrer, 28 h; (*II*) MeOH/NaOH/ 25 °C, stirrer, 50–60 min; (*IIIa* & *IIIb*) EtOH/NaOH/90 °C, reflux, 16 h

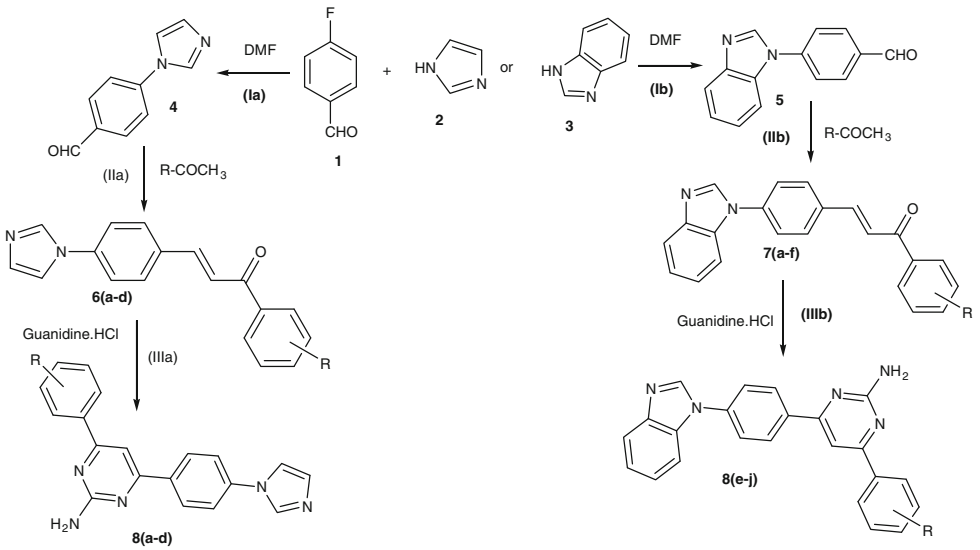
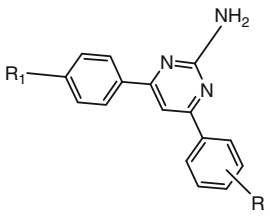


Table 1 General structures of synthesized compounds and anti-leishmanial activity of with respect to control



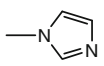
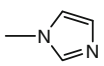
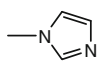
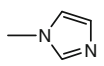
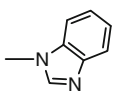
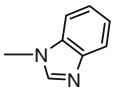
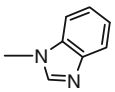
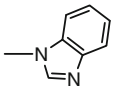
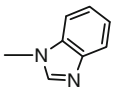
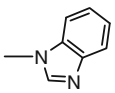
Compound code	Molecular formula	R_1	R	Dose (μg/ml)	Biological activity*
8a	$C_{19}H_{15}N_5$		H	10	32.81
8b	$C_{19}H_{14}ClN_5$		4-Cl	10	46.14
8c	$C_{19}H_{14}BrN_5$		4-Br	10	24.32
8d	$C_{20}H_{17}N_5O$		4-OCH_3	10	18.14
8e	$C_{23}H_{17}N_5$		H	10	41.86

Table 1 continued

Compound code	Molecular formula	R_1	R	Dose (μg/ml)	Biological activity*
8f	$C_{23}H_{16}FN_5$		4-F	10	29.64
8g	$C_{23}H_{16}ClN_5$		4-Cl	10	32.67
8h	$C_{23}H_{15}Cl_2N_5$		2,4-Cl	10	**56.42**
8i	$C_{23}H_{16}BrN_5$		4-Br	10	32.49
8j	$C_{24}H_{19}N_5O$		4-OCH_3	10	**51.18**

* Percentage lysis with respect to control

inhibitor of PTR1 as well as an inhibitor of DHFR and thus act as potential antileishmanial agents.

Chemistry

N-arylation of imidazole or benzimidazole was carried out with para-fluorobenzaldehyde using hexadecyltrimethylammonium bromide as a catalyst to yield compounds 4-(1H-imidazol-1-yl) benzaldehyde (**4**) or 4-(1*H*-benzo[*d*]imidazol-1-yl)benzaldehyde (**5**). Compounds **4** and **5** were reacted with substituted acetophenones in the presence of base (40 % NaOH in methanol) to afford substituted chalcones by Claisen Schmidt condensation (**6a–d**, Scheme 1I, IIa and **7a–f**, 1I, IIb), (Hussain *et al.*, 2009).

The formation of pyrimidine ring from chalcone proceeds via Michael reaction followed by cyclization (Micky *et al.*, 2006). Different substituted chalcones were cyclized by guanidine hydrochloride in the presence of base using ethanol as a solvent to yield pyrimidine derivative (**8a–j**, Scheme 1I, III); (Trivedi *et al.*, 2008; Rashinkar *et al.*, 2009).

Result and discussion

Chemistry

Absorbtion band at 3,323–3,487 cm^{-1} which indicates the presence of aromatic primary amino group (-NH_2). IR data confirms the presence of specific functional groups present in all the synthesized compounds. Absorbtion at 813–740 cm^{-1}; 542–594 cm^{-1}; 1,053–1,230 cm^{-1}, and 1,232–1,255 cm^{-1} indicates the presence of C–Cl, C–Br, C–F, and C–OCH_3 substitution groups in synthesized compound, respectively.

The presence of 7.26–7.15 δ ppm (1H, s, pyrimidine) indicates the formation of pyrimidine ring at synthesized compound. The presence of 5.89–6.45 δ ppm (2H, s, -NH_2) indicates the amino group (-NH_2) at synthesized

compound. 3.84 δ ppm values indicate the presence of methoxy group in synthesized compound.

Biological activity

Biological evaluation was performed against *L. donovani* strain, and percentage lysis inhibition was calculated by maglumine antimoniate as positive control (Table 1). 4-Cl substitution on aromatic phenyl ring increases the biological activity and 2,4 dichloro substitution on aromatic phenyl ring further increases the biological activity. A 4-OCH_3 substituted benzimidazole derivative was found to be more potent than 4-OCH_3 substituted imidazole derivatives. **8h** and **8j** were the most potent compounds of the series and shows more than 50 % inhibition against promastigotes.

Conclusion

2,4,6-Trisubstituted pyrimidine derivatives were synthesized and confirmed by IR, ^{1}H NMR and Mass spectral data and screened for antileishmanial activity. Compounds **8h** and **8j** exhibited 56.42 and 51.18 % inhibition against promastigotes, respectively, thus providing lead structure for antileishmanials.

Experimental

Melting points of synthesized compounds were identified by open capillary method and were uncorrected. Infrared (IR) spectra were recorded using Shimadzu FTIR (SGSITS Indore). Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker Avance II, 400 MHz Spectrophotometer (Panjab University) using DMSO as a solvent and TMS as internal standard (Chemical shift in δ ppm). Spin multiplates are given as *s* (singlet), *d* (doublet), *t* (triplet), and *m* (multiplet). Mass spectra were recorded on GCMS solution\system\tune1\Auto Tuning-EI (IICT Hyderabad). Biological activity of synthesized compounds was performed from Pharmbio Research Center Pvt. Ltd., Indore.

Synthesis of 4-(1H-imidazol-1-yl)benzaldehyde (**4**; Scheme 1I, Ia)

A mixture of imidazole **2** (6.8 g, 100 mmol), anhydrous potassium carbonate (13.80 g, 100 mmol), 4-fluorobenzaldehyde **1** (12.40 g, 100 mmol), hexadecyltrimethylammonium bromide (20 mg), and DMF (50 ml) was stirred for a period of 28 h at 100 °C, and after cooling to room temperature, was poured on to crushed ice (200 ml). Pale yellow crystals obtained were filtered, dried, and recrystallized from methanol (Hussain *et al.*, 2009).

Synthesis of 4-(1H-benzo[d]imidazol-1-yl)benzaldehyde (**5**; Scheme 1I, Ib)

A mixture of benzimidazole **3** (11.8 g, 100 mmol), anhydrous potassium carbonate (13.80 g, 100 mmol), 4-fluorobenzaldehyde **1** (12.40 g, 100 mmol), hexadecyltrimethyl ammonium bromide (20 mg), and DMF (50 ml) was stirred for a period of 28 h at 100 °C, and after cooling to room temperature, was poured on to crushed ice (200 ml). Pale yellow crystals obtained were filtered, dried, and recrystallized from methanol.

Synthesis of 3-(4-(1H-imidazol-1-yl)phenyl)-1-phenylprop-2-en-1-one (**6a–d**; Scheme 1I, IIa)

A methanolic sodium hydroxide solution (40 %, 10 mmol) was added dropwise to a mixture of 4-(1*H*-imidazol-1-yl)benzaldehyde (10 mmol,), acetophenone (10 mmol), and methanol over a period of 50–60 min with continuous stirring till completion of reaction (as indicated by TLC). Precipitates obtained were filtered and washed with cold methanol–water mixture (1:10). Finally, the product was recrystallized from methanol (Hussain *et al.*, 2009).

Synthesis of 3-(4-(1H-benzo[d]imidazol-1-yl)phenyl)-1-phenylprop-2-en-1-one (**7a–f**; Scheme 1I, IIb)

A methanolic sodium hydroxide solution (40 %, 10 mmol) was added dropwise to a mixture of 4-(1*H*-benzo[*d*]imidazol-1-yl)benzaldehyde (10 mmol), acetophenone (10 mmol), and methanol over a period of 50–60 min with continuous stirring till completion of reaction (as indicated by TLC). Precipitates obtained were filtered and washed with cold methanol–water mixture (1:10). Finally, the product was recrystallized from methanol (Hussain *et al.*, 2009).

Synthesis of 4-(4-(1H-imidazol-1-yl)phenyl)-6-phenylpyridine-2-amine (**8a–d**; Scheme 1I, IIIa)

Guanidine hydrochloride (3 mmol) was dissolved in 12 ml 5 % ethanolic solution of NaOH. Synthesized chalcones (**6a–d**) was added to it and refluxed for 16 h. Solution was cooled at room temperature and poured into the crushed ice. Precipitate was filtered and washed with cold water. The crude product was dried and recrystalised from ethanol than methanol (Rashinkar *et al.*, 2009).

4-(4-(1H-imidazol-1-yl)phenyl)-6-(4 chlorophenyl)pyrimidin-2-amine (**8a**)

% Yield: 42. MP (°C):234–236. IR (KBr, cm^{-1}): 3109, 3454, 1520, 1051, 813. ^{1}H NMR (DMSO, 400 MHz) δ: 8.33(2H, d, Ar–H), 8.20(3H, t, Ar–H), 8.05(1H, s, imidazole), 7.71–7.64(3H, m, Ar–H), 7.51(2H, d, imidazole), 7.15(1H, s, pyrimidine), 6.45(2H, s, -NH_2). Mass (*m*/*z*): 347.

4-(4-(1H-imidazol-1-yl)phenyl)-6-phenylpyrimidin-2-amine (**8b**)

% Yield: 40. MP (°C): 136–138. IR (KBr, cm^{-1}): 3128, 3371, 1543, 1053. ^{1}H NMR (DMSO, 400 MHz) δ: 8.36(2H, d, Ar–H), 8.28(3H, t, Ar–H), 8.23(1H, s, imidazole), 7.72(2H, d, imidazole), 7.66–7.61(4H, m, Ar–H), 7.26(1H, s, pyrimidine), 5.89(2H, s, -NH_2). Mass (*m*/*z*): 313.

4-(4-(1H-imidazol-1-yl)phenyl)-6-(4-bromophenyl)pyrimidin-2-amine (**8c**)

% Yield: 46. MP (°C): 230–233. IR (KBr, cm^{-1}): 3113, 3458, 1519, 1051, 586. ^{1}H NMR (DMSO, 400 MHz) δ: 8.31(2H, d, Ar–H), 8.10(3H, t, Ar–H), 7.94(1H, s, imidazole), 7.67–7.59(5H, m, Ar–H), 7.16(1H, s, pyrimidine), 6.31(2H, s, -NH_2). Mass (*m*/*z*): 391.

4-(4-(1H-imidazol-1-yl)phenyl)-6-(4-methoxyphenyl)pyrimidin-2-amine (**8d**)

% Yield: 40. MP (°C): 205–208. IR (KBr, cm^{-1}): 3107, 3454, 1514, 1051, 1255. ^{1}H NMR (DMSO, 400 MHz) δ: 8.33(3H, t, Ar–H), 8.18(2H, d, imidazole), 7.76(3H, d, Ar–H), 7.63(1H, s, imidazole), 7.14(1H, s, pyrimidine), 7.02(2H, d, Ar–H), 6.51(2H, s, -NH_2), 3.85(3H, s, -OCH_3). Mass (*m*/*z*): 343.

Synthesis of 4-(4-(1H-benzo[d]imidazol-1-yl)phenyl)-6-phenylpyridine-2 amine (**8e–j**; Scheme 1I, IIIb)

Guanidine hydrochloride (3 mmol) was dissolved in 12 ml 5% ethanolic solution of NaOH. Synthesized chalcones (**7a–f**) were added to it and refluxed for 16 h. Solution was cooled at room temperature and poured into the crushed ice. Precipitate was filtered and washed with cold water. The crude product was dried and recrystallized from ethanol then methanol (Rashinkar *et al.*, 2009).

4-(4-(1H-benzo[d]imidazol-1-yl)phenyl)-6-(4 fluorophenyl) pyrimidin-2-amine (**8e**)

% Yield: 42. MP (°C): 182–184. IR (KBr, cm^{-1}):3086, 3487, 1510, 1014, 1230. ^{1}H NMR (DMSO, 400 MHz) δ: 8.38(2H, d, Ar–H), 8.33(1H, s, imidazole), 8.28(2H, d, Ar–H), 8.20–8.16(2H, m, Ar–H), 7.84(1H, d, imidazole), 7.75(3H, t, Ar–H), 7.38–7.35(2H, m, Ar–H), 7.26(1H, s, pyrimidine), 6.04(2H, s, -NH_2). Mass (*m*/*z*): 381.

4-(4-(1H-benzo[d]imidazol-1-yl) phenyl)-6-(4-bromophenyl) pyrimidin-2-amine (**8f**)

% Yield: 45. MP (°C): 138–140. IR (KBr, cm^{-1}): 3086, 3410, 1521, 1234, 594. ^{1}H NMR (DMSO, 400 MHz) δ: 8.41(3H, t, Ar–H), 8.12(2H, d, imidazole), 7.81–7.73(3H, m, Ar–H), 7.67–7.63(4H, m, Ar–H), 7.38–7.31(2H, m, Ar–H), 6.38(2H, s, -NH_2). Mass (*m*/*z*): 442.

4-(4-(1H-benzo[d]imidazol-1-yl) phenyl)-6-phenylpyrimidin-2-amine (**8g**)

% Yield: 45. MP (°C): 185–187. IR (KBr, cm^{-1}): 3086, 3323, 1544, 1224. ^{1}H NMR (DMSO, 400 MHz) δ: 8.30(2H, d, Ar–H), 8.19(1H, s, imidazole), 8.10–8.07(2H, m, Ar–H), 7.92–7.8(1H, m, Ar–H), 7.67(2H,d, imidazole), 7.53–7.39 (4H, m, Ar–H), 7.39–7.35(2H, m, Ar–H), 7.26(1H, s, pyrimidine), 5.28(2H, s, -NH_2). Mass (*m*/*z*): 363.

4-(4-(1H-benzo[d]imidazol-1-yl) phenyl)-6-(4-chlorophenyl) pyrimidin-2-amine (**8h**)

% Yield: 46. MP (°C): 161–164. IR (KBr, cm^{-1}): 3101, 3433, 1514, 1010, 742. ^{1}H NMR (DMSO, 400 MHz) δ:8.40(3H, t, Ar–H), 8.18(2H, d, Ar–H), 7.93(1H, s, imidazole), 7.79(2H, d, imidazole), 7.65 (2H, t, Ar–H), 7.50(2H,d, Ar–H), 7.36–7.33(2H, m, Ar–H), 6.33(2H,s, -NH_2). Mass (*m*/*z*): 397.

4-(4-(1H-benzo[d]imidazol-1-yl) phenyl)-6-(4-methoxyphenyl) pyrimidin-2-amine (**8i**)

% Yield: 48. MP (°C): 186–189. IR (KBr, cm^{-1}):3070, 3477, 1512, 1031, 1232. ^{1}H NMR (DMSO, 400 MHz) δ: 8.80(3H, t, Ar–H), 8.14(2H, d, Ar–H), 7.79(1H, d), 7.7(2H, d, imidazole), 7.76(1H, d, Ar–H),7.58 (1H, s, pyrimidine), 7.38–7.30(2H, m, Ar–H),7.02(2H, d, Ar–H), 6.29(2H, s, -NH_2), 3.86(3H, s, -OCH_3). Mass (*m*/*z*): 393.

4-(4-(1H-benzo[d]imidazol-1-yl) phenyl)-6-(2,4-dichlorophenyl) pyrimidin-2-amine (**8j**)

% Yield: 50. MP (°C):128–130. IR (KBr, cm^{-1}): 3070, 3485, 1521, 1105, 736. ^{1}H NMR (DMSO, 400 MHz) δ:8.49(1H, s, imidazole), 8.23(2H, d, imidazole), 7.78(3H, t, Ar–H), 7.76(2H, t, Ar–H), 7.59 (1H, s, pyrimidine), 7.47(1H, d, Ar–H), 7.37–7.29(3H, m, Ar–H), 6.73(2H, s,-NH_2). Mass (*m*/*z*): 431.

Biological activity

The splenic culture *L. donovani* was made in Medium-199 (L-glutamine with Hepes buffer without $NaHCO_3$) supplemented with 10% fetal bovine serum (pH 7.2). The logarithm phases of promastigotes (2×10^6 cells/ml) were incubated with or without the isolates along with Medium-199 at 22 °C. The samples were dissolved in 0.2% DMSO and then added to the culture in doses of 10 μg/ml. After 2 h of treatment, all tubes were centrifuged at 8,000×*g* for 10 min, the supernatant was decanted and the pellets were washed with 20 mM phosphate buffer saline (PBS). Each pellet was dissolved in 100 μl (2 mg/ml) of MTT in phosphate buffer saline.

All the tubes were incubated at 22 °C for 4 h and then centrifuged at 8,000×*g* for 10 min. All the pellets were dissolved in 500 μl DMSO and assessed by UV spectrophotometry (UV 2060 plus—Analytical India) at 570 nm. Percent of lysis of promastigotes by the compounds was calculated using the standard formula of Tim Mosmann. Chloroform (0.1% $CHCl_3$) treatment served as control.

$$\text{Lysis } (\%) = 100 - (T - PC)/(C - PC) \times 100 \qquad (1)$$

T is the test isolate, PC is the positive control—meglumine antimoliate, *C* is the control.

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