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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

# Synthesis, characterization of 4,6-disubstituted aminopyrimidines and their sulphonamide derivatives as anti-amoebic agents

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**Abstract** The present study describes the synthesis and anti-amoebic activity of 4,6-disubstituted aminopyrimidines (**1b–10b**) and their sulphonamide derivatives (**1–20**). All the desired compounds were characterized by spectral data and their purity was confirmed by elemental analysis. The aim of the study was to explore the effect of the target compounds on in vitro growth of HM1:IMSS strain of *Entamoeba histolytica.* In vitro anti-amoebic activity was performed by microdilution method and the results were compared with standard drug metronidazole. The results revealed that sulphonamide derivatives (**1–20**) showed better activity than 4,6-disubstituted aminopyrimidines (**1b–10b**). 5 pyrimidine and 12 sulphonamide derivatives were better inhibitors of the growth of *E. histolytica* than the reference drug metronidazole ($IC_{50}$ = 1.80 μM). The promising in vitro anti-amoebic activity of the compounds make them promising molecules for further lead optimization in the development of novel anti-amoebic agents, therefore, it is hoped that these preliminary results could help in designing better molecules with an enhanced anti-amoebic activity.

**Keywords** Pyrimidine · Sulphonamide · Anti-amoebic activity · HM1:IMSS · *E. histolytica*

## Introduction

Amoebiasis, caused by parasitic protozoan *Entamoeba histolytica*, is an emerging parasitic disease in HIV-infected patients in an area endemic for amoebic infection (Hung *et al*., 1999). Amoebiasis poses a serious health problem, with 100,000 deaths per year, making it the second leading cause of death due to parasitic disease (Ralston and Petri, 2011). However, anti-amoebic chemotherapy relies on 5-nitroimidazoles, but long-term use of these medications produce a number of serious side effects in patients (Ordaz-Pichardo *et al*., 2005). Metronidazole (MNZ) is the medication of choice for invasive amoebiasis; however, it is not known whether its dose or duration require modification in HIV infection when treating invasive amoebiasis (Ohnishi and Uchivama-Nakamura, 2012). Furthermore, it is mutagenic and has been associated with promiscuous side effects (Ordaz-Pichardo *et al*., 2005). Therefore, the search for new effective anti-amoebic compounds is required.

Among various nitrogen containing heterocycles, pyrimidine is promising structural moiety for drug designing. Pyrimidine and their derivatives have been found to be endowed with anti-amoebic activities (Parveen *et al*., 2010, 2011; Siddiqui *et al*., 2013a). The 2-aminopyrimidine, which is a common structural subunit in a number of biologically active natural as well synthetic compounds, has been widely used as a drug like scaffold (Lagoja, 2005). Aminopyrimidine derivatives substituted at N-position are of immense importance because they exhibit multifaceted biological and pharmacological activities. Considering this perspective, we decided to design compounds having aminopyrimidine ring (**1b–10b**) as well as the compounds derived from this bioactive core (**1–20**) (Fig. 1). It was expected that the compounds derived from pyrimidine core incorporating sulphonamide moiety might exhibit better anti-amoebic activity than the compounds having only pyrimidine moiety. The hypothesis behind it is that our recent studies indicate that like pyrimidine,

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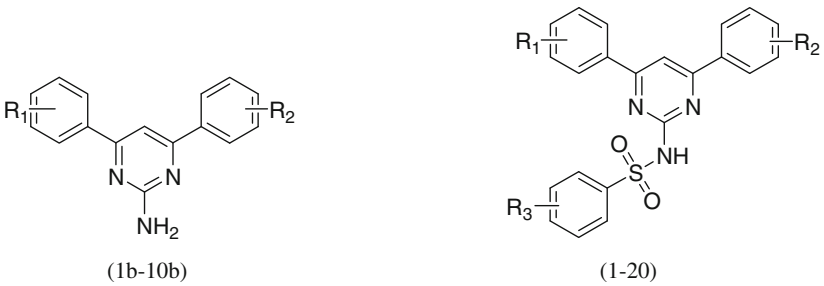
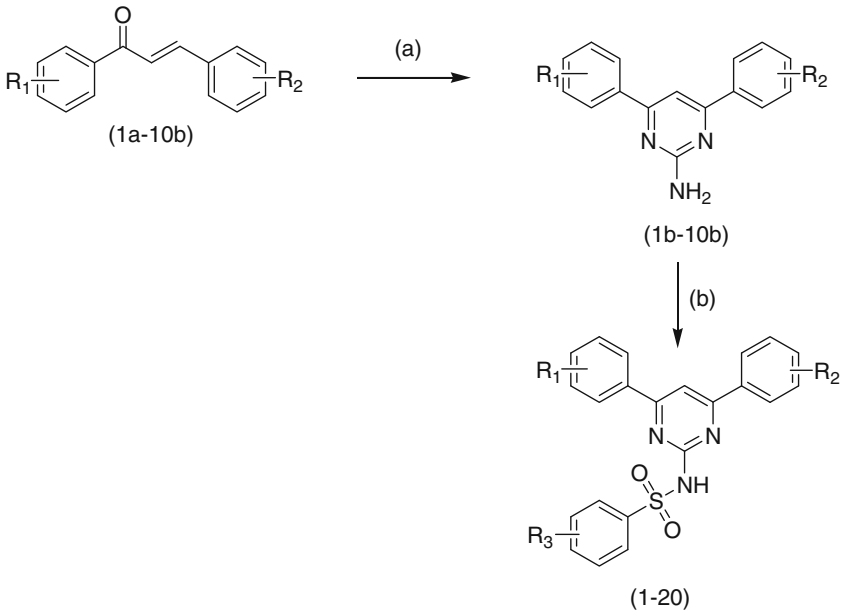



**Fig. 1** 4,6-Disubstituted aminopyrimidines (**1b–10b**) and their sulphonamide derivatives (**1–20**)




**Scheme 1** General method for the synthesis of sulphonamide derivatives of 4,6-disubstituted aminopyrimidines (**1–20**). Reagent and conditions: **a** Guanidine hydrochloride, sodium isopropoxide, isopropanol and reflux; **b** 10 % NaOH, benzenesulphonyl or toluene-*p*-sulphonyl chloride and warm

sulphonamide is crucial structural feature for the display of anti-amoebic activity (Salahuddin *et al*., 2013; Siddiqui *et al*., 2013a), further the introduction of sulphonamide group increases pharmacological potency and/or the absorption, distribution, metabolism and excretion (ADME) attributes of the lead chemical matter (Dai *et al*., 2011). Therefore, the combination of these two moieties is expected to exhibit synergistic effects to display anti-amoebic activity. In view of this, we herein report the synthesis and anti-amoebic activity of 4,6-disubstituted aminopyrimidines (**1b–10b**) and their sulphonamide derivatives (**1–20**).

## Results and discussion

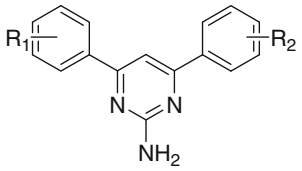
### Chemistry

The synthetic pathway leading to the target compounds is depicted in Scheme 1. 4,6-disubstituted aminopyrimidines (**1b–10b**) were obtained through synthesis of 1,3-diaryl-2-propen-1-ones (**1a–10a**). The synthesis of these compounds (**1a–10a**) was based on Claisen–Schmidt condensation of acetophenone and 4-chloro-acetophenone with different aryl aldehydes by a reported method (Li *et al*., 1995). 1,3-diaryl-2-propen-1-ones (**1a–10a**) were further cyclized with guanidine hydrochloride in the presence of sodium isopropoxide (synthesized in situ by adding sodium metal in isopropanol) to afford 4,6-disubstituted aminopyrimidines (**1b–10b**) (Sunduru *et al*., 2006). Finally, the condensation of 4,6-disubstituted aminopyrimidines (**1b–10b**) with benzene sulphonyl chloride or toluene-*p*-sulphonyl chloride furnished sulphonamide derivatives of 4,6-disubstituted aminopyrimidines (**1–20**) (Furniss *et al*., 1989). The structures of these compounds were elucidated by spectral studies and their purity was confirmed by elemental analyses.

### Anti-amoebic activity

In order to explore the potential of 4,6-disubstituted aminopyrimidines (**1b–10b**) and their sulphonamide derivatives (**1–20**) as possible scaffold for *E. histolytica* inhibitors, all these compounds were evaluated against HM1:IMSS strain of *E. histolytica.* All the experiments were carried out in triplicate at each concentration level and repeated thrice. The anti-amoebic effect was compared

**Table 1** In vitro anti-amoebic activity of 4,6-disubstituted aminopyrimidines (**1b–10b**) against HM1:IMSS strain of *E. histolytica*



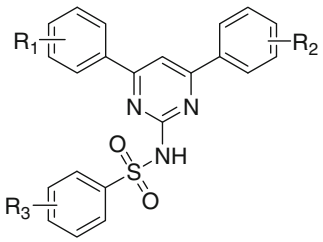
**(1b–10b)**

| Compounds | $R_1$ | $R_2$ | $IC_{50}$ (μM)[a] | S.D. |
|---|---|---|---|---|
| **1b** | H | H | 6.15 | 0.06 |
| **2b** | 4-Cl | H | 2.50 | 0.01 |
| **3b** | H | 2-Cl | **1.72** | 0.05 |
| **4b** | 4-Cl | 2-Cl | **1.54** | 0.05 |
| **5b** | H | 3-Cl | 4.47 | 0.03 |
| **6b** | 4-Cl | 3-Cl | 7.21 | 0.06 |
| **7b** | H | 4-Cl | **1.63** | 0.05 |
| **8b** | 4-Cl | 4-Cl | **1.66** | 0.03 |
| **9b** | H | 3,4-Cl | 5.74 | 0.06 |
| **10b** | 4-Cl | 3,4-Cl | **1.59** | 0.03 |
| MNZ | | | 1.80 | 0.05 |

*S.D.* standard deviation

[a] The values obtained in at least three separate assays done in triplicate

**Table 2** In vitro anti-amoebic activity of sulphonamide derivatives of 4,6-disubstituted aminopyrimidines (**1–20**) against HM1:IMSS strain of *E. histolytica*



**(1–20)**

| Compounds | $R_1$ | $R_2$ | $R_3$ | $IC_{50}$ (μM)[a] | S.D. |
|---|---|---|---|---|---|
| **1** | H | H | H | 2.34 | 0.04 |
| **2** | H | H | 4-$CH_3$ | 1.95 | 0.05 |
| **3** | 4-Cl | H | H | **0.70** | 0.01 |
| **4** | 4-Cl | H | 4-$CH_3$ | **1.35** | 0.06 |
| **5** | H | 2-Cl | H | **0.44** | 0.05 |
| **6** | H | 2-Cl | 4-$CH_3$ | **0.90** | 0.01 |
| **7** | 4-Cl | 2-Cl | H | **0.73** | 0.01 |
| **8** | 4-Cl | 2-Cl | 4-$CH_3$ | **1.44** | 0.04 |
| **9** | H | 3-Cl | H | 3.04 | 0.02 |
| **10** | H | 3-Cl | 4-$CH_3$ | 2.68 | 0.04 |
| **11** | 4-Cl | 3-Cl | H | 5.32 | 0.01 |
| **12** | 4-Cl | 3-Cl | 4-$CH_3$ | 2.70 | 0.06 |
| **13** | H | 4-Cl | H | **1.57** | 0.06 |
| **14** | H | 4-Cl | 4-$CH_3$ | **1.02** | 0.07 |
| **15** | 4-Cl | 4-Cl | H | **1.60** | 0.01 |
| **16** | 4-Cl | 4-Cl | 4-$CH_3$ | **1.42** | 0.04 |
| **17** | H | 3,4-Cl | H | 3.62 | 0.04 |
| **18** | H | 3,4-Cl | 4-$CH_3$ | 4.01 | 0.06 |
| **19** | 4-Cl | 3,4-Cl | H | **1.10** | 0.05 |
| **20** | 4-Cl | 3,4-Cl | 4-$CH_3$ | **0.04** | 0.01 |
| MNZ | | | | 1.80 | 0.05 |

*S.D.* standard deviation

[a] The values obtained in at least three separate assays done in triplicate

with the most widely used medication MNZ. MNZ had a 50 % inhibitory concentration ($IC_{50}$) 1.80 μM in our present experiments which has been found similar to our previous studies (Siddiqui *et al*., 2012, 2013b). The results are given in Table 1 and 2. In terms of structure activity relationship (SAR), it was concluded that sulphonamide derivatives (**1–20**) showed better activity than 4,6-disubstituted aminopyrimidines (**1b–10b**). Out of 10 4,6-disubstituted aminopyrimidines (**1b–10b**), 5 compounds: **3b** ($IC_{50}$ = 1.72 μM), **4b** ($IC_{50}$ = 1.54 μM), **7b** ($IC_{50}$ = 1.63 μM), **8b** ($IC_{50}$ = 1.66 μM) and **10b** ($IC_{50}$ = 1.59 μM) were found to exhibit better activity than MNZ ($IC_{50}$ = 1.80 μM). On the other hand, out of 20 sulphonamides derivatives (**1–20**), 12 compounds: **3** ($IC_{50}$ = 0.70 μM), **4** ($IC_{50}$ = 1.35 μM), **5** ($IC_{50}$ = 0.44 μM), **6** ($IC_{50}$ = 0.90 μM), **7** ($IC_{50}$ = 0.73 μM), **8** ($IC_{50}$ = 1.44 μM), **13** ($IC_{50}$ = 1.57 μM), **14** ($IC_{50}$ = 1.02 μM), **15** ($IC_{50}$ = 1.60 μM), **16** ($IC_{50}$ = 1.42 μM), **19** ($IC_{50}$ = 1.10 μM) and **20** ($IC_{50}$ = 0.04 μM) had $IC_{50}$ value less than MNZ ($IC_{50}$ = 1.80 μM), and therefore were considered to be better inhibitors of in vitro growth of HM1:IMSS strain of *E. histolytica*. It has been reported that sulphonamides block the metabolic pathway of malaria parasite by competing with *p*-aminobenzoic acid. Likewise, we might expect that sulphonamide derivatives of 4,6-disubstituted aminopyrimidines block the *Entamoeba* protein functioning by reaction with the sulphahydryl group present in the parasite in high amount. The $IC_{50}$ values of sulphonamide derivatives support our estimation. Although, the mechanism of the biological activity needs further investigations, which are in progress.

## Experimental protocol

Melting points (mp) were recorded by Capillary method and are uncorrected. Elemental analysis was carried out on Heraeus Vario EL III analyzer by Central Drug Research

Instituted, Lucknow, India and the results were within ±0.3 of the theoretical values. IR spectra were recorded on Perkin Elmer model 1620 FT-IR spectrophotometer as KBr discs. The $^{1}$H NMR and $^{13}$C NMR spectra were recorded on Bruker Spectrospin DPX 300 MHz and Bruker Spectrospin DPX 75 MHz spectrometer, respectively, using DMSO-$d_6$ as a solvent and trimethylsilane (TMS) as an internal standard. During interpretation of $^{1}$H and $^{13}$C NMR a few abbreviations has been used which are s (singlet), d (doublet), m (multiplet), Ar (aromatic) and Ph (phenyl). The FAB-MS spectra of all the compounds were recorded on JEOL SX 102/DA-6000 mass spectrometer using Argon/Xenon 6 kV, 10 mA as the FAB gas, and *m*-nitro benzyl alcohol (NBA) was used as the matrix.

General procedure for preparation of 1,3-diaryl-2-propen-1-ones (**1a**–**10a**)

1,3-diaryl-2-propen-1-ones (**1a**–**10a**) were synthesised by means of Claisen–Schmidt condensation (Li *et al*., 1995).

General procedure for preparation of 4,6-disubstituted aminopyrimidines (**1b**–**10b**)

To a solution of guanidine hydrochloride (1.1 equiv.) in 50 mL isopropanol, sodium metal (1.1 equiv.) was added. The reaction mixture was heated under reflux for 2 h, and the different 1,3-diaryl-2-propen-1-ones (1.0 equiv.) were added to it and further heated under reflux for 8 h. The solvent was removed from reaction mixture in vacuo. Water was added and the aqueous phase was extracted with chloroform. The organic phase was dried over anhydrous $Na_2SO_4$, filtered and concentrated. The crude product was purified by crystallization from ethanol or sometimes by column chromatography on silica gel (2 % methanol in chloroform) to furnish the pure compounds.

*4,6-Diphenylpyrimidin-2-amine (**1b**)*

Yield: 80 %; m.p. 82 °C. Anal. Calc. for ($C_{16}H_{13}N_3$): C 77.71, H 5.30, N16.99 %; Found: C 77.72, H 5.32, N 16.97 %; IR: $\nu_{max}$($cm^{-1}$): 3393 ($NH_2$), 1536, 1480 (C=N); $^{1}$H NMR ($CDCl_3$): ($\delta$, ppm): 5.12 (s, 2H, $NH_2$), 7.23 (s, 1H, Ar–H), 7.81 (m,10H, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 163.6 (C=N pyrimidine), 163.2 (C=C pyrimidine) 162.0 (N=C–N pyrimidine), 134.2 (Ph), 133.2 (Ph), 129.1(Ph), 129.1 (Ph), 128.8 (Ph), 127.6, (Ph) 126.9 (Ph), 126.8 (Ph), 96.0 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 248.34 calc. 247.29.

*4-(4-Chlorophenyl)-6-phenylpyrimidine-2-amine (**2b**)*

Yield: 76 %; m.p. 98 °C. Anal. Calc. for ($C_{16}H_{12}ClN_3$): C 68.21, H 4.29, N 14.91 %; Found: C 68.22, H 4.29, N 14.90 %; IR: $\nu_{max}$($cm^{-1}$): 3389 ($NH_2$), 1519, 1461 (C=N); $^{1}$H NMR ($CDCl_3$): ($\delta$, ppm): 5.16 (s, 2H, $NH_2$), 6.98 (s, 1H, Ar–H), 7.80–7.84 (m, 5H, Ar–H), 7.19 (d, 2H, $J$ = 7.1, Ar–H), 7.23 (d, 2H, $J$ = 7.1, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 163.6 (C=N pyrimidine), 163.3 (C=C pyrimidine), 162.1 (N=C–N pyrimidine), 135.1 (Ph), 133.6, (Ph) 131.2 (Ph), 129.3 (Ph), 129.3 (Ph), 128.8 (Ph), 126.5 (Ph), 86.4 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 282.86 calc. 281.74.

*4-(2-Chlorophenyl)-6-phenylpyrimidin-2-amine (**3b**)*

Yield: 78 %; m.p. 61 °C. Anal. Calc. for ($C_{16}H_{12}ClN_3$): C 68.2, H 4.29, N 14.9 %; Found: C 68.1, H 4.39, N 14.7 %; IR: $\nu_{max}$($cm^{-1}$): 3397($NH_2$), 1549, 1446 (C=N); $^{1}$H NMR ($CDCl_3$): ($\delta$, ppm): 5.21 (s, 2H, $NH_2$), 7.46 (s, 1H, Ar–H), 7.90–7.95 (m, 5H, Ar–H), 7.38–7.40 (m, 4H, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 162.2 (C=N pyrimidine), 161.9 (C=C pyrimidine), 161.3 (N=C–N pyrimidine), 132.3 (Ph), 132.3 (Ph), 130.2 (Ph), 130.2 (Ph), 129.2 (Ph), 129.3 (Ph), 128.7 (Ph), 128.7 (Ph), 127.6 (Ph), 127.4 (Ph), 89.2 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 282.81 calc. 281.74.

*4-(2-Chlorophenyl)-6-(4-chlorophenyl) pyrimidin-2-amine (**4b**)*

Yield: 88 %; m.p. 66 °C. Anal. Calc. for ($C_{16}H_{11}Cl_2N_3$): C 60.78, H 3.51, N 13.29 %; Found: C 60.88, H 3.42, N 13.35 %; IR: $\nu_{max}$($cm^{-1}$): 3418 ($NH_2$), 1515, 1436 (C=N); $^{1}$H NMR ($CDCl_3$): ($\delta$, ppm): 5.23 (s, 2H, $NH_2$), 7.47 (s, 1H, Ar–H), 7.21–7.23 (m, 4H, Ar–H), 7.09 (d, 2H, $J$ = 7.2 Hz, Ar–H), 7.12 (d, 2H, $J$ = 7.2 Hz, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 165.1 (C=N pyrimidine), 164.6 (C=C pyrimidine), 163.9 (N=C–N pyrimidine), 133.3 (Ph), 132.3 (Ph), 131.2 (Ph), 130.2 (Ph), 130.0 (Ph), 129.5 (Ph), 129.3 (Ph), 128.5 (Ph), 127.7 (Ph), 127.4 (Ph), 98.3 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 317.43 calc. 316.18.

*4-(3-Chlorophenyl)-6-phenylpyrimidin-2-amine (**5b**)*

Yield: 68 %; m.p. 72 °C. Anal. Calc. for ($C_{16}H_{12}ClN_3$): C 68.2, H 4.29, N 14.9 %; Found: C 68.2, H 4.32, N 14.3 %; IR: $\nu_{max}$($cm^{-1}$): 3394($NH_2$),1525, 1459 (C=N); $^{1}$H NMR ($CDCl_3$): ($\delta$, ppm): 5.28 (s, 2H, $NH_2$), 7.41 (s, 1H, Ar–H), 7.70–7.74 (m, 5H, Ar–H), 7.08 (s, 1H, Ar–H), 7.11 (d, 1H, $J$ = 7.3 Hz, Ar–H), 7.13 (dd, 1H, Ar–H), 7.11 (d, 1H, $J$ = 7.3 Hz, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 164.6 (C=N pyrimidine), 163.9 (C=C pyrimidine), 163.1 (N=C–N pyrimidine), 133.8 (Ph), 133.1 (Ph), 132.1 (Ph), 130.7 (Ph), 129.2 (Ph), 129.1 (Ph), 128.1 (Ph), 127.3 (Ph), 126.9 (Ph), 125.6 (Ph), 93.1 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 282.96 calc. 281.74.

*4-(3-Chlorophenyl)-6-(4-chlorophenyl) pyrimidin-2-amine (**6b**)*

Yield: 88 %; m.p. 80 °C. Anal. Calc. for ($C_{16}H_{11}Cl_2N_3$): C 60.78, H 3.51, N 13.29 %; Found: C 60.97, H 3.32, N 13.39 %; IR: $\nu_{max}(cm^{-1})$: 3310 ($NH_2$), 1535, 1486 (C=N); $^1$H NMR ($CDCl_3$): ($\delta$, ppm): 5.39 (s, 2H, $NH_2$), 7.41 (s, 1H, Ar–H), 7.23 (d, 2H, $J = 7.2$ Ar–H), 7.14 (d, 2H, $J = 7.2$ Hz, Ar–H), 7.12 (s, 1H, Ar–H), 7.11 (d, 1H, $J = 7.1$ Hz, Ar–H), 7.18 (dd, 1H, Ar–H), 7.18 (d, 1H, $J = 7.1$ Hz, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 163.9 (C=N pyrimidine), 162.1 (C=C pyrimidine), 160.4 (N=C–N pyrimidine), 136.1 (Ph), 134.7 (Ph), 134.3 (Ph), 131.3 (Ph), 130.7 (Ph), 129.7 (Ph), 128.7 (Ph), 128.3 (Ph), 127.4 (Ph), 125.6 (Ph), 95.3 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 317.76 calc. 316.18.

*4-(4-Chlorophenyl)-6-phenylpyrimidin-2-amine (**7b**)*

Yield: 53 %; m.p. 98 °C. Anal. Calc. for ($C_{16}H_{12}ClN_3$): C 68.21, H 4.29, N 14.91 %; Found: C 68.35, H 4.36, N 14.91 %; IR: $\nu_{max}(cm^{-1})$: 3328 ($NH_2$), 1511, 1457 (C=N); $^1$H NMR ($CDCl_3$): ($\delta$, ppm): 5.26 (s, 2H, $NH_2$), 7.65 (s, 1H, Ar–H), 7.41–7.45 (m, 5H, Ar–H), 6.93 (d, 2H, $J = 7.4$ Hz, Ar–H), 7.21 (d, 2H, $J = 7.4$ Hz, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 165.2 (C=N pyrimidine), 163.1 (C=C pyrimidine), 162.9 (N=C–N pyrimidine), 134.3 (Ph), 134.1 (Ph), 129.4 (Ph), 128.9 (Ph), 128.8 (Ph), 128.6 (Ph), 127.6 (Ph), 97.3 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 282.77 calc. 281.74.

*4,6-Bis(4-chlorophenyl)pyrimidine-2-amine (**8b**)*

Yield: 65 %; m.p. 102 °C. Anal. Calc. for ($C_{16}H_{11}Cl_2N_3$): C 60.78, H 3.51, N 13.29 %; Found: C 60.67, H 3.49, N 13.46 %; IR: $\nu_{max}(cm^{-1})$: 3409 ($NH_2$), 1533, 1462 (C=N); $^1$H NMR ($CDCl_3$): ($\delta$, ppm): 5.47 (s, 2H, $NH_2$), 7.52 (s, 1H, Ar–H), 7.11 (d, 2H, $J = 7.2$, Ar–H), 7.12 (d, 2H, $J = 7.2$ Hz, Ar–H), 7.21 (d, 2H, $J = 7.4$, Ar–H), 7.26 (d, 2H, $J = 7.4$ Hz, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 163.2 (C=N pyrimidine), 162.9 (C=C pyrimidine), 162.1 (N=C–N pyrimidine), 134.2 (Ph), 133.9 (Ph), 131.2 (Ph), 131.2 (Ph), 129.2 (Ph), 128.9 (Ph), 128.9 (Ph), 128.6 (Ph), 98.5 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 317.75 calc. 316.18.

*4-(3,4-Dichlorophenyl)-6-phenylpyrimidin-2-amine (**9b**)*

Yield: 61 %; m.p. 101 °C. Anal. Calc. for ($C_{16}H_{11}Cl_2N_3$): C 60.78, H 3.51, N 13.29 %; Found: C 60.77, H 3.49, N 13.31 %; IR: $\nu_{max}(cm^{-1})$: 3328 ($NH_2$), 1524, 1443 (C=N); $^1$H NMR ($CDCl_3$): ($\delta$, ppm): 5.22 (s, 2H, $NH_2$), 7.49 (s, 1H, Ar–H), 7.52–7.57 (m, 1H, Ar–H), 7.21 (s, 1H, Ar–H), 7.24 (d, 2H, $J = 7.3$, Ar–H), 7.28 (d, 2H, $J = 7.3$ Hz, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 163.7 (C=N pyrimidine), 163.6 (C=C pyrimidine), 161.1 (N=C–N pyrimidine), 133.9 (Ph), 133.4 (Ph), 132.5 (Ph), 132.3 (Ph), 130.7 (Ph), 129.1 (Ph), 128.7 (Ph), 127.9 (Ph), 127.6 (Ph), 95.0 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 318.72 calc. 316.18.

*4-(3,4-Dichlorophenyl)-6-(4-chlorophenyl)pyrimidin-2-amine (**10b**)*

Yield: 83 %; m.p. 111 °C. Anal. Calc. for ($C_{16}H_{10}Cl_3N_3$): C 54.81, H 2.87, N 11.98 %; Found: C 54.76, H 2.85, N 11.98 %; IR: $\nu_{max}(cm^{-1})$: 3486 ($NH_2$), 1561, 1461 (C=N); $^1$H NMR ($CDCl_3$): ($\delta$, ppm): 5.31 (s, 2H, $NH_2$), 7.53 (s, 1H, Ar–H), 7.32 (d, 1H, $J = 7.2$ Hz, Ar–H), 7.34 (d, 1H, $J = 7.2$, Ar–H), 7.17 (s, 1H, Ar–H), 7.11 (d, 2H, $J = 7.3$ Hz, Ar–H), 7.15 (d, 2H, $J = 7.3$ Hz, Ar–H); $^{13}$C NMR ($CDCl_3$): ($\delta$, ppm): 164.4 (C=N pyrimidine), 163.3 (C=C pyrimidine), 160.4 (N=C–N pyrimidine), 134.3 (Ph), 133.8 (Ph), 133.5 (Ph), 132.5 (Ph), 131.2 (Ph), 130.8 (Ph), 129.3 (Ph), 128.9 (Ph), 128.8 (Ph), 127.4 (Ph), 95.5 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 351.87 calc. 350.63.

General procedure for preparation of sulphonamide derivatives of 4,6-disubstituted aminopyrimidines (**1**–**20**)

1 g of 4,6-disubstituted aminopyrimidines (**1b**–**10b**) was treated with 4 molar equivalents of 10 % sodium hydroxide solution. 1.5 mol of benzenesulphonyl or toluene-*p*-sulphonyl chloride was added in small portions with constant stirring. The mixture was warmed gently to remove the excess of acid chloride and then acidified with dilute hydrochloric acid to precipitate the sulphonamides (**1**–**20**). Solid obtained was recrystallised from ethanol.

*N-(4,6-Diphenylpyrimidine-2-yl)benzenesulphonamide (**1**)*

Yield: 71 %; m.p. 119 °C. Anal.Calc. for ($C_{22}H_{17}N_3O_2S$): C 68.20, H 4.42, N 10.85 %; Found: C 68.34, H 4.48, N 10.85 %; IR : $\nu_{max}(cm^{-1})$: 3249 (NH), 1570 $_{asym}(SO_2)$, 1025 $_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 10.07 (s, 1H, NH), 7.21–7.34 (m, 5H, $SO_2$–Ar–H); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 164.7 (C=N pyrimidine), 162.6 (C=C pyrimidine), 159.2 (N=C–N pyrimidine), 132.1 ($SO_2$–Ar–C), 131.1 ($SO_2$–Ar–C), 129.7 ($SO_2$–Ar–C), 124.6 ($SO_2$–Ar–C), 96.5 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 388.56 calc. 387.45.

*N-(4,6-Diphenylpyrimidin-2-yl)-4-methylbenzenesulphonamide (**2**)*

Yield: 74 %; m.p. 95 °C. Anal.Calc. for ($C_{23}H_{19}N_3O_2S$): C 68.81, H 4.77, N 10.47 %; Found: C 68.84, H 4.874, N 10.52 %; IR : $\nu_{max}(cm^{-1})$: 3613 (NH), 1508 $_{asy}(SO_2)$, 1024 $_{sy}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 10.11 (s, 1H, NH), 7.31

(d, 2H, $J = 7.4$ Hz, $SO_2$–Ar–H), 7.45 (d, 2H, $J = 7.4$ Hz, $SO_2$–Ar–H), 2.3 (s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 169.9 (C=N pyrimidine), 163.4 (C=C pyrimidine), 162.5 (N=C–N pyrimidine), 142.3 ($SO_2$–Ar–C), 131.7 ($SO_2$–Ar–C), 123.7 ($SO_2$–Ar–C), 127.7 ($SO_2$–Ar–C), 86.8 (C–H pyrimidine), 24.3 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 402.56 calc.401.48.

*N-[4-(4-Chlrophenyl)-6-phenylpyrimidine-2-yl]benzenesulphonamide (**3**)*

Yield: 53 %; m.p. 120 °C. Anal.Calc. for ($C_{22}H_{16}ClN_3O_2S$): C 62.63, H 3.82, N 9.96 %; Found: C 62.82, H 3.87, N 9.84 %; IR : $\nu_{max}$($cm^{-1}$): 3350 (NH), 1372 $_{asym}$($SO_2$), 1102 $_{sym}$($SO_2$); $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 11.12 (s, 1H, NH), 7.6-7.7(m, 5H, $SO_2$–Ar–H); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 162.6 (C=N pyrimidine), 161.6 (C=C pyrimidine), 159.2 (N=C–N pyrimidine), 135.9 ($SO_2$–Ar–C), 132.3 ($SO_2$–Ar–C), 127.3 ($SO_2$–Ar–C), 124.8 ($SO_2$–Ar–C), 89.4 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 422.93 calc. 421.89.

*N-[4-(4-Chlrophenyl)-6-phenylpyrimidine-2-yl]-4-methylbenzenesulphonamide (**4**)*

Yield: 58 %; m.p. 94 °C. Anal.Calc. for ($C_{23}H_{18}ClN_3O_2S$): C 63.37, H 4.16, N 9.64 %; Found: C 63.56, H 4.16, N 9.53 %; IR : $\nu_{max}$($cm^{-1}$): 3691 (NH), 1538 $_{asym}$($SO_2$), 1035 $_{sym}$($SO_2$); $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 11.32 (s, 1H, NH), 7.35 (d, 2H, $J = 7.6$ Hz, $SO_2$–Ar–H), 7.39 (d, 2H, $J = 7.6$ Hz, $SO_2$–Ar–H), 2.5(s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 178.1 (C=N pyrimidine), 167.4 (C=C pyrimidine), 164.6 (N=C–N pyrimidine), 143.6 ($SO_2$–Ar–C), 134.7 ($SO_2$–Ar–C), 126.9 ($SO_2$–Ar–C), 123.5 ($SO_2$–Ar–C), 97.8 (C–H pyrimidine), 23.9 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 436.88 calc. 435.92.

*N-[4-(2-Chlorophenyl)-6-phenylpyrimidin-2-yl]-benzenesulphonamide (**5**)*

Yield: 59 %; m.p. 106 °C. Anal.Calc. for ($C_{22}H_{16}ClN_3O_2S$): C 62.63, H 3.82, N 9.96 %; Found: C 62.71, H 3.82, N 9.78 %; IR : $\nu_{max}$($cm^{-1}$): 3524 (NH), 1334 $_{asym}$($SO_2$), 1086 $_{sym}$($SO_2$); $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 10.67 (s, 1H, NH), 7.17-7.34 (m, 5H, $SO_2$–Ar–H); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 166.3 (C=N pyrimidine), 163.3 (C=C pyrimidine), 161.9 (N=C–N pyrimidine), 139.7 ($SO_2$–Ar–C), 131.1 ($SO_2$–Ar–C), 124.3 ($SO_2$–Ar–C), 123.6 ($SO_2$–Ar–C), 93.5 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 422.88 calc. 421.89.

*N-[4-(2-Chlrophenyl)-6-phenylpyrimidine-2-yl]-4-methylbenzenesulphonamide (**6**)*

Yield: 61 %; m.p. 98 °C. Anal.Calc. for ($C_{23}H_{18}ClN_3O_2S$): C 63.37, H 4.16, N 9.64 %; Found: C 63.46, H 4.13, N 9.66 %; IR : $\nu_{max}$($cm^{-1}$): 3601 (NH), 1401 $_{asym}$($SO_2$), 1124 $_{sym}$($SO_2$); $^1$H NMR (DMSO-$d_6$): $\delta$/ppm:12.30 (s, 1H, NH), 6.98 (d, 2H, $J = 7.5$ Hz, $SO_2$–Ar–H), 6.98 (d, 2H, $J = 7.5$ Hz, $SO_2$–Ar–H), 2.9(s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 173.3 (C=N pyrimidine), 162.4 (C=C pyrimidine), 161.7 (N=C–N pyrimidine), 142.1 ($SO_2$–Ar–C), 136.5 ($SO_2$–Ar–C), 126.7 ($SO_2$–Ar–C), 124.1 ($SO_2$–Ar–C), 94.7 (C–H pyrimidine), 25.6 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 437.01 calc. 435.92.

*N-[4-(2-Chlorophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl]-benzenesulphonamide (**7**)*

Yield: 73 %; m.p. 110 °C. Anal.Calc. for ($C_{22}H_{15}Cl_2N_3O_2S$): C 57.90, H 3.31, N 9.21 %; Found: C 57.81, H 3.49, N 9.22 %; IR : $\nu_{max}$($cm^{-1}$): 2928 (NH), 1380 $_{asym}$($SO_2$), 1053 $_{sym}$($SO_2$); $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 10.76 (s, 1H, NH), 7.26–7.37 (m, 5H, $SO_2$–Ar–H); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 176.1 (C=N pyrimidine), 165.3 (C=C pyrimidine), 161.1 (N=C–N pyrimidine), 135.7 ($SO_2$–Ar–C), 132.9 ($SO_2$–Ar–C), 130.8 ($SO_2$–Ar–C), 125.5 ($SO_2$–Ar–C), 97.6 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 457.41 calc.456.34.

*N-[4-(2-Chlrophenyl)-6-(4-chlorophenyl)pyrimidine-2-yl]-4-methylbenzenesulphonamide (**8**)*

Yield: 85 %; m.p. 123 °C. Anal.Calc. for ($C_{23}H_{17}Cl_2N_3O_2S$): C 58.73, H 3.64, N 8.94 %; Found: C 58.86, H 3.64, N 8.75 %; IR : $\nu_{max}$($cm^{-1}$): 3274 (NH), 1560 $_{asym}$($SO_2$), 1048 $_{sym}$($SO_2$); $^1$H NMR (DMSO-$d_6$): $\delta$/ppm:11.01 (s, 1H, NH), 7.04 (d, 2H, $J = 7.6$ Hz, $SO_2$–Ar–H), 7.06 (d, 2H, $J = 7.6$ Hz, $SO_2$–Ar–H), 2.4 (s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 171.7 (C=N pyrimidine), 165.8 (C=C pyrimidine), 162.6 (N=C–N pyrimidine), 141.7 ($SO_2$–Ar–C), 136.4 ($SO_2$–Ar–C), 129.2 ($SO_2$–Ar–C), 127.3 ($SO_2$–Ar–C), 97.1 (C–H pyrimidine), 27.1 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 471.46 calc. 470.37.

*N-[4-(3-Chlorophenyl)-6-phenylpyrimidine-2-yl]benzenesulphonamide (**9**)*

Yield: 55 %; m.p. 79 °C. Anal.Calc. for ($C_{22}H_{16}ClN_3O_2S$): C 62.63, H 3.82, N 9.96 %; Found: C 62.65, H 3.74, N 9.81 %; IR : $\nu_{max}$($cm^{-1}$): 3463 (NH), 1578 $_{asym}$($SO_2$), 1026 $_{sym}$($SO_2$); $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 10.37 (s, 1H, NH), 7.48-7.58 (m, 5H, $SO_2$–Ar–H); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 172.3 (C=N pyrimidine), 164.3 (C=C pyrimidine), 163.1 (N=C–N pyrimidine), 135.9 ($SO_2$–Ar–C), 132.5 ($SO_2$–Ar–C), 130.7 ($SO_2$–Ar–C), 128.4, ($SO_2$–Ar–C), 95.4 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 422.95 calc. 421.89.

*N-[4-(3-Chlrophenyl)-6-phenylpyrimidine-2-yl]-4-methylbenzenesulphonamide (**10**)*

Yield: 80 %; m.p. 78 °C. Anal.Calc. for ($C_{23}H_{18}ClN_3O_2S$): C 63.37, H 4.16, N 9.64 %; Found: C 63.31, H 4.19, N

9.59 %; IR : $\nu_{max}(cm^{-1})$: 2985 (NH), 1469 $_{asym}(SO_2)$, 1017 $_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm:10.47 (s, 1H, NH), 7.21 (d, 2H, $J = 7.4$ Hz, $SO_2$–Ar–H), 7.29 (d, 2H, $J = 7.4$ Hz, $SO_2$–Ar–H), 2.2(s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 171.5 (C=N pyrimidine), 164.7 (C=C pyrimidine), 162.6 (N=C–N pyrimidine), 141.7 ($SO_2$–Ar–C), 131.3 ($SO_2$–Ar–C), 129.4 ($SO_2$–Ar–C), 127.2 ($SO_2$–Ar–C), 94.1 (C–H pyrimidine), 23.01 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 436.99 calc. 435.92.

*N-[4-(2-Chlorophenyl)-6-(4-chlorophenyl)pyrimidin-2-yl]-benzenesulphonamide (**11**)*

Yield: 79 %; m.p. 140 °C. Anal.Calc. for ($C_{22}H_{15}Cl_2N_3O_2S$): C 57.90, H 3.31, N 9.21 %; Found: C 57.76, H 3.44, N 9.34 %IR : $\nu_{max}(cm^{-1})$: 3467 (NH), 1632 $_{asym}(SO_2)$, 1174 $_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 10.93 (s, 1H, NH), 7.32–7.41 (m, 5H, $SO_2$–Ar–H); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 171.1 (C=N pyrimidine), 165.1 (C=C pyrimidine), 163.2 (N=C–N pyrimidine), 132.8 ($SO_2$–Ar–C), 132.4 ($SO_2$–Ar–C), 130.3 ($SO_2$–Ar–C), 126.8 ($SO_2$–Ar–C), 95.4 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 457.45 calc.456.34.

*N-[4-(3-Chlrophenyl)-6-(4-chlorophenyl)pyrimidine-2-yl]-4-methylbenzenesulphonamide (**12**)*

Yield: 74 %; m.p. 110 °C. Anal.Calc. for ($C_{23}H_{17}Cl_2N_3O_2S$): C 58.73, H 3.64, N 8.94 %; Found: C 58.72, H 3.66, N 8.91 %; IR : $\nu_{max}(cm^{-1})$: 3311 (NH), 1548 $_{asym}(SO_2)$, 1087 $_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm:12.05 (s, 1H, NH), 7.49 (d, 2H, $J = 7.3$ Hz, $SO_2$–Ar–H), 7.52 (d, 2H, $J = 7.3$ Hz, $SO_2$–Ar–H), 2.7 (s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 167.5 (C=N pyrimidine), 163.5 (C=C pyrimidine), 161.3 (N=C–N pyrimidine), 141.6 ($SO_2$–Ar–C), 138.3 ($SO_2$–Ar–C), 130.1 ($SO_2$–Ar–C), 128.9 ($SO_2$–Ar–C), 95.3 (C–H pyrimidine), 26.1 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 471.49 calc. 470.37.

*N-[4-(4-Chlorophenyl)-6-phenylpyrimidine-2-yl]benzenesulphonamide (**13**)*

Yield: 87 %; m.p. 136 °C. Anal.Calc. for ($C_{22}H_{16}ClN_3O_2S$): C 62.63, H 3.82, N 9.96 %; Found: C 62.68, H 3.88, N 9.87 %; IR : $\nu_{max}(cm^{-1})$: 3466 (NH), 1611 $_{asym}(SO_2)$, 1152 $_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 11.08 (s, 1H, NH), 7.53-7.76 (m, 5H, $SO_2$–Ar–H); $^{13}$C NMR(DMSO-$d_6$): $\delta$/ppm: 168.9 (C=N pyrimidine), 164.6 (C=C pyrimidine), 161.6 (N=C–N pyrimidine), 133.1 ($SO_2$–Ar–C), 132.6 ($SO_2$–Ar–C), 130.9 ($SO_2$–Ar–C), 125.7 ($SO_2$–Ar–C), 95.3 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 423.01 calc. 421.89.

*N-[4-(4-Chlrophenyl)-6-phenylpyrimidine-2-yl]-4-methylbenzenesulphonamide (**14**)*

Yield: 46 %; m.p. 95 °C. Anal.Calc. for ($C_{23}H_{18}ClN_3O_2S$): C 63.37, H 4.16, N 9.64 %; Found: C 63.45, H 4.16, N 9.79 %; IR : $\nu_{max}(cm^{-1})$: 3561 (NH), 1393 $_{asym}(SO_2)$, 1119 $_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 11.23 (s, 1H, NH), 6.61 (d, 2H, $J = 7.5$ Hz, $SO_2$–Ar–H), 6.79 (d, 2H, $J = 7.5$ Hz, $SO_2$–Ar–H), 2.2(s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 168.6 (C=N pyrimidine), 163.3 (C=C pyrimidine), 161.4 (N=C–N pyrimidine), 142.6 ($SO_2$–Ar–C), 136.1 ($SO_2$–Ar–C), 130.5 ($SO_2$–Ar–C), 127.6 ($SO_2$–Ar–C), 94.8 (C–H pyrimidine), 24.7 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 436.96 calc. 435.92.

*N-[4-(6-Bis(4-chlorophenyl)pyrimidin-2-yl]-benzenesulphonamide (**15**)*

Yield: 67 %; m.p. 120 °C. Anal.Calc. for ($C_{22}H_{15}Cl_2N_3O_2S$): C 57.90; H 3.31; N 9.21 %; Found: C 57.91; H 3.42; N 9.36 %; IR : $\nu_{max}(cm^{-1})$: 3664 (NH), 1358 $_{asym}(SO_2)$, 1011 $_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 10.86 (s, 1H, NH), 7.34-7.45 (m, 5H, $SO_2$–Ar–H); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 170.5 (C=N pyrimidine), 164.7 (C=C pyrimidine), 162.8 (N=C–N pyrimidine), 132.9 ($SO_2$–Ar–C), 131.8 ($SO_2$–Ar–C), 130.1 ($SO_2$–Ar–C), 125.3 ($SO_2$–Ar–C), 95.4 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 457.49 calc. 456.34.

*N-[4,6-Bis(4-chlorophenyl)pyrimidine-2-yl]-4-methylbenzenesulphonamide (**16**)*

Yield: 59 %; m.p. 118 °C. Anal.Calc. for ($C_{23}H_{17}Cl_2N_3O_2S$): C 58.73, H 3.64, N 8.94 %; Found: C 58.83, H 3.48, N 8.97 %; IR : $\nu_{max}(cm^{-1})$: 3527 (NH), 1457 $_{asym}(SO_2)$, 1073 $_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 11.64 (s, 1H, NH), 7.01 (d, 2H, $J = 7.6$ Hz, $SO_2$–Ar–H), 7.09 (d, 2H, $J = 7.6$ Hz, $SO_2$–Ar–H), 2.1(s, 3H, $CH_3$); $^{13}$C NMR(DMSO-$d_6$): $\delta$/ppm: 171.5 (C=N pyrimidine), 164.2 (C=C pyrimidine), 163.6 (N=C–N pyrimidine), 142.01 ($SO_2$–Ar–C), 137.3 ($SO_2$–Ar–C), 130.5 ($SO_2$–Ar–C), 127.1 ($SO_2$–Ar–C), 94.4 (C–H pyrimidine), 27.8 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 471.45 calc. 470.37.

*N-[4-(3,4-Dichlorophenyl)-6-phenylpyrimidin-2-yl]-benzenesulphonamide (**17**)*

Yield: 74 %; m.p. 105 °C. Anal.Calc. for ($C_{22}H_{15}Cl_2N_3O_2S$): C 57.90, H 3.31, N 9.21 %; Found: C 57.84, H 3.46, N 9.42 %; IR : $\nu_{max}(cm^{-1})$: 3609 (NH), 1396 $_{asym}(SO_2)$, 1096 $_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 12.36 (s, 1H, NH), 7.71-7.65 (m, 5H, $SO_2$–Ar–H); $^{13}$C NMR (DMSO-$d_6$):

$\delta$/ppm: 168.4 (C=N pyrimidine), 164.4 (C=C pyrimidine), 162.01 (N=C–N pyrimidine), 133.2 ($SO_2$–Ar–C), 132.3 ($SO_2$–Ar–C), 130.6 ($SO_2$–Ar–C), 129.9 ($SO_2$–Ar–C), 95.3 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 457.41 calc. 456.34.

*N-[4-(3,4-Dichlorophenyl)-6-phenylpyrimidine-2-yl]-4-methylbenzenesulphonamide (**18**)*

Yield: 87 %; m.p. 87 °C. Anal.Calc. for ($C_{23}H_{17}Cl_2N_3O_2S$): C 58.73, H 3.64, N 8.94 %; Found: C 58.77; H 3.68; N 8.94 %; IR : $\nu_{max}(cm^{-1})$: 3124 (NH), $1583_{asym}(SO_2)$, $1145_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm:12.15 (s, 1H, NH), 6.88 (d, 2H, $J$ = 7.7 Hz, $SO_2$–Ar–H), 6.91 (d, 2H, $J$ = 7.7 Hz, $SO_2$–Ar–H), 2.7(s, 3H, $CH_3$); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 168.4 (C=N pyrimidine), 163.6 (C=C pyrimidine), 161.2 (N=C–N pyrimidine), 143.8 ($SO_2$–Ar–C), 139.2 ($SO_2$–Ar–C), 130.2 ($SO_2$–Ar–C), 125.8 ($SO_2$–Ar–C), 95.6 (C–H pyrimidine), 26.8 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 471.41 calc. 470.37.

*N-[4-(4-Chlorophenyl)-6-(3,4-dichlorophenyl)pyrimidin-2-yl]benzenesulphonamide (**19**)*

Yield: 48 %; m.p. 112 °C. Anal.Calc. for ($C_{22}H_{14}Cl_3N_3O_2S$): C 53.84, H 2.88, N 8.56 %; Found: C 53.93, H 2.89, N 8.56 %; IR : $\nu_{max}(cm^{-1})$: 3316 (NH), $1516_{asym}(SO_2)$, $1178_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm: 10.81 (s, 1H, NH), 7.61-7.74 (m, 5H, $SO_2$–Ar–H); $^{13}$C NMR (DMSO-$d_6$): $\delta$/ppm: 169.5 (C=N pyrimidine), 163.7 (C=C pyrimidine), 162.5 (N=C–N pyrimidine), 135.8 ($SO_2$–Ar–C), 132.1 ($SO_2$–Ar–C), 131.1 ($SO_2$–Ar–C), 129.8 ($SO_2$–Ar–C), 95.6 (C–H pyrimidine); FAB-MS: *m/z* ($M^+$+1) 491.83 calc. 490.78.

*N-[4-(4-Chlorophenyl)-6-(3,4-dichlorophenyl)pyrimidin-2-yl]-4-methylbenzenesulphonamide (**20**)*

Yield: 71 %; m.p. 107 °C. Anal.Calc. for ($C_{23}H_{16}Cl_3N_3O_2S$): C 54.72, H 3.19, N 8.32 %; Found: C 54.85, H 3.14, N 8.34 %; IR : $\nu_{max}(cm^{-1})$: 3568 (NH), $1619_{asym}(SO_2)$, $1024_{sym}(SO_2)$; $^1$H NMR (DMSO-$d_6$): $\delta$/ppm:11.45 (s, 1H, NH), 7.24 (d, 2H, $J$ = 7.3 Hz, $SO_2$–Ar–H), 7.29 (d, 2H, $J$ = 7.3 Hz, $SO_2$–Ar–H), 2.9 (s, 3H, $CH_3$); $^{13}$C NMR(DMSO-$d_6$): $\delta$/ppm: 173.6 (C=N pyrimidine), 163.2 (C=C pyrimidine), 161.7 (N=C–N pyrimidine), 143.8 ($SO_2$–Ar–C), 139.2 ($SO_2$–Ar–C), 130.2 ($SO_2$–Ar–C), 125.8 ($SO_2$–Ar–C), 95.2 (C–H pyrimidine), 26.8 ($CH_3$); FAB-MS: *m/z* ($M^+$+1) 503.93 calc. 504.81.

## In vitro anti-amoebic assay

All the desired compounds were screened for in vitro anti-amoebic activity against HM1:IMSS strain of *E. histolytica* by microdilution method (Wright *et al*., 1988). *E. histolytica* trophozoites were cultured in culture tubes by using Diamond TYIS-33 growth medium. The test compounds (1 mg) were dissolved in DMSO (40 µL, level at which no inhibition of amoeba occurs) (Gillin *et al*., 1982). The stock solutions of the compounds were prepared freshly before the use at a concentration of 1 mg/mL. Two-fold serial dilutions were made in the wells of 96-well microtiter plate (costar). Each test includes MNZ as a standard amoebicidal drug, control wells (culture medium plus amoebae) and a blank (culture medium only). All the experiments were carried out in triplicate at each concentration level and repeated thrice. The amoeba suspension was prepared from a confluent culture by pouring off the medium at 37 °C and adding 5 mL of fresh medium, chilling the culture tube on ice to detach the organisms from the side of flask. The number of amoeba/mL was estimated with the help of a haemocytometer, using trypan blue exclusion to confirm the viability. The suspension was diluted to $10^5$ organism/mL by adding fresh medium and 170 µL of this suspension was added to the test and control wells in the plate, so that the wells were completely filled (total volume, 340 mL). An inoculum of $1.7 \times 10^4$ organisms/well was chosen so that confluent, but not excessive growth, took place in control wells. Plate was sealed and gassed for 10 min with nitrogen before the incubation at 37 °C for 72 h. After the incubation, the growth of amoeba in the plate was checked with a low-power microscope. The culture medium was removed by inverting the plate and shaking gently. Plate was then immediately washed with sodium chloride solution (0.9 %) at 37 °C. This procedure was completed quickly and the plate was not allowed to cool to prevent the detachment of amoeba. The plate was allowed to dry at room temperature and the amoebae were fixed with methanol, and when dried, stained with (0.5 %) aqueous eosin for 15 min. The stained plate was washed once with tap water, then twice with distilled water and then allowed to dry. A 200 µL portion of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader. The % inhibition of amoebal growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best fitting line from which the $IC_{50}$ value was found. The $IC_{50}$ values are reported in Table 1 and 2.

## Conclusion

In the present study, we synthesized 4,6-disubstituted aminopyrimidines (**1b**–**10b**) and their sulphonamide derivatives (**1**–**20**) to evaluate their in vitro anti-amoebic activity

against HM1:IMSS strain of *E. histolytica* by microdilution method. Preliminary results indicates that sulphonamide derivatives (**1**–**20**) showed better activity than 4,6-disubstituted aminopyrimidines (**1b**–**10b**). Out of 10 4,6-disubstituted aminopyrimidines (**1b**–**10b**), 5 had better anti-amoebic activity than the reference drug MNZ ($IC_{50} = 1.80$ μM); while out of 20 sulphonamides derivatives (**1**–**20**), 12 compounds were better inhibitors of the growth of *E. histolytica* than MNZ. In conclusion, the compounds having $IC_{50}$ lesser than MNZ may be subjected to further investigations for the development of novel effective anti-amoebic agents.

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