Synthesis, Characterization, and Anti-Amoebic Activity of *N*-(Pyrimidin-2-yl)benzenesulfonamide Derivatives

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A new series of *N*-(pyrimidin-2-yl)benzenesulfonamide derivatives, **3a**–**3i** and **4a**–**4i**, was synthesized from pyrimidin-2-amines, **2a**–**2i**, with the aim to explore their effects on *in vitro* growth of *Entamoeba histolytica*. The chemical structures of the compounds were elucidated by elemental analysis, FT-IR, ^{1}H- and ^{13}C-NMR, and ESI mass-spectral data. *In vitro* anti-amoebic activity was evaluated against *HM1 : IMSS* strain of *Entamoeba histolytica*. The IC_{50} values were calculated by using the double dilution method. The results were compared with the IC_{50} value of the standard drug 'metronidazole'. The selected compounds were tested for their cytotoxic activities by cell-viability assay using H9C2 cardiac myoblasts cell line, and the results indicated that all the compounds displayed remarkable >80% viabilities to a concentration of 100 μg/ml.

1. Introduction. – Amoebiasis, a disease caused by protozoan parasite *Entamoeba histolytica* [1], is the third leading cause of death worldwide, surpassed by malaria and schistosomiasis only [2][3]. Occasionally, *E. histolytica* trophozoites penetrate the intestinal mucosa, causing amoebic colitis and spread *via* portal circulation to other organs. Amoebic liver abscess is the most common extraintestinal manifestation of invasive amoebiasis [4]. Amoebiasis can be efficiently treated by nitroimidazole derivatives (metronidazole, tinidazole, and ornidazole), but recent studies revealed that several toxic effects such as genotoxicity, gastric mucus irritation, spermatozoid damage *etc*. are associated with their use [5–7]. In addition to the toxic nature, several intestinal protozoan parasites show resistance to these medicines [8][9].

$SO_2N<$ Moiety has been incorporated in many chemotherapeutically important sulfa drugs [10], which have been reported to exhibit antibacterial activities [11][12], HIV protease inhibitors [13], carbonic anhydrase inhibitors [14–16], antiepileptic agents [17], anticonvulsant agents [18][19], anti-amoebic agents [20] and are used as endothelin receptor ETA, being a selective antagonist [21][22]. Pyrimidine ring is present in many biologically significant molecules such as alloxane, barbitone, nucleic acids, such as uracil, thymine, and cytosine, and vitamins, such as thiamine, riboflavin, and folic acids [23–26]. During the last decade, several pyrimidine derivatives have

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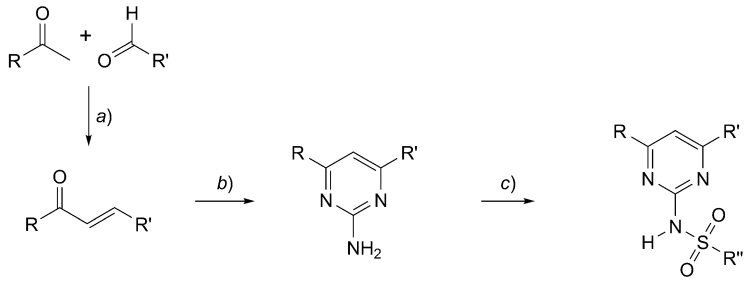
been developed as chemotherapeutic agents, which have been found wide clinical applications [27]. A large number of pyrimidine-based molecules are known for their antimetabolite properties, and are used as antineoplastic and anticancer agents [28]. There are many reports of compounds with pyrimidine moiety which act as antifolates, antibacterials, antiprotozoals, antitubercular, antihistaminic, and analgesics [29–33]. Pyrimidine derivatives of sulfa drugs such as sulfadimidine and sulfamerazine are used in acute urinary tract infections (UTI) and cerebrospinal meningitis [27]. Sulfonamide–trimethoprim combinations are used extensively for opportunistic infections in patients with AIDS [34]. These observations prompted the incorporation of a sulfonamide moiety into the pyrimidine ring to make use of both functionalities to enhance pharmacological activities. In this work, a new series of *N*-(pyrimidine-2-yl)benzenesulfonamide, **3a**–**3i** and **4a**–**4i**, was synthesized and screened *in vitro* for their ability to inhibit the growth of *E. histolytica*. The selected compounds were tested for toxicity profile using H9C2 cardiac myoblasts cell line. This study is an additional effort to develop new chemotherapeutic agents which are anti-amoebic and non-toxic to human cells.

2. Results and Discussion. – 2.1. *Chemistry. N*-(4,6-Substituted pyrimidin-2-yl)sulfonamide derivatives **3a**–**3i** and **4a**–**4i** were synthesized by a multi-step reaction process starting from simple chemical entities. *Claisen–Schmidt* condensation reaction furnish **1a**–**1i** from substituted acetophenone and benzaldehydes/chlorobenzaldehydes in high yields [35]. The cyclization of **1a**–**1i** in the presence of guanidine hydrochloride and isopropoxide, obtained *in situ* by adding Na metal in i-PrOH, yielded 4,6-disubstituted phenylpyrimidin-2-amines **2a**–**2i**, respectively. Sulfonamide derivatives **3a**–**3i** and **4a**–**4i** (*Scheme*) were synthesized by condensation of $PhSO_2Cl$ and TsCl with corresponding pyrimidine derivatives **2a**–**2i**, respectively. The compounds **2a**–**2i**, **3a**–**3i**, and **4a**–**4i** were obtained in good yields and were found stable in solid states. Spectral data such as FT-IR, ^{1}H- and ^{13}C-NMR, and ESI-MS, and elemental analyses were in agreement with the proposed structures of the compounds. The purities were established by sharp melting points and the elemental analysis. Selected diagnostic bands of the FT-IR spectra of **2a**–**2i**, **3a**–**3i**, and **4a**–**4i** provided further support for the formation of the expected compounds. In addition to common bands which arise due to ν(C=C) of aromatic region and benzene, bands which provide a primary information about the conversion of reactants were present in FT-IR spectra. The compounds **2a**–**2i** showed stronger peaks in the regions near 1561–1511 and 1486–1446 cm^{-1}, due to ν(C=N) vibration in aromatic system of the pyrimidine ring. A strong band in the range of 3486–3310 cm^{-1}, attributed to NH_2, rendered support to cyclization reaction. For sulfonamide compounds, **3a**–**3i** and **4a**–**4i**, two bands in the range of 1334–1632 and 1017–1178 cm^{-1} were observed which were ascribed to $\nu_{asym}(SO_2)$ and $\nu_{sym}(SO_2)$ stretching vibrations, respectively. Besides, the shift of the band frequency to 3368–3209 cm^{-1} is attributed to ν(N–H), since the NH_2 functional group indicated a conversion reaction.

The structures of the compounds were further elucidated by ^{1}H-NMR spectroscopy. For **2a**–**2i**, a *singlet* between 5.47 and 5.12 ppm appeared due to the presence of NH_2 group. In **3a**–**3i** and **4a**–**4i**, a *singlet* between 12.30 and 10.19 ppm for NH H-atom suggested the conversion of $NH_2 \rightarrow NH$. The *J* values of some *doublet*s which arise due

Scheme. *Synthesis of Sulfonamide Derivatives* **3a**–**3i** *and* **4a**–**4i**



	R	R'
1a	Ph	Ph
1b	4-Me–C_6H_4	Ph
1c	Ph	2-Cl–C_6H_4
1d	Ph	4-Cl–C_6H_4
1e	4-Me–C_6H_4	4-Cl–C_6H_4
1f	4-Me–C_6H_4	2-Cl–C_6H_4
1g	4-NO_2–C_6H_4	4-Cl–C_6H_4
1h	4-NO_2–C_6H_4	Ph
1i	4-NO_2–C_6H_4	2-Cl–C_6H_4

	R	R'
2a	Ph	Ph
2b	4-Me–C_6H_4	Ph
2c	Ph	2-Cl–C_6H_4
2d	Ph	4-Cl–C_6H_4
2e	4-Me–C_6H_4	4-Cl–C_6H_4
2f	4-Me–C_6H_4	2-Cl–C_6H_4
2g	4-NO_2–C_6H_4	4-Cl–C_6H_4
2h	4-NO_2–C_6H_4	Ph
2i	4-NO_2–C_6H_4	2-Cl–C_6H_4

	R	R'	R''
3a	Ph	Ph	Ph
3b	4-Me–C_6H_4	Ph	Ph
3c	Ph	2-Cl–C_6H_4	Ph
3d	Ph	4-Cl–C_6H_4	Ph
3e	4-Me–C_6H_4	4-Cl–C_6H_4	Ph
3f	4-Me–C_6H_4	2-Cl–C_6H_4	Ph
3g	4-NO_2–C_6H_4	4-Cl–C_6H_4	Ph
3h	4-NO_2–C_6H_4	Ph	Ph
3i	4-NO_2–C_6H_4	2-Cl–C_6H_4	Ph
4a	Ph	Ph	4-Me–C_6H_4
4b	4-Me–C_6H_4	Ph	4-Me–C_6H_4
4c	Ph	2-Cl–C_6H_4	4-Me–C_6H_4
4d	Ph	4-Cl–C_6H_4	4-Me–C_6H_4
4e	4-Me–C_6H_4	4-Cl–C_6H_4	4-Me–C_6H_4
4f	4-Me–C_6H_4	2-Cl–C_6H_4	4-Me–C_6H_4
4g	4-NO_2–C_6H_4	4-Cl–C_6H_4	4-Me–C_6H_4
4h	4-NO_2–C_6H_4	Ph	4-Me–C_6H_4
4i	4-NO_2–C_6H_4	2-Cl–C_6H_4	4-Me–C_6H_4

a) MeOH, NaOH. *b*) Guanidine hydrochloride, ^{i}PrOH, reflux, 8 h. *c*) NaOH soln., $PhSO_2Cl$ or TsCl.

to spin-spin coupling in *para*-substituted Ph rings were informative about the formation of the compounds. In the ^{13}C-NMR spectra, the C=N and C=C C-atoms of pyrimidine ring in **2a**–**2i** resonated in the range of 163.1–159.2 and 105–98.0 ppm, respectively, indicating the presence of the pyrimidine ring. In addition to these, the signals attributed to aromatic and aliphatic C-atoms were also in favor of **2a**–**2i**. In compounds **3a**–**3i** and **4a**–**4i**, a shift in the C=N resonance were found, which can be considered as caused by the induction of electron-withdrawing sulfonyl group. The C=N signals resonated in the range of 176.6–161.6 ppm. Besides, all these compounds were subjected to ESI-MS analysis for further characterization. In the mass spectra of **2a**–**2i**, **3a**–**3i**, and **4a**–**4i**, the characteristic peaks were observed as molecular ion ($[M+1]^+$) peaks that confirmed the molecular weight of compounds.

2.2. *Pharmacology.* 2.2.1. *Anti-Amoebic Activity.* To combine the properties of two different classes of biologically active compounds, we have synthesized a series of pyrimidine sulfa analogs, **3a**–**3i** and **4a**–**4i** and compared their activities with those of the corresponding precursors **2a**–**2i**. All the compounds were screened *in vitro* for their anti-amoebic activities against *HM1:IMSS* strain of *E. histolytica* cultured in TYIS-33

Table. In vitro *Anti-Amoebic Activities of Pyrimidine Derivatives* **2a**–**2i**, N-*(Pyrimidin-2-yl)benzene-sulfonamide Derivatives* **3a**–**3i** and **4a**–**4i** *against HM1:IMSS Strain of* E. histolytica, *and Cytotoxicity Profile of* N-*(Pyrimidin-2-yl)benzenesulfonamide which Showed 2–13-Fold Enhancement of* in vitro *Anti-Amoebic Activity*

Compound	Antiamoebic Activity		Toxicity IC_{50} [μM]	Safety Index (*SI*)*
	IC_{50} [μM]	S.D.		
2a	6.15	0.06	–	–
2b	2.5	0.01	–	–
2c	1.72	0.05	–	–
2d	4.47	0.03	–	–
2e	7.21	0.06	–	–
2f	2.63	0.05	–	–
2g	1.66	0.03	–	–
2h	5.74	0.06	–	–
2i	1.59	0.03	–	–
3a	2.34	0.04	–	–
3b	3.04	0.02	–	–
3c	0.44	0.05	428	974
3d	0.7	0.01	357	510
3e	5.32	0.01	–	–
3f	1.57	0.06	–	–
3g	0.82	0.01	424	518
3h	3.62	0.04	–	–
3i	0.13	0.05	330	2543
4a	1.95	0.05	–	–
4b	2.68	0.04	–	–
4c	0.9	0.01	446	496
4d	1.35	1.06	–	–
4e	2.7	0.06	–	–
4f	1.86	0.07	–	–
4g	0.93	0.04	343	368
4h	4.01	0.06	–	–
4i	0.39	0.01	373	958
MNZ[a])	1.8	0.06	555	308

[a]) Used as standard.

growth medium. The results are compiled in the *Table*. Sulfonamide derivatives showed higher activities than their corresponding pyrimidine precursors. In the pyrimidine series, compounds **2c** (1.72 μM), **2g** (1.66 μM), and **2i** (1.59 μM) exhibited IC_{50} values closer to that of the metronidazole (1.8 μM). However, the anti-amoebic activity was enhanced by the insertion of sulfonamide group. In the sulfonamide series, the compounds **3i** (0.13 μM), **4i** (0.39 μM), and **3c** (0.45 μM) were found 3–13-fold more active. Compounds **3d** (0.7 μM), **3g** (0.82 μM), **4c** (0.9 μM), and **4g** (0.93 μM) displayed a 1.9–2.5-fold difference, while compounds **3f** (1.57 μM), **4d** (1.35 μM), and **4f** (1.86 μM) had IC_{50} values approximately similar to that of the metronidazole. The differences in IC_{50} values between pyrimidine-sulfonamides and their pyrimidine precursors indicate that SO_2NH moiety enhanced the activity. The compound **3i** showed an excellent

activity in terms of its IC_{50} value and is the most active compound in the series. The results were statistically evaluated by variance analyses. The null hypothesis was tested using *t* test, and the significance of the differences between the IC_{50} value(s) of metronidazole *vs.* **3c**, **3d**, **3f**, **3g**, **3i**, **4c**, **4d**, **4f**, **4g**, and **4i** were evaluated. The calculated *t* values were higher than the table values at the 4% level. Hence, the character under study was significantly influenced by the treatment [36].

2.2.2. *Cell-Viability Assay.* Compounds **3c**, **3d**, **3g**, **3i**, **4c**, **4g**, and **4i** with IC_{50} value less or near to that of metronidazole were screened to evaluate the toxic effects on H9C2 cardiac myoblasts. The results were compared with a negative control DMSO and a positive control (metronidazole). A subconfluent population of cardiac myoblast cells was treated with increasing concentrations (1.57–200 μM) of test compounds and positive control, and the number of viable cells was determined after 48 h by MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) cell viability test. For all compounds, *ca*. 80% viable cells were found at 100 μM concentration (*Fig.*). To investigate the selectivity of the compounds, the safety index (*SI*) was calculated and defined as toxicity IC_{50}/protozoal IC_{50}, where toxicity IC_{50} is defined as the concentration of compound that kills 50% of the human (kidney epithelial) cell line, and protozoal IC_{50} is the concentration that kills 50% of amoeba protozoa. This allows us to estimate which compound might be potentially efincacious or toxic against human cells *in vivo*. The numerical results for each compound are collected in the *Table*. An ELISA plate reader (*Labsystems Multiskan RC*, FI-Helsinki) at 570 nm with a reference wavelength of 655 nm was used.

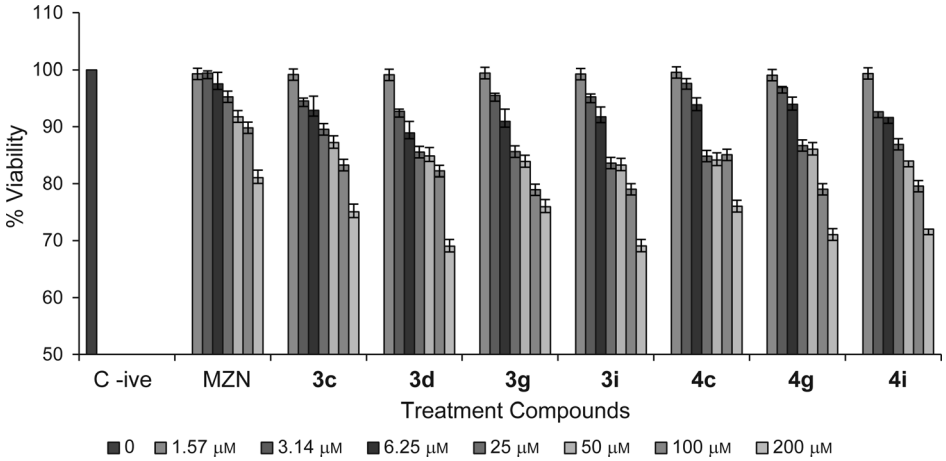


Figure. *Effect of different concentrations of substituted benzene sulfonamide on percent viability of H9C2 cardiac myoblast cell line*

2.2.3. *Structure–Activity Relationships.* Sulfa drugs are synthetic antimicrobial agents with a wide spectrum of microorganisms, and pyrimidine molecules possess a defined place in the world of medicine. The metronidazole is a well-known drug for amoebiasis. The mechanism follows the reduction of NO_2 group by pyruvate : ferredoxin oxidoreductase. Reduced intermediate particle interacts with intracellular

targets. Cytotoxic intermediate particles interact with host cell DNA, resulting in cleavage of the DNA strand and fatal destabilization of the DNA helix [37][38]. In this study, a new series of *N*-(4,6-disubstituted pyrimidin-2-yl)benzenesulfonamide derivatives, **3a**–**3i** and **4a**–**4i**, were compared *in vitro*. Compound **3i** and **4i** were found 4–13 times better than metronidazole. In both compounds, the core pyrimidine ring is substituted with 4-nitrophenyl and 2-chlorophenyl rings. It can be assumed that the NO_2 group is mainly responsible for the activity. However, further studies are needed to disclose the mechanism of action. The compound **4c** which is substituted with Ph and 2-chlorophenyl groups also showed three times higher activity than metronidazole, whereas the compounds with tolyl groups were found least active in the series, indicating that the electron-donating and electron-withdrawing nature of the substituents plays an important role. However, this study is not sufficient to reach a conclusion.

3. Conclusions. – A novel library of *N*-(pyrimidin-2-yl)benzenesulfonamides was obtained, with few reaction steps and low-molecular-weight starting materials, to achieve chemicals that can be used as drug to target the protozoa that cause amoebic infection. Considering the individual drug activities of sulfonamides and pyrimidin-2-amine, structure modifications were performed to obtain products that possess both a pyrimidine moiety and a benzene sulfonamide. All the pyrimidines, *i.e.*, **2a**–**2i**, and benzene sulfonamides, *i.e.*, **3a**–**3i** and **4a**–**4i**, were screened for their anti-amoebic activities against HM1:IMSS strain of *E. histolytica.* Several compounds showed excellent activities higher than that of metronidazole in terms of IC_{50} values. Among them, **3i** and **4i** were the most potent with better inhibitory effects and higher *SI* values.

Experimental Part

General. All chemicals were purchased from *Sigma–Aldrich Chemical Company* (USA) and were used as such. Reactions were monitored on *Merck* pre-coated aluminum plate silica gel *60* F_{254} TLC plates. M.p.: *KSW* melting-point apparatus; uncorrected. IR Spectra: KBr disks; *Perkin Elmer* model *1620 FT-IR* spectrophotometer. ^{1}H- and ^{13}C-NMR spectra: at r.t.; *Brucker Spectroscopic DPX-300* MHz spectrometer in DMSO with TMS as an internal standard; chemical-shift values in ppm. ESI-MS: *Micromass Quattro II* triple quadrupole mass spectrometer. Elemental analysis (C, H, N): *Heraeus Vario EL III* analyzer by *Central Drug Research Instituted*, Lucknow, India; results within ±0.3 of the theoretical values.

1. *Synthesis of Chalcones.* Chalcones **1a**–**1i** were prepared as described in [35].

2. *General Procedure for the Synthesis of Pyrimidine Derivatives* **2a**–**2i**. To a soln. of guanidine hydrochloride (1.1 equiv.) in 50 ml of ^{i}PrOH, Na metal (1.1 equiv.) was added. The mixture was refluxed for 2 h, and the appropriate chalcone (1.0 equiv.) was added, and the mixture was further refluxed for 8 h. The solvent was removed under reduced pressure. H_2O was added, and the aq. phase was extracted with $CHCl_3$. Org. phases were dried (Na_2SO_4), filtered, and concentrated. The crude product was purified by crystallization from MeOH and EtOH, or sometimes by CC (SiO_2; 2% MeOH in $CHCl_3$) to afford the pure compounds.

4,6-Diphenylpyrimidin-2-amine (**2a**). Yield: 80%. M.p. 82°. IR: 745, 729 (benzene), 1536, 1480 (C=N), 3393 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.36–7.21 (*m*, 10 arom. H); 7.09 (*s*, 1 arom. H); 5.12 (*s*, NH_2). ^{13}C-NMR ($CDCl_3$): 163.6, 163.2 (C=N); 162 (N–C=C); 133.2, 131.2, 129.5, 129.1, 128.8, 127.6, 126.9, 126.8, 103.2 (arom. C). ESI-MS: 248.34 ($[M+1]^+$). Anal. calc. for $C_{16}H_{13}N_3$ (247.29): C 77.71, H 5.30, N 16.99; found: C 77.72, H 5.32, N 16.97.

4-(4-Methylphenyl)-6-phenylpyrimidin-2-amine (**2b**). Yield: 76%. M.p. 98°. IR: 713, 742 (benzene), 1519, 1461 (C=N), 2933 (Me), 3389 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.62–7.78 (*m*, 7 arom. H); 7.52 (*d*, *J* = 7.1, 2 arom. H); 6.98 (*s*, 1 arom. H); 5.16 (*s*, NH_2); 2.13 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 163.6, 162.1 (C=N); 160.4 (N–C=C); 135.1, 129.3, 131.2, 133.6, 126.5, 129.3, 128.8, 101.3 (arom. C); 23.7 (alk. C). ESI-MS: 262.76 ($[M+1]^+$). Anal. calc. for $C_{17}H_{15}N_3$ (261.32): C 78.13, H 5.79, N 16.08; found: C 78.36, H 5.68, N 16.39.

4-(2-Chlorophenyl)-6-phenylpyrimidin-2-amine (**2c**). Yield: 78%. M.p. 61°. IR: 762, 717 (benzene), 1549, 1446 (C=N), 3397 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.43–7.35 (*m*, 5 arom. H); 7.27–7.19 (*m*, 4 arom. H); 6.96 (*s*, 1 arom. H); 5.21 (*s*, NH_2). ^{13}C-NMR ($CDCl_3$): 162.2, 161.9 (C=N); 161.3 (N–C=C); 132.3, 130.2, 129.3, 129.2, 128.7, 128.4, 127.6, 127.4, 104.3 (arom. C). ESI-MS: 282.81 ($[M+1]^+$). Anal. calc. for $C_{16}H_{12}ClN_3$ (281.74): C 68.21, H 4.29, N 14.91; found: C 68.1, H 4.39, N 14.7.

4-(4-Chlorophenyl)-6-phenylpyrimidin-2-amine (**2d**). Yield: 68%. M.p. 72°. IR: 789, 721 (benzene), 1525, 1459 (C=N), 3394 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.31 (*d*, *J* = 7.3, 2 arom. H); 7.28–7.19 (*m*, 5 arom. H); 7.14 (*d*, *J* = 7.3, 2 arom. H); 7.11 (*s*, 1 arom. H); 5.28 (*s*, NH_2). ^{13}C-NMR ($CDCl_3$): 164.6, 163.9 (C=N); 163.1 (N–C=C); 133.1, 132.1, 130.7, 129.2, 128.1, 127.3, 126.9, 125.6, 102.6 (arom. C). ESI-MS: 282.96 ($[M+1]^+$). Anal. calc. for $C_{16}H_{12}ClN_3$ (281.74): C 68.21, H 4.29, N 14.91; found: C 68.2, H 4.32, N 14.3.

4-(4-Chlorophenyl)-6-(4-methylphenyl)pyrimidin-2-amine (**2e**). Yield: 88%. M.p. 80°. IR: 781, 739 (benzene), 1535, 1486 (C=N), 2963 (Me), 3310 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.23 (*d*, *J* = 7.2, 2 arom. H); 7.18 (*d*, *J* = 7.4, 1 arom. H); 7.18 (*d*, *J* = 7.1, 1 arom. H); 7.14 (*d*, *J* = 7.2, 2 arom. H); 7.11 (*d*, *J* = 7.1, 2 arom. H); 6.89 (*s*, 1 arom. H); 5.39 (*s*, NH_2); 2.15 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 162.9, 160.4 (C=N); 161.1 (N–C=C); 136.1, 134.7, 134.3, 131.3, 130.7, 128.3, 129.7, 128.7, 127.4, 125.6, 103.1 (arom. C); 24.1 (alk. C). ESI-MS: 318.57 ($[M+Na]^+$). Anal. calc. for $C_{17}H_{14}ClN_3$ (295.77): C 69.03, H 4.77, N 14.21; found: C 68.97, H 4.88, N 14.39.

4-(2-Chlorophenyl)-6-(4-methylphenyl)pyrimidin-2-amine (**2f**). Yield: 53%. M.p. 98°. IR: 709, 827 (benzene), 1511, 1457 (C=N), 2911 (Me), 3328 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.42–7.32 (*m*, 4 arom. H); 7.21 (*d*, *J* = 7.4, 2 arom. H); 7.04 (*d*, *J* = 7.4, 2 arom. H); 6.93 (*s*, 1 arom. H); 5.26 (*s*, NH_2); 2.09 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 163.1, 162.9 (C=N); 159.2 (N–C=C); 134.3, 134.1, 129.4, 128.9, 128.8, 128.6, 127.6, 105.2 (arom. C); 23.7 (alk. C). ESI-MS: 286.73 ($[M+1]^+$). Anal. calc. for $C_{17}H_{14}ClN_3$ (295.77): C 69.03, H 4.77, N 14.21; found: C 68.35, H 4.36, N 14.91.

4-(4-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine (**2g**). Yield: 65%. M.p. 102°. IR: 827, 819 (benzene), 1240 (NO_2(sym.)), 1533, 1462 (C=N), 1610 (NO_2(asym.)), 3409 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.26 (*d*, *J* = 7.4, 2 arom. H); 7.21 (*d*, *J* = 7.4, 2 arom. H); 7.15 (*d*, *J* = 7.2, 2 arom. H); 7.11 (*d*, *J* = 7.2, 2 arom. H); 6.91 (*s*, 1 arom. H); 5.47 (*s*, NH_2). ^{13}C-NMR ($CDCl_3$): 163.2, 162.9 (C=N); 162.1 (N–C=C); 134.2, 133.9, 131.2, 131.2, 129.4, 129.2, 128.9, 128.6, 103.5 (arom. C). ESI-MS: 327.75 ($[M+1]^+$). Anal. calc. for $C_{16}H_{11}ClN_4O_2$ (326.74): C 58.82, H 3.39, N 17.15; found: C 58.76, H 3.49, N 17.31.

4-(4-Nitrophenyl)-6-phenylpyrimidin-2-amine (**2h**). Yield: 61%. M.p. 101°. IR: 817, 743 (benzene), 1245 (NO_2(sym.)), 1524, 1443 (C=N), 1621, (NO_2(asym.)), 3328 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.93 (*d*, *J* = 7.3, 2 arom. H); 7.34–7.21 (*m*, 5 arom. H); 7.28 (*d*, *J* = 7.3, 2 arom. H); 7.09 (*s*, 1 arom. H); 5.22 (*s*, NH_2). ^{13}C-NMR ($CDCl_3$): 163.7, 163.6 (N–C=C); 161.1 (C=N); 133.9, 133.4, 132.5, 132.3, 130.7, 129.1, 128.7, 127.9, 127.6, 98.0 (arom. C). ESI-MS: 293.41 ($[M+1]^+$). Anal. calc. for $C_{16}H_{12}N_4O_2$ (292.29): C 65.75, H 4.14, N 19.17; found: C 65.77, H 4.29, N 19.31.

4-(2-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-amine (**2i**). Yield: 83%. M.p. 111°. IR: 864, 887 (benzene), 1235 (NO_2(sym.)), 1561, 1461 (C=N), 1641 (NO_2(asym.)), 3486 (NH_2). ^{1}H-NMR ($CDCl_3$): 7.92 (*d*, *J* = 7.3, 2 arom. H); 7.56 (*d*, *J* = 7.3, 2 arom. H); 7.38 (*d*, *J* = 7.2, 1 arom. H); 7.32 (*d*, *J* = 7.2, 1 arom. H); 7.17 (*m*, 2 arom. H); 7.11 (*s*, 1 arom. H); 5.31 (*s*, NH_2). ^{13}C-NMR ($CDCl_3$): 164.4, 163.3 (C=N); 161.4 (N–C=C); 134.3, 133.8, 133.5, 132.5, 131.2, 130.8, 129.3, 128.9, 128.7, 127.4, 103.5 (arom. C). ESI-MS: 326.77 ($[M+1]^+$). Anal. calc. for $C_{16}H_{11}ClN_4O_2$ (326.74): C 58.82, H 3.39, N 17.15; found: C 58.76, H 3.41, N 16.98.

3. *General Procedure for the Synthesis of Compounds* **3a**–**3i** *and* **4a**–**4i**. The amines **2a**–**2i** (2.0 equiv.) were dissolved in NaOH soln. (10%, 40 ml), followed by addition of $PhSO_2Cl$ or TsCl (1 equiv.) in small portions. The mixture was heated on H_2O bath to complete the reaction. Dil. HCl was added to precipitate the sulfonamides of the primary amines. Solids obtained were filtered and washed with little cold H_2O.

N-*(4,6-Diphenylpyrimidin-2-yl)benzenesulfonamide* (**3a**). Yield: 71%. M.p. 119°. IR: 1025 (SO_2-(sym.)), 1570 (SO_2(asym.)), 3249 (N–H). ^{1}H-NMR ($CDCl_3$): 10.71 (*s*, NH); 7.64–7.51 (*m*, 5 arom. SO_2-H), 7.29–7.22 (*m*, 10 arom. H); 6.99 (*s*, 1 arom. H). ^{13}C-NMR ($CDCl_3$): 172.6, 169.2 (C=N); 161.3 (N–C=C); 133.2, 132.1, 131.3, 131.1, 129.7 129.5, 129.1, 128.8, 127.6, 126.9, 126.8, 124.6, 103.2 (arom. C). ESI-MS: 388.56 ($[M+1]^+$). Anal. calc. for $C_{22}H_{17}N_3O_2S$ (387.45): C 68.20, H 4.42, N 10.85; found: C 68.34, H 4.48, N 10.85.

N-*[4-(4-Methylphenyl)-6-phenylpyrimidin-2-yl]benzenesulfonamide* (**3b**). Yield: 53%. M.p. 120°. IR: 1102 (SO_2(sym.)), 1472 (SO_2(asym.)), 2931 (Me), 3350 (N–H). ^{1}H-NMR ($CDCl_3$): 10.19 (*s*, NH); 7.91 (*d*, *J* = 6.5, 2 arom. H); 7.74–7.63 (*m*, 8 arom. H); 7.52 (*d*, *J* = 7.3, 2 arom. H); 7.43 (*d*, *J* = 7.3, 2 arom. H); 6.89 (*s*, 1 arom. H); 2.18 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 176.4, 168.1 (C=N); 161.2 (N–C=C); 135.3 (arom. C, Ts); 135.1, 132.7, 129.3, 128.4, 127.5, 127.3, 101.3 (arom. C); 24.7 (alk. C). ESI-MS: 402.73 ($[M+1]^+$). Anal. calc. for $C_{23}H_{19}N_3O_2S$ (401.48): C 68.81, H 4.77, N 10.47; found: C 68.73, H 4.91, N 10.36.

N-*[4-(2-Chlorophenyl)-6-phenylpyrimidin-2-yl]benzenesulfonamide* (**3c**). Yield: 59%. M.p. 106°. IR: 1086 (SO_2(sym.)), 1434 (SO_2(asym.)), 3324 (N–H). ^{1}H-NMR ($CDCl_3$): 10.67 (*s*, NH); 7.67–7.54 (*m*, 5 arom. H); 7.23–7.05 (*m*, 9 arom. H) 6.89 (*s*, 1 arom. H). ^{13}C-NMR ($CDCl_3$): 166.3, 161.9 (C=N); 157.8 (N–C=C); 139.7, 133.3, 132.4, 131.1, 129.8, 129.3, 128.6, 127.4, 123.6, 124.3 103.8 (arom. C). ESI-MS: 422.88 ($[M+1]^+$). Anal. calc. for $C_{22}H_{16}ClN_3O_2S$ (421.90): C 62.63, H 3.82, N 9.96; found: C 62.71, H 3.82, N 9.78.

N-*[4-(4-Chlorophenyl)-6-phenylpyrimidin-2-yl]benzenesulfonamide* (**3d**). Yield: 55%. M.p. 79°. IR: 3263 (N–H), 1578 (SO_2(asym.)), 1026 (SO_2(sym.)). ^{1}H-NMR ((D_6)DMSO): 10.37 (*s*, NH); 7.48–7.58 (*m*, 10 arom. H); 6.91 (*s*, 1 arom. H); 7.32 (*d*, *J* = 7.21, 2 arom. H); 7.21 (*d*, *J* = 7.21, 2 arom. H). ^{13}C-NMR: 164.3, 172.3 (C=N); 161.3 (N–C=C); 135.9 (arom. C, Ts); 132.5, 131.5,130.7, 128.4, 126.4. ESI-MS: 422.95 ($[M+1]^+$). Anal. calc. for $C_{22}H_{16}ClN_3O_2S$ (421.90): C 62.63, H 3.82, N 9.96; found: C 62.65, H 3.74, N 9.81.

N-*[4-(4-Chlorophenyl)-6-(4-methylphenyl)pyrimidin-2-yl]benzenesulfonamide* (**3e**). Yield: 79%. M.p. 140°. IR: 1124 (SO_2(sym.)), 1532 (SO_2(asym.)), 2955 (Me), 3367 (N–H). ^{1}H-NMR ($CDCl_3$): 10.93 (*s*, NH); 7.62 (*d*, *J* = 7.43, 2 arom. H); 7.47–7.32 (*m*, 9 arom. H); 7.21 (*d*, *J* = 7.23, 2 arom. H); 6.83 (*s*, 1 arom. H); 2.13 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 171.1, 165.1 (C=N); 159.4 (N–C=C); 36.4 (arom. C, Ts); 132.8, 131.3, 129.4, 127.3, 126.8 (arom. C); 23.3 (alk. C). ESI-MS: 458.45 ($[M+Na]^+$). Anal. calc. for $C_{23}H_{18}ClN_3O_2S$ (435.92): C 63.37, H 4.16, N 9.64; found: C 63.21, H 4.19, N 9.73.

N-*[4-(2-Chlorophenyl)-6-(4-methylphenyl)pyrimidin-2-yl]benzenesulfonamide* (**3f**). Yield: 87%. M.p. 136°. IR: 1152 (SO_2(sym.)), 1511 (SO_2(asym.)), 2923 (Me), 3266 (N–H). ^{1}H-NMR ($CDCl_3$): 11.08 (*s*, N–H); 7.76–7.53 (*m*, 9 arom. H); 7.23–7.15 (*m*, 4 arom. H); 7.10 (*s*, 1 arom. H); 2.13 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 168.9, 161.6 (C=N); 158.9 (N–C=C); 133.9 (arom. C, Ts); 133.1, 132.6, 130.7, 129.4, 125.7, 103.2 (arom. C); 22.4 (alk. C). ESI-MS: 497.12 ($[M+1]^+$). Anal. calc. for $C_{23}H_{18}ClN_3O_2S$ (435.93): C 63.37, H 4.16, N 9.64; found: C 63.43, H 4.02, N 9.76.

N-*[4-(4-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]benzenesulfonamide* (**3g**). Yield: 67%. M.p. 120°. IR: 1051 (SO_2(sym.)), 1458 (SO_2(asym.)), 3364 (N–H). ^{1}H-NMR ($CDCl_3$): 10.86 (*s*, NH); 7.68 (*d*, *J* = 7.46, 2 arom. H); 7.26 (*d*, *J* = 7.4, 2 arom. H); 7.21 (*d*, *J* = 7.3, 2 arom. H); 7.15 (*d*, *J* = 7.46, 2 arom. H); 7.07–6.93 (*m*, 5 arom. H). ^{13}C-NMR ($CDCl_3$): 170.5, 164.7 (C=N); 158.1 (N–C=C); 135 (arom. C, Ts); 132.9, 131.8,129.6, 129.3, 128.1, 125.3, 106.3 (arom. C). ESI-MS: 467.49 ($[M+1]^+$). Anal. calc. for $C_{22}H_{15}ClN_4O_4S$ (466.90): C 56.59, H 3.24, N 12.00; found: C 56.57, H 3.13, N 11.96.

N-*[4-(4-Nitrophenyl)-6-phenylpyrimidin-2-yl]benzenesulfonamide* (**3h**). Yield: 74%. M.p. 105°. IR: 1096 (SO_2(sym.)), 1496 (SO_2(asym.)), 3209 (N–H). ^{1}H-NMR ((D_6)DMSO): 12.06 (*s*, NH); 7.64 (*d*, *J* = 7.3, 2 arom. H); 7.41–7.35 (*m*, 5 arom. H, Ts); 7.34–7.21 (*m*, 5 arom. H); 7.28 (*d*, *J* = 7.3, 2 arom. H); 7.09 (*s*, 1 arom. H). ^{13}C-NMR ((D_6)DMSO): 171.4, 163.0 (C=N); 161.4 (N–C=C); 136.2 (arom. C, Ts); 133.4, 132.5, 132.3, 130.7, 130.6, 129.1, 128.7,127.9, 127.6, 125.6, 99.3 (arom. C). ESI-MS: 433.41 ($[M+1]^+$). Anal. calc. for $C_{22}H_{16}N_4O_4S$ (432.45): C 61.10, H 3.73, N 12.96; found: C 61.18, H 3.68, N 12.92.

N-*[4-(2-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]benzenesulfonamide* (**3i**). Yield: 48%. M.p. 112°. IR: 1178 (SO_2(sym.)), 1516 (SO_2(asym.)), 3316 (N–H). ^{1}H-NMR ((D_6)DMSO): 10.81 (*s*, NH); 7.92 (*d*, *J* = 7.3, 2 arom. H); 7.56 (*d*, *J* = 7.3, 2 arom. H); 7.34 (*d*, *J* = 7.11, 2 arom. H); 7.38 (*d*, *J* = 7.2, 1 arom. H); 7.32 (*d*, *J* = 7.2, 1 arom. H); 7.17 (*m*, 2 arom. H); 7.16–7.11 (*m*, 4 arom. H). ^{13}C-NMR ((D_6)DMSO): 169.5, 163.7, (C=N); 163.2 (N–C=C); 135.8 (arom. C, Ts); 134.3, 133.8, 133.5, 132.5, 131.2, 132.1, 129.8,

128.9, 128.7, 103.5 (arom. C). ESI-MS: 467.79 ($[M+1]^+$). Anal. calc. for $C_{22}H_{15}ClN_4O_4S$ (466.90): C 56.59, H 3.24, N 12.00; found: C 56.63, H 3.28, N 11.94.

N-*(4,6-Diphenylpyrimidin-2-yl)-4-methylbenzenesulfonamide* (**4a**). Yield: 74%. M.p. 95°. IR: 1024 (SO_2(sym.)), 1508 (SO_2(asym.)), 3313 (N–H). ^{1}H-NMR ($CDCl_3$): 10.23 (*s*, NH); 7.61 (*d*, *J* = 7.4, 2 arom. H, Ts); 7.45 (*d*, *J* = 7.4, 2 arom. H, Ts); 7.36–7.23 (*m*, 10 arom. H); 7.01 (*s*, 1 arom. H); 2.3 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 169.9, 163.4 (C=N); 159.3 (N–C=C); 142.3, 133.2, 131.7, 131.2, 129.5, 129.1, 128.8,127.7 127.6, 126.9, 126.8, 123.7, 103.2 (arom. C); 24.3 (Me). ESI-MS: 402.56 ($[M+1]^+$). Anal. calc. for $C_{23}H_{19}N_3O_2S$ (401.48): C 68.81, H 4.77, N 10.47; found: C 68.84, H 4.874, N 10.52.

4-Methyl-N-*[4-(4-methylphenyl)-6-phenylpyrimidin-2-yl]benzenesulfonamide* (**4b**). Yield: 58%. M.p. 94°. IR: 1035 (SO_2(sym.)), 1538 (SO_2(asym.)), 2964 (Me), 3291 (N–H). ^{1}H-NMR ((D_6)DMSO): 10.47 (*s*, NH); 7.85 (*d*, *J* = 6.5, arom. H); 7.34 (*d*, *J* = 6.5, 2 arom. H); 7.46 (*d*, *J* = 7.31, 2 arom. H); 7.41 (*d*, *J* = 7.31, 2 arom. H); 6.93 (*s*, 1 arom. H); 2.33 (*s*, Me), 2.14 (*s*, Me), 7.41–7.36 (*m*, 5 arom. H). ^{13}C-NMR ((D_6)DMSO): 174.4, 169.2 (C=N); 160.9 (N–C=C); 134.7, 133.6, 131.2, 129.5,129.3, 128.4, 127.2, 126.9 (arom. C); 134.3 (arom. C, Ts); 21.3(alk. C). ESI-MS: 416.88 ($[M+1]^+$). Anal. calc. for $C_{24}H_{21}N_3O_2S$ (415.51): C 69.37, H 5.09, N 10.11; found: C 69.56, H 5.16, N 9.93.

N-*[4-(2-Chlorophenyl)-6-phenylpyrimidin-2-yl]-4-methylbenzenesulfonamide* (**4c**). Yield: 61%. M.p. 98°. IR: 1124 (SO_2(sym.)), 1401 (SO_2(asym.)), 3301 (N–H). ^{1}H-NMR ((D_6)DMSO): 12.30 (*s*, NH); 7.68 (*d*, *J* = 7.5, 2 arom. H); 7.32 (*d*, *J* = 7.5, 2 arom. H, Ts); 7.25–7.11 (*m*, 9 arom. H); 6.93 (*s*, 1 arom. H); 2.19 (*s*, Me). ^{13}C-NMR ((D_6)DMSO): 173.3, 161.7 (C=N); 161.8 (N–C=C); 142.1, 133.5, 132.4, 131.1, 129.8, 129.3, 128.6, 127.4, 126.7, 124.3 123.6, 124.1, 101.93 (arom. C); 25.6 (C). ESI-MS: 437.01 ($[M+1]^+$). Anal. calc. for $C_{23}H_{18}ClN_3O_2S$ (435.93): C 63.37, H 4.16, N 9.64; found: C 63.46, H 4.13, N 9.66.

N-*[4-(4-Chlorophenyl)-6-phenylpyrimidin-2-yl]-4-methylbenzenesulfonamide* (**4d**). Yield: 80%. M.p. 78°. IR: 1017 (SO_2(sym.)), 1469 (SO_2(asym.)), 3285 (N–H). ^{1}H-NMR ($CDCl_3$): 10.47 (*s*, NH); 7.59 (*d*, *J* = 7.01, 2 arom. H); 7.46 (*d*, *J* = 7.01, 2 arom. H); 7.38–7.29 (*m*, 5 arom. H); 7.26 (*d*, *J* = 7.4, 2 arom. H); 7.21 (*d*, *J* = 7.4, 2 arom. H); 6.91 (*s*, 1 arom. H); 2.23 (*s*, Me). ^{13}C-NMR ($CDCl_3$): 173.5, 164.7 (C=N); 160.3 (N–C=C); 135.4 (arom.; C, Ts); 131.5, 131.2, 129.4, 129.1, 128.6, 127.2 (arom. C); 23.01 (Me). ESI-MS: 436.99 ($[M+1]^+$). Anal. calc. for $C_{23}H_{18}ClN_3O_2S$ (435.93): C 63.37, H 4.16, N 9.64; found: C 63.31, H 4.19, N 9.59.

N-*[4-(4-Chlorophenyl)-6-(4-methylphenyl)pyrimidin-2-yl]-4-methylbenzenesulfonamide* (**4e**). Yield: 74%. M.p. 110°. IR: 1087 (SO_2(sym.)), 1548 (SO_2(asym.)), 2961 (Me), 3311 (N–H). ^{1}H-NMR ($CDCl_3$): 12.05 (*s*, NH); 7.72 (*d*, *J* = 7.43, 2 arom. H); 7.47–7.36 (*m*, 6 arom. H); 7.29 (*d*, *J* = 6.9, 2 arom. H); 7.22 (*d*, *J* = 7.18, arom. H); 6.88 (*s*, 1 arom. H); 2.26 (*s*, Me); 2.05 (*s*, Me). ^{13}C-NMR ((D_6)DMSO): 169.5, 168.3 (C=N); 161.4 (N–C=C); 136.3 (arom. C, Ts); 132.6, 131.8. 129.9. 129.6, 128.9, 106.4 (arom. C); 23.6, 21.4 (Me). ESI-MS: 451.19 ($[M+Na]^+$). Anal. calc. for $C_{24}H_{20}ClN_3O_2S$ (449.95): C 64.06, H 4.48, N 9.34; found: C 65.17, H 4.51, N 9.39.

N-*[4-(2-Chlorophenyl)-6-(4-methylphenyl)pyrimidin-2-yl]-4-methylbenzenesulfonamide* (**4f**). Yield: 46%. M.p. 95°. IR: 1119 (SO_2(sym.)), 1443 (SO_2(asym.)), 3261 (N–H). ^{1}H-NMR ($CDCl_3$): 11.23 (*s*, NH); 7.92 (*d*, *J* = 7.5, 2 arom. H); 7.21 (*d*, *J* = 7.31, 2 arom. H); 7.15–7.03 (*m*, 7 arom. H); 6.79 (*d*, *J* = 7.5, 2 arom. H, Ts); 2.2 (*s*, 2 Me). ^{13}C-NMR ($CDCl_3$): 168.6, 164.4 (C=N); 159.4 (N–C=C), 142.6 (arom. C, Ts); 133.1, 132.3, 131.4, 129.9, 129.3, 128, 127.6, 125.5, 106.6 (arom. C); 24.7 (C). ESI-MS: 450.76 ($[M+1]^+$). Anal. calc. for $C_{24}H_{20}ClN_3O_2S$ (449.95): C 64.06, H 4.48, N 9.34; found: C 64.25, H 4.46, N 9.39.

N-*[4-(4-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]-4-methylbenzenesulfonamide* (**4g**). Yield: 59%. M.p. 118°. IR: 1073 (SO_2(sym.)), 1457 (SO_2(asym.)), 3327 (N–H). ^{1}H-NMR ((D_6)DMSO): 11.64 (*s*, NH); 7.71 (*d*, *J* = 7.6, 2 arom. H); 7.26 (*d*, *J* = 7.4, 2 arom. H); 7.21 (*d*, *J* = 7.4, 2 arom. H); 7.15 (*d*, *J* = 7.2, 2 arom. H); 7.11 (*d*, *J* = 7.2, 2 arom. H); 7.09 (*d*, *J* = 7.6, 2 arom. H, Ts); 7.1 (*s*, 1 arom. H); 2.1 (*s*, Me). ^{13}C-NMR ((D_6)DMSO): 171.5, 164.2 (C=N); 159.3 (N–C=C); 137.3 (arom. C, Ts); 132.3, 131.2, 130.4, 129.9, 129.6, 128.7, 125.2, 103.6 (arom. C); 21.8 (Me). ESI-MS: 481.45 ($[M+1]^+$). Anal. calc. for $C_{23}H_{17}ClN_4O_4S$ (480.92): C 57.44, H 3.56, N 11.65; found: C 57.47, H 3.51, N 11.55.

4-Methyl-N-*[4-(4-nitrophenyl)-6-phenylpyrimidin-2-yl]benzenesulfonamide* (**4h**). Yield: 87%. M.p. 87°. IR: 1145 (SO_2(sym.)), 1583 (SO_2(asym.)), 3224 (N–H). ^{1}H-NMR ((D_6)DMSO): 12.15 (*s*, NH); 7.88 (*d*, *J* = 7.7, 2 arom. H); 7.53 (*d*, *J* = 7.3, 2 arom. H); 7.34–7.21 (*m*, 5 arom. H); 7.18 (*d*, *J* = 7.3, 2 arom. H); 7.09 (*s*, 1 arom. H); 7.04 (*d*, *J* = 7.7, 2 arom. H, Ts); 2.7 (*s*, Me). ^{13}C-NMR ((D_6)DMSO): 173.4, 165.2 (C=N); 161.3 (N–C=C); 134.2 (arom. C, Ts); 143.8, 125.8, 130.2, 133.4, 133.9, 132.5, 132.3, 130.7, 127.9,

127.6, 98.0 (arom. C); 23.8 (C). ESI-MS: 447.56 ($[M+1]^+$). Anal. calc. for $C_{23}H_{18}N_4O_4S$ (446.48): C 61.87, H 4.06, N 12.55; found: C 58.77, H 3.68, N 8.94.

N-*[4-(2-Chlorophenyl)-6-(4-nitrophenyl)pyrimidin-2-yl]-4-methylbenzenesulfonamide* (**4i**). Yield: 71%. M.p. 107°. IR: 1024 (SO_2(sym.)), 1519 (SO_2(asym.)), 3368 (N–H). ^{1}H-NMR ((D$_6$)DMSO): 11.45 (*s*, NH); 7.62 (*d*, *J* = 7.31, 2 arom. H); 7.43 (*d*, *J* = 7.37, 2 arom. H); 7.29 (*d*, *J* = 7.37, 2 arom. H); 7.24 (*d*, *J* = 7.21, 2 arom. H); 7.19 (*d*, *J* = 7.21, 1 arom. H); 7.17 (*d*, *J* = 7.31, 2 arom. H); 1.9 (*s*, Me). ^{13}C-NMR ((D$_6$)DMSO): 173.6, 163.2 (C=N); 161.7 (N–C=C); 136.3 (arom. C, Ts); 134.2, 133.4, 133.2, 132.5, 131.2, 132.1, 129.8, 128.9, 128.2, 103.6 (arom. C); 26.8 (C). ESI-MS: 481.93 ($[M+1]^+$). Anal. calc. for $C_{23}H_{17}ClN_4O_4S$ (480.92): C 57.44, H 3.56, N 11.65; found: C 57.55, H 3.44, N 11.58.

Biological Activity. Anti-Amoebic Activity. The compounds **2a**–**2i**, **3a**–**3i**, and **4a**–**4i** were screened *in vitro* for anti-amoebic activity against the HM1: IMSS strain of *E. histolytica* by the microdilution method [39]. *E. histolytica* trophozoites were cultured in TYIS-33 growth medium [40] in wells of 96 microtiter plate (*Costar*).The test compounds were dissolved in DMSO (40 µl), at which level no inhibition of the amoeba occurred [41][42]. The culture medium was added to obtain a concentration of 1 mg/ml. Twofold serial dilutions were made. Each test includes metronidazole as a standard amoebic drug, control wells (culture medium plus amoeba), and a blank (culture medium only). The number of amoeba per mm was estimated with a haemocytometer, and trypan blue exclusion was used to confirm viability. The cell suspension was diluted to 10^5 organisms/ml by adding fresh medium, and 170 µl of this suspension was added to the test and control wells in the plate. An ennoculum of 1.7×10^4 organisms/well was chosen, so that confluent, but not excessive, growth took place. The plates were sealed, gassed for 10 min with N_2, and incubated at 37° for 72 h. After incubation, the growth of the amoeba was checked with a low-power microscope. The culture medium was removed by inverting the plate and shaking gently. The plates were immediately washed with 0.9% aq. NaCl soln. at 37°. This procedure was performed quickly, and the plate was not allowed to cool to prevent the detachment of amoebae. The plate was dried in at r.t., and the amoebae were fixed with chilled MeOH by keeping the plate in an ice bath for 15 min, dried, and stained with 0.5% aq. eosin for 15 min. The stained plate was washed once with tap H_2O and then twice with dist. H_2O and allowed to dry. Then, 0.1N aq. NaOH soln. (200 µl) was added to each well to dissolve the protein and to release the dye (eosine). The optical density of the resulting soln. 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 *vs.* the logarithm of the dose of the drug tested. Linear-regression analysis was used to determine the best-fitting straight line from which IC_{50} values were determined. The experiments were performed thrice for each compound tested.

Cell-Viability Assay. H9C2 Rat cardiac myoblasts were cultured and maintained as monolayer in high-glucose DMEM (*Dulbecco*'s modified *Eagle*'s medium), supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 mg/ml streptomycin, and 2.5 mg/ml amphotericin B, at 37° in a humidified incubator with 5% CO_2 [42]. Cells were incubated with different concentrations of compounds **3c**, **3d**, **3g**, **3i**, **4c**, **4g**, and **4i** in DMSO and metronidazole for 48 h at 37° in 5% CO_2 humidified incubator together with untreated control sample. At appropriate time points, cells were washed in PBS (= phosphate-buffered saline), treated with 600 ml MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) soln. (5 mg/ml) and incubated for 45 min at 37°. After 45 min of incubation at 37°, the cell supernatants were discarded, MTT crystals were dissolved in ^{i}PrOH, and the absorbance was measured at 570 nm. All assays were performed in triplicate. Percent viability was defined as the relative absorbance of treated *vs.* untreated control cells.

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