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Synthesis of 2,4,6-trisubstituted pyrimidines as antimalarial agents

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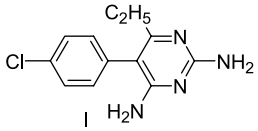
Abstract—A series of 2,4,6-trisubstituted-pyrimidines were synthesized and evaluated for their in vitro antimalarial activity against *Plasmodium falciparum*. Of the 18 compounds synthesized, 14 compounds have shown MIC in the range of 0.25–2 μg/mL. These compounds are in vitro severalfold more active than pyrimethamine.

1. Introduction

Malaria is the most serious and widespread parasitic disease because of its prevalence, virulence and drug resistance, having an overwhelming impact on public health in developing regions of the world. *Plasmodium falciparum* is the main cause of severe clinical malaria and death. Endemic mapping indicates that *P. falciparum* and *P. vivax* account for 95% of the malaria infections.[1–3] Each year, more than 500 million people are infected and close to 2 million die because of malaria. A major thrust in this initiative is the identification of new targets that are critical to the disease process or essential for the survival of the parasite. The dihydrofolate reductase (DHFR) domain of *P. falciparum* is one of the few well-defined targets in malarial chemotherapy. The enzyme catalyzes the nicotinamide adenine dinucleotide phosphate (NADPH) dependent reduction of dihydrofolate to tetrahydrofolate. DHFR has received considerable attention, as it is the target of pyrimethamine (**I**) and other antifolates used for the prophylaxis and treatment of *P. falciparum* infection.[4,5] Because the parasites are developing resistance to conventional antimalarial drugs, the development of drugs attacking crucial targets in the metabolism of the malarial pathogen is imperative.[6]



I

Fatty acid biosynthesis (FAS) is another important target site in the discovery of new antimalarials. Pyridine-4-carboxylic acid hydrazide (Isoniazid), which is a frontline drug in the treatment of tuberculosis, is an inhibitor of an important enzyme (enoyl-ACP reductase) in the fatty acid biosynthesis pathway. Thus pyridine analogues inhibit the biosynthesis of fatty acids, which are fundamental for the survival of *P. falciparum* in the host.[7]

Compounds that act on more than one target sites are more liable to be active. Based on these observations, we have synthesized hybrid derivatives having pyrimidine (DHFR inhibitor) along with pyridine moiety (fatty acid inhibitor).

Antimalarial drugs such as quinine and mefloquine have a piperidine nucleus, while amopyroquine and cycloquine have a pyrrolidine moiety. A large number of compounds having piperidine and pyrrolidine moieties have shown potent antimalarial activity.[8] These results prompted us to synthesize compounds having pyridine moieties along with pyrimidine and these cyclic amines at the 2nd position in the pyrimidine ring.

As part of our ongoing program devoted to the synthesis of diverse heterocycles as anti-infective agents,[9] we had previously reported antimalarial activity in substituted triazines, pyrimidines and quinolines.[10] This communication describes the in vitro antimalarial activity of pyrim-

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idine derivatives substituted with cyclic amines at the 2nd position and pyridine nucleus at the 4th position.

2. Chemistry

To synthesize the 2,4,6-trisubstituted-pyrimidine compounds (**3a**–**i** and **4a**–**i**), 4-acetylpyridine was reacted with different aldehydes (**a**–**i**) in 10% aq NaOH and methanol to yield the corresponding chalcones **2a**–**i**.[11] Piperidine-1-carboxamidine hydrochloride and pyrrolidine-1-carboxamidine hydrochloride were synthesized by refluxing piperidine and pyrrolidine, respectively, with *S*-methyl isothiourea sulfate in water according to a reported procedure.[12] The chalcones **2a**–**i** were further cyclized with imidine hydrochlorides in the presence of sodium isopropoxide (synthesized in situ by adding sodium metal in isopropanol) to afford the 2,4,6-trisubstituted pyrimidines **3a**–**i** and **4a**–**i** as shown in Scheme 1. All the synthesized compounds were well characterized by spectroscopic methods such as IR, mass, NMR and elemental analysis.

3. Biological activity

The in vitro antimalarial assay was carried out in 96-well microtitre plates according to the microassay of Rieckmann et al.[13] The culture of *P. falciparum* NF-54 strain is routinely being maintained in medium RPMI-1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum.[14] The asynchronous parasite of *P. falciparum* was synchronized after 5% D-sorbitol treatment to obtain parasitized cells harbouring only the ring stage.[15] For carrying out the assay, an initial ring stage parasitaemia of ≈1% at 3% haematocrit in a total volume of 200 μL of medium RPMI-1640 was uniformly maintained. The test compound in 20 μL volume at required concentration (ranging between 0.25 μg and 50 μg/mL) in duplicate wells was incubated with parasitized cell preparation at 37 °C in a candle jar. After 36–40 h incubation, the blood smears from each well were prepared and stained with giemsa stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in the presence of different concentrations of compounds. The test concentration that inhibits the complete maturation into schizonts was recorded as the minimum inhibitory concentration (MIC). Pyrimethamine was used as the standard reference drug. Activities of all the tested compounds are shown in Table 1.

4. Results and discussion

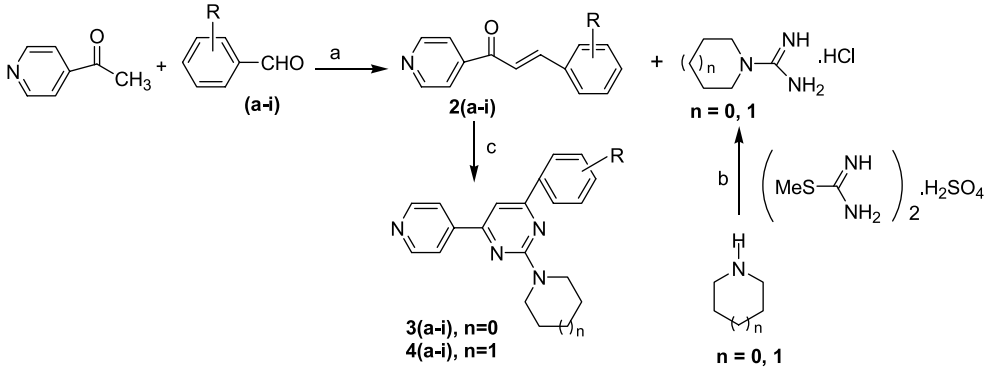
Among all the 18 compounds tested, 1 compound showed MIC of 0.25 μg/mL, 3 compounds showed MIC of 1 μg/mL, and 10 compounds showed MIC of 2 μg/mL.

When the R group was a phenyl ring the pyrrolidine substituted compound (**3a**) showed a MIC of 2 μg/mL. Substitution of the phenyl ring with methoxy group (**3b**), methyl group (**3f**), thiomethyl group (**3g**) and chloro group (**3i**) did not affect the activity. Di- (**3c**) and trisubstitution (**3d**) of the phenyl ring with methoxy group increased the activity. An almost similar trend was also observed for piperidine substituted compounds. The phenyl ring substituted compound showed a MIC of 2 μg/mL. Substitution of methoxy (**4b**), dimethoxy (**4c**) and thiomethyl group (**4g**) on the phenyl ring had no effect on the activity. On substitution of methyl

Table 1. Antimalarial in vitro activity against *P. falciparum*

S. no.	R	MIC (μg/mL)	
		3(**a**–**j**) $n = 0$	**4**(**a**–**j**) $n = 1$
a	C_6H_5	2	2
b	4OMe	2	2
c	2,5-diOMe	1	2
d	2,4,5-triOMe	1	1
e	3,4,5-triOMe	50	50
f	4-Me	2	0.25
g	4-SMe	2	2
h	3,4-DiMe	2	10
i	4Cl	2	10

MIC = Minimum inhibiting concentration for the development of ring stage parasite into the schizont stage during 40 h incubation.
I: standard drug and pyrimethamine have shown a MIC of 10 μg/mL.



Scheme 1. (a) Different aldehydes (**a**–**j**), 10% aq NaOH, methanol, 0 °C–rt, 30 min. (b) (i) Piperidine or pyrrolidine, *S*-methylisothiourea sulfate, water, reflux, 15 min. (ii) Barium chloride, reflux, 15 min. (c) Pyrrolidine-1-carboxamidine.HCl (for **3a**–**j**) or piperidine-1-carboxamidine·HCl (for **4a**–**j**) sodiumisopropoxide, isopropanol, reflux, 8 h.

group at *para* position the compound (**4f**) exhibited an exceptionally drastic increase in activity, having a MIC of 0.25 μg/mL. Trisubstitution of the phenyl ring with methoxy group at 2-, 4- and 5-positions increased the activity, whereas substitution at 3-, 4- and 5-positions decreased the activity of compounds. In general the activity profiles in both the pyrrolidine and piperidine substituted compounds are almost similar.

5. Conclusion

The eighteen 2,4,6-trisubstituted pyrimidines (**3a–i** and **4a–i**) were synthesized as pyrimethamine analogues. In conclusion one compound has shown MIC of 0.25 μg/mL, three compounds showed MIC of 1 μg/mL, whereas ten compounds showed MIC of 2 μg/mL. These compounds are 5–40 times more potent than pyrimethamine. These identified pyrimidines are new leads in antimalarial chemotherapy. These molecules can be very useful for further optimization work in malarial chemotherapy.

6. Experimental details

IR spectra were recorded on Beckman Aculab-10, Perkin Elmer 881 and FTIR 8210 PC, Scimadzu spectrophotometers either on KBr discs or in neat. Nuclear magnetic resonance (NMR) spectra were recorded on either Bruker Avance DRX-300 MHz or Bruker DPX 200 FT spectrometers using TMS as an internal reference. FAB mass spectra were recorded on JEOL SX 102/DA 6000 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas. Chemical analyses were carried out on Carlo-Erba-1108 instrument. The melting points were recorded on an electrically heated melting point apparatus and are uncorrected.

6.1. General procedure for the synthesis of compounds 2a–i

Method A: To a cooled solution of 10% NaOH, 1.0 equiv of liquid aldehydes was added. To this solution 1.0 equiv of 4-acetyl pyridine was added dropwise in about a period of 30 min. The solution was maintained at 0 °C for an hour and then was allowed to stir at rt. After some time a solid started separating out. The solution was further stirred for about 1 h. The solid was filtered out and then recrystallized from methanol or ethanol to give crystals of the chalcone.

Method B: In the case of aldehydes, which were solid, the aldehyde (1 equiv) was first dissolved in minimum quantity of ethanol or methanol (approx 25 mL) and then 10% NaOH solution (approx 100 mL) was added to it to give a clear solution. The solution was cooled up to 0 °C by applying ice bath below it. Then 1 equiv of 4-acetyl pyridine was dropwise added to it, in around a period of 30 min. The solution was maintained at 0 °C for 1 h and then was allowed to stir at room temperature. After some time a solid started separating out. This was stirred for about an hour. The solid was filtered out and then recrystallized from methanol or ethanol to give crystals of the chalcone.

6.1.1. 3-Phenyl-1-pyridin-4-yl-propenone (2a). The compound was synthesized using method A. Yield: 75%; mp 172–174 °C; MS: 210 (M+1); IR (KBr) 3521, 1956, 1673, 1595, 1411, 1225 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.84 (d, 2H, $J = 6.0$ Hz), 8.01 (d, 2H, $J = 7.8$ Hz), 7.86 (d, 1H, $J = 15.1$ Hz), 7.76 (d, 2H, $J = 6.0$ Hz), 7.44–7.40 (m, 3H), 7.36 (d, 1H, $J = 15.1$ Hz). Anal. Calcd for $C_{14}H_{11}NO$: calculated C: 80.36, H: 5.30, N: 6.69. Found: C: 80.56, H: 5.53, N: 6.78.

6.1.2. 3-(4-Methoxy-phenyl)-1-pyridin-4-yl-propenone (2b). The compound was synthesized using method A. Yield: 72%; mp 104–106 °C; MS: 240 (M+1); IR (KBr) 3420, 1946, 1683, 1598, 1489, 1411, 1257 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.83 (d, 2H, $J = 6.1$ Hz), 7.89 (d, 2H, $J = 8.6$ Hz), 7.82 (d, 1H, $J = 15.4$ Hz), 7.75 (d, 2H, $J = 6.1$ Hz), 7.37 (d, 1H, $J = 15.4$ Hz), 6.92 (d, 2H, $J = 8.6$ Hz), 3.88 (s, 3H, OMe). Anal. Calcd for $C_{15}H_{13}NO_2$: calculated C: 75.30, H: 5.48, N: 5.85. Found: C: 75.54, H: 5.59, N: 5.71.

6.1.3. 3-(2,5-Dimethoxy-phenyl)-1-pyridin-4-yl-propenone (2c). The compound was synthesized using method B. Yield: 58%; mp 128–130 °C; MS: 270 (M+1); IR (KBr) 3299, 1956, 1685, 1597, 1456, 1411, 1259 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.85 (d, 2H, $J = 6.0$ Hz), 7.82 (d, 1H, $J = 15.4$ Hz), 7.77 (d, 2H, $J = 6.0$ Hz), 7.39 (d, 1H, $J = 15.4$ Hz), 7.36 (s, 1H), 7.24 (d, 1H, $J = 7.2$ Hz), 6.76 (d, 1H, $J = 7.2$ Hz), 3.88 (s, 3H, OMe), 3.85 (s, 3H, OMe). Anal. Calcd for $C_{16}H_{15}NO_3$: calculated C: 71.36, H: 5.61, N: 5.20. Found: C: 71.58, H: 5.86, N: 4.97.

6.1.4. 1-Pyridin-4-yl-3-(2,4,5-trimethoxy-phenyl)-propenone (2d). The compound was synthesized using method B. Yield: 64%; mp 160–162 °C; MS: 300 (M+1); IR (KBr) 3282, 1939, 1689, 1598, 1468, 1420, 1232 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.82 (d, 2H, $J = 5.9$ Hz), 7.84 (d, 1H, $J = 15.6$ Hz), 7.72 (d, 2H, $J = 5.9$ Hz), 7.64 (s, 1H), 7.37 (d, 1H, $J = 15.6$ Hz), 6.72 (s, 1H), 3.96 (s, 3H, OMe), 3.92 (s, 3H, 2OMe). Anal. Calcd for $C_{17}H_{17}NO_4$: calculated C: 68.21, H: 5.72, N: 4.68. Found: C: 67.98, H: 5.94, N: 4.91.

6.1.5. 1-Pyridin-4-yl-3-(3,4,5-trimethoxy-phenyl)-propenone (2e). The compound was synthesized using method B. Yield: 66%; mp 194–196 °C; MS: 300 (M+1); IR (KBr) 3278, 1943, 1687, 1596, 1457, 1445, 1245 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.79 (d, 2H, $J = 6.0$ Hz), 7.82 (d, 1H, $J = 15.2$ Hz), 7.70 (d, 2H, $J = 6.0$ Hz), 7.37 (d, 1H, $J = 15.2$ Hz), 7.30 (s, 2H), 3.98 (s, 3H, 2OMe), 3.95 (s, 3H, OMe). Anal. Calcd for $C_{17}H_{17}NO_4$: calculated C: 68.21, H: 5.72, N: 4.68. Found: C: 68.47, H: 5.98, N: 4.31.

6.1.6. 1-Pyridin-4-yl-3-*p*-tolyl-propenone (2f). The compound was synthesized using method A. Yield: 76%; mp 138–140 °C; MS: 224 (M+1); IR (KBr) 3290, 1967,

1689, 1598, 1492, 1412, 1238 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.84 (d, 2H, $J = 6.0$ Hz), 7.84 (d, 1H, $J = 15.6$ Hz), 7.74 (d, 2H, $J = 6.0$ Hz), 7.54 (d, 2H, $J = 8.2$ Hz), 7.37 (d, 1H, $J = 15.6$ Hz), 7.24 (d, 2H, $J = 8.2$ Hz), 2.39 (s, 3H). Anal. Calcd for $C_{15}H_{13}NO$: calculated C: 80.69, H: 5.87, N: 6.27. Found: C: 80.82, H: 5.62, N: 6.42.

6.1.7. 3-(4-Methylsulfanyl-phenyl)-1-pyridin-4-yl-propenone (2g). The compound was synthesized using method A. Yield: 70%; mp 94–96 °C; MS: 256 (M+1); IR (KBr) 3412, 1956, 1682, 1595, 1415, 1225 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.83 (d, 2H, $J = 6.1$ Hz), 7.82 (d, 1H, $J = 14.9$ Hz), 7.76 (d, 2H, $J = 6.1$ Hz), 7.55 (d, 2H, $J = 8.6$ Hz), 7.38 (d, 1H, $J = 14.9$ Hz), 7.26 (d, 2H, $J = 8.6$ Hz), 2.52 (s, 3H). Anal. Calcd for $C_{15}H_{13}NOS$: calculated C: 70.56, H: 5.13, N: 5.49. Found: C: 70.74, H: 5.34, N: 5.34.

6.1.8. 3-(3,4-Dimethyl-phenyl)-1-pyridin-4-yl-propenone (2h). The compound was synthesized using method A. Yield: 67%; mp 168–170 °C; MS: 238 (M+1); IR (KBr) 3392, 1952, 1687, 1597, 1424, 1249 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.84 (d, 2H, $J = 6.0$ Hz), 7.89 (d, 2H, $J = 7.8$ Hz), 7.83 (d, 1H, $J = 15.2$ Hz), 7.74 (d, 2H, $J = 6.0$ Hz), 7.51 (s, 1H), 7.40 (d, 1H, $J = 15.2$ Hz), 7.29 (d, 2H, $J = 7.8$ Hz), 2.37 (s, 3H), 2.34 (s, 3H). Anal. Calcd for $C_{16}H_{15}NO$: calculated C: 80.98, H: 6.37, N: 5.90. Found: C: 81.24, H: 6.09, N: 5.68.

6.1.9. 3-(4-Chloro-phenyl)-1-pyridin-4-yl-propenone (2i). The compound was synthesized using method B. Yield: 68%; mp 205–207 °C; MS: 244 (M+1); IR (KBr) 3297, 1948, 1684, 1598, 1489, 1257 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.85 (d, 2H, $J = 6.1$ Hz), 7.86 (d, 1H, $J = 15.6$ Hz), 7.75 (d, 2H, $J = 6.1$ Hz), 8.09 (d, 2H, $J = 8.6$ Hz), 7.38 (d, 1H, $J = 15.6$ Hz), 7.52 (d, 2H, $J = 8.6$ Hz). Anal. Calcd for $C_{14}H_{10}ClNO$: calculated C: 69.00, H: 4.14, N: 5.75. Found: C: 68.74, H: 4.32, N: 5.44.

6.2. General procedure for the synthesis of compounds 3a–i

To a solution of 1.0 equiv of pyrrolidin-1-carboxamidine hydrochloride in 50 mL of isopropanol, 1.1 equiv of sodium metal was added. The reaction mixture was refluxed for 2 h and then different chalcones (**2a–i**, 1.0 equiv) were added to it and refluxed for 8 h. The solvent was removed from the reaction mixture under reduced pressure. Water was added and the aqueous phase was extracted with chloroform and washed with brine solution. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by crystallization from methanol or ethanol or sometimes by column chromatography on silica gel (2% methanol in chloroform) to afford the pure compounds.

6.2.1. (4-Phenyl)-6-pyridin-4-yl-2-pyrrolidin-1-yl-pyrimidine (3a). Yield: 60%; mp 170–172 °C; MS: 302 (M+1); IR (KBr) 2932, 1645, 1574, 1484, 1319, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.76 (d, 2H, $J = 6.1$ Hz), 8.13 (d, 2H, $J = 4.8$ Hz), 7.98 (d, 2H, $J = 6.1$ Hz), 7.50–7.47 (m, 3H), 7.37 (s, 1H), 3.76 (t, 4H, $J = 6.4$ Hz), 2.04 (t, 4H, $J = 6.4$ Hz); ^{13}C ($CDCl_3$, 50 MHz): 165.2, 162.1, 161.7, 150.3, 145.4, 137.2, 127.8, 129.6, 128.9, 120.8, 103.4, 43.7, 25.4. Anal. Calcd for $C_{20}H_{19}N_3$: calculated C: 79.70, H: 6.35, N: 13.94. Found: C: 79.88, H: 6.58, N: 13.64.

6.2.2. 4-(4-Methoxy-phenyl)-6-pyridin-4-yl-2-pyrrolidin-1-yl-pyrimidine (3b). Yield: 68%; mp 164–166 °C; MS: 332 (M+1); IR (KBr) 2924, 1648, 1576, 1486, 1328, 1284 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.73 (d, 2H, $J = 5.8$ Hz), 8.11 (d, 2H, $J = 8.8$ Hz), 7.96 (d, 2H, $J = 5.8$ Hz), 7.31 (s, 1H), 7.02 (d, 2H, $J = 8.8$ Hz), 3.87 (s, 3H, OMe), 3.75 (t, 4H, $J = 6.4$ Hz), 2.06 (t, 4H, $J = 6.4$ Hz); ^{13}C ($CDCl_3$, 50 MHz): 165.4, 162.4, 162.2, 161.9, 150.4, 145.5, 129.9, 128.6, 121.0, 114.0, 101.6, 55.4, 43.9, 25.3. Anal. Calcd for $C_{21}H_{21}N_3O$: calculated C: 76.11, H: 6.39, N: 12.68. Found: C: 76.45, H: 6.66, N: 12.41.

6.2.3. 4-(2,5-Dimethoxy-phenyl)-6-pyridin-4-yl-2-pyrrolidin-1-yl-pyrimidine (3c). Yield: 54%; mp 164–166 °C; MS: 362 (M+1); IR (KBr) 2926, 1638, 1580, 1480, 1325, 1272 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.74 (d, 2H, $J = 6.1$ Hz), 7.97 (d, 2H, $J = 6.1$ Hz), 7.60 (d, 2H, $J = 6.2$ Hz), 7.29 (s, 1H), 6.97 (s, 1H), 3.75 (t, 4H, $J = 6.2$ Hz), 2.05 (t, 4H, $J = 6.2$ Hz) 3.86 (s, 3H, OMe), 3.84 (s, 3H, OMe); ^{13}C ($CDCl_3$, 50 MHz): 164.8, 162.6, 162.3, 154.4, 152.9, 150.8, 146.1, 128.4, 121.6, 116.8, 116.6, 113.7, 107.8, 43.9, 56.8, 56.2, 25.5. Anal. Calcd for $C_{22}H_{23}N_3O_2$: calculated C: 73.11, H: 6.41, N: 11.63. Found: C: 73.56, H: 6.58, N: 11.41.

6.2.4. 4-Pyridin-4-yl-2-pyrrolidin-1-yl-6-(2,4,5-trimethoxy-phenyl)-pyrimidine (3d). Yield: 68%; mp 164–166 °C; MS: 392 (M+1); IR (KBr) 2948, 1636, 1584, 1486, 1325, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.73 (d, 2H, $J = 5.7$ Hz), 7.97 (d, 2H, $J = 5.7$ Hz), 7.82 (s, 1H), 7.73 (s, 1H), 6.61 (s, 1H), 3.96 (s, 3H, OMe), 3.93 (s, 3H, OMe), 3.91 (s, 3H, OMe), 3.74 (t, 4H, $J = 6.5$ Hz), 2.03 (t, 4H, $J = 6.5$ Hz); ^{13}C ($CDCl_3$, 50 MHz): 164.5, 162.5, 162.0, 154.1, 152.3, 150.7, 146.4, 143.9, 121.7, 118.9, 114.3, 107.6, 98.4, 57.2, 57.0, 56.6, 43.8, 25.3. Anal. Calcd for $C_{23}H_{25}N_3O_3$: calculated C: 70.57, H: 6.44, N: 10.73. Found: C: 70.82, H: 6.59, N: 10.48.

6.2.5. 4-Pyridin-4-yl-2-pyrrolidin-1-yl-6-(3,4,5-trimethoxy-phenyl)-pyrimidine (3e). Yield: 70%; mp 158–160 °C; MS: 392 (M+1); IR (KBr) 2932, 1645, 1574, 1484, 1319, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.76 (d, 2H, $J = 6.2$ Hz), 7.98 (d, 2H, $J = 6.2$ Hz), 7.32 (s, 1H), 7.28 (s, 2H), 3.75 (t, 4H, $J = 6.1$ Hz), 3.96 (s, 6H, OMe), 3.91 (s, 3H, OMe), 2.04 (t, 4H, $J = 6.1$ Hz); ^{13}C ($CDCl_3$, 50 MHz): 167.2, 162.8, 163.2, 154.1, 150.8, 145.4, 140.8, 134.7, 121.6, 105.2, 102.7, 61.5, 56.9, 43.9, 25.4. Anal. Calcd for $C_{23}H_{25}N_3O_3$: calculated C: 70.57, H: 6.44, N: 10.73. Found: C: 70.79, H: 6.65, N: 10.44.

6.2.6. 4-Pyridin-4-yl-2-pyrrolidin-1-yl-6-*p*-tolyl-pyrimidine (3f). Yield: 64%; mp 132–134 °C; MS: 316 (M+1); IR (KBr) 2926, 1638, 1580, 1480, 1325, 1272 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.74 (d, 2H, J = 5.6 Hz), 8.04 (d, 2H, J = 7.9 Hz), 7.98 (d, 2H, J = 5.6 Hz), 7.35 (s, 1H), 7.29 (d, 2H, J = 7.9 Hz), 3.79 (t, 4H, J = 6.6 Hz), 2.43 (s, 3H, Me), 2.04 (t, 4H, J = 6.6 Hz); ^{13}C ($CDCl_3$, 50 MHz): 165.5, 162.5, 162.0, 150.2, 145.4, 138.1, 133.6, 130.1, 127.2, 121.2, 101.5, 43.8, 25.4, 18.9. Anal. Calcd for $C_{21}H_{21}N_3$: calculated C: 79.97, H: 6.71, N: 13.32. Found: C: 79.82, H: 6.57, N: 13.41.

6.2.7. 4-(4-Methylsulfanyl-phenyl)-6-pyridin-4-yl-2-pyrrolidin-1-yl-pyrimidine (3g). Yield: 65%; mp 148–150 °C; MS: 348 (M+1); IR (KBr) 2948, 1636, 1584, 1486, 1325, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.74 (d, 2H, J = 6.1 Hz), 8.07 (d, 2H, J = 8.7 Hz), 7.98 (d, 2H, J = 6.1 Hz), 7.36 (s, 1H), 7.33 (d, 2H, J = 8.7 Hz), 3.79 (t, 4H, J = 6.5 Hz), 2.04 (t, 4H, J = 6.5 Hz), 2.54 (s, 3H, SMe); ^{13}C ($CDCl_3$, 50 MHz): 165.6, 163.0, 162.6, 150.7, 145.7, 142.7, 134.4, 127.8, 126.4, 121.4, 102.4, 43.7, 25.3, 16.9. Anal. Calcd for $C_{21}H_{21}N_3S$: calculated C: 72.59, H: 6.09, N: 12.09. Found: C: 72.82, H: 6.42, N: 12.36.

6.2.8. 4-(3,4-Dimethyl-phenyl)-6-pyridin-4-yl-2-pyrrolidin-1-yl-pyrimidine (3h). Yield: 60%; mp 140–142 °C; MS: 330 (M+1); IR (KBr) 1636, 1584, 1486, 1325, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.74 (d, 2H, J = 5.9 Hz), 7.98 (d, 2H, J = 5.9 Hz), 7.89 (s, 1H), 7.85 (d, 1H, J = 7.1 Hz), 7.35 (s, 1H), 7.23 (d, 1H, J = 7.1 Hz), 3.76 (t, 4H, J = 6.4 Hz), 2.04 (t, 4H, J = 6.4 Hz), 2.36 (s, 3H), 2.33 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz): 166.5, 162.8, 162.7, 150.9, 145.9, 140.2, 137.3, 135.6, 130.5, 128.6, 125.0, 121.6, 102.7, 43.7, 25.4, 20.4, 20.2. Anal. Calcd for $C_{22}H_{23}N_3$: calculated C: 80.21, H: 7.04, N: 12.76. Found: C: 80.43, H: 7.35, N: 12.48.

6.2.9. 4-(4-Chloro-phenyl)-6-pyridin-4-yl-2-pyrrolidin-1-yl-pyrimidine (3i). Yield: 67%; mp 174–176 °C; MS: 336 (M+1); IR (KBr) 2948, 1636, 1584, 1486, 1325, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.76 (d, 2H, J = 6.1 Hz), 8.09 (d, 2H, J = 8.9 Hz), 7.98 (d, 2H, J = 6.1 Hz), 7.49 (d, 2H, J = 8.9 Hz), 7.33 (s, 1H), 3.79 (t, 4H, J = 6.6 Hz), 2.06 (t, 4H, J = 6.6 Hz); ^{13}C ($CDCl_3$, 50 MHz): 165.4, 162.3, 161.9, 158.8, 145.6, 135.3, 134.4, 130.1, 128.9, 121.3, 103.6, 43.8, 25.4. Anal. Calcd for $C_{20}H_{18}ClN_3$: calculated C: 71.53, H: 5.40, N: 10.56. Found: C: 71.81, H: 5.57, N: 10.41.

6.3. General procedure for the synthesis of compounds 4a–i

To a solution of 1.0 equiv of piperidin-1-carboxamidine hydrochloride in 50 mL of isopropanol, 1.1 equiv of sodium metal was added. The reaction mixture was refluxed for 2 h and then different chalcones (**1**–**9**, 1.0 equiv) were added to it and refluxed for 8 h. The solvent was removed from the reaction mixture under reduced pressure. Water was added and the aqueous phase was extracted with chloroform and washed with brine solution. The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by crystallization from methanol or ethanol or sometimes by column chromatography on silica gel (2% methanol in chloroform) to afford the pure compounds.

6.3.1. 4-Phenyl-2-piperidin-1-yl-6-pyridin-4-yl-pyrimidine (4a). Yield: 60%; mp 180–182 °C; MS: 316 (M+1); IR (KBr) 2926, 1638, 1580, 1480, 1325, 1272 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.76 (d, 2H, J = 6.1 Hz), 8.12 (d, 2H, J = 7.9 Hz), 7.96 (d, 2H, J = 6.1 Hz), 7.51–7.47 (m, 3H), 7.35 (s, 1H), 4.02 (t, 4H, J = 4.6 Hz), 1.68 (t, 4H, J = 4.6 Hz); ^{13}C ($CDCl_3$, 50 MHz): 165.4, 162.1, 161.6, 150.2, 145.4, 137.3, 127.8, 129.5, 128.9, 120.8, 103.2, 45.5, 26.3, 25.5. Anal. Calcd for $C_{21}H_{21}N_3$: calculated C: 79.97, H: 6.71, N: 13.32. Found: C: 79.82, H: 6.57, N: 13.41.

6.3.2. 4-(4-Methoxy-phenyl)-2-piperidin-1-yl-6-pyridin-4-yl-pyrimidine (4b). Yield: 62%; mp 160–162 °C; MS: 346 (M+1); IR (KBr) 2948, 1636, 1584, 1486, 1325, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.73 (d, 2H, J = 6.0 Hz), 8.09 (d, 2H, J = 8.7 Hz), 7.96 (d, 2H, J = 6.0 Hz), 7.32 (s, 1H), 7.01 (d, 2H, J = 8.7 Hz), 3.94 (t, 4H, J = 4.2 Hz), 3.88 (s, 3H, OMe), 1.69 (t, 4H, J = 4.2 Hz); ^{13}C ($CDCl_3$, 50 MHz): 165.7, 162.7, 162.2, 161.9, 150.7, 146.4, 130.8, 128.9, 121.5, 114.4, 100.9, 55.8, 45.3, 26.3, 25.4. Anal. Calcd for $C_{22}H_{23}N_3O$: calculated C: 76.49, H: 6.71, N: 12.16. Found: C: 76.62, H: 6.54, N: 12.41.

6.3.3. 4-(2,5-Dimethoxy-phenyl)-2-piperidin-1-yl-6-pyridin-4-yl-pyrimidine (4c). Yield: 58%; mp 160–162 °C; MS: 376 (M+1); IR (KBr) 2932, 1645, 1574, 1484, 1319, 1280 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.72 (d, 2H, J = 5.9 Hz), 7.95 (d, 2H, J = 5.9 Hz), 7.35 (s, 1H), 7.30 (s, 1H), 7.11 (d, 1H, J = 6.6 Hz), 6.98 (d, 1H, J = 6.6 Hz), 3.98 (t, 4H, J = 4.4 Hz), 3.87 (s, 3H, OMe), 3.82 (s, 3H, OMe), 1.70 (t, 4H, J = 4.4 Hz); ^{13}C ($CDCl_3$, 50 MHz): 164.9, 162.8, 162.4, 154.4, 152.8, 150.6, 146.1, 128.3, 121.6, 116.9, 116.3, 113.7, 107.6, 57.2, 56.3, 45.5, 26.3, 25.5. Anal. Calcd for $C_{23}H_{25}N_3O_2$: calculated C: 73.57, H: 6.71, N: 11.19. Found: C: 73.76, H: 6.48, N: 11.41.

6.3.4. 2-Piperidin-1-yl-4-pyridin-4-yl-6-(2,4,5-trimethoxy-phenyl)-pyrimidine (4d). Yield: 65%; mp 182–184 °C; MS: 406 (M+1); IR (KBr) 2924, 1648, 1576, 1486, 1328, 1284 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.76 (d, 2H, J = 6.0 Hz), 7.94 (d, 2H, J = 6.0 Hz), 7.74 (s, 1H), 7.68 (s, 1H), 6.60 (s, 1H), 3.96 (s, 3H, OMe), 3.98 (t, 4H, J = 4.4 Hz), 3.94 (s, 6H, 2OMe), 1.68 (t, 4H, J = 4.4 Hz); ^{13}C ($CDCl_3$, 50 MHz): 164.8, 162.8, 162.3, 154.4, 152.4, 150.8, 146.5, 143.8, 121.6, 118.8, 114.1, 106.8, 98.3, 57.2, 57.0, 56.6, 45.6, 26.4, 25.4. Anal. Calcd for $C_{24}H_{27}N_3O_3$: calculated C: 71.09, H: 6.71, N: 10.36. Found: C: 71.45, H: 6.43, N: 10.48.

6.3.5. 2-Piperidin-1-yl-4-pyridin-4-yl-6-(3,4,5-trimethoxy-phenyl)-pyrimidine (4e). Yield: 67%; mp 172–174 °C; MS: 406 (M+1); IR (KBr) 2926, 1638, 1580, 1480,

1325, 1272 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.76 (d, 2H, $J = 6.1$ Hz), 7.95 (d, 2H, $J = 6.1$ Hz), 7.34 (s, 1H), 7.26 (s, 2H), 4.01 (t, 4H, $J = 4.6$ Hz), 3.97 (s, 6H, OMe), 3.94 (s, 3H, OMe), 1.68 (t, 4H, $J = 4.8$ Hz); ^{13}C ($CDCl_3$, 50 MHz): 167.2, 162.8, 163.2, 154.1, 150.8, 145.4, 140.8, 134.6, 121.6, 105.1, 102.7, 60.8, 56.9, 45.6, 26.5, 25.4. Anal. Calcd for $C_{24}H_{27}N_3O_3$: calculated C: 71.09, H: 6.71, N: 10.36. Found: C: 71.32, H: 6.38, N: 10.52.

6.3.6. 2-Piperidin-1-yl-4-pyridin-4-yl-6-*p*-tolyl-pyrimidine (4f). Yield: 72%; mp 144–146 °C; MS: 330 (M+1); IR (KBr) 2936, 1642, 1578, 1488, 1324, 1284 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.73 (d, 2H, $J = 6.1$ Hz), 8.01 (d, 2H, $J = 7.9$ Hz), 7.96 (d, 2H, $J = 6.1$ Hz), 7.32 (s, 1H), 7.25 (d, 2H, $J = 7.9$ Hz), 3.99 (t, 4H, $J = 4.2$ Hz), 2.42 (s, 3H, Me), 1.70 (t, 4H, $J = 4.2$ Hz); ^{13}C ($CDCl_3$, 50 MHz): 166.2, 162.9, 162.6, 150.8, 145.3, 141.2, 135.6, 129.8, 127.4, 121.5, 101.4, 45.3, 26.3, 25.4, 21.8. Anal. Calcd for $C_{22}H_{23}N_3$: calculated C: 80.21, H: 7.04, N: 12.76. Found: C: 80.62, H: 7.34, N: 12.51.

6.3.7. 4-(4-Methylsulfanyl-phenyl)-2-piperidin-1-yl-6-pyridin-4-yl-pyrimidine (4g). Yield: 71%; mp 166-168 °C; MS: 362 (M+1); IR (KBr) 2948, 1636, 1584, 1486, 1325, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.74 (d, 2H, $J = 6.0$ Hz), 8.04 (d, 2H, $J = 8.4$ Hz), 7.95 (d, 2H, $J = 6.0$ Hz), 7.36 (s, 1H), 7.32 (d, 2H, $J = 8.4$ Hz), 3.99 (t, 4H, $J = 4.4$ Hz), 1.69 (t, 4H, $J = 4.4$ Hz), 2.54 (s, 3H, SMe); ^{13}C ($CDCl_3$, 50 MHz): 166.2, 163.4, 162.8, 150.8, 145.8, 142.6, 134.5, 127.7, 126.4, 121.4, 101.9, 45.6, 26.5, 25.4, 16.8. Anal. Calcd for $C_{22}H_{23}N_3S$: calculated C: 73.09, H: 6.41, N: 11.62. Found: C: 73.84, H: 6.67, N: 11.41.

6.3.8. 4-(3,4-Dimethyl-phenyl)-2-piperidin-1-yl-6-pyridin-4-yl-pyrimidine (4h). Yield: 66%; mp 160–162 °C; MS: 344 (M+1); IR (KBr) 2926, 1638, 1580, 1480, 1325, 1272 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.79 (d, 2H, $J = 6.1$ Hz), 7.97 (d, 2H, $J = 6.1$ Hz), 7.87 (s, 1H), 7.82 (d, 1H, $J = 6.9$ Hz), 7.42 (s, 1H), 7.23 (d, 1H, $J = 6.9$ Hz), 4.04 (t, 4H, $J = 4.6$ Hz), 2.36 (s, 3H), 2.33 (s, 3H), 1.71 (t, 4H, $J = 4.6$ Hz); ^{13}C ($CDCl_3$, 50 MHz): 166.2, 163.2, 162.8, 150.8, 145.9, 140.2, 137.4, 135.6, 130.5, 128.7, 125.0, 121.6, 102.8, 45.6, 26.5, 25.4, 20.4, 20.2. Anal. Calcd for $C_{23}H_{25}N_3$: calculated C: 80.43, H: 7.34, N: 12.23. Found: C: 80.62, H: 7.49, N: 12.45.

6.3.9. 4-(4-Chloro-phenyl)-2-piperidin-1-yl-6-pyridin-4-yl-pyrimidine (4i). Yield: 68%; mp 196-198 °C; MS: 351 (M+1); IR (KBr) 2948, 1636, 1584, 1486, 1325, 1265 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ (ppm) 8.76 (d, 2H, $J = 6.1$ Hz), 8.04 (d, 2H, $J = 8.1$ Hz), 7.95 (d, 2H, $J = 6.1$ Hz), 7.47 (d, 2H, $J = 8.1$ Hz), 7.36 (s, 1H), 3.99 (t, 4H, $J = 4.5$ Hz), 1.70 (t, 4H, $J = 4.5$ Hz). ^{13}C ($CDCl_3$, 50 MHz): 166.1, 162.8, 161.9, 159.1, 145.8, 135.2, 134.5, 130.4, 128.8, 121.4, 102.1, 45.5, 26.4, 25.4. Anal. Calcd for $C_{21}H_{20}ClN_3$: calculated C: 72.09, H: 5.76, N: 10.13. Found: C: 71. 82, H: 5.52, N: 10.42.

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