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Synthesis, pharmacological and biological screening of some novel pyrimidine derivatives

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Abstract In the present study a new series of 4-substituted phenyl-6-(pyridin-2-yl)pyrimidin-2-ol (**1–9**) and 4-substituted phenyl-6-(pyridin-2-yl)pyrimidin-2-thiol (**10–18**) have been synthesized by cyclizing 3-substituted phenyl-1-(pyridin-2-yl)prop-2-en-1-one with urea/thiourea in the presence of NaOH as base (**1a–18a**). 3-Substituted phenyl-1-(pyridin-2-yl)prop-2-en-1-one was prepared by condensing 2-acetylpyridine with substituted benzaldehyde in the presence of 20 % NaOH as base. The structures of the newly synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopic studies. Newly synthesized compounds were screened for their anti-inflammatory, analgesic, cytotoxic, and antitubercular activities studies. Few of the compounds exhibited excellent anti-inflammatory activities compared to standard drug diclofenac sodium. Some compounds have shown moderate analgesic activities compared to standard drug pentazocine. Also, some compounds have exhibited moderate cytotoxic and antitubercular activities.

Keywords Chalcones · Pyrimidines · Anti-inflammatory · Analgesic · Cytotoxicity · Antitubercular

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Introduction

Pyrimidine itself is not found in nature but substituted pyrimidines and compounds containing the pyrimidine ring are widely distributed in nature. As pyrimidine is a basic nucleus in DNA and RNA, it has been found to be associated with diverse biological activities. Pyrimidine derivatives are well known for the wide spectrum of biological activities such as antimicrobial (Mohamed *et al*., 2010), antitumor (Linyi and Malhotra, 2012; Sherif *et al*., 2009), analgesic (Nofal *et al*., 2009), anti-inflammatory (El-Gazzar *et al*., 2007), antibacterial (Hilmy *et al*., 2010), antifungal (Sun *et al*., 2011), antiplatelet (Bruno *et al*., 2011), and antitubercular (Mohan *et al*., 2010) activities. Many of the pyrimidine derivatives are reported to possess potential CNS depressant properties (Shishoo *et al*., 1981). A number of nonsteroidal anti-inflammatory drugs (NSAIDs) are available clinically to treat inflammatory disorders. The main mechanism of anti-inflammatory action of NSAIDs is by inhibition of prostaglandin synthesis. Cyclooxygenase (COX; prostaglandin G/H synthase, EC 1.14.99.1) catalyzes the first two steps in the biosynthesis of biological mediator prostaglandin, and there lies a great interest in COX-2 as a key therapeutic target for inflammation (Rouzer and Marnett, 2009; McKellar *et al*., 2008; Jankowski and Hunt, 2008). But side effects associated with chronic use of these NSAIDs are gastrointestinal ulcer. Apart from this, there is evidence to suggest that leukotrienes enhance gastric ulceration which limits the therapeutic utilization of these drugs (Osiri and Moreland, 1999; Lombardino, 1985). Pyrimidine heterocycles possessing hydroxyl group has a unique place in medicinal chemistry and also plays a vital role in biological processes as well as synthetic drugs. Prompted by these literatures, we planned to synthesize some novel pyrimidine derivatives derived

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from chalcones and to explore their biological and pharmacological activities.

Herein, the synthesis of some derivatives of some title structure compounds containing pyridine moiety is reported in an attempt to significantly improve the biological spectrum of pyrimidines. All the novel compounds were evaluated for their anti-inflammatory, analgesic, cytotoxic, and antitubercular activities.

Results and discussion

Chemistry

Various substituted 3-substituted phenyl-1-(pyridin-2-yl)prop-2-en-1-ones were prepared as reported in the literature (Babasaheb *et al*., 2010) by the reaction of 2-acetylpyridine and substituted aromatic aldehydes in the presence of 20 % NaOH as base. These compounds were characterized by TLC, melting point, and IR spectra that showed characteristic absorption band at 1,670 cm^{-1} of CH=CH group. The title compounds 4-substituted phenyl-6-(pyridin-2-yl)pyrimidin-2-ol (**1**–**9**) and 4-substituted phenyl-6-(pyridin-2-yl)pyrimidin-2-thiol (**10**–**18**) derivatives were prepared as reported in the literature (Munawar *et al*., 2008) by cyclization of chalcones with urea/thiourea using ethanol in the presence of NaOH as base. The infrared spectra of the substituted pyrimidines **1**–**18** showing characteristic absorption bands at 3,400–3,450 cm^{-1} were attributed to OH and 2,500–2,550 cm^{-1} were attributed to SH group which is absent in the intermediate chalcones. Similarly, the ^{1}H NMR of the synthesized pyrimidines showed a broad singlet at δ 9.6–10.0 due to OH and at δ 9.2–9.9 due to SH group which was absent in the ^{1}H NMR spectra of the substituted chalcones. Hence, the formation of the title compounds pyrimidines was confirmed and further established by ^{13}C NMR and mass spectra which are in accordance with molecular formula.

Anti-inflammatory activity studies

All the synthesized compounds were tested for their anti-inflammatory activities using carrageenan-induced rat hind paw edema method (Winter *et al*., 1962). Data of anti-inflammatory activity were expressed as mean ± SEM, and the student's *t* test was applied to determine the significance of the difference between the control group and rats treated with the test compounds. The anti-inflammatory activity of the newly synthesized compounds was compared with the standard diclofenac sodium 13.5 mg/kg body weight, showing 64.52 % inhibition of rat paw edema whereas the tested compounds showed inhibition ranging from 31.61 to 58.18 % after 120 min. The compounds **1**, **3**, **9**, and **11** containing 4-chlorophenyl, 3-hydroxyphenyl, 2-bromophenyl (pyrimidin-2-ol derivatives), and 4-fluorophenyl (pyrimidin-2-thiol derivatives) exhibited significant anti-inflammatory activities compared to the standard drug diclofenac. The presence of pyrimidine moiety with electron-withdrawing groups like chloro, fluoro, and bromo accounted for the anti-inflammatory activity.

Analgesic activity studies

All the compounds were tested for their analgesic activities by tail-immersion method (Ramabadran *et al*., 1989) and exhibited percentage inhibition in the range of 12.40–147.74 % after 120 min. The compounds **1**, **2**, **3**, **7**, and **12** containing 4-chlorophenyl, 4-fluorophenyl, 3-hydroxyphenyl, 4-methoxyphenyl (pyrimidin-2-ol derivatives), and 3-hydroxyphenyl (pyrimidin-2-thiol derivatives) substituents showed highly potent analgesic activities compared to the standard drug pentazocine, whereas rest of the tested compounds did not show significant analgesic activity in the model used. The presence of pyrimidine moiety with other substitution and groups like fluoro, chloro, and hydroxy has accounted for their analgesic activities.

Cytotoxic activity studies

The test compounds were subjected to in vitro cytotoxicity against Ehrlich ascites carcinoma (EAC) cells using "trypan blue exclusion method" (Louis and Siegel, 2011). The damaged cells are stained blue by trypan blue stain and can be distinguished from viable cells. Compounds **10** and **11** containing 2-chlorophenyl (pyrimidin-2-ol derivatives) and 2-bromophenyl (pyrimidin-2-thiol derivatives) substituents induced the greatest effect on EAC cells with an activity more than 60 % at a concentration of 250 μg/ml. The presence of pyrimidine moiety with other substitution and groups like chloro and fluoro has accounted for their cytotoxic activity.

Antitubercular activity studies

The test compounds were evaluated for their antitubercular activities against *M. tuberculosis* using microplate alamar blue assay (Chauca *et al*., 2007). A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth. This indicates that the test compound has a potent antitubercular activity in in vitro condition. Compounds **6** and **9** containing 3-nitrophenyl and 2-bromophenyl (pyrimidin-2-ol derivatives) substituents induced the greatest effect on bacterial growth with an activity at a concentration of 25 μg/ml. The presence of pyrimidine moiety with other substitutions and groups like nitro and bromo has accounted for their antitubercular activity.

Conclusion

Several 4-substituted phenyl-6-(pyridin-2-yl)pyrimidin-2-ol and 4-substituted phenyl-6-(pyridin-2-yl)pyrimidin-2-thiol were successfully synthesized in 57–76 % yields and are characterized by ^{1}H NMR, mass spectrometry, and IR studies. All the synthesized compounds were screened for their anti-inflammatory, analgesic, cytotoxic, and antitubercular activities. Anti-inflammatory activity was evaluated by carrageenan-induced paw edema method. Compounds **1**, **3**, **9**, and **11** were found to be biologically active whereas the remaining compounds showed poor anti-inflammatory activities. Analgesic activity was evaluated by tail-immersion method. Compounds **1**, **2**, **3**, **7**, and **12** showed highly potent analgesic activities whereas rest of the tested compounds did not show significant analgesic activities. Cytotoxic activity was evaluated by trypan blue exclusion method. Compounds **10** and **11** induced the greatest effect on EAC cells with an activity more than 60 % at a concentration of 250 μg/ml. Antitubercular activity was evaluated by microplate alamar blue assay. Compounds **6** and **9** induced the greatest effect on bacterial growth with an activity at a concentration of 25 μg/ml.

Experimental

Materials and methods

Melting points were determined by open capillary and were uncorrected. The IR spectra (in KBr pellets) were recorded in Tensor 27 spectrophotometer, Bruker optik (Germany). ^{1}H NMR spectra were recorded ($CHCl_3$) on Bruker (400Mz) spectrometer using TMS as an internal standard. Chemical shift values are given in δ scales. The mass spectra were recorded using a Jeol-D-300 mass spectrometer (70 eV), Shimadzu (Japan) by FAB. Elemental analysis was performed using Vairo Elementar Model, C, H, N analyzer. The completion of the reaction was checked by thin-layer chromatography (TLC) on silica gel-G-coated plates using ethylacetate:chloroform (9:1) as the solvent and observed in UV light. Commercial-grade solvents and reagents were used without further purification.

General procedure for the synthesis of 3-substituted phenyl-1-(pyridin-2-yl)prop-2-en-1-one (**1a**–**18a**)

A mixture of 2-acetylpyridine (0.01 mol) and substituted benzaldehyde (0.01 mol) in absolute ethanol (20 ml) were stirred together for 24 h in the presence of 20 % NaOH (3-4 ml). The reaction mixture was poured into crushed ice and acidified with HCl. The separated solid was filtered, washed with water, and recrystallized from ethanol.

General procedure for the synthesis of 4-substituted phenyl-6-(pyridin-2-yl)pyrimidin-2-ol and 2-thiol **(1–18)**

A mixture of 3-substituted phenyl-1-(pyridin-2-yl)prop-2-en-1-one **(1a–18a)** (0.01 mol) and urea/thiourea was dissolved in 20 ml ethanol in the presence of 4 ml 20 % NaOH and the reaction mixture was refluxed for about 18 h. After the completion of the reaction, the reaction mixture was poured into crushed ice. The separated solid was filtered, washed with cold water, and recrystallized from ethanol (Fig. 1).

*3-(2-Chlorophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (**1a**)*

Yellowish needles (EtOH). This compound was prepared by condensing 2-acetylpyridine with substituted benzaldehyde in the presence of NaOH. It was obtained as a yellow solid; mp 75–77 °C; UV (EtOH) λ_{max} (log ε) 270 (4.25) nm; IR (KBr) ν_{max} 1,506(Ar C=C str), 3,120(Ar C–H bending), 1,690(C=O str), 2,900(aliphatic C–H str), 785(C–Cl str), 1,680(Ar C=N str) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.9 (1H, d, $J = 15.1$ Hz, 3H), 7.1 (1H, d, $J = 15.1$ Hz, 2H), δ 7.2–7.6 (8H, m, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz): 187(C=O, C-1), 121.3, 145.1(CH=CH, C-2, C-3), 134(C, C-2′), 129.9, 129.3, 126.7, 127.8, 120.7, 137.3, 128, 149.7(8 CH aromatic, C-3′, C-4′, C-5′, C-6′ of 2-chlorophenyl, C-3″, C-4″, C-5″, C-6″ of pyridine), 133, 153.3(2C aromatic, C-1′, C-2″); MS: *m/z*: 245 (M+1); Anal. calcd. for $C_{14}H_{11}ClON$: C, 68.70; H, 4.53; N, 5.73. Found: C, 68.62; H, 4.51; N, 5.86.

*3-(4-Fluorophenyl)-1-(pyridin-2-yl)prop-2-en-1-one (**2a**)*

Green needles (EtOH). This compound was prepared by condensing 2-acetylpyridine with substituted benzaldehyde in the presence of NaOH. It was obtained as a green solid; mp 82–84 °C; UV (EtOH) λ_{max} (log ε) 275 (4.28) nm; IR (KBr) ν_{max} 1,502(Ar C=C str), 3,120(Ar C–H bending), 1,692(C=O str), 2,900(aliphatic C–H str), 1,221(C–Br str), 1,680(Ar C=N str) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.9 (1H, d, $J = 15.1$ Hz, H-3), 7.1 (1H, d, $J = 15.1$ Hz, H-2), δ 7.2–7.5 (8H, m, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz): 187(C=O, C-1), 121.3, 145.1(CH=CH, C-2, C-3), 162.1(C, C-4′), 130.4, 115.4, 130.4, 130.8, 137.3, 128, 128, 149.7(8 CH aromatic, C-2′, C-3′, C-5′, C-6′ of 4-fluorophenyl, C-3″, C-4″, C-5″, C-6″ of pyridine), 130.8, 153.3(2C aromatic, C-1′, C-2″); MS: *m/z*: 228 (M+1); Anal. calcd. for $C_{14}H_{11}FON$: C, 73.66; H, 4.86; N, 6.14. Found: C, 73.12; H, 4.88; N, 6.18.

*3-(3-Hydroxyphenyl)-1-(pyridin-2-yl)prop-2-en-1-one (**3a**)*

Dark-brown needles (EtOH). This compound was prepared by condensing 2-Acetylpyridine with substituted benzaldehyde

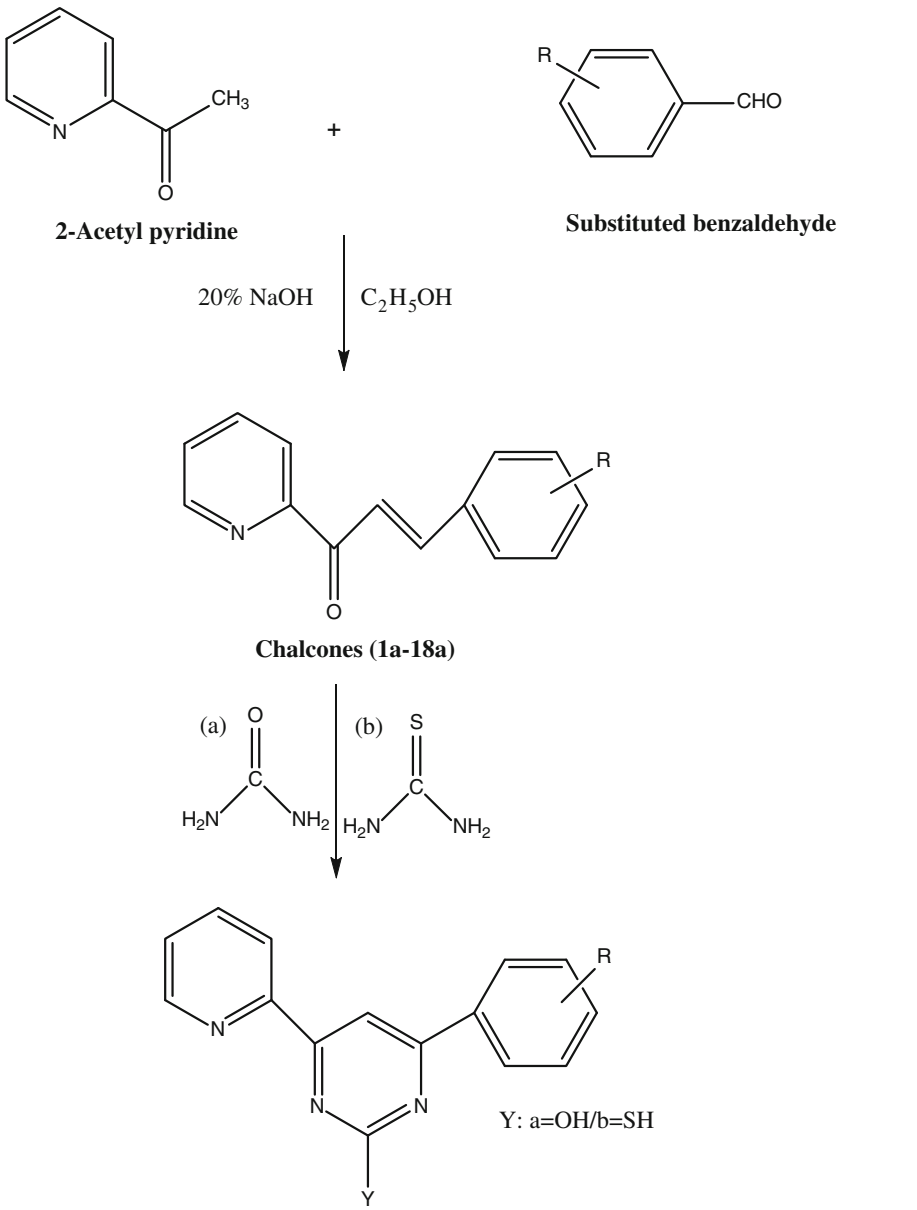

Fig. 1 Scheme for pyrimidine derivatives

R= 4Cl, 4F, 3-OH, 4-N$(CH_3)_2$, 4-CH_3, 3-NO_2, 4-OCH_3, 4-CN, 2Br, 2-Cl

in the presence of NaOH. It was obtained as a dark-brown solid; mp 105–107 °C; UV (EtOH) λ_{max} (log ε) 265 (4.23) nm; IR (KBr) ν_{max} 1,509(Ar C=C str), 3,125(Ar C–H bending), 1,690(C=O str), 2,912(aliphatic C–H str), 3,420(O–H str), 1,680(Ar C=N str) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 6.9 (1H, d, J = 15.1 Hz, H-3), 7.1 (1H, d, J = 15.2 Hz, H-2), δ 7.2–7.6 (8H, m, Ar–H); ^{13}C NMR ($CDCl_3$, 300 MHz): 187(C=O, C-1), 121.3, 145.1(CH=CH, C-2, C-3), 162.1(C, C-3′), 117.6, 115.1, 130, 121, 120.7 137.3, 128, 149.7(8 CH aromatic, C-2′, C-4′, C-5′, C-6′ of 3-hydroxyphenyl, C-3″, C-4″, C-5″, C-6″ of pyridine), 135.4, 153.3(2C aromatic, C-1′, C-2″); MS: *m/z*: 216 (M^+); Anal. calcd. for $C_{14}H_{12}O_2N$: C, 74.31; H, 5.35; N, 6.19. Found: C, 74.21; H, 5.33; N, 6.23.

4-(2-Chlorophenyl)-6-(pyridin-2-yl)pyrimidin-2-ol ***(1)***

Yellow needles (EtOH). This compound was obtained by condensing substituted 2-chloro chalcones with urea. It was obtained as a yellow solid; mp 128–130 °C; yield 72 %; IR (KBr) ν_{max} 1,687(C=N str), 3,058(aromatic C–H str), 1,589(aromatic C=C str), 778 (C–Cl str), 1,350(C–N str), 3,419(O–H str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.51–7.92 (m,

9H, Ar–H), 9.73 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 159.6 (C, C-2), 132.6 (C, C-2″), 93.4, 124.0, 128.9, 130.1, 130.6, 132.5, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-5′, C-6″, C-4″, C-5″, C-3″, C-4′, C-2′, C-6′), 129.9, 133, 155.1, 159.1 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 284 (M+2); Anal. calcd. for $C_{15}H_{11}ClN_3O$: C, 63.27; H, 3.87; N, 14.76. Found: C, 63.19; H, 3.82; N, 14.81.

4-(4-Fluorophenyl)-6-(pyridin-2-yl)pyrimidin-2-ol ***(2)***

Green needles (EtOH). This compound was obtained by condensing substituted 4-fluoro chalcones with urea. It was obtained as a green solid; mp 99–102 °C; yield 75 %; IR (KBr) ν_{max} 1,680(C=N str), 3,058(Ar C–H str), 1,582(Ar C=C str), 1,224(C–F str), 1,346(C–N str), 3,410(O–H str) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.31–7.87 (m, 9H, Ar–H), 9.82 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 159.6 (C, C-2), 162.9 (C, C-4″), 93.4, 116.0, 124.0, 130.6, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-3″, C-5″, C-5′, C-2″, C-6″, C-4′, C-2′, C-6′), 131.4, 133, 155.1, 159.1 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 268 (M+1); Anal. calcd. for $C_{15}H_{11}FN_3O$: C, 67.21; H, 4.11; N, 15.68. Found: C, 67.30; H, 4.08; N, 15.71.

4-(3-Hydroxyphenyl)-6-(pyridin-2-yl)pyrimidin-2-ol ***(3)***

Dark-brown needles (EtOH). This compound was obtained by condensing substituted 3-hydroxy chalcones with urea. It was obtained as a dark-brown solid; mp 90–92 °C; yield 74 %; IR (KBr) ν_{max} 1,683(C=N str), 3,045(Ar C–H str), 1,572(Ar C=C str), 1,350(C–N str), 3,425(O–H str), 1,350(C–N str) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.43–7.91 (m, 9H, Ar–H), 9.83 (s, 2H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 159.6 (C, C-2), 138.9 (C, C-3″), 93.4, 124.0, 124.5, 129, 129.1, 130.4, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-5′, C-6″, C-4″ C-5″, C-2″, C-4′, C-2′, C-6′), 132.9, 133, 155.1, 159.1 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 266 (M^+); Anal. calcd. for $C_{15}H_{12}N_3O$: C, 67.67; H, 4.51; N, 15.80. Found: C, 67.67; H, 4.62; N, 15.89.

4-(4-(Dimethylamino)phenyl)-6-(pyridin-2-yl)pyrimidin-2-ol ***(4)***

Dark-brown needles (EtOH). This compound was obtained by condensing substituted 4-dimethylamino chalcones with urea. It was obtained as a dark-brown solid; mp 145-147 °C; yield 60 %; IR (KBr) ν_{max} 1,678(C=N str), 3,053(Ar C–H str), 1,560(Ar C=C str), 1,338(C–N str), 2,955(aliphatic C–H str), 3,420(O–H str) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.42–7.87 (m, 9H, Ar–H), 9.87 (s, 1H, H-2), 4.83 (s, 6H, H-4″); ^{13}C NMR ($CDCl_3$, 300 MHz): 159.6 (C, C-2), 155.3 (C, C-4″), 93.4, 112.7, 124.0, 128.4, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-3″, C-5″, C-5′, C-2″, C-6″, C-4′, C-2′, C-6′), 125.3, 133, 155.1, 159.1 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 280 M^+); Anal. calcd. for $C_{17}H_{17}N_4O$: C, 69.62; H, 5.80; N, 19.11. Found: C, 69.69; H, 5.72; N, 19.16.

4-(pyridin-2-yl)-6-p-tolyl-pyrimidin-2-ol ***(5)***

Clay-colored needles (EtOH). This compound was obtained by condensing substituted 4-methyl chalcones with urea. It was obtained as a clay-colored solid; mp 86-88 °C; yield 70 %;IR (KBr) ν_{max} 1,682(C=N str), 1,350(C–N aromatic), 3,105(Ar C–H str), 2,985(aliphatic CH str), 3,415(O–H str), 1,572(C=C str) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.11–7.82 (m, 9H, Ar–H), 4.73 (s, 3H, H-4″), 9.91 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 131.7 (C, C-2), 132.6 (C, C-4″), 93.4, 123.2, 124.0, 129.5, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-2″, C-6″, C-3″, C-5″ C-5′, C-4′, C-2′, C-6′), 132.8, 133, 155.1, 159.1 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 264 (M^+); Anal. calcd. for $C_{16}H_{14}N_3O$: C, 72.73; H, 5.80; N, 15.91. Found: C, 72.79; H, 5.39; N, 15.88.

4-(3-Nitrophenyl)-6-(pyridin-2-yl)pyrimidin-2-ol ***(6)***

Brown needles (EtOH). This compound was obtained by condensing substituted 2-chloro chalcones with urea. It was obtained as a brown solid; mp 154–156 °C; yield 68 %; IR (KBr) ν_{max} 1,691(C=N str), 1,354(C–N str), 3,095(Ar C–H str), 3,410(O–H str), 1,565(Ar C=C str) cm^{-1}; ^{1}H NMR (CDCl3): δ 7.12–7.65 (m, 9H, Ar–H), 9.87 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 159.6 (C, C-2), 148.4 (C, C-3″), 93.4, 103.5, 122.7, 123.9, 124.0, 129.5, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-2″, C-5″, C-4″, C-5′, C-6″, C-4′, C-2′, C-6′), 136.8, 133, 155.1, 159.1 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 334 (M^+); Anal. calcd. for $C_{15}H_{11}N_4O_3$: C, 61.02; H, 3.73; N, 18.98. Found: C, 61.12; H, 3.81; N, 18.91.

4-(4-Methoxyphenyl)-6-(pyridin-2-yl)pyrimidin-2-ol ***(7)***

White needles (EtOH). This compound was obtained by condensing substituted 4-methoxy chalcones with urea. It was obtained as a white solid; mp 80–82 °C; yield 76 %; IR (KBr) ν_{max} 1,682(C=N str), 1,350(C–N str), 3,090(Ar C–H str), 3,415(O–H str), 1,560(Ar C=C str), 2,980(aliphatic C–H str) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.13–7.86 (m, 9H, Ar–H), 9.83 (s, 1H, H-2), 3.85 (s, 3H, H-4″); ^{13}C NMR ($CDCl_3$, 300 MHz): 159.6 (C, C-2), 132.6 (C, C-2′), 93.4, 114.8, 124.0, 128.5, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-3″, C-5″, C-5′, C-2″, C-6″, C-4′, C-2′, C-6′), 128.1, 133, 155.1, 159.1 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 278 (M+1); Anal. calcd. for $C_{16}H_{14}N_3O_2$: C, 68.57; H, 5.01; N, 15.00. Found: C, 68.61; H, 5.09; N, 15.12.

4-(2-Hydroxy-6-(pyridin-2-yl)pyrimidin-4-yl)benzonitrile ***(8)***

White needles (EtOH). This compound was obtained by condensing substituted 4-cyano chalcones with urea. It was obtained as a yellow solid; mp 150–152 °C; yield 61; IR (KBr) ν_{max} 1,690(C=N str), 1,360(C–N str), 3,082(Ar C–H str), 3,420(O–H str), 1,555(Ar C=C str), 1,350(aliphatic C–N str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.51–7.89 (m, 9H, Ar–H), 9.94 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 159.6 (C, C-2), 112.6 (C, C-4″), 93.4, 124.0, 126.1, 132.7, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-5′, C-2″, C-6″, C-3″, C-5″, C-4′, C-2′, C-6′), 140.1, 133, 155.1, 159.1 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 289 (M^+); Anal. calcd. for $C_{16}H_{11}N_4O$: C, 69.82; H, 4.00; N, 20.36. Found: C, 69.91; H, 4.09; N, 20.425.

4-(2-Bromophenyl)-6-(pyridin-2-yl)pyrimidin-2-ol ***(9)***

Green needles (EtOH). This compound was obtained by condensing substituted 2-bromo chalcones with urea. It was obtained as a green solid; mp 110–112 °C; yield 69 %; IR (KBr) ν_{max} 1,678(C=N str), 1,365(C–N str), 3,082(Ar C–H str), 3,425(O–H str) 1,562(Ar C=C str), 675 (C–Br str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.13–7.97 (m, 9H, Ar–H), 9.68 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 159.6 (C, C-2), 132.6 (C, C-2″), 93.4, 124.0, 128.2, 129.7, 130.9, 132.1, 134.0, 147.5 149.7 (9 CH aromatic, C-5, C-5′, C-5″, C-6″, C-4″, C-3″, C-4′, C-2′, C-6′), 139.8, 133, 155.1, 159.1 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 330 (M+1); Anal. calcd. for $C_{15}H_{11}ON_3Br$: C, 46.27; H, 3.34; N, 12.77. Found: C, 46.32; H, 3.39; N, 12.82.

4-(2-Chlorophenyl)-6-(pyridin-2-yl)pyrimidin-2-thiol ***(10)***

Green needles (EtOH). This compound was obtained by condensing substituted 2-chloro chalcones with thiourea. It was obtained as a reddish solid; mp 87–89 °C; yield 74 %; IR (KBr) ν_{max} 1,690(C=N str), 1,370(C–N str), 2,052(S–H str), 3,080(Ar C–H str), 1,572(Ar C=C str), 770(C–Cl str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.22–7.96 (m, 9H, Ar–H), 9.37 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 180.2 (C, C-2), 132.2 (C, C-2″), 108.3, 124.0, 128.9, 130.1, 130.6, 132.5, 134.0, 147.5, 147.9 (9 CH aromatic, C-5, C-5′, C-6″, C-4″, C-5″, C-3″, C-4′, C-2′, C-6′), 129.9, 133, 163.3, 161 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 302 (M+2); Anal. calcd. for $C_{15}H_{11}ClN_3S$: C, 59.90; H, 3.66; N, 13.98. Found: C, 59.96; H, 3.71; N, 13.91.

4-(4-Fluorophenyl)-6-(pyridin-2-yl)pyrimidin-2-thiol ***(11)***

Green needles (EtOH). This compound was obtained by condensing substituted 4-fluoro chalcones with thiourea. It was obtained as a green solid, mp 106–108 °C, yield 70 %. IR (KBr) ν_{max} 1,690(C=N str), 3,055(Ar C–H str), 1,585(Ar C=C str), 1,220(C–F str), 1,342(C–N str), 2,046(S–H str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.23–7.95 (m, 9H, Ar–H), 9.47(s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 180.2 (C, C-2), 162.9 (C, C-4″), 108.3, 116.0, 124.0, 130.6, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-5′, C-3″, C-5″, C-2″, C-6″, C-4′, C-2′, C-6′), 131.4, 133, 165, 161 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 284 (M+1); Anal. calcd. for $C_{15}H_{11}FN_3S$: C, 63.40; H, 3.87; N, 14.79. Found: C, 63.48; H, 3.92; N, 14.68.

4-(3-Hydroxyphenyl)-6-(pyridin-2-yl)pyrimidin-2-thiol ***(12)***

Dark-brown needles (EtOH). This compound was obtained by condensing substituted 3-hydroxy chalcones with thiourea. It was obtained as a dark-brown solid; mp 120–122 °C; yield 65 %; IR (KBr) ν_{max} 1,679(C=N str), 3,052(Ar C–H str), 1,572(Ar C=C str), 1,340(C–N str), 3,420(O–H str), 2,046(S–H str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.23-7.95 (m, 9H, Ar–H), 9.86 (s, 1H, H-3″), 9.47 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 180.2 (C, C-2), 157.5 (C, C-3″), 108.3, 115.9, 120.1, 124.0, 130.6, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-2″, C-4″, C-6″, C-5′, C-5″, C-4′, C-2′, C-6′), 138.3, 133, 165, 161 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 282 (M^+); Anal. calcd. for $C_{15}H_{12}N_3SO$: C, 63.82; H, 4.25; N, 1,489. Found: C, 63.89; H, 3.92; N, 14.68.

4-(4-(Dimethylamino)phenyl)-6-(pyridin-2-yl)pyrimidin-2-thiol ***(13)***

Dark-brown needles (EtOH). This compound was obtained by condensing substituted 4-dimethylamino chalcones with thiourea. It was obtained as a dark-brown solid; mp 110–112 °C; yield 58 %. IR (KBr) ν_{max} 1,684(C=N str), 3,062(Ar C–H str), 1,569(Ar C=C str), 1,345(C–N str), 2,061(S–H str), 2,962(aliphatic C–H str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.12–7.76 (m, 9H, Ar–H), 9.97(s, 1H, H-2), 4.83 (s, 6H, H-4″); ^{13}C NMR ($CDCl_3$, 300 MHz): 180.2 (C, C-2), 155.3 (C, C-4″), 108.3, 112.7, 124.0, 128.4, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-3″, C-5″, C-5′, C-2″, C-6″, C-4′, C-2′, C-6′), 125.3, 133, 165, 161 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 309 (M^+); Anal. calcd. for $C_{17}H_{17}N_4S$: C, 63.02; H, 5.50; N, 18.12. Found: C, 66.12; H, 5.59; N, 18.09.

4-(Pyridin-2-yl)-6-p-tolyl-pyrimidin-2-thiol ***(14)***

Yellow needles (EtOH). This compound was obtained by condensing substituted 4-methyl chalcones with thiourea. It was obtained as a yellow solid, mp 160–162 °C; yield 69 %; IR (KBr) ν_{max} 1,682(C=N str), 1,360(C–N str) 3,090(Ar C–H str), 2,062(S–H str), 2,975(aliphatic C–H str), 1,570(Ar

C=C str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.13–7.78 (m, 9H, Ar–H),4.63 (s, 6H, H-4″), 9.54 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 180.2 (C, C-2), 131.7 (C, C-4″), 108.3, 123.2, 124.0, 129.5, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-2″, C-6″, C-5′, C-3″, C-5″, C-4′, C-2′, C-6′), 132.8, 133, 165, 161 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 280 (M^+); Anal. calcd. for $C_{16}H_{14}N_3S$: C, 68.57; H, 5.00; N, 15.00. Found: C, 68.51; H, 5.12; N, 15.13.

4-(3-Nitrophenyl)-6-(pyridin-2-yl)pyrimidin-2-thiol ***(15)***

Yellow needles (EtOH). This compound was obtained by condensing substituted 3-nitro chalcones with thiourea. It was obtained as a black solid; mp 101–103 °C; yield 57 %; IR (KBr)) ν_{max} 1,685(C=N str), 1,350(C–N str), 3,090(Ar C–H str), 2,065(S–H str), 1,562(Ar C=C str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.13–7.73 (m, 9H, Ar–H), 9.46 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 180.2 (C, C-2), 148.4 (C, C-3″), 103.5, 108.3, 122.7, 123.9 124.0, 129.5, 134.0, 147.5, 149.7 (9 CH aromatic, C-2″, C-5, C-5″, C-4″, C-5′, C-6″, C-4′, C-2′, C-6′), 136.8, 133, 165, 161 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 311 (M^+); Anal. calcd. for $C_{15}H_{14}N_4O_2S$: C, 57.32; H, 4.45; N, 17.83. Found: C, 57.19; H, 4.29; N, 17.80.

4-(4-Methoxyphenyl)-6-(pyridin-2-yl)pyrimidin-2-thiol ***(16)***

Yellow needles (EtOH). This compound was obtained by condensing substituted 4-methoxy chalcones with thiourea. It was obtained as a white solid; mp 90–92 °C; yield 70 %; IR (KBr) ν_{max} 1,690(C=N str), 1,345(C–N str), 2,054(S–H str), 3,085(Ar C–H str), 1,554(Ar C=C str), 2,974(aliphatic C–H str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.23–7.95 (m, 9H, Ar–H), 9.48 (s, 1H, H-2), 3.81 (s, 3H, H-4″); ^{13}C NMR ($CDCl_3$, 300 MHz): 180.2 (C, C-2), 160.6 (C, C-4″), 108.3, 114.8, 124.0, 128.5, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-3″, C-5″, C-5′, C-2″, C-6″, C-4′, C-2′, C-6′), 128.1, 133, 165, 161 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 296 (M^+); Anal. calcd. for $C_{16}H_{14}N_3SO$: C, 64.86; H, 4.73 N, 14.19. Found: C, 64.79; H, 4.69; N, 14.77.

4-(2-Mercapto-6-(pyridin-2-yl)pyrimidin-4-yl)benzonitrile ***(17)***

Yellow needles (EtOH). This compound was obtained by condensing substituted 4-cyano chalcones with thiourea. It was obtained as a yellow solid; mp 150–152 °C; yield 65 %; IR (KBr) ν_{max} 1,690(C=N str), 1,358(Ar C–H str), 2,065(S–H str), 3,085(Ar C–H str), 1,575(Ar C=C str), 1,345(aliphatic C–N str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.13–7.85 (m, 9H, Ar–H), 9.57 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 180.2 (C, C-2), 112.6 (C, C-4″), 108.3 124.0, 126.1, 132.7, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-5′, C-2″, C-6″, C-3″, C-5″, C-4′, C-2′, C-6′), 140.1, 133, 165, 161 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 291(M^+); Anal. calcd. for $C_{16}H_{11}N_4S$: C, 65.98; H, 3.78; N, 19.24. Found: C, 65.88; H, 3.84; N, 19.36.

4-(2-Bromophenyl)-6-(pyridin-2-yl)pyrimidin-2-thiol ***(18)***

Yellow needles (EtOH). This compound was obtained by condensing substituted 2-bromo chalcones with thiourea. It was obtained as a green solid; mp 120–122 °C; yield 71 %; IR (KBr) ν_{max} 1,685(C=N str), 1,360(Ar C–H str), 2,058(S–H str), 3,075(Ar C–H str), 1,562(Ar C=C str), 668(C–Br str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.13–7.68 (m, 9H, Ar–H), 9.76 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$, 300 MHz): 180.2 (C, C-2), 120.2 (C, C-2″), 108.2, 124.0, 128.2, 129.7, 130.9, 132.1, 134.0, 147.5, 149.7 (9 CH aromatic, C-5, C-5′, C-5″, C-6″, C-4″, C-3″, C-4′, C-2′, C-6′), 139.8, 133, 163.3, 161 (4C aromatic, C-1″, C-3′, C-4, C-6); MS: *m/z* 345 (M^+); Anal. calcd. for $C_{15}H_{11}N_3SBr$: C, 52.17; H, 3.19; N, 12.17. Found: C, 52.24; H, 3. 23; N, 12.29.

Anti-inflammatory activity

The anti-inflammatory activities of the test compounds was carried out using carrageenan-induced rat paw edema model according to Winter *et al*. (1962) by employing 1 % carrageenan solution as a phlogistic agent. Edema was induced in the left hind paw of Wistar rats (150–200 g) of either sex by the subplantar injection of 0.1 ml of 1 % carrageenan in distilled water. Each group composed of six animals. The animals which were bred in our laboratory were housed under standard conditions and received a diet of commercial food pellets and water ad libitum during the maintenance but they were entirely fasted during the experiment period. Our studies were conducted in accordance with recognized guidelines on animal experimentation. The test compounds were given intraperitoneally 30 min after carrageenan injection. Diclofenac sodium was taken as the standard at a dose of 10 mg/kg body weight (p.o). The rat paw volume was measured after 1, 2, 3, and 4 h, respectively, after carrageenan injection by Plethysmometer. The difference between the paw volume at 4 h and 0 h measurement was calculated and taken as edema volume. Percentage inhibition in the paw edema was calculated using the formula,

$$\% \text{ Edema inhibition} = 100(1 - V_t/V_c),$$

where V_t represents mean increase in paw volume of test and V_c represents mean increase in paw volume of control.

Statistical analysis

All the experimental groups were composed of six animals. Data obtained from animal experiments were expressed as

Table 1 Anti-inflammatory activities of compounds (**1–18**) by carrageenan-induced paw edema in rats

Treatment	Dose (mg/kg)	Increase in the paw volume (ml)			
		1 h	2 h	3 h	4 h
Control	Vehicle	0.2827 ± 0.0318	0.3716 ± 0.0315	0.385 ± 0.0209	0.3867 ± 0.0223
Diclofenac sodium	10	0.1083 ± 0.0296** (61.69)	0.1217 ± 0.0282** (67.24)	0.1362 ± 0.0144** (64.62)	0.1372 ± 0.0145** (64.52)
1	50	0.2067 ± 0.0164 (29.13)	0.1927 ± 0.0145** (48.84)	0.47 ± 0.01** (50.23)	0.54 ± 0.01** (57.33)
2	50	0.1817 ± 0.1817* (37.70)	0.2805 ± 0.0065 (31)	0.2667 ± 0.0098 (30.72)	0.2633 ± 0.0066** (31.61)
3	50	0.1817 ± 0.00094* (37.70)	0.165 ± 0.0076** (56.19)	0.1928 ± 0.0263** (49.92)	0.1617 ± 0.0256** (58.18)
4	50	0.1912 ± 0.0013 (32.36)	0.2013 ± 0.0066 (45.82)	0.2517 ± 0.020 (34.62)	0.22 ± 0.07 (43.10)
5	50	0.1932 ± 0.12 (31.65)	0.1987 ± 0.021 (29.71)	0.22 ± 0.066 (42.85)	0.172 ± 0.067 (55.52)
6	50	0.1617 ± 0.11 (42.80)	0.165 ± 0.063 (55.59)	0.1809 ± 0.022 (53.01)	0.1617 ± 0.023 (51.18)
7	50	0.2067 ± 0.0164 (29.13)	0.2805 ± 0.0065 (24.51)	0.2667 ± 0.0098 (30.72)	0.172 ± 0.067 (55.52)
8	50	0.1912 ± 0.03 (32.36)	0.2613 ± 0.001 (29.68)	0.242 ± 0.064 (37.14)	0.231 ± 0.038 (40.26)
9	50	0.1927 ± 0.0094* (31.83)	0.1752 ± 0.0076** (17.54)	0.188 ± 0.0163** (51.16)	0.162 ± 0.0256** (58.03)
10	50	0.1928 ± 0.0263** (49.92)	0.2667 ± 0.0098 (30.72)	0.231 ± 0.038 (40.10)	0.201 ± 0.0016 (47.29)
11	50	0.1912 ± 0.02 (32.08)	0.175 ± 0.0076** (52.90)	0.1912 ± 0.0263** (50.33)	0.1617 ± 0.0256** (58.18)
12	50	0.1932 ± 0.12 (31.65)	0.1809 ± 0.022 (51.31)	0.22 ± 0.07 (42.85)	0.213 ± 0.0064 (44.91)

All the values are expressed as mean ± SEM ($n = 6$)

* $P < 0.05$ significant compared to control

** $P < 0.01$ significant compared to control

mean ± SEM. The statistical significance of difference between groups was assessed by means of analysis of variance (ANOVA) followed by Dunnett's test. The results of the anti-inflammatory studies are presented in Table 1.

Analgesic activity

All the test compounds were tested for their analgesic activities using tail-immersion method. The animals were weighed and numbered with different identification marks. Animals were divided into ten different groups containing six of each. Albino rats of either sex weighing between 150 and 200 g were used for the experiment. pentazocine at the dose 10 mg/kg body weight (oral) was used as standard which showed percentage analgesic activity of 142.48 %. The lower 5 cm of the tail of animals was marked and this part of the tail was immersed in a cup of freshly filled water at 55 °C. Within few seconds the rat reacts by withdrawing the tail. A withdrawal timing of more than 6 s was regarded as positive response. The reaction time of animals in warm water at 0, 30, 60, 90, and 120 min after the treatment was noted. Increase or decrease in reaction time of the test substance was then compared to that of the standard-drug-treated and solvent-treated animals. The percentage increase or decrease in reaction time at each time interval was calculated as a percentage increase in reaction time = $(R_t/R_c) - 1 \times 100$, where R_t is the mean reaction time of the treated group; R_c is the mean reaction time of the control group. All the values are expressed as mean ± SEM, and percentage analgesic activity shown by the tested compounds are presented in Table 2.

Cytotoxic activity

All the test compounds were studied for short-term in vitro cytotoxicity against EAC cells. The tumor cells aspirated from peritoneal cavity of tumor-bearing mice was washed thrice with normal saline and checked for viability using trypan blue exclusion method. The cell suspension (1 million cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds and volume was made up to 1 ml using phosphate-buffered saline. Control tubes contained only cell suspension. The assay mixtures were incubated for 3 h at 37 °C and then percent of dead cells was evaluated by trypan blue exclusion method. The results of the cytotoxicity studies are presented in Table 3.

Antitubercular activity

The antitubercular activities of the test compounds were assessed against *Mycobacterium tuberculosis* using microplate alamar blue assay. This methodology is non-toxic, uses a thermally stable reagent, and shows good correlation with proportional and BACTEC radiometric method. 200 µl of sterile deionized water was added to all the outer-perimeter wells of sterile 96-well plate to minimize evaporation of medium in the test wells during

Table 2 Analgesic activities of compounds (**1–18**) by tail-immersion method in rats

Treatment	Dose (mg/kg)	Reaction time in sec at time (min)				
		0 min	30 min	60 min	90 min	120 min
Control	–	2.42 ± 0.01	2.49 ± 0.02	2.58 ± 0.03	2.56 ± 0.02	2.66 ± 0.03
Pentazocine	10	2.32 ± 0.01	4.92 ± 0.02* (130.5)	8.14 ± 0.02* (215.50)	8.19 ± 0.01* (219.92)	6.45 ± 0.02** (142.48)
1	50	2.17 ± 0.05	3.82 ± 0.01* (53.41)	6.26 ± 0.01** (142.63)	6.99 ± 0.04** (173.04)	5.43 ± 0.02* (104.13)
2	50	2.66 ± 0.02	3.27 ± 0.14 (31.32)	4.12 ± 0.05* (59.68)	6.32 ± 0.06** (146.87)	5.9 ± 0.04** (121.80)
3	50	2.64 ± 0.03	3.69 ± 0.02* (48.19)	4.12 ± 0.06* (59.68)	6.88 ± 0.03* (168.75)	6.25 ± 0.03** (134.96)
4	50	2.03 ± 0.04	2.31 ± 0.03* (7.22)	3.2 ± 0.04 (24.03)	3.4 ± 0.02 (32.8)	2.26 ± 0.03 (15.03)
7	50	2.47 ± 0.03	3.88 ± 0.01* (55.82)	7.52 ± 0.03** (191.47)	7.18 ± 0.04** (180.46)	6.59 ± 0.03** (147.74)
8	50	2.82 ± 0.06	3.12 ± 0.04 (25.30)	3.10 ± 0.06 (20.15)	4.26 ± 0.03* (66.40)	2.33 ± 0.03 (12.40)
9	50	2.34 ± 0.02	3.66 ± 0.02* (46.98)	5.87 ± 0.03** (127.51)	7.58 ± 0.02** (196.09)	3.80 ± 0.01* (42.85)
10	50	2.12 ± 0.02	2.42 ± 0.03 (2.81)	2.77 ± 0.03 (7.36)	3.69 ± 0.02** (44.14)	3.05 ± 0.03 (14.66)
12	50	2.55 ± 0.03	4.23 ± 0.03* (69.87)	7.45 ± 0.03** (188.75)	7.21 ± 0.03** (181.64)	6.2 ± 0.01** (133.08)

All the values are expressed as mean ± SEM ($n = 6$)

*$P < 0.05$ significant compared to control

** $P < 0.01$ significant compared to control

Table 3 Cytotoxic activities of compounds (**1–18**) by trypan blue exclusion method

Compounds	No. of dead cells (%) at different concentrations (μg/ml)			
	50	100	200	250
Control	–			
1	24	32	52	59
2	25	30	54	60
6	21	30	44	51
10	24	33	57	64
11	18	22	42	69

Table 4 Antitubercular activities of compounds (**1–18**) by micro-plate alamar blue assay

Compounds	Conc. (μg/ml)									
	100	50	25	12.5	6.25	3.125	1.6	0.8	0.4	0.2
Isoniazid (standard)	R	R	R	R	R	R	R	S	R	R
1	S	S	R	R	R	R	R	R	R	R
2	S	S	R	R	R	R	R	R	R	R
6	S	S	R	R	R	R	R	R	R	R
9	S	S	R	R	R	R	R	R	R	R
10	S	S	S	R	R	R	R	R	R	R
11	S	S	S	R	R	R	R	R	R	R

R resistant, *S* sensitive

incubation. The 96-well plate received 100 μl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations of the tested compounds were 0.01–20.0 μl/ml. The plates were covered and sealed with parafilm and incubated at 37 °C for 5 days. After this, 25 μl of freshly prepared 1:1 mixture of alamar blue reagent and 10 % tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth and pink color was interpreted as growth. The minimum inhibitory concentration was defined as the lowest drug concentration which prevented the color change from blue to pink. The results of the antitubercular studies are presented in Table 4.

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