A Study of Anti-inflammatory and Analgesic Activity of New 2,4,6-Trisubstituted Pyrimidines

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Chalcone derivatives (3a—m) were prepared by condensing 4-aminoacetophenone with various substituted aromatic and hetero aromatic aldehydes according to Claisen–Schmidt condensation. These chalcones, on reaction with guanidine hydrochloride under basic alcoholic conditions gave 2,4,6-trisubstituted pyrimidines (5a—m) in quantitative yields. All the newly synthesized pyrimidines were characterized by means of IR, ^{1}H- and ^{13}C-NMR, Electron Ionization (EI)-mass and elemental analyses and screened for anti-inflammatory and analgesic activities by *in vivo*. 2-Amino-4-(4-aminophenyl)-6-(2,4-dichlorophenyl)pyrimidine (5b) and 2-amino-4-(4-aminophenyl)-6-(3-bromophenyl) pyrimidine (5d) were found to be the most potent anti-inflammatory and analgesic activity compared with ibuprofen, reference standard. And also it was found that compound 5b identified as lead structure among all in both the activities. Pyrimidines which showed good anti-inflammatory activity also displayed better analgesic activity.

Key words pyrimidine; anti-inflammatory activity; analgesic activity; chalcone

Chalcones have been very attractive starting materials in medicinal chemistry from the initial years: they are easy to prepare with large variability at the two aromatic rings and the enone provides a bifunctional site for 1,3-dinucleophiles affording several heterocyclic ring-systems while incorporating other diversity elements.[1)] Pyrimidine derivatives have been the subject of substantial attention by synthetic and medicinal chemists because of the role of this heteroaromatic ring in many biological activities such as anticancer,[2)] antiviral,[3)] antitumor,[4)] anti-inflammatory,[5)] antimicrobial,[6)] antifungal,[7)] antihistaminic,[8)] and analgesic activities.[9)]

Novel immunosuppressive agents like 2-aminoquinazolines[10)] and aminopyrimidine amides[11)] were target components of signal transduction pathways mediated by the T cell receptor (TCR) may be useful as therapies for T-cell-mediated inflammatory or autoimmune diseases such as rheumatoid arthritis, polyarthritis scleroderma, inflammatory bowel disease, type 1 diabetes, myasthenia gravis and lupus.[12)] Over the past few years, IκB kinase (IKK) emerged as a prime target for the development of novel anti-rheumatic and anti-inflammatory drugs and also found that IKK2 is a key player in the transduction of pro-inflammatory signals.[13,14)] Adenosine kinase (AK) inhibition is a more effective mechanism for increasing extracellular adenosine (ADO) to limit tissue damage and restore normal function by activating members of the P1 receptor family and these have been found to inhibit AK for controlling metabolic stress and trauma.[15)] Literature revealed that the some substituted pyrimidines act as novel and potent TRPV1 antagonists for the treatment of hyperalgesia.[16,17)]

In view of these references, the synthesis of a new series of 2,4,6-trisubstituted pyrimidines are now reported. The desired target compounds (**5a**—**m**) were prepared from the 1,3-diarylpropenones (**3a**—**m**)[18,19)] and guanidine (**4**) by refluxing them together in a basic alcoholic media (Chart 1). Some authors gave different mechanistic suggestions of their experimental findings, where *e.g.* either hydrogen evolution[20)] or hydride ion migration[21)] was considered for pyrimidines formation and all these synthesized pyrimidines (Table 1) were screened for their analgesic and anti-inflammatory activity.

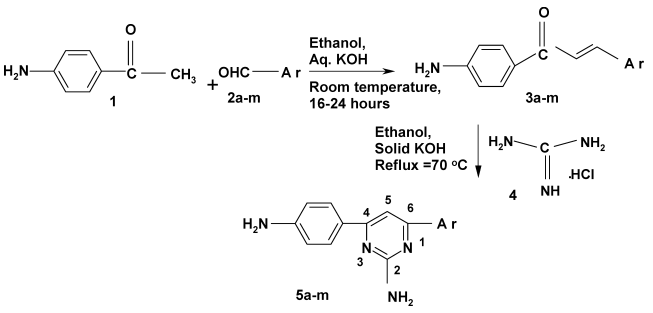

Chart 1

Table 1. 2,4,6-Trisubstituted Pyrimidines

Compound	Ar	Compound	Ar
5a	4-Chlorophenyl	**5h**	4-Methylphenyl
5b	2,4-Dichlorophenyl	**5i**	4-Dimethylaminophenyl
5c	4-Fluorophenyl	**5j**	9-Anthracenyl
5d	3-Bromophenyl	**5k**	2-Pyridinyl
5e	4-Methoxyphenyl	**5l**	4-Pyridinyl
5f	3,4-Dimethoxyphenyl	**5m**	3-Pyridinyl
5g	3,4,5-Trimethoxyphenyl		

Results and Discussion

Chemistry Target compounds, **5a**—**m** were synthesized by the reaction which proceeds either by 1,2-addition and/or 1,4-addition of the amino group of guanidine to the ketone, followed by cyclization to give the corresponding 2-amino-4,6-disubstituted pyrimidine derivatives **5a**—**m**.[22)] The structure of the products, **5a**—**m**, was established spectroscopically. Thus, their IR spectra show two bands in the regions, 1640—1610 cm^{-1} and 1375—1350 cm^{-1} characteristic of the pyrimidine system, and three bands in the regions, 3490—3460 cm^{-1}, 3375—3300 cm^{-1}, and 3200—3100 cm^{-1} (νNH$_2$: free and H-bonded). The ^{1}H-NMR spectra of these com-

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Table 2. Anti-inflammatory Activity of Pyrimidine Derivatives (**5a**—**m**)

Compound	Ar	Percent inhibition ±S.E.M. at various time intervals					
		0.5 h	1.0 h	2.0 h	3.0 h	4.0 h	6.0 h
5a	4-Chlorophenyl	15.23±0.90	41.33±1.04*	82.54±2.62	60.62±2.53	53.54±1.75	44.43±2.73
5b	2,4-Dichlorophenyl	15.22±0.68*	50.45±1.23*	87.23±2.61*	62.51±2.33*	56.94±1.79	48.39±2.65
5c	4-Fluorophenyl	20.01±0.89	40.56±1.21	83.46±2.54*	60.52±2.21	57.22±1.79	37.75±2.61
5d	3-Bromophenyl	18.26±0.68*	49.35±1.41*	86.99±2.62*	62.13±2.25*	53.32±2.01	42.11±2.75
5e	4-Methoxyphenyl	17.32±0.62*	51.32±1.35	83.47±2.45*	62.14±2.02*	54.57±1.68	49.05±2.68
5f	3,4-Dimethoxyphenyl	20.38±0.91	40.49±1.23*	85.60±2.55*	60.12±2.12	54.23±1.82	33.32±2.75
5g	3,4,5-Trimethoxyphenyl	20.13±1.25	42.03±1.45	82.13±2.62	59.02±2.01*	51.20±1.87	42.87±2.76
5h	4-Methylphenyl	20.14±0.92	60.57±1.47	82.82±2.69*	60.25±2.35*	57.24±1.92	43.13±2.78
5i	4-Dimethylaminophenyl	20.06±0.92*	53.05±1.49	83.50±2.51*	77.79±2.42	55.42±1.80*	55.73±2.68
5j	9-Anthracenyl	19.87±0.82	63.09±1.21	91.26±2.35*	76.82±2.26	59.85±1.92	54.61±2.22
5k	2-Pyridinyl	16.23±0.86*	53.41±1.92*	83.53±2.62*	70.79±2.42	61.77±1.97	56.47±2.71
5l	4-Pyridinyl	20.99±0.93	55.84±1.21*	85.12±2.24*	70.74±2.33	55.78±1.76	57.92±2.81
5m	3-Pyridinyl	20.33±0.91	52.13±1.58	83.96±2.52*	72.97±2.48	71.97±2.41	47.97±2.89
	Ibuprofen	20.26±0.90*	53.95±0.97*	97.09±2.86*	79.97±2.38*	67.93±2.22*	58.02±1.87*

All values are represented as mean±S.E.M. ($n=6$). $*p<0.01$ compared to saline control group. One-way ANOVA, Dunnett's *t*-test. Dosage: Ibuprofen-10 mg/kg and test compounds-10 mg/kg body weight by orally.

pounds gave further support for the aminopyrimidine structure, since they show a broad signal at δ 5.47—5.30 (NH_2) which disappeared when the deuteriodimethylsulphoxide solution was shaken with deuterium oxide. And also the characteristic singlet peak observed in the range δ 7.90—7.20 ppm indicates the presence of single proton at C-5 position of pyrimidine ring further confirms the formation of pyrimidine nucleus. In the ^{13}C-NMR spectrum exhibited characteristic peaks between δ 170—160 ppm for ring carbons adjacent to nitrogen atom in pyrimidine nucleus, and δ 150—120 ppm for other ring carbons confirming the pyrimidine structure. The mass spectra showed the corresponding molecular ion peak $[M^+]$ or $[M+H]^+$ as the base peaks and the fragmentation patterns was characteristic of respective pyrimidines. The elemental analyses of all the newly synthesized compounds confirmed their structures.

Anti-inflammatory Activity All the synthesized compounds (**5a**—**m**) were screened for the anti-inflammatory activity by carrageenan induced rat paw edema model. The present study illustrated that the compounds showed dose dependent response. The effect of the test compounds and ibuprofen, as a reference, was measured before and 0.5, 1, 2, 3, 4 and 6 h after carrageenan injection. Percent edema inhibition was calculated as regard to saline control group, as depicted in Table 2. Most of the test compounds showed a reasonable inhibition of edema size ($*p<0.01$) in comparison with ibuprofen. As shown in Table 2, 2-amino-4-(4-aminophenyl)-6-(2,4-dichlorophenyl) pyrimidine (**5b**) and 2-amino-4-(4-aminophenyl)-6-(3-bromophenyl) pyrimidine (**5d**) were found to be the most potent anti-inflammatory compounds, whereas compounds **5e**, **i** and **k** carrying methoxyl and dimethylamino substituent at 4-position on phenyl ring which is present at C-6 position of pyrimidine nucleus respectively and 2-pyridinyl substituent at C-6 position of pyrimidine showed remarkable activity. And also it was found that compound **5b** identified as lead structure among the all.

Analyzing the anti-inflammatory activity of the synthesized compounds **5a**—**m**, the following structure–activity relationship (SAR) was gained. Among four halogen substituted pyrimidine derivatives **5a**—**d**, the potency order was 2,4-Cl_2>*m*-Br>*p*-F>*o*-Cl. Between five electron-donor substituted pyrimidine derivatives **5e**—**i**, the potency order was *p*-$N(CH_3)_2$>*p*-OCH_3>3,4-$(OCH_3)_2$>*p*-CH_3>3,4,5-$(OCH_3)_3$. In addition, among three pyridinyl substituted pyrimidines **5k**—**m**, the potency order was 2-pyridinyl>4-pyridinyl>3-pyridinyl.[12—15]

Analgesic Activity The analgesic activity of the synthesized compounds was also investigated for all representative compounds (**5a**—**m**) by tail flick method, which involves the use of heat as source to induce pain in mice. The increase in the reaction time (time interval) compared to basal is proportional to analgesic activity of the test compounds. The results are summarized in Table 3.

Compounds **5b** and **d** showed dose dependent activity and significantly higher protection at 120 min which is comparable to the standard drug, it indicates that it may exert its action in a same manner as that of well established drug Ibuprofen because they carries 2,4-dichlorophenyl and 3-bromophenyl chemophores at C-6 position of pyrimidine nucleus respectively. In addition it was found that the compounds having 3,4-dimethoxyphenyl (**5f**), 4-dimethylamino (**5i**) and 4-pyridinyl (**5l**) moieties at C-6 position of pyrimidine respectively exhibited moderate analgesic activity and activity has increased at 60 min and reached the maximum peak at 120 min. Analyzing the analgesic activity of all the compounds **5a**—**m**, the following SAR was gained. Among four halogen substituted pyrimidines **5a**—**d**, the potency order was *m*-Br>2,4-Cl_2>*p*-F>*p*-Cl. Between five electron-donor substituted pyrimidines **5e**—**i**, the potency order was 3,4-$(OCH_3)_2$>*p*-$N(CH_3)_2$>*p*-OCH_3>3,4,5-$(OCH_3)_3$>*p*-CH_3. In addition, among the three pyridinyl substituted pyrimidines **5k**—**m**, the potency order was 4-pyridinyl>3-pyridinyl>2-pyridinyl. Among all compound **5d** is found to exhibit significant analgesic activity at 120 min. These results indicated that **5b** and **d** are more promising molecules as anti-inflammatory and analgesic agents and further studies are required to elucidation of exact mechanism of action for their therapeutic potential.

Table 3. Analgesic Activity of Pyrimidine Derivatives (**5a**—**m**)

Compound	Ar	Percent inhibition ±S.E.M. at various time intervals				
		0.5 h	1.0 h	2.0 h	3.0 h	4.0 h
5a	4-Chlorophenyl	46.14±1.57	77.54±1.51	92.24±1.33	65.95±0.87	32.64±1.59
5b	2,4-Dichlorophenyl	45.17±0.62*	85.22±1.69*	97.04±1.37*	75.22±1.08*	29.14±0.55
5c	4-Fluorophenyl	40.13±2.03	78.13±1.83*	90.23±1.77	68.51±1.31	30.53±1.73
5d	3-Bromophenyl	56.46±1.41*	88.41±1.74*	97.83±1.58*	75.78±2.31*	58.62±1.40
5e	4-Methoxyphenyl	43.99±1.86*	71.89±2.81*	88.31±1.87	69.65±1.61	43.03±2.02
5f	3,4-Dimethoxyphenyl	55.18±0.96*	88.50±0.84*	90.45±2.01*	66.50±0.75	56.54±1.03
5g	3,4,5-Trimethoxyphenyl	30.82±1.21	56.04±1.51	78.46±2.13	68.37±1.67	38.87±1.33
5h	4-Methylphenyl	23.13±0.97	52.03±1.31	75.37±1.97	77.12±1.83	33.17±1.21
5i	4-Dimethylaminophenyl	19.88±0.81	82.35±1.31*	88.63±0.69*	67.84±1.97	30.35±1.06
5j	9-Anthracenyl	46.14±2.43	70.94±1.70	75.26±1.31	65.95±1.46	41.78±1.49
5k	2-Pyridinyl	40.23±1.74	51.33±1.83	75.41±2.81	53.38±1.13	39.81±0.97
5l	4-Pyridinyl	27.42±1.22	79.41±1.52*	87.22±0.88*	65.42±1.20	34.30±1.12
5m	3-Pyridinyl	43.31±0.81	61.17±1.31*	84.79±0.93	59.37±1.07	37.17±1.39
	Ibuprofen	55.26±0.90*	89.95±0.97*	99.87±1.86*	79.97±2.38*	58.02±2.22*

All values are represented as mean±S.E.M. ($n=6$). $*p<0.01$ compared to control. One-way ANOVA, Dunnett's t-test. Dosage: Ibuprofen-10 mg/kg and test compounds-10 mg/kg body weight by orally.

Conclusion

These new agents may be act by one of the mechanisms discussed in introduction and can be further utilized for lead optimization purposes and also can be new leads for non-steroidal anti-inflammatory drugs (NSAIDs). It was found that the presence of electron releasing group on phenyl ring system attached at C-6 position of pyrimidine is important for their activity. The aryl group at C-6 position has been replaced by pyridinyl group is also important for those activities.

Experimental

General All reagents and solvents were used as purchased without further purification. Melting points were determined on a standard Boetius apparatus and are uncorrected. The IR spectra were recorded in Perkin-Elmer BXF1 Fourier Transform (FT)-IR spectrophotometer using KBr disc method. ^{1}H- and ^{13}C-NMR spectra were recorded in the indicated solvent on a Bruker AMX 400 and 100 MHz respectively with tetramethylsilane (TMS) as internal standard (chemical shifts in δ ppm). Mass [EI-MS (70 eV)] spectra were recorded on Agilent 1100 EI-mass spectrophotometer. The elemental analyses of the synthesized compounds were recorded on Carlo Erba 1108 elemental analyzer and were within ±0.4% of the theoretical values. Analytical TLC was performed on Silica Gel F_{254} plates (Merck) with visualization by UV (254 nm) chamber with protective filters. All the pyrimidines have been purified by column chromatography performed on silica gel (100—200 mesh, Merck).

General Procedure for the Synthesis of 2-Amino-4-(4-aminophenyl)-6-(substituted or unsubstituted aryl/unsubstituted heteroaryl)pyrimidines 5a—m)[22] To a reaction vial containing 50 μmol of corresponding chalcone (**3**) and 50 μmol of KOH as solid was added 400 μl absolute ethanol. To the reaction mixture was added 200 μl of a 0.25 M solution of guanidine hydrochloride (**4**) in absolute ethanol. The reaction mixture was capped, shaken to ensure mixing and then allowed to reflux at 70 °C for 2 to 6 h. Reaction completion was identified by TLC. Upon completion, the reaction mixture were cooled to room temperature and quenched with 100 μl of a 0.5 M solution of HCl in water. The reaction mixture was shaken to ensure mixing and then concentrated to dryness *in vacuo* to afford the product as a solid. It was purified by column chromatography, using ethylacetate and hexane mixture as mobile phase obtained pure pyrimidine derivatives (**5a**—**m**).

2-Amino-4-(4-aminophenyl)-6-(4-chlorophenyl)pyrimidine (**5a**): Yellow solid, yield 68%, mp 170—172 °C. IR (KBr) cm^{-1}: 3415, 3308, 1632, 1579, 1358, 814. ^{1}H-NMR (DMSO-d_6) δ: 5.64 (2H, s), 6.51 (2H, s), 6.65 (2H, d, J=8.4 Hz), 7.54 (1H, s), 7.56 (2H, d, J=8.4 Hz), 7.97 (2H, d, J=8.8 Hz), 8.22 (2H, d, J=10.0 Hz). ^{13}C-NMR (DMSO-δ_6) δ: 100.02, 113.28, 124.05, 128.30, 128.50, 128.56, 134.78, 136.61, 151.39, 162.56, 163.77, 165.27. EI-MS m/z: 297.5 [{M+H}$^+$]. *Anal.* Calcd for $C_{16}H_{13}N_4Cl$: C, 64.81; H, 4.38; N, 18.89. Found: C, 64.75; H, 4.38; N, 18.88.

2-Amino-4-(4-aminophenyl)-6-(2,4-dichlorophenyl)pyrimidine (**5b**): Yellow solid, yield 53%, mp 175—177 °C. IR (KBr) cm^{-1}: 3445, 3327, 1645, 1588, 824. ^{1}H-NMR (DMSO-d_6) δ: 5.66 (2H, s), 6.60 (2H, s), 6.62 (2H, d, J=8.8 Hz), 7.13 (1H, s), 7.55 (1H, d, J=6.0 Hz), 7.60 (1H, s), 7.74 (1H, d, J=7.8 Hz), 7.85 (2H, d, J=8.2 Hz). EI-MS m/z: 332 [{M+H}$^+$].

2-Amino-4-(4-aminophenyl)-6-(4-fluorophenyl)pyrimidine (**5c**): Orange yellow solid, yield 62%, mp 164—166 °C. IR (KBr) cm^{-1}: 3405, 3308, 1632, 1576, 1361, 1227. ^{1}H-NMR (DMSO-d_6) δ: 5.63 (2H, s), 6.48 (2H, s), 6.64 (2H, d, J=8.4 Hz), 7.33 (2H, d, J=9.2 Hz), 7.52 (1H, s), 7.97 (2H, d, J=8.8 Hz), 8.24 (2H, d, J=8.8 Hz). EI-MS m/z: 281.5 [{M+H}$^+$].

2-Amino-4-(4-aminophenyl)-6-(3-bromophenyl)pyrimidine (**5d**): Yellow solid, yield 67%, mp 187—189 °C. IR (KBr) cm^{-1}: 3401, 3305, 1635, 1575, 1362, 578. ^{1}H-NMR (DMSO-δ_6) δ: 5.65 (2H, s), 6.55 (2H, s), 6.65 (2H, d, J=8.4 Hz), 7.49—7.45 (1H, t), 7.58 (1H, s), 7.69 (1H, d, J=8.8 Hz), 7.99 (2H, d, J=8.4 Hz), 8.20 (1H, d, J=8.0 Hz), 8.41 (1H, s). EI-MS m/z: 343 [{M+2H}$^+$].

2-Amino-4-(4-aminophenyl)-6-(4-methoxyphenyl)pyrimidine (**5e**): Light brown solid, yield 61%, mp 165—167 °C. IR (KBr) cm^{-1}: 3440, 3309, 1614, 1570, 1364, 1238. ^{1}H-NMR (DMSO-d_6) δ: 3.84 (3H, s), 5.60 (2H, s), 6.39 (2H, s), 6.64 (2H, d, J=8.4 Hz), 7.04 (2H, d, J=8.8 Hz), 7.46 (1H, s), 7.95 (2H, d, J=8.4 Hz), 8.15 (2H, d, J=8.8 Hz). EI-MS m/z: 292.5 [M$^+$].

2-Amino-4-(4-aminophenyl)-6-(3,4-dimethoxyphenyl)pyrimidine (**5f**): Brown solid, yield 58%, mp 171—173 °C. IR (KBr) cm^{-1}: 3450, 3362, 1623, 1568, 1358, 1260. ^{1}H-NMR (DMSO-d_6) δ: 3.83 (3H, s), 3.87 (3H, s), 5.61 (2H, s), 6.39 (2H, s), 6.64 (2H, d, J=8.4 Hz), 7.06 (1H, d, J=8.4 Hz), 7.47 (1H, s), 7.75 (1H, s), 7.80 (1H, d, J=10.4 Hz), 7.97 (2H, d, J=8.8 Hz). EI-MS m/z: 323 [{M+H}$^+$].

2-Amino-4-(4-aminophenyl)-6-(3,4,5-trimethoxyphenyl)pyrimidine (**5g**): Brown solid, yield 58%, mp 180—182 °C. IR (KBr) cm^{-1}: 3446, 3358, 1655, 1583, 1328, 1263. ^{1}H-NMR (DMSO-d_6) δ: 3.78 (3H, s), 3.95 (6H, s), 6.77 (2H, d, J=8.8 Hz), 7.58 (2H, s), 7.78 (1H, s), 8.19 (2H, d, J=8.4 Hz), 8.49 (2H, s). EI-MS m/z: 352 [M$^+$].

2-Amino-4-(4-aminophenyl)-6-(4-methylphenyl)pyrimidine (**5h**): Yellow solid, yield 62%, mp 176—178 °C. IR (KBr) cm^{-1}: 3443, 3337, 1575, 1522, 1359. ^{1}H-NMR (DMSO-d_6) δ: 2.38 (3H, s), 5.61 (2H, s), 6.42 (2H, s), 6.64 (2H, d, J=8.8 Hz), 7.31 (2H, d, J=8.0 Hz), 7.48 (1H, s), 7.95 (2H, d, J=8.4 Hz), 8.08 (2H, d, J=8.4 Hz). EI-MS m/z: 278 [{M+H}$^+$].

2-Amino-4-(4-aminophenyl)-6-(4-dimethylaminophenyl)pyrimidine (**5i**): Orange solid, yield 51%, mp 187—189 °C. IR (KBr) cm^{-1}: 3444, 3335, 1614, 1567, 1366. ^{1}H-NMR (DMSO-d_6) δ: 2.99 (6H, s), 5.56 (2H, s), 6.27 (2H, s), 6.64 (2H, d, J=8.4 Hz), 6.78 (2H, d, J=8.8 Hz), 7.38 (1H, s), 7.93 (2H, d, J=8.4 Hz), 8.05 (2H, d, J=8.8 Hz). EI-MS m/z, %: 306 [{M+H}$^+$].

2-Amino-4-(4-aminophenyl)-6-(9-anthracenyl)pyrimidine (**5j**): Dark yellow solid, yield 47%, mp 192—194 °C. IR (KBr) cm^{-1}: 3444, 3357, 1615, 1576, 1375. ^{1}H-NMR (DMSO-d_6) δ: 5.65 (2H, s), 6.60 (2H, s), 6.63 (2H, d, J=7.6 Hz), 7.14 (1H, s), 7.56—7.45 (4H, m), 7.74 (2H, d, J=9.2 Hz), 7.92 (2H, d, J=8.8 Hz), 8.15 (2H, d, J=8.4 Hz,), 8.69 (1H, s). EI-MS m/z: 363

[{M+H}$^+$].

2-Amino-4-(4-aminophenyl)-6-(2-pyridinyl) pyrimidine (**5k**): Greenish yellow solid, yield 47%, mp 162—164 °C. IR (KBr) cm^{-1}: 3338, 1646, 1567, 1363. ^{1}H-NMR (DMSO-d_6) δ: 5.66 (2H, s), 6.55 (2H, s), 6.66 (2H, d, J=8.8 Hz), 7.53—7.50 (1H, m), 7.89 (2H, d, J=8.6 Hz), 7.90 (1H, s), 7.99—7.95 (1H, m), 8.33 (1H, d, J=7.6 Hz), 8.73 (1H, d, J=4 Hz). EI-MS m/z: 264 [{M+H}$^+$].

2-Amino-4-(4-aminophenyl)-6-(4-pyridinyl)pyrimidine (**5l**): Greenish yellow solid, yield 51%, mp 167—169 °C. IR (KBr) cm^{-1}: 3442, 3355, 1575, 1526, 1365. ^{1}H-NMR (DMSO-d_6) δ: 6.18 (2H, s), 7.06 (2H, d, J=8.8 Hz), 7.21 (2H, s), 7.38 (1H, s), 8.16 (2H, d, J=6.0 Hz), 8.35 (2H, d, J=8.8 Hz), 8.90 (2H, d, J=6.0 Hz). ^{13}C-NMR (DMSO-d_6) δ: 102.38, 114.99, 121.97, 126.41, 129.83, 146.50, 151.32, 152.84, 163.34, 165.86, 167.38. EI-MS m/z, %: 264 [{M+H}$^+$]. *Anal.* Calcd for $C_{15}H_{13}N_5$: C, 68.50; H, 4.98; N, 26.63. Found: C, 68.44.; H, 4.97; N, 26.61.

2-Amino-4-(4-aminophenyl)-6-(3-pyridinyl)pyrimidine (**5m**): Greenish yellow solid, yield 48%, mp 167—169 °C. IR (KBr) cm^{-1}: 3332, 3207, 1645, 1566, 1359. ^{1}H-NMR (DMSO-d_6) δ: 6.16 (2H, s), 7.06 (2H, d, J=8.4 Hz), 7.20 (2H, s), 7.34 (1H, s), 7.37—7.79 (3H, m), 8.35 (2H, d, J=8.8 Hz), 9.72 (1H, s). EI-MS m/z: 264 [{M+H}$^+$].

Pharmacology All the synthesized compounds were screened for analgesic and anti-inflammatory activity in rats and mice. Wistar albino rats (150—200 g) and Swiss albino mice (20—25 g) of either sex (M/S Ghosh Enterprises, Calcutta, West Bengal, India) were used and the animals were kept at 26±2 °C with relative humidity 44—56%, with 12 h light/12 h dark cycle. All the animals were fed with standard diet and water *ad libitum*. Permission has been obtained from the IAEC for conducting the above experiments. 18—24 h fasted animals were used for the experiments. 18—24 h fasted animals were used for the experiments. Experimental groups were divided into 15, each containing 6 animals. The test compounds were suspended in 1% sodium carboxymethylcellulose (Na-CMC) and administered at dose of 10 mg/kg of body weight (b.w.) and 10 mg/kg, b.w. of ibuprofen was administered as a reference standard drug for both the activities by orally. The control group received 1% Na-CMC (1 ml/kg, b.w.) in distilled water.

Anti-inflammatory Activity The compounds were tested for anti-inflammatory activity by carrageenan-induced rat paw edema method described by Winter *et al.*[23] and Kulkarni *et al.*[24] method. One hour after the administration of test compounds, rats in all groups were challenged with carrageenan (1% prepared in 0.4% NaCl) in the sub-plantar region of right hind paw. The paw volume was measured at different intervals of time (0.5, 1, 2, 3, 4, 6 h) using digital plethysmometer and 0 h reading, before administration of the carrageenan was taken. The percentage inhibition of paw volume for each test group is calculated using following equation. Percentage of inhibition (%)=[1−volume in ml (test compound)/volume in ml (control)]×100. The results and statistical analysis of anti-inflammatory activity of control, reference drug and the compounds tested are shown in Table 2.

Analgesic Activity Tail flick (tail-withdrawal from the radiant heat) method was conducted according to D'Amour *et al.*[25] and Kulkarni[26] using an analgesiometer was adopted for evaluation of analgesic activity of the test compounds and standard. Basal reaction time has been taken to radiant heat by placing the tip (last 1—2 cm) of the tail of the animals (control, standard and test groups) individually. The tail-withdrawal from the heat is taken as the end point. All the animals were held in position by a suitable restrained with the tail extending out and the tail (up to 2 cm) was then dipped in a water bath maintained at 55±0.5 °C which is present in analgesiometer. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time and was recorded at 0.5, 1, 2, 3 and 4 h after administration of compounds. A cut off point of 10 s was observed to prevent the tail damage. The percentage of protection in the control, standard drug and compound treated animals were calculated using following equation. Percentage of protection (%)=[1−basal reaction time in seconds (control)/basal reaction time in seconds (test compound)]×100. The results and statistical analysis of analgesic activity of control, ibuprofen and the compounds tested are shown in Table 3.

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