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Synthesis, anti-inflammatory and analgesic activities evaluation of some mono, bi and tricyclic pyrimidine derivatives

Sham M. Sondhi,[a,*] Nirupma Singh,[a] Monika Johar[a] and Ashok Kumar[b]

[a]*Department of Chemistry, Indian Institute of Technology Roorkee (IIT R), Roorkee-247667 (U.A), India*
[b]*Department of Pharmacology, LLRM Medical College, Meerut, Meerut (UP), India*

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Abstract—3-Aminobenzonitrile and 2-amino-4-phenyl thiazole on condensation with 4-isothiocyanato-4-methyl pentane-2-one gave condensed monocyclic pyrimidine derivatives **1** and **2**, **3**, respectively. Condensation of 3-aminopropyl imidazole with 3-isothiocyantobutanal gave condensed monocyclic pyrimidine derivative **4**. Bicyclic pyrimidine derivatives **5a** and **5b** have been synthesized by the condensation of diaminomaleonitrile with 4-isothiocyanto-4-methylpentane-2-one and 3-isothiocyanatobutanal, respectively. Condensation of 4-isothiocyanato-4-methyl pentane-2-one with 2,3-diaminopropionic acid hydrochloride yielded another bicyclic compound **7**. 4-Isothiocyanato-4-methyl pentane-2-one, 3-isothiocyanatobutanal and 4-isothiocyanatobutan-2-one on condensation with 2-amino-4-nitro phenol gave tricyclic pyrimidine derivatives **8a**, **8b** and **8c**, respectively. Structures of all the synthesized pyrimidine derivatives are supported by correct IR, ^{1}H NMR and mass spectral data. The anti-inflammatory activity evaluation was carried out using carrageenin-induced paw oedema assay, and compounds **1**, **3** and **5b** exhibited good anti-inflammatory activity, that is, 27.9, 34.5 and 34.3% at 50 mg/kg po, respectively. Analgesic activity evaluation was carried out using phenylquinone writhing assay and compounds **5a**, **5b** and **8b** showed good analgesic activity, that is, 50, 70 and 50% at 50 mg/kg po, respectively.

1. Introduction

In the course of research devoted to the development of new classes of pyrimidine and condensed pyrimidine moieties, we have been interested in the synthesis and evaluation of biological activities of several flat nitrogen heterocycles and condensed nitrogen heterocycles, that is, monocyclic, bicyclic and tricyclic pyrimidine derivatives, which can potentially show anti-inflammatory and analgesic activities. Pyrimidines and condensed pyrimidines are important classes of heterocyclic compounds and exhibit a broad spectrum of biological activities such as anticancer, anti-inflammatory, COX inhibitor, antiallergic, analgesic, etc.[1–6] Prolonged use of NSAIDs causes gastrointestinal ulcers and hence, there is a need to develop safer anti-inflammatory drugs. Pyrimidine[7,8] and condensed pyrimidine[9,10] derivatives possessing anti-inflammatory and analgesic activities are well documented in the literature.[11–15] In continuation of our efforts in search of potential anti-inflammatory and analgesic agents, we have synthesized a number of pyrimidine and condensed pyrimidine derivatives, which we wish to report in this paper.

2. Results and discussion

2.1. Chemistry

3-Aminobenzonitrile (**1a**) on condensation with 4-isothiocyanato-4-methylpentane-2-one (**1b**) by refluxing in methanol and purification by column chromatography over silica gel gave condensed product 3-(4,4,6-trimethyl-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl) benzonitrile (**1**; Scheme 1). The structure of **1** is fully supported by IR, ^{1}H NMR and mass spectral data reported in Section 4. Formation of **1** can be explained by nucleophilic attack of $-NH_2$ of **1a** on isothiocyanato group of **1b** giving a nonisolatable intermediate thiourea **1′** (Scheme 1) and further nucleophilic attack by –NH– to >C═O group giving a ring-cyclized hydrated intermediate **1″** (Scheme 1). Intermediate **1″** then losses water molecule[16] to give pyrimidine derivative **1**. Condensation of 2-amino-4-phenyl thiazole (**2a**) with 4-isothiocyanato-4-methyl pentane-2-one (**1b**) gave product **2** (Scheme 1), whereas

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* Corresponding author. Tel.: +91 1332 285811; fax: +91 1332 273650; e-mail: sondifcy@iitr.ernet.in

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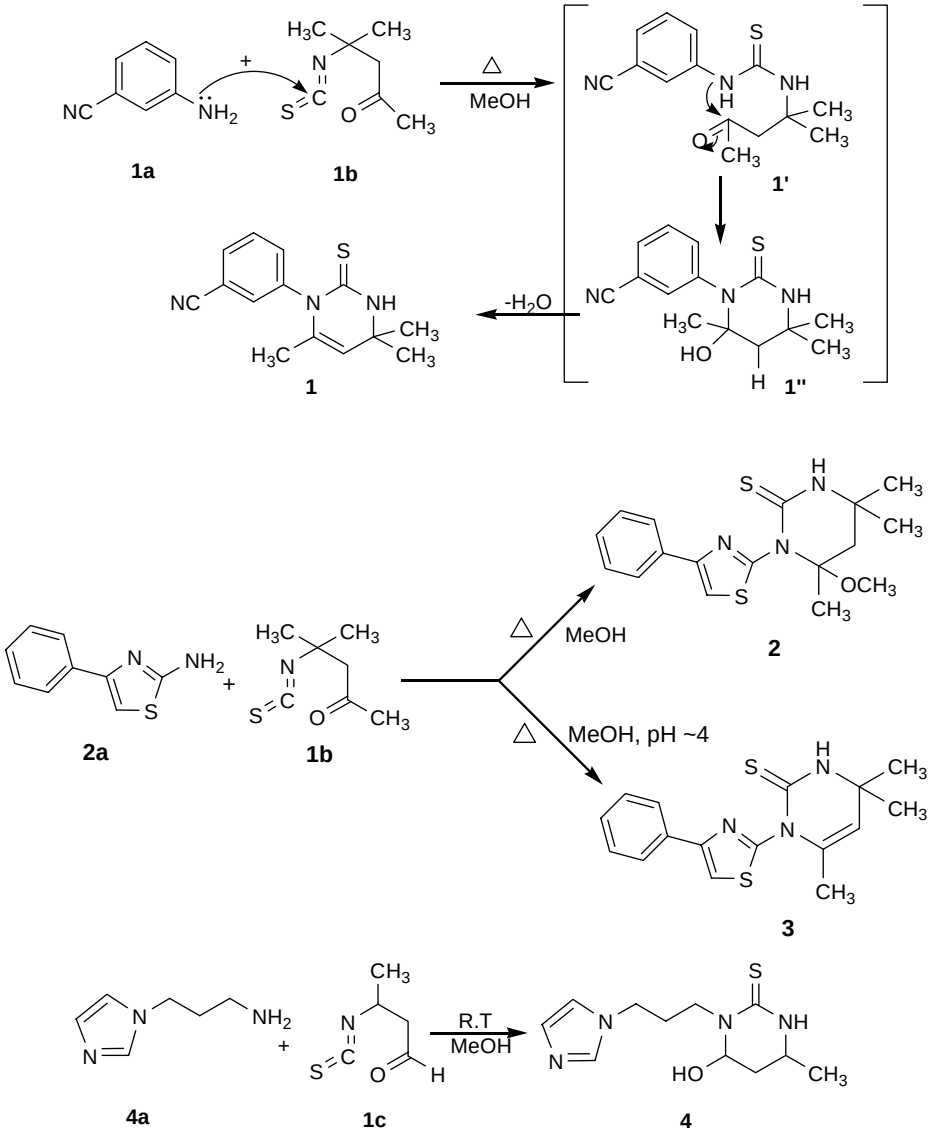


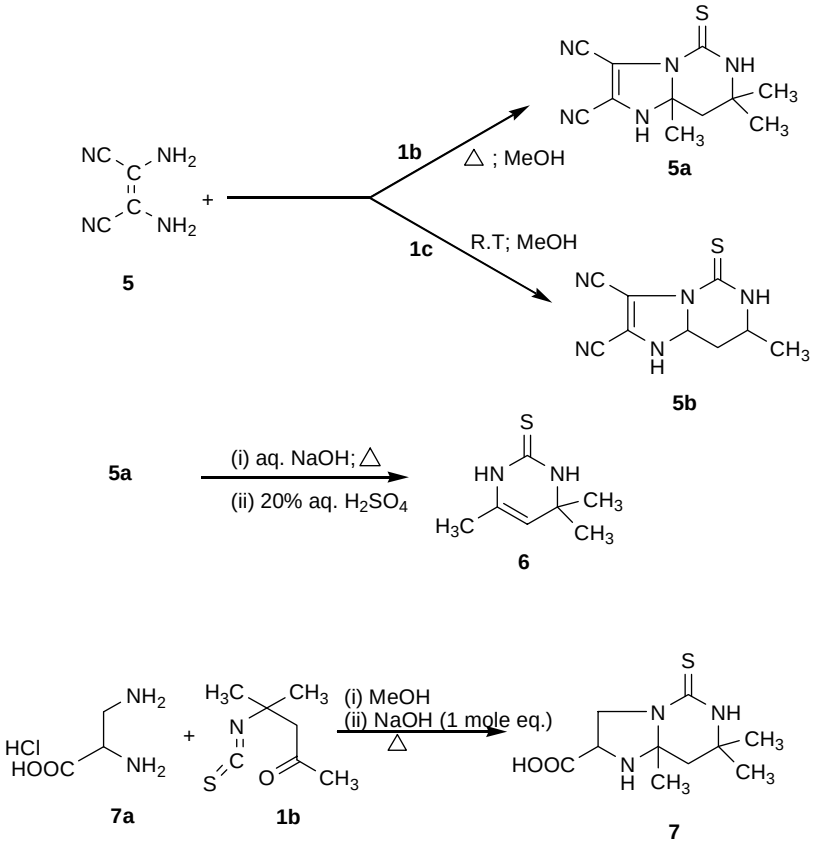
Scheme 1. Synthesis of monocyclic pyrimidine derivatives.

the same reaction at pH ~4 gave product **3** (Scheme 1). ^{1}H NMR of product **2** shows a singlet at δ 3.44 accounting for three protons corresponding to –OCH_3 group, whereas in case of product **3**, a singlet at δ 4.90 accounting for one proton corresponds to >C═CH– of pyrimidine ring. Spectral data of compounds **2** and **3** reported in Section 4 fully support the structures assigned to them.[17] Condensation of **2a** and **1b** at pH ~4 facilitated elimination of water and thus gave pyrimidine derivative **3** (Scheme 1), whereas in the absence of pH adjustment, methoxy derivative **2** was obtained.

Condensation of 3-isothiocyanatobutanal (**1c**) with 3-aminopropyl imidazole (**4a**) at room temperature gave crude product **4** (Scheme 1) which was purified by column chromatography over silica gel. Elution with $CHCl_3$/MeOH (9:1) gave pure condensed product **4**. ^{1}H NMR (DMSO-d_6 + $CDCl_3$) of **4** showed a broad singlet at δ 6.25 accounting for one exchangeable proton, which corresponds to –OH group. FAB-MS of **4** gave MH^+ ion peak at *m*/*z* 255.76 (10%). Structure assigned to compound **4** is in complete agreement with the spectral data reported in Section 4.[18]

Diaminomaleonitrile (**5**) on condensation with 4-isothiocyanato-4-methyl pentane-2-one (**1b**) by refluxing in methanol gave bicyclic product **5a**, whereas condensation of **5** with 3-isothiocyanatobutanal (**1c**) at room temperature using methanol as solvent gave condensation product **5b** (Scheme 2). Both **5a** and **5b** were purified by column chromatography over silica gel to give pure products. Spectral data of compounds **5a** and**5b** reported in Section 4 fully support the structure assigned to them. In case of diamino compounds, one amino group will react with isothiocyanato group giving intermediate similar to **1**″ (Scheme 1) and then, a second amino group attack on carbon bearing –OH group and thus loosing water and forming bicyclic ring structures[19] **5a** and **5b** (Scheme 2).

In an attempt to hydrolyze compound **5a** to give corresponding diacid, compound **5a** was refluxed with 30% NaOH solution for 48 h. The reaction contents were



Scheme 2. Synthesis of bicyclic pyrimidine derivatives.

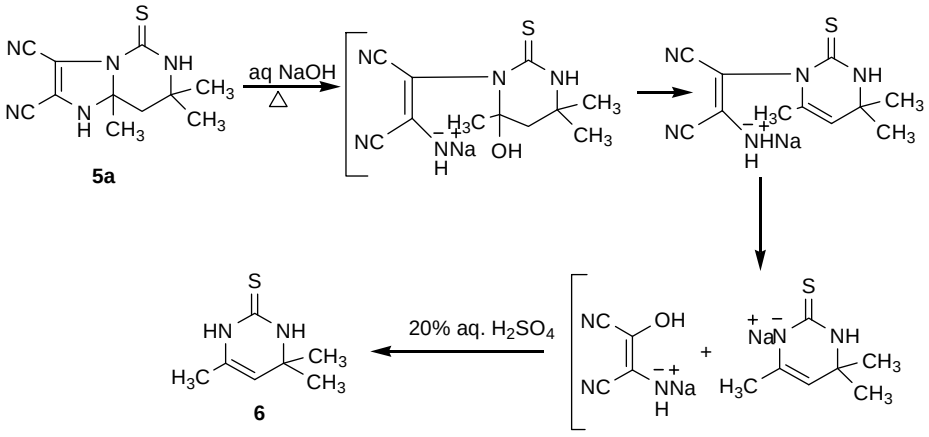
acidified with H_2SO_4 and extracted with ethyl acetate. Organic extract was washed with water and then solvent was removed under reduced pressure, the crude product left behind was subjected to column chromatography over silica gel and elution with $CHCl_3$/EA (4:1) gave pure white product **6** (Scheme 3) having mp 255 °C. ^{1}H NMR (300 MHz; DMSO-d_6) of **6** gave signals at δ 1.15 (singlet, 6H, 2× CH_3), 1.64 (singlet, 3H, CH_3); 4.49 (singlet, 1H, >C═CH–); 8.44 (singlet 1H, NH exch); 9.38 (singlet, 1H, NH, exch). HR-MS of **6** gave M^+ ion peak at 156.07209 (M^+, 51%) calcd for $C_7H_{12}N_2S$ 156.07211. IR spectrum shows a peak at 3200 cm^{-1} (–NH–).

Structure of compound **6** was derived from above-mentioned spectral data and also from undepressed mixed mp with authentic sample[20] and compound **6** was found to be 4,4,6-trimethyl-3,4-dihydropyrimidine-2(1*H*)-thione. A probable mechanism for the formation of **6** is proposed in Scheme 3.

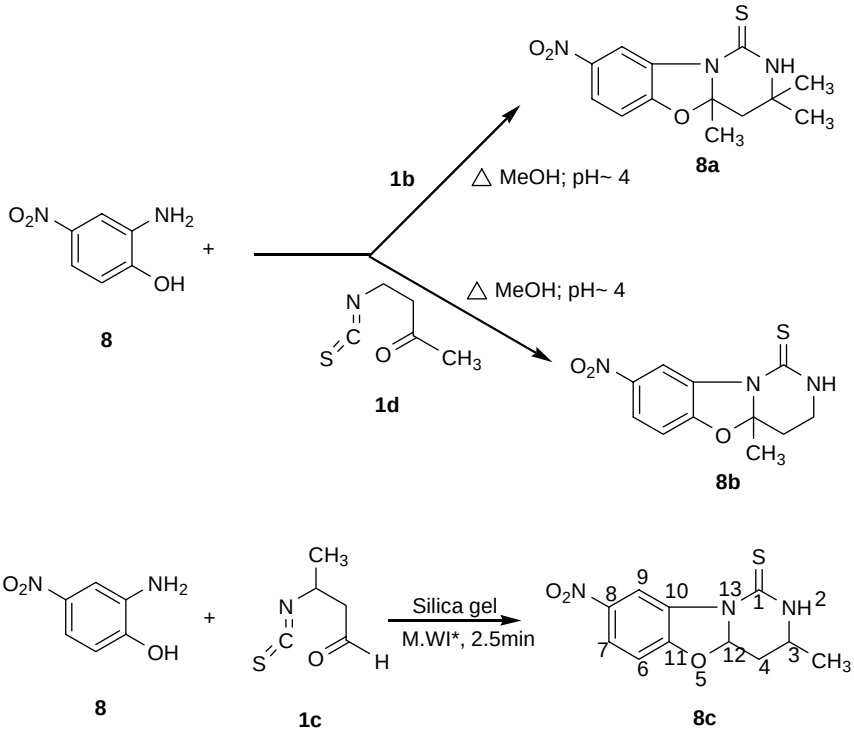
Condensation of 2,3-diaminopropionic acid hydrochloride (**7a**) with 4-isothiocyanato-4-methylpentane-2-one (**1b**) by refluxing in alkaline methanol gave condensation product 7,7,8a-trimethyl-5-thioxo-octahydroimidazo[1,2-f]pyrimidine-2-carboxylic acid (**7**; Scheme 2). Spectral data of compound **7** reported in Section 4 fully support the structure assigned to it.

Condensation of 2-amino-4-nitrophenol (**8**; Scheme 4) with 4-isothiocyanato-4-methylpentane-2-one (**1b**) and 4-isothiocyanato butane-2-one (**1d**) by refluxing in methanol and adjusting the pH of reaction mixture to ~4 (by adding a few drops of 10% sulfuric acid in methanol) gave condensation products **8a** and **8b** (Scheme 4), respectively.

^{1}H NMR of compound **8a** exhibited signals at δ: 1.25–1.35 (2s, looking like a doublet, 6H, CH_3 + CH_3); 1.60 (s, 3H, CH_3); 2.30–2.40 (d, 1H, one H of –CH_2–); 2.65–2.75 (d, 1H, one H of –CH_2–); 7.15 (d, 1H, Ar); 8.0 (dd, 1H, Ar); 9.25 (s, 1H, –NH– exch); 9.45 (d, 1H, Ar). HR-MS of **8a** showed M^+ ion peak at 293.08381 calcd for $C_{13}H_{15}N_3SO_3$ 293.08340. Above-mentioned spectral data fully support the structure assigned to **8a**. Similarly, spectral data of **8b** reported in Section 4, fully support the structure assigned to **8b**. Formation of **8a** and **8b** can be explained on the basis that isothiocyantaoketones react first to –NH_2 group which is meta to NO_2, followed by cyclization involving phenolic –OH group to give tricyclic pyrimidobenzoxazole derivatives.[21] 2-Amino-4-nitrophenol (**8**), 3-isothiocyanatobutanal (**1c**; Scheme 4) and a small amount of silica gel (60–120 mesh) were mixed together and then irradiated in microwave oven for 2.5 min at 850 W. The reaction contents were suspended in methanol and then stirred for 10 min and solvent was decanted off. This process was repeated two more times and then total methanol extract was distilled off under reduced pressure to give crude product **8c**. Crude compound **8c** was purified by column chromatography over silica gel to give pure compound **8c** (Scheme 4). Spectral data of

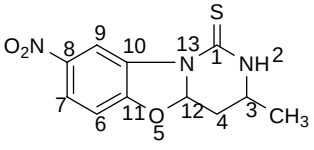


Scheme 3. Probable mechanism for the formation of **6**.



Scheme 4. Synthesis of tricyclic pyrimidine derivatives by conventional and nonconventional method. M.WI* = Microwave irradiation.

8c reported in Section 4 fully support the structure assigned to it.



8c

It is interesting to note that ^{1}H NMR of **8c** exhibits doubling of peaks, that is, a doublet at δ 1.33 and another doublet at 1.40 accounting for 0.75H and 2.25H corresponding to $-CH_3$ group. There are other peaks accounting for fraction of proton, that is, 1.96 (q, 0.75H); 2.28 (m, 0.25H), 2.54 (q, 0.25H), 2.68 (m, 0.75H); 8.32 (s, 0.75H); 8.76 (s, 0.25H); 9.46 (d, 0.75H); 9.50 (d, 0.25H). This behaviour of **8c** can be explained by taking into account that C-3 and C-12 are chiral carbons and at C-3, methyl group can occupy pseudo axial and pseudo equatorial position, which means that compound **8c** is a mixture of two isomers and this has been shown by ^{1}H NMR. This type of observation has also been reported in literature.[22,23]

2.2. Biological results

Compounds **1**–**4**, **5a**–**b**, **7** and **8a**–**b** at 50 mg/kg po were tested for anti-inflammatory activity in the carrageenin-induced paw oedema model[24] and results are summarized in Table 1. Compounds **1**, **2**, **3**, **4**, **5a** and **5b** showed 27.9, 22.3, 34.5, 24.5, 3.0 and 34.3%

Table 1. Anti-inflammatory and analgesic activity of compounds **1**–**4**, **5a**–**b**, **7** and **8a**–**b**

Compound	Dose (mg/kg po)	Anti-inflammatory activity (%)	Dose (mg/kg po)	Analgesic activity (%)
1	50	**27.9**	50	10
2	50	22.3	50	30
3	50	**34.5**	50	30
4	50	24.5	50	20
5a	50	3.0	100	**100**
			50	**50**
			25	**25**
5b	50	**34.3**	50	**70**
7	50	Nil	100	50
			50	25
8a	50	Nil	100	25
8b	50	Nil	100	**75**
			50	**50**
			25	**25**
Ibuprofen	50	38	100	75
			50	50

anti-inflammatory activity, respectively, whereas compounds **7**, **8a** and **8b** were found to be inactive. Ibuprofen, a standard drug, exhibited 38% anti-inflammatory activity at 50 mg/kg po.

Compounds **1**, **2**, **3**, **4**, **5a**, **5b**, **7** and **8b** on analgesic activity evaluation (Table 1) using phenylquinone writhing assay[25] exhibited 10, 30, 30, 20, 50, 70, 25 and 50% activity at 50 mg/kg po, whereas compounds **5a**, **7**, **8a** and **8b** at 100 mg/kg po gave 100, 50, 25 and 75% analgesic activity, respectively. Ibuprofen, a standard drug, exhibited 75% and 50% analgesic activity at 100 and 50 mg/kg po, respectively.

3. Conclusion

Various monocyclic (**1**–**4**), bicyclic (**5a**–**b**, **7**) and tricyclic (**8a**–**c**) pyrimidine derivatives have been synthesized and evaluated for anti-inflammatory and analgesic activities. Compounds **1**, **3** and **5b** exhibited good anti-inflammatory activity, whereas compounds **5a**, **5b** and **8b** exhibited good analgesic activity.

4. Experimental

4.1. General

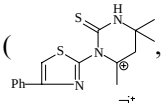
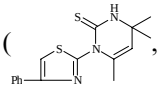
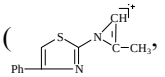
Melting points (mp) were determined on a JSGW apparatus and were uncorrected. IR spectra were recorded using a Perkin-Elmer 1600FT Spectrophotometer. ^{1}H NMR spectra were measured on a Bruker WH-300 Spectrometer in a ca. 5–15% (w/v) solution in DMSO-d_6 (TMS as internal standard). The mass spectrometer peak measurements were made by comparison with perfluorotributylamine using AEI MS-9 double focusing high-resolution mass spectrometer at a resolving power 15,000. FAB-MS was reordered on Jeol SX-120 (FAB) spectrometer. Electron impact (EI) MS was recorded on a Jeol D-300 mass spectrometer. Thin-layer chromatography (TLC) was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapour or by irradiation with ultraviolet light (254 nm). Column chromatography was performed by using Qualigens silica gel for column chromatography (60–120 mesh).

4.2. Synthesis of 3-(4,4,6-trimethyl-2-thioxo-3,4-dihydropyrimidin-1(2*H*)-yl) benzonitrile (1)

3-Aminobenzonitrile (**1a**) (0.118 g, 1 mmol) was dissolved in methanol (15 ml) and to it was added 4-isothiocyanato-4-methylpentane-2-one (**1b**) (0.2 ml, 1.3 mmol). The reaction contents were heated under reflux; after refluxing for 4 h, solid compound started separating out and reaction contents were further refluxed for 8 h. The reaction contents were cooled at room temperature and filtered. The solid product so obtained was washed with cold methanol to give crude condensed product (**1**). Solvent from the filtrate was removed under reduced pressure and to the residue left behind was added cold methanol (3 ml). Solid so separated out was filtered and the combined crude product was dissolved in THF and adsorbed over silica gel. Column was packed with silica gel using pet. ether as a solvent. Crude compound adsorbed over silica was charged on the top of column packed with silica gel. Elution with $CHCl_3$ removed side products and elution with $CHCl_3$/EtOAc (4:1) gave pure white solid product **1**. Yield 0.195 g (75%); mp 225 °C; IR (KBr) ν_{max}: 3448, 2968, 2225, 1585, 1533, 1434, 1282 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) δ: 1.35–1.37 (2s looking like a doublet, 6H, CH_3 + CH_3,), 1.50 (s, 3H, CH_3), 4.87 (s, 1H, >C═CH–), 7.32 (s, 1H, Ar), 7.51–7.59 (m, 2H, Ar), 7.65–7.67 (t, 1H, J = 6 Hz, Ar). Peak for –NH–CS– is expected downfield; FAB-MS m/z 258.57 (MH^+, 100%). Anal. Calcd for $C_{14}H_{15}N_3S$: C, 65.36; H, 5.83; N, 16.34. Found: C, 65.20; H, 5.90; N, 16.31.

4.3. Synthesis of 4-methoxy-4,6,6-trimethyl-3-(4-phenylthiazol-2-yl)-tetrahydropyrimidine-2(1*H*)-thione (2)

2-Amino-4-phenyl thiazole (**2a**) (0.176 g, 1 mmol) was dissolved in methanol (15 ml) and to it was added 4-isothiocyanato-4-methyl pentane-2-one (**1b**) (0.2 ml, 1.3 mmol). The reaction contents were heated under reflux for 11 h and then solvent was removed under reduced pressure. Solid residue left behind was washed

with cold methanol (2 ml) and then crystallized from methanol to give pure white solid condensed product **2**. Yield 0.253 g (73%); mp 200 °C IR (KBr) ν_{max}: 3451, 2959, 1640, 1539, 1443, 1277, 1187 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) δ: 1.38 (s, 6H, CH_3 + CH_3), 1.50 (s, 3H, $–CH_3$), 2.00–2.05 (d, 1H, J_{gem} = 15 Hz, one H of $–CH_2–$), 2.35–2.40 (d, 1H, J_{gem} = 15 Hz, one H of $–CH_2–$), 3.44 (s, 3H, $–OCH_3$), 7.28–7.33 (t, 1H, Ar), 7.37–7.42 (t, 2H, Ar), 7.63 (s, 1H, >C═CH–), 7.87–7.90 (d, 2H, Ar), 8.47 (s, 1H, –NH); FAB-MS *m/z* 370.1043 [$(MNa)^+$, 100%]. *m/z* 316 ( , 20%), 315 ( , 7%), 214 ( , 16%). Anal. Calcd for $C_{17}H_{21}N_3OS_2$: C, 58.78; H, 6.05; N, 12.10. Found: C, 59.01; H, 6.00; N, 12.01.

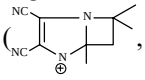
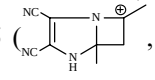
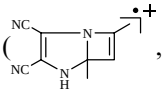
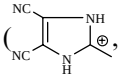
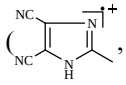
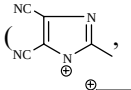
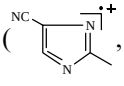
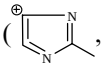
4.4. Synthesis of 4,4,6-trimethyl-1-(4-phenylthiazol-2-yl)-3,4-dihydropyrimidine-2(1*H*)-thione (3)

2-Amino-4-phenyl thiazole (**2a**) (0.176 g, 1 mmol) was dissolved in methanol (15 ml) and to it was added 4-isothiocyanato-4-methylpentane-2-one (**1b**) (0.2 ml, 1.2 mmol). The pH of the reaction contents was adjusted to ~4 by adding a few drops of 10% H_2SO_4 in methanol. The reaction contents were heated under reflux, after 8 h of refluxing, solid product started separating out and refluxing was further continued for 3 h. Solvent was removed under reduced pressure and the crude product so obtained was washed with 10% aq sodium carbonate solution and then with water and air-dried. Crude condensed product **3** was purified by column chromatography over silica gel. Elution with $CHCl_3$ removed side products and further elution with $CHCl_3$/MeOH (9.5:0.5) gave pure condensed product **3**. White solid, 0.236 g (75%); mp 190 °C; IR (KBr) ν_{max}: 3443, 2963, 1639, 1542, 1446, 1278 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) δ: 1.47 (d, 6H, CH_3 + CH_3), 1.70 (s, 3H, $–CH_3$), 4.9 (d, 1H, >C═CH–), 6.86 (bs. 1H, –NH), 7.29–7.53 (m, 3H, Ar), 7.83 (s, 1H, Ar), 7.89–7.90 (d, 2H, Ar); FAB-MS *m/z* 316.94 (MH^+, 30%). Anal. Calcd for $C_{16}H_{17}N_3S_2$: C, 60.95; H, 5.39; N, 13.33. Found: C, 61.05; H, 5.24; N, 13.39.

4.5. Synthesis of 1-[(3-1*H*-imidazol-1-yl)propyl]-6-hydroxy-4-methyl-tetrahydro pyrimidine-2 (1*H*)-thione (4)

3-Aminopropyl imidazole (**4a**) (0.125 g, 1 mmol) was dissolved in dry methanol (10 ml) and to it was added 3-isothiocyanatobutanal (**1c**) (0.2 ml, 1.5 mmol). The reaction contents were allowed to stand at room temperature for 8 days, solvent was removed under reduced pressure and the crude product was subjected to column chromatography over silica gel. Elution with $CHCl_3$ removed the side products and further elution with $CHCl_3$/MeOH (9:1) gave pure condensed product **4**. White solid, 0.100 g (42%); mp 240 °C; IR (KBr) ν_{max}: 3421, 2947, 2869, 1627, 1517, 1446, 1279 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) δ: 1.20–1.22 (d, 3H, *J* = 6 Hz, $–CH_3$), 1.25–1.53 (m, 1H, one H of $–CH_2–$), 1.92-1.96 (d, 1H, one H of $–CH_2–$), 2.10–2.36 (m, 2H, C–CH_2–C), 3.64–3.87 (m, 2H, $–CH_2–$N), 3.99–4.09 (m, 3H, $–CH_2–$N + >CH–CH_3), 4.87 (br s, 1H, –>CH–OH, changes to doublet after D_2O exch), 6.25 (br s, 1H, –OH, exch), 6.97–7.05 (d, 2H, Ar), 7.51 (s, 1H, –NH– exch), 7.57 (s, 1H, Ar). FAB-MS *m/z* 255.76 (MH^+, 10%). Anal. Calcd for $C_{11}H_{18}N_4OS$: C, 51.96; H, 7.08; N, 22.04. Found: C, 51.79; H, 6.91; N, 22.13.

4.6. Synthesis of 7,7,8a-trimethyl-5-thioxo-1,5,6,7,8,8a-hexahydroimidazo[1,2-f] pyrimidine-2,3-dicarbonitrile (5a)

Diaminomaleonitrile (**5**) (0.108 g, 1 mmol) was dissolved in methanol (25 ml) and to it was added 4-isothiocyanato-4-methyl pentane-2-one (**1b**) (0.2 ml, 1.3 mmol). The reaction contents were heated under reflux for 12 h and then solvent was removed under reduced pressure to give crude product **5a**. Crude product **5a** was adsorbed over silica gel and subjected to column chromatography over silica gel. Elution with pet. ether/$CHCl_3$ (1:2) gave pure product **5a**. Brown solid, 0.115 g (46%); mp 165 °C; IR (KBr) ν_{max}: 3427, 2981, 2212, 1630, 1528, 1476, 1283 cm^{-1}. ^1H NMR (300 MHz DMSO-d_6) δ: 1.18 (s, 6H, CH_3 + CH_3), 2.15 (s, 3H, CH_3), 2.63 (s, 2H, $–CH_2–$), 8.39 (s, 1H, NH, exch), other NH is expected downfield; did not give M^+ ion peak in HR-MS but gave *m/z* 188.10576 (M^+–HSCN, 27.08%), 187.09779 ( , 1.05%), 173.08265 ( , 19.94%), 172.07618 ( , 0.82%); 133.05109 ( , 57.84%), 132.04474 ( , 25.58%), 131.03635 ( , 5.85%), 105.03351 ( , 7.33%), 79.03018 ( , 4.33%); 56.06246 ( , 100.00%). Anal. Calcd for $C_{11}H_{13}N_5S$: C, 53.44; H, 5.26; N, 28.34. Found: C, 53.41; H, 5.33; N, 28.41.

4.7. Synthesis of 7-methyl-5-thioxo-1,5,6,7,8,8a-hexahydroimidazo[1,2-f]pyrimidine-2,3-dicarbonitrile (5b)

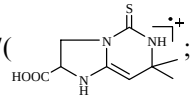
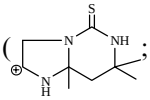
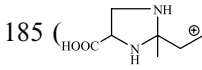
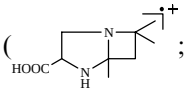
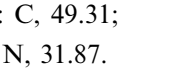
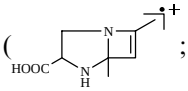
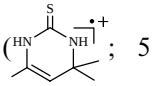
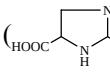
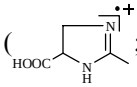
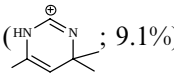
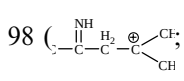
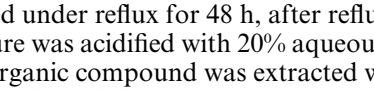
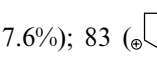
Diaminomaleonitrile (**5**) (0.108 g, 1 mmol) was dissolved in methanol (25 ml) and to it was added 3-isothiocyanatobutanal (**1c**) (0.2 ml, 1.5 mmol). The reaction contents were allowed to stand at room temperature for 4 days and then solvent was removed under reduced pressure to give crude product **5b**. Crude product **5b** was adsorbed over silica gel and then subjected to column chromatography over silica gel. Elution with $CHCl_3$ removed side products and further elution with $CHCl_3$/EtOAc (4:1) gave pure condensed product **5b**. Brown solid, 0.055 g (25%); mp 220 °C. IR (KBr) ν_{max}: 3344, 2977, 2212, 1630, 1552, 1460 cm^{-1}. ^1H NMR (300 MHz DMSO-d_6 + $CDCl_3$) δ: 1.31–1.33 (d, 3H, $–CH_3$), 1.58–1.71 (m, 1H, one H of $–CH_2–$), 2.01–2.08 (dd, 1H, one H of $–CH_2–$), 3.19–3.29 (m, 1H, >CH–

CH_3), 4.41 (br s, changes to quartet after D_2O exchange 1H, ), 5.61 (br s, 1H, –NH–, exch), 6.49 (br s, 1H,  , exch). Anal. Calcd for $C_9H_9N_5S$: C, 49.31; H, 4.11; N, 31.96. Found: C, 49.19; H, 4.01; N, 31.87.

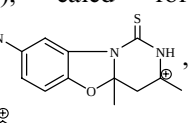
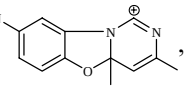
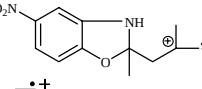
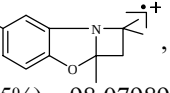
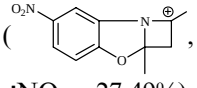
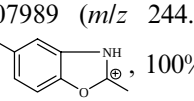
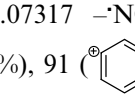
4.8. Synthesis of 4,4,6-trimethyl-3,4-dihydropyrimidine-2 (1*H*)-thione (6)

Compound **5a** (0.500 g, 2 mmol) was added to 30% aqueous sodium hydroxide solution (5 ml). The reaction mixture was heated under reflux for 48 h, after refluxing, the reaction mixture was acidified with 20% aqueous sulfuric acid and the organic compound was extracted with ethyl acetate (3 × 25 ml). Ethyl acetate extract on removal of solvent gave crude product, which was purified by column chromatography over silica gel. Elution with $CHCl_3$ removed side products and further elution with $CHCl_3$/EtOAc (4:1) gave pure product **6**. White solid, 0.100 g (32%); mp 255 °C. IR (KBr) ν_{max}: 3200, 2968 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ: 1.15 (s, 6H, 2× CH_3), 1.64 (s, 3H, CH_3), 4.49 (s, 1H, >C=CH–), 8.44 (s, 1H, NH exch), 9.38 (s, 1H, NH, exch); HR-MS found 156.07209 (M^+, 51%) calcd for $C_7H_{12}N_2S$ 156.07211.

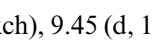
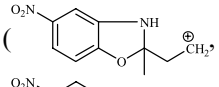
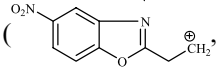
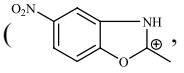
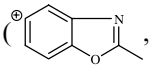
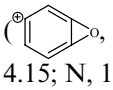
4.9. Synthesis of 7,7,8a-trimethyl-5-thioxo-octahydroimidazo[1,2-f]pyrimidine-2-carboxylic acid (7)

2,3-Diaminopropionic acid hydrochloride (**7a**) (0.700 g, 5 mmol) was dissolved in methanol (75 ml) in the presence of sodium hydroxide (200 mg, 5 mmol) and to this reaction mixture was added 4-isothiocyanato-4-methylpentane-2-one (**1b**) (0.8 ml, 5 mmol). The reaction mixture was heated under reflux, after 4 h of refluxing, some solid started separating out and the reaction contents were further refluxed for 8 h. It was then cooled and the solid separated out was filtered, washed with cold methanol to give crude product **7**, which was purified by crystallization from THF. Yield 600 mg. Filtrate of above reaction was acidified with 10% H_2SO_4 in methanol and then solvent was removed under reduced pressure and to the semisolid residue left behind was added 25 ml water and then it was extracted with ethyl acetate (3 × 25 ml). Ethyl acetate extract on removal of solvent gave crude product **7**, which was purified by column chromatography over silica gel. Elution with $CHCl_3$/pet. ether (1:2) removed side products and further elution with $CHCl_3$/pet. ether (1:1) gave 100 mg pure white solid product **7**. White solid, total yield 0.700 g (60%); mp 200 °C. IR (KBr) ν_{max}: 3436, 2969, 1700, 1529, 1271, 1101 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6 + D_2O) δ: 1.20 (s, 3H, CH_3), 1.29–1.32 (2s looking like a doublet, 6H, CH_3 + CH_3), 1.58–1.62 (d, 1H, J_{gem} = 12 Hz, one H of $–CH_2–$), 2.17–2.21 (d, 1H, J_{gem} = 12 Hz, one H of $–CH_2–$), 2.88–2.95 (q, 1H, one H of $–CH_2–$), 3.40–3.51 (m, 1H, one H of $–CH_2–$), 4.46–4.60 (m, 1H, ); EI-MS *m/z* 244 (M^++1; 4.6%); 243 (M^+, 11.4%); 242 (M^+−H; 100%); 227( ; 37.6%); 198 ( ; 3.9%); 185 ( ; 4.4%) 184 ( ; 36.2%); 169 ( ; 16.9%); 168 ( ; 6.9%) 156 ( ; 5.4%); 129 ( ; 9.0%); 128 ( ; 4.7%) 123 ( ; 9.1%); 98 ( ; 51.5%); 97 ( ; 7.6%); 83 ( ; 46.4%); 82 ( ; 18.8%). Anal. Calcd for $C_{10}H_{17}N_3O_2S$: C, 49.38; H, 6.99; N, 17.28. Found: C, 49.12; H, 7.00; N, 17.41.

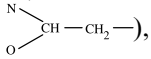
4.10. Synthesis of 2,3,4,4a-tetrahydro-3,3,4a-trimethyl-8-nitro-1(*H*)pyrimido[6,1-b]benzoxazole-1-thione (8a)

2-Amino-4-nitrophenol (**8**) (0.154 g, 1 mmol) was dissolved in methanol (15 ml) and to it was added 4-isothiocyanato-4-methyl pentane-2-one (**1b**) (0.2 ml, 1.3 mmol). The pH of the reaction contents was adjusted to about ∼4 by adding a few drops of 10% H_2SO_4 in methanol. The reaction contents were heated under reflux, after 2 h of refluxing, some solid started separating out and the reaction contents were further refluxed for 8 h. Solvent was removed under reduced pressure and the residue left behind was basified by adding 10% aq sodium carbonate solution (10 ml). Solid separated out was filtered, washed with with water and air-dried to give crude product **8a**. Crude product **8a** was purified by crystallization from methanol to give pure condensed product **8a**. Yellow solid, 0.245 g (83%); mp 190 °C; IR (KBr) ν_{max}: 3481, 2981, 1499 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 1.25–1.35 (2s, looking like a doublet, 6H, CH_3 + CH_3), 1.60 (s, 3H, CH_3), 2.30–2.40 (d, 1H, one H of $–CH_2–$), 2.65–2.75(d, 1H, one H of $–CH_2–$), 7.15 (d, 1H, Ar), 8.0 (dd, 1H, Ar), 9.25 (s, 1H, –NH– exch), 9.45 (d, 1H, Ar); HR-MS Found 293.08381 (M^+, 58.02%), calcd for $C_{13}H_{15}N_3SO_3$ 293.08340. 278.06144 ( , 12.65%), 244.07317 ( , 56.52%), 235.10609 ( , 10.51%), 234.09963 ( , 2.22%), 219.07647 ( , 14.75%), 98.07989 (*m/z* 244.07317 –·NO_2, 27.49%), 179.04699 ( , 100%), 91 ( , 14.15%). Calcd for $C_{13}H_{15}N_3O_3S$: C, 53.24; H, 5.12; N, 14.33. Found: C, 53.50; H, 5.29; N, 14.17.

4.11. Synthesis of 2,3,4,4a-tetrahydro-4a-methyl-8-nitro-1(*H*)pyrimido[6,1-b]benzoxazole-1-thione (8b)

2-Amino-4-nitrophenol (**8**) (0.154 g, 1 mmol) was dissolved in methanol (15 ml) and to it was added 4-isothiocyanatobutan-2-one (**1 d**) (0.2 ml, 1.5 mmol). The pH of the reaction mixture was adjusted to ~4 by adding a few drops of 10% H_2SO_4 in methanol. The reaction contents were heated under reflux for 8 h, solvent was removed under reduced pressure and the residue left behind was basified by adding 10% aq sodium carbonate solution (10 ml). Solid separated out was filtered, washed with water and air-dried to give crude product **8b**. Crude product **8b** was purified by crystallization from methanol to give pure product **8b**. Brown solid, 0.200 g (79%); mp 203 °C. IR (KBr) ν_{max}: 3427, 2980, 1474, 1202 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6) δ: 1.65 (s, 3H, CH_3), 2.15–2.30 (m, 1H, one H of –CH_2–), 2.55–2.65 (m, 1H, one H of –CH_2–), 3.30–3.50 (m, 2H, –CH_2–), 7.15 (d, 1H, Ar), 8.0 (dd, 1H, Ar), 9.20 (s, 1H,  exch), 9.45 (d, 1H, Ar); HR-MS found 265.05118 (M^+, 75.15%) calcd for $C_{11}H_{11}N_3O_3S$ 265.05212. 250.03091 (M^+—·CH_3, 11.28%), 207.07794 ( , 33.93%), 191.04366 ( , 15.33%), 179.04475 ( , 100.00%), 132.04323 ( , 3.00%), 91.01799 ( , 8.90%). Calcd for $C_{11}H_{11}N_3O_3S$: C, 49.81; H, 4.15; N, 15.84. Found: C, 50.00; H, 4.10; N, 15.91.

4.12. Synthesis of 2,3,4,4a-tetrahydro-3-methyl-8-nitro-1(*H*)pyrimido[6,1-b] benzoxazole-1-thione (8c)

2-Amino-4-nitrophenol (**8**) (0.154 g, 1 mmol), 3-isothiocyanatobutanal (**1c**) (0.2 ml, 1.5 mmol) and silica gel (60–120 mesh) (2 g) were mixed together thoroughly and this reaction mixture was subjected to microwave irradiation (by keeping in microwave oven) for 2.5 min at a power of 850 W. To this reaction mixture was added 50 ml methanol, stirred for 10 min and then filtered. This process was repeated two more times and the combined filtrate was distilled off under reduced pressure to give crude product **8c**. Crude product **8c** was purified by column chromatography over silica gel. Elution with $CHCl_3$ removed side products and further elution with $CHCl_3$/EtOAc (4:1) gave pure product **8c**. Yellow solid, 0.065 g (25%); mp 260 °C. IR (KBr) ν_{max}: 3443, 1499, 1239, 1113 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6 + $CDCl_3$) ^1H NMR shows doubling of some peaks in the ratio of 1:3 indicating that this compound is a mixture of two isomers in the ratio of 25:75, which can arise due to chiral carbon present in the molecule. ^1H NMR δ: 1.32–1.34 (d, 0.75H, CH_3), 1.39–1.42 (d, 2.25H, CH_3), 1.92–2.03 (q, 0.75H, one H of –CH_2–), 2.21–2.35 (m, 0.25H, one H of –CH_2–), 2.50–2.59 (q, 0.25H, one H of –CH_2–), 2.65–2.71 (m, 0.75H, one H of –CH_2–), 3.74–3.83 (m, 1H, >CH–CH_3), 6.05–6.11 (m, 1H, ), 6.88–6.93 (q, 1H, Ar), 7.91–8.02 (m, 1H, Ar), 8.32 (s, 0.75H, SH, exch), 8.76 (s, 0.25H, SH, exch), 9.45–9.46 (d, 0.75H, Ar), 9.50–9.51 (d, 0.25H, Ar). FAB-MS *m/z* 266.87 (MH^+; 8%). Calcd for $C_{11}H_{11}N_3O_3S$: C, 49.81; H, 4.15; N, 15.84. Found: C, 50.09; H, 3.99; N, 16.01.

4.13. Anti-inflammatory activity screening[24]

Anti-inflammatory activity screening was carried out using carrageenin induced paw oedema in albino rats. Oedema in one of the hind paws was induced by injection of carrageenin solution (0.1 ml of 1%) into planter apponeurosis. The volume of the paw was measured plethysmographyically immediately after and 3 h after the injection of the irritant. The difference in volume gave the amount of oedema developed. Percent inhibition of the oedema between the control group and the compound treated group was calculated and compared with the group receiving a standard drug. Compounds **1**–**4** and **5a**–**b**, **7** and **8a**–**b** at 50 mg/kg po exhibited 27.9, 22.3, 34.5, 24.5, 3.0, 34.3, 0.0, 0.0 and 0.0%, respectively, anti-inflammatory activity (Table 1) compared to ibuprofen which showed 38% activity at 50 mg/kg po.

4.14. Analgesic activity screening[25]

Analgesia was measured by the writhing assay using Swiss mice (15–20 g). Female mice were screened for writhing on day 1 by injecting intraperitonially 0.2 ml of a 0.02% aqueous solution of phenylquinone. They were kept on flat surface and the number of writhes of each mouse was recorded for 20 min. The mice showing significant (>10) writhes were sorted out and used for analgesic assay on the following day. The mice consisting of 5 in each group and showing significant writhing were given orally a 25 or 50 or 100 mg/kg po dose of the test compounds 15 min. prior to phenylquinone challenge. Writhing was again recorded for each mouse in a group and a percentage protection was calculated using following formula:

$$\text{Protection} = 100 - [\{(\text{No. writhings for treated mice})/ \times (\text{No. of writhings for untreated mice})\} \times 100].$$

This was taken as percent analgesic response and was averaged in each group of mice. Percent of animals exhibiting analgesia was determined with each dose. Compounds **1**, **2**, **3**, **4**, **5a**, **5b**, **7** and **8b** were screened for analgesic activity at 50 mg/kg po. All these compounds exhibited 10, 30, 30, 20, 50, 70, 25 and 50%, respectively, analgesic activity as compared to ibuprofen, which exhibited 50% activity. Compounds **5a**, **7**, **8a** and **8b** were also screened at 100 mg/kg po and they exhibited 100, 50, 25 and 75%, respectively, analgesic activity (Table 1), whereas ibuprofen at 100 mg/kg po exhibited 75% activity.

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