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Short communication

Anti-inflammatory, analgesic and antiamoebic activity evaluation of pyrimido[1,6-*a*]benzimidazole derivatives synthesized by the reaction of ketoisothiocyanates with mono and diamines

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Abstract

(UN) substituted *o*-phenylenediamines **1a**–**g** reacted with 3-isothiocyanatobutanal to give pyrimidobenzimidazole derivatives, **2a**–**g**, respectively. Products **4**, **6** and **8**, **10** were obtained by condensation of 3-isothiocyanatobutanal with 2,3-diaminopyridine, 1,4-diaminobutane and 3-isothiocyanatopropanal with 4,5-dimethyl-1,2-phenylenediamine, *o*-nitroaniline, respectively. S-Methylation of **2f** and **11b** gave products **12a** and **12b**, respectively. Anti-inflammatory and analgesic activity evaluations of **2a**–**g** and **12b** were carried out at 50 mg kg^{-1} p.o. Compound **2c** exhibited good anti-inflammatory (46%) and mild analgesic activity (50%). Antiamoebic activity evaluations (using microdilution method) of **2a**–**g** against *Entamoeba-histolytica* (strain HM1: IMSS) were carried out and compounds **2a**, **2b**, **2d** and **2g** exhibited good antiamoebic activity in vitro.

Keywords: Pyrimido[1,6-*a*]benzimidazoles; Anti-inflammatory; Analgesic; Antiamoebic

1. Introduction

Inflammatory diseases and amoebiasis are major health problems of mankind. Many anti-inflammatory drugs are available but cannot be used continuously for long time as they have ulcerogenic activity as major side effect [1]. Pyrimidine derivatives possessing anti-inflammatory [2–6] and analgesic activities [7] have been reported in literature. Amoebiasis caused by the protozoan parasite *Entamoeba-histolytica* remains a major world health problem and caused up to 100,000 deaths per annum [8], placing it as third parasitic disease. Common side effects of most commonly used antiamoebic drug metronidazole are nausea, is mutagenic in bacteria [9] and high doses in rodents may cause carcinoma. In addition the possibility of the future development of resistant strains as well demonstrated by other protozoa cannot be excluded.

In continuation of our efforts in search of potential anti-inflammatory [10–17] and antiamoebic [18] agents we have synthesized pyrimidobenzimidazole derivatives and screened them in vitro for their ability to inhibit the growth of *E. histolytica*. The compounds were also screened in vivo for anti-inflammatory and analgesic activity to develop better antiamoebic, anti-inflammatory and analgesic agents.

2. Chemistry

3-Isothiocyanatobutanal on condensation with *o*-phenylenediamine by refluxing in methanol at ca. pH 5 gave pyrimidobenzimidazole derivative **2a** (Fig. 1) in 34% yield. The structure of **2a** is fully supported by correct IR, ^{1}H-NMR and HRMS spectral data reported in Section 4. It is interesting to note that ^{1}H-NMR of **2a**

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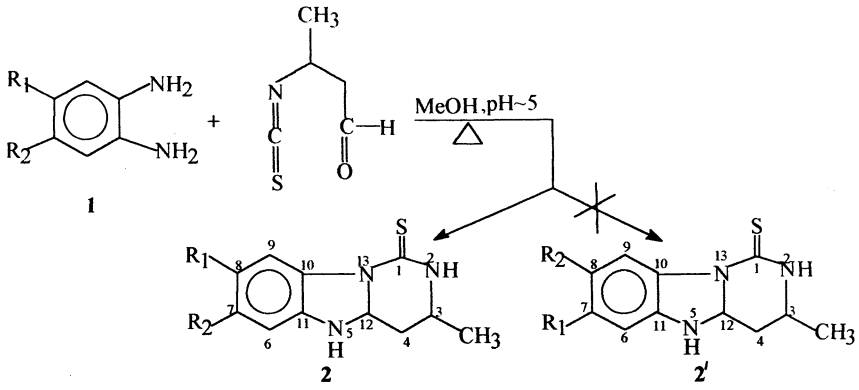


a; R_1=H, R_2=H
b; R_1=H, R_2=CH_3
c; R_1=NO_2, R_2=H
d; R_1=COOH, R_2=H
e; R_1=H, R_2=Cl
f; R_1=C_6H_5CO, R_2=H
g; R_1=CH_3, R_2=CH_3

Fig. 1. Synthesis of **2a**–**g**

shows doubling of peaks i.e. double doublet at δ 1.1 (dd, 3H, CH_3) and peaks accounting for half proton i.e. δ 1.6 (q, 0.5H), 1.80 (m, 0.5H), 2.10 (d, 0.5H), 2.40 (d, 0.5H), 8.25 (s, 0.5H exch.) and 8.60 (s, 0.5H, exch.). This behaviour of **2a** can be explained by considering 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 **2a** is a mixture of two isomers and this has been shown by ^{1}H-NMR. This type of observation have also been reported in literature [19]. Similarly condensation of various substituted *o*-phenylenediamines (**1b**–**g**) with 3-isothiocyanatobutanal was carried out to give corresponding condensed products **2b**–**g** (Fig. 1). Yield. m.p. solvent of elution or crystallization and spectral data of **2b**–**g** are reported in Section 4. ^{1}H-NMR data of **2b**–**g** indicate that they are all mixtures of isomers. Assignment of structure **2b**–**f** where alternative structures **2′b**–**f** are also possible is based on the fact that most basic amino group will react first with 3-isothiocyanatobutanal and then less basic amino group will react to give tricyclic products [20] **2b**–**f** and not **2′b**–**f** (Fig. 1).

Condensation of 2,3-diaminopyridine with 3-isothiocyanatobutanal by refluxing in methanol at ca. pH 5 gave mixture of **4** (Fig. 2) and not tricyclic mixture of **4′**. Formation of **4** can be explained from fact that in case of 2,3-diammopyridine amino group at position 3 w.r.t. ring nitrogen is more basic than amino group at position 2 w.r.t ring nitrogen [21,22] and thus amino group at position 3 undergoes condensation with 3-isothiocyanatobutanal at ca. pH 5 to form intermediates 3′ and 3″.

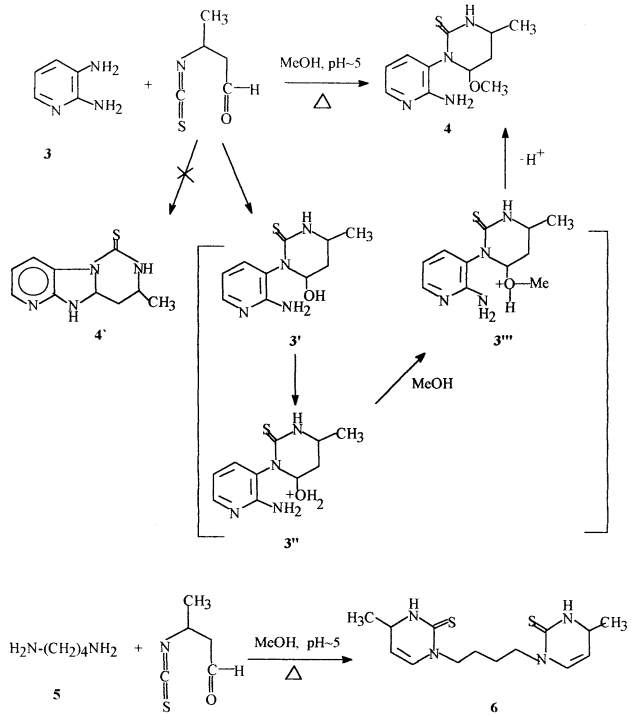


Fig. 2. Synthesis of **4** and **6**

After formation of 3″ methanol attacks on the carbon to form 3‴ which then losses a proton to give mixture of **4** (Fig. 2) and not **4′**. The structure assigned to **4** is fully supported by correct IR. ^{1}H-NMR and HRMS spectral data reported in Section 4. ^{1}H-NMR spectra of **4** gave peaks accounting for less than one proton and this can

be explained on the basis that **4** is a mixture of isomers, which can arise due to the presence of two chiral carbons in the structure.

Condensation of 1,4-diaminobutane with 3-isothiocyanatobutanal at ca. pH 5 under reflux condition in methanol gave bis pyrimidine derivative **6** (Fig. 2) in 31% yield. The structure of compound **6** is supported by correct IR, ^{1}H-NMR and HRMS spectral data reported in Section 4. After doing various reactions of 3-isothiocyanatobutanal, a few reactions of 3-isothiocyanatopropanal were also studied. It is interesting to note that while preparation of 3-isothiocyanatobutanal was not a problem and could be obtained by addition of HSCN to crotonaldehyde, in the case of addition of HSCN to acrolein was accompanied by polymerization of aerolein to large extend and 3-isothiocyanatopropanal was obtained in low yield. Condensation of 4,5-dimethyl-1,2-phenylenediamine with 3-isothiocyanatopropanal gave bicyclic product **8** (Fig. 3) and not tricyclic product similar to **2g**. Formation of **8** can occur via intermediate **7′**. Compound **8** gave satisfactory elemental analysis for C and H. IR (KBr; cm^{-1}) spectrum of **8** show peaks at 3273, 3133 (–NH–) and 1624 (Ar) ^{1}H-NMR (300 MHz; DMSO-d_6) of **8** show signals at δ 2.22 (s, 6H, 2 × CH_3) 6.91 (s, 2H, Ar) and 12.29 (s, 2H, 2 × NH). IR, ^{1}H-NMR and elemental analysis are consistent with the structure assigned to compound **8**. Condensation of *o*-nitroaniline (**9**) with 3-isothiocyanatopropanal gave product **10** (Fig. 3). The structure of compound **10** is supported by correct ^{1}H-NMR reported in Section 4. When compounds **2f** and **11b** [20] were heated under reflux in methanol after adjusting the pH of the reaction to ca. 1 by adding concentrated sulphuric acid, S-methyl derivatives **12a** and **12b** (Fig. 4) were obtained in 50 and 35% yields, respectively. The structure of **12a** and **12b** are supported by correct IR, ^{1}H-NMR and HRMS spectral data reported in Section 4. Formation of **12a** and **12b** can be explained from the fact that conc. sulfuric acid may generate methyl carbonium ion (CH_3) which can attack on >C=S⇌>C–SH tautomer, because sulphur has good nucleophilic character so it can give rise to the formation of S-methyl derivative.

3. Pharmacological results and discussion

Compounds **2a**–**g** and **12b** were tested for anti-inflammatory activity [23] in carrageenin induced paw oedema model at 50-mg kg^{-1} p.o. and results are summarized in Table 1. Compounds **2a**, **2b**, **2c**, **2d**, **2e**, **2f**, **2g** and **12b** showed 19, 21, 46, 22, 16, 28, 15 and 5% anti-inflammatory activity, respectively, as compared to standard drug ibuprofen which showed 51% activity at 50 mg kg^{-1} p.o. A look at Table 1 indicates that compound **2c** exhibited good anti-inflammatory activity. Analgesic activity evaluations [24] in the phenylquinone writhing assay of **2a**–**g** and **12b** were carried at 50 mg kg^{-1} p.o. and results are summarized in Table 1. A look at Table 1 indicates that compound **2c** possesses

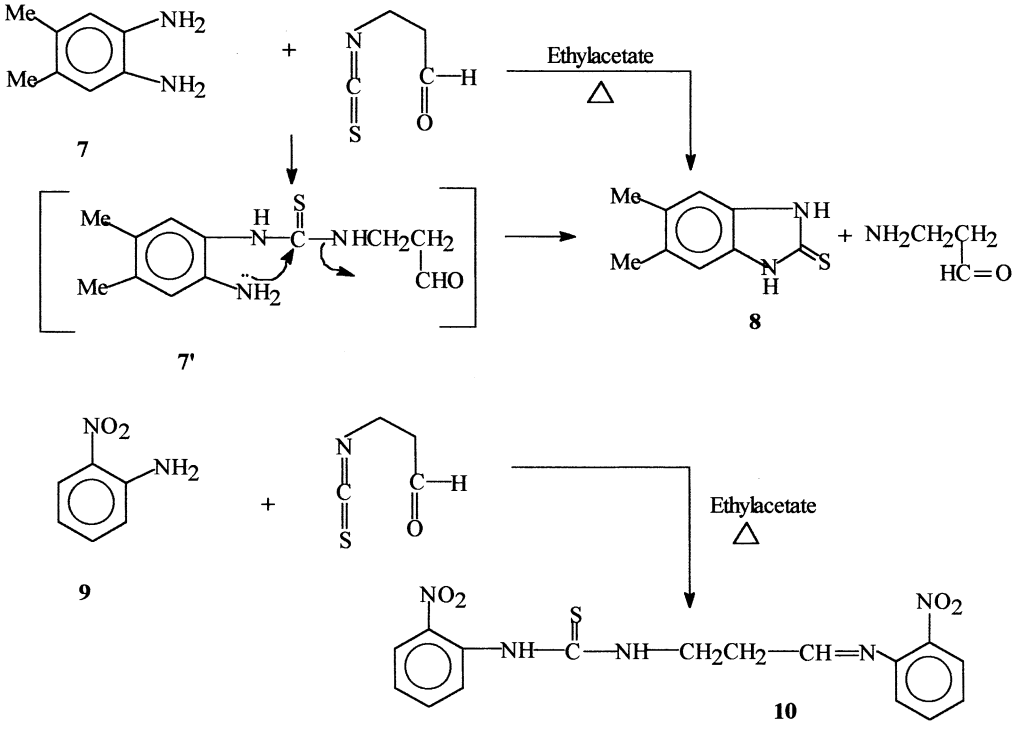
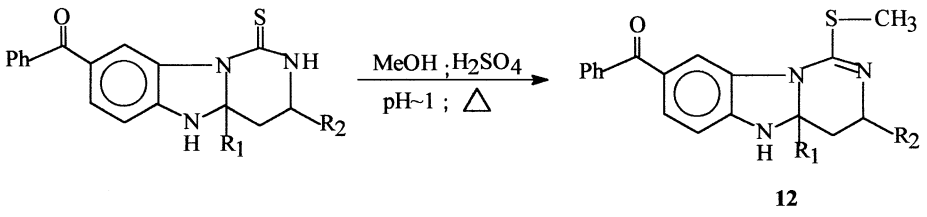


Fig. 3. Synthesis of **8** and **10**



2f R_1=H, R_2=CH_3 **12a** R_1=H, R_2=CH_3

11b R_1=CH_3, R_2=H **12b** R_1=CH_3, R_2=H

Fig. 4. Synthesis of **12a**,**b**

mild analgesic activity. Antiamoebic activity [25–28] evaluations of compounds **2a**–**g** were carried out in vitro against *E. histolytica* (strain HM1: IMSS) and IC_{50} values obtained are reported in Table 1. Metronidazole had 50% inhibitory concentration (IC_{50}) of 1.22 μM in our experiment. It was interesting to note that compound **2g** showed good antiamoebic activity having IC_{50} value 1.82 μM.

4. Experimental

4.1. Chemistry

Melting points (m.p.) were determined on a JSGW apparatus and are uncorrected. Only principal sharply defined IR peaks are reported. ^{1}H-NMR spectra were recorded in a 5–15% (w/v) solution in DMSO-d_6 using Bruker WH-300 MHz types spectrometer. The MS spectrometer peak measurements were made by comparison with perfluorotributylamine using an AEIMS-9 double focusing high resolution mass spectrometer at a resolving power of 15 000. Thin layer chromatography (TLC) was performed on silica gel G for TLC (Merck) and spot were visualized by iodine vapour or by irradiation with UV light (254 nm). Column chromatography was performed by using Qualigens silica gel for column chromatography (60–120 mesh).

4.1.1. General procedure for synthesis of 2

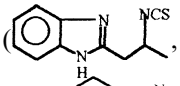
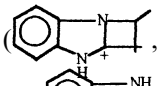
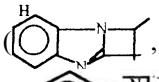
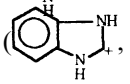
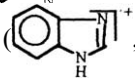
*4.1.1.1. Synthesis of 3,4,4a,5-tetrahydro-3-methylpyrimido[1,6-a]benzimidazol-1(2H)thione (**2a**).* *o*-Phenylenediamine (540 mg; 5 mmol) was dissolved in MeOH (25 ml) and to it was added 3-isothiocyanatobutanal (0.7 mL, 5 mmol) and pH of the reaction contents was adjusted to ca. 5 by adding a few drops of 10% sulphuric acid (10% H_2SO_4 in MeOH). The reaction mixture was heated under reflux for 8 h and then solvent was removed under reduced pressure and the residue left behind was basified with 10% aqueous sodium bicarbonate (ca. 10 mL). Solid separated out was filtered, washed with water and air dried to give crude product **2a**. Crude **2a** was purified by column chromatography over silica gel and elution was done with $CHCl_3$–EtOAc (9.5:0.5) to give pure condensed produce 3,4,4a,5-tetrahydro-3-methylpyrimido[1,6-*a*]-benzimidazol-1(2*H*)thione (**2a**).

M.p. 208–210 °C (0.372 g, 34%), IR (KBr, *v*, cm^{-1}), 3477, 3160 (–NH–), 1604, 1499 (Ar); ^{1}H-NMR:

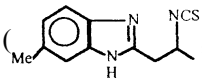
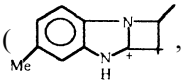
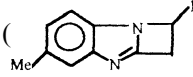
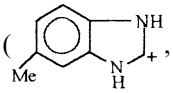
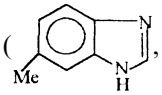
Table 1
Anti-inflammatory, analgesic and antiamoebic activity evaluation of **2a**–**g** and **12b**

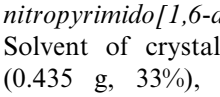
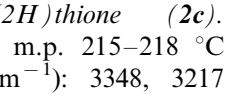
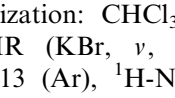
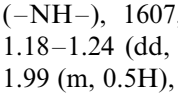
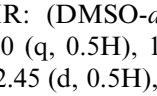
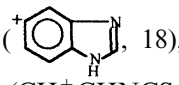
Compounds no.	Anti-inflammatory activity% 50 mg kg^{-1} p.o	Analgesic activity% 50 mg kg^{-1} p.o	Antiamoebic activity IC_{50} μM (S.D.)
2a	19	10	3.56 (0.53)
2b	21	10	2.96 (0.46)
2c	46	50	9.69 (0.93)
2d	22	20	2.62 (0.21)
2e	16	10	15.61 (2.16)
2f	28	20	3.99 (0.66)
2g	15	30	1.82 (0.19)
12b	5	0.0	NT
Ibuprofen	51	75	NT
Metronidazole	NT	NT	1.22 (0.26)

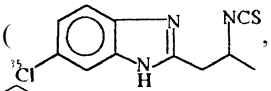
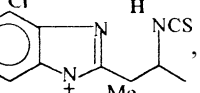
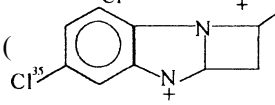
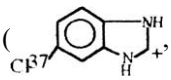
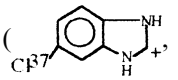
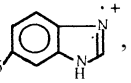
NT, not tested; S.D., Standard deviation.

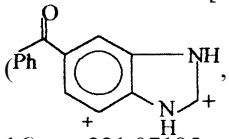
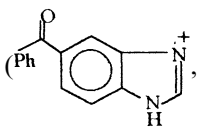
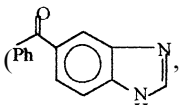
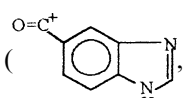
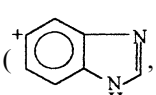
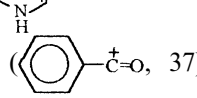
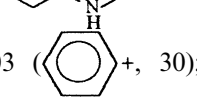
(DMSO-d_6) δ 1.10 (dd, 3H, $-CH_3$), 1.60 (q, 0.5H), 1.80 (m, 0.5H), 2.10 (d, 0.5H), 2.40 (d, 0.5H), 3.5 (m, 1H), 5.4 (dd, 1H), 6.30–6.65 (s+m, 3H, 1H exch+2HAr), 6.80 (m, 1H, Ar), 8.20 (s, 0.5H, exch, –NH), 8.50 (m, 1H, Ar), 8.60 (s, 0.5H, exch-NH–); HRMS (*m*/*z*, rel. int.%): Found: 219.08307 [M^+, 61] Calc. for $C_{11}H_{13}N_3S$ 219.08302; 218.07269 [M^+-H, 9], 217.06756 ( , 28); 159.09209 ( , 29); 158.08191 ( , 3); 119.06076 ( , 100); 118.05308 ( , 63); Anal. Calc. for $C_{11}H_{13}N_3S$: C, 60.27; H, 5.93; N, 19.17. Found: C, 60.52; H, 6.10; N, 19.03%.

Similarly were prepared compound **2b**–**c**, **e**–**g**.

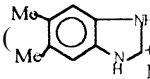
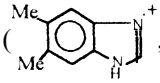
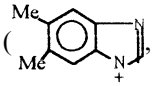
*4.1.1.2. 3,4,4a,5-Tetrahydra-3,7-dimethylpyrimido[1,6-a]benzimidazol-1(2H)thione (**2b**).* Solvent of crystallization: $CHCl_3$; m.p. 212–215 °C (0.337 g, 29%), IR (KBr, ν, cm^{-1}). 3190 (–NH–), 1612, 1502 (Ar); ^{1}H-NMR: (DMSO-d_6) δ 1.14–1.21 (dd, 3H, $-CH_3$), 1.60 (m, 0.5H), 1.90 (m, 0.5H), 2.17 (s, 3H, CH_3), 2.30 (m, 1H), 3.70 (m, 1H), 5.38 (d, 1H), 6.25–6.50 (s+d, 2H, Ar), 7.25 (bs, 1H, NH exch.); 8.19 (s, 1H, (C=S)NH exch.), 8.50 (dd, 1H, Ar): HRMS Found: 233.09848 [M^+, 72] Calc. for: $C_{12}H_{15}N_3S$ 233.039866, 231.08280 ( , 27); 173.10763 ( , 29), 172.09918 ( , 9), 133.07628 ( , 100); 132.06860 ( , 54); Anal. Calc. for $C_{12}H_{15}N_3S$: C, 61.80; H, 6.43; N, 18.02. Found: C, 61.63; H, 6.57; N, 18.21%.

*4.1.1.3. 3,4,4a,5-Tetrahydro-3-methyl-8-nitropyrimido[1,6-a]benzimidazol-1(2H)thione (**2c**).* Solvent of crystallization: $CHCl_3$; m.p. 215–218 °C (0.435 g, 33%), IR (KBr, ν, cm^{-1}): 3348, 3217 (–NH–), 1607, 1513 (Ar), ^{1}H-NMR: (DMSO-d_6) δ 1.18–1.24 (dd, 3H, $-CH_3$), 1.66–1.70 (q, 0.5H), 1.95–1.99 (m, 0.5H), 2.20–2.24 (d, 0.5H), 2.45 (d, 0.5H), 3.65 (m, 1H), 5.73–5.76 (d, 1H), 6.55 (t, 1H, Ar), 7.85 (m, 1H, Ar), 8.07 (s, 1H, Ar), 8.07 (bs, 1H, exch; NH), 8.72 (s, 0.5Hexch; SH), 8.85 (s, 0.5H, exch SH), 9.44 (dd, 1H, Ar); HRMS (*m*/*z*, rel. int.%); Found: 264.06681 [M^+, 54] Calc. for $C_{11}H_{12}N_4SO_2$ 264.06808; 262.05231 ( , 37) 261.05231 ( , 5), 164.04576 ( , 100); 163.03791 ( , 22) 118.05297 ( , 37); 117.04549 ( , 18), 90.03382 ( , 13); 86.00568 (CH_3^+CHNCS, 67); Anal. Calc. for $C_{11}H_{12}N_4SO_2$: C, 50.00; H, 4.54; N, 21.21. Found: C, 50.31; H, 4.33; N, 21.43%.

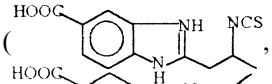
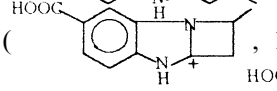
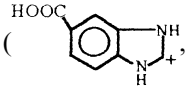
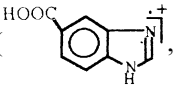
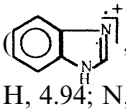
*4.1.1.4. 3,4,4a,5-Tetrahydro-7-chloro-3-methypyrimido[1,6-a]benzimidazol-1(2H)thione (**2e**).* Solvent of elution: CCl_4–EtOAc (1:1); m.p. 240–242 °C (0.433 g, 34%); IR (KBr, ν, cm^{-1}): 3207 (–NH–), 1604, 1511 (Ar), ^{1}H-NMR: (DMSO-d_6) δ 1.00–1.30 (dd, 3H, $-CH_3$), 1.40–1.65 (q, 0.5H), 1.70–2.00 (m, 0.5H), 2.20 (d, 0.5H), 2.40 (d, 0.5H), 3.50 (m, 1H), 5.40–5.60 (d, 1H), 6.50–6.80 (m, 3H, oneH exch+2H Ar), 8.30–8.80 (m, 2H, oneH exch, 1H, Ar); HRMS (*m*/*z*, rel. int.%). Found: 255.03920 [M^+, 17] Calc. for $C_{11}H_{12}N_3SCl^{37}$ 255.04109; 251.02811 ( , 54), 250.01992 ( , 8); 193.05139 ( , 39), 155.01901 ( , 31); 153.02178 ( , 100), 152.01374 ( , 48); 86.00644 (CH_3^+CHNCS, 46); Anal. Calc. for $C_{11}H_{12}N_3SCl$: C, 52.07; H, 4.73; N, 16.56. Found: C, 51.90; H, 4.91; N, 16.29%.

*4.1.1.5. 3,4,4a,5-Tetrahydro-8-benzoyl-3-methylpyrimido[1,6-a]benzimidazol-1(2H)thione (**2f**).* Solvent of crystallization: MeOH; m.p. 213–215 °C (0.742 g, 46%); IR (KBr, ν, cm^{-1}): 3382, 3248 (–NH–), 1650 (>C=O), 1573, 1505 (Ar); ^{1}H-NMR (DMSO-d_6) δ 1.0–1.25, (dd, 3H, $-CH_3$), 1.50 (q, 0.5H), 1.90 (m, 0.5H), 2.20 (d, 0.5H), 2.40 (d, 0.5H), 3.50 (m, 1H), 5.60 (dd, 1H), 6.60(d, 1H, Ar), 7.20–7.90 (m, 7H, oneH exch, NH+6H, Ar), 8.50 (s, 0.5H, exch), 8.70 (s, 0.5H, exch), 9.15 (d, 1H, Ar); HRMS (*m*/*z*, rel. int.%). Found: 323.10873 [M^+, 68] Calc. for $C_{18}H_{17}N_3OS$ 323.10922, 321.092751 [M^+-2H, 24]; 223.08724 ( , 100); 222.07940 ( , 16); 221.07185 ( , 3), 145.04038 ( , 38); 117.04598 ( , 10), 105.03418 ( , 37), 77.03903 ( , 30);

Anal. Calc. for $C_{18}H_{17}N_3OS$: C, 66.87; H, 5.26; N, 13.00. Found: C, 67.02; H, 5.10; N, 13.27%.

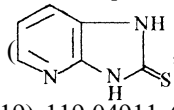
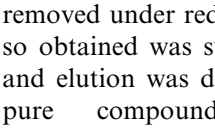
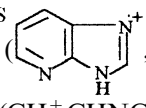
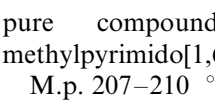
*4.1.1.6. 3,4,4a,5-Tetrahydro-3,7,8-trimethylpyrimido[1,6-a]benzimidazol-1(2H)thione (**2g**).* Solvent of crystallization MeOH; m.p. 198–200 °C (0.395 g, 32%), IR (KBr, ν, cm^{-1}); 3192 (–NH–), 1615, 1503 (Ar), ^{1}H-NMR: (DMSO-d_6) δ 1.12–1.20 (dd, 3H, $-CH_3$), 1.83–1.89 (m, 1H), 2.07–2.14 (s+d, 6.5H, $2 \times CH_3 + 0.5H$), 2.29–2.30 (d, 0.5H), 3.48–3.53 (q, 1H), 5.27–5.33 (m, 1H), 6.04–6.05 (d, 1H), 6.38–6.41 (2s, 1H), 8.37–8.43 (2s, 2H, Ar); HRMS (*m/z*, rel. int.%): Found: 247.1141 [M^+, 72] Calc. for $C_{13}H_{17}N_3S$ 247.11432; 246.10497 [M^+-H, 8], 245.09856 [M^+-2H, 13], 187.12345 [$M^+-(H+HSCN)$;23], 147.09227 ( , 100), 146.08448 ( , 49), 145.07676 ( , 16), 131.06099 (*m/z* 146.08448 $-CH_3$,15); Anal. Calc. for $C_{13}H_{17}N_3S$: C, 63.15; H, 6.88; N, 17.00. Found: C, 63.41; H, 7.01; N, 16.82%.

*4.1.1.7. Synthesis of 3,4,4a,5-tetrahydro-8-carboxyl-3-methylpyrimido[1,6-a]benzimidazol-1(2H)thione (**2d**).* 3,4-Diaminobenzoic acid (0.760 g, 5 mmol) was dissolved in MeOH (80 mL) and 3-isothiocyanatobutanal (0.7 mL, 5 mmol) was added to it and the reaction contents were heated under reflux for 8 h. Solvent was removed under reduced pressure and the crude product so obtained was subjected to column chromatography and elution was done with C_6H_6–EtOAc (3:1) to give pure compound 3,4,4a,5-tetrahydro-8-carboxyl-3-methylpyrimido[1,6-*a*]benzimidazol-1(2*H*)thione (**2d**).

M.p. 207–210 °C (0.236 g, 18%), IR (KBr, ν, cm^{-1}): 3192 (–NH–), 1679 (–COOH), 1627 (Ar); ^{1}H-NMR: (DMSO-d_6) δ 1.16–1.30 (dd, CH_3), 1.57–1.68 (q, 0.5H), 1.86–1.99 (m, 0.5H), 2.16–2.20 (dd, 0.5H), 2.37–2.43 (dd, 0.5H), 3.54–3.67 (m, 1H), 5.57–5.61 (m, 1H), 6.51–6.57 (t, 1H, Ar), 7.21–7.22 (d, 1H, –NH); 7.49–7.52 (dd, 1H, Ar), 8.44 (s, 0.5H), 8.64–8.66 (d, 0.5H), 9.13–9.18 (d, 1H, Ar), 12.20 (s, 1H, COOH); HRMS (*m/z*, rel. int.%): Found: 263.0723 [M^+, 57], Calc. for $C_{12}H_{13}N_3SO_2$ 263.07285, 261.05669 ( , 14), 203.08151 ( , 19), 163.05035 ( , 100); 162.04272 ( , 34), 118.05268 ( , 5); Anal. Calc. for $C_{12}H_{13}N_3SO_2$: C, 54.75; H, 4.94; N, 15.96. Found: C, 54.59; H, 5.03; N, 16.20%.

*4.1.2. Synthesis of 3-(1,2,3,4,5,6-hexahydro-4-methyl-6-methoxy-2-thioxo-1-pyrimidinyl)-2-aminopyridine (**4**)*

2,3-Diaminopyridine (218 mg; 2 mmol) was dissolved in MeOH (10 mL), and to it was added 3-isothiocyanatobutanal (0.3 mL, 2 mmol). To the reaction mixture a few drops of dilute sulphuric acid (10% H_2SO_4 in MeOH) was added to adjust the pH of the reaction contents to ca. 5, and reaction contents were heated under reflux for 13 h. Solvent was removed under reduced pressure and the residue was basified with 10% aq. sodium bicarbonate solution. Solid separated out was filtered washed with water and air dried to give crude product which was subjected to column chromatography over silica gel. Elution with CCl_4–EtOAc (1:1) gave product 3-(1,2,3,4,5,6-hexahydro-4-methyl-6-methoxy-2-thioxo-1-pyrimidinyl)-2-aminopyridine (**4**).

M.p. 172–175 °C (0.025 g, 5%); IR (KBr, ν, cm^{-1}); 3202 ($-NH_2$), 1524 (Ar); ^{1}H-NMR: (DMSO-d_6) δ 1.20 (d, 3H, CH_3), 1.90–2.05 (m, 1H, one H of CH_2), 2.10–2.20 (m, 1H, one H of CH_2), 3.10 (s, $2/3 \times 3H$; OCH_3), 3.15 (s, $1/3 \times 3H$, OCH_3), 3.50 (m, 1H), 4 30 (m, 2/3H), 4.70 (m, 1/3H), 5.50 (s, 0.6H, exch, NH_2), 5.70 (s, 1.4H, exch, $-NH_2$), 6.50–6.65 (dt, 1H, Ar), 7.15–7.30 (dq-1H, Ar), 7.90 (dd, 1H, Ar), 8.65 (s, 0.7H, exch-SH), 8.75 (s, 0.3H, exch,-SH); HRMS (*m/z*, rel. int.%): Found: 252.10537 [M^+, 100] Calc. for $C_{11}H_{16}N_4SO$, 252.10448, 237.98118 [M^+-CH_3, 17], 221.08554 [M^+-OCH_3, 6] 220.07671 [M^+-CH_3OH, 4]; 219.12436 [M^+-SH, 13], 219.07022 [$M^+-(CH_3OH+H)$, 4]; 162.10312 [$M^+-(CH_3OH+NCS)$, 10]; 151.02034 ( , 47), 120.05609 ( , 19) 119.04911 ( , 5), 93.04550 ( , 20); 86.00574 (CH_3^+CHNCS, 15). Anal. Calc. for $C_{11}H_{16}N_4SO$: C, 52.38; H, 6.34; N, 22.22. Found: C, 52.49; H, 6.43; N, 22.01%.

*4.1.3. Synthesis of 1,4-bis(1′,2′,3′,4′-tetrahydro-4′-methyl-2′-thioxo-1′-pyrimidinyl)butane (**6**)*

1,4-Diaminobutane (0.6 mL, 6 mmol) was dissolved in MeOH (20 mL) and to it was added 3-isothiocyanatobutanal (1.6 mL; 12 mmol) and pH was adjusted to ca. 5 by adding a few drops of dilute sulphuric acid (10% H_2SO_4 in MeOH). The reaction contents were heated under reflux, for 8 h and then solvent was removed under reduced pressure and the reduced basified with 10% aqueous sodium bicarbonate. Solid product separated out was filtered washed with water and air dried to give crude product **6** which was purified by column chromatography over silica gel. Elution with CCl_4–EtOAc (1:4) gave pure product 1,4-bis(1′,2′,3′,4′-tetrahydro-4′-methyl-2′-thioxo-1′-pyrimidinyl)butane (**6**).

M.p. 150 °C (0.576 g, 31%); IR (KBr, ν, cm^{-1}): 3204 (–NH–), ^{1}H-NMR: (DMSO-d_6) δ 1.12–1.14 (d, 6H, 2 × –CH_3), 1.23–1.30 (t, 2H), 1.58 (s, 4H), 2.10–2.14 (d, 2H), 4.17 (m, 2H), 4.60 (t, 2H), 8.20 (s, 2H) other protons are expected to be buried under H_2O peak at δ 3.30–3.33, HRMS (*m/z*, rel. int.%): Found: 310.12843 [M^+, 34] Calc. for $C_{14}H_{22}N_4S_2$: 310.12886. Anal. Calc. for $C_{14}H_{22}N_4S_2$: C, 54.19; H, 7.09; N, 18.09. Found: C, 54.00; H, 7.21; N, 18.22%.

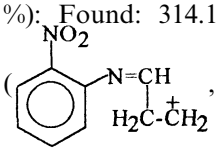
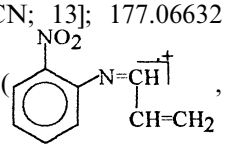
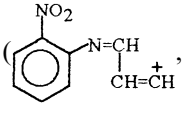
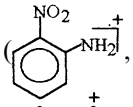
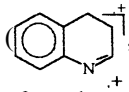
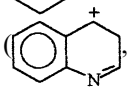
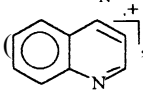
4.1.4. Synthesis of 5,6-dimethylbenzimidozole-(1H)thione (8)

4,5-Dimethyl-*o*-phenylenediamine (800 mg, 6 mmol) was dissolved in EtOAc (40 mL), 3-isothiocyanatopropanal (0.8 mL; 7 mmol) was dissolved in EtOAc (40 mL) and filtered to remove polymeric material. Clear solutions of both compounds were mixed and heated under reflex for 8 h. Solvent was removed under reduced pressure and the residue left behind was subjected to column chromatography over silica gel. Elution with $CHC1_3$–EtOAc (7:3) gave 5,6-dimethylbenzimidazole-2(1*H*)thione (**8**).

M.p. 225 °C (0.100 g, 10%); IR (KBr, ν, cm^{-1}) 3273, 3133 (–NH–) and 1624 (Ar). ^{1}H-NMR (300 MHz; DMSO-d_6) δ 2.22(s, 6H, 2 × CH_3); 6.91 (s, 2H, Ar) and 12.29 (s, 2H, 2 × NH–). Anal. Calc. for $C_9H_{10}N_2S$: C, 60.67; H, 5.61. Found: C, 60.51; H, 5.44%.

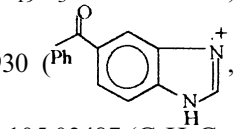
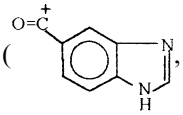
4.1.5. Synthesis of N-(o-nitrophenyl)-N′[3″-(1″-o-nitroiminophenyl)propyl]thiourea (10)

o-Nitroaniline (0.690 mg; 5 mmol) was dissolved in EtOAc (10 mL). 3-Isothiocyanatopropanal (0.600 mL, 5 mmol) was dissolved in EtOAc (10 mL) and polymeric material was filtered off. Both solutions were mixed and heated under reflux for 8 hrs. Solvent was removed under reduced pressure and the residue left behind was subjected to column chromatography over silica gel. Elution with petroleum ether–$CHCl_3$ (7:3) gave *N*-(*o*-nitrophenyl)-N′-[3″-(1″-*o*-nitrominophenyl)propyl]thiourea (**10**).

M.p. 155 °C (0.093 g, 10%); IR (KBr, ν, cm^{-1}) 3440, (–NH–) and 1621 (–CH=N–), 1511 (Ar); ^{1}H-NMR: (DMSO-d_6) δ 2.0 (m, 2H), 3.2–3.5 (m, 3H), 5.12 (bd, 1H) 6.60 (t, 1H, Ar), 6.70 (t, 1H, Ar), 7.31 (d, 1H, Ar), 7.44 (d, 1H, Ar), 7.61 (t, 1H, Ar), 8.01 (d, 1H, Ar), 8.09 (d, 2H, Ar), 8.57 (s, 1H, –NH–); HRMS (*m/z*, rel. int. %): Found: 314.10129 [M^+ −HSCN; 13]; 177.06632 ( , 100), 176.05819 ( , 27), 175.05051 ( , 20), 138.04284 ( , 8), 131.07313 ( , 25), 130.06516 ( , 22) 129.05778 ( , 24); Anal. Calc. for $C_{16}H_{15}N_5SO_4$: C, 51.47; H, 4.02; N, 18.76. Found: C, 51.52; H, 4.00; N, 18.93%.

4.1.6. Synthesis of 3,4,4a,5-tetrahydro-8-benzoyl-1-(S-methylpyrimido[1,6-a]benzimidazole (12a)

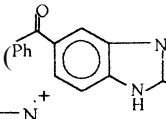
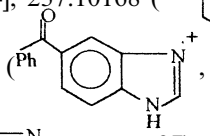
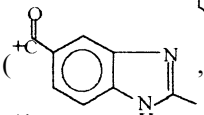
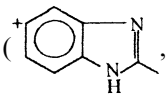
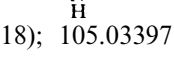
Pyrimidobenzimidazole (**2f**) (323 mg; 1 mmol) was dissolved in MeOH (20 mL) and to it was added conc. H_2SO_4 (1 mL). Reaction contents having ca. pH 1 were heated under reflux for 8 h and then solvent was removed under reduced pressure. The residue left behind was basified with (50%) aq. sodium bicarbonate solution. Solid product separated out was filtered, washed with water and air dried to give crude 3,4,4a,5-tetrahydro-8-benzoyl-1-(*S*-methyl)-3-methylpyrimido[1,6-*a*]benzimidazole (**12a**). Crude product was purified by column chromatography over silica gel. Elution with $CHCl_3$–EtOAc (1:1) gave pure product **12a**.

Solvent of crystallization MeOH, M.p. 185–188 °C (0.169 g, 50%); IR (KBr, ν cm^{-1}) 3431 (–NH–), 1686 (>C=O), 1610 (C=N), 1506 (Ar); ^{1}H-NMR (DMSO-d_6) δ 1.3 (dd, 3H, C–CH_3), 1.65 (m, 0.5H); 2.0 (m, 0.5H), 2.35–2.45 (dd, 1H), 3.40 (s, 3H, –SCH_3), 3.80 (m, 1H); 5.70 (d, 1H): 6.60–6.8 (dd, 1H, Ar); 7.30 (dd, 1H, Ar); 7.50–8.10 (m, 6H, Ar), HRMS (*m/z*, rel. int.%). Found: 337.12280 [M^+, 14] Calc. for $C_{19}H_{19}N_3SO$ 337.12488, 335.10961 [M^+ −2H, 17]; 222.07930 ( , 31); 145.03962 ( ,100); 105.03487 (C_6H_5C=O, 32); 77.03752 ($C_6H_5^+$, 17); Anal Calc. for $C_{19}H_{19}N_3SO$: C, 67.65; H, 5.63; N, 12.46. Found: C, 67.49; H, 5.22; N, 12.61%.

Similarly pyrimidobenzimidazole **11b** [20] was S-methylated to give 3,4,4a,5-tetrahydro-8-benzoyl-1-(*S*-methyl)-4a-methylpyrimido[1,6-*a*]benzimidazole (**12b**)

4.1.7. Synthesis of 3,4,4a,5-tetrahydro-8-benzoyl-1-(S-methyl)-4a-methylpyrimido[1,6-a]benzimidazole (12b)

Solvent of elution $CHCl_3$–EtOAc (1:1); m.p. 180 °C (0.117 g, 35%) IR (KBr, ν, cm^{-1}): 3264 (–NH–) 1684 (>C=O), 1666 (–C=N), 1504 (Ar); ^{1}H-NMR (DMSO-d_6) δ 1.4 (s, 3H, CH_3), 1.9 (m, 1H), 2.1 (m, 1H), 3.2 (m, 2H), 3.4 (m, 3H, –SCH_3), 6.5 (d, 1H, Ar), 7.0 (s, 1H, –NH–, exch), 7.2 (d, 1H, Ar); 7.60 (m, 5H, Ar); 7.90 (s, 1H, Ar): HRMS (*m/z*, rel. int.%): Found: 337.12389 [M^+, 16] Calc. for $C_{19}H_{19}N_3SO$ 337.12488; 322.10084 [M^+ −CH_3, 20); 290.12845 [M^+ −SCH_3, 4]; 274.09827

$[M^+ - (SMe + CH_4)$ 4]; 237.10168 ( , 32), 236.09449 ( , 26), 159.05541( , 37), 131.6083 ( , 18); 105.03397 ( , 44) 77.03964 ($C_6H_5^+$, 31). Anal. Calc. for $C_{19}H_{19}N_3SO$: C, 67.65; H, 5.63; N, 12.46. Found: C, 67.52; H, 5.70; N, 12.31%.

4.2. Pharmacology

4.2.1. Anti-inflammatory activity screening [23]

Anti-inflammatory activity testing was carried out using carrageenin induced paw ocdema in albino rats. The oedema in one of the hind paws was induced by injection of 0.1 mL of 1% carrageenin solution into planter aponeurosis. The volume of the paw was measured plethysmographically 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 control group and the compound treated group was calculated and compared with the group receiving standard drug. At 50 mg kg^{-1} p.o. compound **2c** possessed good 46% anti-inflammatory activity whereas compounds **2a**, **b**, **d**, **e**, **f**, **g**, and **12b** inhibited the carrageenin induced hind paw oedema by 19, 21, 22, 16, 28, 15 and 5%, respectively as compared to the standard drug ibuprofen which showed 51% activity at 50 mg kg^{-1} p.o.

4.2.2. Analgestic activity screening [24]

Analgesic activity was evaluated in albino mice using the phenylquinone writhing assay. Female swiss mice (15–20 g) were injected 0.2 mL of 0.02% aq. phenylquinone (2-phenyl-1,4-benzoquinone) and observed for writhing far 20 min. Number of writhes produced by each mouse was counted during this period. A minimum of 10 writhes produced by a mouse was considered positive and used in the analgesic testing on the following day.

The mice consisting of five in each group and showing significant writhing were given orally 50 mg kg^{-1} p.o. dose of test compound, 15 min prior to phenylquinone challenge. Writhing was again recorded for each mouse in a group and a percentage protection was calculated using the following formula:

$$\%\text{Protection} = 100 - [(\text{No. of writhing in treated})/(\text{No. of writhing in untreated}) \times 100]$$

This was taken as percent of analgesic response and was averaged in each group of mice, percent of animals exhibiting analgesia was determined with each dose. Compound **2a**–**g** and **12b** were screened for analgesic activity. Compound **2c** showed good analgesic activity i.e. 50% at 50 mg kg^{-1} p.o. Compound **2a**, **2b**, **2d**, **2e**, **2f**, **2g** and **12b** showed 10, 10, 20, 10, 20, 30 and 0% at 50 mg kg^{-1} p.o. analgesic activity, respectively.

4.2.3. Antiamoebic activity screening

4.2.3.1. In vitro testing against E. histolytica [25–28]. Axenic culture of HM: LMSS strain of *E. histolytica* was maintained in Diamond's medium [25]. All the compounds including metronidazole were dissolved in dimethylsulphoxide (DMSO). The maximum concentration of DMSO in the test did not exceed 0.1% at which level no inhibition of amoebal growth occurred [26,27]. Activity against *E. histolytica* (strain HM1: IMSS) in vitro was assessed using microplate method [28]. DMSO (40 μL) was added to the sample (1 mg) followed by enough culture medium to obtain concentration of 1 mg mL^{-1}. Samples were dissolved or suspended by mild sonication in a sonicleaner bath (Julabo, USRI, Germany) for a few minutes and then further diluted with medium to concentration of 0.1 mg mL^{-1}. Twofold serial dilutions were made in the wells of 96-well microtitre plate (Nunc) in 170 μL of medium. Each test includes metronidazole as a standard amoebicidal drug, control wells (culture medium plus amoebae) were prepared from a confluent culture by pouring off the medium, adding 2 mL of medium and chilling the culture on ice to detach the organisms from the side of the flask. The number of amoeba per mL was estimated with haemocytometer and trypan blue exclusion was used to confirm viability. Fresh culture medium was added to dilute the suspension to 10^5 organism and 170 μL of this suspension was added to the test and control wells in the plate so the wells were completely filled (total volume, 340 μL). An inoculum of 1.7×10^4 organisms/well was chosen so that confluent, but not excessive growth took place in control wells. Plate was sealed with expanded polystyrene (0.5 mm thick). Secured with tape, placed in a modular incubating chamber (Flow Laboratories. High Wycombe, UK) and gassed for 10 min, with nitrogen before incubation at 37 °C for 72 h.

4.2.3.2. Assessment of antiamoebic activity. After incubation, the growth of amoeba in the plate was checked with a low power microscope. The culture medium was removed by inverting the plate and shaking gently. Plate was then immediately washed once in sodium chloride solution (0.9%) at 37 °C. This procedure was completed quickly, and the plate was not allowed to cool in order to prevent the detachment of amoeba. The plate was allowed to dry at room temperature and the amoeba

were fixed with methanol and when dry, stained with (0.5%) aqueous eosine for 15 min. Stained plate was washed once with tape water and then twice with distilled water and allowed to dry. A 200 μL portion of 0.1 N sodium hydroxide solution was added to each well to dissolve the protein and release the dye. The optical density of the resulting solution in each well was determined at 490 nm with a microplate reader (Labsystem Multiskane Bichromatic, UK). The % inhibition of amoeba growth was calculated from the optical densities of the control and test wells and plotted against the logarithm of the dose of the drug tested. Linear regression analysis was used to determine the best-fitting straight line from which the IC_{50} value was found.

Compounds **2a**–**g** were screened for antiamoebic activity against *E. histolytica* (strain HM-1: IMSS) and IC_{50} (50% inhibitation concentration) values were determined. Best IC_{50} value was shown by compound **2g** and was found to be 1.82 μM as compared to standard drug metronidazole which shows IC_{50} value of 1.22 μM.

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