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Synthesis, Anti-Inflammatory, Analgaesic and Anti-Amoebic Activity Evaluation of Some Pyrimidobenzimidazole and Pyrimidopyridoimidazole Derivatives

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o-Phenylenediamine, 4-nitro-1,2-phenylenediamine, 3,4-diaminobenzophenone, 3,4-diaminotoluene, 4,5-dimethylphenylenediamine and 3,4-diaminobenzoic acid (1a–f) react with 4-isothiocyanatobutan-2-one to give the pyrimidobenzimidazole derivatives (2a–f) respectively. Condensation of 4-nitro-1,2-phenylenediamine with 4-isothiocyanatobutan-2-one at room temperature gave the thiourea derivative (3). *o*-Nitroaniline, on condensation with 4-isothiocyanatobutan-2-one at pH ca. 5 and under reflux conditions, gave the thiourea derivative (4). 2,3-Diaminopyridine, on condensation with 4-isothiocyanatobutan-2-one, gave product (5). Condensation of 2,3-diaminopyridine with 4-isothiocyanato-4-methylpentan-2-one using acetic acid as solvent gave compounds (6)–(8), whereas compounds (7) and (9) were isolated from the same reagents in dimethylformamide (DMF). 4-Isothiocyanato-4-methylpentan-2-one, on refluxing in acetic acid, gave compound (6). Anti-inflammatory activity evaluation was carried out at 100 mg/kg p.o. (paw oedema) for compounds (2a–f), (7) and (8). Compounds (2b), (2d) and (7) showed good anti-inflammatory and analgesic activities. Anti-amoebic activity evaluation of (2a–f) and (7) against *Entamoeba histolytica* (strain HM-1: IMSS) was carried out, and compound (2b) exhibited anti-amoebic activity similar to metronidazole in vitro.

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Introduction

In the design of biomolecular tools and drugs, it is important to obtain high target specificity. The target could be an entire organ, or a type of tissue, or it could be molecular. Thus, compounds that could be directed not only towards DNA but even towards a particular nucleotide sequence in DNA might exhibit high specific biological activity. Such compounds would be potential drugs against viruses,[1,2] bacteria[3] and parasites.[4] A literature survey reveals that the biological activity of amino heterocyclic derivatives has been widely demonstrated, and such compounds have a long history in the treatment of, for example, malaria[1] and bacterial infections.[3] Amoebiasis is caused by the protezoan parasite *Entamoeba histolytica* and remains a major world health problem; it causes up to 100000 deaths per annum,[4] placing it third amongst deadly parasitic diseases. Metronidazole is considered to be the drug of choice but it has common side effects including nausea, it is mutagenic in bacteria,[5] 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. Many anti-inflammatory drugs have been used, but these drugs frequently have ulcerogenic activity,[6] and hence cannot be continuously used for long periods. Pyrimidine derivatives possessing anti-inflammatory[7–11] and analgesic[12] activities have been reported in the literature.

In a continuation of our efforts in search of potential anti-inflammatory[13–19] and anti-amoebic[20] agents, we have synthesized various pyrimidobenzimidazole and pyrimidopyridoimidazole derivatives and screened them in vitro for their ability to inhibit the growth of *Entamoeba histolytica*. These compounds were also screened in vivo for anti-inflammatory and analgesic activity, in order to develop better agents.

Results and Discussion

4-Isothiocyanatobutan-2-one, on condensation with *o*-phenylenediamine by refluxing in methanol at pH ca. 5, gave the

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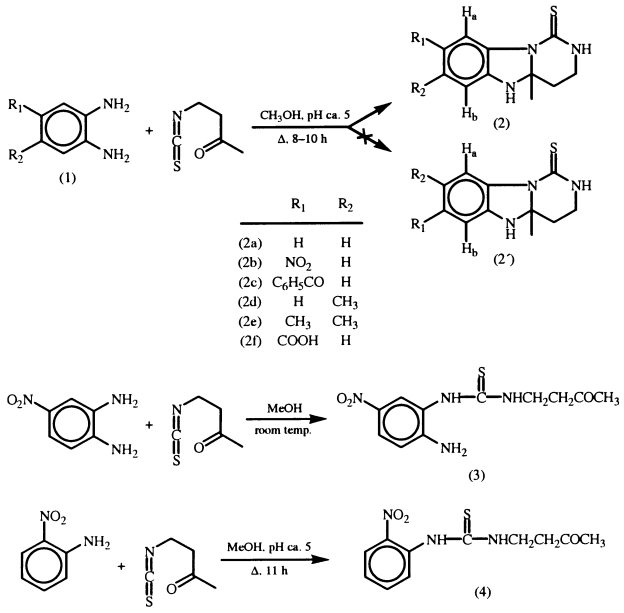
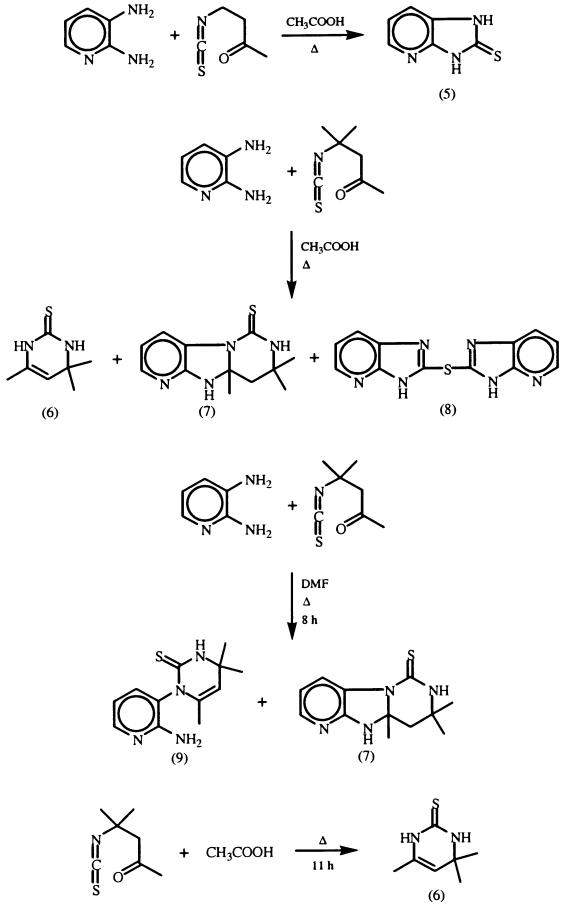


Scheme 1

pyrimidobenzimidazole derivative (2a) in 40% yield (Scheme 1). The structure of compound (2a) is fully supported by the infrared (IR), ^{1}H nuclear magnetic resonance (NMR) and high-resolution mass spectroscopy (HRMS) data reported in Table 1. Condensation of 4-nitro-1,2-phenylenediamine with 4-isothiocyanatobutan-2-one, at pH ca. 5 under reflux conditions in methanol, gave the pyrimidobenzimidazole derivative (2b) in 45% yield. The structure of (2b) is supported by the IR, ^{1}H NMR and HRMS spectroscopic data found in Table 1. 4-Isothiocyanatobutan-2-one is presumed to react first with the amino group *meta* to the nitro group, and then the amino group *para* to the nitro group undergoes cyclization to give the pyrimidobenzimidazole derivative (2b) (Scheme 1). In 4-nitro-*o*-phenylenediamine all the aromatic protons appear below δ 7.8, but in the product (2b) a doublet appears at δ 9.5 corresponding to one proton. This downfield shift of H_a could be due to the influence of C=S on H_a. A further coupling constant of *J* 1 Hz corresponds to *meta*-coupling. *meta*-Coupling of H_a will be possible only if compound (2b) has the structure shown in Scheme 1. The downfield shift of H_a is that which is expected on the basis of what is reported in the literature.[16,18] In the case of 3,4-diaminobenzophenone (1c) and 3,4-diaminobenzoic acid (1f), since benzoyl and carboxyl are electron-withdrawing groups, the amino group *meta* to benzoyl in the case of (1c) and *meta* to the carboxyl group in the case of (1f) will react first with 4-isothiocyanatobutan-2-one, and then the second amino group will complete the cyclization to give the final products (2c) and (2f). The ^{1}H NMR spectrum of (2c) shows the aromatic proton at lowest field (H_a) at δ 9.2 having a coupling constant, J_m, of 2 Hz, whereas in the case of (2f) the aromatic proton at lowest field (H_a) at δ 8.5 exhibited a coupling constant, J_m, of 1 Hz. These observations support the structures assigned to (2c) and (2f). The spectroscopic data for (2c) and (2f) are reported in Table 1. In the case of (1d), the methyl group has an electron-releasing effect so the most basic amino group will be the one situated at the position *para* to the methyl group. During condensation of 3,4-diaminotoluene with 4-isothiocyanatobutane-2-one, the most basic amino group will react first and then the other amino group will react second to give final product (2d). The lowest-field aromatic proton (H_a) in the ^{1}H NMR spectrum of (2d) appears at δ 8.45 as a doublet with a coupling constant, J_o, of 8 Hz, thus confirming the structure assigned to (2d). Condensation of 4,5-dimethylphenylenediamine with 4-isothiocyanatobutan-2-one gave product (2e). The structure of (2e) is fully supported by the IR, ^{1}H NMR and HRMS data reported in Table 1. The spectroscopic data (IR, ^{1}H NMR and HRMS) for (2a–f) are consistent with their assigned structures and do not support the isomeric structures (2′a–f) (Scheme 1).

Condensation of 4-isothiocyanatobutan-2-one with 4-nitro-1,2-phenylenediamine at room temperature using methanol as solvent gave compound (3). The IR, ^{1}H NMR and HRMS data for compound (3) are reported in Table 1 and fully support the assigned structure. *o*-Nitroaniline, on condensation with 4-isothiocyanatobutan-2-one under reflux in methanol and at pH ca. 5, gave compound (4). The structure of compound (4) is fully supported by its IR, ^{1}H NMR and HRMS data (Table 1).



Scheme 2

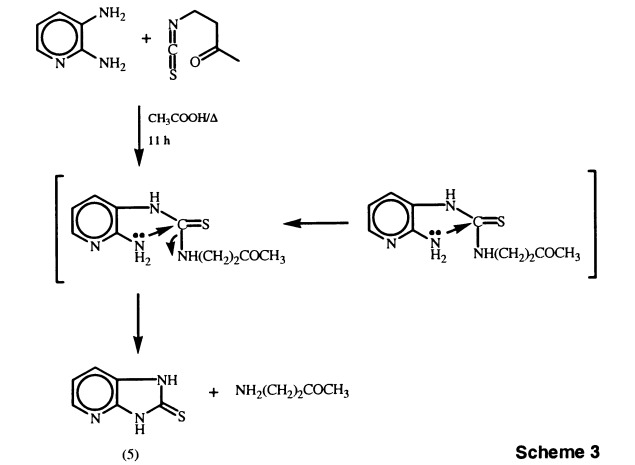
In order to extend the methodology developed for the synthesis of pyrimidobenzimidazole to the synthesis of pyrimidopyridoimidazole, we studied several reactions of

Table 1. Physical constants and spectroscopic data for (2a–f) and (3)–(9)

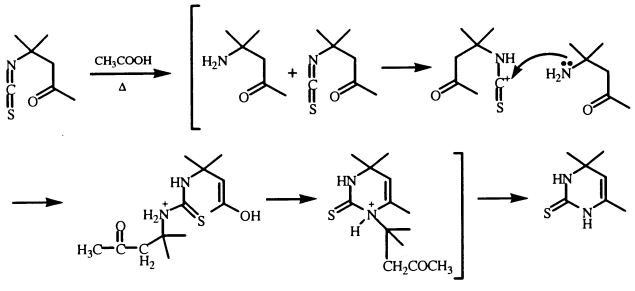
Cpd No.	Solvent of crystallization/elution	M.p. (°C)	Yield[A] (%)	IR (KBr) ν (cm^{-1})	^{1}H NMR[B] δ (*J* in Hz)	HRMS or EI-MS, *m/z* (%)
(2a)	9:1 $CHCl_3$/EtOAc	155	40	3428 (–NH–) 1498 (Ar)	1.35 (s, 3H, CH_3), 2.00 (m, 1H, CH_2), 2.2 (dd, 1H, CH_2), 3.25 (m, 2H, CH_2), 6.46 (s, 1H, NH), 6.59 (t, 2H, Ar), 6.83 (t, 1H, J_o 8, Ar), 8.37 (s, 1H, NH), 8.62 (d, 1H, J_o 8 Hz, Ar)	Found: 219.08283 (M^+, 94%) Calc. for $C_{11}H_{13}N_3S$: 219.08302 *m/z* 218.07458 (M–H, 6), 204.05954 (M–CH_3, 100)
(2b)	1:1 $CHCl_3$/EtOAc	241	45	3400 (–NH–) 1602, 1516 (Ar)	1.4 (s, 3H, CH_3), 2.05 (m, 1H, CH_2), 2.30 (m, 1H, CH_2), 3.30 (m, 2H, CH_2), 6.55 (d, 1H, J_o 9, Ar), 7.90 (dd, 1H, J_o 9, J_m 1, Ar), 8.20 (exch. s, 1H, NH), 8.80 (exch. s 1H, NH), 9.5 (d, 1H, J_m 1, Ar)	Found: 264.06836 (M^+, 86%) Calc. for $C_{11}H_{12}N_4SO_2$: 264.06808 *m/z* 249.04535 (M–CH_3, 100), 203.05199 [M–(CH_3+NO_2), 29], 190.06210 [M–(CH_3+HSCN), 18]
(2c)	1:1 $CHCl_3$/EtOAc	202	40	3463 (–NH–) 1711 (C=O) 1570, 1502 (Ar)	1.5 (s, 3H, CH_3), 1.95 (m, 1H, CH_2), 2.25 (m, 1H, CH_2), 3.30 (m, 2H, CH_2), 6.6 (d, 1H, J_o 8, Ar), 7.3 (dd, J_o 8, J_m 2, Ar), 7.6 (m, 6H, Ar+NH, 1H exch.), 8.5 (exch. s, 1H, NH), 9.2 (d, 1H, J_m 2, Ar)	Found: 323.10928 (M^+, 89%) Calc. for $C_{18}H_{17}N_3SO$: 323.10922 *m/z* 322.10131 (M–H, 6), 308.08617 (M–CH_3, 100), 263.11691 [M–(H+HSCN), 3], 249.10292 [M–(CH_3+HSCN), 21], 77.03896 (C_6H_5, 61)
(2d)	4:1 $CHCl_3$/EtOAc	187	45	3439 (–NH–) 1502 (Ar)	1.33 (s, 3H, >C–CH_3), 1.96 (m, 1H, CH_2), 2.21 (m, s, 4H, CH_3, 1H of CH_2), 3.23 (m, 2H, CH_2), 6.21 (s, 1H, NH), 6.46 (d, 2H, J_o 8, Ar), 8.31 (s, 1H, NH), 8.45 (d, 1H, J_o 8, Ar)	Found: 233.09914 (M^+, 99%) Calc. for $C_{12}H_{15}N_3S$: 233.09866 *m/z* 232.09085 (M–H, 8), 218.07545 (M–CH_3, 100), 159.09263 [M–(CH_3+HSCN), 36]
(2e)	4:1 $CHCl_3$/EtOAc	200–205	40	3559 (–NH–) 1618, 1510 (Ar)	1.30 (s, 3H, CH_3), 1.95 (m, 1H, CH_2), 2.00 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.20 (m, 1H, CH_2), 3.25 (m, 2H, CH_2), 6.10 (exch. s, 1H, NH), 6.40 (s, 1H, Ar), 8.25 (exch. s, 1H, NH), 8.40 (s, 1H, Ar)	Found: 247.11453 (M^+, 87%) Calc. for $C_{13}H_{17}N_3S$: 247.11432 *m/z* 246.10656 (M–H, 7.5), 232.09093 (M–CH_3, 100), 213.12607 (M–H_2S, 15), 187.12240 [M–(H+HSCN), 6], 173.10803 [M–(CH_3+HSCN), 30]
(2f)	9:1 $CHCl_3$/MeOH	216–218	35	3330 (–NH–) 1651 (COOH) 1594, 1531 (Ar)	1.35 (s, 3H, CH_3), 2.0 (m, 1H, CH_2), 2.25 (m, 1H, CH_2), 3.2 (m, 2H, CH_2), 6.55 (d, 1H, J_o 8, Ar), 7.3 (exch. s, 1H, NH), 7.5 (dd, 1H, J_o 8, J_m 1, Ar), 8.5 (d, 1H, J_m 1, Ar), 9.15 (exch. s, 1H, NH), 12.2 (exch. br s, 1H, COOH)	Found: 263.07295 (M^+, 86%) Calc. for $C_{12}H_{13}O_2N_3S$: 263.07285 *m/z* 262.06490 (M–H, 7), 248.04935 (M–CH_3, 99), 189.06615 [M–(CH_3+HSCN), 37]
(3)	MeOH	143	20	3616, 3479, 3377 (–NH_2, –NH–) 1707 (C=O) 1624 (Ar)	2.1 (s, 3H, CH_3), 2.7 (m, 2H), 3.1 (m, 1H), 3.4 (m, 1H), 6.8 (d, 1H, Ar), 7.9 (m, 2H, Ar)[D]	Found: 282.07892 (M^+, 6%) Calc. for $C_{11}H_{14}N_4O_3S$: 282.07867
(4)	1:4 pet. spirits/$CHCl_3$	54	19	3400 (–NH–) 1700 (C=O) 1620, 1590 (Ar)	2.11 (s, 3H, CH_3), 2.9 (t, 2H, CH_2), 3.5 (t, 2H, CH_2), 6.65 (t, 1H, Ar), 7.01 (d, 1H, Ar), 7.50 (t, 1H, Ar), 8.00 (d, 1H, Ar)[D]	*m/z* 208.08461 (M–HSCN, 56)
(5)	1:1 $CHCl_3$/EtOAc	>245	25	3485 (–NH–) 1619 (>C=N–) 1507 (Ar)	7.12 (dd, 1H, J_o 6, J_o 8, Ar), 7.45 (dd, 1H, J_o 8, J_m 1.5, Ar), 8.09 (dd, 1H, J_o 6, J_m 1.5, Ar), 12.69 (exch. s, 1H, NH), 13.11 (exch. s, 1H, NH)	Found: 151.02111 (M^+, 100%) Calc. for $C_6H_5N_3S$: 151.02042
(6)	4:1 $CHCl_3$/EtOAc	256 (dec.)[C]	2 (35)	3200 (–NH–)	1.15 (s, 6H, 2×CH_3), 1.64 (s, 3H, CH_3), 4.49 (s, 1H, C=CH), 8.44 (exch. s, 1H, NH), 9.38 (exch. s, 1H, NH)	Found: 156.07209 (M^+, 51%) Calc. for $C_7H_{12}N_2S$: 156.07211 *m/z* 141.04846 (M–CH_3, 100)
(7)	1:1 $CHCl_3$/EtOAc	230	27 (14)	3186 (–NH–) 1606 (C=N–) 1510 (Ar)	1.21 (s, 3H, CH_3), 1.31 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 2.12 (d, 1H, CH_2), 2.35 (d, 1H, CH_2), 6.48 (dd, 1H, J_o 8, 6, Ar), 7.54 (d, 1H, J_o 6, Ar), 7.67 (exch. s, 1H, NH), 8.57 (d, 1H, J_o 8, Ar), 8.61 (exch. s, 1H, NH)	*m/z* 248 (M, 10%), 233 (M–CH_3, 6), 174 [M–(CH_3+HSCN), 20]
(8)	MeOH/EtOAc	220	8	3445 (–NH–) 1599 (Ar)	5.6 (br s, 2H, 2×NH), 7.05 (dd, 1H, J_o 6, 8, Ar), 7.11 (dd, 1H, J_o 6, 8, Ar), 7.47 (dd, 1H, J_o 8, J_m 1.2, Ar), 7.85 (dd, 1H, J_o 8, J_m 1.2, Ar), 8.04 (dd, 1H, J_o 6, J_m 1.2, Ar), 8.20 (dd, 1H, J_o 6, J_m 1.2, Ar)	*m/z* 151.02040 (M–$C_6H_3N_3$, 100), 117.03298 (M–$C_6H_3N_3S$, 1)[E]
(9)	1:4 $CHCl_3$/EtOAc	220–225	12	3340, 3177 (–NH_2, –NH–) 1632 (C=N–) 1513 (Ar)	1.28 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 4.9 (s, 1H, C=CH), 5.55 (exch. s, 2H, NH_2), 6.60 (dd, 1H, J_o 5, 8), 7.20 (dd, 1H, J_o 8, J_m 2, Ar), 7.87 (dd, 1H, J_o 5, J_m 2, Ar), 8.90 (exch. s, 1H, NH)	Found: 248.11037 (M^+, 46%) Calc. for $C_{12}H_{16}N_4S$: 248.10957 *m/z* 233.08666 (M–CH_3, 100), 216.05988 [M–(CH_3+NH_3), 32], 199.09873 [M–(CH_3+H_2S), 14], 174.10344 [M–(CH_3+HSCN), 29]

[A] Values in parentheses refer to yields obtained from an alternative procedure. [B] NMR spectra were run in $(CD_3)_2SO$. [C] Lit. 254–255°C. [D] Spectrum recorded after D_2O exchange. [E] M^+ ion not found.

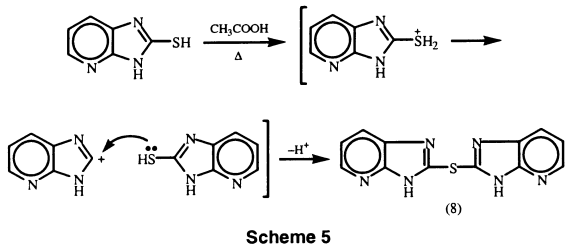
2,3-diaminopyridine with certain isothiocyanato ketones (Scheme 2). 2,3-Diaminopyridine, on condensation with 4-isothiocyanatobutan-2-one in refluxing acetic acid, gave compound (5). The structure of compound (5) is fully supported by the IR, ^{1}H NMR and HRMS data reported in Table 1. 2,3-Diaminopyridine, on condensation with 4-isothiocyanato-4-methylpentan-2-one in refluxing acetic acid, gave, upon workup and chromatographic separation, compounds (6)–(8). The structure of compound (6) is supported by its IR, ^{1}H NMR and HRMS data, which are reported in Table 1.The formation of product (7) can be explained by the fact that the ring nitrogen of pyridine decreases the nucleophilic character [21, 22] of the amino groups present at positions 2 and 4 and thus the more basic amino group, i.e. the amino group at position 3, will react first followed by the amino group at position 2. The structure of compound (7) is supported by its IR, ^{1}H NMR and HRMS data, which are reported in Table 1. Spectroscopic data for compound (8) supported the assigned structure. The formation of compound (7) is similar to that of (2a–f), but the formation of compounds (5), (6) and (8) needs some explanation. It is reported in the literature[23] that



Scheme 3



Scheme 4



Scheme 5

isothiocyanato ketones undergo acid hydrolysis to give amino ketones, and thus it is expected that compound (5) can be formed by following the reaction path mentioned in Scheme 3. Formation of compound (6) can arise from partial hydrolysis of 4-isothiocyanato-4-methylpentan-2-one to 4-amino-4-methylpentan-2-one and then condensation of 4-isothiocyanato-4-methylpentan-2-one and 4-amino-4-methylpentan-2-one as mentioned in Scheme 4. Formation of (8) can arise via formation of compound (5) and then loss of H_2S from two molecules to give compound (8) as mentioned in Scheme 5. In order to confirm that 2,3-diaminopyridine plays no role in the formation of compound (6), 4-isothiocyanato-4-methylpentan-2-one was dissolved in acetic acid, the solution refluxed for 11 h, and then the solvent was removed under reduced pressure. The solid residue was basified with aqueous sodium carbonate, and the residue was crystallized from methanol to give compound (6), of which the melting point, mixed melting point, TLC and co-TLC were identical to an authentic sample synthesized by the method reported in literature.[24] Condensation of 4-isothiocyanato-4-methylpentan-2-one with 2,3-diaminopyridine using dimethyl formamide (DMF) as the solvent gave compounds (7) and (9). Compound (7) obtained from the reaction using DMF as the reaction solvent was the same as that obtained from the reaction using acetic acid as the solvent. The IR, ^{1}H NMR and HRMS data for compound (9) reported in Table 1 fully support the assigned structure.

Compounds (2a–f), (7) and (8) were tested for anti-inflammatory activity[25] in the carrageenin-induced paw oedema model at 100 mg/kg p.o. and the results are summarized in Table 2. Compounds (2a–d), (7) and (8) showed 6, 43, 24, 12, 34 and 16% anti-inflammatory activity, respectively, as compared to the standard drug ibuprofen, which showed 51% activity at 50 mg/kg p.o. However, compounds (2e) and (2f) were found to be inactive. The data in Table 2 indicate that compounds (2b), (2c) and (7) exhibited good anti-inflammatory activity

Analgesic activity evaluation[26] in the phenylquinone writhing assay for (2a–f), (7) and (8) was carried out at 100, 50 and 25 mg/ kg p.o., and the results are summarized in Table 2. The data in Table 2 indicate that compounds (2a), (2d) and (7) possess strong analgesic activity, while compounds (2b), (2c) and (8) possess mild analgesic activity, and compounds (2e) and (2f) possess poor analgesic activity.

Anti-amoebic activity[27–29] evaluation of compounds (2a–f) and (7) was carried out in vitro against *E. histolytica* (strain HK-9), and IC_{50} (50% inhibition concentration) values obtained are reported in Table 2. All the compounds showed anti-amoebic activity, but the best IC_{50} value was shown by compound (2b) (the IC_{50} value for this compound was very close to the standard drug metronidazole. Introduction of a nitro group (i.e. (2b)), a benzoyl group (i.e. (2c)) and a caboxylic group (i.e. (2f)) to the pyrimidobenzimidazole ring system enhanced the anti- amoebeic activity considerably, whereas introduction of one or two methyl groups in the pyrimidobenzimidazole ring system has only slightly changed the IC_{50} values relative to metronidazole.

Table 2. Anti-inflammatory, analgesic and anti-amoebic activity evaluation of compounds (2a–f), (7) and (8)

Compound tested	Anti-inflammatory activity (%) (100 mg/kg p.o.)	Analgesic activity (%) 100 mg/kg p.o.	50 mg/kg p.o.	25 mg/kg p.o.	Anti-amoebic activity IC_{50} (μg/mL)[A]
(2a)	6	80	60	20	0.67 (0.075)
(2b)	43	60	25	25	0.32 (0.070)
(2c)	24	60	40	20	0.45 (0.045)
(2d)	12	90	50	20	0.63 (0.080)
(2e)	0	20	20	—	0.63 (0.061)
(2f)	0	20	10	—	0.40 (0.058)
(7)	34	80	60	30	0.94 (0.065)
(8)	16	40	20	—	—
Ibuprofen	51	100	75	50	—
Metronidazole	—	—	—	—	0.21 (0.044)

[A] Values in parentheses refer to the standard deviation.

Conclusion

Condensation of a number of phenylenediamines and 2,3-diaminopyridine with 4-isothiocyanatobutan-2-one and 4-isothiocyanato-4-methylpentan-2-one has been carried out and the resulting products were screened for anti-inflammatory, analgesic and anti-amoebic activity. Compounds (2b), (2d) and (7) showed good anti-inflammatory and analgesic activity, while compound (2b) exhibited anti-amoebic activity similar to metronidazole.

Experimental

General Procedures

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 ca. 5–15% (w/v) solution in $(CD_3)_2SO$. The MS peak measurements were made by comparison with perfluorotributylamine using an AEIMS-9 double-focusing high-resolution mass spectrometer at a resolving power of 15000. 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 (UV) light (254 nm). Column chromatography was performed by using Qualigens silica gel (60–120 mesh). The yields, melting points and spectroscopic data for all compounds are reported in Table 1.

General Procedure for the Formation of (2): Condensation of 1,2-Phenylenediamine with 4-Isothiocyanatobutan-2-one

4-Isothiocyanatobutan-2-one (0.65 mL, 5 mmol) was added to a solution of 1,2-phenylenediamine (540 mg, 5 mmol) in methanol (50 mL). The pH of the reaction mixture was adjusted to about 5 by adding a few drops of 10% H_2SO_4 in methanol, the reaction mixture was heated under reflux for 11 h, and then the solvent was removed under reduced pressure. The residue left behind was basified with 10% aqueous sodium carbonate solution (10 mL). The semi-solid residue so obtained was filtered off, washed with water and the crude product was dissolved in methanol. The resulting solution was adsorbed on silica gel and subjected to column chromatography over silica gel. Elution with $CHCl_3$ removed any side products, and elution with $CHCl_3$/ethyl acetate (9:1) gave pure 4a-methyl-3,4,4a,5-tetrahydropyrimido[1,6-*a*]benzimidazol-1(2*H*)thione (2a).

Similarly prepared were 4a-methyl-8-nitro-3,4,4a,5-tetrahydropyrimido[1,6-*a*]benzimidazol-1(2*H*)thione (2b), 8-benzoyl-4a-methyl-3,4,4a,5-tetrahydropyrimido[1,6-*a*]benzimidazol-1(2*H*)thione (2c), 4a,7-dimethyl-3,4,4a,5-tetrahydropyrimido[1,6-*a*]benzimidazol-1(2*H*)thione (2d), and 4a,7,8-trimethyl-3,4,4a,5-tetrahydropyrimido[1,6-*a*]-benzimidazol-1(2*H*)thione (2e). In the case of 8-carboxy-4a-methyl-3,4,4a,5-tetrahydropyrimido[1,6-*a*]benzimidazol-1(2*H*)thione (2f), the residue was not basified with aqueous sodium carbonate solution during workup. Physical constants, spectroscopic data and solvents of elution/crystallization are reported in Table 1.

Condensation of 2-Amino-4-nitroaniline with 4-Isothiocyanatobutan-2-one

2-Amino-4-nitroaniline (765 mg, 5 mmol) was dissolved in methanol (100 mL), and 4-isothiocyanatobutan-2-one (0.65 mL, 5 mmol) was added. The reaction contents were allowed to stand for 15 days at room temperature and the product that separated out was filtered off and washed with chilled methanol to give *N*′-(2′-amino-5′-nitrophenyl)-*N*-(4-butyl-2-oxo)thiourea (3), which was recrystallized from methanol.

Condensation of o-*Nitroaniline with 4-Isothiocyanatobutan-2-one*

4-Isothiocyanatobutan-2-one (0.65 mL, 5 mmol) was added to a solution of *o*-nitroaniline (690 mg, 5 mmol) in MeOH (100 mL). The pH of reaction mixture was adjusted to about 5 by adding a few drops of 10% H_2SO_4 in methanol. The reaction contents were heated under reflux for 11 h, the solvent was removed under reduced pressure and the residue was basified with 10% aqueous sodium carbonate (10 mL). The sticky residue left behind was washed with water and the crude product was subjected to column chromatography [elution with light petroleum/$CHCl_3$ (1:4)] to give N-*(4-butyl-2-oxo)*-N-*(2′-nitrophenyl)thiourea* (4) (Found: C, 49.6; H, 5.0; N, 15.5. $C_{11}H_{13}N_3O_3S$ requires C, 49.4; H, 4.9; N, 15.7%).

Condensation of 2,3-Diaminopyridine with 4-Isothiocyanatobutan-2-one

4-Isothiocyanatobutan-2-one (0.30 mL, ca. 2 mmol) was added to 2,3-diaminopyridine (218 mg, 2 mmol) in acetic acid (10 mL). The mixture was heated under reflux in a sand bath for 11 h and then the solvent was distilled off under vaccum. The residue left behind was basified with 10% aqueous sodium carbonate (ca. 5 mL) to give a sticky product, which was washed with water and then dissolved in $CHCl_3$. The $CHCl_3$ solution was dried over anhydrous sodium sulfate and then the solvent was removed under vaccum. The crude product was subjected to column chromatography over silica gel. Elution with $CHCl_3$/ethyl acetate (1:1) gave pyrido[2,3-*d*]imidazol-2(1*H*)thione (5).

Condensation of 4-Isothiocyanato-4-methylpentan-2-one with 2,3-Diaminopyridine

2,3-Diaminopyridine (2.18 g, 0.02 mol) was dissolved in acetic acid (25 mL) and 4-isothiocyanato-4-methylpentan-2-one (3.20 mL, 0.02 mol) was then added. The mixture was heated under reflux in a sand bath for 8 h and then the solvent was removed under reduced pressure. The semi-solid residue was basified with 10% aqueous sodium carbonate (20 mL), and the solid product was filtered off, washed with water (20 mL) and air-dried. The crude product so obtained was dissolved in methanol, adsorbed over silica gel and subjected to column chromatography over silica gel. Elution with

$CHCl_3$ gave 4,4,6-trimethyl-1,4-dihydropyrimidine-2(3*H*)thione (6). Further elution with $CHCl_3$/ethyl acetate (1:1) gave *3,3,4a-trimethyl-3,4,4a,5-tetrahydropyrimido[6,1-*b*]pyrido[2,3-*d*]imidazole* (7) (Found: C, 58.0; H, 6.7; N, 22.6; $C_{12}H_{16}N_4S$ requires C, 58.1; H, 6.5; N, 22.6%). The filtrate from the above reaction mixture was extracted with ethyl acetate (50×5 mL). The combined ethyl-acetate extracts were washed with water and dried over sodium sulfate. Ethyl acetate was distilled off and the solid product left behind was washed with chilled methanol to give *2,2′-thiodipyrido[2,3-*d*]imidazole* (8) (Found: C, 53.9; H, 2.9; N, 31.2; $C_{12}H_8N_6S$ requires C, 53.7; H, 3.0; N, 31.3%).

Condensation of 2,3-Diaminopyridine with 4-Isothiocyanato-4-methylpentan-2-one

2,3-Diaminopyridine (218 mg, 2 mmol) was dissolved in DMF (0.5 mL), and 4-isothiocyanato-4-methylpentan-2-one (0.33 mL, 2 mmol) was added. The reaction mixture was heated in a water bath for 8 h and then the solvent was removed under vaccum. Chilled methanol (5 mL) was added to the semi-solid residue left behind, and the solid product that separated out was filtered off and washed with chilled methanol (1 mL). The solid product so obtained was air-dried to give compound (7). The filtrate was concentrated and subjected to column chromatography over silica gel. Elution with $CHCl_3$/ethyl acetate (1:4) gave 2-amino-3-(4,4,6-trimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-1-yl)pyridine (9).

Acid-Catalysed Condensation of 4-Isothiocyanato-4-methylpentan-2-one

4-Isothiocyanato-4-methylpentan-2-one (1 mL, 6 mmol) and acetic acid (10 mL) were heated under reflux for 11 h, the solvent was removed under reduced pressure and the solid residue left behind was washed with aqueous sodium carbonate (10%, 10 mL) and then with water. The crude product was dissolved in methanol and treated with charcoal to remove coloured impurities. Removal of the solvent gave a white solid, which was recrystallized from methanol to give 4,4,6-trimethylpyrimidine-1,4-dihydro-2(3*H*)thione (6) (350 mg, 40%). The spectroscopic data and m.p. for this compound were identical to those reported in Table 1.

Anti-Inflammatory Activity Screening[25]

Anti-inflammatory activity testing was carried out using carrageenin-induced paw oedema in albino rats. Oedema in one of the hind paws was induced by injection of 0.1 mL of a 1% carrageenin solution into planter aponeurosis. The volume of the paw was measured plethysmographically both immediately after and 3 h after injection of the irritant. The difference in volume gave the amount of oedema that had developed. Percentage inhibition of the oedema between the control group and the group treated with test compound was calculated and compared with the group receiving the standard drug. At 100 mg/kg p.o., compounds (2b) and (7) possessed good (43 and 34%, respectively) anti-inflammatory activity, whereas compounds (2a), (2c), (2d) and (8) inhibited the carrageenin-induced hind paw oedema by 6, 24, 12 and 16%, respectively, as compared with the standard drug ibuprofen (which showed 51% activity at 50 mg/kg p.o.).

Analgesic Activity Screening[26]

Analgesic activity was evaluated in albino mice using the phenylquinone writhing assay. Female swiss mice (15–20 g), bred in the Animal House of Central Drug Research Institute, Lucknow, and maintained under standard laboratory conditions, were used in the study. Mice were injected with 0.2 mL of 0.02% aqueous phenylquinone (2-phenyl-1,4-benzoquinone) and writhing was observed for 20 min. The 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 these mice were used in the analgesic testing on the following day.

These mice, five per group and showing significant writhing, were given orally 50 and 100 mg/kg p.o. doses of test compounds 15 min prior to the phenylquinone challenge. Writhing was again recorded for each mouse in a group and the percentage protection was calculated using the following formula, where N_{TM} refers to the number of writhes in the treated mouse, and N_{UM} refers to the number of writhes in the untreated mouse:

$$\% \text{ protection} = 100-[(N_{TM})/(N_{UM})\times 100]$$

This was taken as percentage of analgesic response and was averaged in each group of mice.

The percentage of animals exhibiting analgesia was determined with each dose. Compounds (2a–f), (7) and (8) were screened for analgesic activity. Compounds (2a), (2d) and (7) showed good analgesic activity (80, 90 and 80%, repectively, at 100 mg/kg p.o.; 60, 50 and 60%, respectively, at 50 mg/kg p.o.; and 20, 20 and 30%, respectively, at 25 mg/k.g p.o.). Compounds (2b), (2c), (2e), (2f) and (8) showed repectively 60, 60, 20, 20 and 40% analgesic activity at 100 mg/kg p.o.; 25, 40, 20, 10 and 20% at 50 mg/kg p.o.; and 25, 20, 0, 0 and 0% at 25 mg/k.g. p.o.

Anti-Amoebic Activity Screening

In vitro testing against Entamoeba histolytica.[27–29] Activity against *Entamoeba histolytica* (strain HM-1: IMSS) in vitro was assessed using the microplate method. Dimethyl sulfoxide (DMSO) (40 µl) was added to the sample (1 mg) followed by enough culture medium to obtain a concentration of 1 mg/mL. Samples were dissolved or suspended by mild sonication in a sonicleaner bath (Julabo, USRI, West Germany) for a few minutes and then further diluted with medium to a concentration of 0.1 mg/mL. Twofold serial dilutions were made in the wells of a 96-well microtitre plate (Nunc) in 170 µL of medium. Each test included 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 a haemocytometer, and Trypan Blue exclusion was used to confirm viability. Fresh culture medium was added to dilute the suspension to 10^5 organisms, and 170 µL of this suspension was added to the test and control wells in the plate so that 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 the control wells. Each plate was sealed with expanded polystyrene (0.5-mm thick), secured with tape, placed in a modular incubating chamber (Flow Laboratories, High Wycombe, U.K.) and gassed for 10 min with nitrogen before incubation at 37°C for 72 h.

Assessment of anti-amoebic 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 it gently. The 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 amoebae. The plate was allowed to dry at room temperature, the amoeba were fixed with methanol and, when dry, stained with (0.5%) aqueous eosine for 15 min The stained plate was washed once with tap water and then twice with distilled water and allowed to dry. A 200-µL portion of 0.1 M 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, U.K.). The percentage inhibition of amoeba growth was calculated from the optical densities of the control and test wells, and was 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–f), (7) and (8) were screened for anti-amoebic activity against *Entamoeba histolytic* (strain HM-1: IMSS) and their IC_{50} (50% inhibition concentration) values were determined. The most promising IC_{50} value was shown by compound (2b) [0.32µg/mL, as compared with the standard drug metronidazole (IC_{50} 0.21µg/mL)].

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References

[1] D. W. Henry, in *The Acridines* 2nd Edn (Ed. R. M. Acheson) **1973**, p. 829 (Wiley Interscience: NewYork).
[2] P. Chandra, M. Waltersdorf, *Biochem. Pharmacol*. **1976**, *25*, 237.
[3] A. C. R. Dean, in *The Acridines* 2nd Edn (Ed. R. M. Acheson) **1973**, p. 789 (Wiley Interscience: New York).
[4] WHO report on the consultation on Amoebiasis, WHO, *Epidemiol. Record* **1997**, *14*, 4.
[5] P. A. Martinez, *Biology of* E. histolytica, in *Amoebiasis* (Ed. P. A. Martinez) **1986**, pp.12–43 (Elsevier: Amsterdam).
[6] M. E. Wolff (Ed.), *Burger's Medicinal Chemistry, Part III* **1981**, pp. 1205–1273 (Wiley: New York).
[7] M. Inoue, K. Hashimoto, *Jpn Kokai Tokyo Koho Jp*. (*Jap. Pat. No.* 03204877 [91,204,877]; *Chem. Abstr.* **1992**, *116*, 6580z).
[8] K. Goto, *Jpn Kokai Tokyo Koho Jp*. (*Jap. Pat. No.* 03215488 [91,215,488]; *Chem. Abstr.* **1992**, *116*, 128962w).
[9] K. Goto, K. Hashimoto, K. Kanai, *Jpn Kokai Tokyo Koho Jp*. (*Jap. Pat. No.* 63,168,685 [88,198,685]; *Chem. Abstr.* **1989**, *110*, 23911b).
[10] A. Santagati, M. Modica, M. Santagati, A. Caruso, V. Cutuli, *Pharmazie* **1994**, *49*, 64 (*Chem. Abstr.* **1994**, *120*, 323518r).
[11] R. W. Vou Borstel, M. K. Bamat, B. M. Hiltrand, *PCT Int. Appl. WO* 690,11,15 (*Chem. Abstr.* **1996**, *124*, 250921n).
[12] R. Russo, G. Romoeo, N. A. Santagati, A. Caruso, V. Cutuli, D. Amore, *Eur. J. Med. Chem*. **1994**, *29*, 569.
[13] D. R. Shridhar, C. V. R. Sastri, K. B. Lal, O. P. Bansal, S. M. Sondhi, *Gazette of India* March 28, **1987**, p. 213 (*Indian Pat.* 159118; *Chem. Abstr.* **1988**, *108*, 167289m).
[14] D. R. Shridhar, C. V. R. Sastri, K. B. Lal, O. P. Bansal, S. M. Sondhi, *Gazette of India* March 28, **1987**, p. 214 (*Indian Pat.* 159119; *Chem. Abstr.* **1988**, *108*, 131573k).
[15] S. M. Sondhi, A. Magan, V. K. Mahesh, R. C. Srimal, A. K Goel, *Indian J. Chem*. **1994**, *33B*, 1144.
[16] S. M. Sondhi, A. Magan, R. Sahu, V. K. Mahesh, R. Shukla, G. K. Patnaik, *Synthesis* **1994**,1175.
[17] S. M. Sondhi, N. Singhal, R. P. Verma, R. Raghubir, A. K. Goel, G. K. Patnaik, *Indian J. Chem*. **1997**, *36B*, 620.
[18] S. M. Sondhi, V. K. Sharma, R. P. Verma, N. Singhal, R. Shukla, R. Raghubir, M. P. Dubey, *Synthesis* **1999**, 878.
[19] S. M. Sondhi, V. K. Sharma, N. Singhal, R. P. Verma, R. Shukla, R. Raghubir, M. P. Dubey, *Phosphorous Sulfur Silicon Relat. Elem*. **2000**, *156*, 21.
[20] S. M. Sondhi, R. Sahu, A. Magan, D. K. Ghosh, R. M. Mukhopadhya, G. K. Chatterjee, A. K. Dass, S. K. Chaudhuri, *Indian Drugs* **1994**, *31*, 317.
[21] J. March, *Advanced Organic Chemistry* (4th Edn) **1992**, p. 649 (Wiley: NewYork).
[22] R. T. Morrision, R. N. Boyd, *Organic Chemistry* 6th Edn, **1999**, pp.1068–1071 (Prentice Hall of India: New Delhi).
[23] B. V. Unkovskii, L. A. Iqnatova, M. M. Donskaya, M. G. Zaitseva, *Chem. Abstr.* **1966**, *64*, 9719b.
[24] R. A. Mathes, F. D. Stewart, F. J. R. Swedish, *J. Am. Chem. Soc*., **1948**, *70*, 1452.
[25] C. A. Winter, E. A. Risley, G. W. Nuss, *Proc. Soc. Exp. Biol. Med.* **1962**, *111*,544.
[26] P. P. Singh, A. Y. Junnarkar, R. C. Seshagiri, R. K. Varma, D. R. Shridhar, *Meth. Find. Explt. Clin. Pharmcol*. **1983**, *5*, 601.
[27] C. W. Wright, M. J. O'Neill, J. D. Phillipson, D. C. Warhurst, *Antimicrob. Agents Chemother.* **1988**, *32*, 1725.
[28] F. D. Gillin, D. S. Reiner, M. Suffness, *Antimicrob. Agents Chemother.* **1982**, *22*, 342.
[29] A. T. Keen, A. Harris, J. D. Phillipson, D. C. Warhurst, *Planta Med*. **1986**, 278.