

Synthesis and biological evaluation of some novel pyrazolopyrimidines incorporating a benzothiazole ring system

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In the present study, a series of benzothiazol derivatives **3a-l** containing pyrazolo[3,4-*d*]pyrimidine moiety at the second position were synthesized and characterized by analytical and spectral data. The compounds were tested for their *in vitro* antimicrobial activity. Compounds 1-(1,3-benzothiazol-2-yl)-3-methyl-4-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**3a**), 1-(1,3-benzothiazol-2-yl)-4-(4-chlorophenyl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**3d**) and 1-(1,3-benzothiazol-2-yl)-3-methyl-4-substituted phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines (**3h-j**) showed significant inhibitory activity against *P. aeruginosa* whereas compounds 1-(1,3-benzothiazol-2-yl)-4-(2-chlorophenyl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**3b**), 2-[1-(1,3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]phenol (**3e**), 1-(1,3-benzothiazol-2-yl)-4-(3,4-dimethoxyphenyl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**3h**), 4-[1-(1,3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]-*N,N*-dimethylaniline (**3j**) and 1-(1,3-benzothiazol-2-yl)-3-methyl-4-[2-phenylvinyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine (**3k**) were found to be active against *C. albicans*. Some of these synthesized compounds were evaluated for their *in vivo* acute toxicity, analgesic, anti-inflammatory, and ulcerogenic actions. The tested compound 4-[1-(1,3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]-*N,N*-dimethylaniline (**3j**) exhibited maximum analgesic and anti-inflammatory activities. Compounds 1-(1,3-benzothiazol-2-yl)-3-methyl-4-(3-nitrophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (**3i**) and **3j** showed a significant gastrointestinal protection compared to the standard drug diclofenac sodium.

Keywords: benzothiazoles, pyrazolo[3,4-*d*]pyrimidine, antimicrobial activity, anti-inflammatory activity

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A major mechanism of action of nonsteroidal anti-inflammatory drugs (NSAIDs) is the lowering of prostaglandin (PG) production through inhibition of the cyclooxygenase (COX) enzyme, which catalyzes the conversion of arachidonic acid into PG (1). Since PG

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has a dual function, mediation of inflammation (2) and cytoprotection (3) in the stomach and intestine, long-term usage of NSAIDs to relieve the symptoms of inflammation and pain always results in gastrointestinal (GI) disorders and renal toxicity (4). It is known that bacterial infections often produce pain and inflammation. In normal practice, chemotherapeutic, analgesic, and anti-inflammatory drugs are prescribed simultaneously, which increases the risk of developing NSAIDs-related complications, especially in the elderly, patients with a prior history of peptic ulcer disease and patients with impaired kidney functions. Hence, there is a pressing need for drugs having both antimicrobial and analgesic/anti-inflammatory activities with minimum adverse effects.

Pyrazolo[3,4-*d*]pyrimidine and related fused heterocycles are of interest as potential bioactive molecules and have received a great deal of attention due to their useful antibacterial (5), antifungal (6) and anti-inflammatory (7) properties. In addition, many benzothiazole derivatives exhibit a wide variety of biological activities [antimicrobial (8, 9) and anti-inflammatory (10, 11)]. Based on the above observations and with the aim of developing new dual acting antimicrobial/anti-inflammatory agents with minimum ulcerogenic effects, it appeared of interest to link the benzothiazole nucleus at the second position to some pyrazolo[3,4-*d*]pyrimidine moiety. This is an attempt to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules. In the present investigation, we aimed to synthesize novel benzothiazole incorporated pyrazolo[3,4-*d*]pyrimidine derivatives and evaluate their antimicrobial, analgesic, anti-inflammatory and ulcerogenic activities.

EXPERIMENTAL

Requisite chemicals were of reagent grade and were purified when necessary. Melting points were determined in open glass capillaries and are uncorrected. The reaction progress was routinely monitored by thin layer chromatography (TLC) on silica gel plates (Merck 60 F₂₅₄, Germany). The IR spectra were recorded on a Shimadzu 8400S FT-IR spectrometer (Japan) in KBr pellets and band positions are given in reciprocal centimeters (cm⁻¹). The ¹H NMR and ¹³C NMR spectra were recorded using a Bruker AV-III 400 spectrometer (Germany) with dimethyl sulfoxide (DMSO)-*d*₆ as solvent. Chemical shifts were reported in ppm using the solvent as internal standard. Mass spectra were measured on a Shimadzu LC-MS 2010A mass spectrometer (Japan) equipped with a manual injector, a photodiode array detector, an electrospray ionization source and a single-quadrupole mass analyzer in both positive and negative ion modes. Elemental analyses were carried out on a Flash EA 1112 series instrument (Thermo Finnigan, Italy).

2-Hydrazino-1,3-benzothiazole (**1**) and 1-(1,3-benzothiazol-2-yl)-3-methyl-1,4-dihydro-5*H*-pyrazol-5-one (**2**) were prepared by the procedure described in the literature (12, 13).

*Synthesis of 1-(1,3-benzothiazol-2-yl)-3-methyl-4-substituted phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives (3a-1). General procedure*

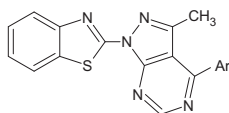
A mixture of 1-(1,3-benzothiazol-2-yl)-3-methyl-1,4-dihydro-5*H*-pyrazol-5-one (**2**) (10 mmol, 2.31 g), urea (10 mmol, 0.60 g) and appropriate aldehyde (10 mmol, for

benzaldehyde 1.06 g, 2,3 or 4-chlorobenzaldehyde 1.40 g, 2 or 4-hydroxybenzaldehyde 1.22 g, 4-methoxybenzaldehyde 1.36 g, 3,4-dimethoxybenzaldehyde 1.66 g, 3-nitrobenzaldehyde 1.51 g, 4-dimethylaminobenzaldehyde 1.49 g, cinnamaldehyde 1.32 g, 2-furfuraldehyde 0.96 g) in absolute ethanol (30 mL) was heated under reflux on a water bath for 7–8 h. The mixture was concentrated *in vacuo* and cooled to room temperature. The separated solid was filtered, washed several times with water, dried and recrystallized from appropriate solvent to give the title compounds **3a-l**.

Physicochemical and spectral data for the synthesized compounds are given in Tables I and II. Synthetic route is represented in Scheme 1.

Solvent for crystallization: for **3a**, **3c-f**, **3h**, **3i** acetone, for **3b**, **3j** chloroform and methanol 1:1, for **3g**, **3l** methanol, for **3k** chloroform and petroleum ether 1:1

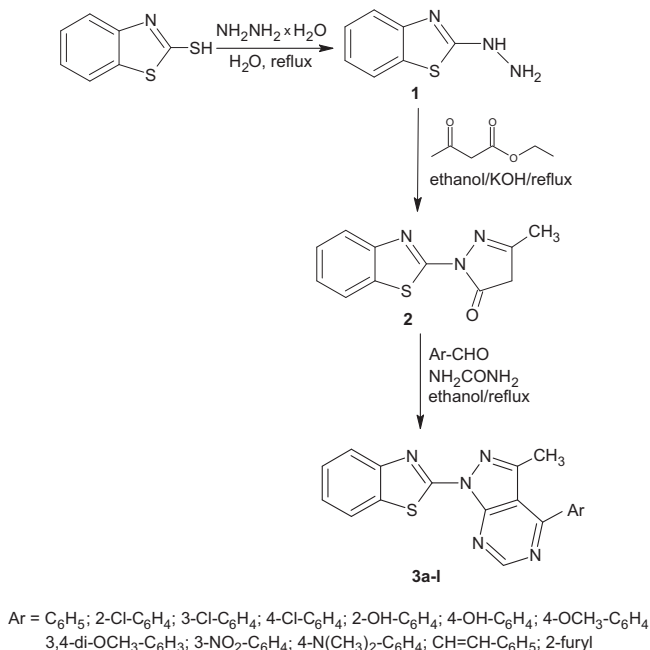
Table I. Physicochemical properties of the synthesized compounds **3a-l**



Compd.	Ar	Formula (M _r)	Yield (%)	M.p. (°C)	Analysis (calcd./found, %)		
					C	H	N
3a	C ₆ H ₅	C ₁₉ H ₁₃ N ₅ S (343.40)	66	165	66.47/66.50	3.79/3.82	20.40/20.46
3b	2-Cl-C ₆ H ₄	C ₁₉ H ₁₂ ClN ₅ S (377.85)	67	228	60.31/60.29	3.17/3.20	18.51/18.56
3c	3-Cl-C ₆ H ₄	C ₁₉ H ₁₂ ClN ₅ S (377.85)	61	203	60.31/60.36	3.17/3.13	18.51/18.49
3d	4-Cl-C ₆ H ₄	C ₁₉ H ₁₂ ClN ₅ S (377.85)	70	185	60.31/60.28	3.17/3.13	18.51/18.56
3e	2-HO-C ₆ H ₄	C ₁₉ H ₁₃ N ₅ OS (359.40)	66	225	63.50/63.46	3.62/3.68	19.49/19.54
3f	4-HO-C ₆ H ₄	C ₁₉ H ₁₃ N ₅ OS (359.40)	61	280	63.50/63.53	3.62/3.58	19.49/19.45
3g	4-CH ₃ O-C ₆ H ₄	C ₂₀ H ₁₅ N ₅ OS (373.43)	69	165	62.53/62.49	4.21/4.19	18.77/18.81
3h	3,4-di-CH ₃ O-C ₆ H ₃	C ₂₁ H ₁₇ N ₅ O ₂ S (403.45)	62	198	62.53/62.49	4.21/4.19	17.36/17.31
3i	3-NO ₂ -C ₆ H ₄	C ₁₉ H ₁₂ N ₆ O ₂ S (388.40)	72	171	58.76/58.80	3.09/3.13	21.64/21.69
3j	4-N(CH ₃) ₂ -C ₆ H ₄	C ₂₁ H ₁₈ N ₆ S (386.47)	67	240	65.28/65.32	4.66/4.69	21.76/21.81
3k	C ₆ H ₅ -CH=CH	C ₂₁ H ₁₅ N ₅ S (369.44)	61	195	68.29/68.33	4.06/4.11	18.97/18.92
3l	2-furyl	C ₁₇ H ₁₁ N ₅ OS (333.36)	62	180	61.26/61.31	3.30/3.36	21.02/21.08

Table II. Spectral characterization of the new compounds

Compd.	IR (KBr) (ν , cm^{-1})	^1H NMR (δ , ppm)	^{13}C NMR (δ , ppm)	MS (m/z)
3a	3057 (Ar-CH), 2972 (CH_3), 1633 (C=N), 1600 (Ar-C=C), 756 (C-S-C)	8.21 (s, 1H, N-CH=N), 8.03–7.69 (m, 4H, ArH), 7.54–7.21 (m, 5H, ArH), 2.48 (s, 3H, CH_3)		343 (M ⁺)
3b	3048 (Ar-CH), 2970 (CH_3), 1627 (C=N), 1599 (Ar-C=C), 752 (C-S-C), 727 (C-Cl)	8.28 (s, 1H, N-CH=N), 7.99–7.81 (m, 4H, ArH), 7.79–7.26 (m, 4H, ArH), 2.45 (s, 3H, CH_3)		376 (M ⁺ +2)
3c	3057 (Ar-CH), 2979 (CH_3), 1631 (C=N), 1595 (Ar-C=C), 755 (C-S-C), 729 (C-Cl)	8.31 (s, 1H, N-CH=N), 8.17–7.86 (m, 4H, ArH), 7.76–7.38 (m, 4H, ArH), 2.39 (s, 3H, CH_3)		378 (M ⁺)
3d	3070 (Ar-CH), 2976 (CH_3), 1626 (C=N), 1610 (Ar-C=C), 756 (C-S-C), 727 (C-Cl)	8.34 (s, 1H, N-CH=N), 8.11–7.83 (m, 4H, ArH), 7.72–7.31 (m, 4H, ArH), 2.15 (s, 3H, CH_3)		380 (M ⁺ +2)
3e	3064 (OH), 3034 (Ar-CH), 2971 (CH_3), 1610 (C=N), 1595 (Ar-C=C), 758 (C-S-C)	8.44 (s, 1H, N-CH=N), 8.18–7.76 (m, 4H, ArH), 7.69–7.38 (m, 4H, ArH), 5.23 (brs, 1H, OH, D ₂ O exchangeable), 2.35 (s, 3H, CH_3)		359 (M ⁺)
3f	3066 (OH), 3030 (Ar-CH), 2981 (CH_3), 1630 (C=N), 1579 (Ar-C=C), 763 (C-S-C)	8.28 (s, 1H, N-CH=N), 7.88–7.78 (m, 4H, ArH), 7.64–7.27 (m, 4H, ArH), 5.02 (brs, 1H, OH, D ₂ O exchangeable), 2.29 (s, 3H, CH_3)		358 (M ⁺ -1)
3g	3010 (ArH), 2966 (CH_3), 1622 (C=N), 1580 (Ar-C=C), 1271, 1021 (C-O-C), 758 (C-S-C)	8.41 (s, 1H, N-CH=N), 8.11–7.70 (m, 3H, ArH), 7.56–6.68 (m, 5H, ArH), 3.93 (s, 3H, OCH_3), 2.42 (s, 3H, CH_3)		373 (M ⁺)
3h	3057 (ArH), 2970 (CH_3), 1633 (C=N), 1585 (Ar-C=C), 1273, 1020 (C-O-C), 754 (C-S-C)	8.42 (s, 1H, N-CH=N), 8.18–7.73 (m, 4H, ArH), 7.69–6.71 (m, 3H, ArH), 3.86 (s, 6H, 2x OCH_3), 2.38 (s, 3H, CH_3)		404 (M ⁺ +1)
3i	3063 (Ar-CH), 2965 (CH_3), 1645 (C=N), 1598 (Ar-C=C), 1526, 1349 (NO_2), 757 (C-S-C)	8.39 (s, 1H, N-CH=N), 8.13–7.60 (m, 4H, ArH), 7.58–7.19 (m, 4H, ArH), 2.45 (s, 3H, CH_3)	152.28, 151.13, 150.66, 150.37, 148.18, 147.59, 134.84, 134.56, 133.82, 133.08, 131.69, 130.88, 129.66, 129.41, 126.88, 126.42, 123.97, 123.44, 32.98	390 (M ⁺ +2)
3j	3057 (Ar-CH), 2985 (CH_3), 1620 (C=N), 1597 (Ar-C=C), 759 (C-S-C)	8.33 (s, 1H, N-CH=N), 8.07–7.71 (m, 4H, ArH), 7.67–7.21 (m, 4H, ArH), 2.45 (s, 6H, N(CH_3) ₂), 2.28 (s, 3H, CH_3)	155.58, 153.21, 151.53, 149.59, 146.44, 141.39, 135.23, 133.98, 132.98, 131.26, 129.98, 128.93, 127.38, 125.88, 124.16, 122.34, 35.23, 32.98	385 (M ⁺ -1)
3k	3059 (Ar-CH), 2886 (CH_3), 1647 (C=N), 1610 (C=C alkene), 1589 (Ar-C=C), 754 (C-S-C)	8.29 (s, 1H, N-CH=N), 8.12–7.70 (m, 4H, ArH), 7.62–7.30 (m, 5H, ArH), 6.23–5.96 (m, 2H, CH=CH), 2.45 (s, 3H, CH_3)		369 (M ⁺)
3l	3064 (Ar-CH), 2977 (CH_3), 1634 (C=N), 1598 (Ar-C=C), 1273, 1013 (C-O-C), 756 (C-S-C)	8.36 (s, 1H, N-CH=N), 8.02–7.76 (m, 4H, ArH), 7.68–7.46 (m, 3H, ArH), 2.37 (s, 3H, CH_3)		332 (M ⁺ -1)



Scheme 1

Biological study

Antimicrobial activity. – Standard strains were procured from the National Collection of Industrial Microorganism, National Chemical Laboratory, India. Synthesized compounds **3a-l** were tested [50 and 100 µg mL⁻¹ in sterile dimethyl sulfoxide (DMSO)] for their *in vitro* antimicrobial activity by the cup plate diffusion method (14). The antibacterial activity was evaluated on nutrient agar (Hi-media) plates (37 °C, 24 h) against Gram-positive bacteria *Staphylococcus aureus* NCIM 2079, *Bacillus megaterium* NCIM 2187, Gram-negative bacteria *Escherichia coli* NCIM 2567 and *Pseudomonas aeruginosa* NCIM 2036. Test compounds were also evaluated for their antifungal potential on Sabouraud dextrose agar (Hi-media) plates (26 °C, 48–72 h) against *Candida albicans* NCIM 3100. Ciprofloxacin and ketoconazole were used as control drugs as 10 and 20 µg mL⁻¹ solutions, respectively, in sterile dimethyl sulfoxide. Average diameter of inhibition zone (three independent evaluations) of bacterial or fungal growth around the disk in mm (Table III) were recorded.

Animals. – Experimental animals were procured from the M/S Venkatesh Enterprises, India. The animals were fed a standard rodent diet (Hindustan Liver Ltd., India) with water *ad libitum* and were housed in rooms maintained at 23 ± 2 °C with a 12 h light/dark cycle and relative humidity 45–50 %. The Animal Ethics Committee of the J. S. S. College of Pharmacy, Ootacamund, India, approved all experimental protocols, in accordance with the Indian Government Legislation on Animal Care. Synthesized com-

Table III. Antimicrobial activity of synthesized compounds **3a–l**^a

Compd.	Zone of inhibition (mm)				
	<i>Staphylococcus aureus</i>	<i>Bacillus megaterium</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
3a	13	–	19	23	16
3b	13	–	17	19	24
3c	–	–	18	19	22
3d	12	12	21	27	18
3e	12	–	20	19	24
3f	13	12	19	19	23
3g	11	–	21	18	22
3h	13	–	19	23	26
3i	12	13	21	24	21
3j	12	12	22	22	26
3k	13	–	19	18	24
3l	12	12	21	23	13
Ciprofloxacin	30	35	41	50	–
Ketoconazole	–	–	–	–	32
DMSO	–	–	–	–	–

– no activity.

^a Average of three independent determinations. Concentration tested: compounds **3a–l** 100 µg mL⁻¹, ciprofloxacin 10 µg mL⁻¹, ketoconazole 20 µg mL⁻¹.

pounds **3a–l** were evaluated for their acute toxicity, analgesic and anti-inflammatory activities. Data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Student's *t*-test to assess the statistical significance.

Acute toxicity. – Acute oral toxicity was checked for compounds **3a–l** following the Organization of Economic Cooperation and Development (OECD-423) guidelines (15) (acute toxic class method). Swiss albino mice (*n* = 3) of either sex (38–45 g), selected by random sampling, were used for the study. The animals were fasted for 3–4 h with water *ad libitum*, after which the test compounds were administered orally as a suspension in 1 % carboxymethylcellulose (CMC) (*m/V*, in water) at doses of 50, 100, 250, 500 and 1000 mg kg⁻¹ body mass. The mice were observed for three days.

Analgesic activity by acetic acid induced writhing method. – Analgesic activity of the synthesized compounds was evaluated by the acetic acid induced writhing method (16) using Swiss albino mice (*n* = 6) of either sex (35–48 g). The test compounds and diclofenac sodium were suspended in 1.0 % CMC and administered orally. One hour after administration of the test compounds, 0.10 mL acetic acid solution (1.0 %, *m/V*, in sterile saline) was injected intraperitoneally and stretching movements comprising of arching of the back, body elongation and extension of hind limbs were counted for 15 min. The analgesic activity was expressed in terms of percentage inhibition (Table IV).

Table IV. Analgesic activity of some selected compounds in mice by the acetic induced writhing method

Compd.	3a	3d	3h	3i	3j	3l	Diclofenac sodium
Inhibition (%) ^a	41.7 ± 1.3 ^c	37.3 ± 0.8 ^c	55.2 ± 1.2 ^c	56.3 ± 1.2 ^d	59.6 ± 0.9 ^c	36.6 ± 0.6 ^b	62.6 ± 1.3 ^c

Test compounds and diclofenac sodium were tested at 0.30 and 0.06 mmol kg⁻¹ body mass, respectively.

^a Mean ± SEM, *n* = 6.

Significance levels compared to the control: ^b *p* < 0.05, ^c *p* < 0.01 and ^d *p* < 0.001.

Analgesic activity by Eddy's hot plate method. – Analgesic activity of the synthesized compounds was also evaluated by Eddy's hot plate method (17) using Swiss albino mice (*n* = 6) of either sex (44–52 g). The test compounds and paracetamol were administered orally suspended in 1.0 % CMC. One hour after administration of the test compounds, animals were individually placed on hot plate maintained at 55 ± 0.5 °C. The response time was noted as the time at which animals reacted to the pain stimulus either by paw licking or jump response, whichever appeared first. The reaction time in control and treated animals was recorded at 0.5, 1, 2 and 3 h after the treatment. The cut off time for the reaction was 15 seconds. Results are presented in Table V.

Anti-inflammatory activity. – The acute anti-inflammatory activity of the synthesized compounds was determined following the carrageenan induced paw oedema method (18) in Wistar albino rats (*n* = 6) of either sex (155–180 g). The animals were fasted for 24 h before the experiment with free access to water. The test compounds and diclofenac sodium were administered orally as a suspension in 1.0 % CMC. Control rats received appropriate volumes of 1.0 % CMC solution orally. Thirty minutes after administration of the test compounds, 0.1 mL carrageenan solution (1.0 %, *m/V*, in sterile saline) was injected into the sub-plantar tissue of the right hind paw of each rat. Paw volume was measured at time intervals of 0.5, 1, 2 and 3 h after carrageenan injection by means of a plethysmometer (UGO Basile 7140, India). The anti-inflammatory activity results are summarized in Table VI.

Table V. Analgesic activity of some selected compounds in mice by Eddy's hot plate method

Compd.	Analgesic activity ^a			
	0.5 h	1 h	2 h	3 h
3a	32.1 ± 0.9 ^c	60.3 ± 1.2 ^d	39.7 ± 0.8 ^c	24.6 ± 1.0 ^c
3d	30.8 ± 0.9 ^c	64.4 ± 1.0 ^c	51.5 ± 1.2 ^c	26.1 ± 1.2 ^d
3h	50.0 ± 1.1 ^c	73.2 ± 1.1 ^c	75.0 ± 1.5 ^b	61.4 ± 1.0 ^c
3i	58.4 ± 1.3 ^c	43.4 ± 0.8 ^c	35.1 ± 1.1 ^d	27.0 ± 1.1 ^d
3j	40.4 ± 1.3 ^c	54.9 ± 1.2 ^d	51.7 ± 1.1 ^c	39.6 ± 0.9 ^b
3l	49.8 ± 1.2 ^d	51.7 ± 1.2 ^b	67.5 ± 1.1 ^c	61.6 ± 1.3 ^c
Paracetamol	61.3 ± 1.0 ^c	70.2 ± 0.9 ^c	86.9 ± 0.8 ^c	58.7 ± 0.7 ^c

Test compounds and paracetamol were tested at 0.30 and 0.66 mmol kg⁻¹ body mass, respectively.

^a Mean ± SEM, *n* = 6.

Significance levels compared to the respective control: ^b *p* < 0.05, ^c *p* < 0.01 and ^d *p* < 0.001.

Table VI. Anti-inflammatory activity of some selected compounds by the carrageenan induced rat paw oedema method

Compd.	Protection ^a			
	30 min	1 h	2 h	3 h
3a	17.0 ± 0.9 ^b	36.7 ± 0.7 ^b	38.4 ± 0.8 ^c	27.2 ± 1.1 ^c
3d	12.5 ± 1.1 ^d	14.8 ± 1.2 ^d	21.7 ± 1.2 ^d	30.0 ± 1.1 ^b
3h	13.3 ± 1.3 ^c	20.7 ± 0.8 ^d	41.6 ± 1.0 ^c	35.9 ± 0.7 ^c
3i	44.6 ± 1.2 ^c	29.1 ± 1.1 ^c	28.4 ± 1.3 ^c	23.9 ± 1.2 ^c
3j	56.8 ± 1.0 ^d	67.7 ± 1.2 ^b	74.7 ± 1.0 ^d	47.6 ± 0.7 ^d
3l	19.5 ± 1.1 ^b	29.5 ± 1.4 ^b	17.6 ± 1.3 ^c	17.5 ± 1.1 ^b
Diclofenac sodium	29.9 ± 1.2 ^d	49.3 ± 0.9 ^b	61.1 ± 1.2 ^c	72.0 ± 0.9 ^d

Test compounds and diclofenac sodium were tested at 0.30 and 0.06 mmol kg⁻¹ body mass, respectively.

^a Mean ± SEM, *n* = 6.

Significance levels compared to the respective control: ^b *p* < 0.05, ^c *p* < 0.01 and ^d *p* < 0.001.

Table VII. Ulcerogenic activity of some selected compounds

Compd.	1 % CMC	3h	3i	3j	Diclofenac sodium
Severity index ^a	1.1 ± 0.8 ^d	3.7 ± 0.7 ^b	3.5 ± 1.2 ^c	3.0 ± 0.9 ^c	6.1 ± 1.1 ^c

Test compounds and diclofenac sodium were tested at 0.60 and 0.12 mmol kg⁻¹ body mass, respectively.

^a Mean ± SEM, *n* = 6.

Significance levels compared to the control: ^b *p* < 0.05, ^c *p* < 0.01 and ^d *p* < 0.001.

Ulcerogenic effects. – Test compounds **3e**, **3h** and **3j** were evaluated for their acute ulcerogenic effects according to the method of Cioli *et al.* (19) using Wistar albino rats (*n* = 6) of either sex (175–190 g). The test compounds and diclofenac sodium were administered orally as a suspension in 1 % CMC solution. Control group received appropriate volumes of CMC solution orally. Food, but not water, was removed 24 h before administration of the test compounds. After compound treatment, the rats were fed a standard diet for 17 h and then sacrificed. Their stomachs were removed, cut out along the greater curvature, washed with distilled water and then gently cleaned by dipping in normal saline. Mucosal damage was examined by means of a magnifying glass. For each stomach, the mucosal damage was assessed according to the following scoring system: 0.5 redness; 1.0 spot ulcers; 1.5 hemorrhagic streaks; 2.0 ulcers > 3 but ≤ 5; 3.0 ulcers > 5 (20). The mean score of each treated group minus the mean score of the control group was regarded as the severity index of gastric mucosal damage. Results are presented in Table VII.

RESULTS AND DISCUSSION

The synthetic route for the preparation of title compounds **3a-l** is outlined in Scheme 1. The intermediate 2-hydrazino-1,3-benzothiazole (**1**) was prepared (**12**) by refluxing 2-mercaptobenzothiazole with hydrazine hydrate in water. 1-(1,3-Benzothiazol-2-yl)-3-methyl-1,4-dihydro-5*H*-pyrazol-5-one (**2**) was prepared (**13**) by the treatment of compound (**1**) with ethyl acetoacetate in boiling ethanolic potassium hydroxide. Further condensation of compound (**2**) with urea and araldehydes in refluxing absolute alcohol furnished the corresponding title compounds 1-(1,3-benzothiazol-2-yl)-3-methyl-4-substituted phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**3a-l**) in 61–72 % yield. Structures of the newly synthesized compounds were confirmed by analytical and spectral data.

The infrared spectra of compounds (**3a-l**) revealed absorption bands within 2986–2965 cm^{-1} for CH_3 , 1647–1610 cm^{-1} for $\text{C}=\text{N}$ and 763–752 cm^{-1} for $\text{C}-\text{S}-\text{C}$ stretch. Formation of pyrazolopyrimidine ring in **3i** was supported by its ^1H NMR spectrum (**21**), which showed a singlet signal at δ 8.19 ppm due to the $\text{CH}=\text{N}$ fragment of the pyrazolopyrimidine ring. Two multiplet signals that appeared at δ 8.13–7.60 and 7.58–7.19 ppm were assigned to four aromatic protons each. Furthermore, the signal for the CH_3 fragment was ascribed to a singlet at δ 2.45 ppm. Its mass spectrum showed an $\text{M}+2$ peak at m/z 390, which is in conformity with the molecular formula $\text{C}_{19}\text{H}_{12}\text{N}_6\text{O}_2\text{S}$. It exhibited a base peak at m/z 232 due to the $\text{C}_{11}\text{H}_{10}\text{N}_4\text{S}^+$ fragment, which is consistent with the structure of this compound **3i**. In the ^{13}C NMR spectrum of compound **3i**, azomethine carbon of the benzothiazole ring resonated as a singlet signal at δ 152.28 ppm, whereas two signals observed at δ 151.13 and 150.66 ppm were assigned to azomethine carbons of the pyrimidine ring. The $\text{C}=\text{N}$ fragment of the pyrazole ring exhibited a signal at δ 150.37 ppm. The appearance of signals between 148.18–123.44 ppm supported the presence of fourteen sp^2 carbon atoms. A signal at 32.98 ppm was assigned to the CH_3 fragment.

In vitro antimicrobial activity of synthesized compounds by the cup plate method (**14**) was first tested at 50 $\mu\text{g mL}^{-1}$ concentration, but all compounds failed to produce the antimicrobial effect against the tested microorganisms. However, at 100 $\mu\text{g mL}^{-1}$, tested compounds **3a**, **3d**, **3h-j** and **3l** displayed significant inhibitory activity (inhibition zone 22–27 mm, Table III) against *Pseudomonas aeruginosa*, whereas the other compounds showed moderate activity (inhibition zone 18–22 mm) against the same organism. In this regard, compound **3d** with 4- $\text{Cl}-\text{C}_6\text{H}_4$ group at the fourth position of the pyrazolopyrimidine ring showed the highest activity (inhibition zone 27 mm). When the position of the chloro substituent group was changed either to the second (**3b**) or third position (**3c**) in the phenyl ring, a marked decrease in activity against *P. aeruginosa* was observed. It was also observed that substitution with 2- $\text{OH}-\text{C}_6\text{H}_4$ (**3e**), 4- $\text{OH}-\text{C}_6\text{H}_4$ (**3f**) or $\text{CH}=\text{CH}-\text{C}_6\text{H}_5$ (**3k**) groups at the fourth position of the pyrazolopyrimidine ring resulted in a marked decrease in activity against the same microorganism. All the tested compounds exhibited either moderate or weak inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus megaterium*.

Antifungal screening revealed moderate to weak inhibitory activity against *Candida albicans*. In this regard, compounds with 3,4-di- $(\text{OCH}_3)_2$ (**3h**) or 4- $\text{N}(\text{CH}_3)_2$ (**3j**) bulky groups in the phenyl ring present at the fourth position of the pyrazolopyrimidine ring exhibited maximum activity (inhibition zone 26 mm in both cases). In the present investigation, activities of the tested compounds were much weaker than that of the standard drug.

Compounds **3h-j** showed significant inhibition against the acetic acid induced writhing (55.2–59.6 %), compared to the reference drug diclofenac sodium (62.6 % inhibition at a dose of 0.06 mmol kg⁻¹ body mass). In this regard, highest activity (59.6 % inhibition) was observed for compound **3j**. It was also observed that compounds with C₆H₅ (**3a**), 4-Cl-C₆H₄ (**3d**) or 2-furyl (**3l**) groups at the fourth position of the pyrazolopyrimidine ring resulted in a marked decrease in analgesic activity (36.6–41.7 % inhibition). It is evident that substitution of 3-NO₂-C₆H₄, 4-N(CH₃)₂-C₆H₃ or 3,4-(OCH₃)₂-C₆H₃ (in **3i**, **3j** and **3h**) bulky groups in the phenyl ring present at the fourth position of the pyrazolopyrimidine ring is optimal for inhibition against the acetic acid induced writhing. However, all the tested compounds were found to be significantly less potent compared to the standard drug.

Analgesic activity of the synthesized compounds was also evaluated by Eddy's hot plate method (17). Screening results at the 0.30 mmol kg⁻¹ (body mass) dosage are summarized in Table V. Derivatives **3h**, **3i** and **3l** exhibited fast analgesic activity (49.8–58.4 % analgesia), as evident from the observation at 30 min following oral administration (0.30 mmol kg⁻¹). After 1 h, compound **3h** with 3,4-(OCH₃)₂-C₆H₃ group at the fourth position of the pyrazolopyrimidine ring showed maximum activity (73.2 % analgesia) compared to the reference drug paracetamol (70 % analgesia at 0.66 mmol kg⁻¹), while after 2 and 3 h compounds **3h** and **3l** showed marked activity (61.4–75.0 % analgesia) against thermal stimuli compared to the reference drug (86.9 and 58.7 % analgesia, respectively). It is evident from the results that the presence of either C₆H₄ or 3-NO₂-C₆H₄ group markedly decreased the analgesic activity. It is also important to note that, except for compounds **3h** and **3l**, a marked decrease in analgesic activity was observed after 3 h following oral administration of test compounds. The promising analgesic activity shown by compounds **3h** and **3j** in both models indicated that these compounds might possess centrally and peripherally mediated analgesic properties.

At first, the anti-inflammatory activity of the synthesized compounds was studied (18) at a 0.15 mmol kg⁻¹ body mass dose, but it failed to affect the oedematogenic response. The anti-inflammatory activity results determined at the 0.30 mmol kg⁻¹ b.m. dosage are summarized in Table VI. Compounds **3f** and **3j** showed a rapid onset of action (44.6 and 56.8 % inhibition, respectively), as evident from the observation 30 min after carrageenan injection. After 1 h, maximum activity (67.7 % protection) was observed in compound **3j** having 4-N(CH₃)₂-C₆H₄ group at the fourth position of the pyrazolopyrimidine ring and after 2 h compound **3j** exhibited potent activity (74.7 % protection) when compared to the reference drug diclofenac sodium (61.1 % at 0.06 mmol kg⁻¹ b.m.). In general, a sharp decline in anti-inflammatory activity was observed 3 h after carrageenan injection. The other compounds showed moderate to weak activity (12.5–35.9 % inhibition) at all time points and were less potent compared to the standard drugs.

Carrageenan-induced oedema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandin and slow reacting substances with peaks at 3 h (21, 22). In the present study, previous oral treatment with the test compounds was effective in reducing the carrageenan induced paw oedema in rats between 30 min and the second hour after the injection. This suggests that the compounds probably inhibited the release of histamine, serotonin and kinins in the first phase.

Compounds **3h–j** were evaluated for their ulcerogenic potential in rats according to the method reported by Cioli *et al.* (19). The results indicated low ulcerogenic potential of the tested compounds (severity index 2.5–3.7). The lowest reduction in ulcerogenic potential (severity index 3.0) was observed for compound **3j** (Table VII). The other tested compounds, **3h** and **3i**, also exhibited a better gastrointestinal safety profile (severity index 3.7 and 3.5, respectively) compared to the standard drug diclofenac sodium (severity index 6.1).

No behavioral changes in animals were observed during the experiment and at the end hematological parameters were estimated and there were no observable changes. In the present study mortality was not observed and the tested compounds were well tolerated by the experimental animals up to 1000 mg kg⁻¹.

CONCLUSIONS

In summary, various benzothiazole derivatives were prepared with the objective of developing dual antimicrobial-anti-inflammatory agents with minimum ulcerogenic effects. Among these compounds, 4-[1-(1,3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]-*N,N*-dimethylaniline (**3j**) showed the most prominent and consistent activity and the lowest ulcerogenicity. Therefore, compound **3j** could represent a fruitful matrix for the development of a new class of dual antimicrobial-anti-inflammatory agents.

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