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Synthesis, characterization and pharmacological studies of biologically active benzimidazole derivatives

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ABSTRACT

The reaction of aniline derivatives with ammonium thiocyanate yield 4-thiocyanoaniline. The thiocyano-aniline was condensed with o-phenylenediamine and carbon disulphide to get benzimidazole. These compounds were synthesized in good yield and their structures were confirmed by IR,[1]H-NMR and [13]C-NMR .Antimicrobial activity against bacteria and fungi ,anti-inflammatory activity and analgesic activity were studied for the synthesized compounds.

Keywords: benzimidazole, thiocyanate, o-phenylenediamine, pharmacological activity.

INTRODUCTION

Benzimidazole derivatives are very useful intermediates for the development of pharmaceutical interest [1]. Benzimidazole derivatives have found the application in diverse therapeutic areas including antihypertensive[2], antiviral[3], antifungal[4], anticancer[5,6], anti-histaminic[7], antitubercular[8], antiallergic[9,10], antioxidant[11,12,13] and antimicrobial activities[14-20]. The 1-*H*-benzimidazole ring, which, exhibit remarkable basic characteristics due to their nitrogen content and comprises the active substances for several drugs. In the present study, novel benzimidazoles are synthesized from aniline compounds (Scheme 1).

MATERIALS AND METHODS

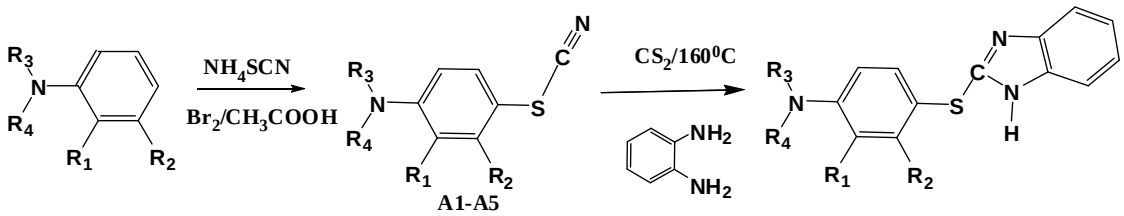
All the melting points were taken in open capillaries and are uncorrected. Elemental analysis was performed on a Perkin-Elmer analyzer. IR spectra are recorded in KBr on Shimadzu spectrometer, ^{1}H-NMR and ^{13}C-NMR in DMSO-d6 on a Bruker AC-400 spectrometer using TMS as an internal standard. The microorganisms were obtained from National Chemical Laboratory, Pune.

General procedure for the synthesis of thiocyanate (A1-A5)
The substituted/unsubstituted aniline (0.5 mol) was dissolved in acetic acid (125 ml) and the solution was added to the solution of ammonium thiocyanate (1.05mol, 80 g) in glacial acetic acid (250 ml). This solution was cooled to 10-20º C. To this well stirred solution, a solution of bromine (0.5 mol, 25.7 ml) in acetic acid (250 ml) was added dropwise for thirty minutes and the temperature was maintained below 20ºC. After the addition of bromine, it was kept at room temperature for ten minutes and then it was diluted with an equal amount of water. The solid material was filtered, washed, dried and recrystallized from ethanol.

General procedure for the synthesis of benzimidazoles (Compound B1-B5)

A mixture of thiocyanate A1-A5 (0.01 mol), o-phenylenediamine (0.01mol, 1.08g) and carbon disulphide (0.1 mol, 8 ml) was heated in an oil bath at 160° C for 6 hours. The resultant benzimidazole was cooled and recrystallised from ethanol.

Scheme 1



	R1	R2	R3	R4
A1,B1	**-NO_2**	**-H**	**-H**	**-H**
A2,B2	**-COOH**	**-H**	**-H**	**-H**
A3,B3	**-H**	**-NO_2**	**-H**	**-H**
A4,B4	**-H**	**-COOH**	**-H**	**-H**
A5,B5	**-H**	**-H**	**-C_2H_5**	**-C_2H_5**

Table 1 Analytical data of thiocyanate (A1-A5)

Thiocyanate	Yld (%)	M. Pt (° C)	Molecular formula	Elemental Analysis (%) Reported (Calculated)					M wt
				C	H	N	O	S	
A1	76	115-116	$C_7H_5SN_3O_2$	43.18 (43.07)	2.51 (2.58)	21.29 (21.53)	16.32 (16.39)	16.39 (16.43)	195
A2	62	218-219	$C_8H_6SN_2O_2$	49.41 (49.47)	3.08 (3.11)	14.38 (14.42)	16.40 (16.48)	16.45 (16.50)	194
A3	97	139-140	$C_8H_6SN_2O_2$	49.42 (49.47)	3.05 (3.11)	14.35 (14.42)	16.39 (16.48)	15.90 (16.05)	194
A4	75	85-86	$C_{11}H_{14}N2S$	63.99 (64.04)	6.80 (6.84)	13.47 (13.58)	–	15.48 (15.54)	206
A5	80	91-92	$C_7H_5SN_3O_2$	43.01 (43.07)	2.40 (2.58)	21.29 (21.35)	16.42 (16.39)	16.38 (16.43)	195

IR data for the thiocyanate (A1-A5)

A1 (2-nitro-4-thiocyanatoaniline) - $\nu_{C\equiv N}$: 2170 cm^{-1}
A2 (5-amino-2-thiocyanatobenzoicacid) - $\nu_{C\equiv N}$: 2150 cm^{-1}
A3 (2-amino-5-thiocyanatobenzoicacid) - $\nu_{C\equiv N}$: 2155 cm^{-1}
A4 (N,N-diethyl-4-thiocyanatoaniline) - $\nu_{C\equiv N}$: 2210 cm^{-1}
A5 (3-nitro-4-thiocyanatoaniline) - $\nu_{C\equiv N}$: 2257 cm^{-1}

Compound B1(4-(1*H*-benzo[d]imidazol-2-ylthio)-2-nitroaniline): IR(KBr) cm^{-1} : 3429(NH_2), 1504(NO_2), 1623(C=N str), 3350(NH), 3089(aromatic) .^{1}H-NMR : δ 6.99 – 7.89 (Ar-H, multiplet),δ 3.5 (Ar-NH_2 , singlet). ^{13}C-NMR : δ 122.7 (Ar-C) , δ 147 (C=N).

CompoundB2(2-(1*H*-benzo[d]imidazol-2-ylthio)-5-aminobenzoic acid):IR(KBr)cm^{-1}: 3429(NH_2), 1631(C=Nstr), 3318(NH), 2491(OH str). ^{1}H-NMR : δ 7.0 – 7.1 (Ar-H, multiplet), δ 12.5 (-COOH , singlet). ^{13}C-NMR : δ 122.7 (Ar-C) .

Compound B3(5-(1*H*-benzo[d]imidazol-2-ylthio)-2-aminobenzoic acid): IR(KBr)cm^{-1} : 3329(NH_2), 1600(C=N str), 3155(NH), 2987(aromatic) ,2570(OH str), 827(C=C bending) .^{1}H-NMR : δ 6.99 – 7.89 (Ar-H, multiplet),δ 3.5 (Ar-NH_2 , singlet).^{13}C-NMR : δ 122.7 (Ar-C) , δ 147 (C=N).

Compound B4(4-(1*H*-benzo[d]imidazol-2-ylthio)-*N*,*N*-diethylaniline): IR(KBr) cm^{-1} : 3438(NH_2), 1607(C=N str), 3114(aromatic) .^{1}H-NMR : δ 6.6 – 7.8 (Ar-H, multiplet),δ 3.6 (Ar-NH_2 , singlet). ^{13}C-NMR : δ 122-132 (Ar-C) , δ 144 (C=N).

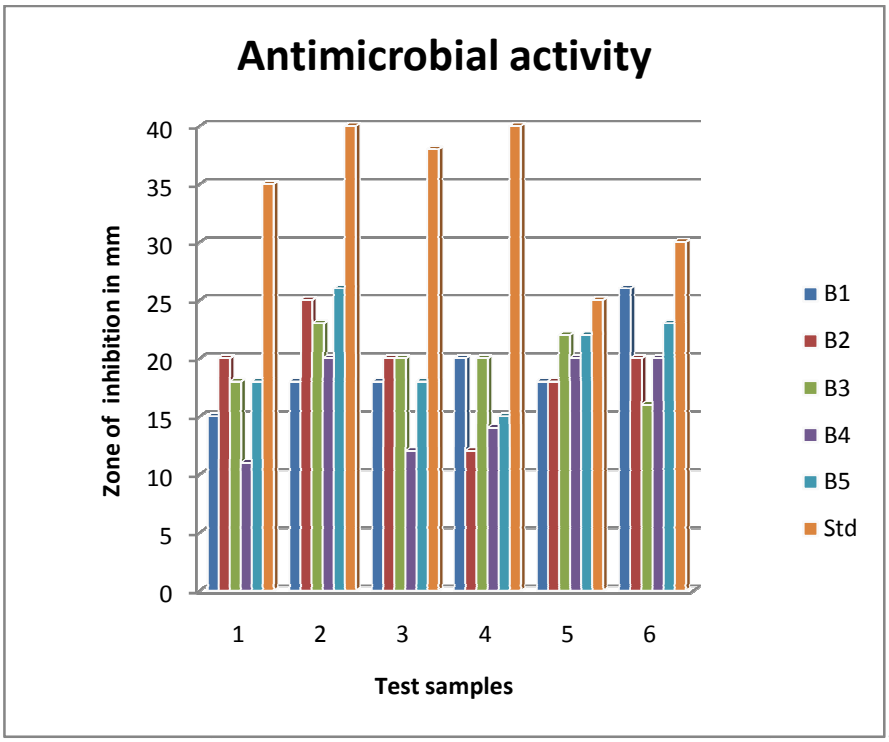
Compound B5(4-(1*H*-benzo[d]imidazol-2-ylthio)-3-nitroaniline): IR(KBr) cm^{-1} : 3429(NH_2), 1504(NO_2), 1623(C=N str), 3350(NH), 3089(aromatic) .^{1}H NMR : δ 7.22 – 7.4 (Ar-H, multiplet),δ 2.5 (Ar-NH_2 , singlet), δ 12.5 (NH, singlet). ^{13}C-NMR : δ 117.6-122.7 (Ar-C) , δ 140(C=N).

Table 2 Analytical data of benzimidazole (B1-B5)

Benzimidazole	Yld (%)	M. Pt (° C)	Molecular formula	Elemental Analysis (%) Reported (Calculated)					M wt
				C	H	N	O	S	
B1	77	360-361	$C_{13}H_{10}SN_4O_2$	54.49 (54.54)	3.48 (3.52)	19.54 (19.57)	11.21 (11.18)	11.28 (11.20)	286
B2	66	283-284	$C_{14}H_{11}SN_3O_2$	58.90 (58.93)	3.84 (3.89)	4.75 (14.73)	1.23 (11.22)	11.18 (11.24)	285
B3	80	219-220	$C_{14}H_{11}SN_3O_2$	58.97 (58.93)	3.76 (3.89)	14.68 (14.73)	11.19 (11.22)	11.20 (11.24)	285
B4	71	243-244	$C_{17}H_{19}N_3S$	68.62 (68.65)	6.40 (6.44)	14.18 (14.13)	–	10.76 (10.78)	297
B5	72	311-312	$C_{13}H_{10}SN_4O_2$	54.59 (54.54)	3.54 (3.52)	19.51 (19.57)	11.22 (11.18)	11.15 (11.20)	286

Anti-microbial Activity

The anti-microbial activity for the sample was carried out by Disc Diffusion Technique[21]. The test microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus niger*) maintained by periodical subculturing on nutrient agar and sabouraud dextrose agar medium for bacteria and fungi respectively. The effects produced by the samples were compared with the effect produced by the positive control (Reference standard ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 units/disc for fungi).



1.*Staphylococcus aureus* **2.***Bacillus Subtilis* **3.***Escherichia Coli* **4.***Pseudomonas aeruginosa*
4.*Candida Albicans* **5.***Aspergillus Niger*

Table 2 Antimicrobial activities of the synthesized compounds

S.No	Name of the microorganisms	Zone of Inhibition in mm					
		B1	B2	B3	B4	B5	Std
1.	*Staphylococcus aureus*	15	20	18	11	18	35
2.	*Bacillus Subtilis*	18	25	23	20	26	40
3.	*Escherichia Coli*	18	20	20	12	18	38
4.	*Pseudomonas aeruginosa*	20	12	20	14	15	40
5.	*Candida Albicans*	18	18	22	20	22	25
6.	*Aspergillus Niger*	26	20	16	20	23	30

Anti-inflammatory Activity

Carrageenan induced hind paw edema

Albino rats of either sex weighing 150-200 gms were divided into six groups of six animals each. The dosage of the drugs administrated to the different groups were as follows: Group 1 – Control received normal saline, Group 2 to 16 received test in a dose of 50 mg/kg and Group 17-Indomethacin(10mg/Kg). All the drugs were administrated orally.

After one hour of the administration of the drugs, dose 0.1 ml of 1% w/v carrageenan solution in normal saline was injected into the subplantar tissue of the left hind paw of the rat and the right hind paw served as the control. The paw volume of the rats were measured in the digital plethysmograph(Ugo basile, Italy) at the end of 0, 60, 120 and 180 min.The increase in paw edema of the treated group was compared with that of the control and the inhibitory effect of the drugs were studied. The relative potency of the drugs under investigations were calculated based upon the percentage inhibition of the inflammation.

$$\text{Percentage Inhibition} = \frac{\text{Control(increase in paw volume in 3}^{rd}\text{ hour)} - \text{Test(increase in paw volume in 3}^{rd}\text{ hour)}}{\text{Control (increase in paw volume in 3}^{rd}\text{ hour).}} \times 100$$

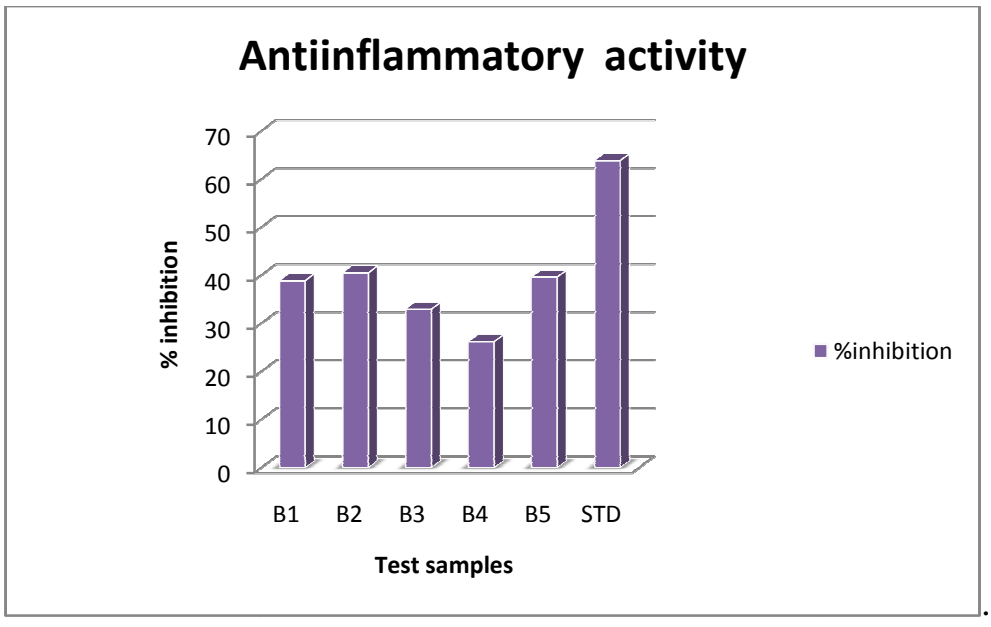


Table 3 Anti-inflammatory activity of the synthesized compounds

Treatment	Dose mg/kg p.o.	Paw volume increase after 3 hours(ml)	Percentage inhibition
control	5 ml/kg	111.61 ± 10.56	-
B1	50	68.27 ± 5.26	38.83
B2	50	66.42 ± 3.58	40.48
B3	50	74.86 ± 8.46	32.92
B4	50	82.38 ± 7.12	26.18
B5	50	67.62 ± 5.72	39.59
Indomethacin	**10 mg/kg**	**0.4 ± 3.62**	**63.80**

P< 0.001 values are expressed as ±SEM.
Number of animals using are 6 in each group.

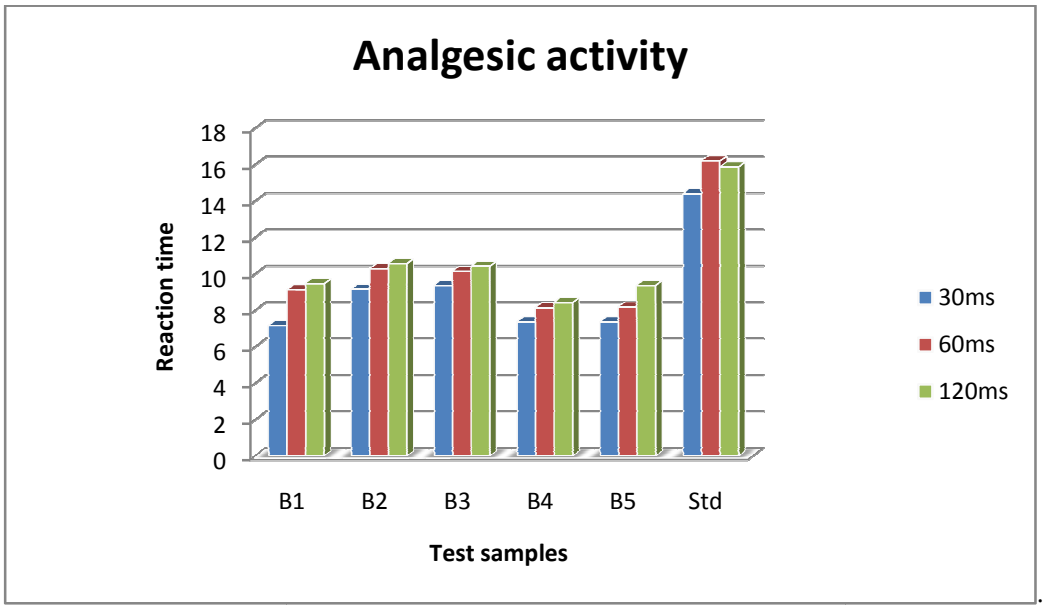
Analgesic activity

The analgesic activity of the given sample was evaluated by using Hotplate method. The albino mice of either sex were used, the animals were divided into nine groups of 5 animals each. Group 1 received normal saline(1ml/kg), group 2 received standard (pentazocine 10 mg/kg) intraperitonealy, groups 3 to 9 received the given extract (50 mg/kg) orally. Before administrating the drug, basal reaction time was studied by placing the animals in hotplate and the parameters such as paw licking, jumping response were noted. The maximum cutoff time is 15 sec. After half an hour of administration of the drug, the reaction time was noted and compared.

Table 4 Analgesic activity of the synthesized compounds

S.No	Groups	Drug	Dose (mg/kg)	Reaction time(in sec)			
				Before administratn of drug	After administration of drug		
					30 mins	60 mins	120 mins
1.	Control	Saline	1ml/kg	4.41±0.16	4.42±0.20	4.48±0.20	4.43±0.17
2.	Test-1	B1	50	4.52±0.24	7.16±0.34	9.12±0.22	9.46±0.16
3.	Test-2	B2	50	4.32±0.24	9.16±0.18	10.28±0.26	10.56±0.32
4.	Test-3	B3	50	4.92±0.24	9.36±0.22	10.14±0.32	10.42±0.18
5.	Test-4	B4	50	4.34±0.24	7.36±0.12	8.14±0.24	8.42±0.26
6.	Test-5	B5	50	4.32±0.26	7.36±0.28	8.18±0.46	9.36±0.12
7.	Standard	Pentazocine	10	5.42±0.16	14.4±0.32	16.2±0.18	15.86±0.28

Mean ±S.E.M, n=5.



DISCUSSION

The thiocyanate**s** (A1-A5) were synthesized in good yield by the reaction of aniline derivatives with ammonium thiocyanate and Br_2/CH_3COOH under ice-cold condition. Compounds A1-A5 on reaction with o-phenylenediamine in the presence of carbon disulphide afforded compounds B1-B5. The purity of all the synthesized compounds were confirmed by their sharp melting points (uncorrected) and column chromatography. The chemical structures were confirmed by IR, ^{1}H-NMR and ^{13}C_NMR techniques. The aromatic (Ar-H) stretching frequencies for all the derivatives were found to be at the range of 2900-3100 cm^{-1}.The presence of NH stretching was confirmed by the peaks at 3100-3550 cm^{-1}. Also ^{1}H-NMR spectra were useful for identifying protons. The peaks at the frequency range 6.0 – 8.0 confirm the aromatic protons and 2.0-3.9 confirms the NH_2 protons. From the microbiological data, it was observed that compounds B3 and B5 showed marginal activity, while compound B1 proved to be the most active among the tested compounds. The anti-inflammatory activity study showed that compound B2 has significant effect over carrageenan induced hind paw edema. On percentage protection basis, compound B2 showed 40.48%, while Indomethacin showed 63.80% when compared to control. Compound B2 proved to possess potential analgesic activity .

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