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Synthesis and Antibacterial Activity of Some Substituted Benzimidazole Analogue

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ABSTRACT

In the present scheme, we have an attempt to synthesize some novel benzimidazole derivatives by substituting triazole moiety at N-1 position of benzimidazole by fusion reaction of benzimidazole-1-acetic acid with thiocarbohydrazide. The substituted triazole was refluxed with different aromatic carboxylic acid in the presence of $POCl_3$ yield different benzimidazole derivatives, respectively. The synthesized compounds were characterized by IR, ^{1}H-NMR and Mass spectroscopy. The compounds were screened for antibacterial (gram +ve, gram –ve bacteria) activities.

Key words: Benzimidazole, thiocarbohydrazide, substituted benzoic acid, benzimidazole- 1-acetic acid

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INTRODUCTION

The incorporation of an imidazole nucleus, a biologically accepted pharmacophore, in the benzimidazole molecule has made it a versatile heterocycle possessing wide spectrum of biological activity. Moreover, benzimidazole derivatives are structural isosteres of naturally occurring nucleotides, which allows them to interact easily with the biopolymers of the living systems. Therefore, numerous biological activities and functions have been described: antihelminthic,[1,2] antifungal,[3,4] antimicrobial[5,6,7], abtihypertensive[8,9] anxiolytic[10,11] anticancer, [12,13] antiviral,[14,15] anticonvulsant,[16] and antitumor,[17] antiprotozoal activity[18].

MATERIAL AND METHOD:

The melting points were taken in open capillary tube and are uncorrected. The IR spectra of the compounds were recorded on ABB Bomen FTIR spectrometer MB 104 with KBr Pellets. ^{1}H-NMR spectra were recorded on 300 MHz–Bruker DPX 200. The chemical shifts are reported as parts per million down fields from tetramethylsilane. Mass spectra were recorded on LC-MS. The purity of the compounds was checked by TLC on precoated SiO_2 gel (HF254 200 mesh) aluminium plates (E Merck).

General procedures:

The synthetic strategy leading to the target compounds are illustrated in figure1 scheme and number of substitution is mentioned in Table 1. Benzimidazole synthesized by an equimolar quantities of *o*-phenylenediamine (0.01 mol), formic acid (0.01 mol) was refluxed for 2 hr. The mixture was cooled and filtered off. The product was recrystallized by water. This compounds was obtained as white crystalline solid; yield 90%; m.p. 172°C

General method of synthesis of Benzimidazole-1-acetic acid:

Chloroacetic acid (0.05 mol) was dissolved in 20 ml of dry chloroform and 4 ml pyridine. To which an equimolar amount of benzimidazole (0.05 mol,) was added and reaction mixture was refluxed for 4 hr., after cooling the viscous residue obtained was washed with DCM/acetone and recrystallized from ethanol; yield 65% ; m.p. 123°C

General method of synthesis of thiocarbohydrazide:

29 ml (25 ml hydrazine hydrate + 4 ml water)[0.5 mol] were placed in 250 ml three necked flask equipped with thermometer, efficient agitator, dropping funnel, and reflux condenser connected to caustic trap. The temperature was lowered to 10°C and 0.1 mol of carbon disulfide (7.6 g, 6.01 ml) are dropped in while maintaining temperature below 15°C. 75 ml of water are added and

temperature raised 85°C and held there for 1.5 hours. The temperature is then lowered to 10°C., the product was filtered and washed with water and recrystallized it by methanol.

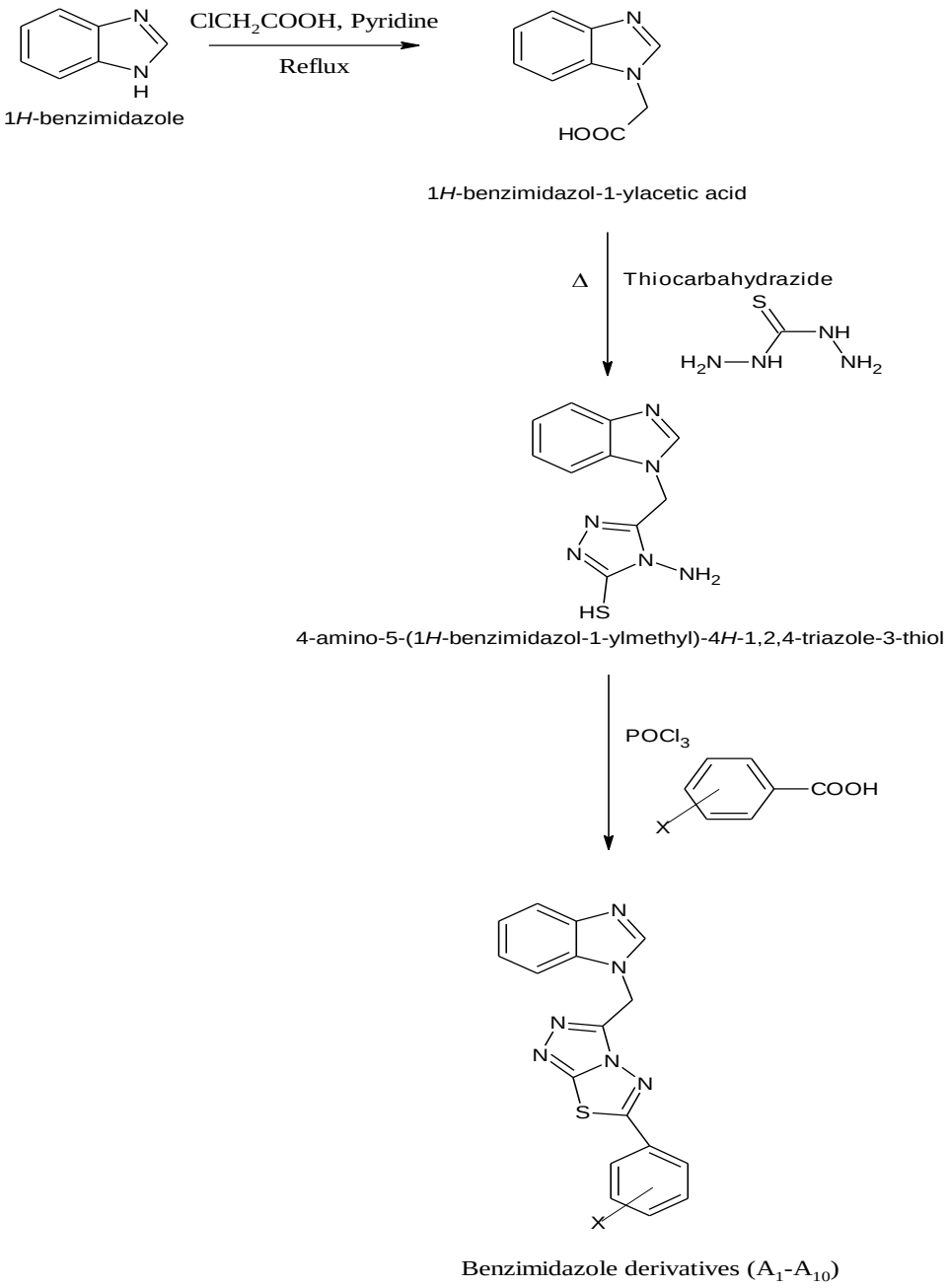


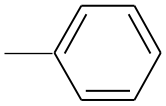
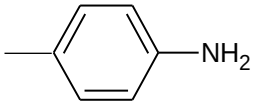
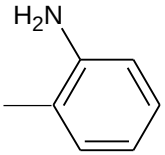
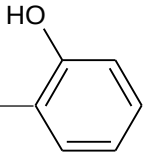
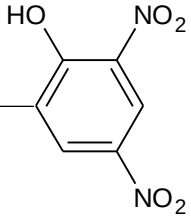
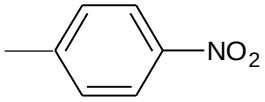
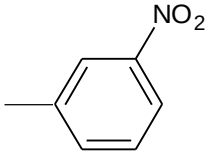
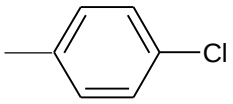
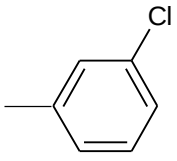
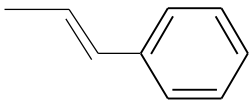
Figure1: Scheme

General method of synthesis of 4-amino-5-(1*H*-benzimidazol-1-ylmethyl)-4*H*-1,2,4-triazole-3-thiol:

An equimolar mixture of an acid and thiocarbohydrazide was heated on an oil bath till the contents melted. The reaction mixture was maintained at this temperature for 3 h. Then it was allowed to cool and treated with dilute sodium bicarbonate solution in order to remove any

unreacted acid left. The solid was filtered, washed with water, dried and recrystallized from ethanol to obtain the pure triazole.

Table 1: Substitution

Compound	Substitutent (-X)
A1	
A2	
A3	
A4	
A5	
A6	
A7	
A8	
A9	
A10	

General method synthesis of derivatives of benzimidazole bearing triazole moiety (A1-A10):

A mixture of triazole (0.01 mol), substituted benzoic acids (0.01 mol) and phosphorus oxychloric acid (10 ml) was heated for 8 hr. Excess of phosphorus oxychloric acid was removed under reduced pressure. The resulting reaction mass was cooled and poured into cold water with vigorous stirring. The solid thus obtained was filtered, washed with dilute sodium bicarbonate solution followed by water, dried and recrystallized from ethanol.(A1-A10) Physical characterization of derivatives (A1-A10) is mentioned in Table 2

RESULT AND DISCUSSION:

1-[(6-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl]-1H-benzimidazole (A1) (Table 2): IR data: -Ar(3066 cm^{-1}), -C=N(1550 cm^{-1}) ^{1}H NMR data (DMSO)(d/ppm):7.119-8.073 (m, 9H,-Ar-H), 1.196 (s, 2H, -CH_2-) Mass spectroscopy data: m/z 332.2 (M^{+1})

4-[3-(1H-benzimidazol-1-ylmethyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]aniline (A2): IR data: -NH_2(3398cm^{-1}), -C=N (1469cm^{-1}), -Ar(2924 cm^{-1})

4-[3-(1*H*-benzimidazol-1-ylmethyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]aniline (A3): IR data: -NH_2 (3427 cm^{-1}), -C=N (1425 cm^{-1}), -Ar (2924.09 cm^{-1}) ^{1}H NMR data (DMSO)(d/ppm): 8.114-8.367 (m, 8H, Ar-H), 4.5 (s, 2H, -NH_2) Mass data: m/z 347.4 (M^+)

2-[3-(1*H*-benzimidazol-1-ylmethyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]phenol (A4): IR data**:**-OH (3356 cm^{-1}), -C=N (1454 cm^{-1}), -Ar (2926.01 cm^{-1})

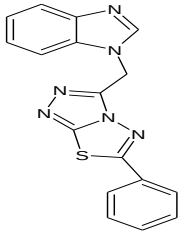
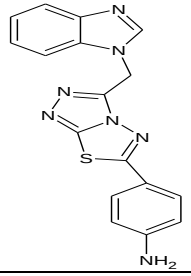
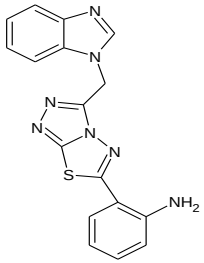
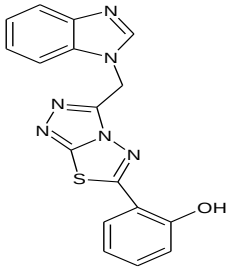
2-[3-(1*H*-benzimidazol-1-ylmethyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]-4,6-dinitrophenol (A5): IR data: -OH (3400 cm^{-1}), -NO_2 (sym 1537 cm^{-1}, Asym 1346 cm^{-1}), -Ar (3086 cm^{-1}), -C=N (1608 cm^{-1}), ^{1}H NMR data (DMSO)(d/ppm): 7.088-7.219 (m, 4H, Ar-H), 7.081 (s, 1H, Ar-H), 7.084 (s, 1H, Ar-H), 8.909 (s, 1H, Ar-OH), Mass data; m/z 438.0 (M^+)

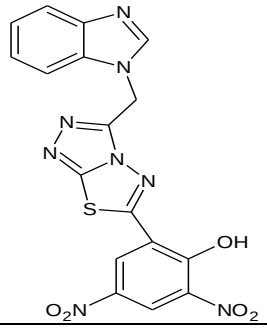
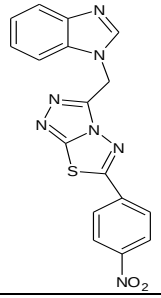
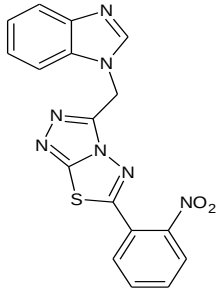
1-{[6-(4-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}-1*H* benzimidazole (A6): IR data: -Ar (2877 cm^{-1}), -C=N (1469 cm^{-1})1591 (symmetric –NO_2), 1091 (Asymmetric –NO_2)(^{1}H NMR data (DMSO)(d/ppm): 7.498-7.528 (m, 4H,-Ar-H), 8.161(s,1H,Ar-H),7.590(m,4H,Ar-H),2.252 (s, 2H, -CH_2-) Mass spectroscopy data: m/z 377.2 (M^+)

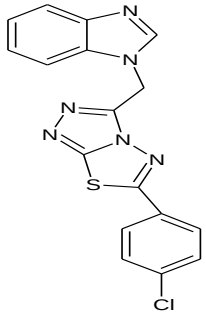
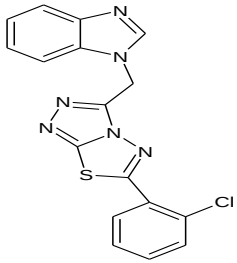
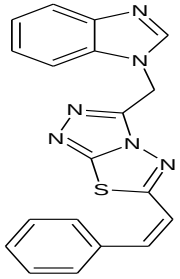
1-{[6-(2-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}-1*H*-benzimidazole (A7): IR data: -Ar(2927 cm^{-1}), -C=N (1481cm^{-1}), 1687 (symmetric –NO_2), 1348 (Asymmetric –NO_2)

1-{[6-(4-chlorophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}-1*H*-benzimidazole (A8): IR data: -Cl (833 cm^{-1}), -C=N (1481 cm^{-1}), -Ar (2924.09 cm^{-1})

Table-2: Physical Characterisation Of Derivatives (A1-A10)

Sr. no	Com	Chemical structure	Chemical name	M.F.	M.W.	% Yield	Colour & Nature	MP	Mobile Phase	R_F Value
1.	A1		1-[(6-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)methyl]-1*H*-benzimidazole	$C_{17}H_{12}N_6S$	332	67%	Brown amorphous	264 °C	Ethyl acetate: *n*-hexane (8: 2)	0.67
2.	A2		4-[3-(1*H*-benzimidazol-1-ylmethyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]aniline	$C_{17}H_{13}N_7S$	347	73%	Black amorphous	254 °C	Ethyl acetate: n-hexane (7:3)	0.76
3.	A3		2-[3-(1*H*-benzimidazol-1-ylmethyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]aniline	$C_{17}H_{13}N_7S$	347	49%	Brown amorphous	263 °C	Ethyl acetate: chloroform (6: 4)	0.81
4.	A4		2-[3-(1*H*-benzimidazol-1-ylmethyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]phenol	$C_{17}H_{12}N_6OS$	348	67%	Black amorphous	276 °C	Acetone: n-hexane (7:3)	0.7

5.	A5		2-[3-(1*H*-benzimidazol-1-ylmethyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl]-4,6-dinitrophenol	$C_{17}H_{10}N_8O_5S$	438	79%	Yellowish Brown amorphous	259 °C	Ethyl acetate: *n*-hexane (8: 2)	0.58
6.	A6		1-{[6-(2-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}-1*H*-benzimidazole	$C_{17}H_{11}N_7O_2S$	377	63%	Blackish brown amorphous	289 °C	Ethyl acetate: ether (6: 4)	0.75
7.	A7		1-{[6-(2-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}-1H-benzimidazole	$C_{17}H_{11}N_7O_2S$	377.3	71%	Blackish brown amorphous	273 °C	Chloroform: *n*-hexane	0.68

8.	A8		1-{[6-(4-chlorophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}-1*H*-benzimidazole	$C_{17}H_{11}ClN_6S$	366.8	55%	Yellowish brown amorphous	282 °C	Acetone: *n*-hexane (7: 3)	0.82
9.	A9		1-{[6-(2-chlorophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}-1*H*-benzimidazole	$C_{17}H_{11}ClN_6S$	366.8	64%	Yellowish brown	255 °C	Ethyl acetate: ether (8: 2)	0.71
10	A10		1-({6-[(Z)-2-phenylvinyl][1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl}methyl)-1*H*-benzimidazole	$C_{19}H_{14}N_6S$	358.4	62%	Brown amorphous	277 °C	Chloroform: *n*-hexane (9: 1)	0.8

1-{[6-(2-chlorophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]methyl}-1*H*-benzimidazole (A9): IR data**:**-Cl (856 cm^{-1}), -C=N (1454 cm^{-1}), -Ar (2926.01 cm^{-1}), ^{1}H NMR data (DMSO)(d/ppm): 7.431-7.510 (m, 4H, Ar-H), 7.537-7.790 (m, 4H, Ar-H)2.506 (s, 2H, -CH_2-), Mass data: m/z 367.2 (M^+)

1-({6- [(Z)-2 -phenylvinyl] [1,2,4] triazolo [3,4-b] [1,3,4] thiadiazol-3-yl} methyl) -1*H*-benzimidazole (A10): IR data: -Ar (3086 cm^{-1}), -C=N (1608 cm^{-1}),1627 (-C=C-)

Antibacterial activity[19, 20]**:**

Antibacterial activity assessment All bacterial strains were obtained from the Nathajirao G. Halgekar Institute of dental science & science & research cntre Belgaum and were as follows: *E. coli, klebsiella, Staphylococcus aureus, Enterococcus faecalis.* All the newly synthesized compounds were dissolved in Dimethylsulphoxide (DMSO) or ethanol to prepare chemicals stock solution of 10 mg/ ml.

Agar-well diffusion method: Simple susceptibility screening test using agar-well diffusion method as adapted earlier was used. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately 106 colony forming unit (cfu) per mL. They were ‘‘flood-inoculated’’ onto the surface of nutrient agar media (Difco, Detroit, MI) and then dried. For *S.aureus* and *E.faecalis* Nutrient agar media was used and For *E.coli* and *klebesiella* Brain Heart Infusion agar was used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 mL of the extract substances were delivered into the wells. The plates were incubated for 18 hours at 35°C. Antibacterial activity was evaluated by measuring the zone of inhibition against the test organism. Ciprofloxacin (10 mg) was standard drugs. Dimethylsulphoxide and ethanol were used as solvent control. The antibacterial activity results are summarized in Table 3.

TABLE 3: SCREENING FOR ANTIBACTERIAL ACTIVITY.

Comp	**Bacteria, concern. (µg) and Inhibition zone (mm)**							
No.	***E.coli***		***Klebesiella***		***S.aureus***		***E.fecalis***	
	75	**50**	**75**	**50**	**75**	**50**	**75**	**50**
A1	10	9	10	8	10	-	12	10
A2	11	8	11	6	12	9	10	8
A3	12	10	12	9	12	10	10	7
A4	10	9	10	8	11	9	12	8
A5	11	9	10	6	6	-	18	15
A6	10	8	11	8	12	10	10	8
A7	11	9	R	-	8	0	10	-
A8	9	8	8	-	10	6	11	8
A9	9	8	10	-	10	8	10	-
A10	10	-	8	-	11	9	10	-

CONCLUSION:

We have shown that reaction of 4-amino-5-(1*H*-benzimidazol-1-ylmethyl)-4*H*-1,2,4-triazole-3-thiol with substituted aromatic acids on heating in $POCl_3$ involve cyclization with formation o f 1,3,4-thiadiazole rings. This reaction may be useful for combinational syntheses of N-1 substituted benzimidazole analogue having various X substitution with a view to test for biological activity. Compound A3 show good activity against both gram positive and gram negative bacteria and compound A1, A2, A4, A5, A7 and A10 show moderate activity against gram positive and gram negative. Compounds A8, A9 show weak activity against gram positive bacteria.

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