Research Article

SYNTHESIS, CHARACTERIZATION AND PHARMACOLOGICAL SCREENING OF SOME NOVEL BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

The reaction of o-phenylenediamine with anthranillic acid yield compound 2-(1H benzo[d]imidazol-2-yl) aniline (1). The compound (1) was condensed with Chloroacetyl chlorides in the presence of dry benzene to get compound 2-chloro-N-[2-(2,3-dihydro-1H-benzimidazol-2-yl)phenyl]acetamide. Further it to then treated with KSCN to getan intermediate compound then reacted with corresponding aromatic aldehyde yield compound 5 benzylidene-3-[2-(2,3-dihydro-1H-benzimidazol-2-yl)phenyl]-2-imino-1,3-thiazolidin-4-one. The compounds were synthesized in good yields and their structures were confirmed by IR, ¹H-NMR-spectral data and elemental analysis. Antimicrobial activity against bacteria and fungi was studied. Investigation of antimicrobial activity of the compounds was done by disc diffusion method using Gram-positive (*S. aureus* and *B. subtilis*), Gram-negative (*E. coli*, *S. typhi*) bacteria. Some derivatives showed remarkable activity comparable to that of standard against Gram-positive and Gram-negative bacteria. The results of preliminary biological tests showed that of these compounds possess good biological activities.

Keywords: benzimidazole, anthranillic acid, o-phenylenediamine, KSCN, biological activity.

INTRODUCTION

Benzimidazole derivatives are very useful intermediates or subunits of the development of pharmaceutical or biological interest¹. Benzimidazole derivatives are an important class of bioactive molecules in the field of drugs and pharmaceuticals². The literature has revealed that the substitutions at the 1, 2 and 5 positions of the benzimidazole moiety are crucial for exhibiting a wide range of pharmacological activities. Specifically, 2-substituted analogs of benzimidazole are known to be potent biologically active compounds. Benzimidazoles are regarded as a promising class of bioactive heterocyclic compounds that exhibit a range of biological activities. Specifically, this nucleus is a constituent of vitamin-B₁₂. Benzimidazole derivatives play important role in medical field with diverse therapeutic areas including antiulcer³⁻⁵, antihypertensive^{6,7}, antiviral⁸, antifungal^{9,10}, anticancer^{10,11}, antitubercular¹², antioxidant^{13,14}, antimicrobial¹⁵ and benzimidazoles also showed anticancer activity against DNA topoisomerase I^{16,17} and colon cancer cell lines³⁰, anti-inflammatory¹⁸, activities etc. The potency of these clinically useful drugs in treatment of microbial infections and other activities encouraged the development of some more potent and significant compounds. Biologically active benzimidazoles have been known for a long time and they can act as bacteriostats or bactericides, as well as fungicides¹⁹⁻²⁴. A compound containing benzimidazole and benzene rings have been used extensively for pharmaceutical purpose since 1960. 1-H-Benzimidazole rings, which exhibit remarkable basic characteristics due to their nitrogen content, comprise the active substances for several drugs. A number of biological activities have been attributed to these compounds²⁵. The NHCs are usually used as ligands for transition metal complexes. Various functional groups, such as, chloro, fluoro, nitro, methoxy, and so on, have an important significance in medicinal chemistry. Especially the

dihalogen-like dichloro, as in antifungal azoles, provides a potent antifungal activity for drugs such as oxiconazole, ketoconazole, terconazole, itraconazole etc. Anti-fungal activity of benzimidazole is due to, binding to the fungal microtubules and stopping hyphal growth. It also binds to the spindle microtubules and blocks nuclear division.

Structural Modification of Benzimidazole

The use of Benzimidazole dates many years back. In 1990 various benzimidazole derivatives were synthesized with substitution of fluorine, propylene, tetrahydroquinoline and cyclized compound which resulted in compounds with increased stability, bioavailability and significant biological activity. It was also showed that substitution on pyridine by electron donating group increases activity. In 1991, benzimidazole derivatives were synthesized by derivatization at N-H of benzimidazole by electron donating group and substitution with long chain of propyl, acetamido, thio, thiazole-amino, tetra methyl piperidine on pyridine resulting in good antiulcer activity. Due to the structural similarity to purine, antibacterial ability of benzimidazoles is explained by their competition with purines resulting in inhibition of the synthesis of bacterial nucleic acids and proteins.

MATERIALS AND METHODS

All the chemicals and reagents were of synthetic grade and commercially procured from S.D. Fine Chem. Ltd. (Mumbai, India). The melting points were determined using open capillary tubes and are uncorrected. Purity of the all synthesized compounds was checked by thin layer chromatography technique (0.2 mm thickness of silica gel G plates) and iodine was used as visualizing agent. The λ_{max} of the compounds was measured by IR spectra were recorded on FT-IR 8400S, Fourier Transform (Shimadzu) Infrared spectrophotometer using KBr disk method. ¹H NMR spectra

were recorded on JEOL (JNM-ECS400, 400 MHz) in dimethyl sulfoxide (DMSO-d₆) using Tetramethylsilane as an internal standard. The mass spectra were recorded on a Micro mass Q-TOF and Shimadzu LC-MS 2010A Mass spectrometer and CHN elemental analysis was performed at Perkin Elmer Auto system XL analysis

General Procedure for the Preparation of 2-(2-Amino Phenyl) benzimidazole

Heated together mixture of *o*-phenylenediamine (6.27 g, 0.03 mole), 20 mL of water, anthranilic acid (12.34 g, 0.09 mole) under reflux for 45 minutes. Made the cooled reaction mixture distinctly basic by the gradual addition of the concentrated ammonia solution, the precipitated product was collected and re crystallized from ethanol gave white crystals, Yield: 86.55 %. m.p 248-250°C, R_f value: 0.250, (Found C: 74.62, H: 5.30, N: 20.08: C₁₃H₁₁N₃), IR(KBr) cm⁻¹: 3384(N-H), 2996, 2918 (C-H), 1702, 1733 (C=O), 1455 (C=C), 1657 (C=N), Mass spectra *m/z*, 209.04(M)⁺.

2-Chloro-N-[2-Phenyl-(1H-Benzimidazole-2-yl)]-Acetamide

A solution of 2-(1H-benzimidazol-2-yl) aniline (4.18 g, 0.02 mole) in dry benzene (60 mL) was cooled to 0-5°C. Chloroacetyl chloride (5 mL, 0.04 mole) dissolved in dry benzene (20 mL) was slowly added to the solution with vigorous stirring. When the addition was complete, the reaction mixture was refluxed for 3 h. Benzene was removed *in vacuo*. The residue was washed with 5 % NaHCO₃, and subsequently with water. The crude product was dried and crystallized from ethanol to give colorless crystals, yield 74 %. m.p 235-236°C, (Found C:63.05, H:4.23, Cl:12.41, N:14.71, O:5.60, C₁₅H₁₂ClN₃O), R_f 0.29, IR(KBr) cm⁻¹: 3434 (NH), 2896 (CH), 1762 (C=O), 1443 (C=C), 1625 (C=N), 685(C-Cl), Mass spectra *m/z*, 286.1 (M)⁺, 288(M+2)⁺

3-[2-(1H-Benzimidazol-2-yl)-Phenyl]-2-Imino-1, 3-Thiazolidin-4-One

A mixture of 2-chloro-N-[2- phenyl-(1H-benzimidazol-2-yl)]-acetamide (8.5 g, 0.03 mole), KSCN (6.0 g, 0.06 mole) and dry acetone (100 mL) was refluxed for 3 h. Excess acetone was removed *in vacuo* and the residue was stirred with water (50 mL). The solid product was filtered, washed with water, and dried. The thiazolidinone was obtained by crystallization from EtOH:Water, yield 70 %, m.p. 173-175°C, (Found C:62.32, H:3.92, N:18.17, O:5.19, S:10.40, C₁₆H₁₂N₄OS), R_f 0.31, IR(KBr) cm⁻¹: 3180 (N-H), 3055.7 (C-HAr), 1719(C=O), 1605(C=N), 1437.7 (C-N), ¹H NMR :7.97 (m, 4H, aromatic), 7.81 (m,4H, aromatic), 7.26 (s,1H,thiazole), 4.20 (m, 2H, -CH₂),12.38 (s,1H, NH).

3-[2-(1H-Benzimidazol-2-yl)Phenyl]-2-Imino-5-Nitrobenzylidene-1, 3-Thiazolidin-4-One

3-[2-(1H-benzimidazol-2-yl)-phenyl]-2-imino-1, 3-thiazolidin-4-one (3.08 g, 0.01 mole) and 3-nitrobenzaldehyde (3.0 g, 0.02 mole) were added to a solution of anhydrous NaOAc (2.0 g, 0.02 mole) in AcOH (30 mL). The mixture was refluxed for 5 h at 120°C and cooled to room temp. The solid product was filtered from the mixture, washed with water, dried and crystallized from EtOH to form yellow crystals, IR(KBr) cm⁻¹: 3400(N-H), 1773(C=O),1611(C=N), 1428(C-N), 1516 (Ar-NO₂),770, 710(C-H), 618(C-S-C), ¹H NMR:7.34 (m, 4H,aromatic),10.66 (s, 1H, NH benzimidazole), 7.93

(m,4H,aromatic), 7.61 (s,1H, thiazole-5), 7.95 (s,1H, =CH) 7.97 (s,4H,aromatic). Mass spectra *m/z*, 442.12(M)⁺

3-[2-(1H-Benzimidazol-2-yl)Phenyl]-2-Imino-5-(Nitrobenzylidene)-1, 3-Thiazolidin-4-One

IR (KBr) cm⁻¹: 3374 (N-H), 1767 (C=O), 1668 (C=N), 1535 (Ar-NO₂), 795 (C-H)655(C-S-C); ¹H NMR: 7.55 (m,4H, aromatic), 7.59 (m, 4H, aromatic), 7.85 (s,1H,thiazole), 11.28 (s,1H,NH benzimidazole), 7.40 (s,1H, =CH), 7.89 (s,4H,aromatic). Mass spectra *m/z*, 443.78 (M+1)⁺

3-[2-(1H-Benzimidazol-2-yl)Phenyl]-2-Imino-5-(Nitrobenzylidene)-1, 3-Thiazolidin-4-One

IR (KBr) cm⁻¹: 3486 (N-H), 1694 (C=O), 1585 (C=N), 1322.0 (Ar-NO₂), 782 (C-H), 671 (C-S-C); ¹H NMR : 6.79-7.32 (m,4H,aromatic), 7.28 (m,4H,aromatic), 7.39 (s,1H,=CH), 10.98 (s,1H,NHbenzimidazole), 7.92 (s,1H,thiazole), Mass spectra *m/z*, 443.21 (M+2)⁺

3-[2-(1H-Benzimidazol-2-yl)-Phenyl]-2-Imino-5-Chlorobenzylidene-1, 3thiazolidin-4-One

IR (KBr) cm⁻¹: 3328 (NH), 1714 (C=O), 1564 (C=N),1455 (C-N),747 (C-Cl), 668 (C-S-C),¹H NMR: 6.76-7.52 (m, 9H,aromatic), 12.31 (s, 1H, NHbenzimidazole),7.90 (s,1H,thiazole).Mass spectra *m/z*,430.41, (M)⁺.

3-[2-(1H-Benzimidazol-2-yl)-Phenyl]-2-Imino-5-(O-Hydroxybenzylidene)-1, 3-Thiazolidin-4-One

IR (KBr) cm⁻¹: 3453 (OH), 3225 (NH), 1658 (C=O), 1268 (C-N), 1590 (C=N), 784 (C-H), 645(C-S-C); ¹H NMR: 6.96-7.37 (m,9H,aromatic), 9.41 (s,1H,OH), 11.29 (s,1H,NHbenzimidazole), 7.94 (s,1H,thiazole). Mass spectra *m/z*, 412.46(M)⁺.

3-[2-(1H-Benzimidazol-2-yl)Phenyl]-2-Imino-5-(Pmethoxybenzylidene)-1, 3-Thiazolidin-4-One

IR (KBr) cm⁻¹: 3330 (N-H), 1738 (C=O), 1573 (C=N), 1400 (C-N), 788 (C-H), 635 (C-S-C); ¹H NMR: 2.36 (s, 3H, OCH₃), 6.84-7.53 (m, 9H, aromatic), 11.88 (s, 1H, NH benzimidazole), 7.92 (s, 1H, thiazole). Mass spectra *m/z*, 396 (M)⁺.

3-[2-(1H-Benzimidazol-2-yl)Phenyl]-5-Benzylidene-2-Imino-1, 3-Thiazolidin-4-One

IR (KBr) cm⁻¹: 3327 (N-H), 1722 (C=O), 1621 (C=N), 1393 (C-N), 894 (C-H), 664(C-S-C)

3-[2-(1H-Benzimidazol-2-yl)-Phenyl]-2-Imino-5-[3-Phenylprop-2-EN-1-Ylidene]-1, 3-Thiazolidin-4-One

IR (KBr) cm⁻¹: 3416 (N-H), 1698 (C=O), 1486 (C=C),1645 (C=N), 1185 (C-N), 857(C-H), 659(C-S-C), ¹H NMR: 7.28-8.18 (m,1H-CH,9H, aromatic), 11.35 (s,1H,NHbenzimidazole), 7.92 (s,1H,thiazole),

3-[2-(1H-Benzimidazol-2-yl)Phenyl]-2-Imino-5-(2phenylethylidene)-1, 3-Thiazolidin-4-One

IR (KBr) cm⁻¹: 3062(N-H), 1700(C=O), 2884(CH₂), 1584(C=N), 1128(C-N), 840(C-H), 661(C-S-C).

Animals

Adult Swiss albino mice (20–25 g) and albino rats weighing (150–200 g) of either sex were used as experimental animals. All the animals were housed in groups of 4–8 per cage at a temperature of 25 ± 1°C and a relative humidity of 45–55 %. A 12 h dark and 12 h light cycle was followed during the

experiments. Animals were allowed free access to food and water *ad libitum*. During the study period, guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animals Ethics Committee (IAEC) were followed for the maintenance of animals.

Acute Toxicity Studies

The acute toxicity studies were carried out in groups of six Swiss albino mice, weighing 20–25 g which were fasted overnight and treated orally with the test compounds. The dosage was varied from 100–1000 mg/kg body weight orally. All the animal experiments were performed with the approval of Institutional Animal Ethics Committee, Assam Down Town University, Panikhati, Guwahati, Assam, India.

Analgesic Activity by Tail-Flick Method in Mice

The analgesic activity was carried out by Tail-flick method using Swiss albino mice. In this method, heat is used as a source of pain. Overnight fasted healthy and adult male Swiss albino mice weighing between 20 g and 25 g, in a group of six each were taken for the investigation. The animals were kept into a small cage with an opening for the tail at the rear wall. The tail was held gently and a light beam exerting radiant heat was directed to the proximal third of the tail. The tip of the tail of the mice was individually placed on the radiant heat source at constant temperature 55°C. The cut-off reaction time was fixed at 15 s to avoid tissue damage. The tail flick response was measured at 0 h, 1 h, 2 h, 3 h and 4 h

after treatment of test compounds by digital analgesimeter (INCO, Ambala, India). The drug pentazocine (3.9 mg/kg, i.p.) was used as standard drug for comparison and test groups received synthesized benzimidazole derivatives at 100 mg/kg p.o.

Anti-inflammatory Activity by Carrageenan-induced Rat Paws Edema Method

The anti-inflammatory activity of the test compounds was evaluated by carrageenan induced rat paw edema model of Winter *et al.* (1962). Rats of either sex were treated with benzimidazole derivatives (100 mg/kg p.o.) and standard drug Diclofenac sodium (100 mg/kg p.o.), one hour prior to the 1 % w/v solution injection of 0.1 mL carrageenan into the plantar region of left hind paw. The marking was just made beyond the tibia-tarsal junction of (knee joint) left hind paw in each animal of all groups. Paw volume was measured by Plethysmometer (Model 520, IITC, Life sciences, USA) at 0 h, 1 h, 2 h, 3 h and 4 h after carrageenan injection. The difference between the paw volume at 4th h and 0 h measurement was calculated and taken as edema volume. Percentage inhibition in the paw was calculated by using the formula,

$$\text{Percentage inhibition} = 100 (1 - V_t/V_c),$$

Where V_t = mean increase in paw volume of test, and
 V_c = mean increase in paw volume with the control²⁶.

Table 1: Physiochemical properties of the compounds (4a-4i)

Comp.	Ar	Mol. Formula	Mol. Wt.	M.P	R _f value	% Yield	Elemental Analysis (%) Calcd./ Found		
							C	H	N
4a		C ₂₃ H ₁₅ N ₅ O ₃ S	441.46	210-213	0.355	73.4	62.58 62.46	3.42 3.38	15.86 15.76
4b		C ₂₃ H ₁₅ N ₅ O ₃ S	441.46	223-225	0.344	78	62.58 62.48	3.42 3.33	15.86 15.72
4c		C ₂₃ H ₁₅ N ₅ O ₃ S	441.46	238 -241	0.387	80	62.58 62.50	3.42 3.39	15.86 15.81
4d		C ₂₃ H ₁₅ ClN ₄ OS	430.90	255-257	0.252	78	64.11 64.01	3.51 3.48	13.00 12.97
4e		C ₂₃ H ₁₆ N ₄ O ₂ S	412.46	110 -112	0.390	67	66.97 66.91	3.91 3.85	13.58 13.49
4f		C ₂₄ H ₁₈ N ₄ O ₂ S	426.49	167-170	0.403	88	67.59 67.51	4.25 4.18	13.14 13.10
4g		C ₂₃ H ₁₆ N ₄ O ₂ S	396.46	195 -198	0.285	59	69.68 69.58	4.07 4.01	14.13 14.07
4h		C ₂₅ H ₁₈ N ₄ OS	422.50	149-151	0.261	77	71.07 71.01	4.29 4.20	13.26 13.19
4i		C ₂₄ H ₁₈ N ₄ OS	410.49	188-189	0.272	91	70.22 70.11	4.42 4.35	13.65 13.58

Table 2: Analgesic activity of benzimidazole derivatives on mice by using tail-flick method

Comp. code	Tail withdrawing time in second (Mean $\pm$ SEM)				
	0 h	1 h	2 h	3 h	4h
Control	1.56 $\pm$ 0.16	2.16 $\pm$ 0.16	2.33 $\pm$ 0.21	2.66 $\pm$ 0.21	2.82 $\pm$ 0.72
Std	2.16 $\pm$ 0.16	8.5 $\pm$ 0.34	11.33 $\pm$ 0.21**	10.16 $\pm$ 0.30*	10.83 $\pm$ 0.30**
4a	2.0 $\pm$ 0.25	4.66 $\pm$ 0.21	6.53 $\pm$ 0.33	8.73 $\pm$ 0.21**	9.66 $\pm$ 0.33**
4b	2.16 $\pm$ 0.16	5.33 $\pm$ 0.21	7.5 $\pm$ 0.22	9.10 $\pm$ 0.22*	9.16 $\pm$ 0.30**
4c	2.0 $\pm$ 0.25	8.66 $\pm$ 0.10	8.44 $\pm$ 0.21*	9.33 $\pm$ 0.21*	9.46 $\pm$ 0.33**
4d	2.0 $\pm$ 0.25	5.0 $\pm$ 0.25	7.16 $\pm$ 0.33*	10.5 $\pm$ 0.22**	10.83 $\pm$ 0.33**
4e	1.61 $\pm$ 0.30	4.5 $\pm$ 0.22	3.23 $\pm$ 0.09*	4.63 $\pm$ 0.21	4.36 $\pm$ 0.33
4f	2.16 $\pm$ 0.30	3.33 $\pm$ 0.21	5.33 $\pm$ 0.42**	4.33 $\pm$ 0.33	4.16 $\pm$ 0.30
4g	2.16 $\pm$ 0.16	3.16 $\pm$ 0.30	5.0 $\pm$ 0.36	4.0 $\pm$ 0.30	5.50 $\pm$ 0.42**
4h	1.0 $\pm$ 0.25	3.53 $\pm$ 0.21	4.83 $\pm$ 0.30*	3.73 $\pm$ 0.21	6.50 $\pm$ 0.30**
4i	1.16 $\pm$ 0.16	2.83 $\pm$ 0.30	5.16 $\pm$ 0.47**	4.83 $\pm$ 0.21**	5.9 $\pm$ 0.30**

n = 6 animals in each group.
All synthesized compounds tested at a dose of 100 mg/kg p.o. body weight, Std-pentazocine (3.9 mg/kg i.p.), Control-vehicle (0.5 % CMC), *p < 0.05 vs control, **p < 0.01 vs control.

Table 3: Anti-inflammatory activity of synthesized compounds on carrageenan-induced acute paws edema in rats

Comp. code	Tail withdrawing time in second (Mean $\pm$ SEM) % Inhibition				
	0 h	1 h	2 h	3 h	4h
Control	0.15 $\pm$ 0.01	0.22 $\pm$ 0.01	0.23 $\pm$ 0.02--	0.25 $\pm$ 0.01	0.25 $\pm$ 0.01
Std	0.15 $\pm$ 0.01	0.12 $\pm$ 0.01	0.10 $\pm$ 0.01	0.08 $\pm$ 0.01	0.08 $\pm$ 0.01
4a	0.13 $\pm$ 0.01	0.11 $\pm$ 0.01	0.10 $\pm$ 0.01	0.11 $\pm$ 0.01	0.12 $\pm$ 0.01
4b	0.11 $\pm$ 0.01	0.07 $\pm$ 0.01	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	0.10 $\pm$ 0.01
4c	0.14 $\pm$ 0.01	0.13 $\pm$ 0.01	0.11 $\pm$ 0.01	0.10 $\pm$ 0.01	0.11 $\pm$ 0.01
4d	0.10 $\pm$ 0.01	0.10 $\pm$ 0.01	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01	0.09 $\pm$ 0.01
4e	0.17 $\pm$ 0.01	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01	0.15 $\pm$ 0.01	0.15 $\pm$ 0.01
4f	0.12 $\pm$ 0.01	0.12 $\pm$ 0.01	0.13 $\pm$ 0.01	0.13 $\pm$ 0.01	0.10 $\pm$ 0.01
4g	0.18 $\pm$ 0.01	0.16 $\pm$ 0.01	0.15 $\pm$ 0.01	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01
4h	0.16 $\pm$ 0.01	0.20 $\pm$ 0.01	0.21 $\pm$ 0.01	0.18 $\pm$ 0.01	0.18 $\pm$ 0.01
4i	0.18 $\pm$ 0.01	0.16 $\pm$ 0.01	0.19 $\pm$ 0.01	0.16 $\pm$ 0.01	0.19 $\pm$ 0.01

n = 6 animals in each group.
All synthesized compounds tested at a dose of 100 mg/kg p.o. body weight, Std-pentazocine (3.9 mg/kg i.p.), Control-vehicle (0.5 % CMC), *p < 0.05 vs control, **p < 0.01 vs control.

Table 4(A): Antibacterial activity of compounds

Compd. code	*Zone of inhibition in (mm)					
	Bacteria				Fungi	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>B. subtilis</i>	<i>A. niger</i>	<i>C. albicans</i>
1	-----	15	-----	18	15	19
2	-----	17	12	11	20	13
3	15	18	15	10	17	18
4a	38	24	13	37	28	30
4b	37	13	35	30	30	32
4c	25	28	37	33	38	30
4d	38	12	27	31	32	28
4e	31	23	19	14	14	12
4f	39	33	36	28	38	37
4g	20	-----	11	16	18	19
4h	12	-----	20	10	14	17
4i	14	-----	15	09	11	12
Amoxycilline	31	36	39	38	-----	-----
Ketoconazole	-----	-----	-----	-----	15	18
DMSO	-----	-----	-----	-----	-----	-----

*Average three reading

Table 4(B): Antibacterial activity of compounds

Compd. code	*MIC values in μ g/ml					
	Bacteria				Fungi	
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>B. subtilis</i>	<i>A. niger</i>	<i>C. albicans</i>
1	25	25	25	25	25	50
2	25	25	25	25	25	25
3	25	25	25	25	25	25
4a	50	50	25	25	50	50
4b	25	50	25	50	50	50
4c	25	25	50	25	50	50
4d	50	50	50	25	50	50
4e	50	25	25	25	25	25
4f	25	50	50	50	25	25
4g	25	25	25	25	25	25
4h	25	25	25	25	25	25
4i	25	25	25	25	25	25
Amoxycilline	25	50	50	50	-----	-----
Ketoconazole	-----	-----	-----	-----	50	50
DMSO	-----	-----	-----	-----	-----	-----

Scheme 1: The synthetic route of the target compounds

Figure 1: Structure of Bezimidazole (IUPAC Name: 1H-benzimidazole)

The suggested mechanism for the formation of these compounds can be as follow in

Antimicrobial Activity

Zone of Inhibition

The synthesized compounds were screened for their antibacterial activity against two gram positive organisms such as *Staphylococcus aureus*, *Bacillus subtilis*, and two gram negative organisms such as *Escherichia coli* and *Salmonella typhi* by cup plate method²⁷.

Minimum Inhibitory Concentration

Eight MIC tubes were taken and labeled it as number 1-8. 1 ml of broth was transferred to each tube, 1 ml (100 µg) of synthesized compound were added to first tube and then 1 ml from first tube was transferred to second, so similarly 1 ml from each tube was transferred to second tube up to 7th tube and 8th tube kept as controlled i.e. without drug. 0.1 ml of culture (bacterial and fungal) was added to all 8 tubes. All the tubes were incubated at 37°C for 24 h. After incubation turbidity was observed. The tube which shows turbidity and the tube which is before that tube and whose conc. are called as minimum inhibitory concentration^{28,29}.

RESULTS AND DISCUSSION

Benzimidazole derivatives were synthesized and the structures of the compounds were established by means of IR, ¹H-NMR and elemental analysis. All the compounds were evaluated for their *in-vivo* analgesic activity (Table 2), anti-inflammatory activity (Table 3), anti microbial activity (Table 3).

Acute Toxicity Studies

Acute toxicity and gross behavior studies revealed that the tested compounds in the present investigation were found to be nontoxic up to 1000 mg/kg p.o.

Analgesic Activity

Table 2, revealed that almost all the compounds showed very potent analgesic activity when compared with standard pentazocine. Among the tested compounds 4a, 4b, 4c and 4d showed profound analgesic activity. The rest of the compounds 4g, 4h and 4i showed moderate activity when compared with the control.

Screening of Anti-inflammatory Activity

From the Table 3, it was found that most of the tested compounds showed significant results in comparison with standard diclofenac sodium. Amongst all the compounds 4b, 4f, 4c and 4d showed potent anti-inflammatory activity and the rest of the compounds showed moderate activity.

Antimicrobial Evaluation

The *in vitro* antimicrobial activity was performed using the Cup plate method with different strains of bacteria and fungi. Amoxicillin and Ketoconazole were used as positive control for bacteria and fungi. The results of the final compounds for preliminary antibacterial testing are shown in Table 4. The results revealed that the majority of the synthesized compounds showed varying degree of inhibition against the tested microorganisms as evident from their minimal inhibitory concentration (MIC). The chloro group at 2, methoxy group at 4 position and nitro at 2, 3 and 4 position of phenyl ring in the molecule of benzimidazole has the best overall antibacterial profile as the 2-hydroxy and without substituents on phenyl ring at benzimidazole displayed moderate activity. The compounds showed activity which is comparable with control against bacterial strains in

increasing order of 4-NO₂ > 4-OCH₃ > 4-Cl > 2- OH. This shows that para position with lipophilic group may be important to exhibit significant activity as antimicrobial agents. It is quite evident from the above sequence that the compound 4c which contain nitro group (strong electron withdrawing group) at para position and a 4-chloro substituent is highly active. The activity decreasing as the electron withdrawing ability of the substituent decreased. The derivatives 4c and 4f exhibited good activity in comparison to the standard.

CONCLUSION

All derivatives of benzimidazole have been successfully synthesized and screened for analgesic activity by tail-flick method in mice, anti-inflammatory activity by carrageenan-induced rat paws edema method, antimicrobial activity by cup plate and serial dilution method. The pharmacological activities exhibited by synthesized novel benzimidazole derivatives have confirmed that these compounds may serve the purpose of being accepted as the novel therapeutic agents. Furthermore, an extensive toxicological study of these derivatives are highly recommended to assess the safety and pharmacological efficacy of the compounds studied.

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