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Synthesis, antimicrobial and anthelmintic activity of some novel benzimidazole derivatives

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ABSTRACT

New benzimidazole derivatives containing 4-chloropyridine-2-carbonyl and N-methyl picolinamide moieties in one side chain at 1H position of benzimidazoles (2-((benzylthio)methyl)-1H-benzo[d]imidazol-1-yl)(4-chloropyridin-2-yl)methanones(3) have been synthesized as depicted in scheme-1. The intermediates and final compounds were purified and their chemical structures have been confirmed by IR, ^{1}H NMR, and Mass spectral data. All the derivatives were examined for their anthelmintic activity against Indian adult earthworms (pheretima posthuma) at various concentrations (0.2% and 0.5%), antibacterial activity against B.subtilis, B.cereus, S.epidermidis, S.typhi, P.aeruginosa and K.pneumoniae and antifungal activity against A.flavus, F.oxysporium and P.notatum at a concentration of 2mg/ml. Most of the compounds tested have shown promising activities when compared with the standard drugs.

Keywords: Benzimidazoles, Anthelmintic activity, Albendazole, Antimicrobial activity, Amikacin.

INTRODUCTION

Anthelmintics or antihelminthics are drugs that expel helminth parasitic worms (helminths) from the body, either by stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing). However they have shown the development of resistance to some broad spectrum anthelmintics (benzimidazoles, levamisole, avermectins) and also some narrow spectrum dewormers such as the salicylanilides (closantel). Some type of dangerous helminthes infections like filariasis has only a few therapeutic modalities at present [1]. The continuous and long-term reliance on a small range of compounds has led to the development of drug resistance in many helminthic strains. In addition, after treatment with albendazole or mebendazole, several

side effects have been reported in hosts such as gastrointestinal symptoms (epigastric pain, diarrhea, nausea, vomiting), nervous system symptoms (headache, dizziness), and allergic phenomena (edema, rashes, urticaria). Some anthelmintic drugs, such as praziquantel and albendazole are contraindicated for certain groups of patients like pregnant and lactating woman. These drugs have also to be used with caution in hepatitis patients and in children below 2 years of age [2]. To overcome the development of drug resistance it is crucial to synthesize a new class of compounds possessing different chemical properties from those of used commonly.

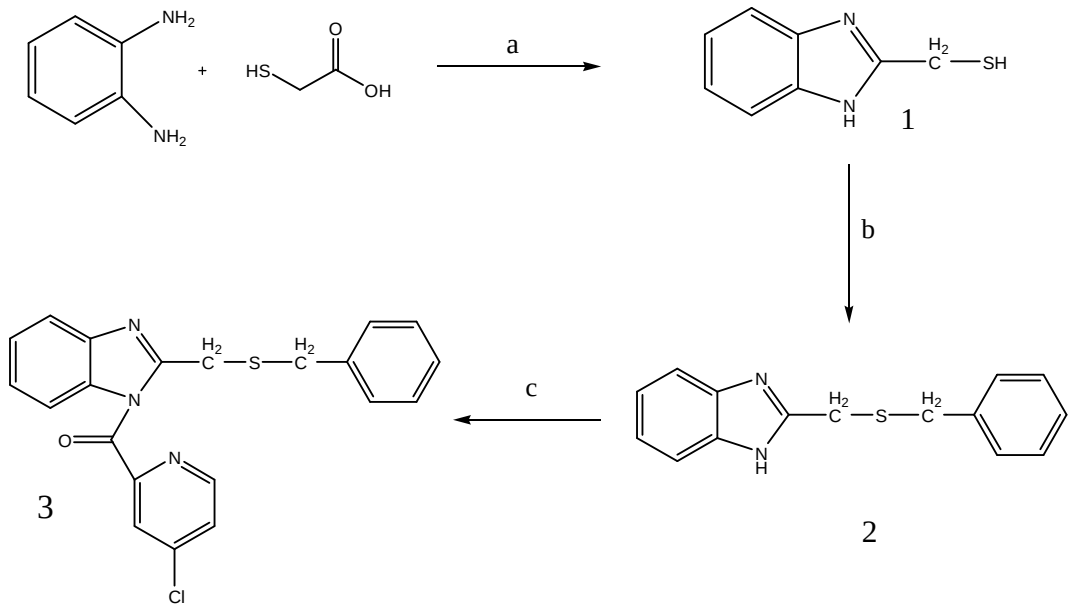
A survey of literature reveals that some of the picolinic acid derivatives possess various biological activities like antimicrobial, antibacterial, anthelmintic and antitubercular activities. In the present study, an attempt was made towards the incorporation of picolinic acid derivative moiety, to probe how this moiety will influence the anthelmintic activity along with benzimidazole derivatives.

In view of these valid observations and as a continuation of our work, prompted us to synthesize new benzimidazole derivatives and the synthesized compounds were screened for their antibacterial, antifungal and anthelmintic activity.

MATERIALS AND METHODS

The chemicals and solvents used for the experimental work were commercially procured from E. Merck, India, S.D. Fine Chem, India and Qualigens, India. Silica gel G used for analytical chromatography (TLC) was obtained from S.D. Fine Chem, India.

SCHEME-1



Reagents : (a) 4N HCl; (b) anhydrous K_2CO_3, benzyl chloride, dry acetone;
(c) t-BuOK, DMF, anhydrous K_2CO_3, 4- chloropyridine-2-carbonyl chloride.

Melting points were determined in an open glass capillary using a Kjeldahl flask containing liquid paraffin and are uncorrected. The proton magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker 300 MHz instrument (Bruker, Germany) in DMSO/$CDCl_3$ using TMS as internal standard. Chemical shifts (δ) are expressed in ppm. The infrared spectra of compounds were recorded in KBr on a FTIR- -8400S, Fourier Transform (Shimadzu), Japan infrared spectrophotometer. Mass spectra were recorded on LC-MS/MS (API-4000 TM), Applied BioSystems, MDS SCIEX (Canada).

Experimental

Synthesis of (1H-benzo[d]imidazol-2-yl)methanethiol (1):

A mixture of 4gm orthophenylene diamine, 36 ml of 4 N HCL and 3.4 ml of thioglycollic acid was taken in a round bottom flask and the solution was boiled under reflux for 3 hours until reaction completes which is checked by T.L.C analysis [3]. Further the solution was cooled on ice and made alkaline by the addition of 30% NH_3 solution. The precipitate formed was filtered, dried and recrystallized from suitable solvents. M.P; 156-158^{o}C Yield; 72 %.

Synthesis of 2-((benzylthio)methyl)-1H-benzo[d]imidazole (2):

(1H-benzo[d]imidazol-2-yl)methanethiol (0.005 mol) was added to suspension of benzyl chloride (0.005 mol) and anhydrous potassium carbonate (0.005 mol) in dry acetone (15 ml). The reaction mixture was stirred for 6 – 8 hrs at ambient temperature and acetone was then evaporated. Distilled water was added to the residue and the formed precipitate was filtered, washed with water, dried and recrystallized from appropriate solvent. The purity of the compound was checked by TLC and spectral data. M.P; 170-172^{o}C Yield; 64 %. IR (KBr)(cm^{-1}): 3070(N-H str.), 3165 (Ar-H str.),640 (C-S str.), 1640-1530 (C=C & C=N str.). H^1 NMR (DMSO-*d6*): δ 4.8, (s, 1H, NH), 8.0-7.1 (m, 9H, Ar-H), 3.6 (s, 4H, -CH_2-S-CH_2-). EI-MS: *m/z* = 255.2(M^+).

Synthesis of (2-((benzylthio)methyl)-1H-benzo[d]imidazol-1-yl)(4-chloropyridin-2-yl)methanone (3):

A solution of **2** (0.005 mol) in dry N,N-dimethylformamide was treated with potassium *tert*-butoxide and the reddish brown mixture was stirred at room temperature for 2 hr. The contents were treated with 4-chloropyridine-2-carbonyl chloride (0.005 mol) and potassium carbonate and then heated to 80^{o}C for 6 hr. The mixture was cooled to room temperature and poured into ethyl acetate. The combined organics were washed with brine, dried over sodium sulphate and concentrated to give *(2-((benzylthio)methyl)-1H-benzo[d]imidazol-1-yl)(4-chloropyridin-2-yl)methanone*. M.P; 224-226^{o}C Yield; 60 %. IR (KBr) (cm^{-1}): 3165 (Ar-H str.), 1620-1540 (C=C & C=N str.), 650 (C-S str.), 1240 (C-N), 1650 (C=O), 730 (C-Cl). H^{1}NMR (DMSO-*d6*): δ 9.2 -7.1 (m, 12H, Ar-H), 3.4 (s, 4H, -CH_2-S-CH_2-). EI-MS: *m/z* = 394.1(M+1).

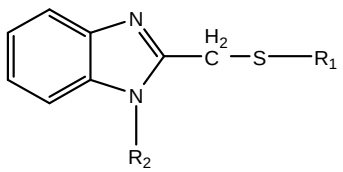
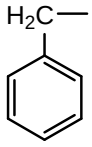
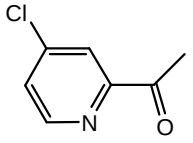
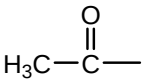
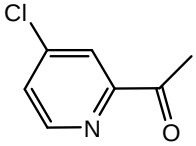
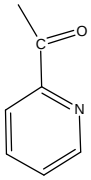
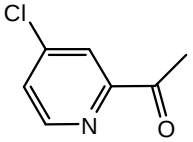
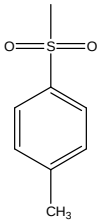
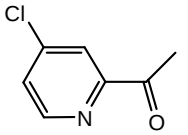
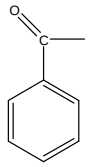
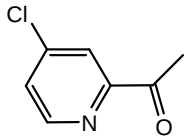
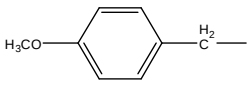
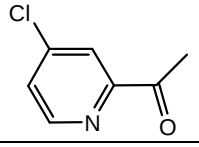
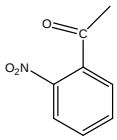
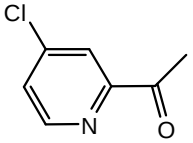
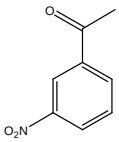
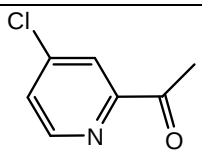
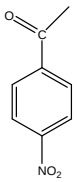
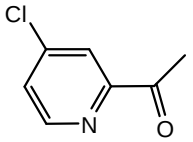
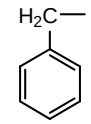
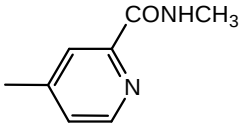
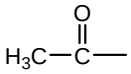
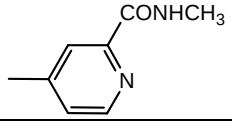
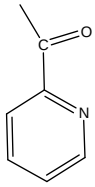
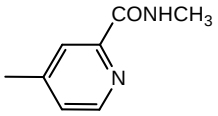
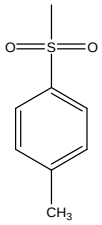
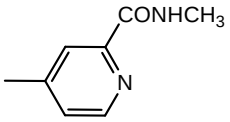
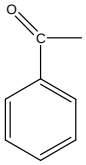
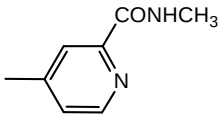
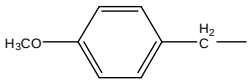
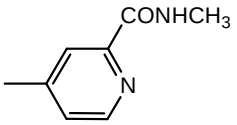
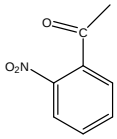
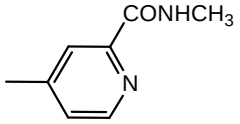
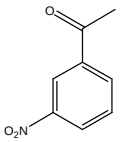
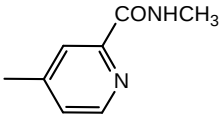
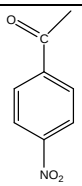
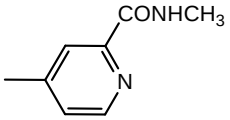


Table 1: Physical data of synthesized compounds (3-20)

Comp.	R_1	R_2	Mol. Formula	Mol. Wt.	m.p. (^oC)	Yield%
3			$C_{21}H_{16}ClN_3OS$	393.89	226	60
4			$C_{16}H_{12}ClN_3O_2S$	345.8	260	75
5			$C_{20}H_{13}ClN_4O_2S$	408.86	225	72
6			$C_{21}H_{16}ClN_3O_3S_2$	457.95	250	68
7			$C_{21}H_{14}ClN_3O_2S$	407.87	230	72
8			$C_{22}H_{18}ClN_3O_2S$	423.92	215	62
9			$C_{21}H_{13}ClN_4O_4S$	452.87	247	72
10			$C_{21}H_{13}ClN_4O_4S$	452.87	255	75
11			$C_{21}H_{13}ClN_4O_4S$	452.87	263	70

12			$C_{22}H_{20}N_4OS$	388.49	232	61
13			$C_{17}H_{16}N_4O_2S$	340.4	208	62
14			$C_{21}H_{17}N_5O_2S$	403.46	215	76
15			$C_{22}H_{20}N_4O_3S_2$	452.55	287	68
16			$C_{22}H_{18}N_4O_2S$	402.47	240	74
17			$C_{23}H_{22}N_4O_2S$	418.51	235	62
18			$C_{22}H_{17}N_5O_4S$	447.47	265	68
19			$C_{22}H_{17}N_5O_4S$	447.47	260	72
20			$C_{22}H_{17}N_5O_4S$	447.47	255	65

Biological Evaluation

Antibacterial Activity

Antibacterial activity of the synthesized compounds was determined, using a slightly modified cup plate method [4,5,6]. Muller Hinton agar was used for the growth of bacterial strains (*B.subtilis* (MTCC 121), *B.cereus* (ATCC 14579), *S.epidermidis* (ATCC 25923), *S.typhi* (MTCC

733), *P.aeruginosa* (MTCC 741) and *K.pneumoniae* (ATCC 29212). Each organism was suspended in normal saline solution and transmittance (T) of 75 to 77% at 530 nm was made, which is equal to 10^6 CFU/ml. All the test compounds were dissolved in DMSO at a concentration of 2 mg/ml. Each plate was inoculated with 20 µl of microbial suspension. 100 µl of the test compounds was added to each cup. The plates containing bacteria were incubated at 37^o C for 24 hrs, the positive antimicrobial activity were read based on the growth inhibition zone and compared with the solvent as a negative control and Amikacin as comparative drug, shown in Table-2.

Table 2: Antibacterial activity of synthesized benzimidazole derivatives. (3-20)

Comp.	Zone of inhibition (mm)					
	B.subtilis	***B.cereus***	***S.epidermidis***	***S.typhi***	***P.aeruginosa***	***K.pneumoniae***
3	6.8	7	11.4	8.2	10.5	7
4	7	10.5	9	7.2	6.4	6.8
5	11.5	10.6	11	9.7	9.2	7
6	11	6.9	11	12.5	12	8
7	9	7.2	12.4	9.6	9.2	8
8	8.1	11.5	9	7.8	8.2	8
9	7.8	7	10	7.2	9.4	7.9
10	7.6	9	9.8	7.5	10	8
11	8	10	11	7.8	10.2	8.2
12	12	12.2	12	12	12.6	11
13	9	15	11.2	13.5	13.5	10
14	10.2	13.3	13.8	10	15.8	10
15	9.6	14	13.2	14.6	12.7	9.5
16	7	15.6	11	15.5	15.2	9
17	8.5	14	12.6	11	11.2	8.7
18	9.3	13.8	16.2	16.8	16.6	10
19	12	12.2	17.2	17.5	18	11
20	12.8	14.2	17.4	18.4	18.2	12.2
Negative Ctrl.	--	--	--	--	--	--
Standard (Amikacin)	21	20	21	23	20	19.5

-- No activity, Negative Control - DMSO

Anthelmintic Activity

Indian adult earthworms (*pheretima posthuma*) were used to study anthelmintic activity. The earthworms (collected from the water logged areas of soils, Jangaon, Warangal, Andhra Pradesh) were washed with normal saline to remove all fecal materials. The earthworms in 4-5 cm. in length and 0.1 - 0.2 cm in width were used for all experimental protocol. The earthworm resembles both anatomically and physiologically to the intestinal roundworm parasites of human beings, hence can be used to study anthelmintic activity [7].

The newly synthesized compounds were tested for anthelmintic activity. *Pheretima posthuma* of nearly equal size were selected randomly for present study. The worms were acclimatized to the laboratory condition before experimentation. The earthworms were divided into four groups of six earthworms in each. Albendazole diluted with normal saline solution to obtain 0.2% w/v and 0.5% w/v served as standard and poured into petridishes. The synthesized compounds were prepared in minimal quantity of DMSO and diluted to prepare two concentrations i.e. 0.2% w/v, 0.5% w/v for each compound. Normal saline served as negative control as shown in Table 3. Six

earthworms nearly equal size are taken for each concentration and placed in petridishes at room temperature. The time taken for complete paralysis and death are recorded. The mean paralysis time and mean lethal time for each sample was calculated. The time taken for worms to become motionless was noted as paralysis time and to ascertain death, each worm was frequently applied with external stimuli which stimulates and induce movement in the earthworms, if alive [8] .

Table 3 Anthelmintic activity of synthesized Compounds

Comp.	Time for paralysis (min)		Time for death (min)	
	% of Concentration		% of Concentration	
	0.2%	**0.5%**	**0.2%**	**0.5%**
3	15:10	9:20	25:35	16:10
4	11:28	8:38	22:40	12:08
5	2:09	1:48	8:20	4:55
6	3:08	2:05	12:20	9:30
7	11:46	8:20	22:50	13:08
8	38:05	22:58	56:20	31:04
9	5:10	4:45	15:45	9:46
10	6.05	4:18	12:35	8:35
11	1:12	0:48	6:05	3:50
12	16	13:09	21:10	15:05
13	5	3:30	16:45	12:40
14	6:10	3:16	15:25	10:25
15	8:45	5:20	17:26	11:18
16	5:22	3:16	11:18	8:19
17	17:40	12:40	21:08	18:30
18	3:35	2:48	10:55	6:50
19	2:40	2:10	7:18	5:16
20	1:45	1:20	5:20	3:58
Negative Control	--	--	--	--
Standard (Albendazole)	0:30	0:22	0:36	0:34

-- No Activity, Negative Control- Normal Saline

Antifungal activity

The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121^{o}C/15lbs for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160^{o}C for 1 ½ hours. 30 ml of sterile SDA was seeded by organisms (about 2 ml according to Mc Farland's standard), in semi hot conditions (40^{o}C) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Bores were made on the medium using sterile borer and 0.1 ml of the solution of synthesized compounds at 2mg/ml concentration in DMF were added to respective bores and 0.1ml of the standard Ampotericin B at a concentration of 200 µg/0.1ml was used as standard as shown in Table 4. The Petri plates seeded with fungal organisms, containing solution of synthesized compounds and the standard drug were kept in a refrigerator at 4^{o}C for 1 hour to facilitate the diffusion of the compounds and the standard in to the media. After diffusion the Petri plates were incubated at 28^{o}C for one week and later the zone of inhibition was observed and measured using a scale [9].

Table 4: Antifungal activity of synthesized benzimidazole derivatives. (3-20)

Comp.	Zone of inhibition in(mm) (200µg/100µl)		
	A.flavus	***F.oxysporium***	***P.notatum***
3	--	--	--
4	4	6	6
5	12	14	10
6	11	6	6
7	--	--	--
8	--	11	11
9	6	6	13
10	7	6.9	2
11	6	8	7
12	10	6	14
13	9.4	13	4
14	--	--	--
15	8	--	12
16	2	12	4
17	--	--	--
18	12	11	9
19	10	9	--
20	14	12	10.5
Negative Control(DMF)	--	--	--
Standard (Amphotericin B)	19	16	19

-- No activity; the antifungal activity was assayed by cup plate method; 100 µl/cup (disc diameter is 6mm) Amphotericin B (100µl/cup) was used as +ve ctrl.

RESULTS AND DISCUSSION

From the antibacterial screening it was observed that all the compounds exhibited activity against all the organisms employed as indicated in Table 2. The compounds 18, 19 and 20 have showed highest activity against both the Gram-positive and Gram-negative bacteria. The compound 20 showed maximum zone of inhibition (18.4mm) against *S.typhi.* Compounds 19 and 20 showed high antibacterial activity, compounds 12, 15 and 16 showed good activity and compounds 5, 6 and 7 showed moderate activity against all the organisms. Compounds 19 and 20 showed good activity against *S.typhi, S.epidermidis, P.aeruginosa* and *K.pneumoniae,* compound 15 and 16 showed good activity against *B.cereus.* Compounds 3, and 9 showed less activity among all the synthesized compounds. But all the derivatives have shown less antibacterial activity when compared to the standard drug Amikacin.

The result of anthelmintic activity exhibited by compounds on *Pheretima posthuma* is shown in Table 3. A closer inspection of data from this table indicates that compounds 11 and 20 showed very high activity than all the other synthesized compounds. The compounds 5, 6, 18 and 19 showed good activity and compounds 9, 10, 13, 14 and 16 showed moderate activity while 8 showed very less activity at both the concentrations. But all the compounds have shown less anthelmintic activity when compared to the standard drug Albendazole.

The antifungal activity of the compounds studied against *A.flavus, F.oxysporium and P.notatum* is shown in Table 4**.** Amphotericin B was used as reference for inhibitory activity against fungi. It was observed that the compound 20 showed maximum zone of inhibition (14mm) against

A.flavus compound 5 and 13 showed maximum zone of inhibition (14mm and 13mm) against *F.oxysporium* and compound 9 and 12 showed maximum zone of inhibition (13mm and 14mm) against *P.notatum.*

CONCLUSION

The proposed benzimidazole derivatives were synthesized successfully. All the compounds were evaluated for antibacterial, antifungal and anthelmintic activity. All the synthesized compounds were found to have good activity, among all the active compounds of benzimidazole derivatives, 19 and 20 showed good antibacterial activity against all the organisms employed for the antibacterial activity, compound 20 showed good antifungal activity against all the organisms employed for the antifungal activity and compound 11 and 20 showed very high anthelmintic activity than all the other synthesized compounds at both the concentrations. Amongst the various compounds synthesized, the compounds (12-20) containing N-methylpicolinamide moiety possess greater activity compared to similar analogs containing 4-chloropyridine-2-carbonyl moiety (3-11).

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