Synthesis, Anthelmintic and Antimicrobial Activity of Some Novel (4- (2 - ((Substituted) Methyl))) -1H-Benzo[D] Imidazol-1-YL) Pyridin-2-YL) (1H-Imidazol-1-YL) Methanones

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Abstract **- Anthelmintic drugs resistance has created a major problem over the world. As per World Health Organization only few drugs are used for the therapy of helminth infections in the human population. Taking this into consideration we have made attempts to synthesize and evaluate some novel benzimidazole molecules having side chain of (4-chloropyridin-2-yl) (1H-imidazol-1-yl) methanone at the 1H position of the benzimidazoles. Few novel 4- (2 - ((substituted) methyl) -1H-benzo [d] imidazol-1-yl) -N-methylpyridine-2-carboxamide (Va-Vq) have been synthesized as given in the scheme. All the intermediate compounds and the final molecules were purified, and the chemical structures are confirmed by IR, 1H NMR and Mass spectral data. All the newly synthesized molecules have been tested for their anthelmintic activity on adult Indian earthworms (pheretima posthuma) at various concentrations (0.2% and 0.5%) and antibacterial activity on *B. subtilis, B. cereus, S. epidermidis, S. typhi, P. aeruginosa and K. pneumonia.* All the newly synthesized molecules showed good activities when compared to the standard molecules.**

Index Terms **- Benzimidazoles, Anthelmintic activity, Albendazole, Amikacin.**

INTRODUCTION

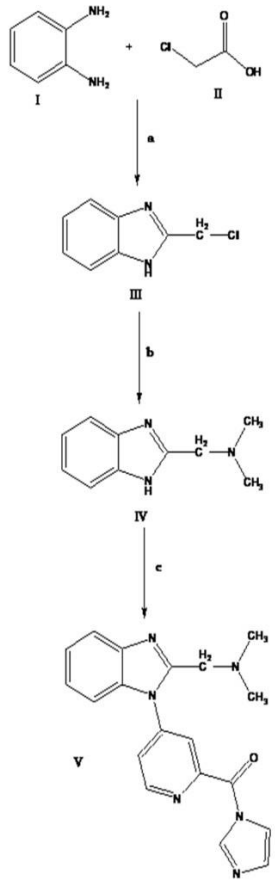
Anthelmintic drugs are those which expel the helminth parasitic worms from the body. They are also called as vermifuges or vermicides. The parasitic worms have developed resistance gradually to some broad spectrum like benzimidazoles, levamisole, avermectins and some narrow spectrum dewormers like salicylanlilides[1]. Various side effects such as gastrointestinal symptoms (epigastric pain, diarrhea, nausea, vomiting), symptoms affecting the nervous system (headache, dizziness) and allergic phenomena (edema, rashes, urticaria) were reported in the host after treatment with albendazole or mebendazole[2]. It is essential to synthesize some novel molecules which have chemical properties from the drugs that are used commonly to overcome the development of resistance to drugs.

Literature study reveals that few picolinic acid derivatives possess various pharmacological activities like antibacterial, antimicrobial, anthelmintic and antitubercular activities. We have made an attempt in the present study to attach moiety derived from picolinic acid to benzimidazoles and test how the molecule affects the anthelmintic activity.

MATERIALS AND METHODS

The solvents and chemicals used for the research work were procured commercially from E. Merck India, S.D. Fine Chem India and Qualigens India. Silica gel G used for the TLC was procured from S.D. Fine Chem India. Melting points were determined in open glass capillary (Kjeldahl flask having liquid paraffin) and are uncorrected. The Proton magnetic resonance spectra were recorded on Brukers 300 MHz (Bruker Germany) in CDCl3 / DMSO using TMS as internal standard. The IR spectra were recorded suing KBr on FTIR-8400S Fourier transform (Shimadzu Japan). Mass spectra were recorded on LC-MS / MS (API-400), Applied Biosystems MDS SCIEX – Canada.

SCHEME



Reagents : (a) 4N HCl, (b) anhydrous K_2CO_3, dimethyl amine(secondary amines), dry acetone.
(c) t-BuOK, DMF, anhydrous K_2CO_3, (4-chloropyridin-2-yl)(1H-imidazol-1-yl)methanone.

EXPERIMENTAL

Synthesis of 2-(chloromethyl)-1H-benzo[d]imidazole (III)

A round bottom flask was taken and o-phenylenediamine (0.03mol), 36 ml of 4N Hydrochloric acid and chloroacetic acid (0.03mol) was added and heated the solution under reflux for 3hrs. Further cooled the solution in ice bath made it alkaline by addition of dilute ammonia[3,4]. The product that was formed was filtered, dried and was recrystallized by use of suitable solvents. M.P.: 140°C, Yield: 80%.

Synthesis of (1H-benzo[d]imidazol-2-yl)-N,N-dimethylmethanamine (IV)

A flat bottom flask was taken and 2-(chloromethyl)-1H- benzo[d]imidazole(III) (0.005mol) was added to suspension of different secondary amines (dimethyl amine)(0.005mol and anhydrous potassium carbonate (0.005mol) in 15 ml of dry acetone. At room temperature the reaction mixture was stirred for about 6-8hrs and evaporated the remaining acetone. To the residue distilled water was added to give precipitate that was filtered and then washed with water and then recrystallized with suitable solvent[5,6]. The compounds were checked for purity by TLC and spectral data. M.P.:160°C, Yield: 68%. IR(KBr)(in cm^{-1}) at : 3075 (N-H str), 3155 (Ar-H str), 1650-1540 (C=C and C=N str). H^1 NMR(DMSO-*d6*):(δ,ppm) 5.2, (s, 1H, NH), 8.1-7.2 (m, 4H, Ar-H), 3.4(s, 2H, $-CH_2-$), 2.3 (s, 6H, $-N(CH_3)_2$). EI-MS: *m/z* 175 (M^+) and an M+1 peak at 176.

Synthesis of (4-(2-((dimethylamino)methyl)-1H-benzo[d]imidazol-1-yl)pyridine-2-yl)(1H-imidazol-1-yl)methanone (Va – Vq):

A flat bottom flask was taken and a solution of different compounds of IV(0.005mol) in anhydrous N,N-dimethylformamide and treated with potassium tert-butoxide and the resultant reddish-brown mixture was stirred for 2hrs at room temperature. The contents of the flask were then treated with (4-chloropyridin-2-yl) (1H-imidazol-1-yl)methanone(0.005mol) and potassium carbonate and then it was heated to 80°C for 6hrs. The final mixture was cooled to room temperature and then poured into ethyl acetate[7,8]. The organic extracts were combined and washed with brine, dried over sodium sulfate and concentrated to give the (4-(2-((dimethylamino)methyl)-1H- benzo[d] imidazol-1-yl)pyridine-2-yl) (1H-imidazol- 1-yl) methanone. M.P. 210°C. Yield: 61%. IR(KBr)(incm^{-1}) at3122 (Ar-H str), 1600-1510 (C=C and C=N str), 1239 (C-N), 1662 (C=O), 745(C-Cl). H^1 NMR(DMSO-*d6*):(δ,ppm): 9.4-7.2(m, 10H, Ar-H), 3.8(s, 2H, $-CH_2-$), 2.5(s, 6H, $-N(NH_3)_2$). EI-MS: *m/z* = M+1 peak at 347.2. The physical data of the newly prepared molecules is given in Table-1

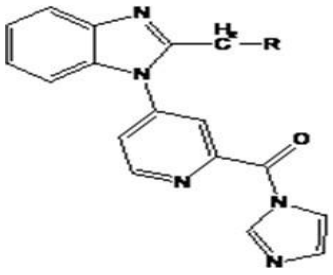
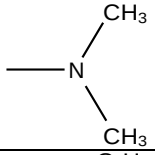
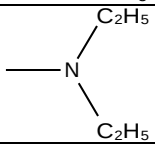
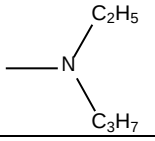
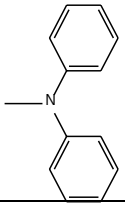
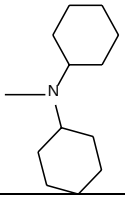
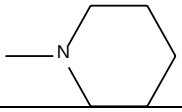
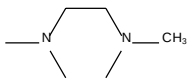
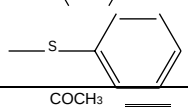
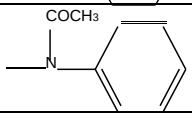
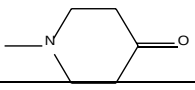
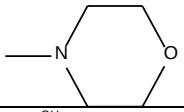
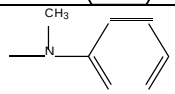
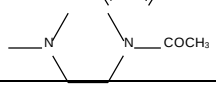
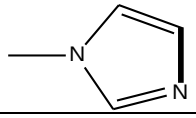
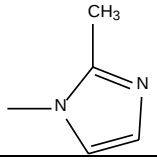
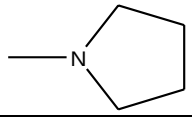
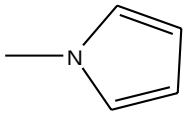


Table -1 The Physical data of newly synthesized molecules (Va – Vq)

S.No	Comp	-R	Mol. Formula	M.P. (°C)	% Yield
1	Va		$C_{19}H_{18}N_6O$	210	61
2	Vb		$C_{21}H_{22}N_6O$	195	70
3	Vc		$C_{22}H_{24}N_6O$	230	66
4	Vd		$C_{29}H_{22}N_6O$	235	58
5	Ve		$C_{29}H_{34}N_6O$	225	59
6	Vf		$C_{22}H_{22}N_6O$	180	68
7	Vg		$C_{22}H_{23}N_7O$	226	58
8	Vh		$C_{23}H_{17}N_5OS$	240	63
9	Vi		$C_{25}H_{20}N_6O_2$	208	62
10	Vj		$C_{22}H_{20}N_6O_2$	180	60
11	Vk		$C_{21}H_{20}N_6O_2$	175	66
12	Vl		$C_{24}H_{20}N_6O$	220	63
13	Vm		$C_{23}H_{23}N_7O_2$	228	73

14	Vn		$C_{20}H_{15}N_7O$	190	58
15	Vo		$C_{21}H_{17}N_7O$	221	60
16	Vp		$C_{21}H_{20}N_6O$	200	60
17	Vq		$C_{21}H_{16}N_6O$	196	65

BIOLOGICAL ACTIVITY

ANTIBACTERIAL ACTIVITY

The antibacterial activity of the newly synthesized molecules was determined by slightly modified cup method[9,10,11]. Muller Hington agar was used for the growth of the 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). Transmittance (T) of 75 to 77% at 530nm was obtained by suspending the organisms in normal saline solution, equal to 106 CFU/ml. All the molecules under activity were dissolved in DMSO at a concentration of 2mg/ml. 20µl of microbial suspension was inoculated into each plate. Each cup was added with 100 µl of test compounds. The bacteria containing plates were incubated at 37°C for 24hrs. The growth inhibition zone of the positive antimicrobial activity was read and compared with the solvent used as negative control. Amikacin was used as standard drug. The antimicrobial activity of the newly synthesized molecules is given in Table 2

Table 2 : Antibacterial activity of newly synthesized molecules (Va – Vq)

Comp.	Zone of inhibition (mm)					
	B.subtilis	*B.cereus*	*S.epidermidis*	*S.typhi*	*P.aeruginosa*	*K.pneumoniae*
Va	10	14.2	13.6	14.1	13	9.6
Vb	8	16	12	16	15	9.5
Vc	12	18	16	13	14	9.5
Vd	8.2	12	9.5	8.4	8.6	8.2
Ve	8.5	16.4	12.6	15	15.4	9.8
Vf	6	8	12	8	11	7.8
Vg	12	12	12.2	12	12.4	10
Vh	10	16	11	14	14	11
Vi	10.4	13.5	14	10.4	16.2	10.2
Vj	7	11	9	12.1	10.4	8.8
Vk	15.5	16.8	17.8	17.2	17.6	11
Vl	8	11.5	12	7	9	9
Vm	10	8	8	7.5	11	8
Vn	14.5	15.2	18	17.4	18.2	12
Vo	12	18	16	13	14	9.5
Vp	10	8	11.2	13	13	8.5
Vq	13	17	16	13	15	9.5
Negative Ctrl.	--	--	--	--	--	--
Standard (Amikacin)	22	19	20	22	20	18

- No activity, Negative control - DMSO

ANTHELMINTIC ACTIVITY

The earthworms (Indian – *pheretima posthuma)* were used for the anthelmintic activity study. They were washed by normal saline and removed the fecal matter. The earthworms of 4-5cm in length and 0.1-0.2cm of width have been used for the experimental activity[12]. The earthworms of same size have been selected randomly for the experiment. They had been acclimatized for the conditions of the laboratory before to the experiment. The earthworms had been divided into 4 groups and each group having 6 earthworms. Albendazole used as standard was diluted with normal saline to give 0.2%w/v and 0.5%w/v and poured into the petridishes. The newly prepared molecules were taken in minimal quantity of DMSO and diluted to give two concentrations of 0.2%w/v and 0.5%w/v of each molecule. The normal saline solution acted as negative control. 6 earthworms of almost equal size, taken and placed in petridishes for each concentration at room temperature. Time taken for complete paralysis and death was recorded. For each sample the average time for paralysis and lethal time had been calculated[13,14].

Table 3: Anthelmintic activity of newly synthesized molecules (Va – Vq)

Comp.	Time for paralysis(min)		Time for death(min)	
	% of Concentration		% of Concentration	
	0.2%	0.5%	0.2%	0.5%
Va	8:40	6:20	19:20	11:00
Vb	4:20	3	10:15	8:10
Vc	12:45	7:40	21:0	18:30
Vd	23:10	16:50	31:05	26:55
Ve	38:05	22:58	56:20	31:04
Vf	18:06	10:45	24:35	18:10
Vg	8	6:09	21:10	15:05
Vh	5:0	3:30	16:45	12:40
Vi	6:05	3:02	18:25	9:25
Vj	11:46	8:20	22:50	13:08
Vk	0:30	0:25	4:20	2:30
Vl	5:08	3:45	17:30	9:20
Vm	0:50	0:40	7:05	3:40
Vn	3:10	2:08	12:05	9:35
Vo	11:12	9:25	17:05	14:08
Vp	2:09	1:48	8:20	4:55
Vq	15:06	9:45	26:35	16:10
Negative Control	--	--	--	--
Standard (Albendazole)	0:25	0:18	0:31	0:29

- *No activity, Negative control - DMSO*

RESULTS AND DISCUSSIONS

It was observed that all the molecules antibacterial activity on screening against all the organisms that were used as given in table2. Molecules Vn, Vk, Vc, Vq and Va showed good activity on both Gram positive and Gram-negative bacteria. Highest zone of inhibition of 18.2mm was shown by molecule Vn on *P.aeruginosa*. Good activity was shown by the molecules Vc, Ve and Vg and moderate activity was seen against all the organisms by the Va, Vh, Vi and Vo molecules. Good activity was seen against *B.cereus, S.epidermidis, S.typhi*, *P.aeruginosa* and *K.pneumoniae* by molecule Vn and good activity was seen against *B.cereus* by molecules Vc and Vo. Among all the molecules Vf and Vj had showed low activity. But none of the molecule showed a comparative activity to that of Amikacin.

The table3 gives the anthelmintic activity shown by the molecules on earthworms. High activity was shown by molecules Vk and Vm among all the newly synthesized molecules. Good activity was shown by Vb, Vh, Vl, Vn and Vp and moderate activity by Va,

Vg and Vi and less activity by Vd and Ve at both the concentrations. All the molecules have shown less activity when compared to the standard Albendazole drug.

CONCLUSION

The benzimidazole molecules were synthesized successfully. The anthelmintic and antibacterial activity of all the molecules was evaluated. Good activity was shown by all the molecules, of all the molecules Vn and Vk have shown good activity on all the organisms used for the antibacterial activity and Vk and Vm have shown good anthelmintic activity among all other molecules at both the concentrations

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