Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2015, Volume 3, Issue 5
ISSN: 2321–3647(online)

Synthesis and Evaluation of 4-(2-Substituted-1h-Benzo [D]Imidazol-1-Yl)-N-Methylpyridine-2-Carboxamide Derivatives for their Antimicrobial and Anthelmintic Activity

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ABSTRACT

The resistance to anthelmintic drugs has created a major hitch throughout the world over the decades. As per the WHO only a few drugs are used as anthelmintic agents in the recent days for human beings in the treatment of helminth infestations. An attempt has been made to synthesize some novel benzimidazole derivatives with N-methyl picolinamide moiety at 1H position of the benzimidazoles. And, some novel derivatives of 4-(2-substituted-1h-benzo[d]imidazol-1-yl)-n-methylpyridine-2-carboxamide have been synthesized as given in the scheme. All the newly synthesized final compounds and intermediates were purified and their IR, ^{1}H NMR, and Mass spectral data have confirmed the chemical structures. All the newly synthesized molecules were examined for their anthelmintic activity against Indian adult earthworms (*pheretima posthuma*) at different concentrations (0.2% and 0.5%) and antibacterial activity against *S.epidermidis, S.typhi, B.subtilis, B.cereus, P.aeruginosa and K.pneumoniae.* Many of the newly synthesized molecules which were tested for the biological activity have shown promising activities when compared with that of the standard drugs.

Keywords: Picolinic acid, Benzimidazoles, Antimicrobial agents, Anthelmintic drugs.

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Received 07 March 2015, Accepted 24 March 2015

Please cite this article as: Lingala S, Synthesis and Evaluation of 4-(2-Substituted-1h-Benzo [D]Imidazol-1-Yl)-N-Methylpyridine-2-Carboxamide Derivatives for their Antimicrobial and Anthelmintic Activity. American Journal of Pharmacy & Health Research 2015.

INTRODUCTION

The drugs that expel helminth parasitic worms (helminths) from the body are called as anthelmintics, these compounds show their action either by stunning or by killing them. These may also be called as vermifuges (stunning) or vermicides (killing). Resistance has been developed to some broad spectrum anthelmintic agents like benzimidazoles, levamisole, avermectins and also towards some narrow spectrum dewormers such as the salicylanilides (closantel) (Anshul Chawla *et al*., 2012)[1]. Development of drug resistance in many helminthic strains was due to continuous reliance on a small range of compounds for a longer period. In addition to this, several side effects have been reported after treatment with albendazole or mebendazole, (Doddarasinakere K R *et al.,* 2012)[2]. An attempt has been made in the present study, 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 in our present study, we had reported the synthesis of some novel benzimidazole derivatives and antibacterial and anthelmintic activity were screened for the newly synthesized compounds.

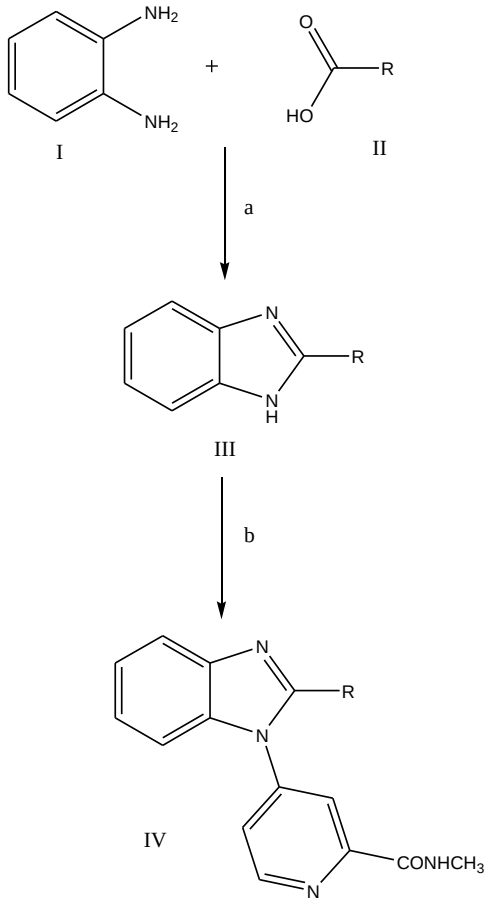
MATERIALS AND METHOD

The solvents and chemicals used for the experimental work were commercially procured from S.D. Fine Chem, India, E. Merck, India and Qualigens, India. Silica gel G used for the analytical chromatography (TLC) was obtained from S.D. Fine Chem, India. A Kjeldahl flask containing liquid paraffin with an open glass capillary was used for determining the melting points of the compounds and are uncorrected. The proton magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker 300 MHz instrument (Bruker, Germany) in DMSO/CDCl3 using TMS as internal standard. The 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 LCMS/ MS (API-4000 TM), Applied BioSystems, MDS SCIEX (Canada).

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

A mixture of *o*-phenylenediamine (0.03 mol), 36 ml of 4 N HCl and different carboxylic acids (0.03 mol) were taken in a round bottom flask and the solution was refluxed for 3 hours. Further the solution was cooled on ice bath and made alkaline by the addition of dilute ammonia solution. The product formed was filtered, dried and recrystallized from suitable solvents (V.Rajini Priya *et al.*, 2010)[3]. M.P ; 170^{o}C, Yield; 79 %.

SCHEME



Reagents : (a) 4N HCl and different carboxylic acids
(b) t-BuOK, DMF, anhydrous K_2CO_3,
4-chloro-N-methylpyridine-2-carboxamide.

Synthesis of N-methyl-4-(2-substituted-1H-benzo [d]imidazol-1-yl) pyridine-2-carboxamide (IVa-IVk)

A solution of various 2-(substituted)-1H-benzo[d]imidazoles (0.005 mol) in dry N,N-dimethylformamide were treated with potassium *tert*-butoxide and the reddish brown mixture was stirred at room temperature for 2 hr. The contents were treated with 4-chloro-N-methylpyridine-2-carboxamide (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 N-methyl-4-(2-substituted-1H-benzo[d]imidazol-1-yl) pyridine-2-carboxamide (Vivek Daniel *et al.*, 2010; Umesh K. Patil *et al.*, 2004)[4,5]. The Physical data of all the newly synthesized compounds is given in Table-1. M.P; 225^{o}C, Yield; 75 %. **IR (KBr) (cm^{-1}):** 3142 (Ar-H str.), 1612-1536 (C=C & C=N str.), 1248 (C-N), 1640 (C=O). **^{1}HNMR (DMSO-*d6*): δ** 5.2, (s, 1H, NH), 8.4 -7.6 (m, 7H, Ar-H), 3.8 (s, 2H, -CH2-), 2.5 (s, 6H, -N (CH3)2). **EI-MS: *m/z*** = 252 **(M^{+})**.

BIOLOGICAL EVALUATION

Antibacterial Activity

Antibacterial activity of the synthesized compounds was determined, using a slightly modified cup plate method. 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 106 CFU/ml (Becker, B.M *et al.,* 1964)[5]. 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. All the synthesized compounds have shown antibacterial activity (A. Sebiomo *et al.*, 2011)[7].

Anthelmintic Activity

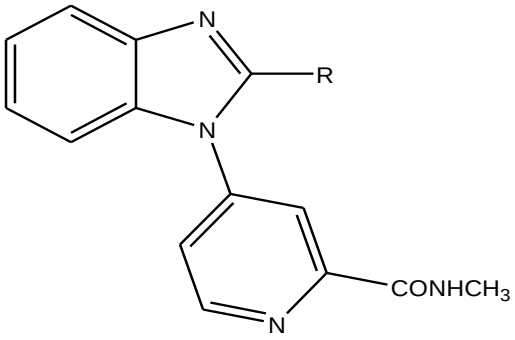
Indian adult earthworms (*pheretima posthuma*) were used to study anthelmintic activity. The earthworms (collected from the water logged areas of soils, Jangaon, Warangal, Andhra Pradesh) were washed with normal saline to remove all fecal materials. The earthworms in 4 - 5 cm. in length and 0.1 - 0.2 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. 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 (Dutta S *et al.,* 2010; Rajesh R *et al.,* 2010)[8,9]. 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. 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 (Mohammed M S *et al.*, 2005; Srikanth L *et al.*, 2011)[10,11], shown in Table-3.

RESULTS AND DISCUSSION

The antibacterial activity data of N-methyl-4-(2-substituted-1H-benzo[d]imidazol-1-yl) pyridine-2-carboxamide derivatives (IVa - IVk) synthesized by given scheme indicates that all the compounds have shown moderate antibacterial activity against the organisms employed. Among all the compounds, the compound IVk (R = methyl) has relatively highest inhibitory effect against *B.cereus, S.epidermidis, S.typhi* and *P. aeruginosa* with zone of inhibition of 14.8, 16.5, 16.7 and 16.8 mm respectively. This is followed by compound IVh (R = 4-nitrophenyl) which has significant inhibitory effect against *S.epidermidis* and *S.typhi* with the zone of inhibition of 16.2 and 15.5 mm while the compound IVe was showing good activity on *S.typhi* and *P. aeruginosa* with zone of inhibition of 15.5 and 15.2 mm. The compound IVa was showing least activity on *B. subtilis* with zone of inhibition of 5 mm. The rest of the compounds in this series have exhibited moderate to mild antibacterial activity. The anthelmintic activity of newly synthesized N-methyl-4-(2-substituted-1H-benzo[d]imidazol-1-yl)pyridine-2-carboxamide derivatives (IVa - IVk) by following the procedures is presented in Table 3. The compound IVh (R = 4-nitrophenyl) has shown significant activity causing paralysis and death of the worms in 3.07±0.02 min and 12.30±0.04 min at concentration of 0.2% and 2.05±0.01 min. and 9.30±0.07 min. at a concentration of 0.5%. which is followed by compound IVg and IVk. The compounds IVc, IVe, IVi and IVj were showing moderate activity. The compound IVb (R = 4-methylphenyl) was least active and the time taken at concentration of 0.2% was 30.90±1.64 min. and 52.31±1.27 min and at a concentration of 0.5% the time taken was 20.05±0.33 min. and 34.16±1.48 min.

Table 1: Physical data of synthesized compounds (IVa - IVk)



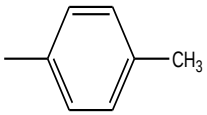
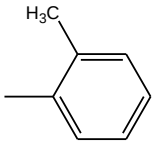
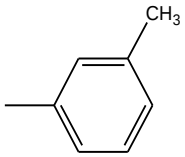
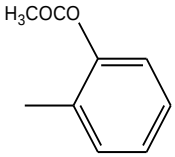
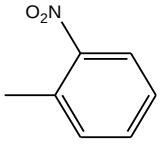
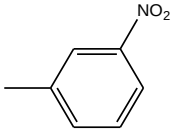
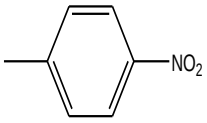
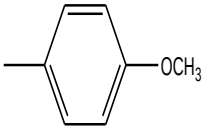
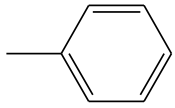
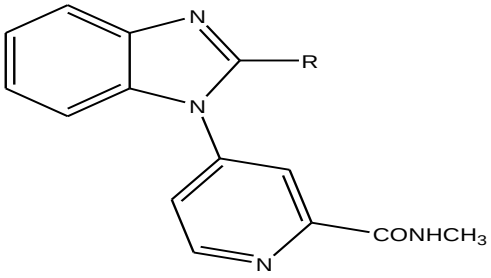
S.No.	Comp.	R	Mol. Formula	m.p. (°C)	Yield%
1	IVa	—H	$C_{14}H_{12}N_4O$	225	75
2	IVb		$C_{21}H_{18}N_4O$	238	62
3	IVc		$C_{21}H_{18}N_4O$	266	78
4	IVd		$C_{21}H_{18}N_4O$	212	75
5	IVe		$C_{22}H_{18}N_4O_3$	218	68
6	IVf		$C_{20}H_{15}N_5O_3$	215	65
7	IVg		$C_{20}H_{15}N_5O_3$	247	60
8	IVh		$C_{20}H_{15}N_5O_3$	234	74
9	IVi		$C_{21}H_{18}N_4O_2$	195	62
10	IVj		$C_{20}H_{16}N_4O$	257	60
11	IVk	—CH3	$C_{15}H_{14}N_4O$	240	65

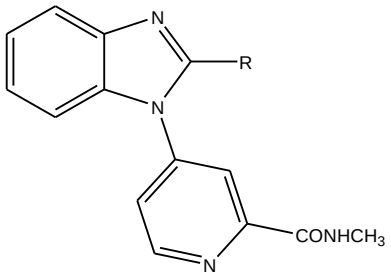
Table 2: Antibacterial activity of synthesized compounds. (IVa - IVk)



S.No.	Comp.	Zone of inhibition (mm)					
		B.subtilis	***B.cereus***	***S.epidermidis***	***S.typhi***	***P.aeruginosa***	***K.pneumoniae***
1	IVa	5	9	11	8	8.4	7.5
2	IVb	7.8	8.6	9	7.5	11	9
3	IVc	8	7.4	9	6.8	8.2	9
4	IVd	9	11.2	12.4	10.6	9.7	9.2
5	IVe	7	12.6	8	15.5	15.2	9
6	IVf	8.5	14	12.6	11	11.2	8.7
7	IVg	9	8.4	8	7.8	8.3	8
8	IVh	11	12	16.2	15.5	12	10
9	IVi	10	9.8	7	7.4	8	6.4
10	IVj	8.2	8.5	7	5.6	9.8	7.5
11	IVk	9.4	14.8	16.5	16.7	16.8	12
12	Neg. Ctrl.	--	--	--	--	--	--
13	Std	20	21	21.2	22.6	21	19.8

Standard – Amikacin, -- No activity, Negative Control - DMF

Table 3: Anthelmintic activity of synthesized compounds. (IVa - IVk)



S.No.	Comp.	Time for paralysis (min)		Time for death (min)	
		Concentration			
		0.2%	**0.5%**	**0.2%**	**0.5%**
1	IVa	11.07±1.83 [d]	8.19±1.30 [d]	21.53±3.60 [d]	16.02±2.38 [d]
2	IVb	30.90±1.64 [d]	20.05±0.33 [d]	52.31±1.27 [d]	34.16±1.48 [d]
3	IVc	7.99±1.74 [a]	5.35±1.59 [a]	20.79±3.09 [d]	15.27±2.76 [d]
4	IVd	11.12±1.81 [d]	7.70±1.39 [d]	22.30±3.35 [d]	17.45±2.75 [d]
5	IVe	7.57±0.80 [a]	5.00±0.66	17.74±1.84 [c]	12.26±1.21 [b]
6	IVf	17.23±2.67 [d]	12.90±1.41 [d]	32.73±3.89 [d]	27.18±2.77 [d]
7	IVg	3.35±0.04	2.48±0.04	10.55±0.04	6.50±0.04
8	IVh	3.07±0.02	2.05±0.01	12.30±0.04	9.30±0.07

9	IVi	7.87±1.03[a]	5.73±0.56[a]	21.17±2.74 [d]	16.46±1.93 [d]
10	IVj	6.98±1.74	5.15±1.25[a]	18.22±3.44[c]	12.98±2.92[b]
11	IVk	4.57±0.86	3.07±0.70	10.07±1.70	7.87±1.70
12	Negative Control	--	--	--	--
13	Standard (Albendazole)	0.34 ± 0.01	0.25 ± 0.01	0.37 ± 0.01	0.29 ± 0.01

All the results are expressed as Mean±SEM. (n = 6). P value was calculated by one-way ANOVA followed by Bonferroni's multiple t-test. [a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001 and [d]*P*<0.0001 Significantly different when compared with reference compound, Albendazole. Control worms were alive up to 24 hrs of observation. -- No Activity, Negative Control- Normal Saline.

CONCLUSION

All the new benzimidazole derivatives were synthesized successfully as per the given scheme. All the compounds were screened for their antibacterial and anthelmintic activity. All the compounds synthesized newly were found to show good activity, among all the active compounds of benzimidazole derivatives, IVk and IVh have shown good antibacterial activity against all the organisms that were employed for the antibacterial activity and compound IVg and IVh have shown high anthelmintic activity than all the other compounds synthesized at both the concentrations.

ACKNOWLEDGEMENTS

The author is thankful to Mr. V.Prasad Rao, Chairman, and Mr. V. Vinay Kumar, Director, Prasad Institute of Pharmaceutical Sciences, Jangaon, Warangal, for providing laboratory facilities and financial support.

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