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SYNTHESIS AND COMPARATIVE ANTIMYCOBACTERIAL EVALUATION OF CERTAIN SUBSTITUTED BENZIMIDAZOLES

K. UMAA*, K. KANNAN[a] and K. KRISHNAKUMAR[b]

PSG College of Pharmacy, COIMBATORE – 641004 (T.N.) INDIA
[a]Department of Pharmacy, Annamalai University, CHIDAMBARAM (T. N.) INDIA
[b]St. James College of Pharmacy, CHALAKUDY (Kerala) INDIA

ABSTRACT

A series of 2-methyl-1H-benzimidazole hydrazide derivatives have been synthesized, purified and the structures of these heterocycles have been characterized on the basis of spectral analysis (IR, ^{1}H NMR, ^{13}C NMR and Mass). Test compounds were tested for antimycobacterial potentials using alamar blue assay method (ABA) and luciferase reporter phage assay (LRPA) against the pathogen *Mycobacterium tuberculosis*. Some of the compounds are found to possess remarkable antimycobacterial potentials proving their equipotency with that of the standard marketed chemotherapeutic agent, isoniazid.

Key words : Benzimidazoles, Characterization, Alamar blue assay, Luciferase reporter phage assay.

INTRODUCTION

Research in medicinal chemistry renders a vital role in the discovery of newer therapeutic agents to tackle the diseases created by the mutational changes of microorganisms. Benzimidazoles constitute an interesting class of heterocyclic compounds with diverse biological and pharmacological potentials such as antimicrobial, anthelmintic, antihistaminic, antiulcer and anti-inflammatory properties apart from the numerous drugs marketed under various trade names. In view of these reports, efforts were made to explore the newer derivatives of 2-methyl-1H-benzimidazole hydrazides.

The final analogs **(4a-4l)** of the parent compound, N', N''-[1-(1H-benzimidazole-2-yl)-2-(4-substituted phenyl ethane-1-2-diyl)] substituted aromatic hydrazide derivatives, were synthesized by treating with different substituted aromatic aldehydes **(R_1)** and

* Author for correspondence; Email : umadevi97@yahoo.co.in, Mobile : 9843466987

substituted hydrazides **(R_2)** of **Scheme 1.** All the compounds were characterized by physical and spectral techniques. The newly synthesized compounds were screened for antimycobacterial potentials using alamar blue assay and luciferase reporter phage assay.

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. IR spectra (cm^{-1}) were recorded on a Fourier Transform IR spectrophotometer (Shimadzu 8700) using KBr disc method. ^{1}H NMR and ^{13}C NMR were reported on AMX 500 MMZ spectrophotometer using TMS as the internal standard. Mass spectra were recorded on a LC-MS Triple quadruple mass spectrometer. The purity of the test compounds was determined by thin layer chromatography. The antibacterial screening was carried out at PSG College of Pharmacy, Coimbatore. The antimycobacterial screening was performed by Alamar blue assay method and Luciferase reporter phage assay technique at Rajiv Gandhi Institute of Science and Technology, Trivandrum and Tuberculosis Research Centre, Chennai.

General procedure for synthesis[7,8]

Synthesis of 2- methyl-1H-benzimidazole (1)

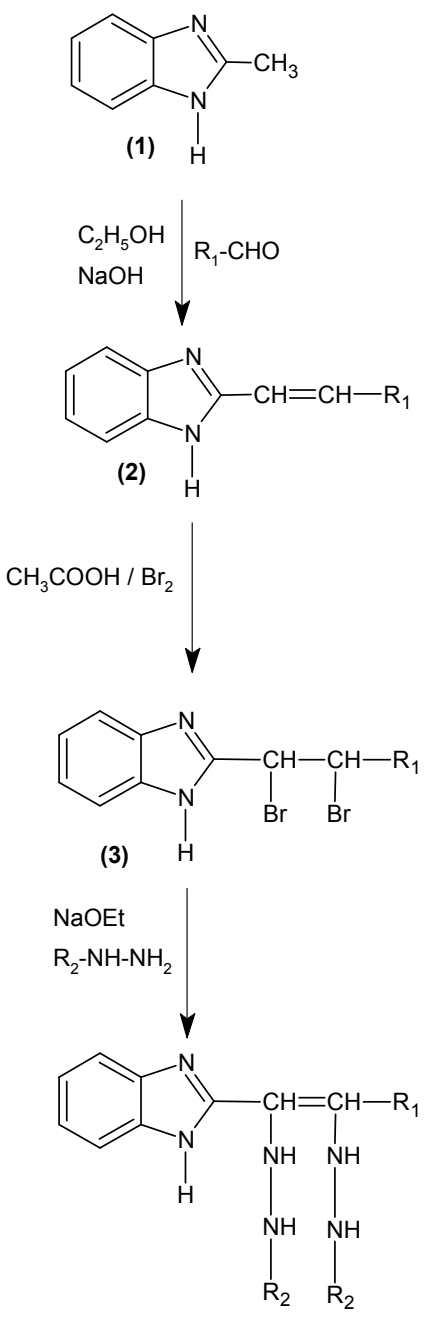
o-Phenylenediamine dihydrochloride (2.19 g, 0.15 mole) dissolved in 10 mL water was extracted with acetic acid (2.74 g, 0.45 mole) and refluxed for 45 min. It was cooled and concentrated ammonia solution was slowly added, filtered and recrystallized from ethanol.

Synthesis of 2-[(2-substituted phenyl) vinyl]-1H-benzimidazole (2)

Sodium hydroxide solution (30 mL, 10%) was added to 2-methyl benzimidazole (1.76 g, 0.01 mole) in ethanol. To this, unsubstituted methoxy and chloro substituted aromatic aldeydes (0.01mole) were dissolved in a minimum quantity of ethanol, stirred for 4-5 hrs and left overnight. Concentrated hydrochloric acid was added dropwise. The solid separated was filtered and recrystalized from ethanol.

Synthesis of 2-[1,2-dibromo-2-(4-substituted phenyl) ethyl-1H-benzimidazole (3)

The above compound **(2)** (2.98 g, 0.01 mole) was dissolved in glacial acetic acid (10.0 mL) containing bromine 6 mL, 0.03 mole), stirred for 3 hrs and left overnight. Crushed ice was added to remove the product and recrystalized from 50% ethanol.



Comp.	- R_1	- R_2
(4a)	- C_6H_5	- H
(4b)	- C_6H_4-OCH_3	- H
(4c)	- C_6H_4-Cl	- H
(4d)	- C_6H_5	- C_6H_5
(4e)	- C_6H_4-OCH_3	- C_6H_5
(4f)	- C_6H_4-Cl	- C_6H_5
(4g)	- C_6H_5	-CO-C_6H_5
(4h)	- C_6H_4-OCH_3	-CO-C_6H_5
(4i)	- C_6H_4-Cl	-CO-C_6H_5
(4j)	- C_6H_5	-CO-C_5H_4N
(4k)	- C_6H_4-OCH_3	-CO-C_5H_4N
(4l)	- C_6H_4-Cl	-CO-C_5H_4N

Scheme 1

Synthesis of [1-(1H-benzimidazole-2-yl)-2-(4-substituted phenyl ethane-1,2-diyl)] substituted aromatic hydrazide derivatives (4a-l)

Compound **(3)** (0.01 mole, 4.15 g) was taken and sodium (0.15 mole) in 50 mL absolute alcohol was added. (0.02 moles) of hydrazine or different substituted hydrazides like phenyl hydrazine, benzoyl hydrazide and isonicotinic hydrazide were added and refluxed for 24 hrs, concentrated and poured on crushed ice. Product separated was crystallized by using 50% ethanol.

Biological evaluation

The newly synthesized compounds were screened for antibacterial activity using Kirby bauer method against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*. Dimethyl sulphoxide was used as the solvent and Gentamycin as the standard.

The novel compounds were evaluated for their antimycobacterial potentials using alamar blue assay technique. *Mycobacterium tuberculosis* H_{37} RV strain maintained on Lowenstein Jensen medium was used as test organism. Stock solutions of the newer compounds were prepared in dimethyl formamide , filtered, sterilized and were added to 450 µL of middle brook TB broth to achieve concentrations of 1,0.5,0.25 and 0.1 µg/mL. 50 mL of the vortexed culture was inoculated, mixed and incucated at 37ºC using isoniazid as standard. On the seventh day, 25 µL of alamar blue solution was added and observed for 6 hours. Blue colour in the tube indicates sensitivity of *Mycobacterium tuberculosis* to the newly synthesized compounds and pink colour indicates resistance of the organism. Luciferase reporter phage assay was also carried out. The relative log unit reduction of mycobacterial population was estimated using this technique.

RESULTS AND DISCUSSION

The data of physical characteristics of the newer compounds are given in Table 1. Formation of compounds **(4a-4l)** was confirmed by the appearance of prominent peaks in IR (cm^{-1}) at 3436 (–NH stretch), 2832 (aliphatic –CH stretch), 1596 (–C=N stretch) and 1359 (–C=C stretch)of aromatic group. The compounds **(4g-4l)** possessed peak at the range 1684-1720 cm^{-1} for C=O group.

The ^1H NMR ($CDCl_3$ + DMSO) (δ ppm): singlet at 12.18 for –NH group of imidazole, doublet at 2.0 for –NH group attached to –CH, doublet at 3.9 for -NH attached to phenyl group. Aromatic protons are confirmed by appearance of peaks between 7.3-8.3.

Compounds **(4g-4l)** possessed a doublet at 8.1 showing the occurrence of two NH groups attached to C=O groups.

The ^{13}C NMR showed peak at 62 for aliphatic 'C' attached to substituted phenyl group, 141 for 'C' of –CN and –NH, 110-130 for aromatic 'C' of benzimidazole and phenyl rings. Mass spectrum of **(4l)** has a molecular ion peak at m/z 525, base peak at m/z 481 along with other peaks.

Thus, the structures of the newer compounds were analyzed with differences in melting point and R_f values using the solvent system- benzene : chloroform : methanol (60 : 20 : 20). These were confirmed by spectral evidences. All the above compounds were screened for antibacterial activity by two different techniques.

Table 1 : Physical characterization data of compounds (4a-4l)

Compound	M.P. (°C)	R_f vlaue	Yield (%)
4a	213	0.48	67
4b	228	0.51	51
4c	221	0.49	62
4d	130	0.59	76
4e	152	0.51	73
4f	141	0.63	64
4g	200	0.49	81
4h	219	0.51	70
4i	206	0.66	72
4j	203	0.41	71
4k	199	0.48	63
4l	193	0.58	67

Solvent system - Benzene ; Chloroform : Methanol = 60 : 20 : 20

Table : 2 Results of antimycobacterial activity of newer substituted benzimidazoles

Compound	Alamar blue assay	LRPA % RLU reduction	
		50	100
4a	25	62.64	77.54
4b	--	-	-
4c	25	64.12	72.32
4d	5	77.97	85.60
4e	2.5	81.12	83.07
4f	0.5	97.07	97.49
4g	50	12.98	6.10
4h	50	15.70	9.21
4i	1	-	-
4j	2.5	83.16	89.78
4k	50	14.60	10.32
4l	0.5	98.34	98.42
INH (std)	0.5	99.89	100

RLU - Relative log unit
LRPA- Luciferase reporter phage assay

Biological screening[2,3]

Initially, the compounds were screened for antibacterial activity by Kirby bauer method using Gentamycin as the standard. The compounds exhibited no significant effect on the organisms *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.*

All the synthesized compounds were screened *in vitro* for their antimycobacterial activity against *Mycobacterium tuberculosis* H_{37} RV strain using isoniazid as the standard. A comparative study was carried out by using 2 techniques.

(i) LRPA (Luciferase reporter phage assay)

(ii) ABA (Alamar blue assay).

An examination of the data revealed that most of the compounds exhibited significant antimycobacterial potentials. Compounds **(4e)-** 2[2-(4-methoxy phenyl)-1, 2-bis (2-phenyl hydrazinyl)ethyl]-1H-benzimidazole and **(4j)**–N',N''-[1-(1H-benzimidazol-2-yl-2-phenylethane-1, 2-diyl] di-isonicotino hydrazide proved to have inhibitory concentration of 2.5 μg/mL as per ABA values and 80-90% relative log unit reduction as per LRPA method. **(4f)** - 2[2-(4-chloro phenyl)-1, 2-bis (2-phenyl hydrazinyl)ethyl]-1H-benzimidazole and **(4l)** –(N',N''-[1-(1H-benzimidazol-2yl)-2-(4-chlorophenyl)ethane-1, 2-diyl] di-isonicotino hydrazide proved to be the most superior compounds possessing 0.5 μg/mL inhibitory concentration as per ABA technique and 97-98.5% relative log unit reduction of mycobacterial population as per LRPA technique proving to be equivalent to the famous marketed drug, isoniazid.

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