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Research Article

Synthesis of some new benzimidazole acetic acid derivatives and evaluation for their antimicrobial and antitubercular activities

Kale S.C*[1], Kale M.K[2], Biyani K.R[1]

[1]*Anuradha College of Pharmacy, Chikhli, Dist.Buldhana, Maharashtra,India*
[2]*K.G.R.D.College of Pharmacy and Research Institute, Karjat, Raigad, India*

***Correspondence Info:**
Mr.S.C.Kale
Assistant Professor;
Anuradha College of Pharmacy,
Chikhli,Dist-Buldhana.
Maharastra State, India
E.mail: sachin_kale83@rediffmail.com

Abstract

A new series of benzimidazoles acetic acid derivatives were synthesized by reacting 2(2-substituted phenyl ethenyl) 1H benzimidazole with chloroacetic acid under reflux.

Despite the availability of highly potential antitubercular agents, tuberculosis remains primary cause of comparatively high mortality worldwide. The statistics shows that around three million people through out the world die annually from tuberculosis and today more people die from tuberculosis than ever before. Therefore, the development of new drugs with activity against multi drug- resistant (MDR) TB, extensively drug –resistant (XDR) TB, and latent TB is a priority task. Although new agents that will shorten the duration of current chemotherapy are also needed.

A literature survey indicates that benzimidazole derivatives possess different biological activities such as anti-microbial activity, anti-ulcer, antiparasitic, antiprotozoal antiviral and antitubercular and physical properties shown that, benzimidazoles have high melting points, benzimidazoles are usually soluble in polar solvents and sparingly soluble in non polar solvent,benzimidazoles are weakly basic, being some what less basic than imidazole, benzimidazoles are also sufficiently acidic to be generally soluble in aqueous alkali. The acidic properties of benzimidazoles, like those of imidazole, seem to be due to stabilization of the ion by resonance. The pKa value of benzimidazoles (pKa=5.30).

The structures of these compounds were established by means of IR, ^{1}H-NMR and elemental analysis. All compounds were evaluated for antibacterial, antifungal and antitubercular activities. Most of the compounds have shown significant antibacterial, antifungal and antitubercular activity when compared with the standard drug.

Keywords: Benzimidazole Acetic Acid, Antitubercular, Antimicrobial

1. Introduction

Tuberculosis, termed the “captain of the men of death” is a major public health problem. Tuberculosis (TB) is still a major public health problem, compounded by the human immunodeficiency virus (HIV)-TB co-infection and recent emergency of multidrug - resistant (MDR) and extensively drug resistant (XDR)-TB. Novel anti-TB drugs are urgently required M. tuberculosis is responsible for most cases of tuberculosis; the reservoir of infection is humans with active tuberculosis.

Despite the availability of highly potential antitubercular agents, tuberculosis remains primary cause of comparatively high mortality worldwide. The statistics shows that around three million people through out the world die annually from tuberculosis and today more people die from tuberculosis than ever before. Therefore, the development of

new drugs with activity against multi drug- resistant (MDR) TB, extensively drug –resistant (XDR) TB, and latent TB is a priority task. Although new agents that will shorten the duration of current chemotherapy are also needed.[1] A new dimension was added in the year 1980 due to the spread of HIV with high prevalence of tuberculosis and *mycobact avium* complex infection among the patients[2].

The increasing global tuberculosis burden due to the curse of HIV, MDR and XDR-TB has lead to search of newer therapeutic agents to tackle the menance. The present first line drugs like INH, pyrazinamide, ethambutol, and rifampicin are potent antitubercular agent. They act by inhibition of mycolic acid and RNA / DNA synthesis. They possess numerous adverse reactions[3]. To avoid these effects it seemed promising to look for more selective compounds, at other targets to suppress the activity[4]

The extensive literature survey shown that;

Presence of benzimidazole nucleus in numerous categories of therapeutic agents such as antimicrobials, antivirals, antiparasites, anticancer, anti-inflammatory, antioxidants, proton pump inhibitors, antihypertensives, anticoagulants, immunomodulators, hormone modulators, CNS stimulants as well as depressants, lipid level modulators, antidiabetics, etc. has made it an indispensable anchor for development of new therapeutic agents. Varied substitutes around the benzimidazole nucleus have provided a wide spectrum of biological activities. Importance of this nucleus in some activities like, Angiotensin I (AT(1)) receptor antagonism and proton-pump inhibition.[31]

Benzimidazole plays an important role in the medicinal chemistry with many pharmacological activities such as antimicrobial, antiviral, antiulcer, etc. has made an indispensable anchor for discovery of novel therapeutic agents. Substitution of benzimidazole nucleus is an important synthetic strategy in the drug discovery. on the basis of substitution pattern around the nucleus with an aim to help medicinal chemists for developing an SAR on benzimidazoles for each activity. The aim is to study the work reported, chemistry and pharmacological activities of benzimidazole derivatives during past years.[9]

Ligand-induced stabilization of G-quadruplex structures formed by the human telomeric DNA is an active area of research. The compounds which stabilize the G-quadruplexes often lead to telomerase inhibition.[33]

Benzimidazole's unique base-selective DNA recognition property has been studied widely. However, most of the early benzimidazole systems have been targeted towards the binding of duplex DNA. The design and synthesis of new benzimidazole systems towards selective recognition of the double-stranded DNA then to achieve selective recognition of the G-quadruplex DNA [3].

Various derivatives of benzimidazole[5-7] exhibit interesting biological properties hence attempt was made to synthesized benzimidazole acetic acid derivatives for promising antitubercular activity.

2. Material and Methods [19-26, 31-37]

Melting points were determined in open capillary method and are uncorrected. IR spectra were recorded on Thermo Nicolet IR 200 spectro-photometer using KBr disc method. The ^{1}H-NMR spectra were recorded on sophisticated multinuclear FT-NMR Spectrometer model Avance-III (Bruker), using dimethylsulfoxide-d_6 as solvent and tetramethylsilane as internal standard.

2.1 General method for preparation of cinnamic acids: (Doebners Method) [36]

Aromatic aldehyde (0.02 mole) and malonic acid (0.42 mole) was dissolved in a mixture of dry pyridine (75 ml) and piperidine (1.3 ml) was added. The reaction mixture was heated under reflux for 2 hrs. A rapid evolution of carbon dioxide took place. Cooled, poured into excess of water containing hydrochloric acid (1N) to combine with pyridine. The solid that separated was filtered and recrystallized from hot water[10-14]. Yield -91%, m.p. -134-135^{0}C.

2.2 Synthesis of 2-(2-Phenyl ethenyl) – 1 H- benzimidazole:[36]

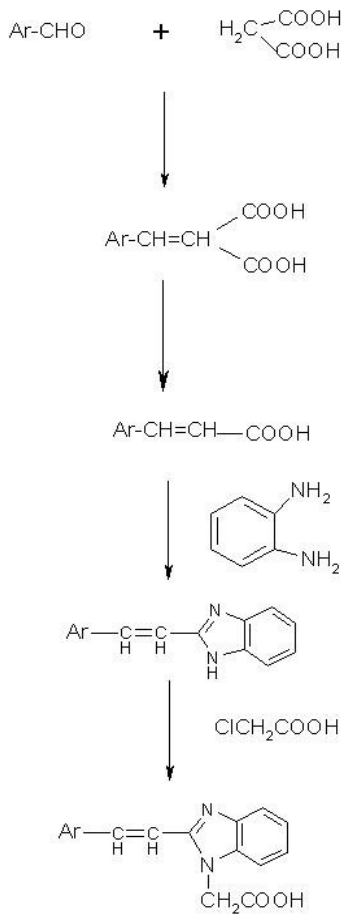
A mixture of aryl cinnamic acid (0.05 mol) and o-Phenylenediamine (0.05 mol) was treated with 4 N Hydrochloric acid and stirred at room temperature for 1hour. Until it goes into a solution. The reaction mixture refluxed further for 4-6 hours cooled and neutralized with dilute ammonia. The precipitate that separated was filtered and washed with water and

crystallized from methanol to get solid crystal of pure (BZ_1) [11-15] yield - 96%, m.p. –119-120^{0}C.

2.3 Synthesis of 2-(2-Phenyl ethenyl) – 1 carboxy- benzimidazole:[36]

A mixture of 2 (2-Phenyl ethenyl)–1H benzimidazole (0.01 mol) and chloroacetic acid (0.01mol) in 30 ml of dry benzene was refluxed for 7-8 hour using dean stark apparatus. The residue was washed with Sodium bicarbonate solution and the product was washed with water thoroughly and crystallized from alcohol to get solid crystals of pure (BC_1) [12-13] Yield- 82%, m.p. – 125-126^0 C.

PROPOSED SCHEME



Ar=phenyl

4-Chlorophenyl

4-Nitrophenyl

4-Methylphenyl

4-Methoxyphenyl

4-Hydroxyphenyl

Furaldehyde

2.4 Antimicrobial activity[8]

The antimicrobial activity of the synthesized compounds was determined by cup-plate method. The antibacterial activity was determined against gram-positive organism *Staphylococcus aureus* and gram-negative organism *Escherichia coli* at 50-mcg/ml and 75mcg/ml concentration of sample compounds. Dimethyl Formanide was used as control. The bacteria were subcultured on nutrient agar broth and incubated at 37^0 C for 18-24 hrs. Standard antibacterial drug Ciprofloxacin was also screened under similar conditions at 50-mcg/ml and 75mcg/ml concentration for comparison.

The antifungal activity was carried out against the fungi *Candida albicans* and *A. fumigatus* at 50 mcg/ml and 75mcg/ml concentration of sample compounds. The fungi were subcultured in Sabourod's dextrose agar medium. The fungal susceptibility testing was done by cup-plate method using Fluconazole (50 mcg/ml and 75mcg/ml concentration) as standard. The petri dishes were incubated a 37^0 C for18-24 hrs.

2.5 Antitubercular evaluation[9]

The antitubercular screening of synthesized compounds was carried out by middle brook 7H9 broth base (M198) medium against.

$H_{37}Rv$ strain at100 mcg/ml, 125 mcg/ml and 250mcg/ml. L J Medium containing standard drug as well as control. middle brook 7H9 broth base (M198) medium was inoculated with mycobacterium tuberculosis of $H_{37}Rv$ strain. The inoculated medium was incubated for 37^0 C for 6 weeks. At the end of 6 weeks the growth of mycobacterium tuberculosis was read.

Streptomycin (100 mcg/ml, 125 mcg/ml and 250mcg/ml) was used as a standard drug.

3. Result and Discussion[27-30]

The title compounds (BC_1- BC_7) were prepared from 2(2-substituted phenyl ethenyl) 1H benzimidazole by various steps. The structures of the compounds were confirmed by spectra and analytical studies. All the compounds synthesized matched with spectral data.

The compounds synthesized were screened mainly for the antitubercular activity by using middle brook 7H9 broth base media method using $H_{37}Rv$ strain. Compounds BC_1, BC_2, BC_3 and BC_4 have shown significant antitubercular activity when compared with the standard drug Streptomycin. Remaining compounds have also shown moderate antitubercular activity. (Table-2).

The compounds synthesized were also screened for antibacterial and antifungal activities by cup-plate method. Compounds BC_1, BC_5 and BC_6 have shown significant antibacterial activity against gram-negative organism *Escherichia coli* standard drug Ciprofloxacin was used and BC_1 and BC_6 have shown significant antifungal activity against *Candida albicans* standard drug was Fluconazole used. (Table-1).

It was noted that the compound which showed antitubercular activity were not good antibacterial and antifungal agents this may be explained on the basis of organism mycobacterial tuberculosis has different cell wall structure compared to bacteria and fungi.

All compounds found to be very good antitubercular agents & present synthesized compound can definitely as lead compound for future molecular manipulation studies.

Table-I Antibacterial and antifungal activity of synthesized compounds.

Compounds Code.	Zone of inhibition at 75 & 50 mg/ml (in mm.)							
	E.Coli		***S.aureus***		***A. Fumigatus***		***C. albicans***	
	75	50	75	50	75	50	75	50
BC_1	18	14	16	17	19	17	20	15
BC_2	10	5	11	10	10	08	11	06
BC_3	10	8	R	R	R	R	15	10
BC_4	6	8	11	8	R	R	11	10
BC_5	19	18	13	17	R	R	11	9
BC_6	18	17	12	19	19	15	16	14
BC_7	10	9	11	9	R	R	11	10
Ciprofloxacin	21	21	22	20	-	-	-	-
Fluconazole	-	-	-	-	23	24	23	23

Table-II Anti tubercular activity of the synthesized compounds.

Compound Code	100 mcg/ml	125 mcg/ml	250 mcg/ml
BC_1	S	S	S
BC_2	S	S	S
BC_3	S	S	S
BC_4	S	S	S
BC_5	R	S	S
BC_6	R	R	S
BC_7	R	S	S
Streptomycin	S	S	S

S denotes Significant and **R** denotes Resistant

3.1 Analytical Data Of The Synthesized Compounds

$\underline{BC_1}$ **:** IR(KBr) cm^{-1} : 3023(-O-H Str.), 2603 (-Ar-C-H Str.), 1689 (-C=O str.), 1414(-C-N Str.), 761(-Ar).

^1H NMR ($CDCl_3$) : 7.20-7.59 (9H of Ar), 3.32 (2H of CH_2). 12.36 (1H of OH) m.p. 125-126°C, yield 82%, mol. Wt. 278. $C_{17}H_{14}N_2O_2$. (Found C: 73.37 H:5.04 N: 10.06 required C : 73.38 H: 5.04 N : 10.07).

$\underline{BC_2}$: IR(KBr) cm^{-1} : 3031(-O-H Str), 2362(-Ar-C-H Str.), 1690 (-C=O str.), 1413 (-C-N Str.), 816 (-C-Cl- Str.).

^1H NMR ($CDCl_3$) : 7.38-7.64 (8H of Ar), 4.88 (2H of CH_2). m.p. 218-219°C, yield 80%, mol. Wt. 312.45 $C_{17}H_{13}N_2O_2Cl$. (Found C: 65.29 H:4.16 N:8.90 required C : 65.29 H: 4.16 N : 8.96).

$\underline{BC_3}$: IR(KBr) cm^{-1} : 3181 (-O-H Str), 2850 (-Ar-C-H str.), 1690(-C=O str.), 1521(-C-NO_2 Str.), 1425 (C-N Str) m.p. 186-187°C, yield 62%, mol. Wt. 323. $C_{17}H_{13}N_3O_4$ (Found C: 63.17 H:4.02 N:13.1 required C : 63.15 H: 4.02 N : 13.0).

$\underline{BC_4}$: IR(KBr) cm^{-1} : 3188 (-O-H free), 2963 (-Ar-C-H Str), 1666 (-C=O str.), 1447 (-C-N Str.) m.p. 159-160°C, yield 76%, mol. Wt. 294. $C_{17}H_{14}N_2O_3$. (Found C: 69.32 H:4.75 N:9.51 required C : 69.38 H: 4.76 N : 9.52).

$\underline{BC_5}$: IR(KBr) cm^{-1}: 2929 (-O-H free), 2841(-Ar-C-H Str), 1683(-C=O str.), 1431 (-C-N Str.), 1252 (-O-CH_3 Str)

^1H NMR : 4.84 (2H of CH_2), 3.81(3H of OCH_3), 6.99-7.63 (9H of Ar). m.p. 149-150°C, yield 63%, mol. Wt. 308. $C_{18}H_{16}N_2O_3$. (Found C: 70.12 H:5.18 N:9.09 required C : 70.12 H: 5.19 N : 9.09).

$\underline{BC_6}$: IR(KBr) cm^{-1} : 2922(-O-H Str.), 2363(-Ar-C-H Str), 1681(-C=O str.), 1420(-C-N Str.). m.p. 165-167°C, yield 94%, mol. Wt.292. $C_{18}H_{16}N_2O_2$ (Found C: 73.91, H: 5.42, N:9.58 required C : 73.97 H: 5.48 N : 9.58).

$\underline{BC_7}$: IR(KBr) cm^{-1} : 3357(-O-H Str.), 2927(-Ar-C-H Str.), 1682 (-C=O str.), 751(-C-H Def.)

^1H NMR : 7.56-7.72 (3H of Furan, 7.38-7.59 (4H of Ar), 4.86 (2H of CH_2). m.p. 228-229°C, yield 94%, mol. Wt.268. $C_{15}H_{12}N_2O_3$ (Found C: 67.15 H:4.45 N:10.43 required C : 67.16 H: 4.48 N : 10.44).

Table-III Characterization of data of compounds. [16-18]

Sr. No.	Compounds	Melting point	Yield %
1	BC_1	125-126	82
2	BC_2	218-219	80
3	BC_3	186-187	62
4	BC_4	159-160	76
5	BC_5	149-150	63
6	BC_6	165-167	94
7	BC_7	228-229	94

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