Microbiological Evaluation of 4-substituted-imidazolidine Derivatives

M. S. Y. KHAN, A. HUSAIN*, S. SHARMA AND M. RASHID
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi-110 062, India

In the present studies, two series of 4-substituted-imidazolidines (IIIa-i and IIIj,k) were synthesized by reacting different tetrahydro-di-Schiff bases (IIa-i and IIj,k) with *p*-diethylaminobenzaldehyde/ dimethylaminobenzaldehyde. The title compounds were evaluated for their antibacterial and antifungal actions against some selected microbes. The results of microbiological evaluation revealed that two compounds, 4-(1,3-bis(benzo[d][1,3]dioxol-5-ylmethyl)-4-methylimidazolidin-2-yl)-*N,N*-diethyl aniline (IIIj), 4-(1,3-bis(benzo[d][1,3]dioxol-5-ylmethyl)-4-methylimidazolidin-2-yl)-N,N-dimethyl aniline (IIIk) were good in their antibacterial as well as antifungal actions. Minimum inhibitory concentration values (*MIC*) of the compounds are reported.

Key words: Antibacterial, antifungal, di-Schiff base, imidazolidines

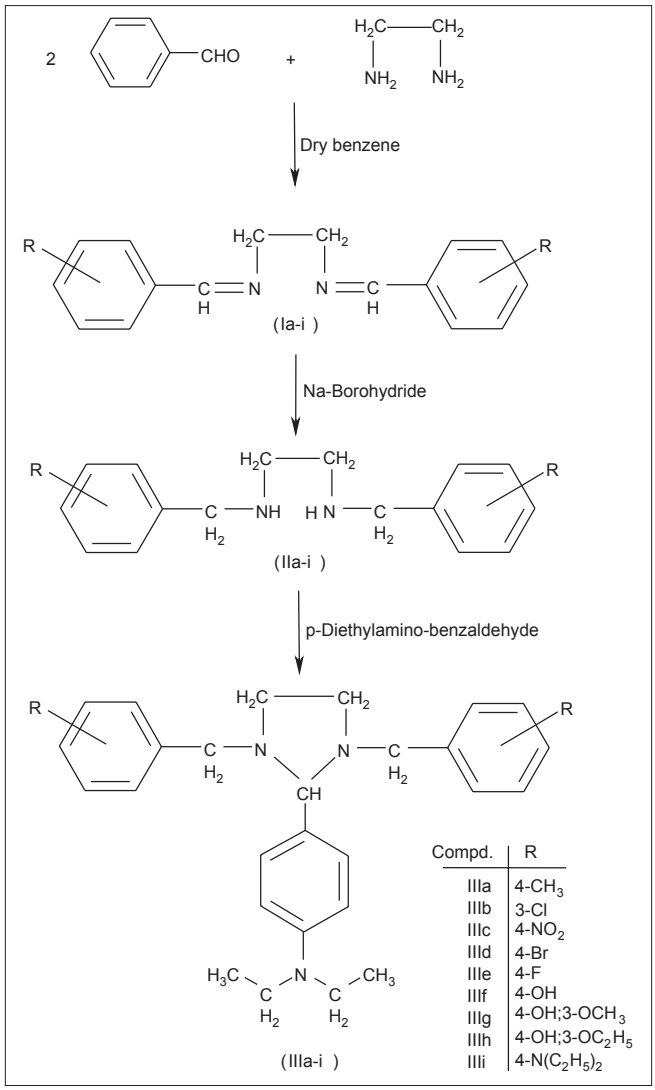
The demand for new antimicrobial agents is increasing due to the developing resistance towards commonly used antimicrobial agents. Also, the incidence of systemic bacterial and fungal infections has been increasing rapidly over the past few decades due to an increase in the number of immuno-compromised hosts[1]. Moreover, Immunosuppression due to malignancy, immunosuppressive therapies, HIV-infection, broad-spectrum antimicrobial treatment and age, as well as invasive procedures and mucosal barriers places patients at risk for bacterial and fungal infections[1,2]. These observations clearly indicate the need of as well as search for alternative new and more effective antimicrobial agents with a broad spectrum of activity.

Imidazole derivatives including saturated imidazoles (imidazolidines/ tetrahydroimidazoles) are important heterocycles due to their antibacterial, antitubercular, antifungal, antiHIV, antiinflammatory, analgesic, anticancer and anticonvulsant activities[3-9]. They have also been utilized as a versatile template for the synthesis of compounds with potential enzyme inhibition activities[10]. Literature survey revealed that not much work has been done on biologically active tetrahydroimidazoles[6-9]. Since imidazolidines show antimicrobial actions, and in continuation of our work on this nucleus[7-9], it was considered worthwhile to study antimicrobial potential of some newer 4-substituted-imidazolidines.

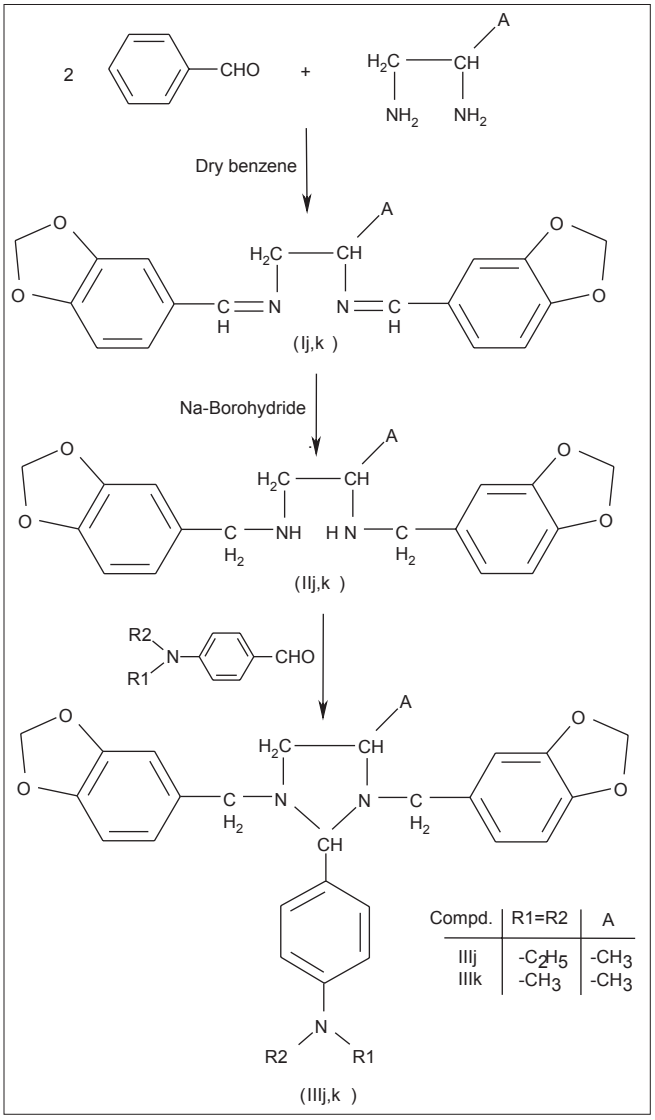
As shown in Schemes 1 and 2, synthesis of title compounds is based on the formation of di-Schiff bases (Ia-i and Ij,k) prepared by condensing two moles of arylaldehydes with ethylenediamine/1,2-diaminopropane. These di-Schiff bases on reduction with sodium borohydride furnished tetrahydro-di-Schiff bases (IIa-i and IIj,k) which on subsequent condensation with *p*-dimethylaminobenzaldehyde/*p*-diethylaminobenzaldehyde gave the corresponding 4-substituted-imidazolidines (IIIa-i and IIIj,k). The structures of the synthesized compounds were supported by spectroscopic data (^{1}H-NMR and MS) and elemental analysis results.

Melting points were taken on a liquid paraffin bath in open capillary tubes and are uncorrected. Progress of the reactions was monitored using TLC plates (silica gel G) in the solvent system benzene-ethanol (8:2). The spots were located by exposure to iodine vapors or under UV light. ^{1}H-NMR spectra of the compounds were recorded on a Bruker spectropsin DPX-300 MHz in $CDCl_3$. Mass spectra were recorded on LCMS/MS (Perkin-Elmer and LABINDIA, Applied Biosystem). Elemental analyses were performed on a Perkin-Elmer 240 analyzer and were in range of ±0.4% for each element analyzed (C,H,N). Dry solvents were used throughout. Di-Schiff bases (Ia-i and Ij,k) and tetrahydro-di-Schiff

***Address for correspondence**
E-mail: drasifhusain@yahoo.com



Scheme 1: Protocol for synthesis of title compounds (IIIa-i)



Scheme 2: Protocol for synthesis of title compounds (IIIj,k)

bases (IIa-i and IIj,k) were synthesized according to the literature method[7,8].

Tetrahydro-di-Schiff base IIa (2 mmol) was dissolved in ethanol (15 ml) and *p*-diethylamino-benzaldehyde was added in equimolar ratio. The reaction mixture was shaken for 5 h on a wrist action shaker and then left in a refrigerator for overnight. However, no crystalline product could be obtained and on processing it by concentrating to dryness the contents were poured into ice cold water which gave a solid mass. It was crystallized from methanol to give TLC pure 4-(1,3-Bis(4-methylbenzyl) imidazolidin-2- yl)-*N,N*-diethylaniline IIIa. Similarly compounds IIIb-i and IIIj,k were prepared (Table 1).

In general, ^{1}H-NMR spectra of the title compounds showed peaks of aromatic, diethyl/dimethyl, methylenes (2×CH_2 and CH) of imidazolidines ring and benzylic methylene protons. The multiplets at δ 2.4 and 3.1 and a singlet at δ 3.6 showed the presence of 2×CH_2 and CH of imidazolidines ring. Two doublets, each at δ 3.2 and 3.7, could be accounted for two benzylic methylenes. Protons of three substituted phenyl rings appeared in the region of δ 6.4-7.5. Mass spectra of the compounds showed molecular ion peaks in reasonable intensities. Microanalysis data were in range of ± 0.4% for the theoretical values of the element analyzed (C,H,N).

Antibacterial activity of the compounds was evaluated against *Staphylococcus aureus* (ATCC-29737), *Escherichia coli* (ATCC-8739), and *Pseudomonas aeruginosa* (NCLM-2035) bacterial strains at a concentration of 100 µg/ml by cup plate method[11].

TABLE 1: PHYSICAL CONSTANTS OF THE TITLE COMPOUNDS (IIIA-I AND IIIJ,K)

Compd	Substituent			MP (°)	Yield (%)	Molecular formula	Molecular weight
	R	R_1=R_2	A				
IIIa	4-CH_3	-	-	100-102	70	$C_{29}H_{37}N_3$	399
IIIb	3-Cl	-	-	148-150	58	$C_{27}H_{31}Cl_2N_3$	467
IIIc	4-NO_2	-	-	168-170	53	$C_{27}H_{31}N_5O_4$	489
IIId	4-Br	-	-	135-138	71	$C_{27}H_{31}Br_2N_3$	557
IIIe	4-F	-	-	143-145	56	$C_{27}H_{31}F_2N_3$	435
IIIf	4-OH	-	-	136-138	68	$C_{27}H_{33}N_3O_2$	431
IIIg	4-OH; 3-OCH_3	-	-	133-135	65	$C_{29}H_{37}N_3O_4$	491
IIIh	4-OH; 4-OC_2H_5	-	-	121-123	53	$C_{31}H_{41}N_3O_4$	519
IIIi	4-N(C_2H_5)$_2$	-	-	125-126	72	$C_{35}H_{51}N_5$	541
IIIj	-	-C_2H_5	-CH_3	106-108	60	$C_{30}H_{35}N_3O_4$	501
IIIk	-	-CH_3	-CH_3	74-76	67	$C_{28}H_{31}N_3O_4$	473

Compounds inhibiting growth of one or more of the above microorganisms were further tested for minimum inhibitory concentration (MIC). MICs were determined by broth dilution technique. A solution of the compounds was prepared in dimethylformamide (DMF) and a series of doubling dilutions prepared with sterile pipettes. To each of a series of sterile test tubes a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. The inoculum consisting of an overnight broth culture of microorganisms was added to the tubes. The tubes were incubated at 37° for 24 h and examined for turbidity. The lowest concentration (highest dilution) required to inhibit the growth of bacteria was regarded as MIC. Ciprofloxacin was used as standard drug for comparison (Table 2).

TABLE 2: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE COMPOUNDS (IIIA-I AND IIIJ,K)

Compound	Antibacterial activity[a]			Antifungal activity[a]	
	S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
IIIa	>100	>100	50	>100	>100
IIIb	25	50	>100	25	>100
IIIc	>100	>100	50	50	50
IIId	>100	>100	>100	50	>100
IIIe	50	>100	50	>100	50
IIIf	50	>100	50	>100	25
IIIg	>100	25	25	50	>100
IIIh	>100	50	>100	50	>100
IIIi	>100	>100	>100	50	>100
IIIj	50	25	>100	12.5	50
IIIk	25	12.5	25	12.5	25
Standard-1[b]	6.25	6.25	6.25	nt	nt
Standard-2[b]	nt	nt	nt	6.25	6.25

[a]The activity is expressed as corresponding minimum inhibitory concentration (MIC); [b]Standard-1 = Ciprofloxacin, Standard-2 = Griseofulvin; nt = not tested.

Antifungal activity of the synthesized compounds was determined against *Candida albicans* and *Aspergillus niger* by agar diffusion method[12]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 ml) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of corresponding species. Agar media (20 ml) was poured into each petridish. Excess of suspension was decanted and the plates were dried by placing in an incubator at 37° for 1 h. Wells were made using an agar punch and, each well was labelled accordingly. A control was also prepared in triplicate and maintained at 37° for 3-4 days. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6×10^4-6×10^4 c.f.u./ml. The cultures were incubated for 48 h at 37° and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentration (Table 2). The antifungal activity of the compounds was compared with the standard drug; Griseofulvin.

The antimicrobial screening data (Table 2) showed that compound IIIk showed good activity against *E. coli* and *C. albicans* with MIC of 12.5 µg/ml, and significant activity against *S. aureus*, *P. aeruginosa* and *A. niger* with MIC of 25 µg/ml. Another compound, IIIj, was good in its action against *C. albicans* with MIC of 12.5 µg/ml, and significant in its action against *E. coli* with MIC of 25 µg/ml. Similar type of activity was shown by the compound IIIb against *S. aureus* and *C. albicans*, by the compound IIIg against *E. coli* and *P. aeruginosa* and by the compound IIIf against *A. niger* with MIC of 25 µg/ ml. Rest of

compounds were moderate in their antimicrobial actions.

An analysis of antimicrobial test results revealed that the compounds having trisubstituted imidazolidine heterocyclic ring (IIIj and IIIk; Scheme 2) showed better activity as compared to that of the disubstituted imidazolidines (Scheme 1). Further, disubstituted phenyl rings on the imidazolidine heterocyclic ring showed slightly better activity as compared to that of the mono-substituted phenyl rings. Among the mono-substituted phenyl rings on the imidazolidine ring, presence of chloro, nitro and hydroxyl group showed significant activity.

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