Original Research Article

SYNTHESIS AND ANTIMICROBIAL EVALUATION OF CERTAIN ARYLAZO IMIDAZOLES

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ABSTRACT

Seven novel arylazo imidazoles were synthesized and structures have been elucidated on the basis of elemental analysis, IR and ^{1}H NMR spectral data. Antibacterial activity studies was done against two gram positive bacteria (*S. aureus, P.aeruginosa,*) and two gram negative bacteria (*E.coli and B.subtili*), while antifungal activity studies was done against *C.albicans and A.nigerz*. Minimum inhibitory concentration was found out by Serial dilution method. Antibacterial and antifungal activity studies of the title compounds (IIIa-IIIe) was compared with standards namely Furacin and Flucanazole. All the synthesized arylazo imidazoles have shown significant activity against the tested microbes. The compounds IIIb, IIIc and IIId exhibit relatively higher activity than the standards.

***Keywords*:** Arylazo imidazoles, Elemental analysis, IR spectra, ^{1}H NMR spectra, Antimicrobial screening.

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INTRODUCTION

Amino alkylation of an acidic proton next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine is called Mannich reaction. The final product β-amino carbonyl compound is known as a Mannich base [1]. Mannich bases and their derivatives have applications in many areas. They have been used in paint and polymer chemistry as hardeners, cross linkers, reaction accelerators [2,3]. However, the most important applications are in the field of pharmaceutical products [4,5]. Mannich bases can either directly be employed or used as intermediates in the synthesis of compounds of pharmaceutical importance.

A series of novel Mannich bases derived from secondary amines and cinchophen amide have been found to exhibit antimicrobial activity [6]. Pandeya et al.[7-9] have reported the synthesis and anti-HIV activity of Mannich bases of isatin. Sridhar et al.[10] have described the synthesis, antibacterial, antifungal and anti-HIV activities of norflaxacin Mannich bases. Koksal et al.[11] synthesised novel series Mannich bases of 5-nitro-3-substituted piperazino-methyl-2-benzoxazolinones that were found to demonstrate analgesic activities and anti inflammatory activities.Gahane et al.[12] have described the synthesis and anticonvulsant activity of 3-arylidene-2-phenylimino-4-thiazolidinones. The newly synthesized Mannich bases were tested for their anti-inflammatory and ulcerogenic activity. Barlin and Ireland [13] prepared di-Mannich bases which were found to be active antimalarials especially against chloroquine resistant isolate (K-I) of *Plasmodium falciparum*. Mannich bases are found to exhibit antihelmintic activity [14], CNS activity [15], anti-inflammatory activity [16], analgesic

activity [16], antitumor activity [17], cytotoxicity [18] etc. Prompted by above observations, the authors have synthesized a series of Mannich derivatives carrying imidazole moiety and evaluated for their antimicrobial activity. The details are presented in the following lines.

EXPERIMENTAL

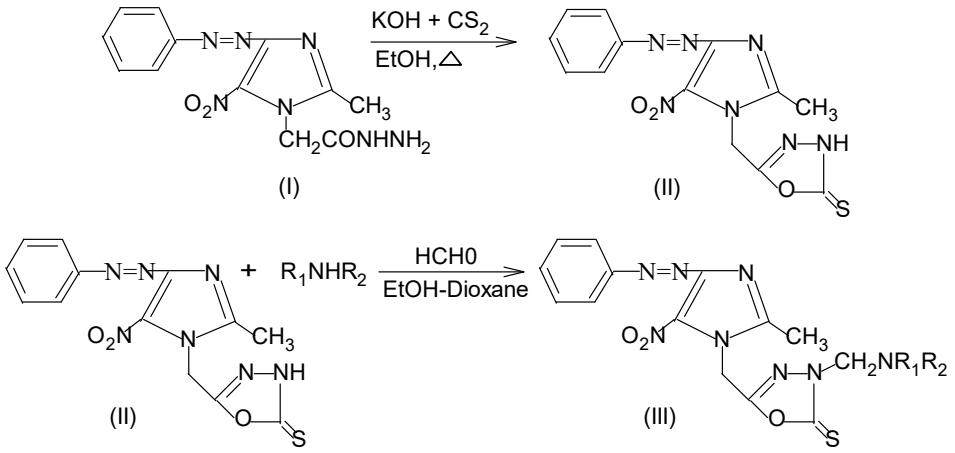
All the chemicals and reagents used were procured from Merck (India) Limited. The melting points of the newly synthesized compounds were determined in open capillaries and were uncorrected. The IR spectra were recorded on a Perkin-Elmer 983 IR spectrometer. The ^{1}H-NMR spectra were recorded on a Bruker AC 300F (200 MHz) NMR spectrometer using DMSO – d_6 or $CDCl_3$ as solvent and TMS as an internal standard. Mass spectra of the compounds were recorded on a Jeol JMS-D300 mass spectrometer operating at 70 eV. The purity of all the compounds was confirmed by TLC.

Synthetic procedure employed in the present studies

(2-methyl-5-nitro-4-phenyl azo-imidazol-1-yl)- aceticacid hydrazide I is synthesized by the procedure mentioned in the literature [19].

A mixture of (2-methyl-5-nitro-4-phenyl azo-imidazole-1-yl)-acetic acid hydrazide I (30.3gm, 0.1 mol), KOH (5.5 gm, 0.1 mol), ethanol (100 mL) and carbondisulphide (6.00 mL, 0.1 mol) taken in a round bottomed flask fitted with a water condenser was refluxed on a water bath till the evolution of hydrogen sulphide ceased. The excess of alcohol was removed by distillation. The reaction mixture was cooled to room temperature and the contents were poured into ice cold water and neutralized with dilute hydrochloric acid. The solid precipitated was filtered, washed with water and dried. The product so obtained was purified by recrystallization from ethanol-dioxane mixture to give 5-(2-methyl-5-nitro-4-phynylazo-imidazole-1-yl-methyl)-3H-{1,3,4} oxadiazole-2-thione II. (Scheme I).

A solution of 5-(2-methyl-5-nitro-4-phynylazo-imidazole-1-yl-methyl)-3H-{1,3,4} oxadiazole-2-thione II (0.01 mol) in absolute ethanol-dioxane mixture (1:1, 20 mL) was treated with formaldehyde 40% (1.5 mL). To this, appropriate amine (0.01 mol) in ethanol (10 mL) was added and reaction mixture was stirred over night. The precipitated Mannich base was collected by filtration, dried and recrystallization using ethanol-DMF mixture (1:1). The other compounds III b-g were obtained by similar procedure using the appropriate amine. Characterization data of these compounds are given in table 3.4. (Scheme I)



R_1=H, R_2=Phenyl, *p*-anisyl, *p*-fluorophenyl, *p*-chlorophenyl, *p*-bromophenyl, *p*-nitrophenyl, morpholinyl.

Scheme 1: Synthesis of Mannich bases III.

RESULTS AND DISCUSSION

The compound II and compounds of III series are characterized by elemental analysis, IR and ^{1}H NMR spectral data.

Characterization of 5-(2-methyl-5-nitro-4-phynylazo-imidazole-1-yl-methyl)-3H-{1,3,4} oxadiazole-2-thione II

The IR(KBr) spectrum of 5-(2-methyl -5-nitro-4-phenylazo-imidazol-1-yl methyl)-3H-[1,3,4]-oxadiazol-2-thione II exhibited characteristic band at around 3136 cm^{-1} (NH, str), 2873 cm^{-1} (CH, str), 1134 cm^{-1} (C=S), 1546 cm^{-1}(asymmetric str, nitro group), 1325 cm^{-1} (NO_2, symmetric str), 1615 cm^{-1} (N=N, str), and 3058 cm^{-1} (C_6H_5, str). The IR spectral data is furnished in Table1.

The ^{1}HNMR spectrum (200 MHz) of II was recorded in $CDCl_3$ + DMSO-d_6. The formation of 5-(2-methyl -5-nitro-4-phenyl azo-imidazol -1-yl methyl)-3H-[1,3,4]-oxadiazol-2-thione II was further confirmed by ^{1}HNMR spectral assignments. A broad signal due to thiol-thione tautomeric proton was observed at around δ 14.7. A sharp singlet was observed at 2.30 integrating for three protons of the methyl group while the CH_2 protons connecting the oxadiazole and imidazole moiety appeared as a singlet at 5.45 integrating for two protons. The aromatic protons appeared as a singlet at 6.60-7.20 integrating for five proton. The ^{1}HNMR data of II is compiled in Table1.

Table 1: Characterization data of 5-(2-methyl -5-nitro-4-phenylazo-imidazol-1-yl methyl)-3H-[1,3,4]oxadiazol-2-thione II

Elemental analysis data						
Molecular Formula	**Yield % m.p(^{O}C)**	**Elemental analysis found (Calculated)**				
		C	**H**	**N**	**O**	**S**
$C_{13}H_{11}N_7O_3S$	74 213-214	44.72 (45.21)	3.01 (3.21)	27.89 (28.39)	14.01 (13.90)	9.42 (9.29)
IR spectral data(υ max in cm^{-1})						
NH	**CH_3**	**C=S**	**NO_2**	**N=N**	**C_6H_5**	
3136	2873	1134	1546 1325	1615	3058	
^{1}HNMR spectral data (200 MHz)(solvent..$CDCl_3$+DMSO-d_6)(δ ppm)						
δ 2.30 (s, 3H, CH_3), 5.45 (s, 2H, NCH_2), 14.70 (s, thiol-thione tautomeric proton), 7.0 (m, 5H, C_6H_5).						

Characterization of 5-(2-methyl -5-nitro-4-phenylazo-imidazol-1-yl methyl)-3-phenyl amino methyl -3H-[1,3,4]oxadiazol-2-thione III a-g

The characterization data in particular elemental analysis data is given in Table 2.

The structures of these compounds III a-g were determined by IR, and ^{1}HNMR spectra.

The IR (KBr) spectra of 5-(2-methyl -5-nitro-4-phenyl azo-imidazol-1-yl methyl)-3-phenyl amino methyl -3H-[1,3,4]oxadiazol-2-thione IIIa exhibited characteristic bands around 1610; 1546, 1325; 2940; 1113; 3142; 1597 cm^{-1} due to N=N, asymmetric NO_2 stretching, symmetric NO_2 stretching, CH stretching, C=S, NH and C=N groups respectively. The IR spectral data is furnished in Table 3.

Table 2: Characterization data of 5-(2-methyl -5-nitro-4-phenyl azo-imidazol-1-yl methyl)-3-phenyl amino methyl -3H-[1,3,4]oxadiazol-2-thione III

Compd	-R_1	-R_2	Molecular Formula	Yield % m.p(^{0}C)	Elemental analysis found (Calculated)				
					C	H	N	S	Halogen
IIIa	H	phenyl	$C_{20}H_{18}N_8O_3S$	54 162	52.80 (53.32)	3.83 (4.03)	24.37 (24.87)	6.83 (7.12)	----
IIIb	H	*p*-anisyl	$C_{21}H_{20}N_8O_4S$	62 181	51.90 (52.49)	4.00 (4.20)	22.82 (23.32)	6.90 (6.67)	----
IIIc	H	*p*-fluorophenyl	$C_{20}H_{17}FN_8O_3S$	65 156	50.78 (51.28)	3.46 (3.66)	23.52 23.92)	6.96 (6.84)	4.16 (4.06)
IIId	H	*p*-chlorophenyl	$C_{20}H_{17}ClN_8O_3S$	46 143	49.04 (49.54)	3.33 (3.53)	22.61 (23.11)	6.91 (6.61)	7.21 (7.31)
IIIe	H	*p*-bromophenyl	$C_{20}H_{17}BrN_8O_3S$	52 173	44.88 (45.38)	3.04 (3.24)	20.63 (21.17)	6.12 (6.06)	15.16 (15.09)
IIIf	H	*p*-nitrophenyl	$C_{20}H_{17}N_9O_5S$	59 169	47.98 (48.48	3.26 (3.46	24.94 (25.44	6.55 (6.47)	----
IIIg	-	Morpholinyl	$C_{18}H_{20}N_8O_4S$	56 174	48.14 (48.64)	4.32 (4.54)	24.81 (25.21)	7.15 (6.86)	----

Table 3: IR spectral data of Mannich bases

compd	R_1	R_2	υ max in cm^{-1}					
			NO_2	CH (str)	C=S	N=N	C=N	NH
IIIa	H	C_6H_5	[1546 1325]	2945	1113	1610	1599	3147
IIIb	H	p-Anisyl	[1546 1325]	2950	1113	1610	1601	3152
IIIc	H	*p*-fluorophenyl	[1546 1325]	2955	1113	1610	1603	3157
IIId	H	*p*-chlorophenyl	[1546 1325]	2960	1113	1610	1601	3162
IIIe	H	*p*-bromophenyl	[1546 1325]	2965	1113	1610	1605	3167
IIIf	H	*p*-nitrophenyl	[1546 1325]	2948	1113	1610	1609	3172
IIIg	--	morpholinyl	[1546 1325]	2956	1113	1610	1602	3177

The ^{1}HNMR spectra (200 MHz) of III a-g were recorded in $CDCl_3$+DMSO-d_6. The data is shown in the Table 4. The formation of Mannich base is indicated by the signal at around δ 11-13 due to thiole-thione tautomeric proton. The ^{1}HNMR spectrum of IIIb showed a sharp signal

at δ 2.40 integrating for three protons of the methyl group. The N-CH_2-N protons appeared at δ 5.60 as a singlet was integrating for two protons, while the CH_2 protons connecting the oxadiazole and imidazole moiety appeared as a singlet at δ 4.95 integrating for two protons. The signal due to NH proton appeared as broad singlet at δ 11.10 integrating for one proton. The phenyl group attached to azo group has appeared as a singlet at δ 7.25 integrating for five protons. The 4-methoxyphenyl group in the Mannich base was noticed as two doublets at δ 6.97 and δ 7.47 and each doublet corresponds to two protons. The ^{1}HNMR data of typical compounds of IIIa-g is compiled in Table 4.

Table 4: ^{1}HNMR data of Mannich bases III

compd	R_1	R_2	^{1}HNMR(200 MHz)(solvent..$CDCl_3$+DMSO-d_6) (δ ppm)
IIIa	H	C_6H_5	δ 2.50 (s, 3H, CH_3), 5.50 (s, 2H, N-CH_2-N), 4.96 (s, 2H,N-CH_2), 10.23 (s, 1H, NH), 7.50 (m, 5H, C_6H_5), 7.25 (s, 5H, C_6H_5).
IIIb	H	*p*-anisyl	δ 2.40 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 5.62 (s, 2H, N-CH_2-N), 5.06 (s, 2H, N-CH_2), 11.10 (s, 1H, NH) 6.97 (d, 2H, Aromatic protons), 7.47 (d, 2H, Aromatic protons), 7.25 (m, 5H, C_6H_5).
IIIc	H	*p*-fluoroanisyl	δ 2.49 (s, 3H, CH_3), 5.47 (s, 2H, N-CH_2-N), 4.95 (s, 2H, N-CH_2), 11.22 (s, 1H, NH), 7.26 (m, 5H, C_6H_5), 7.4 (d, 2H, Aromatic protons),7.50 (d, 2H, Aromatic protons).
IIIf	H	*p*-nitrophenyl	δ 2.55 (s, 3H, CH_3), 5.55 (s, 2H, N-CH_2-N), 4.98 (s, 2H, N-CH_2), 11.25 (s, 1H, NH), 7.29 (m, 5H, C_6H_5), 7.48 (d, 2H, Aromatic protons),7.60 (d, 2H, Aromatic protons).
IIIg	-	morpholinyl	δ 2.50 (s, 3H, CH_3),5.49 (s, 2H, N-CH_2-N), 4.95 (s, 2H, N-CH_2), 11.19 (s, 1H, NH), 7.24 (m, 5H, C_6H_5), 2.62 (t, 4H, -CH_2-N-CH_2), 3.70 (t, 4H, -CH_2-O-CH_2).

Evaluation of antimicrobial activity

The antibacterial activity of the newly synthesized Mannich bases was carried out against four different pathogenic organisms, namely Staphylococcus aureus and Bacillus subtilis(Gram positive) and Escherichia coli and Pseudomonas aeruginosa (Gram negative). Antifungal activity study was carried out against the fungi namely, *Candida albicans* and ***A. niger*** The MIC values of the compounds in the present investigation have been assessed by serial dilution method [20].

Determination of Minimum Inhibitory Concentration

The test compound is dissolved in dimethylformamide (5 mL) to prepare a stock solution of concentration 1000 µg/mL. One loop full of an 18 hour broth culture was inoculated into 5 mL of nutrient broth and was incubated at 37°C for 4 hours. An assay was prepared by diluting with labeled test tubes numbered 1-11. An aliquot of 0.5 mL stock solution of test compound was added to the first tube. The solution was mixed well and 0.5 mL of this solution was transferred into second tube. This process was repeated serially to obtain the quantities indicated in each of the test tubes. The eleventh tube was taken as growth control. Drops of diluted broth culture of the test organism (approximately 0.5 mL) were added into all tubes using a sterilized pasteur pipette. The solutions were mixed gently and the

incubation was carried out at 37°C for 16-18 hours. The concentration at which there was no turbidity was taken as minimum inhibitory concentration. The results of antimicrobial studies are also given in Table 5.

Table 5: Antibacterial and Antifungal activity data of compounds III

Compound	Antibacterial activity (MIC in μg/mL)				Antifungal activity data	
	S. aureus	***P.aeruginosa***	***E.coli***	***B.subtilis***	***C. albicans***	***A. niger***
IIIa	--	--	--	--	--	--
IIIb	0.25	0.25	0.50	0.25	0.25	0.25
IIIc	0.25	0.25	0.50	0.25	0.125	0.125
IIId	0.25	0.25	0.125	0.25	0.125	0.125
IIIe						
IIIf	0.25	0.25	0.25	0.25	0.25	0.25
IIIg	0.25	0.25	0.25	0.25	0.25	0.25
Furacin (Std)	0.25	0.25	0.25	0.5	-----	----
Flucanazole (Std)	---	---	----	----	0.25	0.25
Solvent control (DMF)	----	----	----	----	----	----

Among the compounds tested for antibacterial activity, all the compounds showed significant activity comparable with that of standard and in the case of compound III d, the activity was much higher against *E. coli*. Similarly, all the compounds showed activity comparable to that of the standard drug furacin. Compounds IIIb and IIIc showed the activity at a much lower concentration than that of the standard drug flucanazole.

CONCLUSION

Antibacterial (against *S. aureus, P.aeruginosa, E.coli and B.subtili*) and antifungal (against *C.albicans and A.nigerz*) activity studies of the synthesized Mannich bases (IIIa-IIIe) was compared with Furacin and Flucanazole to reveal the potency of the synthesized compounds. Antimicrobial screening revealed that compounds exhibit moderate activity when compared to standard. The compounds IIIb, IIIc and IIId exhibit relatively higher activity against than the standards.

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