Synthesis and antimicrobial studies of some Mannich bases carrying imidazole moiety

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Starting from 2-methyl-4-nitro-imidazole, new 5-(2-methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazole-2-thione (3) was synthesized and was subjected to Mannich reaction with appropriate amines to yield a new series of 3-substituted aminomethyl-5-(2-methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazole-2-thiones (4a-j). The structure of the title compounds was elucidated by elemental analysis and spectral data. The newly synthesized Mannich bases were screened for their antibacterial and antifungal activity. Many of these compounds exhibited potent antifungal activity.

Keywords: imidazole derivatives, Mannich bases, oxadiazole, antibacterial activity, antifungal activity

The most important applications of Mannich bases are in the field of pharmaceutical products (1, 2). Studies have revealed that Mannich bases show good anticancer (3), antimycobacterial (4), remarkable anti-HIV and antitubercular activities (5). The presence of the basic Mannich side-chain has shown marked antimalarial (6), anti-inflammatory, analgesic (7, 8) and antimicrobial activities (9). Imidazole nucleus is also a major component of a variety of drugs such as angiotensin II receptor antagonists, oral antiflammatory agents, protein kinase inhibitors and fungicides (10). It is frequently found as a part of a large number of biologically and medicinally significant substances (11, 12).

There are not many reports describing the synthesis and biological studies of Mannich bases bearing imidazole moiety. From this point of view, the present study successfully describes a general and convenient method for the synthesis of a series of 3-substituted aminomethyl-5-(2-methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazole-2-thiones and depicts the antimicrobial activity of the newly synthesized Mannich bases.

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EXPERIMENTAL

Melting points were determined in open glass capillaries using a VEEGO, VMP-D digital melting point apparatus (Veego, India) and are uncorrected. Elemental analyses were performed on a Perkin-Elmer 2400 analyzer (Perkin-Elmer, USA) and were found within \pm 0.4 % of the theoretical values. The IR spectra were recorded as KBr discs using a JASCO FTIR 4100 spectrometer (Jasco, Japan). ¹H NMR spectra were recorded on a Bruker Avance II 400 NMR spectrometer (Bruker Instruments Inc., USA) using CDCl₃ or DMSO-*d*₆ as solvent and TMS as internal standard. Mass spectra were measured on a JEOL GCMATE II mass spectrometer (Jeol, Japan).

2-Methyl-4-nitro imidazole, ethyl choloroacetate, ciprofloxacin and fluconazole were procured from Sigma-Aldrich, USA. All other chemicals used in the study were of analytical grade.

Synthetic procedures

2-Methyl-4-nitro-1-imidazo-ethyl acetate (1). – A mixture of 2-methyl-4-nitro-imidazole (12.7 g, 0.1 mol), ethyl chloroacetate (10.7 mL, 0.1 mol) and potassium carbonate (20.4 g, 0.15 mol) in dry acetone (200 mL) was refluxed for 50 h. The reaction mixture was filtered hot and the solvent was distilled off from the filtrate. The crude ester 1 thus obtained was purified by recrystallization from absolute ethanol.

2-Methyl-4-nitro-1-imidazo-acethydrazide (2). – A mixture of ester (1) (21.3 g, 0.1 mol) and 99 % hydrazine hydrate (4.9 mL, 0.1 mol) in absolute ethanol (50 mL) was refluxed for 8 h. On cooling, the solution gave a solid mass of hydrazide **2**, which was collected by filtration, and recrystallized from absolute ethanol.

5-(2-Methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazole-2-thione (3). – A mixture of 2-methyl-4-nitro-1-imidazo-acethydrazide (2) (19.9 g, 0.1 mol), KOH (5.6 g, 0.1 mol), carbon disulphide (6.0 mL, 0.1 mol) in absolute ethanol (100 mL) was placed in a round-bottomed flask fitted with a water cooled condenser and 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 to ice-cold water and neutralized with dilute hydrochloric acid. The solid precipitated was filtered, washed thoroughly with water and dried. The product was further purified by recrystallization from the ethanol-dioxane mixture (1:1) to give **3**.

3-Substituted aminomethyl-5-(2-methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazole-2-thiones (4*a*–*j*). – A mixture of the solution of 5-(2-methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazole-2-thione (3) (2.41 g, 0.01 mol) in absolute ethanol and dioxane mixture (1:1, 20 mL) was treated with formaldehyde (40 %, 1.5 mL, 0.05 mol). Later, the appropriate amine (0.01 mol) in absolute ethanol (10 mL) was added with stirring and the reaction mixture was stirred overnight. The precipitated Mannich base was collected by filtration and dried. Recrystallization was done from the ethanol-DMF mixture (1:1) to give compounds 4*a*–*j*.

Antimicrobial studies

The antimicrobial activity of the newly synthesized Mannich bases was checked against three fungal strains, viz., Candida albicans, Trichophyton rubrum and Trichophyton

mentagrophytes and two pathogenic bacteria, *viz., Staphylococcus aureus* and *Streptococcus pyogenes*. Fluconazole and ciprofloxacin were used as standards. The synthesized compounds and standards were dissolved in DMSO to give a concentration of 1000 µg mL⁻¹. The minimum inhibitory concentration (*MIC*) was determined by the two-fold tube dilution method (13). Dilutions of test and standard compounds were prepared in Sabouraud dextrose broth for fungi and nutrient broth for bacteria. The samples were incubated at 25 °C for 48 hours for *C. albicans*, 5–7 days at 20–22 °C for *T. rubrum* and *T. mentagrophytes* and at 37 °C for 24 hours for bacteria. At the end of the incubation period, *MIC* values were recorded as the lowest concentrations of substances that showed no visible turbidity.



Scheme 1.

RESULTS AND DISCUSSION

Chemistry

In the present work, a series of Mannich bases has been synthesized. Elimination reaction of 2-methyl-4-nitro-1*H*-imidazole with ethyl chloroacetate in the presence of po-

Compd.	R ₁	R ₂	Molecular formula (M _r)	Yield (%) M. p. (°C)	Elemental analysis (%) Found/calcd.		
					С	Н	Ν
1			C ₈ H ₁₁ N ₃ O ₄	80	45.05/	5.15/	19.69/
1	_	-	(213.19)	109–111	45.03	5.16	19.70
2		-	$C_6H_9N_5O_3$	66	36.17/	4.53/	35.13/
			(199.17)	197–199	36.15	4.52	35.15
3	-	-	$C_7H_7N_5O_3S$	65	34.82/	2.90/	29.02/
			(241.24)	242-243	34.83	2.91	29.04
4a	н	C_6H_5	$C_{14}H_{14}N_6O_3S$	87	48.52/	4.03/	24.24/
	11		(346.36)	150-152	48.50	4.04	24.25
4b	Η	$C_6H_4C_2H_5$ -	$C_{16}H_{18}N_6O_3S$	63	51.29/	4.80/	22.43/
		р	(374.42)	136–137	51.28	4.81	22.44
4c	Η	C_6H_4F -p	$\mathrm{C}_{14}\mathrm{H}_{13}\mathrm{N}_{6}\mathrm{O}_{3}\mathrm{FS}$	71	46.12/	3.59/	23.04/
			(364.36)	199–121	46.11	3.57	23.05
4d	Η	C_6H_4Cl-p	$C_{14}H_{13}N_6O_3ClS$	65	44.11/	3.42/	22.05/
			(380.81)	135–137	44.12	3.41	22.06
4e	Н	C_6H_4Br-p	$\mathrm{C}_{14}\mathrm{H}_{13}\mathrm{N}_{6}\mathrm{O}_{3}\mathrm{BrS}$	51	39.50/	3.05/	19.73/
			(425.26)	142–144	39.51	3.06	19.75
4f	Н		СНИОЗ	54	11 12 /	3 71 /	24.18/
			(405.39)	200-202	44 40	3.70	24.107
		NO2	(100.07)	200 202	11110	011 0	
4g	_	$(C_6H_5)_2$	$C_{20}H_{18}N_6O_3S$	60	56.82/	4.25/	19.87/
			(422.46)	125–127	56.81	4.26	19.88
4h	_	NM	C12H17N7O2S	51	42.45/	5.02/	28.86/
			(339.37)	204-206	42.43	5.01	28.88
4i	_	N_N-CH ₃	$C_{13}H_{19}N_7O_3S$	50	44.15/	5.36/	27.72/
			(353.40)	193–195	44.14	5.38	27.73
		\frown	C14Ha1N=OaS	55	45.71/	5.73/	26.66/
4j	-	N_C2H5	(367.43)	130–132	45.72	5.72	26.67

Table I. Physical and analytical properties of the new compounds

tassium carbonate and dry acetone led to the formation of ethyl (2-methyl-4-nitro-1*H*-imidazol-1-yl) acetate (1). The resulting ester was subjected to hydrazinolysis to yield a solid mass of 1-[2-(hydrazinooxy)-2-oxoethyl]-2-methyl-4-nitro-1*H*-imidazole (2), which when refluxed with KOH and carbon disulphide in ethanol gave new 5-[(2-methyl-4-nitro-1*H*-imidazol-1-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (3). New Mannich bases (4a-j) were obtained by the reaction of 1,3,4-oxadiazole with appropriate amines in the presence of 40 % formaldehyde (Scheme 1) in moderate to good yields.

The newly synthesized compounds were characterized by spectral data and elemental analysis. In the IR spectra of compounds **4a-j**, the absorption band due to the thione (C=S) group was observed at 1100–1150 cm⁻¹. In the ¹H NMR spectra of these Mannich bases, the signal due to thione proton around δ 13–15 ppm was absent, indicating the formation of a Mannich base. In a typical example, the ¹H NMR spectrum of **4d** showed a sharp singlet at δ 2.26 ppm integrating for three protons of the methyl group of imidazole moiety. The -N-CH₂-N- protons appeared at δ 5.66 ppm as a singlet integrating for two protons, while the CH₂ protons connecting the oxadiazole and imidazole moiety appeared as a singlet at δ 5.21 ppm integrating for two protons. The *ortho-* and *meta*-protons of *p*-chlorophenyl moiety appeared as two doublets centered at δ 7.38 and 7.67 ppm, each integrating for two protons. The imidazole 5-H proton appeared as a singlet at δ 8.09 ppm integrating for one proton. The signal due to the NH proton appeared as a broad singlet at δ 10.13 ppm integrating for one proton.

Further evidence for the proposed structure of Mannich bases was obtained by recording their mass spectra. In most cases, the molecular ion peaks were very weak indicating that the molecular ions were not stable (Scheme 2). The fragmented peaks observed in these cases are in conformity with their respective assigned structures. The fragmentation paths for these compounds are shown in Scheme 2. Elemental analysis data together with IR, ¹H NMR and MS data supported the proposed structures for compounds **1-3** and **4a-j** (Tables I and II).

Structure and antimicrobial activity

Newly synthesized 3-substituted aminomethyl-5-(2-methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazole-2-thiones (**4a-j**) were examined for antimicrobial activity and the reults are summarized in Table III. The antifungal screening results revealed that Mannich base **4b-j** exhibited higher to moderate activity against *C. albicans* and *T. mentagrophytes* compared to standard fluconazole. Compound **4a** with phenyl substituent showed the least activity.

All aminomethyl-5-(2-methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazole-2-thiones possessing different substituent groups (**4b-j**), except **4a**, showed higher inhibitory activity against *C. albicans* than the reference fluconazole. It is worth noting that compound **4c** with *p*-fluorophenyl substituent and **4f** with 2-methyl-4-nitrophenyl substituent showed 5-fold better *in vitro* activity against *C. albicans with MIC* 172 and 154 µmol L⁻¹, respectively, compared to the standard drug fluconazole (*MIC* 816 µmol L⁻¹). Besides, compound **4h** (*MIC* 368 µmol L⁻¹) integrating piperazinyl group exhibited 2-fold better activity than the standard drug fluconazole.

Furthermore, the compounds with ethyl, fluoro and bromo groups at *para* position in phenyl substituent (**4b**, **4c** and **4e**), with 2-methyl-4-nirophenyl substituent (**4f**), and

Compd.	IR $(v_{\max,} \text{ cm}^{-1})$	¹ H NMR (400 MHz, δ , ppm) (DMSO-d ₆)	DART MS (m/z)
1	2980 (CH), 1728 (C=O), 1533 (N=C)	1.28 (t, 3H, CH ₃), 2.34 (s, 3H, CH ₃), 4.22 (q, 2H, CH ₂), 5.01 (s, 2H, N-CH ₂), 8.19 (s, 1H, H-5 imidazole)	213 (M+H)+
2	3151 (NH), 2962 (CH), 1686 (C=O), 1598 (N=C)	2.33 (s, 3H, CH ₃), 4.71 (s, 2H, N-CH ₂), 5.08 (s, 2H, NH ₂), 8.18 (s, 1H, H-5 imidazole), 9.50 (s, 1H, CONH)	199 (M+H)+
3	3142 (NH), 2952 (CH), 1620 (N=C), 1135 (C=S)	2.40 (s, 3H, CH ₃), 5.38 (s, 2H, N-CH ₂), 8.16 (s, 1H, H-5 imidazole), 14.33 (s, 1H, NH)	242 (M+H, bp)+
4a	3151 (NH), 2976(CH), 1595 (N=C), 1133 (C=S)	2.34 (s, 3H, CH ₃), 5.09 (s, 2H, N-CH ₂), 5.61 (s, 2H, N-CH ₂ -N), 7.25 (t, 1H, ArH), 7.38 (t, 2H, ArH), 7.60 (d, 2H, ArH), 8.04 (s, 1H, H-5 imidazole), 10.23 (s, 1H, NH)	346 (M+H)+
4b	3133 (N-H), 2959 (C-H), 1542 (N=C), 1139 (C=S)	2.33 (s, 3H, CH ₃), 2.51 (q, 2H, CH ₂), 2.63 (t, 3H, CH ₃), 5.27 (s, 2H, N-CH ₂), 5.63 (s, 2H, N-CH ₂ -N), 7.32 (d, 2H, ArH), 7.60 (d, 2H, ArH), 8.25 (s, 1H, H-5 imidazole), 10.13 (s, 1H, NH)	374 (M+H)+
4c	3150 (N-H), 2946 (C-H), 1551 (N=C), 1133 (C=S)	2.33 (s, 3H, CH ₃), 5.11 (s, 2H, N-CH ₂), 5.66 (s, 2H, N-CH ₂ -N), 7.15 (t, 2H, ArH),7.65 (d, 2H, ArH), 8.11 (s, 1H, H-5 imidazole), 10.78 (s, 1H, NH)	365 (M+H)+
4d	3152 (N-H), 3049 (C-H), 1539 (N=C), 1131 (C=S)	2.26 (s, 3H, CH ₃), 5.21 (s, 2H, N-CH ₂), 5.66 (s, 2H, N-CH ₂ -N), 7.38 (d, 2H, ArH), 7.71 (d, 2H, ArH), 8.09 (s, 1H, H-5 imidazole), 10.13 (s, 1H, NH)	381 (M+H)+
4e	3132 (N-H), 2956 (C-H), 1543 (N=C), 1140 (C=S)	2.33 (s, 3H, CH ₃), 5.26 (s, 2H,N-CH ₂), 5.67 (s, 2H, N-CH ₂ -N), 7.38 (d, 2H, ArH), 7.71 (d, 2H, ArH), 8.23 (s, 1H, H-5 imidazole), 10.21 (s, 1H, NH)	425 (M+H)+
4f	3121 (N-H), 3076 (C-H), 1534 (N=C), 1147 (C=S)	2.20 (s, 3H, CH ₃), 2.34 (s, 3H, CH ₃), 5.49 (s, 2H, N-CH ₂), 5.52 (s, 2H, N-CH ₂ -N), 7.03 (s, 1H, ArH), 7.48 (s, 1H, ArH),7.96 (s, 1H, ArH), 8.37 (s, 1H, H-5 imidazole), 10.24 (s, 1H, NH)	405 (M+H)+
4g	2940 (C-H), 1593 (N=C), 1107 (C=S)	2.36 (s, 3H, CH ₃), 5.18 (s, 2H, N-CH ₂), 5.84 (s, 2H, N-CH ₂ -N), 7.20 – 7.89 (m, 10H, ArH), 8.05 (s, 1H, H-5 imidazole)	423 (M+H)+
4h	2939 (C-H), 1596 (N=C), 1112 (C=S)	2.46 (s, 3H, CH ₃), 2.55 (t, 4H, CH ₂ -piperazine), 3.26 (t, 4H, CH ₂ -piperazine), 4.97 (s, 2H, N-CH ₂), 5.53 (s, 2H, N-CH ₂ -N), 8.09 (s, 1H, H-5 imidazole), 8.34 (s, 1H, NH-piperazine)	339 (M+H)+
4i	2935 (C-H), 1595 (N=C), 1102 (C=S)	1.36 (s, 3H, CH ₃), 5.03 (s, 3H, NCH ₃), 2.42 (m, 4H, CH ₂ -piperazine), 2.75 (m, 4H, CH ₂ _ piperazine), 4.97 (s, 2H, N-CH ₂), 5.53 (s, 2H, N-CH ₂ -N), 8.19 (s, 1H, H-5 imidazole)	353 (M+H)+
4j	2940 (C-H), 1591(N=C), 1116 (C=S)	1.37 (s, 3H, CH ₃), 4.90 (q, 2H, piperazine-N-CH ₂), 1.20 (t, 3H, piperazine-CH ₃), 2.41 (m, 4H, CH ₂ -piperazine), 2.77 (m, 4H, CH ₂ _ piperazine), 4.95 (s, 2H, N-CH ₂), 5.53 (s, 2H, N-CH ₂ -N), 8.18 (s, 1H, H-5 imidazole)	368 (M+H)+

Table II. Spectral data of new compounds

with methyl piperazinyl (4i) or ethyl piperazinyl unit (4j), showed higher activity against *T. mentagrophytes* (*MIC* values ranging between 86–340 µmol L^{-1}) compared to fluconazole (*MIC* 408 µmol L^{-1}). Among these, compound 4c exhibited about 5-times better antifungal activity with *MIC* value 86 µmol L^{-1} . Nevertheless, all the tested compounds exhibited weaker activity than fluconazole against *T. rubrum*. In case of antibacterial activity, almost all the compounds showed weaker activity than



Mass fragmentation pattern

Scheme 2.

	MIC (µmol L ⁻¹)						
Compd.	L	Antifungal acti	Antibacterial activity				
-	C. albicans	T. rubrum	T. mentagrophytes	S. pyogenes	S. aureus		
4a	1443	1443	1443	2887	1443		
4b	668	334	334	1335	334		
4c	172	343	86	686	343		
4d	657	657	1313	657	1313		
4e	588	294	294	1175	294		
4f	154	308	308	308	308		
4g	592	1184	1184	592	1184		
4h	368	737	737	368	184		
4i	707	354	354	354	354		
4j	680	340	340	340	340		
Ciprofloxacin	_	-	-	94	189		
Fluconazole	816	200	408	_	_		
Solvent control (DMSO)	_	-	_	_	_		

Table III. Antimicrobial activity of synthesized Mannich bases

ciprofloxacin against *S. pyogenes* and *S. aureus*. However, piperazinyl substituted compound **4h** with *MIC* value 184 µmol L⁻¹ showed promising activity against *S. aureus* compared to the standard antibiotic ciprofloxacin (*MIC* 189 µmol L⁻¹).

CONCLUSIONS

A series of new 3-substituted aminomethyl-5-(2-methyl-4-nitro-1-imidazomethyl)-1,3,4-oxadiazole-2-thiones (**4a-j**) have been synthesized and tested for their antifungal and antimicrobial activities. The results revealed that most of the compounds exhibited moderate to good activity against *C. albicans*, *T. mentagrophytes* and *S. aureus*. Compounds **4b-j** exhibited excellent antifungal activity against *C. albicans* with low *MIC* values. In addition, compound **4c** with fluoro substitution exhibited about 5-fold enhanced activity against *T. mentagrophytes* compared to fluconazole. The antifungal strength of certain tested compounds was more pronounced than their antibacterial activities. Thus, the promising activities and easy synthesis of Mannich bases carrying imidazole moiety make them very attractive antifungal leads. Further, a complete structure activity relationship and mechanistic approach could contribute to the development of new antimicrobial drugs.

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