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Synthesis and Antimicrobial Evaluation of Some New Mannich Bases Bearing 1,3,4-Oxadiazoline Ring System

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Abstract: *A Series of 3-[(phenylamino)methyl]-5-(2,3,4-trichlorophenyl)-1,3,4-oxadiazole-2(3H)-thione (6a-l) and 3-[(alkylamino)methyl]-5-(2,3,4-trichlorophenyl)-1,3,4-oxadiazole-2(3H)-thione (7a-l)* ***(Scheme 3)*** *were synthesized by treating 1,3,4-oxadiazol-2-thione(5) with primary/secondary amines and formaldehyde. 1,3,4-oxadiazol-2-thione(5)* ***(Scheme 2)*** *was prepared by refluxing hydrazide with carbon disulfide and KOH in ethanol. 2,3,4-trichlorophenyl Hydrazide* ***(Scheme 1)*** *was synthesized from 2,3,4-trichloroacetophenone using the procedure reported in literature. The structures of newly synthesized compounds are characterized by elemental analysis, IR, ^{1}H-NMR and mass spectroscopic studies and were screened for their antimicrobial activities. The preliminary results revealed that some of the compounds exhibited promising antimicrobial activities.*

Keywords: *Mannich Bases, antibacterial, antifungal, 1, 3, 4-oxadiazole-5-thione*

1. INTRODUCTION

1,3,4-oxadiazole derivatives constitute an important class of heterocyclic compounds. They possess anti-inflammatory[1,2] antibacterial[3,4], antifungal[5,6], anticonvulsant[7] analgesic[8], anticancer[9,10,11] anti-tubercular[12] and[13] and hypolipidemic[14] activities.

1,3,4-oxadiazoles posses wide variety of uses, in particular as biologically active compounds in medicine and in agriculture as dye stuffs, UV absorbing and fluorescent materials, heat resistant polymers and scintillators. Substituted 1,3,4-oxadiazole derivatives are of considerable pharmaceutical interest.eg, 2-Amino-1,3,4-oxadiazoles act as muscle relaxants[15]. 5-Aryl-2-hydroxymethyl-1,3,4-oxadiazole derivatives[16] have shown analgesic, anti-inflammatory, anticonvulsant, diuretic and antiematic properties. 2-Hydroxyphenyl-1,3,4-oxadiazole acts as a hypnotic and a sedative[17] drug. Some medicinal applications of 1,3,4-oxadiazoles are in the area of photosensitizers[18], liquid crystals[19] and organic light emitting diodes[20] (OLED).

Therapeutic agents such as Raltegravir a HIV-integrase inhibitor, Furamizole an antibacterial, Tiodazosin and Nesapidil which are antihypertensive agents are based on 1,3,4-oxadiazole moiety

Mannich bases have been reported as potential biological agents. They find application as antitubercular[21] antimalarial[22] vasorelaxing[23] anticancer[24] and analgesic drugs[25]. They are also used in polymer industry as paints and surface active agents [26]

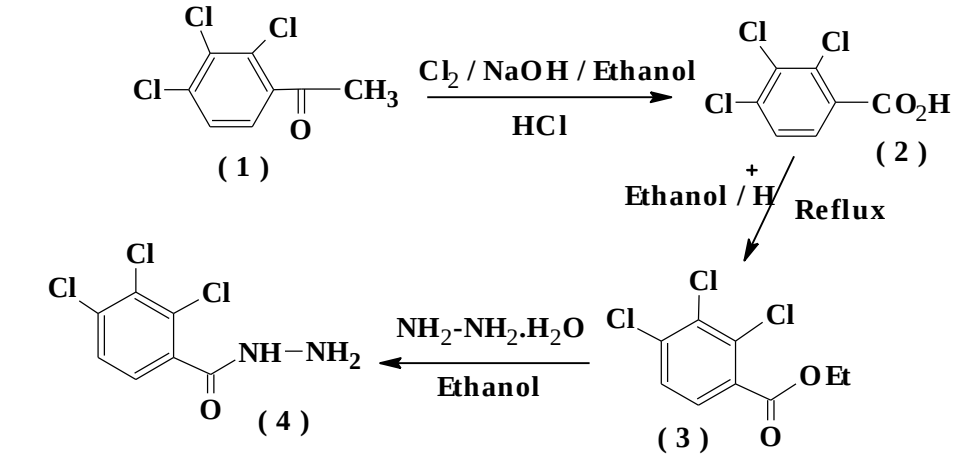
Prompted by the varied biological activities of 1,3,4-oxadiazole derivaties and Mannich bases, it was decided to synthesize some Mannich bases derived from 1,3,4-oxadiazol-2-thione bearing 2,3,4-trichlorophenyl moiety.

2. MATERIALS AND METHODS

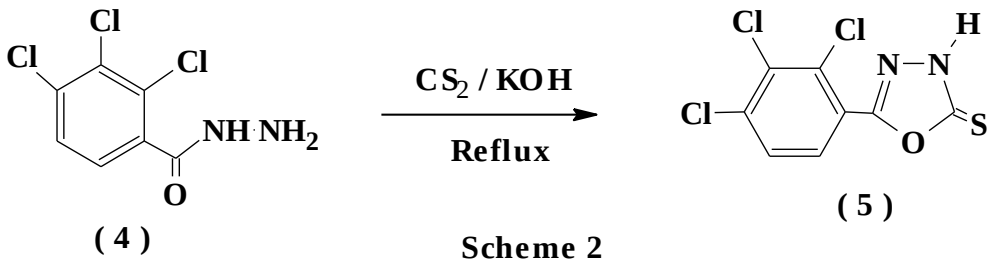
The chemicals 2,3,4-trichloroacetophenone, hydrazine hydrate, carbondisulfide and primary and sec. amines used for the synthesis of novel oxadiazolthione mannich bases were procured from Sigma-Aldrich, Bengaluru, India. Melting points of the compounds **(6a-l & 7a-e)** were determined in open capillary tubes and are uncorrected. The purity of synthesized compounds was checked by TLC observing single spot on Merck silica gel 60 F_{254} coated alumina plates. The structures of (**6a- l &7a-e**) were confirmed by spectral studies. The IR spectra (cm^{-1}) were recorded on a **Shimadzu-FTIR 577** Infrared spectrophotometer in KBr pellets. The ^{1}H-NMR was recorded on a **Brucker AMX-400(400 MHz)** spectrometer using $CdCl_3$-*d* as solvent and TMS as the internal standard.

FAB Mass spectra were recorded on a JEOL SX 102/DA-6000 Mass Spectrometer using argon/xenon (6 kv, 10 mA) as the FAB gas

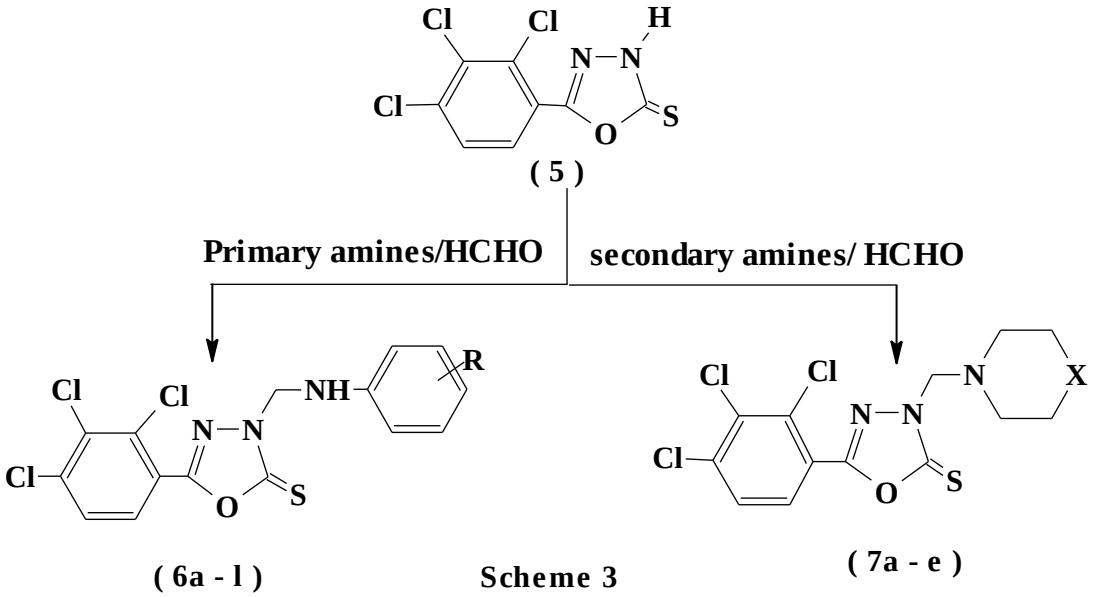
2.1. Reaction Scheme



Scheme 1



Scheme 2



Scheme 3

Table1. *Characterization Data of The Compounds (6a-L)*

Compd.	R_1	MF	M.W	Yield (%)	M.P (^{0}C)	Found (Calculated)		
						C	H	N
28	--	$C_8H_3N_2OSCl_3$	280	85	185-190	35.73 34.28	2.22 1.07	11.15 10.00
6a	4--H	$C_{15}H_{10}Cl_3N_30S$	386.68	76	156-159	48.33 46.58	2.74 2.58	10.96 10.87
6b	2-CF_3	$C_{16}H_9N_3Cl_3OSF_3$	454.68	81	117-12	42.41 42.25	2.17 1.98	9.42 9.24
6c	4-Br	$C_{15}H_9N_3OSBr$	465.58	88	174-178	38.79 38.68	2.14 1.93	9.27 9.02
6d	4--F	$C_{15}H_9N_3Cl_3OSF$	404.67	91	143-147	44.67 44.51	2.41 2.22	10.45 10.38
6e	4-Cl	$C_{15}H_9C_4N_3OS$	421.12	73	187-191	42.91 42.77	2.33 2.13	10.26 9.98
6f	4-NO_2	$C_{15}H_9C_{l3}N_4O_3S$	431.68	68	166-171	41.93 41.72	2.25 2.08	13.24 12.98
6g	2,5-Cl_2	$C_{15}H_8N_3C_{l5}OS$	455.57	77	152-155	40.26 39.53	1.91 1.75	9.47 9.22
6h	2,4,5-Cl_3	$C_{15}H_7C_{l6}N_3OS$	490	59	160-164	36.92 36.75	1.63 1.42	8.75 8.57
6i	4-CH_3	$C_{16}H_{12}Cl_3N_3OS$	400	74	112-115	48.32 47.95	3.27 2.99	10.64 10.49
6j	4-OCH_3	$C_{16}H_{12}Cl_3N_3OS$	416	83	183-185	46.34 46.11	2.97 2.88	10.25 10.08
6k	2-NO_2-4-Cl	$C_{15}H_8C_{l4}N_4O_3S$	465.81	88	171-174	40.03 39.83	2.01 1.77	12.52 12.39
6l	3-Cl-4-F	$C_{15}H_8Cl_4N_3OSF$	438.81	69	159-162	41.35 41.02	2.04 1.82	9.73 9.57

Table2. *Characterization Data Of The Compounds (7a-E)*

Compd	X	MF	M.W	Yield (%)	M.P (^{0}C)	Analysis (%)Found (Calculated)		
						C	H	N
7a	CH-CO_2Et	$C_{17}H_{18}Cl_3N_3O_3S$	450.35	73	192-195	45.34 45.29	4.34 3.99	9.55 9.32
7b	O	$C_{13}H_{12}Cl_3N_3O_2S$	380.35	81	177-183	42.23 41.01	3.31 3.15	11.26 11.04
7c	CH_2	$C_{14}H_{14}Cl_3N_3OS$	378.35	78	133-137	44.61 44.40	3.98 3.70	11.23 11.10
7d	N-CH_3	$C_{13}H_{15}Cl_3N_4OS$	381.35	76	166-172	41.98 40.90	4.31 3.93	14.79 14.68
7e	NH	$C_{13}H_{13}Cl_3N_4O_3S$	379.35	87	145-149	42.77 41.12	3.63 3.42	14.91 14.76

2.2. General procedure for the preparation of 2,3,4-trichlorobenzoic acid (2)

2,3,4-Trichloroacetophenone **(24)** (0.1 mol) and alcoholic KOH (0.1 mol) was taken in the conical flask. Chlorine gas was passed in to the flask with continuous stirring for 12h. It was then neutralized with conc. HCl, filtered and dried. It was recrystallised from ethanol. Yield 89%. m.p.187-188^{0}C [CAS;50-75-9]

2.3. General procedure for the synthesis of Ethyl 2,3,4-trichlorobenzoate (3)

2,3,4-Trichlorobenzoic acid **(26**) (0.01mol) was refluxed with absolute alcohol in presence of two drops of conc. Sulfuric acid for 24 h. Later it was neutralized with sodium bicarbonate and extracted

with ether. The solvent was distilled off and the product was recrystallised from ethanol. Yield 76%. m.p.166-167^{0}C [CAS;86569-80-4]

2.4. General procedure for the synthesis of 2,3,4-Trichlorobenzoyl hydrazine (4)

Ethyl 2,3,4-trichloroethylbenzoate **(26)** (0.01 mol) was refluxed with hydrazine hydrate (0.01 mol) in ethanol for 4 h. Excess of solvent was distilled off and poured in to water to get the title compound **(27**). It was recrystalised from ethanol. Yield 83%. m.p.76-78 ^{0}C

2.5. General preparation of 5-(2,3,4-trichlorophenyl)-1,3,4-oxadiazol-2-thione (5)

A mixture of 2,3,4-trichlorobenzoyl hydrazine **(27)** (0.1 mol), carbon disulphide (0.2 mol) and potassium hydroxide solution (30%, 5 ml) was refluxed on a water bath for 2h. The reaction mixture was cooled, acidified and the product separated was purified by recrystallization from ethanol. Yield 85%, m.p.185-190 ^{0}C

2.6. Synthesis of 3-(aminomethyl)-5-(2,3,4-trichlorophenyl)-1,3,4-oxadiazol-2-thiones (6a-l) & (7a-f).

A mixture of 5-(2,3,4-trichlorophenyl)-1,3,4-oxadiazol-2-thione **(28)** (1mmol), formaldehyde (2 mmol) and a primary/secondary amine (1 mmol) was taken in ethanol-diaoxane mixture and stirred at room temperature for 6h. Later the reaction mixture was poured in to ice cold water and the separated solid was recrystalised from ethanol. The characterization data of these compounds are given in Table4.

Compound(4). IR (KBr, cm^{-1}): 3306, 3207(NH_2 & NH), (Ar-H), 1659(C=O), 1631 & 1578(C=C) 825 & 803(C-Cl); ^{1}H-NMR ($CDCl_3$, δ): δ 3.46(s, 2H, NH_2), 9.21(s, 1H, NH), δ 7.33 & 7.45(2d, 2H, *J*=8 Hz, 2,3,4-trichlorophenyl); FAB MS (*m/z*, %): 238(M^+, 13), 239(M^++1, 100), 240 (M+2, 25), 242 (M+4, 12) & 244(M+6, 6).

Compound(5). IR (KBr, cm^{-1}): 3032(Ar-H), 3062 & 2916 (NH/SH), 1587& 1498 (C=N, C=C) 829 & 767(C-Cl); ^{1}H-NMR ($CDCl_3$, δ): 14.20(s, 1H, NH/SH), 7.52 & 7.75(2d, 2H, *J*=8.8 Hz, 2,3,4-trichlorophenyl); FAB MS (*m/z*, %): 280(M^+, 25), 281(M^++1, 100), 282(M+2, 31), 284(M+4, 26) & 286(M+6, 9), 249(15), 238(11), 207(26).

Compound(6a). IR (KBr, cm^{-1}): 3313(NH), 3031(Ar-H), 2925 & 2854(C-H, CH_2), 1602 & 1527(C=N, C=C) 821 & 747(C-Cl); ^{1}HNMR ($CDCl_3$, δ): 6.83(s, 1H, NH), 6.81-7.25(m, 5H, phenyl), 7.48 & 7.67(2d, 2H, *J*=8.8 Hz, 2,3,4-trichlorophenyl); FAB MS(*m/z*, %): 385(M^+, 46), 387(M+2, 50), 389(M+4, 69), 369(100), 391(M+6, 59), 307(47).

Compound(6c). IR (KBr, cm^{-1}): 3335(NH), 3095 & 3023(Ar-H), 2925 & 2854(C-H), 1596 & 1517(C=N, C=C) 821 & 747(C-Cl); ^{1}HNMR ($CDCl_3$, δ): 6.83(s, 1H, NH), 5.54(d, 2H, *J*=2.8 Hz, N-CH_2-N), 6.82 and 7.29(2d, 4H, 4-bromophenyl), 7.52 & 7.70 (2d, 2H, *J*=8.8 Hz, 2,3,4-trichlorophenyl); FABMS(*m/z*, %): 463(M^+, 46), 465(M+2, 48), 467(M+4, 40) & 469(M+6, 26), 391(100), 307(45), 289(22), 281(22), 219(18).

Compound(6e). IR (KBr, cm^{-1}): 3315(NH), 3025(Ar-H), 2923(C-H), 1599, 1518 & 1493(C=N, C=C) 818 & 770(C-Cl); ^{1}H-NMR ($CDCl_3$, δ): 5.54(s, 1H, NH), 5.15(d, 2H, *J*=7.6 Hz, N-CH_2-N), 6.85 & 7.17(2d, 4H, 4-chlorophenyl), δ7.51 & 7.70(2d, 2H, *J*=8.4 Hz, 2,3,4-trichlorophenyl); FAB MS(*m/z*, %): 419(M^+, 89**)**, 421(M+2, 94), 423(M+4, 50) & 425(M+6, 21), 418(M-1, 49), 391(100), 391(92), 307(67), 289(54), 283(33).

Compound(7a); IR (KBr, cm^{-1}): 3081(Ar-H), 2983 & 2858(C-H), 1721(C=O), 1589 & 1442(C=N, C=C) 858, 820 & 767(C-Cl); ^{1}H-NMR ($CDCl_3$, δ): 5.11(s, 2H, N-CH_2-N), 4.14 (q, 2H, CH_2 of ethyl), δ 1.23(t, 3H, CH_3 of ethyl), 1.67-3.75 (m, 9H, piperidine), 7.54 & 7.76 (2d, 2H, *J*=8.8 Hz, 2,3,4-trichlorophenyl); FAB MS(*m/z*, %): 449(M^+, 53), 451(M+2, 37), 453(M+4, 25) & 455(M+6, 10), 323(100),184(22), 170(100), 448(M-1, 26), 323(38).

Compound(7b). IR (KBr, cm^{-1}): 3067(Ar-H), 2963 & 2847(C-H), 1596 & 1444(C=N, C=C) 860, 829 & 767(C-Cl); ^{1}H-NMR ($CDCl_3$, δ): 5.10(s, 2H, N-CH_2-N), 2.87 & 3.71(2t, 8H, morpholine), 7.55 & 7.77(2d, 2H, *J*=8.8 Hz, 2,3,4-trichlorophenyl); FAB MS(*m/z*, %): 379(M^+, 92), 381(M+2, 58), 383(M+4, 51), 385(M+6, 36), 378(M-1, 50), 307(100), 289(18), 253(37).

3. BIOLOGICAL ACTIVITY STUDIES

3.1. Antibacterial Activity

The newly synthesized compounds **(6a-l)** and **(7a-f)** were screened for their antibacterial activities against *E. coli* (ATTC-25922), *S. aureus* (ATTC-25922), *P. aeruginosa (ATTC-27853)* and *K. pneumoniae* bacterial strains by the disc diffusion method[27, 28] Discs measuring 6.25 mm in diameter were punched from Whatman no.1 filter paper. Batches of 100 discs were dispensed to each screw capped bottles and sterilized by dry heat at 140 ^{0}C for an hour. The test compounds were prepared with different concentrations using *N, N* dimethyl formamide. Exactly 1 mL containing 100 times the amount of chemical in each disc was added to each bottle, which contains 100 discs. The discs of each concentration were placed in nutrient agar medium inoculated with fresh bacterial strains separately. The plates were incubated at 37^{o}C for 24h. Ciprofloxacin was used as a standard drug. Solvent and growth controls were kept separately and the zone of inhibition was measured. The results of such studies are given in Table 3

3.2. Antifungal Activity

All the newly synthesized compounds **(6a-l)** and **(7a-f)** were screened for their antifungal activity against *C. albicans* **(**NICM No.300*), A. fumigatus* (NICM No.902), *A. flavus* (NICM No.524) and *T. mentagrophytes* (recultured) in DMSO by serial dilution method[29, 30] Sabourauds agar (prepared by dissolving peptone (1g), D-glucose (4g) and agar (2g) in distilled water (100 ml) and adjusting the pH to 5.7) was used as medium for fungal growth. Normal saline was used to make spore suspension of fungal strains (i.e., a loopful of particular fungal stain was transferred to 3 ml of saline in order to obtain a suspension of corresponding species). Prepared Sabourauds agar media (20 ml) was poured in to each Petri dish. Excess of media was decanted and the plates were dried by placing in an incubator for 1h. Wells were made on these seeded agar plates using an agar punch and labeled. A 10µg/ml solution of the test compound in DMSO was then added in to each of these labeled wells. A control was also prepared in the same way using DMSO. The Petri dishes were then incubated at 37 ^{0}C for 3-4 days. The diameter of the inhibition zone and the minimum inhibitory concentrations (MIC) were determined in comparison with the standard drug Ciclopiroxolamine. The results of antifungal studies are given in Table 4

Table3. *Antibacterial Activity Data Of Compounds (6a-L) And (7a-F)*

Compound	Diameter of the inhibition zone (in mm)			
	S. aureus	***P. aeruginosa***	***K. Pneumoniae***	***E. coli***
6a	16	15	21	16
6b	**18**	**17**	**24**	**19**
6c	12	14	10	10
6d	17	16	20	13
6e	16	11	14	17
6f	**19**	**17**	**23**	**20**
6g	15	15	10	13
6h	14	17	11	10
6i	17	10	10	12
6j	13	10	15	12
6k	**18**	**17**	**25**	**18**
6l	**19**	**18**	**22**	**19**
7a	**18**	**18**	**24**	**18**
7b	16	15	19	11
7c	12	10	13	17
7d	**19**	**25**	**25**	**18**
7e	13	15	16	17
Standard	19	18	25	20

Table4. *Antifungal activity data of compounds (6a-l) and (7a-f)*

Compound	Diameter of the inhibition zone (in mm)			
	A. fumigatus	***A. flavus***	***C. albicans***	***P. marneffei***
6a	13	15	11	17
6b	**22**	**17**	**19**	**20**
6c	17	11	15	13
6d	13	10	17	12
6e	15	11	14	11
6f	**22**	**18**	**20**	**18**
6g	14	17	13	13
6h	**19**	**16**	**15**	**17**
6i	11	11	15	13
6j	**22**	**17**	**20**	**19**
6k	12	17	12	17
6l	**22**	**17**	**19**	**18**
7a	**21**	**18**	**19**	**19**
7b	17	11	12	12
7c	**20**	**17**	**19**	**20**
7d	**22**	**17**	**18**	**20**
7e	13	18	11	12
Standard	22	18	20	20

4. RESULT AND DISCUSSION

The antibacterial screening revealed that among the tested compounds **(6a-l) and (7a-f)**, **6b, 6f, 6k, 6l, 7a** and **7d** showed excellent antibacterial activity against all the bacterial strains. The remaining compounds were found to be resistant or moderately active against all the bacterial strains. Similarly the antifungal screening data revealed that among the tested compounds **6b, 6f, 6h, 6j, 6l, 6a, 6c** and **7d** showed excellent antifungal activity against all the tested fungal strains. The remaining compounds were found to be resistant or moderately active against all the tested fungal strains.

5. CONCLUSION

This article reports the successful synthesis and antimicrobial evaluation of some new Manncih bases derived from 1,3,4-oxadiazoline ring system having 2,3,4-trichlorophenyl moiety. The antimicrobial activity results indicated that **6f, 6k, 6l, 7a** and **7d** showed the most promising antibacterial and **6b, 6f, 6h, 6j, 6l, 6a, 6c** and **7d** antifungal activity. Thus these compounds can be recommended antimicrobial agents.

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