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Synthesis, Characterization and Antimicrobial activity of some new Mannich bases derived from 4-amino triazoles.

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ABSTRACT

The increasing clinical importance of drug-resistant fungal and bacterial pathogens has lent additional urgency to microbiological research and new antimicrobial compound development. For this purpose, new a series of Mannich base such as N- [(di substituted-amino) methyl]-N- (4H-1, 2, 4-triazole-4-yl) benzamide 2(a-f) were synthesized by condensation of the 4H-1, 2,4-triazole-4-yl) benzamide with formaldehyde and secondary amine. The chemical structures of these compounds were elucidated by IR, 1H-NMR and elemental analysis. All compounds were screened in vitro for their antibacterial and antifungal activity. The compound 2(c), 2(d) was found to have potent antibacterial and anti-fungal activity.

Keywords: 4-amino Triazole, Mannich bases, Antibacterial activity, Antifungal activity, MIC value

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INTRODUCTION

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decade's revealed substantial medical need for new classes of anti microbial agents. There is real perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms of action, which are distinct from those of well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant. 1,2,4-triazoles represent an overwhelming and rapid developing field in modern heterocyclic chemistry. From literature it is predictable that 1, 2, 4-triazoles represent important pharmacophore, and play a vital role as medicinal agents. A degree of respectability has been bestowed for 1, 2, 4-triazoles derivatives due to their wide range of biological activities such as antimicrobial [l, 2, 3], analgesic, anti-inflammatory [4, 5], anticancer [6] and antioxidant properties [7]. Ribavirin (antiviral) [8], Rizatriptan (antimigraine) [9] Posaconazole, Fluconazole and Itraconazole **[**10, 11], that are efficient antifungal drugs used in current treatment of fungal infection.

Therefore we have synthesized a new class of mannich bases containing triazole ring and evaluated for antimicrobial and antifungal activities. The structures of the compounds synthesized were assigned on the basis of elemental analysis and spectral analysis.

MATERIALS AND METHODS

All chemicals were obtained from Jain chemicals Moradabad. All chemicals and solvents used were of analytical grade.

Experimental Section

All the reagents and solvents used were of laboratory grade Melting point were determined in open capillaries and are uncorrected. The reactions were monitored by TLC and spots are detected by UV chamber and also using iodine as visualizing agent . The IR spectra were recorded on a Dike miracle FT-IR spectrophotometer from Alkem pharmaceutical Baddi. The 1HNMR were recorded on a Bruker Avance II 400 MHz spectrometer using DMSO-d6 as a solvent and a tetra methyl silane (TMS) as an internal standard and expressed in δ ppm from Panjab University (Chandigarh). The compounds were also subjected to C, H and N analysis (Carlo-Erba) at CDRI Luck now. All the solvents (AR grade) and reagents were purified and dried according to the procedures given in Vogel's Textbook of Practical Organic Chemistry. The synthesis of compounds shown in **Scheme 1.**

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Synthesis and Characterization of Compounds

Synthesis of 4H - (1, 2, 4-triazole-4-yl) benzamide (1)

4-Amino triazole (0.01mole) was dissolved in ethyl methyl ketone. Benzoyl chloride (0.01mole) and 10% NaOH solution was added dropwise with constant stirring for 2 hrs on ice bath. The reaction mixture was poured in ice cold water. The product was filtered , dried and recrystallized from ethanol to give compound **(1).** The completion of the reaction was monitored by TLC and purified by coloumn chromatography on silica gel (60-120 mesh) eluting with ethyl acetate: methanol (5:3). The yield was found to be 78%.

General method for the synthesis of N - (di substituted amino) methyl (4H-1,2,4-triazole-4-yl) benzamide (2a-f)

4H-(1,2,4-triazole -4-yl)benzamide (0.01mole) was dissolve in ethanol (25 ml). Formaldehyde (5 ml) and 2^0 amine (0.01mole) was added dropwise and refluxed for 12 hrs. The crude product was filtered, dried and recrystallized from ethanol to furnish compound (2a-f).

N-(di phenyl amino) methyl - N- (4H-1,2,4-triazole-4-yl) benzamide, (2a)

IR (KBr Cm^{-1}), 1773 (C=O), 3000-3200 (CH_2), 1449 -1565 (C=N, C=C), 3050-3000 (Ar-H)

^{1}HNMR δ 7.8-8.0(s, 2H, CH triazole), 3.3 (s, 2H, CH2), 6.6-7.1 (m, 10H, biphenyl), 7.4-7.8(m, 5H, Aromatic ring).

N-(di methylamino) methyl - N- (4H-1, 2, 4-triazole-4-yl) benzamide, (2b)

1716 (C=O), 1450-1534 (C=C,C=N), 2996-3284 (CH_2), 2850-2960 [N(CH3)2]
^{1}HNMR δ 7.9-8.0 (s, 2H, CH triazole), 3.4-3.5 (s, 2H, CH2) , 0.8-1.1 (s, 6H, methyl), 7.4-7.6 (m, 5H, Aromatic ring),

N-(morphilino methyl) - N - (4H-1, 2, 4-triazole-4-yl) benzamide, (2c)

IR (KBr Cm^{-1}), 1716 (C=O), 1456-1590 (C=C, C=N), 2886-3296 (CH_2)
^{1}HNMR δ 8.03 (s, 2H, CH triazole), 4.2 (s, 2H, CH2) , 2.1-2.8 (m, 8H, morpholine), 7.2-7.6 (m, 5H, Aromatic ring),

N-(piperazine-1-yl) methyl-N-(4H-1, 2, 4 - triazole-4-yl) benzamide, (2d)

IR (KBr Cm^{-1}), 1712 (C=O), 1435-1572 (C=C, C=N), 2880-3290 (CH_2), 3649 (NH)
^{1}HNMR δ 7.9-8.0 (s, 2H, CH triazole), 3.5(s, 2H, CH2) , 2.0-2.9 (m, 8H, piperazine), 7.4-7.5 (m, 5H, Aromatic ring)

N-(4-ethyl piperazin-1-yl) methyl-N-(4H-1, 2, 4-triazole-4-yl) benzamide, (2e)

IR (KBr Cm^{-1}), 1679(C=O), 2668-2828 (CH2), 3071 (N-C2H5)
^{1}HNMR δ 8.0 (s, 2H, CH triazole), 3.8 (s, 2H, CH2) , 2.1 (s, 8H, piperazine),7.4-7.9 (m, 5H, Aromatic ring),1.0-2.0 (m,5H, CH2CH3)

N-(di ethyl amino) methyl-N-(4H-1, 2, 4-triazole-4-yl) benzamide, (2f)

IR (KBr Cm^{-1}), 1682(C=O), 3071[$N(CH_3CH_2)_2$], 2668-2828 (CH_2), 1419-1582 (C=C,C=N)
^{1}HNMR δ 8.1 (s, 2H, CH triazole), 4.0 (s, 2H, CH2), 1.02-2.64 (m, 10H, C2H5), 7.4-7.9 (m, 5H, Aromatic ring)

RESULTS AND DISCUSSION

The starting compound namely 4H-(1, 2, 4-triazole-4-yl) benzamide synthesized from the reaction of 4 amino triazole and benzoyl chloride. The reaction of (1) with formaldehyde and different secondary amine was carried out to get of N-(di substituted amino)methyl (4H-1,2,4-triazole-4-yl)benzamide 2(a-f).

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Table 1. Physical and analytical data of compounds

S.No.	NR_1R_2	Rf value	M.P. ^0C	% yield
2a	Di Phenyl amine	0.93	280-282	68%,
2b	Di methyl amine	0.68	220-223	66%
2c	Morpholine	0.72	179-81	73%
2d	Piperazine	0.65	238-242	75%
2e	N-ethyl piperazine	0.61	243-247	70%
2f	Di ethyl amine	0.82	252-256	63%

Table 2. Elemental analysis data of compounds

S.No.	Molecular formula	Molecular weight	% Analysis Found (calcd)		
			C%	H%	N%
2a	$C_{22}H_{19}N_5O$	369.41	69.52(69.49)	4.18(4.15)	16.96(15.94)
2b	$C_{12}H_{15}N_5O$	245.28	56.76(56.72)	5.14(5.08)	25.55(25.52)
2c	$C_{14}H_{17}N_5O_2$	287.31	56.52(56.50)	4.96(4.93)	23.37(23.35)
2d	$C_{14}H_{18}N_6O$	286.33	55.73(54.71)	5.34(5.32)	27.32(27.28)
2e	$C_{16}H_{22}N_6O$	314.38	51.13(51.09)	6.05(6.03)	25.73(25.71)
2f	$C_{14}H_{19}N_5O$	273.33	50.52(48.52)	6.01(5.54)	24.62(24.58)

ANTIMICROBIAL STUDIES

The zone of inhibition of the synthesized compound 2(a-f) were determined by agar diffusion technique [12,13]. The organism tested were Streptococus pyrogen (NCIM-2608), Bacillus aureus (NCIM-2797), Micrococus leuteus (NCIM-2704), Streptococus epidermis (NCIM-2493), Clostridium sporogen (NCIM-2559), Klebsiella pneumonia (NCIM-2957), Salmonella typhimurium (NCIM-2501), Pseudomonas aeruginosa (NCIM-2863), Serratia marcesens (NCIM-2078) and Proteus vulgaris (NCIM-2813) for antibacterial activity and Gibberella fujikuroi (NCIM-655), Rhizopus oligosporus (NCIM-1215), Neurospora crassa (NCIM-908), Aspergillus niger (NCIM-618), Candida albican (NCIM-3557) for antifungal activity. The agar media were inoculated with test organism and a solution of test compound 50 µg/ml and 100 µg/ml in DMSO. DMSO 10 µg/ml was separately in cups (8 mm diameter) in the agar medium. Streptomycin (50 µg /ml) and (100 µg /ml), ampicillin (50 µg /ml) and (100 µg /ml), and fluconazole (5 µg /ml) and (10 µg /ml), were used as a reference for antibacterial and antifungal activity respectively. The zones of inhibition were measured after 24 hrs incubation.

For determination of MIC value [14] by serial plate dilution method five milligrams of each test compounds were dissolved in 1 mL of dimethylsulfoxide (DMSO) separately to prepare stock solution. From stock solution different concentrations 50 µg /ml, 25 µg /ml, 12.5 µg /ml, 6.25 µg /ml, 3.12 µg /ml and 1.56 µg /ml of each compound were prepared. Thus, proper amounts of the different concentrations of compounds were pipette on the blank disks, which were placed on the plates. The plates were incubated at 37^0C for 24 h. The minimum

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inhibitory concentrations (MICs), the lowest concentration (mg/mL) of the test compound that resulted no visible growth on the plates were recorded. DMSO was used as a solvent control to ensure that solvent had no effect on bacterial growth. Streptomycin, ampicillin and fluconazole were used as a control drug. The results of the anti microbial activities are summarized in table 3, table 4, table 5, table 6, and table 7.

Table 3. Antibacterial studies of N-(substituted aryl -1,3,4-oxadiazole -2 yl) methyl-N-(4H-1,2,4 –triazol-4 yl) benzamide derivative against gram (+ ve bacteria) . Zone of inhibition is expressed in mm.

S.No.	Sp		Ba		Ml		Se		Cs	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
2a	12	22	16	25	15	20	17	22	12	22
2b	14	23	18	22	17	23	14	25	16	25
2c	15	23	14	24	15	26	17	21	19	23
2d	12	26	13	26	16	25	20	26	16	26
2e	10	25	18	27	12	24	19	25	18	27
2f	11	23	13	24	11	20	15	23	14	24
Standard	22	26	23	28	21	28	25	30	24	28

Streptococus pyrogen (Sp), Bacillus aureus (Ba), Micrococus leuteus (Ml), Streptococus epidermis (Se), Clostridium sporogen (Cs).

Table 4. Antibacterial studies of N-(substituted aryl -1,3,4-oxadiazole -2 yl) methyl-N-(4H-1,2,4 –triazol-4 yl) benzamide derivative against gram (- ve bacteria) . Zone of inhibition is expressed in mm.

S.No.	Kp		St		Pa		Sm		Pv	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
2a	18	26	25	18	22	30	18	20	18	22
2b	20	25	24	16	25	28	16	18	18	19
2c	20	24	20	17	23	25	12	16	16	17
2d	22	28	25	19	22	28	18	20	18	22
2e	23	26	24	23	24	24	15	19	16	19
2f	19	24	26	21	23	26	16	18	15	18
standard	26	28	26	25	27	30	20	26	22	27

Klebsiella pneumonia (Kp), Salmonella typhimurium (St), Pseudomonas aeruginosa (Pa), Serratia marcesens (Sm) and Proteus vulgaris (Pv).

Table5. MICs (µg /ml) value of N-(substituted aryl -1,3,4-oxadiazole -2 yl) methyl-N-(4H-1,2,4 –triazol-4 yl) benzamide derivative against gram (+ ve bacteria) & gram (- ve bacteria)

S.No.	Sp	Ba	Ml	Se	Cs	Kp	St	Pa	Sm	Pv
2a	50	>50	50	25	50	>50	50	25	>50	25
2b	25	50	>50	>50	50	50	50	25	50	25
2c	1.56	6.25	25	50	12.5	3.12	3.12	25	6.25	50
2d	25	25	50	50	6.25	25	25	>50	25	>50
2e	25	>50	25	50	50	25	50	50	25	25

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2f	12.5	6.25	12.5	>50	>50	50	25	>50	50	25
Standard	1.56	3.12	6.25	1.56	3.12	6.25	1.56	6.25	3.12	1.56

Streptococus pyrogen (Sp), Bacillus aureus (Ba), Micrococus leuteus (Ml), Streptococus epidermis (Se), Clostridium sporogen (Cs), Klebsiella pneumonia (Kp), Salmonella typhimurium (St), Pseudomonas aeruginosa (Pa), Serratia marcesens (Sm) and Proteus vulgaris (Pv).

Table 6. Antifungal studies of N-(substituted aryl -1,3,4-oxadiazole -2 yl) methyl-N-(4H-1,2,4 –triazol-4 yl) benzamide derivative against fungi. Zone of inhibition is expressed in mm.

S.No.	Gf		Ro		Nc		An		Ca	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
2a	13	21	17	15	18	22	15	18	18	20
2b	15	22	16	16	18	23	12	16	22	22
2c	14	26	17	19	16	24	14	18	20	21
2d	16	24	18	20	20	25	11	22	22	26
2e	15	23	20	22	18	22	18	20	20	24
2f	13	23	16	20	16	23	12	21	18	20
standard	19	25	20	24	22	26	19	24	20	26

Gibberella fujikuroi (Gf), Rhizopus oligosporus (Ro), Neurospora crassa (Nc), Aspergillus niger (An), Candida albican (Ca).

Table 7. MICs (µg /ml) value of N-(substituted aryl -1, 3, 4-oxadiazole -2 yl) methyl-N-(4H-1, 2, 4 –triazol-4 yl) benzamide derivative against fungi.

S.No.	Gf	Ro	Nc	An	Ca
2a	25	>50	>50	25	50
2b	50	25	25	>50	25
2c	25	50	12.5	1.56	25
2d	>50	25	>50	50	6.25
2e	25	12.5	50	12.5	25
2f	12.5	25	25	6.25	12.5
Standard	1.56	6.25	3.12	1.56	6.25

Gibberella fujikuroi (Gf), Rhizopus oligosporus (Ro), Neurospora crassa (Nc), Aspergillus niger (An), Candida albican (Ca).

SUMMARY AND CONCLUSION

A new series of new N- [(di substituted-amino)methyl]-N- (4H-1,2,4-triazole-4-yl)benzamide derivative were synthesized by the steps mentioned in experimental part. The structure of the synthesized compounds was confirmed by IR and ^{1}HNMR method. All the compounds were evaluated for antibacterial and antifungal activity. Compounds have shown promising antibacterial and antifungal activity. The compound 2c showed very promoting activity with MIC 1.56 µg/ml and 3.12 µg/ml against both Gram + ve Streptococus pyrogen and Gram – ve

Klebsiella pneumonia. Whereas rest of the compounds showed moderate activity with MIC 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml.

Compound 2c, 2d showed pronounced antifungal activity against Aspergillus niger with MIC 6.25 µg/ml. Standard drug MIC = 1.56 µg/ml, 3.12 µg/ml and 6.25 µg/ml. Thus the compound 2(c), 2(d) was found to have potent antibacterial and anti-fungal activity.

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