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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF NEW MANNICH BASE LIGANDS DERIVED FROM ACETAMIDE, ACRYLAMIDE, BENZAMIDE AND PHTHALIMIDE

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ABSTRACT
The present work describes the synthesis, characterization and *in vitro* antimicrobial studies of the three component condensation of secondary amines, aldehydes and organic compounds containing at least one active hydrogen atom by Mannich reaction. All the synthesized compounds were characterized by elemental analyses, IR, UV, NMR and mass spectral studies. The antibacterial and antifungal activities were evaluated by agar disc diffusion method. They showed some interesting antibacterial and antifungal activities. The compound *N*-[Morpholino (methyl)]phthalimide, has high antibacterial as well as antifungal activity.

KEYWORDS
Chelation theory, disc diffusion, Mannich condensation, toxicity, immunomodulatory.

INTRODUCTION
Mannich bases are a special class of ligands with a variety of donor atoms exhibiting interesting coordination modes towards various metals[I]. The azomethine linkage in Mannich bases is responsible for the biological activities such as antitumor, antibacterial, antifungal and herbicidal activities[II,III]. Mannich bases have often been used as chelating ligands in the field of coordination chemistry[IV]. Their metal complexes are found to be of great interest during recent years. It is well known that N, O and S atoms play an important role in the coordination of metals at the active sites of numerous metallobiomolecules[V,VI]. Among the various classes of nitrogen-containing heterocyclic compounds, amide/imide derivatives are very important in pharmacological activities. In addition, these compounds are also associated with a wide spectrum of biological activities ranging from antitubercular, analgesic, anti-inflammatory, immunomodulatory, antipsychotic and antiallergic activities[VII-X]. They possess activities as vasopressin antagonistic, vasodilating, hypotensive, inhibiting growth of mesagium cells, water diuretic, platelet agglutination inhibitory activity

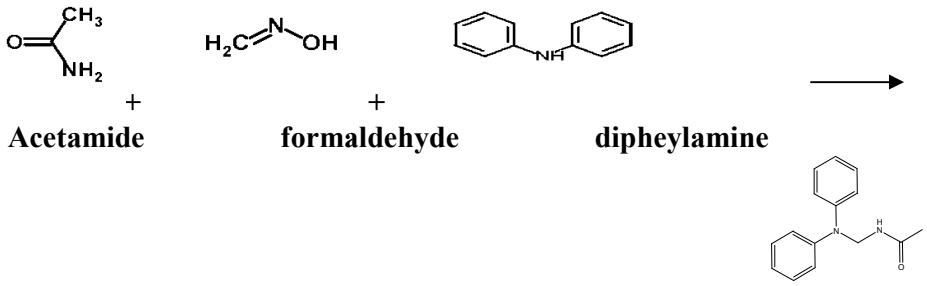
etc.[XI-XV]. Here, we report the synthesis, characterization and biological studies of compounds of morpholine, pyrrolidine and diphenylamine with some amide and imide derivatives.

RESULTS AND DISCUSSION

In the present study, seven new heterocyclic compounds have been synthesized by using Mannich condensation reaction[XVI]. They have a lone pair of electrons in their structures; hence they are called ligands. In general, they have a tendency to form a complex with metal ions. The structures of the newly synthesized heterocyclic compounds have been confirmed by elemental analyses, IR, UV, ^{1}H &^{13}C NMR and mass spectral studies[XVII].

General procedure for the preparation of the new heterocyclic compounds

The synthesis of *N*-[(Diphenylamino)methyl]acetamide is described in **Scheme-I.** *N*-[(Diphenylamino)methyl]acetamide, *N*-[(Diphenylamino)methyl]acrylamide *N*-[Phenyl(pyrrolidin-1-yl)methyl]acetamide, *N*-[Phenyl(pyrrolidin-1-yl)methyl]benzamide, *N*-[Morpholino(phenyl)methyl]acetamide, *N*-[Morpholino(phenyl)methyl]benzamide and *N*-[Morpholino(methyl)]phthalimide were synthesized by Mannich condensation reaction between acetamide/acrylamide/benzamide/phthalimide, formaldehyde/benzaldehyde and diphenylamine/pyrrolidine/morpholine respectively in 1:1:0.5 mol ratio.



Scheme I: Synthesis of *N*-[(Diphenylamino)methyl]acetamide(DPAMAce)

Ethanolic solution of the respective amides/imide was mixed with corresponding aldehydes and amines and stirred in an ice bath. After 10 days, a colourless/yellow coloured solid formed in each case was filtered, washed with water and acetone(Scheme-I). The compounds were dried at 60^{o}C in an air oven and recrystallised from ethanol. All the compounds were insoluble in water, but completely soluble in methanol, ethanol, propanol, butanol, chloroform, carbon tetrachloride, acetone, dimethyl formamide, ether, benzene etc. The elemental analysis and physical data of the synthesized compounds are shown in Table-I.

Table I. Physical and analytical data of the synthesized heterocyclic compounds

Comp. code	Formula	Mol. wt	M.P (^{o}C)	Yield (%)	Elemental Analysis					
					%C		%H		%N	
					Calc.	Found	Calc.	Found	Calc.	Found
DPAMAce	$C_{15}H_{16}N_2O$	240	110	92	75.0	73.01	6.60	6.19	11.60	11.58
DPAMAcry	$C_{16}H_{16}N_2O$	252	112	87	76.19	74.35	6.30	6.05	11.11	11.24
PBA	$C_{13}H_{18}N_2O$	218	120-124	90	71.60	71.53	8.26	8.31	12.84	12.97
PBB	$C_{18}H_{20}N_2O$	280	78-80	96	77.14	76.91	7.14	7.28	10.00	9.06

MBA	$C_{13}H_{18}N_2O_2$	234	148-150	91	66.67	66.41	7.69	7.74	11.97	11.65
MBB	$C_{18}H_{20}N_2O_2$	296	162-165	89	72.97	73.05	6.76	6.80	9.46	9.53
MMP	$C_{13}H_{14}N_2O_3$	246	114-116	79	63.41	63.05	5.69	5.61	11.38	11.29

Antibacterial and antifungal studies

The antibacterial and antifungal activities of the synthesized compounds were screened *in vitro* against six bacterial species viz *E.coli, P.aeruginosa*, *S.typhi, B.subtilis, S.pyogenes, S.aureus* and two fungal species *A.niger* and *A.flavus* by disc diffusion method[XIX] using agar nutrient as medium and gentamycin as control. The paper disc containing compound was placed on the surface of the nutrient agar plate, previously spread with sterilized culture of microorganism. After incubating this, at 37^{o}C for 24 hrs, the diameter of inhibition zone around the paper disc was measured.

Spectral data of the synthesized heterocyclic compounds

N-[(Diphenylamino)methyl]acetamide (DPAMAce)

FT-IR (KBr ν_{max}, cm^{-1}): 3258(NH), 3061(CH-aromatic ring), 2959(CH-aliphatic group) 1637(C=O), 1241 & 1067(C-N-C), 889-622(C-H o.p.b. of aromatic ring), 1360-1067(C-H o.p.b. of aromatic ring), 751(monosubstituted aromatic ring). ^{1}H NMR (300MHz, DMSO-d_6): δ 8.46(s, NH), 5.06 & 5.05(d, CH_2), 7.30 – 6.96(m, 5H phenyl ring), 1.84(s, CH_3). ^{13}C NMR (300MHz, DMSO-d_6): δ 169.60(s, C=O), 146.61 – 120.91(m, 5C, benzene ring), 56.12(s, CH_2), 22.57(s, CH_3). UV-Vis.(DMF): 301(n→π*), 274(π→π*). FABMS: m/z = 240($C_{15}H_{16}N_2O$), m/z = 197($C_{13}H_{13}N_2^+$), m/z = 182($C_{13}H_{12}N^+$), m/z = 168($C_{12}H_{10}N^+$).

N-[(Diphenylamino)methyl]acrylamide (DPAMAcry)

FT-IR (KBr ν_{max}, cm^{-1}): 3295(NH), 3042(CH-aromatic ring), 2912(CH-aliphatic group) 1655(C=O), 1218 & 1088(C-N-C), 898-689(C-H o.p.b. of aromatic ring), 1383-1088(C-H o.p.b. of aromatic ring), 755(monosubstituted aromatic ring). ^{1}H NMR (300MHz, DMSO-d_6): δ 8.66(s, NH), 7.31 – 6.92(m, 5H phenyl ring), 6.31(s, vinyl CH), 5.17 & 5.16(d, CH_2), 6.17 & 5.63(d, vinyl CH_2). ^{13}C NMR (300MHz, DMSO-d_6): δ 164.78(s, C=O), 146.58 – 120.95(m, 5C, benzene ring), 56.17(s, CH_2), 125.91(s, terminal C of vinyl), 131.45(s, vinyl C with CO). UV-Vis(DMF): 302(n→π*), 250(π→π*). FABMS: m/z = 252($C_{16}H_{16}N_2O$), m/z = 197($C_{13}H_{13}N_2^+$), m/z = 182($C_{13}H_{12}N^+$), m/z = 168($C_{12}H_{10}N^+$).

***N*-[Phenyl(pyrrolidin-1-yl)methyl]acetamide (PBA)**

FT-IR (KBr ν_{max}, cm^{-1}): 3306(NH), 1646(C=O), 3053, 3035(ν_{CH} aromatic), 2971, 2876(ν_{CH} alicyclic), 2821(ν_{CH} aliphatic), 1149(C-N-C), 1601($\nu_{C=C}$, ν_{C-N}), 1531(δ_{NH} secondary amide), 1029(δ_{CH} i.p.b benzene), 986 (δ_{CH} + o.p.b of pyrrolidine), 879, 851(δ_{NH} wagging and twisting), 732(δ_{CH} o.p.b benzene), 704(o.p.b ring C=C). ^{1}H NMR (300MHz, DMSO-d_6): δ 8.42(s, NH), 5.86 & 5.83 (d, CH), 7.39 - 7.26(m, CH benzene ring) 2.51(s, $N(CH_2)_2$ at α, α^1), 1.65(s, $(CH_2)_2$ at β, β^1). ^{13}C NMR (300MHz, DMSO-d_6): 169.62 (s, C=O), δ141.62-127.68(m, 6C phenyl ring), 49.23(s, pyrrolidine $N(CH_2)_2$ at α, α^1), 23.59(s, pyrrolidine $(CH_2)_2$ at β, β^1). UV-Vis.(DMF): 287(n→π*), 226(π→π*). FABMS: m/z = 218 ($C_{13}H_{18}N_2O$), m/z = 127 ($C_6H_{11}N_2O^+$), m/z = 99 ($C_5H_{10}N_2^+$), m/z = 70 ($C_4H_8N^+$).

***N*-[Phenyl(pyrrolidin-1-yl)methyl]benzamide (PBB)**

FT-IR (KBr ν_{max}, cm^{-1}): 3346(NH), 1634(C=O), 3057, 3033(ν_{CH} aromatic), 2965, 2873(ν_{CH} alicyclic), 2801(ν_{CH} aliphatic), 1147(C-N-C), 1578($\nu_{C=C}$, ν_{C-N}), 1515(δ_{NH} secondary amide), 1029(δ_{CH} i.p.b benzene), 930(δ_{CH} + o.p.b of pyrrolidine), 908, 868(δ_{NH} wagging and twisting), 719(δ_{CH} o.p.b benzene), 540($\pi_{C=O}$), 505(o.p.b ring C=C). ^{1}H NMR (300MHz, DMSO-d_6): δ 8.92(s, NH), 5.86 & 5.83 (d, CH), 7.93 - 7.54(m, 5H amido phenyl ring), 7.48 -

7.25(m, 5H aldehydic phenyl ring), 2.56(s, $N(CH_2)_2$ at α, α^1), 1.69(s, $(CH_2)_2$ at β, β^1). ^{13}C NMR (300MHz, DMSO-d_6): δ 168.39 (s,C=O), 141.58- 127.75(m, 6C aldehydic phenyl ring), 134.83- 127.95(m, 6C, amido phenyl ring), 70.44(s, methine C), 49.60 (s, pyrrolidine $N(CH_2)_2$ at α, α^1), 23.71 (s, pyrrolidine $(CH_2)_2$ at β, β^1). UV-Vis.(DMF): 263(n→π*), 235(π→π*). FABMS: m/z = 280($C_{18}H_{20}N_2O$), m/z = 204($C_{12}H_{15}N_2O^+$), m/z = 126($C_6H_{10}N_2O^+$), m/z = 70($C_4H_8N^+$).

***N*-[Morpholino(phenyl)methyl]acetamide (MBA)**

FT-IR (KBr ν_{max}, cm^{-1}): 3297(NH), 1647(C=O), 1493, 1449, 1206(CH_2), 1116(C-N-C), 1049, 1034(C-O-C), 749(monosubstituted morpholine). ^1H NMR (300MHz, DMSO-d_6): δ 8.44(s, NH), 5.61(s, CH_2), 7.44 - 7.28(m, 5H phenyl ring), 2.51(m, $CH_2)_2$), 3.58(m, $O(CH_2)_2$). ^{13}C NMR (300MHz, DMSO-d_6): δ 170.13(s,C=O), 139.71 - 127.76(m, 5C, benzene ring), 66.70(s, $O(CH_2)_2$), 48.98 (s, $N(CH_2)_2$). UV-Vis(DMF): 286(n→π*), 242(π→π*). FABMS: m/z = 234($C_{13}H_{18}N_2O_2$), m/z = 143($C_6H_{11}N_2O_2^+$), m/z = 114 ($C_5H_{10}N_2O^+$), m/z = 86($C_4H_8NO^+$).

***N*-[Morpholino(phenyl)methyl]benzamide (MBB)**

FT-IR (KBr ν_{max}, cm^{-1}): 3292(NH), 1637(C=O), 3063, 3030(ν_{CH} aromatic), 2962, 2914, 2893(ν_{CH} alicyclic), 2852(ν_{CH} aliphatic), 1602 ($\nu_{C=C}$, ν_{C-N}), 1522(δ_{CH2} morpholine ring), 1489, 1447(δ_{NH} secondary amide), 1354, 1330, 1310(ν_{CN} mixed with δ_{NH}), 1210(ν_{ring}), 1136, 1071($\nu_{CNC)}$, 1111(C-N-C), 1024($\nu_{C\text{-}O\text{-}C\ as}$ + $\nu_{C\text{-}O\text{-}C\ sy}$ morpholine), 1007,948(δ_{CH}+o.p.b morpholine), 915(π_{CH}+ δ_{CH}+ o.p.b ring), 747-897(δ_{NH} wagging and twisting) , 747(δ_{CH} o.p.b benzene ring), 698(o.p.b of ring C=C-H benzene), 634(i.p.b of benzene), 409(o.p.b ring C=C). ^1H NMR (300MHz, DMSO-d_6): δ 8.82(s, NH), 5.93 & 5.90(d, CH), 7.94 - 7.27(m, CH benzene ring) 3.65 (s, $O(CH_2)_2$ of morpholine), 2.51(s, $N(CH_2)_2$ of morpholine). ^{13}C NMR (300MHz, DMSO-d_6): δ 167.62 (s, C=O), δ134.73-127.90(m, C phenyl ring), 66.74(s, $O(CH_2)_2$ of morpholine), 49.33(s, $N(CH_2)_2$ of morpholine). UV-Vis(DMF): 274(n→π*), 236(π→π*). FABMS: m/z = 296 ($C_{18}H_{20}N_2O_2$), m/z = 120($C_7H_6NO^+$), m/z = 105($C_7H_5O^+$), m/z = 77($C_6H_5^+$).

***N*-[Morpholino(methyl)]phthalimide (MMP)**

FT-IR (KBr ν_{max}, cm^{-1}): 3292(NH), 1637(C=O), 2962(ν_{CH} aromatic), 2926, 2881(ν_{CH} alicyclic), 2853(ν_{CH} aliphatic), 1771($\nu_{C=C}$, ν_{C-N}), 1640(δ_{CH2} morpholine ring), 1531, 1507(δ_{NH} secondary amide), 1409, 1397, 1368(ν_{CN} mixed with δ_{NH}), 1345(ν_{ring}), 1152, 1097(ν_{CNC}), 1164(C-N-C), 1097($\nu_{C\text{-}O\text{-}C\ as}$ + $\nu_{C\text{-}O\text{-}C\ sy}$ morpholine), 1032, 998(δ_{CH}+o.p.b morpholine), 927(π_{CH}+ δ_{CH}+ o.p.b ring), 785-912(δ_{NH} wagging and twisting), 739(δ_{CH} o.p.b benzene ring), 801, 773(ortho disubstituted benzene), 680(o.p.b of ring C=C-H benzene), 646 (i.p.b of benzene), 421(o.p.b ring C=C). ^1H NMR (300MHz, DMSO-d_6): δ 8.71(s, NH), 7.85 - 7.32(m, CH benzene ring), 4.43(s,CH_2) 3.54(s, $O(CH_2)_2$ of morpholine), 2.50(s, $N(CH_2)_2$ of morpholine). ^{13}C NMR (300MHz, DMSO-d_6): δ 168.68(s, C=O), δ134.50-123.13(m, C phenyl ring), 65.94(s, $O(CH_2)_2$ of morpholine), 50.34(s, $N(CH_2)_2$ of morpholine). UV-Vis(DMF): 302(n→π*), 282(π→π*). FABMS: m/z = 246 ($C_{13}H_{14}N_2O_3$), m/z = 202 ($C_{11}H_{10}N_2O_2^+$), m/z = 160 ($C_9H_6NO_2^+$), m/z = 77 ($C_6H_5^+$).

A comparison of diameters of the inhibition zones of the compounds investigated and listed in Table II and III, shows that all the synthesized compounds exhibit satisfactory antibacterial and antifungal activity against all the bacterial and fungal species studied. They have larger diameters of inhibition zones than the control: gentamycin at the same concentration and identical conditions. A possible mechanism of toxicity may be speculated in the light of chelation theory[XX]. Chelation is the formation of complex with metal ion already present in a membrane of bacteria or fungus. It reduces considerably the polarity of the compound mainly because of partial sharing of its charge with other groups and possible π-delocalization of electron over the compound ring. This increases the liphophilic character of the compound

which favours its permeation through lipoid layers of bacteria and fungus membranes. Furthermore, the mechanism of action of the compounds may involve the formation of hydrogen bond through the uncoordinated hetero atoms O, S and N with the active centers of the cell constituents resulting in the interference with the normal cell process. Presence of lipophilic and polar substituents like C=O, NH, etc., are expected to enhance the toxicity.

Table II Antibacterial activity of the synthesized heterocyclic compounds

Compound	Escherichia coli			Pseudomonas aeruginosa			Salmonella typhi			Bacillus subtilis			Streptococcus pyogenes			Staphylococcus aureus		
Conc.(µg/disc)	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Control	09	11	14	08	10	13	08	09	12	09	11	12	08	11	13	08	09	11
DPAMAce	12	16	21	14	16	20	11	17	23	13	15	19	10	15	22	13	17	20
DPAMAcry	15	19	24	16	20	31	14	19	28	12	18	25	14	19	23	12	18	24
PBA	18	25	31	14	20	28	18	24	36	15	21	23	14	22	31	15	18	25
PBB	20	27	34	15	24	30	18	26	37	17	24	29	16	25	36	17	23	29
MBA	15	24	28	13	20	27	16	22	33	14	18	22	14	19	26	13	18	23
MBB	17	26	30	13	19	28	15	26	35	16	22	28	15	20	27	14	19	28
MMP	27	32	46	23	29	40	26	32	45	19	27	39	21	26	37	20	31	43

Table III Antifungal activity of the synthesized heterocyclic compounds

Compound	A. niger			A. flavus		
Conc. (µg/disc)	10	20	30	10	20	30
Control	08	09	12	05	08	10
DPAMAce	11	14	17	13	18	21
DPAMAcry	12	14	19	15	21	26
PBA	14	18	25	17	24	30
PBB	18	26	32	18	23	28
MBA	13	24	28	15	22	25
MBB	14	25	29	14	23	30
MMP	23	31	40	25	34	47

EXPERIMENTAL

All the chemicals and solvents were of AR grade. Ethanol, methanol and the solvents were dried by the standard procedures[XVIII]. The elemental analyses were performed using Carlo Erba 1108 elemental analyzer at RSIC, CDRI, Lucknow. IR spectroscopy analyses were recorded on Spectrum-one Perkin Elmer FT-IR spectrometer by using KBr pellets. The UV visible spectra were recorded on a Schimadzu UV spectrometer in the wavelength range 200-800 nm. The ^{1}H and ^{13}C NMR were recorded on a Bruker instrument and on a JEOL-GSX 400 spectrometer. The FAB mass recorded by using a JEOL–GC mate mass spectrometer. The antimicrobial activity was determined with the disc diffusion method.

CONCLUSION

The new heterocyclic compounds synthesized by Mannich synthetic route are obtained in good yield. The structures of the compounds have been confirmed by elemental analyses and

various spectral studies. All the compounds have excellent antibacterial and antifungal activities.

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