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Synthesis and Characterization of Some Mannich Bases as Potential Antimicrobial Agents

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A series of Mannich base derived from the substituted acetophenone were synthesized under the acidic condition and evaluated for the antimicrobial activity by the disc diffusion method. Chemical structures of synthesized Mannich base were determined by FTIR, ^{1}H NMR, FAB-MS spectroscopic analysis. The results showed that the Mannich bases were successfully synthesized under the acidic condition and compound **4** in the series possess the significant antimicrobial activity.

Key Words: Mannich base, Antibacterial activity, Antifungal activity.

INTRODUCTION

The infectious and parasitic diseases exert a devastated effect on human health, nutrition, quality of life, civilization and nation's economics and are the one of leading cause for the death worldwide. Present scenario became worrisome due to the emergence of new infectious diseases, increasing incidence of drug resistance cases, reappearance of old diseases and drug side effects.

This has led to resurgence in the antimicrobial research to complete the imperative need of continuous design and development of new antimicrobial agents[1,2]. Mannich base is a β-amino-ketone and formed as a result of the reaction between an amine, aldehyde and a carbon acid[3]. Mannich bases are reported to have antimicrobial[4-7], cytotoxic[8,9], diuretic[10], antiinflammatory[11], antimalarial[12-14] and anticonvulsant[15,16] activities. It has been found that biological activities of Mannich bases are due to the libration of α,β-unsaturated ketones during their *in vivo* deamination process and *in vitro* conditions of similar environment[4].

In this study, a series of Mannich bases were synthesized from substituted acetophenone using classical Mannich reaction under acidic condition. Mannich bases (**1-8**) were obtained in the good yield and purity. Proposed structures of compounds (**1-8**) were confirmed by the spectroscopic analysis *viz.*, IR, ^{1}H NMR, FAB-Mass and compounds were evaluated for the antimicrobial activity. Among all compounds, compound **4** found exhibited significant antimicrobial activity against the *E. coli*, *B. subtilis*, *S. aureus* and *C. albicans*.

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EXPERIMENTAL

All reagents were LR grade and purchased from Merck. The melting points of the synthesized compounds were determined by the open capillary method and were uncorrected. The IR spectra were recorded on JASCO FT/IR plus using KBr, ^{1}H NMR spectra were recorded in Bruker Avance DRX-2000 (300 MHz, FT NMR) and TMS was used as an internal standard. Mass spectra were recorded on Jeol SX102/DA-6000 FAB mass spectrometer, using nitrobenzylalcohol as matrix. Microanalyses for C, H, N and O were done by using Carlo Erba EA-1108 element analyzer, which were within 0.4 % of the calculated values.

Microorganism strain: Antimicrobial activity of the series was evaluated by using bacterial strains of *Basicilus subtilis* (NCIM 2063), *S. epidermitis* (NCIM 2493), *S. aureus* (NCIM 2079), *E. coli* (NCIM 2931) and *Candida albicance* (NCIM 347) as fungal strain.

General procedure: Substituted amine (0.02 mol) was dissolved slowly in ethanol (50 mL) in an ice bath (0-5 °C) and conc. HCl (0.0082 mol) was added drop wise with stirring for 15 min. Substituted acetophenone (0.02 mol) was then added to the reaction mixture and was brought to the room temperature. The reaction mixture was refluxed with *p*-formaldehyde (0.02 mol, 1.72 g) on heated oil bath at 60-101 °C for 8-24 h, with continuous stirring until reaction mixture became clear. TLC was used to monitor the progress of the reaction. Reaction mixture was concentrated to its 1/5th of volume at a temperature of 50 ºC. The coloured product was washed with acetone, filtered and dried in oven at 45-50 ºC temperature and recrystallized with ethanol.

1-Phenyl-3-(phenylamino)propan-1-one (1): Yellow colour crystalline, m.p. 252 ºC, soluble in water and DMSO; IR (KBr, cm^{-1}): 3542, 2703, 1679, 599, 1439, 1224, 755; ^{1}H NMR (D_2O, δ, ppm): 2.594 (m, -CH_2), 3.170 (w, -CH_2), 4.708 (m, -NH), 7.285-8.152 (w, -ArH); FABMS (m/z): 223 (M^+ peak, calculated for molecular formula $C_{15}H_{15}NO$), 209, 158, 149, 121 (base peak), 117, 107, 93, 77; Elemental analysis (%): found C = 78.15, H = 6.57, N = 5.98; calculated C = 78.47, H = 6.60, N = 6.01.

1-(2-Hydroxyphenyl)-3-(phenyl amino)propan-1-one (2): Cream yellow colour crystalline, m.p. 330 ºC (decompose), soluble in water and DMSO; IR (KBr, cm^{-1}) 3538, 2897, 1638, 1572, 1206, 759; ^{1}H NMR (D_2O, δ, ppm): 2.602 (m, -CH_2), 3.269 (w, -CH_2), 4.028 (m, -NH_2), 4.759 (s, -OH), 7.351-7.465 (m, -ArH); FABMS (m/z): 237 (M^+ peak, calculated for molecular formula $C_{15}H_{15}NO_2$), 223, 211, 150, 138, 121, 106, 94, 77; Elemental analysis (%): found C = 74.15, H = 6.18, N = 5.18; calculated C = 74.67, H = 6.27, N = 5.81.

3-(Naphthalen-2-ylamino)-1-phenyl propan-1-one (3): Dark brown crystalline, m.p. 340 ºC, soluble in water and pyridine; IR (KBr, cm^{-1}): 3439, 2897, 1607, 1517, 1164, 762; ^{1}H NMR (pyr, δ, ppm): 2.013 (s, -CH_2), 3.424 (m, -CH_2), 4.353 (w, -NH_2), 7.384-8.722 (s, -ArH); FABMS (m/z): 273 (M^+ peak, calculated for molecular formula $C_{19}H_{17}NO$), 260, 209, 120, 107; Elemental analysis (%): found C = 82.59, H = 6.18, N = 5.19; calculated C = 82.88, H = 6.22, N = 5.09.

3-(3-Oxo-3-phenylpropylamino)naphthalene-1-sulfonic acid (4): Light cream-pink colour crystalline, m.p. 307 ºC, soluble in water, DMSO, pyridine, DMF; IR (KBr, cm^{-1}): 3226, 2902, 1608, 1526, 1354, 709; ^1H NMR (DMSO, δ, ppm): 2.498 (w, $-CH_2$), 2.509 (m, $-CH_2$), 3.188 (w, $-NH_2$), 6.979-8.381 (m, -ArH), 11.033 (w, -S-OH); FABMS (m/z): 353 (M^+ peak, calculated for molecular formula $C_{19}H_{17}NO_4S$), 325, 273, 260, 225, 165, 107; Elemental analysis (%): found C = 64.02, H = 4.62, N = 3.69; calculated C = 64.21, H = 4.82, N = 3.94.

3-(Diphenylamino)-1-phenylpropan-1-one (5): Dark greenish crystilline, m.p. 230 ºC, soluble in water, DMSO; IR (KBr, cm^{-1}): 3026, 2902, 1679, 1511, 1105; ^1H NMR: 2.694 (m, $-CH_2$), 3.370 (w, $-CH_2$), 7.185-8.052 (w, -ArH); FABMS (m/z): 289 (M^+ peak, calculated for molecular formula $C_{21}H_{19}NO$), 227, 120, 107; Elemental analysis (%): found C = 83.12, H = 6.55, N = 4.66; calculated C = 83.69, H = 6.35, N = 4.65.

N-(3-Oxo-3-phenylpropyl)-N-phenyl acetamide (6): Yellow colour crystalline, m.p. 257 ºC, soluble in pyridine, DMSO; IR (KBr, cm^{-1}): 2572, 1687, 1518, 1210, 754; ^1H NMR (DMSO, δ, ppm): 2.311(m, $-CH_3$), 3.532(m, $-CH_2$), 4.003 (w, $-CH_2$), 7.34-7.925 (s -ArH); FABMS (m/z): 269 (M^+ peak, calculated for molecular formula $C_{17}H_{17}NO_2$), 238, 226, 209, 120, 107; Elemental analysis (%): found C = 76.09, H = 6.38, = N = 5.21; calculated C = 76.38, H = 6.41, N = 5.24.

1-(3-(2-Hydroxyphenyl)-3-oxopropyl) thiourea (7): White colour crystalline, m.p. 189 ºC, soluble in water, DMSO, pyridine, DMF; IR (KBr, cm^{-1}): 3309, 3043, 1649, 1544, 664; ^1H NMR (DMSO, δ, ppm): 2.497 (m, $-NH_2$), 2.509 (m, $-CH_2$), 3.586 (m, $-CH_2$), 5.052 (m, -OH), 8.136-7.925 (-ArH); FABMS (m/z): 228 (M^+ peak, calculated for molecular formula $C_{10}H_{12}N_2O_2S$), 209, 192, 176, 120, 107, Elemental analysis (%): found C = 52.55, H = 5.19, N = 11.99; calculated C = 53.55, H = 5.39, N = 12.49.

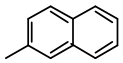
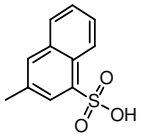
3-(Diphenylamino)-1-(2-hydroxy phenyl)propan-1-one (8): Dark greenish powder, m.p. 242 ºC, soluble in water, DMSO; IR (KBr, cm^{-1}): 3391, 3024, 2901, 1603, 1509, 1107, 820; ^1H NMR: 3.558 (m, $-CH_2$), 3.113 (w, $-CH_2$), 4.519 (s, -OH), 7.351-8.245 (m, -ArH); Elemental analysis (%): found C = 79.4, H = 5.93, N = 4.29; calculated C = 79.47, H = 6.03, N = 4.41.

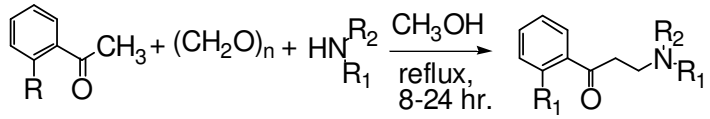
Antimicrobial activity: Antimicrobial activity of the compounds (**1-8**) was evaluated by the disc diffusion method[17]. Ofloxacin and micanozole were used as reference drugs in the study. Synthesized compounds and reference drugs were dissolved in the DMSO at 5 mg/mL solution and sterilized by filtration using 0.45 μm Millipore filter paper. 10 μL of this solution was applied over the sterile discs (6 mm diameter), dried and placed over inoculated agar media with suspension of respective microorganism. DMSO was used as control. Plates were inoculated with the test and standard discs on them were incubated for bacteria at 37 ± 1 ºC for 24 h; and for fungal 25 ± 1 ºC for 24 h. Observations were made for the zone of inhibition around the synthesized compounds and compared with standard.

RESULTS AND DISCUSSION

Structural formula of a series of new Mannich bases (1-8) is given in the Table-1. Compounds were successfully synthesized, according to **Scheme-I**, under the acidic condition and obtained in good yield. Recrystallization with ethanol afforded compounds with high purity.

TABLE-1
PHYSICAL DATA OF SYNTHESIZED COMPOUNDS (**1-8**)

Compd.	R	R_1	R_2	m.f.	Temp. of reaction (ºC)	Time of reaction (h)	Yield (%)	CLogP
1	H	H		$C_{15}H_{15}NO$	80	8	93.0	3.079
2	OH	H		$C_{15}H_{15}NO_2$	100	12	84.0	1.503
3	H	H		$C_{19}H_{17}NO$	80	4	91.0	4.253
4	H	H		$C_{19}H_{17}NO_4S$	80	6	94.0	1.466
5	H			$C_{21}H_{19}NO$	60	6	93.0	5.363
6	H			$C_{17}H_{17}NO_2$	100	16	77.0	2.881
7	OH	H		$C_{10}H_{12}N_2O_2S$	100	24	74.0	2.981
8	OH			$C_{21}H_{19}NO_2$	60	6	88.0	5.697



Scheme-I

In general, infrared spectrum of all compounds (**1-8**) showed the absorption band at 3600-3500, 3025-2700, 1600-1500 and 1680-1690 cm^{-1} which were originated due to the characteristic N-H, C-H, C=C and C=O stretching vibration, respectively. Presence of three carbon atom chain in compounds (**1-8**) was characterized by the rocking vibration at 800-650 cm^{-1}. Broad bands at 3600-3500 cm^{-1} were shown by compound (**2**, **7** and **8**) due the O-H stretching. Low intensity of these band were observed which suggest that -OH group forms intramolecular hydrogen bonding

with C=O present at the adjacent carbon atom of the phenyl ring. Considering the example of compound **1**, the values of absorption band in IR spectrum were found to be 3542, 2703, 1679 cm^{-1} for the N-H, C-H and C=O stretching vibration respectively[18].

Moreover, the multiplicity patterns and chemical shift values obtained for the series are in accordance with the proposed structure of compounds. For example in compound **2**, the presence of five proton of aromatic ring was assigned by the multiplet at δ 7.285- 8.152 in ^1H NMR. Singlet at δ 4.028 can be ascribed for proton of secondary amine. However, chemical shift produced by the proton of -OH group was found relatively in the downfield (δ 4.759) relative to TMS. Further, weak multiplet at δ 2.602 and 3.424 showed the proton of alkyl chain at C-2 and C-3 atom[18]. Molecular ion peak of compound **2** was found to be at 237 and it was well concurred with its molecular weight ($C_{15}H_{15}NO_2$). Molecular mass of compound **2** was again confirmed by elemental analysis. In chemical analysis, carbon, hydrogen and nitrogen were lying in the close proximity (in the range of ± 0.4) with the percentage values calculated for $C_{15}H_{15}NO_2$. Similarly, no anomalies were found in results of spectroscopic data and elemental analysis of other compounds in the series.

Further, antimicrobial activity of compounds was evaluated using disc diffusion method[17] against selected pathogenic bacteria and fungi causative for known common infections. Ofloxacin and micanozole were used as positive control for standard antibacterial and antifungal activity. Zone of inhibition was used to measure the antimicrobial activity of the compounds (**1-8**) and data is presented in Table-2. Antimicrobial activity of the series was studied in the range of 10-22 mm. In general, compound **2**, **3**, **4** and **8** showed appreciable antibacterial activity. However, antibacterial activity of compound **4** was found to be good against the *E. coli, B. subtilis* and *S. aureus*. Interestingly, it was found more active as antifungal (21 mm) than antibacterial and its antifungal activity was comparable to the micanozole (22.0 mm).

TABLE-2
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED COMPOUNDS
(ZONE OF INHIBITION IN mm AT CONC. 50 µg/mL)

Compound	*E. coli*	*B. subtilis*	*S. aureus*	*S. epidermitis*	*C. albicans*
1	15	12	12	–	–
2	16	15	15	12	13
3	12	16	15	12	14
4	20	18	22	15	21
5	10	12	13	11	12
6	12	10	12	11	10
7	12	11	12	10	14
8	12	15	11	15	13
Ofloxacin	22	24	27	23	–
Micanozole	–	–	–	–	22

From the study, it may be concluded that the secondary amine is essential for the antimicrobial activity for the Mannich bases. Introduction of further substitution at N atom results in the deprive activity (**5**, **6** and **8**). Further, the substitution by the aromatic ring is favourable for the antimicrobial activity. The substitution of electron withdrawing group at the aromatic ring (near to N atom) significantly increases the activity. In addition, the presence of hydroxyl group at 2nd positionin the aromatic ring (near to C=O) group is also favourable for the activity of Mannich bases. However, no significant correlation was observed between the calculated C LogP and antimicrobial activity of the compounds.

Conclusion

Findings of this study suggested that 3-(3-oxo-3-phenylpropylamino)naphthalene-1-sulfonic acid possess significant antimicrobial activity. Further, this study can be utilized for the design of more potent antimicrobial agents.

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