SYNTHESIS AND MICROBIOLOGICAL EVALUATION OF MANNICH BASES DERIVED FROM 4,6-DIACETYLRESORCINOL

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SUMMARY

In the present investigations 4,6-diacetyl resorcinol **1** on condensation with formaldehyde and some selected amines following the Mannich reaction conditions yielded eight new Mannich bases **2a-h**. It was observed that the reaction did not take place at the acetyl function but occurred at the 2-position into the aromatic ring. The compounds were characterized on the basis of elemental analysis as well as ^{1}H NMR and Mass spectral data. The antibacterial and antifungal activities of the title compounds were tested by the disc diffusion method using nutrient agar medium against various microorganisms such as gram positive *Staphylococcus aureus* and *Bacillus subtilis*, gram negative *Escherichia coli* and the fungi *Aspergillus flavus* and *Candida albicans.* Ofloxacin and voriconazole at 20 µg/mL were used as standard drugs for antibacterial and antifungal activities, respectively.

Keywords : Mannich base, resorcinol, antibacterial, antifungal.

INTRODUCTION

The presence of nitrogen atom alongwith other features impart interesting biological activities to the parent compounds[1]. Mannich bases, including those derived from different acetophenones, possess diverse biological activities including potent antibacterial[2,4], antimycobacterial[3], antifungal[2,4] and anti-HIV[4] activities. Resorcinol is a simple and important aromatic chemical (1,3-benzenediol) that has been chemically incorporated into various compounds to enhance their pharmacological profile[5].

The essential feature of Mannich reaction is the replacement of the active hydrogen by an aminomethyl or substituted aminomethyl group. However, it is also well known that with phenols the reaction proceeds on a nuclear position. Hence it was considered worthwhile to study this reaction on 4,6-diacetyl resorcinol having both $COCH_3$ and nuclear position available. This moiety was used earlier to build benzodipyrone derivatives and found to have a good antibacterial activity[6].

Over the past few decades the bacterial resistance to antibiotics has become one of the most important problems of infections treatment. Although there are antimicrobial agents having different structures and mechanisms are frequently used in the treatment of microbial infections, even then these agents are associated with resistance. To overcome the development of drug resistance, it is necessary to synthesize a new class of antimicrobial compounds possessing different mechanism or chemical properties from those that are used commonly. Derivatives of 4,6-diacetyl resorcinol show potential antibacterial activity[6]. In view of these points and in continuation of our work on novel resorcinol derivatives[6], it was planned to synthesize Mannich bases derived from 4,6-diacetyl resorcinol.

In the present work we report the Mannich reaction proceeds at the 2-position of 4,6-diacetyl resorcinol and several new such compounds have been prepared by varying amine component and the products have been evaluated for their antibacterial and antifungal activities.

EXPERIMENTAL

Chemistry

Melting points were determined in open capillary tubes and are uncorrected. Purity of the compounds was checked by thin layer chromatography (TLC) on silica gel G plates, with the solvent system: toluene-ethyl acetate-formic acid (5:4:1, v/v/v). The spots were located under iodine vapours and UV light. ^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer (internal reference-tetramethyl silane) and Mass spectra were recorded on a JEOL JMS-D 300 instrument. Elemental analyses were performed on a Perkin-Elmer 240 analyzer and the values were in range of ±0.4% for each element analyzed (C, H, N).

Preparation of 1,1'-(4,6-dimethyl-1,3-phenylene)diethanone (1)

It was synthesized according to the method reported in literature[6].

Preparation of ***Mannich bases (2a-h)***

Compound **1** (0.002 mol) was dissolved in chloroform (20 ml) and to it were added formaldehyde (0.004 mol), an amine (0.004 mol) and tetrabutyl ammonium bromide (0.002 mol) followed by distilled water (10 ml). After stirring the contents for 24 h, chloroform layer was separated and washed with aqueous sodium bicarbonate (5% w/v) followed by washing with water. The organic layer was then dried over anhydrous sodium sulphate, filtered and evaporated to dryness. The residue was crystallized from an appropriate solvent to give TLC pure crystals of **2a-h** (Table I). Each one of these compounds gave a violet colour with ethanolic ferric chloride solution (test for phenol).

1,1'-{5-[(Dimethylamino)methyl]-4,6-dihydroxy-1,3-phenylene}diethanone (**2a**): Yield: 63 %; m.p. 148-150 °C; ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.32 (s, 6H, 2x-CH_3), 2.61 (s, 6H, 2x-$COCH_3$), 4.13 (s, 2H, -CH_2-, benzylic), 8.27 (s, 1H, aromatic proton), 13.18 (s, 2H, 2xOH); MS (*m/z*): 251 (M^+), 207, 193, 190, 44; Anal. $C_{13}H_{17}NO_4$, Calcd. C, 62.14; H, 6.82; N, 5.57; found C, 62.18; H, 6.86; N, 5.45.

1,1'-{5-[(Diethylamino)methyl]-4,6-*dihydroxy-1,3-phenylene}diethanone* (**2b**): Yield: 60 %; m.p. 135-137 °C; ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.35 (t, 6H, 2x-$CH_2\underline{CH_3}$), 2.87 (q, 4H, 2x-$\underline{CH_2}CH_3$), 2.60 (s, 6H, 2x-$COCH_3$), 4.08 (s, 2H, -CH_2-, benzylic), 8.35 (s, 1H, aromatic proton), 13.25 (s, 2H, 2xOH). MS (*m/z*): 279 (M^+), 207, 193, 190, 72. Anal. $C_{15}H_{21}NO_4$, calcd. C, 64.50; H, 7.58; N, 5.01; found C, 64.62; H, 7.54; N, 5.07.

1,1'-{5-[(4-Methylphenylamino)methyl]-4,6-*dihydroxy-1,3-phenylene} diethanone* (**2c**): Yield: 52 %; m.p. 167-169 °C; ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.35 (s, 3H, -CH_3), 2.67 (s, 6H, 2x-$COCH_3$), 4.14 (s, 2H, -CH_2-, benzylic), 7.03 & 7.46 (d, each, A_2B_2, *p*-substituted phenyl ring), 8.37 (s, 1H, aromatic proton), 9.02 (s, 1H, -NH-), 13.18 (s, 2H, 2xOH); MS (*m/z*): 313 (M^+), 207, 91, 77; Anal. $C_{18}H_{19}NO_4$, calcd. C, 69.00; H, 6.11; N, 4.47; found C, 68.88; H, 6.20; N, 4.48.

1,1'-{5-[(4-Methoxylphenylamino)methyl]-4,6-*dihydroxy-1,3-phenylene} diethanone* (**2d**): Yield: 58 %; m.p. 172-174 °C; ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.73 (s, 6H, 2x-$COCH_3$), 3.91 (s, 3H, -OCH_3), 4.18 (s, 2H, -CH_2-, benzylic), 7.14 & 7.57 (d, each, A_2B_2, *p*-substituted phenyl ring), 8.52 (s, 1H, aromatic proton), 9.06 (s, 1H, -NH-), 13.22 (s, 2H, 2xOH); MS (*m/z*): 329 (M^+), 207, 193, 190, 122; Anal. $C_{18}H_{19}NO_5$, calcd. C, 65.64; H, 5.81; N, 4.25; found C, 65.57; H, 5.76; N, 4.31.

1,1'-{5-[(3-Methoxylphenylamino)methyl]-4,6-*dihydroxy-1,3-phenylene} diethanone* (**2e**): Yield: 55 %; m.p. 160-162°C; ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.69 (s, 6H, 2x-$COCH_3$), 3.88 (s, 3H, -OCH_3), 4.16 (s, 2H, -CH_2-, benzylic), 7.08-7.33 (m, 4H, *m*-substituted phenyl ring), 8.29 (s, 1H, aromatic proton), 8.97 (s, 1H, -NH-), 13.17 (s, 2H, 2xOH); MS (*m/z*): 329 (M^+), 207, 193, 190, 122, 121; Anal. $C_{18}H_{19}NO_5$, calcd. C, 65.64; H, 5.81; N, 4.25; found C, 65.66; H, 5.78; N, 4.22.

1,1'-{5-[(Morpholin-4-yl)methyl]-46-dihydroxy-1,3-phenylene}

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diethanone (**2f**): Yield: 64 %; m.p. 134-136 °C; ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.68 (b, 10H, 2´-$COCH_3$ and 2x-CH_2-, a to nitrogen), 3.87 (b, 6H, 2´-CH_2-, a to oxygen + -CH_2-, benzylic), 8.35 (s, 1H, aromatic proton), 13.16 (s, 2H, 2xOH); MS (*m/z*): 293 (M^+), 207, 193, 190, 86; Anal. $C_{15}H_{19}NO_5$, calcd. C, 61.42; H, 6.53; N, 4.78; found C, 61.40; H, 6.51; N, 4.83.

1,1´-{5-[(Piperidin-1-yl)methyl]-4,6-*dihydroxy-1,3-phenylene}diethanone* (**2g**): Yield: 66 %; m.p 138-140 °C; ^{1}H-NMR (DMSO-d_6, δ, ppm): 1.67 (bs, 6H, 2´-CH_2, meta to N + -CH_2, para to N), 2.82 (s, 4H, 2´-CH_2, ortho to N), 2.66 (s, 6H, 2´-$COCH_3$), 3.97 (s, 2H, -CH_2-, benzylic), 8.34 (s, 1H, aromatic proton), 13.23 (s, 2H, 2xOH); MS (*m/z*): 291 (M^+), 207, 193, 190, 84. Anal. $C_{16}H_{21}NO_4$, calcd. C, 65.96; H, 7.26; N, 4.81; found C, 65.92; H, 7.25; N, 4.86.

1,1´-{5-[(4-Methylpiperazin-1-yl)methyl]-4,6-*dihydroxy-1,3-phenylene}diethanone* (**2h**): Yield: 57 %; m.p. 162-164 °C; ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.33 (s, 3H, -CH_3), 2.51 (bs, 8H, 4x CH_2), 2.72 (s, 6H, 2´-$COCH_3$), 4.09 (s, 2H, -CH_2-, benzylic), 8.24 (s, 1H, aromatic proton), 13.86 (s, 2H, 2xOH); MS (*m/z*): 306 (M^+), 207, 193, 190, 99. Anal. $C_{16}H_{22}N_2O_4$, calcd. C, 62.73; H, 7.24; N, 9.14; found C, 62.65; H, 7.26; N, 9.12.

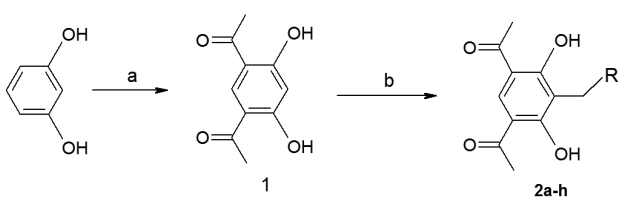
Antibacterial and antifungal activity

All the synthesized compounds were screened for *in vitro* antibacterial and antifungal activities at the concentration of 100 and 200 µg/mL by cup-plate method[7] in nutrient agar (Hi-media) (antibacterial activity) and Sabouraud dextrose agar (Hi-media) (antifungal activity). The bacterial strains gram positive (*Bacillus subtilis* ATCC 6633 & *Staphylococcus aureus* ATCC 25923), gram negative (*Escherichia coli* ATCC 8739) and fungal strains (*Candida albicans* NCIM 300 & *Aspergillus flavus* NCIM 524) were used. The sterilized media was poured into sterile petridishes and allowed to solidify for 30 minutes. On the surface of the media, 0.1 ml microbial suspension was spread and after 10 minutes, cups were made by punching into agar surface with a sterile cork borer (6 mm diameter) and scooping out the punched part of the agar. Four cups were made in each petridish and into these cups was added 0.1 ml of the drug solution. The drug solution was allowed to diffuse for about an hour and plates were then incubated at 37 ± 0.5 °C for 24 h (antibacterial activity) and at 28 °C for 48-72 h (antifungal activity). A solvent control dimethylformamide (DMF) was also run to know the activity of the blank. The diameter of zone of inhibition (mm) was measured. Ofloxacin (20 µg/mL) and voriconazole (20 µg/mL) were used as reference drugs for comparison. The results are presented in **Table-II**.

RESULTS AND DISCUSSION

Chemistry

Titled compounds (**2a-h**) were synthesized through one-pot reaction as depicted in Scheme-**1**. The starting material 4,6-diacetyl resorcinol **1** was



Scheme-1. Protocol for synthesis, (a) $Ac_2O/ZnCl_2$; (b) Tetrabutyl amm.bromide/HCHO/amine

synthesized by the reported procedure[6].

It (**1**) was condensed with different amines and formaldehyde, however, the yields were poor and hence the reaction was carried out in the presence of tetrabutyl ammonium bromide, a phase transfer catalyst, in chloroform solution to give Mannich bases **2a-h** in satisfactory yields. The structures assigned to the compounds were supported by the results of elemental analysis as well as ^{1}H NMR and Mass spectral data (**Table-I**).

The formation of Mannich bases could be inferred by the presence of a methylene signal around d 4.0±0.2 instead of the signals for CH_2-CH_2 and the presence of acetyl signal consistently around d 2.6±0.2. Further mass spectral data of all these compounds showed molecular ion peaks of reasonable intensity besides the diagnostic peaks at m/z 207, 193 and 190 arising from diacetyl resorcinol moiety and can be formulated as shown in **Fig. 1**. Beside these, there was a fragment arising from the amine moiety, which was dependent on the amine used.

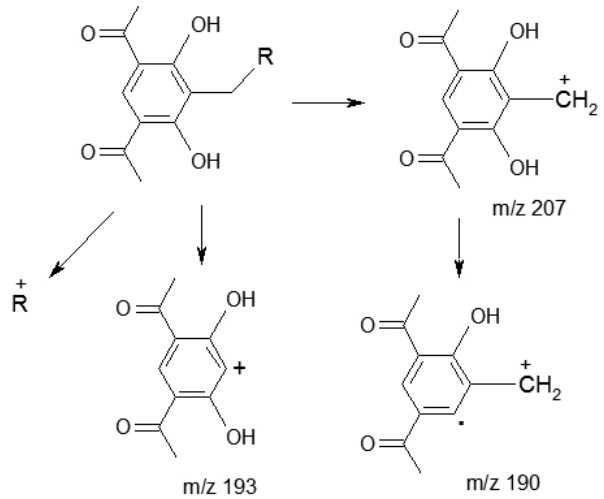
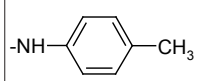
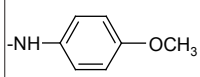
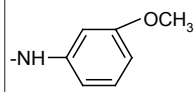
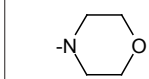
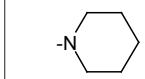
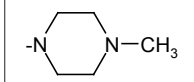


Fig.1: Mass fragmentation pattern of the compounds **2a-h.**

Table-I: Physical data of the compounds 2a-h.

Compd	R	M.P. (°C)	Yield (%)	Molecular Formula	Molecular Weight
2a	-$N(CH_3)_2$	148-50	63	$C_{13}H_{17}NO_4$	251.28
2b	-$N(C_2H_5)_2$	135-137	60	$C_{15}H_{21}NO_4$	279.33
2c		166-68	52	$C_{18}H_{19}NO_4$	313.35
2d		172-174	58	$C_{18}H_{19}NO_5$	329.35
2e		160-62	55	$C_{18}H_{19}NO_5$	329.35
2f		134-36	64	$C_{15}H_{19}NO_5$	293.32
2g		138-40	66	$C_{16}H_{21}NO_4$	291.34
2h		162-64	57	$C_{16}H_{22}N_2O_4$	306.36

Antibacterial and antifungal activity

All the synthesized compounds were screened for *in vitro* antibacterial and antifungal activities at the concentration of 100 and 200 µg/mL. The bacterial

strains gram positive (*Bacillus subtilis* ATCC 6633 & *Staphylococcus aureus* ATCC 25923), gram negative (*Escherichia coli* ATCC 8739) and fungal strains (*Candida albicans* NCIM 300 & *Aspergillus flaveous* NCIM 524) were used. Ofloxacin and voriconazole were used as reference drugs for comparison. Among the tested compounds, 1,1'-{5-[(4-methylpiperazin-1-yl)methyl]-46-dihydroxy-1,3-phenylene}diethanone (**2h**) showed very good activity against *S. aureus*, *C. albicans* and *A. flaveous*, similar type of activity exhibited by 1,1'-{5-[(4-methoxylphenylamino)methyl]-46-dihydroxy-1,3-phenylene} diethanone (**2d**) against *S. aureus* and *A. flaveous*. Both the compounds, **2h** and **2d**, also showed good activity against *E. coli* and *B. subtilis.* Rests of the compounds were moderate to low in their antimicrobial action (**Table-II**).

An analysis of results showed that the Mannich bases derived from methylpiperazine and *p*-anisidine (**2h** & **2d**) were excellent in their antimicrobial action against the tested organisms. Highest activity was observed in the methylpiperazine derivative (**2h**), while replacing methylpiperazine with *p*-anisidine (**2d**) slightly decreased the activity and it was further observed that replacing it with *m*-anisidine (**2e**), morpholine (**2f**), piperidine (**2g**) and *p*-toludene (**2c**) the activity decreased significantly. Among the synthesized Mannich bases, the bases derived from dimethylamine (**2a**) and diethylamine (**2b**) showed lowest antibacterial activity. However, the activities of tested compounds are less than that of the standard agents used.

Table-II: Antibacterial and antifungal activities of the compounds 2a-h.

Compounds	Conc. (µg\ mL)	Diameter of zone of inhibition (mm)				
		E. coli	*S. aureus*	*B. subtilis*	*C. albicans*	*A. flaveous*
Voriconazole	20	nt	nt	nt	27	31
Ofloxacin	20	27	30	29	nt	nt
2a	100	11	13	10	12	9
	200	16	18	13	16	12
2b	100	10	12	9	8	6
	200	13	16	11	12	10
2c	100	10	12	11	12	10
	200	16	15	14	16	13
2d	100	17	20	18	19	18
	200	22	24	21	25	23
2e	100	15	18	14	16	15
	200	20	21	19	22	18
2f	100	12	15	13	12	14
	200	18	19	17	18	20
2g	100	14	15	12	16	11
	200	17	19	15	21	16
2h	100	18	23	15	20	21
	200	21	26	22	27	25

nt = not tested

CONCLUSIONS

As concluding remarks we obtained herein eight new Mannich bases from 4,6-diacetyl resorcinol. Findings revealed that Mannich reaction did not take place at the acetyl function but occurred at the 2-position into the aromatic ring. Among the synthesized compounds, two compounds (**2h** and **2d**) showed significant antibacterial and antifungal activity against the tested microbes.

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