NEW 4,6-DIACETYL RESORCINOL MANNICH BASES: SYNTHESIS AND BIOLOGICAL EVALUATION

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Abstract: In the present study, a series of Mannich bases was synthesized by condensing 4,6-diacetylresorcinol with formaldehyde and some selected secondary amines following the Mannich reaction conditions. Findings revealed that Mannich reaction did not take place at the acetyl function but occurred on the aromatic ring position between the two hydroxyl groups. It was also observed that in one case instead of the desired base (**3a**), the dimer (**4**) of the starting material (4,6-diacetylresorcinol) having a methylene bridge could be isolated. The newly synthesized compounds were characterized on the basis of elemental analysis as well as 'H NMR and mass spectral data. The products have been evaluated for antiinflammatory, ulcerogenic and lipid peroxidation actions with some significant results.

Key words: Mannich bases, resorcinol, antiinflammatory, ulcerogenic

Mannich reaction is widely used for the construction of nitrogen containing compounds (1). The presence of nitrogen atom along with other features may impart interesting biological activities to the parent compound (2-4). Mannich bases, including those derived from different acetophenones, possess diverse biological activities including antiinflammatory (5), analgesic (5), antibacterial (6-8), antimycobacterial (9, 10), antifungal (8), anticonvulsant (11), anthelmintic (12), anticancer (13-15) and anti-HIV (8) activities. Resorcinol (1,3-benzenediol) is a simple and important aromatic chemical that has been chemically incorporated into various compounds to enhance their pharmacological profile (16-21). Resorcinol derivatives show profound antioxidant activities (21, 22). A variety of bioactive coumarin derivatives have also been synthesized from resorcinol (23).

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 Mannich reaction proceeds in the ring (nuclear position). Hence, it was considered worthwhile to study this reaction on 4,6-diacetylresorcinol 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 (24). In view of these points and in continuation of our work on novel resorcinol derivatives (24), herein we report the Mannich reaction of 4,6-diacetylresorcinol, which proceeds at the 2-position of this molecule, and several new Mannich bases have been prepared by varying amine component and the products have been evaluated for antiinflammatory, ulcerogenic and lipid peroxidation activities.

EXPERIMENTAL

All melting points are uncorrected and were recorded in a liquid paraffin bath using open-end capillaries. ¹H NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer (internal reference: tetramethylsilane). The splitting pattern abbreviations are as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; m, multiplet. Mass spectra were recorded on a JEOL JMS-DX 303 instrument. The progress of the reactions was monitored on silica gel G plates in the solvent system: toluene-ethyl acetateformic acid (5:4:1, v/v/v) using iodine vapors as visualizing agent. Microanalyses were performed on Perkin-Elmer 240 analyzer and the values were found within \pm 0.4% of the theoretical values.

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4,6-Diacetyl resorcinol (1)

It was synthesized according to the method reported in literature (24).

General method for the synthesis of Mannich bases (2a-j) and dimer (4)

Compound 1 (0.002 mole) was dissolved in chloroform (20 mL) and to it were added formaldehyde (0.004 mole), an amine (0.004 mole) and tetrabutylammonium bromide (0.002 mole) 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 sulfate, filtered and evaporated to dryness. The residue was crystallized from an appropriate solvent to give TLC pure crystals of **2a-j** and **4**. Each one of these compounds gave a violet color with ethanolic ferric chloride solution (the test for phenol).

1-[5-Acetyl-2,4-dihydroxy-3-(dimethylaminomethyl)phenyl]-1-ethanone (**2a**)

Yield 63%; m.p. 148-50°C. ¹H NMR (DMSO-d₆, δ , ppm): 1.32 (s, 6H, 2× -CH₃), 2.61 (s, 6H, 2×-COCH₃), 4.13 (s, 2H, -CH₂-, benzylic), 8.27 (s, 1H, arom.), 13.18 (s, 2H, 2× OH). MS (m/z): 251 (M⁺), 207, 193, 190, 44. Analysis: (C₁₃H₁₇NO₄, 251.28), found: C 62.18, H 6.86, N 5.45%; calcd.: C 62.14, H 6.82, N 5.57%.

1-[5-Acetyl-2,4-dihydroxy-3-(diethylaminomethyl)phenyl]-1-ethanone (**2b**)

Yield 60%; m.p. 135-37°C. ¹H NMR (DMSOd₆, δ , ppm): 1.35 (t, 6H, 2× -CH₂CH₃), 2.87 (q, 4H, 2× -CH₂CH₃), 2.60 (s, 6H, 2× -COCH₃), 4.08 (s, 2H, -CH₂-, benzylic), 8.35 (s, 1H, arom.), 13.25 (s, 2H, 2× OH). MS (m/z): 279 (M⁺), 207, 193, 190, 72. Analysis: (C₁₅H₂₁NO₄, 279.33), found: C 64.62, H 7.54, N 5.07%; calcd.: C 64.50, H 7.58, N 5.01%.

1-[5-Acetyl-2,4-dihydroxy-3-(3-toluidinomethyl)phenyl]-1-ethanone (**2c**)

Yield 53%; m.p. 148-50°C. ¹H NMR (DMSOd₆, δ , ppm): 1.41 (s, 3H, -CH₃), 2.73 (s, 6H, 2× -COCH₃), 4.26 (s, 2H, -CH₂-, benzylic), 6.96-7.31 (m, 4H, *m*-substituted phenyl ring), 8.33 (s, 1H, arom.), 12.93 (s, 2H, 2× OH). MS (m/z): 313 (M⁺), 207, 193, 190, 105, 91, 77. Analysis: (C₁₈H₁₉NO₄, 313.35), found: C 68.91, H 6.15, N 4.50%; calcd.: C 69.00, H 6.11, N 4.47%.

1-[5-Acetyl-2,4-dihydroxy-3-(4-toluidinomethyl)phenyl]-1-ethanone (**2d**)

Yield 58%; m.p. 167-69°C. ¹H NMR (DMSO-d₆, δ , ppm): 1.35 (s, 3H, -CH₃), 2.67 (s, 6H, 2×

-COCH₃), 4.14 (s, 2H, -CH₂-, benzylic), 7.03 and 7.46 (d, each, A_2B_2 , *p*-substituted phenyl ring), 8.37 (s, 1H, arom.), 9.02 (s, 1H, -NH-), 13.18 (s, 2H, 2× OH). MS (m/z): 313 (M⁺), 207, 91, 77. Analysis: (C₁₈H₁₉NO₄, 313.35), found: C 68.88, H 6.20, N 4.48%; calcd.: C 69.00, H 6.11, N 4.47%.

1-[5-Acetyl-2,4-dihydroxy-3-(4-methoxyanilinomethyl)phenyl]-1-ethanone (**2e**)

Yield 58%; m.p. 172-74°C. ¹H NMR (DMSOd₆, δ , ppm): 2.73 (s, 6H, 2× -COCH₃), 3.91 (s, 3H, -OCH₃), 4.18 (s, 2H, -CH₂-, benzylic), 7.14 and 7.57 (d, each, A₂B₂, *p*-substituted phenyl ring), 8.52 (s, 1H, arom.), 9.06 (s, 1H, -NH-), 13.22 (s, 2H, 2× OH). MS (m/z): 329 (M⁺), 207, 193, 190, 122. Analysis: (C₁₈H₁₉NO₅, 329.35), found: C 65.57, H 5.76, N 4.31%; calcd.: C 65.64, H 5.81, N 4.25%.

1-[5-Acetyl-2,4-dihydroxy-3-(3-methoxyanilinomethyl)phenyl]-1-ethanone (**2f**)

Yield 55%; m.p. 160-62°C. 'H NMR (DMSOd₆, δ , ppm): 2.69 (s, 6H, 2× -COCH₃), 3.88 (s, 3H, -OCH₃), 4.16 (s, 2H, -CH₂-, benzylic), 7.08-7.33 (m, 4H, *m*-substituted phenyl ring), 8.29 (s, 1H, arom.), 8.97 (s, 1H, -NH-), 13.17 (s, 2H, 2× OH). MS (m/z): 329 (M⁺), 207, 193, 190, 122, 121. Analysis: (C₁₈H₁₉NO₅, 329.35), found: C 65.66, H 5.78, N 4.22%; calcd.: C 65.64, H 5.81, N 4.25%.

1-(5-Acetyl-2,4-dihydroxy-3-morpholinomethylphenyl)-1-ethanone (**2g**)

Yield 64%; m.p. 134-36°C. 'H NMR (DMSOd₆, δ , ppm): 2.68 (bs, 10H, 2× -COCH₃ and 2× -CH₂-, α to nitrogen), 3.87 (b, 6H, 2× -CH₂-, α to oxygen + -CH₂-, benzylic), 8.35 (s, 1H, arom.), 13.16 (s, 2H, 2× OH). MS (m/z): 293 (M⁺), 207, 193, 190, 86. Analysis: (C₁₅H₁₉NO₅, 293.31), found: C 61.40, H 6.51, N 4.83%; calcd.: C 61.42, H 6.53, N 4.78%.

1-(5-Acetyl-2,4-dihydroxy-3-piperidinomethyl-phenyl)-1-ethanone (**2h**)

Yield 66%; m.p. 138-40°C. ¹H NMR (DMSOd₆, δ , ppm): 1.67 (bs, 6H, 2× -CH₂, *meta* to N + -CH₂, *para* to N), 2.82 (s, 4H, 2× -CH₂, *ortho* to N), 2.66 (s, 6H, 2′ -COCH₃), 3.97 (s, 2H, -CH₂-, benzylic), 8.34 (s, 1H, arom.), 13.23 (s, 2H, 2× OH). MS *m*/*z*: 291 (M⁺), 207, 193, 190, 84. Analysis: (C₁₆H₂₁NO₄, 291.34), found (%): C 65.92, H 7.25, N 4.86%; calculated: C 65.96, H 7.26, N 4.81%.

1-(5-Acetyl-2,4-dihydroxy-3-tetrahydro-1*H*-1-pyrrolylmethylphenyl)-1-ethanone (**2i**)

Yield 53%; m.p. 148-50°C. ¹H NMR (DMSOd₆, δ, ppm): 1.75 (s, 4H, 2× -CH₂, *meta* to N), 2.58 (s, 4H, 2× -CH₂, *ortho* to N), 2.71 (s, 6H, 2× -COCH₃), 4.01 (s, 2H, -CH₂-, benzylic), 8.13 (s, 1H, arom.), 13.41 (s, 2H, 2×OH). MS m/z: 277 (M⁺), 207, 193, 190, 70. Elemental analysis (C₁₅H₁₉NO₄, 277.32), found (%): C 64.92, H 7.03, N 5.11%; calculated: C 64.97, H 6.91, N 5.05%.

1-[5-Acetyl-2,4-dihydroxy-3-(4-methylpiperazinomethyl)phenyl]-1-ethanone (2j)

Yield 57%; m.p. 162-164°C. ¹H NMR (DMSO-d₆, δ , ppm): 2.33 (s, 3H, -CH₃), 2.51 (b s, 8H, 4x CH₂), 2.72 (s, 6H, 2×-COCH₃), 4.09 (s, 2H, -CH₂-, benzylic), 8.24 (s, 1H, aromatic proton), 13.86 (s, 2H, 2xOH). MS *m*/*z*: 306 (M⁺), 207, 193, 190, 99. Elemental analysis (C₁₆H₂₂N₂O₄, 306.36), found (%): C 62.65, H 7.26, N 9.12; calculated: C 62.73, H 7.24%, N 9.14. %

2-(3,5-Diethyl-2,6-dihydroxybenzyl)-4,6-diethyl-1,3-benzenediol (4)

Yield: 61%; m.p. >330°C. ¹H NMR (DMSOd₆, δ , ppm): 2.59 (s, 12H, 4× -COCH₃), 4.32 (s, 2H, -CH₂-), 8.10 (s, 2H, 2× arom.), 13.25 (s, 4H, 4× -OH). MS (m/z): 400 (M⁺), 383, 207, 193, 190, 77. Analysis: (C₂₁H₂₀O₈, 400.38), found: C 61.41, H 6.55, N 4.82%; calcd.: C 61.42, H 6.53, N 4.78%.

Biological evaluation

Wistar rats of either sex weighing 180-200 g were used for biological evaluation of the synthesized compounds. The animals were housed in groups of six at room temperature of $25 \pm 2^{\circ}$ C under 12 h light/12 h dark cycle, with free access to food and water, and utmost care was taken to ensure that the animals are treated in the most humane and acceptable manner. Animals were obtained from central animal house facility, Hamdard University, New Delhi, India.

Antiinflammatory activity

Antiinflammatory activity of the synthesized compounds (**2b**, **2d**, **2e**, **2g**, **2h**, **2j** and **4**) was determined by following carrageenan-induced rat paw edema method (26). The rats were randomly divided into groups of six. One group was kept as a control, and received only 0.5% carboxymethylcellulose (CMC) solution and the animals of other groups were treated with test compounds and standard drug (indomethacin) at a dose level of 10 mg/kg *p.o.* Indomethacin was obtained as a gift sample from ARBRO Pharmaceuticals, Kirti Nagar, New Delhi, India. Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1 mL was injected

subcutaneously into the sub-plantar region of the right hind paw of each rat, 30 min after the administration of the test compounds and the standard drug. The paw volume was measured by plethysmometer after 3 h of carrageenan injection. The edema volume in control group (V_c) and edema volume in groups treated with test compounds (V_t) was measured and the percentage inhibition of edema was calculated using the formula:

Antiinflammatory activity (% inhibition) = $[(V_{c} - V_{t})/V_{c}] \times 100$

Acute ulcerogenesis

Ulcerogenic activity (27) was evaluated after p.o. administration of test compounds and standard drug (indomethacin) at the dose of 60 mg/kg. Control rats received *p.o.* vehicle (suspension of 1%) methylcellulose). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and then sacrificed. Each stomach was removed and opened along the greater curvature, washed with distilled water and cleaned carefully by dipping in normal saline. The mucosal damage was examined with a magnifying glass (10×) and assessed according to the following scoring system: 0.5: redness, 1.0: spot ulcers, 1.5: hemorrhagic streaks, 2.0: ulcers > 3 but \leq 5, 3.0: ulcers > 5. The mean score of each treated group minus the mean score of control group was regarded as a severity index of ulcerogenesis.

Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the reported method (28). After screening for ulcerogenic activity, the gastric mucosa were scrapped with two glass slides, weighed (100 mg) and homogenized in 1.8 mL of 1.15% cold potassium chloride solution. The homogenate was supplemented with 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of acetate buffer (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid. The mixture was heated at 95°C for 1 h. The reactants were supplemented after cooling with 5 mL of the mixture of *n*-butanol : pyridine (15:1 v/v), shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm. The absorbance of the supernatant organic layer was measured at 532 nm on a UV spectrophotometer. The results were expressed as nmol MDA/100 mg tissue, using extinction coefficient $1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$.



Scheme 1. Protocol for the synthesis of Mannich bases (2a-f, 2g-j, 4)

RESULTS AND DISCUSSION

Chemistry

Title compounds were synthesized according to Scheme 1. The starting material, 4,6-diacetylresorcinol 1, was synthesized by the reported procedure (24). It was condensed with different amines and formaldehyde, however, the yields were poor and hence the reaction was carried out in the presence of tetrabutylammonium bromide, a phase transfer catalyst, in chloroform solution, to give the bases 2a-j. It is interesting to note that in one case, while using diethanolamine instead of the desired compound 3a, dimer 4 of the starting material was formed. The structures assigned to the compounds were supported by the results of elemental analysis as well as 'H NMR and mass spectral data.

The occurrence of Mannich reaction on the aromatic ring position between the two hydroxyl groups could be inferred by the presence of a methylene signal around d 4.1 \pm 0.2 instead of the signals for CH₂-CH₂ and the presence of acetyl signal consistently around d 2.6 \pm 0.2. Had the reaction proceeded at the acetyl function, there would be signals for CH₂-CH₂. 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 diacetylresorcinol moiety and can be formulated as shown in Figure 1. There was a fragment arising from the amine moiety which was dependent on the amine used.

However, the product with diethanolamine gave the CH_2 signal at d 4.32 and did not show any signal derived from diethanolamine moiety. From the molecular ion peak in the mass spectrum the compound could be inferred to be a dimer with CH_2 group as a bridge. Its NMR spectrum also did not show any signal arising from the diethanolamine moiety. The failure with diethanolamine to give a Mannich base analogue may not be unusual as it has been reported earlier also with ketones (25).

Biological evaluation

Antiinflammatory activity

In this test, the most active compounds were 2g and 2j which showed 56.26% and 51.80% inhibition, respectively, and their activity was comparable with that of the standard indomethacin (66.24%) at



Figure 1. Mass fragmentation pattern of Mannich bases (2a-j, 4)

Compound	Antiinflammatory activity ^a (% inhibition)	Ulcerogenic activity ^a (Severity index)	Lipid peroxidation ^{a,b}
2b	18.89 ± 1.69**		
2d	27.6 ± 1.96**		
2e	40.76 ± 1.79**	1.16 ± 0.25**	$4.42 \pm 0.52 **$
2g	56.26 ± 3.03*	0.91 ± 0.35**	3.26 ± 0.23**
2h	32.27 ± 2.19**		
2j	51.8 ± 1.84**	0.83 ± 0.25**	3.15 ± 0.46**
4	34.82 ± 1.6**		
Control	-	0.00	2.59 ± 0.20**
Ind.	66.24 ± 2.1	2.25 ± 0.21	7.95 ± 0.56

Table 1. Biological evaluation data of the title compounds.

^aValues are represented as the mean \pm SEM, Relative to respective standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for n = 6; ** p < 0.01; *p < 0.05. ^bLipid peroxidation values are presented as nmol MDA content/ 100 mg tissue. Ind. = indomethacin

the same dose level, 10 mg/kg *p.o.* The rest of the compounds showed moderate action. Mannich bases derived from morpholine and methylpiperazine were good in their antiinflammatory action. Maximum activity (56.26%) was seen in the morpholine derivative (2g), while replacing morpholine with methylpiperazine (2j) decreased the activity (51.80%) and it was further observed that replacing

it with substituted phenyl (2d and 2e) or piperidine moiety (2h) the activity decreased significantly. The results are presented in Table 1. *Acute ulcerogenic activity*

The compounds which showed antiinflammatory activity > 40% (**2e**, **2g** and **2j**) were screened for their ulcerogenic activity in albino rats at the dose of 60 mg/kg. The tested compounds showed low ulcerogenic activity (1.16, 0.91 and 0.83, respectively), whereas indomethacin showed high severity index of 2.25 (Table 1).

Lipid peroxidation study

The compounds tested for ulcerogenic activity (2e, 2g, 2j) were also tested for their lipid peroxidation action. It is evident that compounds showing less ulcerogenic activity also show reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation (28). Therefore, an attempt was made to correlate the low ulcerogenesis of the compounds with that of lipid peroxidation. The activity was determined according to the method of Ohkawa (28). The lipid peroxidation is measured as nmol of MDA/100 mg of tissue. Indomethacin (standard drug) showed high lipid peroxidation, 7.95, whereas the control group showed 2.59 nmole/100 mg of tissue (Table 1). It was found that all the three compounds showing low ulcerogenic activity also showed reduction in lipid peroxidation.

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