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Research Article

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF MANNICH BASES CONTAINING MORPHOLINE MOIETY

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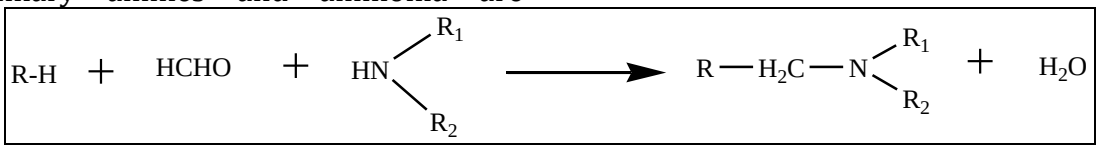
Keywords: 4-nitro acetophenone, substituted benzaldehydes, morpholine, mannich reaction, in vitro antibacterial activity.

ABSTRACT

A variety of morpholine derivatives as mannich bases were prepared through mannich reaction by reacting 4-nitro acetophenone as compound containing active hydrogen, substituted benzaldehyde and morpholine as secondary amine compound. All the synthesized compounds structures were characterized by physical analysis data and spectral analysis data (IR and ^{1}H-NMR spectral analysis). The newly synthesized compounds were evaluated for their antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa* in comparision with standard drug Streptomycin. However the antibacterial activity of the synthesized compounds against the tested organisms was found to possess good to moderate activity.

INTRODUCTION

The mannich reaction is an example of nucleophilic addition of an amine to a carbonyl group followed by dehydration to the schiff base. The mannich reaction is also considered as a condensation reaction. In the mannich reaction, primary or secondary amines or ammonia, are employed for the activation of formaldehyde. The mannich reaction is a three component condensation reaction in which a compound containing an active hydrogen atom is allowed to react with formaldehyde and an amine derivative. Secondary amines rather than primary amines and ammonia are employed; the resulting product (mannich base) is an amine compound having the N atom linked to the R substrate through a methylene group. The mannich reaction can be presented by the following reaction. The essential feature of the reaction is the replacement of the active hydrogen atom by an aminomethyl or substituted aminomethyl group. The R-H moiety symbolizes the active hydrogen component which includes ketones, aldehydes, acids, esters, phenols, acetylenes, α-picolines, nitroalkanes and quinolines.



Mannich bases have gained importance due to their application in antibacterial activity[1,2] and other applications are in agro chemicals such as plant growth regulators[3]. Moreover N-bridged heterocyclic derivatives show important antibacterial activity[4]. The aminoalkylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds[5]. Mannich bases have several biological activities such as antimicrobial[6] and anticancer[7]. Morpholine derivatives were

reported to possess antimicrobial[8], anti-inflammatory[9] and central nervous system activities[10]. Therefore, bearing in mind the above observation, we were led to synthesize and test the antimicrobial activity of a new series of mannich base derivatives.

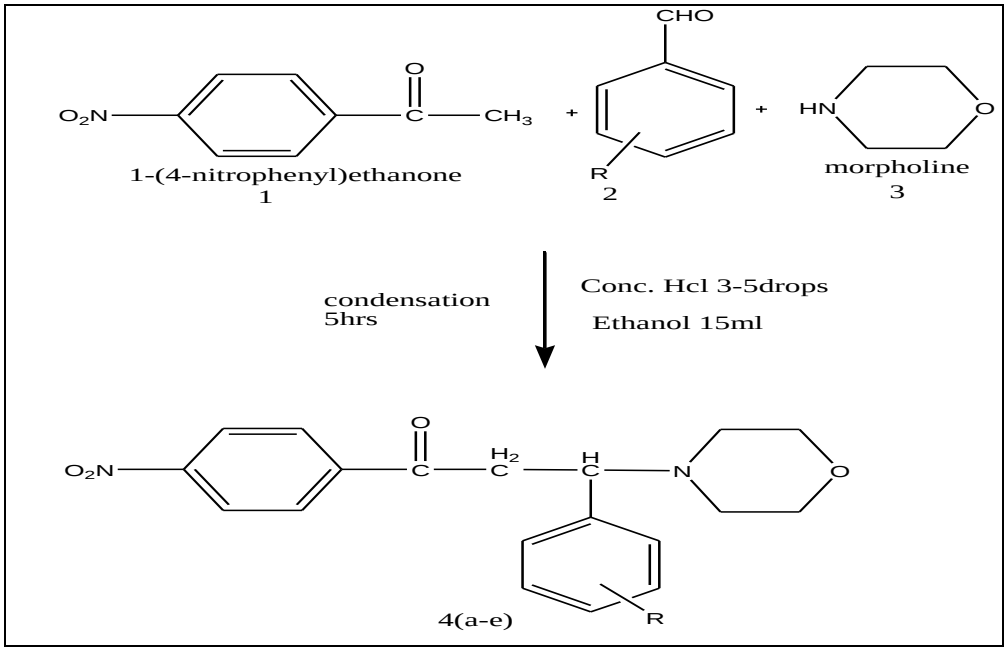
MATERIALS AND METHODS

All the chemicals were procured from commercial suppliers Merck grade and further they were used without purification. Melting points were determined in open capillary tubes on electrical melting point apparatus and are uncorrected. The ^{1}H-NMR spectra chemical shifts in δ, ppm were recorded on Bruker NMR 400 MH_Z using spectrophotometer using DMSO-d_6 as solvent. The IR spectra of the synthesized compounds were recorded on Bruker FT-IR spectrophotometer with KBr pellets. The progress of the reaction and purity of the compounds was checked by TLC on pre-coated silica gel G plates by using *n*-hexane:ethylacetate (9:1) v/v as a mobile phase and visualized in UV cabinet.

EXPERIMENTAL

General procedure for synthesis of compounds 4a-4e[11]

To a mixture of 4-nitroacetophenone (1.6gm), aryl aldehyde (1.2ml) and morpholine (1ml), add 15ml of ethanol, concentrated hydrochloric acid 3-5 drops in a round bottom flask. The mixture is condensed for 5 hrs at room temperature. The completion of the reaction was confirmed by TLC (using Silica Gel-G stationary phase and *n*-hexane:ethylacetate, 9:1 v/v as mobile phase). The reaction mixture was poured into crushed ice and the product was precipitated as a solid. The contents were filtered and it was dried and recrystallised from 95% ethanol. The above procedure was followed by all the remaining compounds. Experimental scheme was given in the **Figure 1**.



Compound	R	Compound	R
4a	4-hydroxy	**4d**	2,4-dichloro
4b	4-methyl	**4e**	3,4,5-tri methoxy
4c	3-fluoro		

Figure 1: Experimental scheme

ANTIBACTERIAL ACTIVITY[12,13,14]

Antibacterial property involves in the measurement of the relative potency or activity of compounds by determining the amount of test material required for producing stipulated effect on suitable organism under standard conditions.

The procedures employed in microbial assay were,

a. Cylinder plate method or cup plate method

b. Turbidimetric or tube assay method (two fold serial dilution method).

In the present study, antimicrobial screening was carried out by cup plate method. In cup plate method, the antimicrobial substance diffuses from the cup through a solidified agar layer in a petridish or a plate to an extent so that the growth of added micro-organism is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antimicrobial substance. The antibacterial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader.

All the synthesized compounds were screened for antibacterial activity against gram positive and gram negative micro organisms and the activity was compared with appropriate reference standard. Microorganisms were grown in nutrient agar medium. Methanol and distilled water were used as control and the drug vehicles for the samples and reference standards respectively.

Test organisms: The microorganisms used for the experiment were procured from MTCC, IMTECH, Chandighar. Gram-positive organisms: *Staphylococcus aureus, Bacillus subtilis.* Gram-negative organisms: *Escherichia coli, Pseudomonas aeruginosa.*

Culture Media: Nutrient agar for bacteria**-** Beef extract 0.3%, Sodium chloride 0.5%, Peptone 0.5%, Agar 2.0%, pH 7.2-7.4.

Sterilization: Sterilization of the media, water, etc. was carried out at 120ºC by autoclaving at 15 lbs/inch^2 for about 20 minutes. The glassware like syringes, petridishes, pipettes, empty test tubes were sterilized by dry heat in an oven at a temperature of 160°C for one hour. The sterilized medium was cooled to 40°C and poured into the petridishes to contain 6 mm thickness. The media was allowed to solidify at room temperature.

Preparation of test and standard solutions: The stock solution of test compounds was prepared by dissolving the samples at a concentration of 1mg/ml in methanol. The stock solution of reference standard Streptomycin was prepared at a concentration of 1mg/ml in sterile water. Antibacterial activity was screened by adding 0.05 ml stock solution to each cup by using micropipette. All the test compounds were dissolved in methanol at a concentration of 1mg/ml. Each plate was inoculated with 20 µl of microbial suspension. 100 µl of the test compounds was added to each cup. The plates containing bacteria were incubated at 37ºC for 24 hrs, the positive antimicrobial activity were read based on the growth inhibition zone and compared with Streptomycin drug.

Determination of zone of inhibition by cup plate method[15]

The cup plate assay of drug potency is based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds. A sterile borer was used to prepare four cups of 6 mm diameter in the agar medium spread with the micro-organisms and 0.1 ml of inoculum was spread on the agar plate by spread plate technique. Accurately measured (0.05 ml) solution of each concentration and reference standard was added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37°C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of methanol and water which were used as drug vehicles. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

RESULTS AND DISCUSSION

Morpholine derivatives as mannich bases were synthesized using the appropriate synthetic procedure i.e. reaction of compound containing active hydrogen (1), aryl aldehyde (2) and

secondary amine compound morpholine (3) in presence of ethanol as solvent and conc. HCl catalyst. The reactants 4-nitro acetophenone, substituted aromatic aldehyde and morpholine were taken in a RBF containing ethanol and catalytic amount of conc. HCl and heated at refluxing temperature for 5-6 hrs. The reactants were heated at 80-90ºC and the progress of reaction was monitored by TLC. Finally the reaction mixture was poured onto the crushed ice and then recrystallized from ethanol. The melting point of the compound was found to be same as that of reported. Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded in KBr discs on a Bruker (300 FT-IR). Thin layer chromatography was performed on silica gel-G (Merck). ^{1}H-NMR spectra were recorded on a Bruker 400 spectrometer operating at 400.13 MHz for 1H in DMSO. Physical characterization data of all the synthesized compounds we given in Table 1.

Table 1: Physical characterization data of synthesized compounds *4a-4f*

Compd.	R	m.p. (ºC)	Molecular formula	m.w.	% yield	Elemental analysis (%) C, H, N-Calculated
4a	4-hydroxy	170	$C_{19}N_2O_5H_{20}$	356	88.24	63.24, 5.87, 7.76
4b	4-methyl	190	$C_{20}N_2O_4H_{22}$	354	80.12	66.26, 6.15, 8.13
4c	3-fluoro	170	$C_{19}H_{19}N_2O_4F$	357.9	60.01	66.65, 6.48, 8.18
4d	2,4-dichloro	178	$C_{19}H_{18}N_2O_4Cl_2$	409	66.84	67.40, 6.79, 7.86
4e	3,4,5-trimethoxy	60	$C_{22}H_{26}N_2O_7$	430	87.43	57.73, 5.10, 7.09

Spectral data

3-(4-hydroxyphenyl)-3-morpholino-1-(4-nitrophenyl)propan-1-one (4a):

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3016.24, Aromatic C=C stretch: 1543.22, C=O stretch: 1710, Phenolic –OH stretch: 3510.11, Aromatic C-O-C stretch: 1209.84, NO_2 stretch: 1525, C-N stretch: 1112.21, Alkyl C-H stretch: 2849.1. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 8.34 (d,4H,C_6H_4-NO_2), 2.81-3.06(d,2H, -$COCH_2$), 4.44 (t,1H,-CH_2-C**H**), 6.70(d,2H, Phenolic C-3 & C-5 protons), 7.12 (d,2H, phenolic C-1 & C-6 protons), 2.67 (t, 4H, C-3 & C-5 Morpholine protons), 3.59 (t, 4H, C-2 & C-6 Morpholine protons), 5.35 (s, 1H, phenolic-OH).

3-morpholino-1-(4-nitrophenyl)-3-tolypropan-1-one (4b):

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3025.65, Aromatic C=C stretch: 1556.73, C=O stretch: 1712, Aromatic C-O-C stretch: 1215.65, NO_2 stretch: 1522, C-N stretch: 1312.21, Alkyl C-H stretch: 2979.85. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 8.34 (d,4H,C_6H_4-NO_2), 2.81-3.06(d,2H, -$COCH_2$), 4.44 (t,1H,-CH_2-C**H**), 7.18 (d,2H, Tolyl C-3 & C-5 protons), 7.17 (d,2H, tolyl C-1 & C-6 protons), 2.67 (t, 4H, C-3 & C-5 Morpholine protons), 3.59 (t, 4H, C-2 & C-6 Morpholine protons), 2.34 (s, 3H, C_6H_4-C**H$_3$**).

3-(3-flurophenyl)-3-morpholino-1-(4-nitrophenyl)propan-1-one (4c):

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3029.45, Aromatic C=C stretch: 1536.53, C=O stretch:1714, Aromatic C-O-C stretch: 1240.65, NO_2 stretch: 1322, C-N stretch: 1310.31, Alkyl C-H stretch: 2849.85, C-F stretch: 1349.21. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 8.34 (d,4H,C_6H_4-NO_2), 2.81-3.06(d,2H, -$COCH_2$), 4.44 (t,1H,-CH_2-C**H**), 6.83 (s,1H, C-2 proton of C_6H_4F), 7.06 (d,2H,C-4 & C-6 protons of C_6H_4F), 7.38 (t,1H, C-5 proton of C_6H_4F), 2.67 (t, 4H, C-3 & C-5 Morpholine protons), 3.59 (t, 4H, C-2 & C-6 Morpholine protons).

3-(2,4-dichlorophenyl)-3-morpholino-1-(4-nitrophenyl)propan-1-one (4d):

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3050.45, Aromatic C=C stretch: 1585.23, C=O stretch:1715, Aromatic C-O-C stretch: 1230.65, NO_2 stretch: 1345, C-N stretch: 1323.31, Alkyl C-H stretch: 2949.63, C-Cl stretch: 845.22. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 8.34 (d,4H,C_6H_4-NO_2), 2.81-3.06(d,2H, -$COCH_2$), 4.44 (t,1H,-CH_2-C**H**), 7.75 (s,1H, C-3 proton of $C_6H_3Cl_2$), 7.32 (d,1H, C-5 proton of $C_6H_3Cl_2$), 7.04 (d,1H, C-6 proton of $C_6H_3Cl_2$), 2.67 (t, 4H, C-3 & C-5 Morpholine protons), 3.59 (t, 4H, C-2 & C-6 Morpholine protons).

3-(3,4,5-trimethoxyphenyl)-3-morpholino-1-(4-nitrophenyl)propan-1-one (4e):

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3075.45, Aromatic C=C stretch: 1595.32, C=O stretch:1715, Aromatic C-O-C stretch: 1120.65, NO_2 stretch: 1290, C-N stretch: 1345.13, Alkyl C-H stretch: 2850.63. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 8.34 (d,4H,C_6H_4-NO_2), 2.81-3.06(d,2H, -$COCH_2$), 4.44 (t,1H,-CH_2-C**H**), 3.83 (s,9H, -OCH_3), 6.52 (s,2H, C-1 & C-6 protons of trimethoxy benzene), 2.67 (t, 4H, C-3 & C-5 Morpholine protons), 3.59 (t, 4H, C-2 & C-6 Morpholine protons).

***In vitro* antibacterial activity**

Antimicrobial screening was carried out against gram+ve and gram-ve micro organisms by cup plate method. In cup plate method, the antimicrobial substance diffuses from the cup through a solidified agar layer in a petridish or a plate to an extent so that the growth of added micro-organism is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of antimicrobial substance. The antibacterial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader and were depicted in Table 2. Graphical representation of comparative antibacterial activity of the synthesized compounds were shown in the Figure 2.

Table 2: Zone of inhibition (mm) of the tested samples and reference compound

Compound (100 µg/ml)	Gram+ve bacteria		Gram-ve bacteria	
	S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*
4a	14	13	15	13
4b	16	12	18	15
4c	9	7	5	10
4d	8	11	9	11
4e	7	9	6	8
Control	-	-	-	-
Streptomycin	25	19	16	24

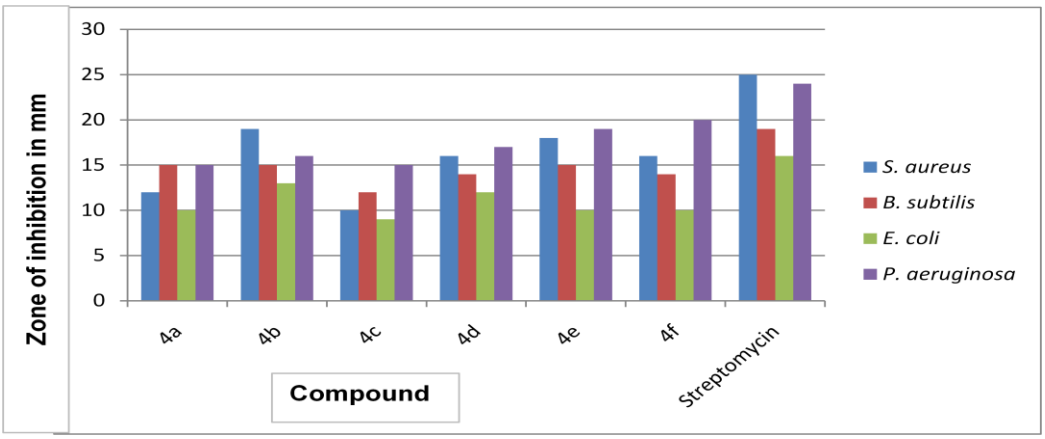


Figure 2: Comparative antibacterial activity of the synthesized compounds

CONCLUSION

In the present work various substituted aryl aldehydes were used to prepare substituted morpholine derivatives as mannich bases in good yields. A facile one-pot method under mild conditions has been developed for the synthesis of the title compounds. All the compounds synthesized were characterized by physically (R_f values, Melting point, Molecular weight, Molecular formula) and few compounds were characterized by spectral data (^{1}H-NMR, IR spectra). All the compounds were evaluated for their antibacterial activity against gram+ve and gram-ve micro-organisms by cup plate method. Among the synthesized compounds 3-(4-hydroxyphenyl)-3-morpholino-1-(4-nitrophenyl)propan-1-one 4a gives high % yield. The antibacterial screening results states that compound 4b shown significant activity against S. aureus, 4a compound shown significant activity against *B. subtilis*, compound 4b shown significant activity against *E. Coli* and *P. aeruginosa.*

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