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Original Article

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SYNTHESIS OF PARACETAMOL DERIVATIVES AS MANNICH BASES AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract

A variety of Paracetamol derivatives as mannich bases were prepared through mannich reaction by reacting Paracetamol as compound containing active hydrogen, substituted benzaldehyde, morpholine as secondary amine compound and small amount of conc. HCl as catalyst. A simplistic one-pot method under mild conditions has been developed for the synthesis of all the compounds and they were characterized by physically (Rf values, Melting point, Molecular weight, Molecular formula) and by spectral data (IR and 1H-NMR spectral analysis). Antibacterial activity was carried out by using cup plate method. All the newly synthesized compounds were screened for antibacterial activity against gram positive and gram negative microorganisms i.e. Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa in comparison with standard drug Streptomycin. However the antibacterial activity of the synthesized compounds against the tested organisms was found to possess good to moderate activity. The 1H-NMR spectra chemical shifts in δ, ppm were recorded on Bruker NMR 400 MHZ using spectrophotometer using DMSO-d6 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:ethyl acetate (9:1) v/v as a mobile phase and visualized in UV cabinet. A facile one-pot method under mild conditions has been developed for the synthesis of the title compounds. All the compounds were evaluated for their antibacterial activity against gram +ve and gram -ve micro-organisms by cup plate method. 3-(4-chlorophenyl)-3-(morpholine-4-yl)-N-(4-hydroxyphenyl) propanamide 4a gives high % yield. The antibacterial screening results states that compound 4b shown significant activity against S. aureus, 4a and 4b compounds shown significant activity against B. subtilis, compound 4b shown significant activity against E. coli and compound 4f shown significant activity against P. aeruginosa.

Keywords: Paracetamol, Substituted benzaldehydes, Morpholine, Mannich reaction, In vitro antibacterial activity

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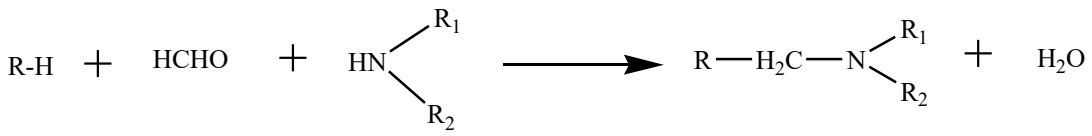
INTRODUCTION

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 symbolizes the active hydrogen component which includes ketones, aldehydes, acids, esters, phenols, acetylenes, a-picolines, nitroalkanes and quinolines.



Mannich bases have gained importance due to their application in antibacterial activity [Holla, 2010, Chakravarthy, 2006]and other applications are in agrochemicals such as plant growth regulators [Mannich, 1912]. Moreover N-bridged heterocyclic derivatives show important antibacterial activity [Turan-Zitouni, 2005]. The amino alkylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds[Tramontini, 1990]. Mannich bases have several biological activities such as antimicrobial [Medić-Šarić, 1980] and anticancer[Borenstein, 1987]. Morpholine derivatives were reported to possess antimicrobial [Tramontini, 1973], anti-inflammatory [Thompson, 1968] and central nervous system activities [Cummings, 1960]. 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.

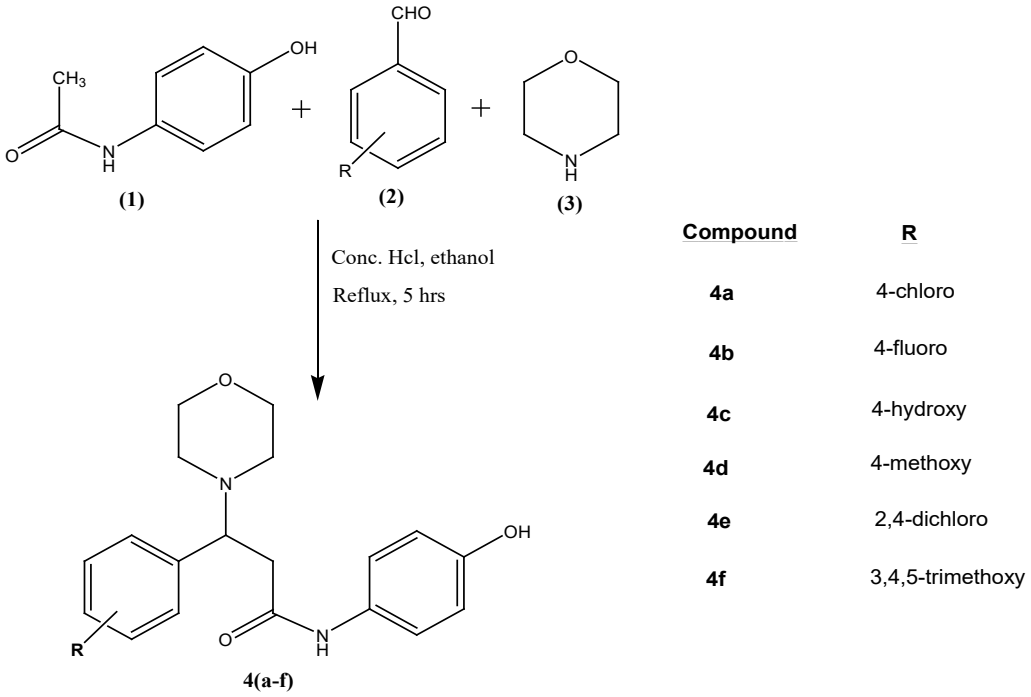
MATERIALS AND METHODS

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 1H-NMR spectra chemical shifts in δ, ppm were recorded on Bruker NMR 400 MHZ by spectrophotometer using DMSO-d6 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:ethyl acetate (9:1) v/v as a mobile phase and visualized in UV cabinet.

EXPERIMENTAL

General procedure for synthesis of 3-(4-chlorophenyl)-3-(morpholine-4-yl)-N-(4-hydroxyphenyl)propanamide(4a) [Idhayadhulla, 2014]:

A mixture of 4-chlorobenzaldehyde (0.01 mol), morpholine (0.01 mol) and paracetamol (0.01 mol) in 10 ml of ethanol was prepared. Small amount of Conc. HCl 3-5 drops was added to the reaction mixture. The resulting mixture was refluxed for 5 hrs at 110ºC. The completion of the reaction was confirmed by TLC (using Silica Gel-G stationary phase and n-hexane:ethyl acetate, 9:1 v/v as mobile phase). The reaction mixture was poured into ice-cold water and the product was precipitated as a pale yellow solid. The contents were filtered and the product was washed with cold water, dried and purified by recrystallization from 95% ethanol. The above procedure was followed by all the remaining compounds. Experimental scheme was given in the Figure



Compound	R
4a	4-chloro
4b	4-fluoro
4c	4-hydroxy
4d	4-methoxy
4e	2,4-dichloro
4f	3,4,5-trimethoxy

Figure 1: Experimental scheme

3-(4-chlorophenyl)-3-(morpholin-4-yl)-N-(4-hydroxyphenyl)propanamide (4a)

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3009.15, C=C stretch: 1530.24, NHCO stretch: 1654.84, Phenolic OH stretch: 3510.12, C-Cl stretch: 874.52. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 10.221 (s, 1H,-CONH-), 6.535-7.125 (d, 4H, Ph-OH), 9.658 (s, 1H, Ph-OH), 7.165-7.439 (d, 4H, Ph-OCH_3), 2.632-2.759 (d, 2H, $COCH_2$), 4.204-4.368 (t, 1H, $COCH_2CH$), 3.658-3.847 (t, 4H, CH_2-O-CH_2), 2.554-2.684 (t, 4H, CH_2-N-CH_2).

3-(4-fluorophenyl)-3-(morpholin-4-yl)-N-(4-hydroxyphenyl)propanamide (4b)

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3015.44, C=C stretch: 1542.28, NHCO stretch: 1663.12, Phenolic OH stretch: 3508.14, C-F stretch: 1225.57. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 10.245 (s, 1H,-CONH-), 6.425-7.146 (d, 4H, Ph-OH), 9.742 (s, 1H, Ph-OH), 7.109-7.398 (d, 4H, Ph-OCH3), 2.587-2.697 (d, 2H, $COCH_2$), 4.225-4.374 (t, 1H, $COCH_2CH$), 3.584-3.742 (t, 4H, CH_2-O-CH_2), 2.565-2.674 (t, 4H, CH_2-N-CH_2).

3-(4-hydroxyphenyl)-3-(morpholin-4-yl)-N-(4-hydroxyphenyl)propanamide (4c)

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3006.32, C=C stretch: 1554.26, NHCO stretch: 1659.62, Phenolic OH stretch: 3521.10, 3584.16. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 10.462 (s, 1H,-CONH-), 6.561-7.245 (d, 4H, Ph-OH), 9.537 (s, 1H, Ph-OH), 9.758 (s, 1H, Ph-OH), 7.256-7.489 (d, 4H, Ph-OCH_3), 2.546-2.684 (d, 2H, $COCH_2$), 4.310-4.426 (t, 1H, $COCH_2CH$), 3.512-3.708 (t, 4H, CH_2-O-CH_2), 2.542-2.651 (t, 4H, CH_2-N-CH_2).

3-(4-methoxyphenyl)-3-(morpholin-4-yl)-N-(4-hydroxyphenyl)propanamide (4d)

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3016.24, C=C stretch: 1543.22, NHCO stretch: 1668.24, Phenolic OH stretch: 3508.45, C-O-C stretch: 1209.84. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 10.684 (s, 1H,-CONH-), 6.534-7.037 (d, 4H, Ph-OH), 9.757 (s, 1H, Ph-OH), 7.265-7.574 (d, 4H, Ph-OCH_3), 3.859 (s, 3H, OCH_3), 2.658-2.765 (d, 2H, $COCH_2$), 4.228-4.371 (t, 1H, $COCH_2CH$), 3.665-3.832 (t, 4H, CH_2-O-CH_2), 2.546-2.675 (t, 4H, CH_2-N-CH_2).

3-(2,4-dichlorophenyl)-3-(morpholin-4-yl)-N-(4-hydroxyphenyl)propanamide (4e)

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3025.65, C=C stretch: 1556.73, NHCO stretch: 1687.25, Phenolic OH stretch: 3511.04, C-O-C stretch: 1215.65, C-Cl stretch: 775.23. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 10.552 (s, 1H,-CONH-), 6.712-7.324 (d, 4H, Ph-OH), 9.445 (s, 1H, Ph-OH), 7.234 (s, 1H, 3'=CH, 2,4-dichloro phenyl), 7.125-7.167 (d, 1H, 5'=CH, 2,4-dichloro phenyl), 6.945-6.994 (d, 1H, 6'=CH, 2,4-dichloro phenyl), 2.744-2.775 (d, 2H, $COCH_2$), 4.152-4.264 (t, 1H, $COCH_2CH$), 3.612-3.842 (t, 4H, CH_2-O-CH_2), 2.455-2.614 (t, 4H, CH_2-N-CH_2).

3-(3,4,5-trimethoxyphenyl)-3-(morpholin-4-yl)-N-(4-hydroxyphenyl)propanamide (4f)

IR (KBr, cm^{-1}): Aromatic C-H stretch: 3031.27, C=C stretch: 1563.42, NHCO stretch: 1646.56, Phenolic OH stretch: 3518.41, C-O-C stretch: 1195.46, 1209.84, 1213.24. ^{1}H-NMR (400 MHz, DMSO-D_6) δ: 10.941 (s, 1H,-CONH-), 6.634-7.139 (d, 4H, Ph-OH), 9.643 (s, 1H, Ph-OH), 7.442 (s, 2H, Ph-OCH_3), 3.754 (s, 6H, 3'&5'-OCH_3), 3.623 (s, 3H, 4'-OCH3), 2.735-2.839 (d, 2H, $COCH_2$), 4.246-4.382 (t, 1H, $COCH_2CH$), 3.635-3.810 (t, 4H, CH_2-O-CH_2), 2.612-2.726 (t, 4H, CH_2-N-CH_2).

ANTIBACTERIAL ACTIVITY [Idhayadhulla, 2014; Saroj Kumar, 2016; Nagarajaa, 2011, prabu, 2011] :

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 the 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 the cup plate method. In cup plate method, the antimicrobial substance diffuses from the cup through a solidified agar layer in a Petri dish 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 the 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 microorganisms and the activity was compared with an appropriate reference standard. Microorganisms were grown in nutrient agar medium. Methanol and distilled water were used as a 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/inch2 for about 20 minutes. The glassware like syringes, Petri dishes, pipettes, empty test tubes was 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 Petri dishes 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 1 mg/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 1 mg/ml. Each plate was inoculated with 20 μl of microbial suspension. 100 μl of the test compounds were added to each cup. The plates containing bacteria were incubated at 37ºC for 24 hrs, the positive antimicrobial activity was read based on the growth inhibition zone and compared with Streptomycin drug.

Determination of zone of inhibition by cup plate method (Indian Pharmacopoeia, 1996).

The cup plate assay of drug potency is based on measurement of the diameter of the 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 a 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

CHEMISTRY

Paracetamol derivatives as mannich bases were synthesized using the appropriate synthetic procedure i.e. reaction of a compound containing active hydrogen (1), aryl aldehyde (2) and secondary amine compound morpholine (3) in presence of ethanol as solvent and conc. HCl as a catalyst. The reactants, Paracetamol, substituted aromatic aldehyde, and morpholine were taken in an RBF containing ethanol and a 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 the 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 were uncorrected. IR spectra were recorded in KBr discs on a Bruker (300 FT-IR). Thin layer chromatography was performed on silica gel-G (Merck). 1H-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 were 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-chloro	212-214	$C_{19}N_2O_3H_{21}Cl$	360	79.25	63.24, 5.87, 7.76
4b	4-fluoro	228-230	$C_{19}N_2O_3H_{21}F$	344	63.34	66.26, 6.15, 8.13
4c	4-hydroxy	198-200	$C_{19}H_{22}N_2O_4$	342	71.08	66.65, 6.48, 8.18
4d	4-methoxy	234-236	$C_{20}H_{24}N_2O_4$	356	67.50	67.40, 6.79, 7.86
4e	2,4-dichloro	258-260	$C_{19}H_{20}N_2O_3Cl_2$	394	71.35	57.73, 5.10, 7.09
4f	3,4,5-trimethoxy	244-246	$C_{22}H_{28}N_2O_6$	416	65.60	63.45, 6.78, 6.73

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	12	15	10	15
4b	19	15	13	16
4c	10	12	9	15
4d	16	14	12	17
4e	18	15	10	19
4f	16	14	10	20
Control	-	-	-	-
Streptomycin	25	19	16	24

IN VITRO ANTIBACTERIAL ACTIVITY

The antimicrobial screening was carried out against gram+ve and gram-ve microorganisms by cup plate method. In cup plate method, the antimicrobial substance diffuses from the cup through a solidified agar layer in a Petri dish or a plate to an extent so that the growth of

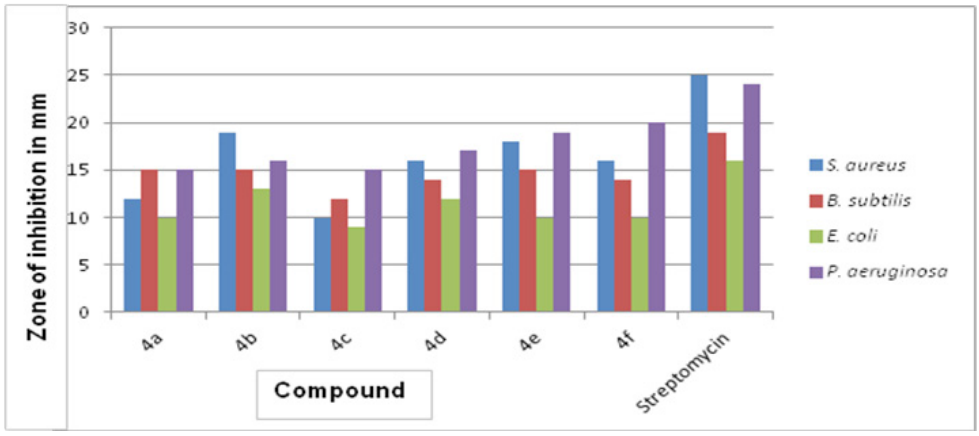


Figure 2: the Comparative antibacterial activity of the synthesized compounds

added micro-organism is inhibited entirely in a circular area or zone around the cavity containing the solution of a known quantity of the 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 the comparative antibacterial activity of the synthesized compounds was shown in Figure 2.

CONCLUSION

In the present work, variously substituted aryl aldehydes were used to prepare substituted Paracetamol 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 physically (Rf values, Melting point, Molecular weight, Molecular formula) and few compounds were characterized by spectral data (1H-NMR, IR spectra). All the compound were evaluated for their antibacterial activity against gram+ve and gram-ve micro-organisms by cup plate method. Among the synthesized compounds 3-(4-chlorophenyl)-3-(morpholin-4-yl)-N-(4-hydroxyphenyl) propanamide 4a gives high % yield. The antibacterial screening results state that compound 4b shown significant activity against S. aureus, 4a and 4b compounds shown significant activity against B. subtilis, compound 4b shown significant activity against E. coli and compound 4f shown significant activity against P. aeruginosa.

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