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Research Article

Synthesis, Characterization and Antioxidant Activity of Morpholine Mannich Base Derivatives

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ABSTRACT
A series of morpholine mannich base derivatives were synthesized by a single step reaction of morpholine with substituted benzaldehydes and N-phenylacetamide. Progress of reaction was monitored by TLC using ethylacetate and n-hexane as mobile phase. The compounds were characterized by ATR and ^{1}HNMR spectral analysis. Antioxidant assay of the synthesized compounds was done by DPPH and ABTS assay method using Ascorbic acid as standard. The synthesized morpholine mannich base derivatives demonstrated significant radical scavenging property.

Keywords: Morpholine, mannich base, antioxidant

INTRODUCTION

Free radicals which are the products of normal cellular metabolism play an important role in the survival and death of a cell. These are reactive chemical species with a single unpaired electron in the outer orbit and are capable of independent existence. The unstable configuration of free radicals results in abstracting of electron from adjacent substances which in turn become free radicals and ultimately result in damage to the cell[1]. Two types of free radicals are ROS(Reactive oxygen species) and RNS(Reactive nitrogen species)[2]. ROS and RNS at optimum concentration are involved in various physiological functions such as cellular signaling pathways, redox regulation, immune function, in mitogenic response[3,4]. Under normal circumstances they are scavenged by antioxidants which act as defense mechanism. Insufficient levels of antioxidants lead to oxidative stress and nitrosative stress due to increase in production of free radicals. Oxidative stress which is defined as an imbalance between reactive oxygen species and defensive antioxidant system, deregulates number of cellular functions[5]. It is responsible for various pathological states such as ageing[6],atherosclerosis[7], aids[8], cancer[9], asthma[10], autoimmune diseases such as rheumatic disease[11], cardiovascular dysfunction[12], cataract[13], diabetes[14,15], neurodegenerative disease such as Alzheimer's[16] and Parkinson disease[17].

Antioxidants

Antioxidants are substances required at low concentrations for delaying or inhibiting oxidation of a substrate. Thereby, act as scavangers of free radicals[18]. Monohydroxy/polyhydroxyphenol groups present in the structure of antioxidants slow down the pace of lipid peroxidation[19].

Classification of antioxidants

The antioxidants are classified in the following ways:
Gutteridge and Halliwell divided antioxidants into three categories
Primary antioxidants: These are the agents which prevent the formation of oxidants.
Secondary antioxidants: Act as scavangers of ROS.
Tertiary antioxidants: They utilize dietary source or other antioxidants to repair the already oxidized molecules [20].

Antioxidants according to their nature and action

Enzymatic antioxidants: Glutathione peroxidase, catalase, superoxide dismutase.
Non –enzymatic antioxidants:
Nutrient antioxidants: Carotenoids, tocopherol, ascorbic acid.
Metabolic antioxidants: Glutathione,thioredoxin, lipoic acid[18].

On the basis of origin

Endogenous antioxidants: Further classified into primary antioxidants which form inactive ROS intermediates (Glutathione, superoxide dismutase, catalase and secondary antioxidants (glutathione reductase, glucose 6 phosphate dehydrogenase) which directly detoxify ROS without formation of any intermediate[21,22].
Exogenous/dietary antioxidants: These are obtained from food and various other dietary sources such as vegetables, herbs, spices and vitamins.

Mode of action of antioxidants

Primary or chain breaking antioxidants: Prevent the propagation of the reaction due to formation of less reactive radicals (superoxide dismutase, Vit E).

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Reaction Scheme

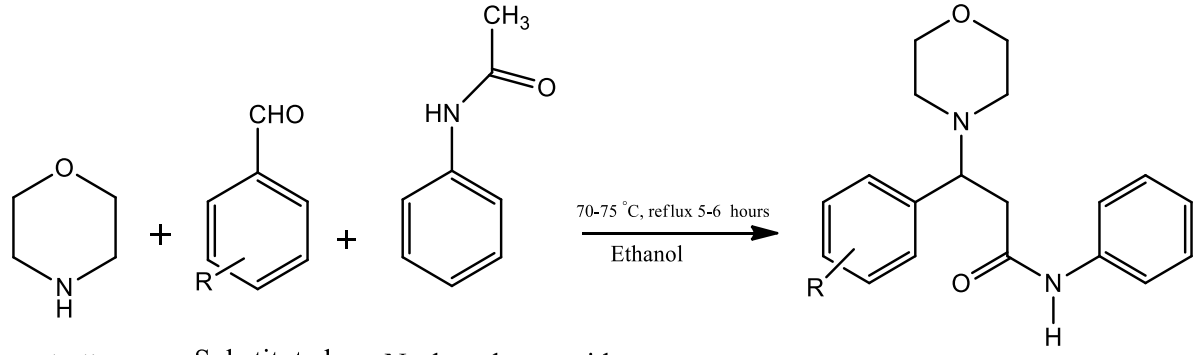
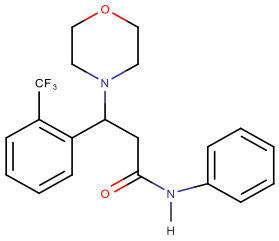
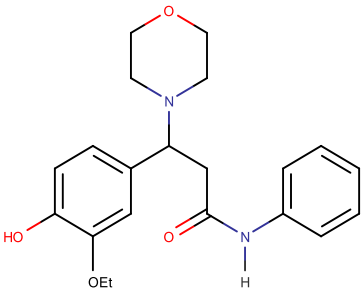
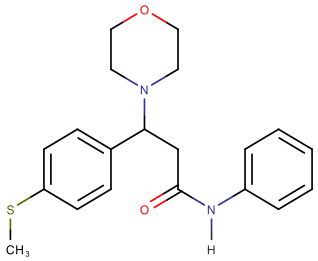
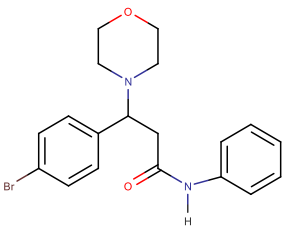
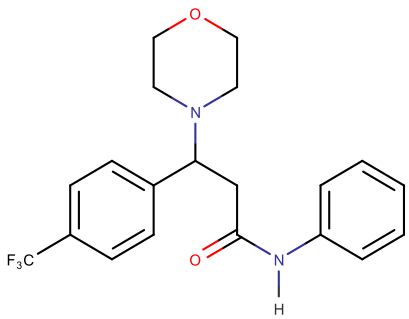
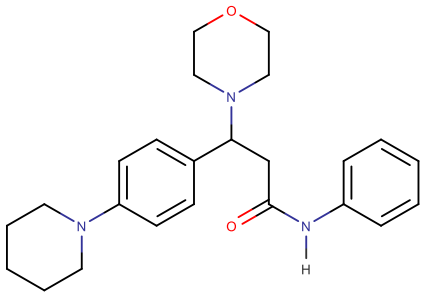
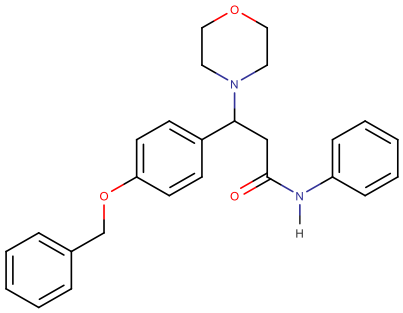
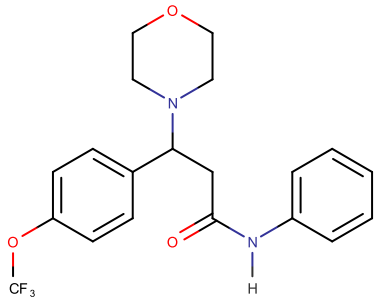
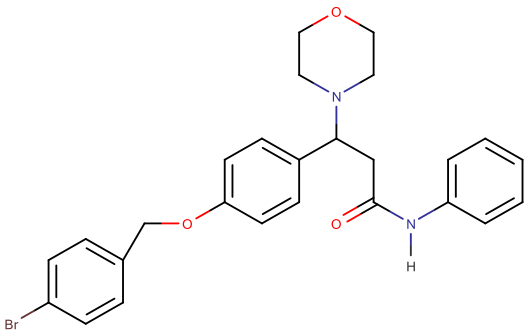


Table 1: List of synthesized compounds.

Name	Structure	IUPAC Name
MB-1		3-(morpholin-4-yl)-N-phenyl-3-[2-(trifluoromethyl)phenyl]propanamide
MB-2		3-(3-ethoxy-4-hydroxyphenyl)-3-(morpholino-4-yl)-N-phenylpropanamide
MB-3		3-(4-(methylthio)phenyl)-3-morpholino-N-phenylpropanamide
MB-4		3-(4-bromophenyl)-3-(morpholin-4-yl)-N-phenylpropanamide

MB-5		3-(morpholin-4-yl)-N-phenyl-3-[4-(trifluoromethyl)phenyl] propanamide
MB-6		3-(morpholin-4-yl)-N-phenyl-3-[4-(piperidin-1-yl)phenyl]propanamide
MB-7		3-[4-(benzyloxy)phenyl]-3-(morpholin-4-yl)-N-phenylpropanamide
MB-8		3-(morpholin-4-yl)-N-phenyl-3-[4-(trifluoromethoxy)phenyl]propanamide
MB-9		3-4-[(4-bromophenyl)methoxy]phenyl-3-(morpholin-4-yl)-N-phenylpropanamide

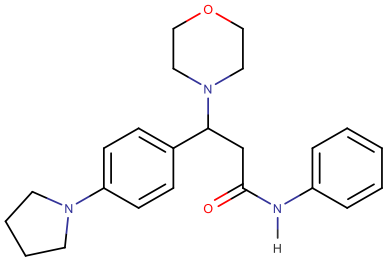
MB-10		3-(morpholin-4-yl)-N-phenyl-3-[4-(pyrrolidin-1-yl)phenylpropanamide

Table 2: Physical properties of synthesized compounds.

Name	Molecular formula	Molecular weight	Percentage Yield	Melting Point° C	Solubility
MB-1	$C_{20}H_{21}F_3N_2O_2$	378.39	74.3%	180-184	Chloroform, DMSO, Ethanol
MB-2	$C_{21}H_{26}N_2O_4$	370.44	36.8%	293-296	Chloroform, DMSO, Ethanol, Methanol
MB-3	$C_{20}H_{24}N_2O_2S$	356.16	51.2%	160-168	Chloroform, DMSO, Ethanol, Methanol
MB-4	$C_{19}H_{21}BrN_2O_2$	389.29	79.3%	143-148	Chloroform, DMSO, Ethanol, Methanol
MB-5	$C_{20}H_{12}F_3N_2O_2$	378.39	82.1%	210-216	Chloroform, DMSO, Ethanol, Methanol
MB-6	$C_{24}H_{31}N_3O_2$	393.52	29.4%	239-244	Chloroform, DMSO, Ethanol
MB-7	$C_{26}H_{28}N_2O_3$	416.51	60.2%	325-328	Chloroform, DMSO, Ethanol, Methanol
MB-8	$C_{20}H_{21}F_3N_2O_3$	394.39	69.7%	274-279	Chloroform, DMSO, Ethanol
MB-9	$C_{26}H_{27}BrN_2O_3$	495.41	28.3%	306-309	Chloroform, DMSO, Ethanol, Methanol
MB-10	$C_{23}H_{29}N_3O_2$	379.50	45.8%	210-211	Chloroform, DMSO, Ethanol

Secondary or preventive antioxidants: Block early production of free radicals (catalase, peroxidase)[18].

MATERIALS AND METHODS

All chemicals and solvents for the synthesis were supplied by Sigma Aldrich, Loba Chemie and CDH under certificate of purity. TLC of the newly synthesized compounds was done on Silica gel 60 F-254 coated glass plates purchased from Merck. Scientech-2211 digital auto melting/boiling point apparatus was used for the determination of melting point. Proton magnetic resonance (^{1}HNMR) spectra were recorded on Bruker 400 MHz NMR spectrometer using $CDCl_3$ as solvent. Chemical shifts were reported in parts per million relative to tetramethylsilane (TMS) as an internal standard. IR spectra of the compounds were recorded on Bruker- Alpha 1005151/06 ATIR spectrophotometer.

Synthetic Procedure

An equimolar quantity of morpholine and benzaldehyde were dissolved into ethanol and kept aside for about10 minutes. To, this solution equimolar concentration of N-phenylacetamide was added. The reaction mixture was refluxed for a period of 5-6 hours at a temperature of 70-75 °C. The progress of the reaction was monitored by TLC, using n-Hexane:Ethyl acetate in the ratio of 2:3 as the mobile phase. The reaction mixture was cooled to room temperature and then poured into ice cold water. The precipitate so obtained was collected by filtration except (MB-3, MB-5, MB-8) which seperated as precipitates in the reaction mixture. The precipitates were recrystallized with ethanol.

Antioxidant Assay

The antioxidant assay of the compounds was performed using DPPH and ABTS Assay method.

DPPH Assay

The antioxidant assay was performed by the method of *Mensor et al*[23]. 0.01mM solution of DPPH in methanol was perpared. Methanolic solutions of all the compounds in the following concentration ranges were prepared 40µg/mL, 60µg/mL, 80µg/mL and 100µg/mL. Similarly solutions of ascorbic acid in the same concentration range were prepared. 1mL DPPH solution was added to 1mL sample solution and to ascorbic acid solution as well. Finally volume was made upto 3mL using methanol. The test tubes containing the assay mixture were incubated at a dark and cool place for period of 30 minutes. After incubation the absorbance of the DPPH solution, blank (methanol), samples and ascorbic acid containing DPPH reagent were recorded at 517 nm on a UV-VIS spectrophotometer.

The anti-oxidant activity was measured using the formula

% scavenging or inhibition = $[(A_o-A_s)/A_o]*100$

Where,

A_o= Absorbance of the DPPH solution without sample

A_s= Absorbance of DPPH solution with sample

ABTS assay

7 mM of ABTS stock solution was prepared in water. 2.45mM solution of potassium persulfate was added to it in 1:1 ratio (volume/volume).The reaction mixture was left in a dark place for generation of free radicals for period of about 16 hours. This solution was further diluted using methanol so that it has a stable absorbance of 0.700±0.05 at 734nm. Methanolic solutions of all compounds including ascorbic acid were prepared in the concentration ranges of 40µg/mL, 60µg/mL, 80µg/mL and 100µg/mL. To 0.1ml of the test solutions 1mL of diluted ABTS solutions was added. Absorbance of the test and the standard solutions were taken immediately at 734 nm[24].

Table 3: Spectral data of synthesized compounds.

Name	IR spectra data	^{1}HNMR spectra data ($CDCl_3$)
MB-1	3064 ν (CH str in phenyl ring), 1660 ν (NHCO), 1315 ν (C-N), 1262 ν (C-O), 1044 ν (C-F)	δ7.50-7.48(5H,Ar-H), 7.33-7.28(3H,Ar-H), 7.12-7.07(1H,NH), 6.65(1H,Ar-H), 4.31(1H,methine),3.70-3.67(4H,O-CH_2-morpholine),2.37-2.34(4H,N-CH_2-morpholine, 2.16-2.12(2H,CH_2)
MB-2	3367 ν (OH), 3082 ν (CH str in phenyl ring), 1601 ν (NHCO), 1317 ν (C-N), 1276 ν (C-0),1039 ν (C-O-C)	δ7.51-7.41(3H,Ar-H)),7.33-7.25(2H,Ar-H),7.12-7.07(1H,NH),6.43(3H,Ar-H),5.33(1H,OH),4.60-4.56(2H,methylene),4.32(1H,methine),3.61-3.71(4H,O-CH_2-morpholine),2.64(2H,CH_2),2.17-2.16(4H,N-CH_2-morpholine),1.51-1.41(3H,CH_3)
MB-3	3064 ν (CH str in phenyl ring), 1622 ν (NHCO), 1305 ν (C-N), 1263 ν (C-O), 661 ν (S-C)	δ7.69(4H,Ar-H),7.67(1H,NH),7.25-7.00(5H,Ar-H), 4.16(1H,methine),3.73-3.69(4H,O-CH_2-morpholine),2.80(3H,CH_3),2.68(2H,CH_2), 2.48-2.40(4H,N-CH_2-morpholine)
MB-4	3069 ν (CH str in phenyl ring), 1622 ν (NHCO), 1320 ν (C-N), 1216 ν (C-O), 663 ν (C-Br)	δ7.78-7.55(5H,Ar-H), 7.33(1H,NH),7.12-7.02(4H,Ar-H), 4.62 (1H,methine), δ3.57-3.39(4H,O-CH_2-morpholine),2.63-2.58(2H,CH_2),2.44-2.19(4H,N-CH_2-morpholine)
MB-5	3017 ν (CH str in phenyl ring), 1661 ν (NHCO), 1327 ν (C-N), 1264 ν (C-O), 1069 ν (C-F)	δ7.63-7.60(4H,Ar-H), 7.50-7.47(1H,NH),7.33-7.26(3H,Ar-H), δ7.23-7.08(2H,Ar-H),4.71-4.47 (1H,methine),3.71-3.66(4H,O-CH_2-morpholine),2.89-2.86(2H,CH_2), δ2.44-2.41(2H,N-CH_2-morpholine), 2.31-2.17(2H,N- CH_2-morpholine)
MB-6	3045 ν (CH str in phenyl ring), 1669 ν (NHCO), 1316 ν (C-N), 1227 ν (C-O), 1441 ν (C-N)	δ7.73-7.70(2H,Ar-H),7.51-7.48(5H,Ar-H),7.34(1H,NH), 6.90-6.87(2H,Ar-H),4.34(1H,methine), 3.41-3.39(4H,O-CH_2-morpholine), 3.66(4H,piperidine), 2.34(2H,CH_2), 2.17(4H,N-CH_2-morpholine), 1.67-1.58(6H,piperidine)
MB-7	3050 ν (CH str in phenyl ring), 1680 ν (NHCO), 1317 ν (C-N), 1255 ν (C-O)	δ7.85-7.82(1H,NH), 7.64-7.60(2H,Ar-H),7.42-7.40(5H,Ar-H), 7.26-7.24 (5H,Ar-H),6.38(2H,Ar-H),5.51(2H,O-CH_2), 4.15(1H,methine), 3.51(4H,O-CH_2-morpholine), 2.72(2H,CH_2), 2.22-2.15(4H,N-CH_2-morpholine)
MB-8	3006 ν (CH str in phenyl ring), 1669 ν (NHCO), 1314 ν (C-N), 1254 ν (C-O)	δ7.50(1H,NH), 7.33-7.07(7H,Ar-H), 6.22(2H,Ar-H), 4.50(1H,methine), 3.66-3.59(4H,O-CH_2-morpholine),2.69-2.66(2H,CH_2), 2.43-2.36(4H,N-CH_2-morpholine)
MB-9	3010 ν (CH str in phenyl ring), 1650 ν (NHCO), 1340 ν (C-N), 1201 ν (C-O)	δ7.41(1H,NH), 7.38-7.31(4H,Ar-H), 7.25-7.18(4H,Ar-H), 6.90-7.03(5H,Ar-H),5.68(2H,O-CH_2), 4.43(1H,methine) 3.31-3.29(4H,O-CH_2-morpholine),2.41(2H,CH_2), 2.33-2.29(4H,N-CH_2-morpholine)
MB-10	3021 ν (CH str in phenyl ring), 1660 ν (NHCO), 1303ν (C-N), 1216 ν (C-O)	δ7.73-7.70(2H,Ar-H),7.51-7.48(5H,Ar-H),7.34(1H,NH), 6.90-6.87(2H,Ar-H),4.41(1H,methine), 3.44-3.39(4H,O-CH_2-morpholine), 3.54(4H,pyrrolidin), 2.43(2H,CH_2), 2.71-2.67(4H,N-CH_2-morpholine), 1.85-1.81(4H,pyrrolidin)

Antioxidant Assay

Table 4: Percentage scavenging of DPPH radical by compounds and ascorbic acid.

Compounds	Dilutions(µg/mL)			
	40µg/mL	60µg/mL	80µg/mL	100µg/mL
MB-1	52.48%	67.70%	75.20%	80.19%
MB-2	40.69%	55.89%	68.34%	72.53%
MB-3	31.85%	43.40%	51.65%	60.40%
MB-4	46.50%	59.60%	68.93%	80.62%
MB-5	54.12%	65.28%	77.0%	84.30%
MB-6	30.65%	42.48%	58.90%	69.14%
MB-7	8.80%	21.67%	39.37%	43.70%
MB-8	36.87%	58.30%	72.34%	87.01%
MB-9	15.45%	27.79%	51.18%	62.95%
MB-10	10.43%	19.70%	25.26%	38.76%
Ascorbic acid	65.28%	72.14%	86.52%	92.38%

The antioxidant activity of the samples were determined using the equation

$\%E = [(A_c - A_t)/A_c] * 100$,

Where

E= Antioxidant activity

A_c=Absorbance of the ABTS stock solution

Table 5: Percentage scavenging of ABTS radical by compounds and ascorbic acid.

Compounds	Dilutions			
	40µg/mL	60µg/mL	80µg/mL	100µg/mL
MB-1	38.90%	49.20%	64.80%	77.50%
MB-2	25%	39.17%	50.60%	58.80%
MB-3	23.40%	35.41%	44.50%	56.20%
MB-4	29.70%	45%	62.70%	75.50%
MB-5	39.50%	51.20%	68.30%	72.70%
MB-6	18.60%	30.66%	45.17%	53.02%
MB-7	10.71%	18.30%	30.52%	41.64%
MB-8	30.38%	41.50%	61.7%	80%
MB-9	19.57%	30.10%	43%	52.80%
MB-10	13.60%	24.14%	31.70%	42.60%
Ascorbic acid	41.80%	53.27%	70.20%	89.47%

A_t= Absorbance of the test compounds

RESULTS AND DISCUSSION

In brief 10 morpholine mannich base derivatives were synthesized. The synthesized compounds were characterized by ATR and ^{1}HNMR spectral analysis and were found in accordance with the assigned structure. The derivatives were screened for antioxidant activity by DPPH and ABTS assay method against ascorbic acid as a standard. The results are indicated in Table 4 for DPPH Assay and Table 5 for ABTS Assay method. MB-1 and MB-8 were found to possess comparable activity to ascorbic acid using DPPH Assay method. Radical scavenging activity of compound MB-8 was significant in ABTS Assay method.

More selective compounds can be produced if further derivatization of the above molecules is carried out.

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