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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF MORPHOLINE MANNICH BASE DERIVATIVES

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Keywords:

Morpholine, Mannich base, Antibacterial, Antifungal

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ABSTRACT: In the present study 10 morpholine mannich base derivatives were synthesized by three component reaction of morpholine, N-phenylacetamide and various substituted benzaldehydes. Progress of the reaction was monitored by thin layer chromatography and the compounds synthesized were characterized by ATR and ^{1}HNMR techniques. Antimicrobial activity of the synthesized compounds was evaluated against gram positive strains *Staphylococcus aureus* and *Bacillus subtilis* and gram negative strains *Pseudomonas aeruginosa* and *Escherichia coli* by cup plate method. *Candida albicans* was used for evaluation of antifungal activity. Three dilutions of the test compounds were taken and compared against ciprofloxacin and fluconazole used as standard. The test compounds were more active towards gram positive than gram negative bacteria. Considerable antifungal activity was shown by the compounds.

INTRODUCTION: Microbial resistance and the emergence of the multi-resistant bacterial strains known as super bugs pose a great threat to the currently available antimicrobial regime and thus increasing the demand for the development of newer antimicrobial agents with novel mechanism of action, better in efficacy and safety profile [1]. After 1970s the discovery of new antimicrobials has taken a slower pace, which necessitated the development of better antimicrobials. Only four new antibiotic classes have been approved by Food and Drug Administration from last ten years. In 2000 and 2003 drugs such as oxazolidinone, linezolid, daptomycin, in 2007 pleuromultin, retapamulin and in 2011 macrolactone, fidaxomicin, synthesized from the earlier discovered lead molecules.

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All the drugs mentioned above are produced naturally by microorganisms except linezolid produced synthetically [2 - 5]. Number of chemical reactions are used for the synthesis of new chemical entities or for the modification of existing ones, such as Mannich reaction and it's variants which has gained immense importance in the field of medicinal chemistry for the preparation of various bioactive skeletons [6]. The method offers a great choice in the use of various components and so far has been the one of the commonest reaction employed in the Pharmaceutical field [7]. Extensive research is been carried out for the use of Mannich bases as antibiotics [8], antihypertensive [9], anticoagulant [10], hypoglycemics [11], antiprotozoal [12] and in the biosynthetic pathways of secondary metabolites such as alkaloids [13].

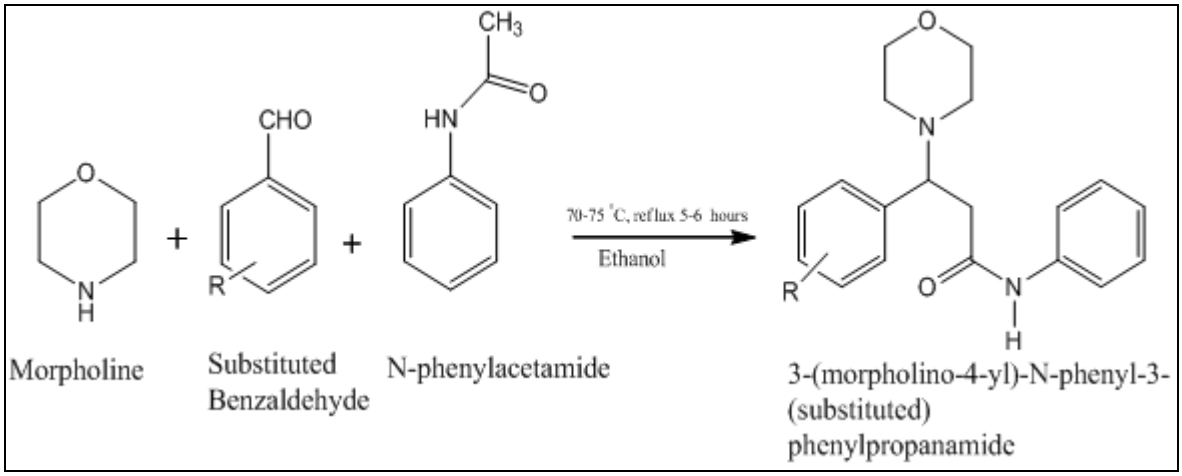
Heterocyclic compounds form building blocks in organic and pharmaceutical chemistry because of their well established pharmacological properties. Morpholine which is a heterocyclic amine has been studied extensively for its chemical, structural and pharmacological properties.

It has a broad spectrum of biological properties and as such is an important pharmacophore in the drug discovery process [14]. The drugs available containing morpholine scaffolds are: linezolid [15], gefetinib [16], phenadoxone [17], timolol [18].

MATERIALS AND METHODS: All chemicals and solvents were supplied by Sigma Aldrich, Loba Chemie and CDH under certificate of purity. The melting range of the synthesized compounds was determined by Scientech - 2211 digital auto melting/boiling point apparatus. Progress of reaction was checked by TLC using Merck Silica gel 60 F-254 coated glass plates. 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 were recorded on Bruker- Alpha 1005151/06 ATIR spectrophotometer.

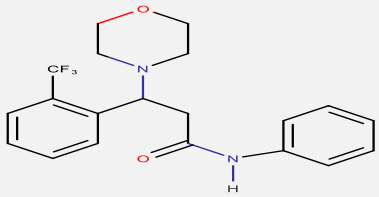
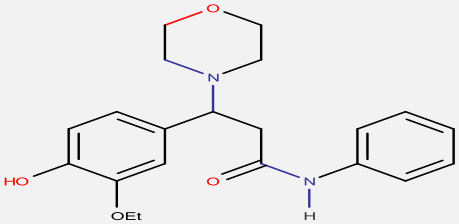
Synthetic Procedure: An equimolar quantity of morpholine and benzaldehyde were dissolved into ethanol and kept aside for about 10 minutes. To, this solution equimolar concentration of N-phenylacetamide was added. The reaction mixture was refluxed for a period of 5 - 6 hours at a temperature of 70 - 75 ºC. 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 precipitate obtained was recrystallised with ethanol.

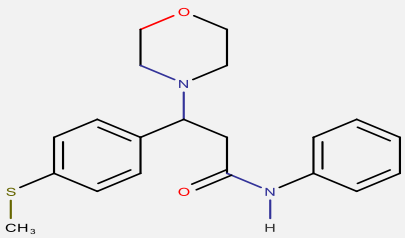
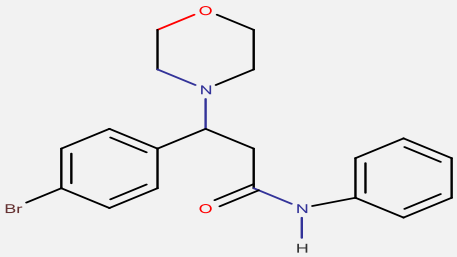
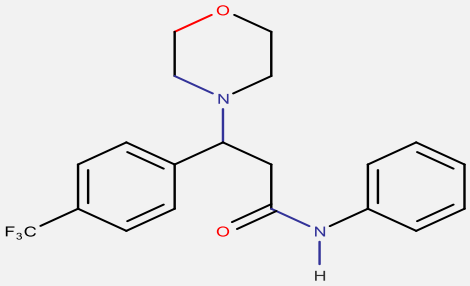
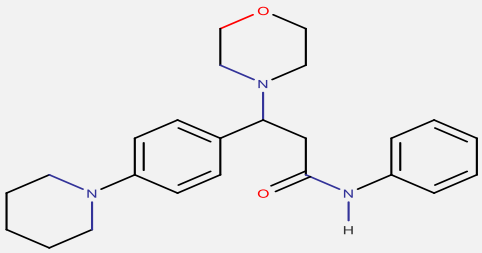
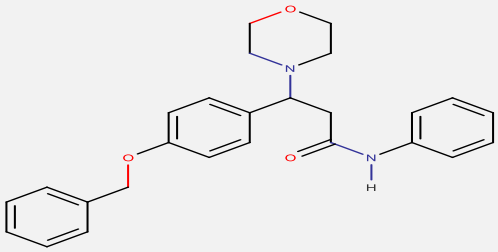
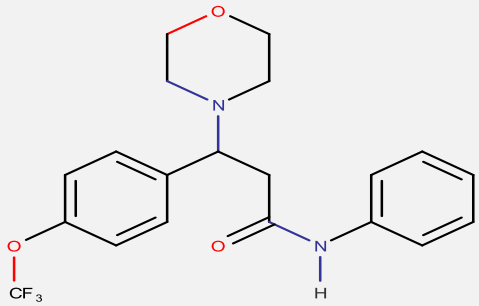
Reaction Scheme:



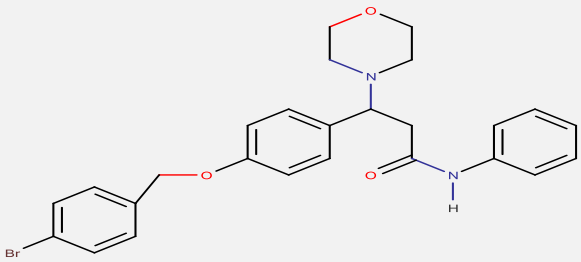
SCHEME 1: REACTION SCHEME

TABLE 1: LIST OF SYNTHESIZED COMPOUNDS

Compound	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

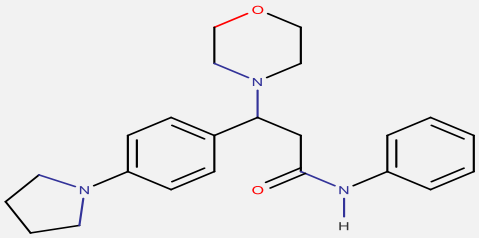
MB-3		3-(4-(methylthio)phenyl)-3-morpholino-N-phenylpropanamide
MB-4		3-(4-bromophenyl)-3-(morpholin-4-yl)-N-phenylpropanamide
MB-5		3-(morpholin-4-yl)-N-phenyl-3-[4-(trifluoromethyl)phenyl] propanamide
MB-6		3-(morpholin-4-yl)-N-phenyl-3-[4-(piperidin-1-yl)phenyl]propanamide
MB-7		3-[4-(benzyloxy)phenyl]-3-(morpholin-4-yl)-N-phenylpropanamide
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

Compound	Molecular formula	Molecular weight	Percentage Yield	Melting Point ºC	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

Biological Evaluation: All the synthesized compounds were screened for *in vitro* antibacterial activity against *Staphylococcus aureus* (Gram positive), *Bacillus subtilis* (Gram positive), *Escherichia coli* (Gram negative) and *Pseudomonas aeruginosa* (Gram negative) by cup plate method. Ciprofloxacin was used as the reference antibacterial drug. Antifungal assay was screened against *Candida albicans* using Fluconazole as standard drug.

Antimicrobial Assay: The nutrient agar medium was prepared by dissolving 23 g of nutrient agar in 1000 ml distilled water and autoclaved at 121 ºC, 15 Psig for 30 minutes and then cooled to 45 - 50ºC. Inoculation of the medium was done aseptically with 0.5 ml of strains of *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* at room temperature. For *C. albicans* Sabouraud agar was used and inoculated in same manner as for bacterial strains. Into each sterile petridish 15 ml inoculated molten agar medium was poured. Solidification of the plates was done at room temperature. After solidification, the cups of 6 mm diameter were made by scooping out the medium with the sterilised corn borer and were labelled. All the synthesized compounds and reference were dissolved in DMSO to get required concentration of 60 µg/ml, 80 µg/ml and 100 µg/ml. The solution of each compound, reference and a control (DMSO) were added separately into each cup. For antibacterial assay, the plates were incubated for about 24 hours, for antifungal activity incubation of the plates was done for period of 48 hours. The diameter of zone of inhibition was measured.

RESULTS AND DISCUSSION:

TABLE 3: SPECTRAL DATA OF SYNTHESIZED COMPOUNDS

Compound	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)

The synthesized compounds were characterized by ATR and ^{1}HNMR spectral analysis. The spectral data of the synthesized compounds was found in accordance to the molecular structure.

Antimicrobial Assay:

TABLE 4: DIAMETER OF ZONE OF INHIBITION (mm) OF COMPOUNDS AGAINST *STAPHYLOCOCCUS AUREUS*

Compound	Dilutions		
	60 µg/mL	80 µg/mL	100 µg/mL
MB-1	-	12	14
MB-2	-	8	10
MB-3	-	-	12
MB-4	12	18	22
MB-5	18	20	24
MB-6	-	-	10
MB-7	-	16	20
MB-8	10	16	20
MB-9	-	12	14
MB-10	-	10	16
Ciprofloxacin	20	24	30

TABLE 5: DIAMETER OF ZONE OF INHIBITION (mm) OF COMPOUNDS AGAINST *BACILLUS SUBTILIS*

Compound	Dilutions		
	60 µg/mL	80 µg/mL	100 µg/mL
MB-1	12	18	22
MB-2	16	20	24
MB-3	-	12	14
MB-4	14	16	18
MB-5	10	14	20
MB-6	-	14	22
MB-7	12	18	24
MB-8	-	-	10
MB-9	-	12	16
MB-10	-	10	12
Ciprofloxacin	20	26	30

TABLE 6: DIAMETER OF ZONE OF INHIBITION (mm) OF COMPOUNDS AGAINST *ESCHERICHIA COLI*

Compound	Dilutions		
	60 µg/mL	80 µg/mL	100 µg/mL
MB-1	-	-	10
MB-2	10	14	22
MB-3	-	-	8
MB-4	12	16	24
MB-5	14	20	26
MB-6	-	16	18
MB-7	16	22	24
MB-8	-	14	18
MB-9	-	8	12
MB-10	-	6	12
Ciprofloxacin	20	24	28

TABLE 7: DIAMETER OF ZONE OF INHIBITION (mm) OF COMPOUNDS AGAINST *PSEUDOMONAS AERUGINOSA*

Compound	Dilutions		
	60 µg/mL	80 µg/mL	100 µg/mL
MB-1	-	-	12
MB-2	-	6	10
MB-3	-	6	8
MB-4	10	16	20
MB-5	-	10	14
MB-6	-	10	12
MB-7	12	16	18
MB-8	16	18	20
MB-9	-	12	14
MB-10	-	8	12
Ciprofloxacin	22	26	28

TABLE 8: DIAMETER OF ZONE OF INHIBITION (mm) OF COMPOUNDS AGAINST *CANDIDA ALBICANS*

Compound	Dilutions		
	60 µg/mL	80 µg/mL	100 µg/mL
MB-1	10	14	20
MB-2	-	9	10
MB-3	-	10	14
MB-4	10	14	18
MB-5	10	16	20
MB-6	10	18	22
MB-7	-	14	17
MB-8	6	10	14
MB-9	-	-	10
MB-10	-	-	8
Fluconazole	24	34	40

MB-4, MB-5, MB-7, MB-8 were found to be active against *Staphylococcus aureus*. *S. aureus* was found to be resistant to most of the derivatives at concentration of 40µg/ml. MB-5 was found to be most active against *S. aureus*. *Bacillus subtilis* was found to be most susceptible towards the synthesized derivatives. MB-1, MB-2, MB-5, MB-6 and MB-7 were found be active against *Bacillus subtilis.* MB-2 and MB-7 were found to be most active among all the derivatives against *B. subtilis*. MB-2, MB-4, MB-5, MB-7 were active against *E. coli*. MB-5 was found to be most active against *E. coli*. Only compounds MB-4 and MB-8 were found to inhibit significantly *Pseudomonas aeruginosa*. This strain was found to be least susceptible.

The synthesized compounds were also tested for their antifungal activity against *Candida albicans*, taking fluconazole as the standard drug. Susceptibility of the synthesized compounds towards *Candida albicans* was determined according to the zone of inhibition [19]. MB-1, MB-5 and MB-6 were found to be active against *C. albicans*. MB-6 was most active against *C. albicans*.

CONCLUSION: In summary 10 morpholine mannich base derivatives were synthesized and characterized by ATR and ^{1}HNMR spectral analysis. The synthesized compounds were evaluated for their antimicrobial property. The antibacterial activity was evaluated against Gram positive and Gram negative strains using cup plate method. The compounds were found to be more active towards gram positive bacterial strains which may be attributed due to the presence of morpholine nucleus. Considerable inhibition of the fungus *Candida albicans* was also determined which may be possibly due to blockade of ergosterol synthesis. An extensive study is warranted for the further establishment of these molecules in clinical trials.

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CONFLICT OF INTEREST: None are declared.

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