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ORIGINAL ARTICLE

Synthesis of some Mannich base derivatives and their antimicrobial activity study

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Abstract A series of 2-(phenyl)-2-(morpholin-4-yl)-*N*-phenylacetamide **I–VII** were synthesized by Mannich base method. Synthesized compounds **I–VII** were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass and elemental analyses. Synthesized compounds **I–VII** were screened for antibacterial activity against various bacterial strains and compared with standard Ciprofloxacin at concentration 100 μg/mL and for antifungal activity against various fungal strains and compared with Clotrimazole at concentration 100 μg/mL; particularly 3-(4-chlorophenyl)-3-(morp holin-4-yl)-*N*-phenylpropanamide **III** that has high antibacterial activity against *Streptococcus epidermidis* was compared with standard Ciprofloxacin.

1. Introduction

In recent years series attention has been directed toward the discovery and development of new antifungal drugs. Mostly caused by *Candida albicans,* these infections are often spread through the use of broad-spectrum of antibiotics agents, anti-cancer, and anti-AIDS drugs. The main problem in the treatment of fungal infection is the increasing drug resistance subjected to antimycotic therapy such as persons infected with HIV (Wildfeuer et al., 1998). Azoles (imidazole and triazole) are present in many effective antifungal drugs, widely used for the treatment of topical or inner mycoses, in particular AIDS-related mycotic pathologies (Koltin, 1990).

Their main effect is to block fungal ergosterol biosynthesis by preventing the access of natural substrate lanosterol to the active site of the cytochrome P-450-dependent enzyme 14α-lanosterol demethylase (Odds, 2003). Since the identification of clotrimazole in 1972 (Buchel et al., 1972), a number of antifungal imidazole agents have been studied and now are used in clinical practice; that are miconazole and bifonazole (Fromtling, 1988). Mannich bases have gained importance due to their application in antibacterial activity (Holla et al., 1998; Sarangapani and Reddy, 1994) and other applications are in

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agro chemicals such as plant growth regulators (Mannich and Krosche, 1912). Moreover *N*-bridged heterocyclic derivatives show important antibacterial activity (Turan-Zitouni et al., 2005). The aminoalkylation of aromatic substrates by the Mannich reaction is of considerable importance for the synthesis and modification of biologically active compounds (Tramontini and Angliolini, 1990). Mannich bases have several biological activities such as antimicrobial (Edic-Saric et al., 1980) and anticancer (Borenstein and Doukas, 1987). Morpholine derivatives were reported to possess antimicrobial (Tramontini, 1973), anti-inflammatory (Thompson, 1968) and central nervous system activities (Cummings and Shelton, 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.

2. Experimental section

2.1. General procedures

Melting points were recorded in open capillary tubes and were uncorrected. The IR spectra (KBr) were recorded on a Shimadzu 8201pc (4000–400 cm^{-1}). The ^1H NMR and ^{13}C NMR were recorded on a Bruker DRX-400 MHz. Mass spectra (EI) were recorded on a Jeol JMS D-300 spectrometer operating at 70 eV. The elemental analysis (C, H, N and S) was recorded using an Elementer analyzer model (Varian EL III). The purity of the compounds was checked by thin layer chromatography (TLC).

2.1.1. 3-(Furan-2-yl)-3-(morpholin-4-yl)-N-phenylpropanamide I

To prepare the mixture of furfuraldehyde (0.1 mol, 10.6 mL), morpholine (0.1 mol, 8.7 mL) and phenylacetamide (0.1 mol, 13.5 g) in ethanol, the reaction mixture was refluxed for 5 h. The reaction mixture were cooled and poured into ice-cold water. The precipitate was collected by filtration. The precipitate was dried and recrystallised from absolute ethanol. The above procedure was followed by all the remaining compounds **III–VII**.

IR (cm^{-1}): 3047 (CHstr in phenyl ring), 1680 (NHCO); ^1H NMR (DMSO-d_6, 400 MHz): δ 10.21 (s, 1H, CONH), 7.44–7.19 (m, 5H, Ph-H), 6.44–6.47 (d, 2H in furyl ring), 7.60 (s, 1H, furyl ring), 4.30 (t, 1H, CH), 3.58 (t, 4H, CH_2–O–CH_2), 2.60 (t, 4H, CH_2–N–CH_2), 2.71 (d, 2H, $COCH_2$). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 176 (CONH), 110.3, 108.6, 144.9, 153.8 (furyl ring), 56.0 (N–CH), 37.3 ($COCH_2$), 121.4, 129.4, 129.0, 137.9 (phenyl ring), 66.8 (CH_2–O–CH_2), 46.0 (CH_2–N–CH_2). MS (EI): m/z (%) = 301.12 ($M^+ + 1$, 12%), 224.25, 209.24, 167.32, 87.17.

2.1.2. 3-(Morpholin-4-yl)-N,3-diphenylpropanamide II

IR (cm^{-1}): 3022 (CHstr in phenyl ring), 1647 (NHCO); ^1H NMR (DMSO-d_6, 400 MHz): δ 10.29 (s, 1H, CONH), 7.67–7.22 (m, 5H, Ph-H), 7.39–7.24 (m, 5H, phenyl), 4.45 (t, 1H, CH), 3.45 (t, 4H, CH_2–O–CH_2), 2.89 (t, 4H, CH_2–N–CH_2), 2.64 (d, 2H, $COCH_2$). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 174.2 (CONH), 137.5, 122.3, 129.5, 128.0 (phenyl), 63.6 (N–CH), 38.5 ($COCH_2$), 138.9, 129.3, 127.5, 126.9 (phenyl ring), 67.3 (CH_2–O–CH_2), 47.8 (CH_2–N–CH_2). MS (EI): m/z (%) = 311.45 ($M^+ + 1$, 24%), 234.76, 158.20, 143.89, 116.90, 87.23.

2.1.3. 3-(4-Chlorophenyl)-3-(morpholin-4-yl)-N-phenylpropanamide III

IR (cm^{-1}): 3028 (CHstr in phenyl ring), 1648 (NHCO), 848 (C–Cl); ^1H NMR (DMSO-d_6, 400 MHz): δ 10.64 (s, 1H, CONH), 7.64–7.17 (m, 5H, Ph-H), 7.54–7.44 (d, 2H, phenyl), 4.25 (t, 1H, CH), 3.64 (t, 4H, CH_2–O–CH_2), 2.54 (t, 4H, CH_2–N–CH_2), 2.64 (d, 2H, $COCH_2$). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 177.2 (CONH), 134.7, 127.4, 127.9, 138.2 (phenyl), 60.2 (N–CH), 38.3 ($COCH_2$), 139.2, 122.4, 129.5, 127.0 (phenyl ring), 67.8 (CH_2–O–CH_2), 48.5 (CH_2–N–CH_2). MS (EI): m/z (%) = 345.92 ($M^+ + 1$, 56%), 311.43, 235.76, 157.34, 144.20, 116.76, 86.09.

2.1.4. 3-(4-Hydroxyphenyl)-3-(morpholin-4-yl)-N-phenylpropanamide IV

IR (cm^{-1}): 3032 (CHstr in phenyl ring), 1629 (NHCO), 1438 (C–OH); ^1H NMR (DMSO-d_6, 400 MHz): δ 10.74 (s, 1H, CONH), 9.49 (s,1H, Ph-O*H*), 7.66–7.21 (m, 5H, Ph-H), 7.15–6.78 (d, 4H, phenyl), 4.64 (t, 1H, CH), 3.74 (t, 4H, CH_2–O–CH_2), 2.68 (t, 4H, CH_2–N–CH_2), 2.79 (d, 2H, $COCH_2$). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 174.6 (CONH), 154.2, 116.8, 129.7, 130.9 (phenyl ring), 63.8 (N–CH), 38.5 (*C*OCH_2), 135.4, 121.3, 127.2, 128.9 (phenyl ring), 65.4 (CH_2–O–CH_2), 48.9 (CH_2–N–CH_2). MS (EI): m/z (%) = 311.45 ($M^+ + 1$, 34%), 234.76, 158.20, 143.89, 116.90, 87.23.

2.1.5. 3-(Morpholin-4-yl)-3-(4-nitrophenyl)-N-phenylpropanamide V

IR (cm^{-1}): 3027 (CHstr in phenyl ring), 1617 (NHCO), 1527 (C–NO_2); ^1H NMR (DMSO-d_6, 400 MHz): δ 10.24 (s, 1H, CONH), 7.60–7.15 (m, 5H, Ph-H), 8.26–7.47 (d, 4H, phenyl), 4.51 (t, 1H, CH), 3.65 (t, 4H, CH_2–O–CH_2), 2.48 (t, 4H, CH_2–N–CH_2), 2.87 (d, 2H, $COCH_2$). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 177.0 (CONH), 123.8, 124.9, 146.3, 145.9 (phenyl ring), 64.4 (N–CH), 39.1 (*C*OCH_2), 120.8, 128.6, 127.9, 138.4 (phenyl ring), 67.1 (CH_2–O–CH_2), 48.0 (CH_2–N–CH_2). MS (EI): m/z (%) = 356.76 ($M^+ + 1$, 76%), 311.34, 235.67, 159.01, 144.45, 114.63, 88.91.

2.1.6. 3-(4-Methoxyphenyl)-3-(morpholin-4-yl)-N-phenylpropanamide VI

IR (cm^{-1}): 3042 (CHstr in phenyl ring), 1687 (NHCO); ^1H NMR (DMSO-d_6, 400 MHz): δ 10.44 (s, 1H, CONH), 7.59–7.21 (m, 5H, Ph-H), 6.98–7.20 (d, 4H, phenyl), 4.20 (t, 1H, CH), 3.54 (t, 4H, CH_2–O–CH_2), 3.80 (s, 3H, OCH_3), 2.61 (t, 4H, CH_2–N–CH_2), 2.54 (d, 2H, $COCH_2$). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 176 (CONH), 131.1, 112.9, 148.3, 127.9 (phenyl ring), 61.6 (N–CH), 38.4 (*C*OCH_2), 121.7, 128.3, 128.1 (phenyl ring), 67.9 (CH_2–O–CH_2), 48.1 (CH_2–N–CH_2), 40.9 ($N(CH_3)_2$). MS (EI): m/z (%) = 341.83 ($M^+ + 1$, 17%), 309.43, 234.67, 159.09, 142.94, 116.98, 88.46.

2.1.7. 3-(4-(Dimethylamino)phenyl)-3-morpholino-N-phenylpropanamide VII

IR (cm^{-1}): 3081 (CHstr in phenyl ring), 1628 (NHCO); ^1H NMR (DMSO-d_6, 400 MHz): δ 10.98 (s, 1H, CONH), 7.69–7.15 (m, 5H, Ph-H), 6.79–7.16 (d, 4H, phenyl), 4.67 (t, 1H, CH), 3.74 (t, 4H, CH_2–O–CH_2), 3.12 (s, 6H, $N(CH_3)_2$)), 2.34 (t, 4H, CH_2–N–CH_2), 2.67 (d, 2H, $COCH_2$). ^{13}C NMR (DMSO-d_6, 400 MHz): δ 176 (CONH), 131.1, 112.9, 148.3, 127.9 (phenyl ring), 61.6 (N–CH), 38.4 (*C*OCH_2), 121.7,

128.3, 128.1 (phenyl ring), 67.9 (CH_2–O–CH_2), 48.1 (CH_2–N–CH_2), 40.9 ($N(CH_3)_2$). MS (EI): *m*/*z* (%) = 355.93 ($M^+ + 1$, 20%), 310.34, 235.85, 154.72, 140.92, 116.72, 86.92.

2.2. Biological evaluation

2.2.1. In vitro antibacterial screening

The compounds **I–VII** were evaluated for their in vitro antibacterial activity against *Escherichia coli* (MTCC-739), *Proteus mirabilis, Nonhemolytic streptococcus, Pseudomonas aeruginosa* (MTCC-2435), *Micrococcus luteus* (MTCC-106), *Enterococcus faecalis, Streptococcus epidermidis, Bacillus* spp., *Klebsiella pneumoniae (recultured),* and *Staphylococcus aureus* (MTCC-96), by disc diffusion method (Bauer et al., 1966; Petersdorf and Sherris, 1965). It was performed using a Mueller–Hinton agar (Hi-Media) medium. Each compound and standard were used at a concentration of 100 μg/mL in DMSO. The zone of inhibition was measured after 24 h incubation at 37 °C.

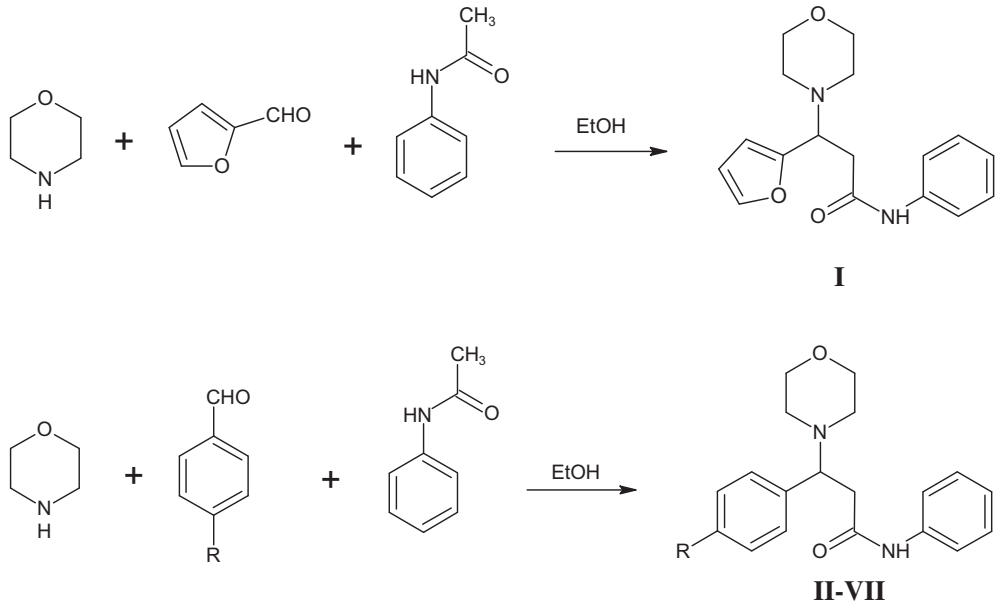
2.2.2. In vitro antifungal screening

The compounds **I–VII** were evaluated for their *in vitro* antifungal activity such as *Aspergillus niger, C. albicans, Microsporum audouinii* and *Cryptococcus neoformans* (recultured) using a disc diffusion method (Gillespie, 1994; Collins, 1976; Verma et al., 1998) with sabouraud's dextrose agar (Hi-Media). Each compound and standard were used at a concentration of 100 μg/mL in DMSO. The zone of inhibition (mm) was measured incubated at 37 °C.

3. Results and discussion

3.1. Chemistry

The compounds **I–VII** were synthesized by Mannich base method (Scheme 1), the method described in literature (Jamal Abdul Nasser et al., 2009, 2008). Physicochemical data of the compounds **I–VII** are given in Table 1. The formation of all the compounds was confirmed by recording the IR, ^{1}H NMR, ^{13}C NMR and elemental analyses. The IR spectrum of compound **I** showed absorption bands at 3047 and 1680 cm^{-1} corresponding to aromatic C–H str and NHCO groups, respectively. The ^{1}H NMR spectra of compound **I** shows a singlet observed at δ 10.21, 4.30 and 2.71 corresponding to CONH, –CH– and $CONH_2$ protons, respectively. ^{13}C NMR spectrum of the compound **I** shows peaks at δ 176.0, 56.0 and 37.3 corresponding to CONH, N–CH– and $CONH_2$ carbons, respectively. Mass spectra of compound **I** show the molecular ion peak *m*/*z* 301.12 ($M^+ + 1$, 12%), which is confirmed by the molecular mass of compound **I**.



Scheme 1 Scheme of the synthetic route **I–VII**.

Table 1 Physical characterization of compounds **I–VII**.

Compd. No.	R	m.p.	m.w.	Yield (%)	M.F.	Elemental analysis, calculated (found) (%)		
						C	H	N
I	–	79	300.35	91	$C_{17}H_{20}N_2O_3$	67.98(67.87)	6.71(6.70)	9.33(9.28)
II	–H	55	310.39	87	$C_{19}H_{12}N_2O_2$	73.52(73.54)	7.14(7.10)	9.03(9.05)
III	–Cl	80	344.83	91	$C_{19}H_{21}ClN_2O_2$	66.18(66.28)	6.14(6.10)	8.12(8.10)
IV	–OH	92	326.38	88	$C_{19}H_{22}N_2O_3$	69.92(69.87)	6.79(6.69)	8.58(8.55)
V	$–NO_2$	88	355.38	96	$C_{19}H_{21}N_2O_4$	64.21(64.31)	5.96(5.84)	11.82((11.78)
VI	$–OCH_3$	68	340.41	94	$C_{20}H_{24}N_2O_3$	70.56(70.50)	7.11(7.09)	8.23(8.31)
VII	$–N(CH_3)_2$	76	353.45	91	$C_{21}H_{27}N_3O_2$	71.36(71.29)	7.70(7.65)	11.89(11.95)

Table 2 Antibacterial activity of compounds **I–VII**.

Test organisms	Compound **I**	Compound **II**	Compound **III**	Compound **IV**	Compound **V**	Compound **VI**	Compound **VII**	Ciprofloxacin
E. coli	6	8	9	–	–	–	–	27
P. mirabilis	8	–	–	–	–	12	–	19
Non streptococcus	5	–	–	16	10	16	–	17
P. aeruginosa	9	–	–	18	–	–	10	20
M. luteus	–	5	–	–	–	–	12	32
E. faecalis	12	–	–	–	12	15	–	26
S. epridermidis	–	6	20	–	14	–	–	15
K. pneumoniae	–	–	–	–	18	–	–	19
Bacillus spp.	–	–	–	–	–	12	12	20
S. aureus	14	6	13	–	9	–	18	22

The compounds were used at concentration 100 μg/mL.
Ciprofloxacin used as the standard.
Zone of inhibition measured at (mm).

3.2. Biological screening

3.2.1. Antibacterial activity

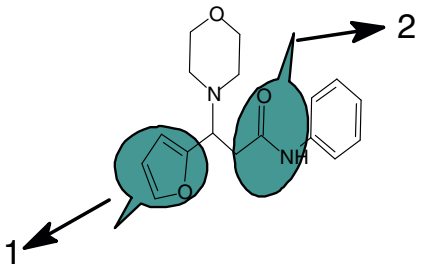
Compound **III** is highly active against *S. epridermidis* whereas compound **VI** has equipotent activity against *Non hemolytic streptococcus*, and compound **V** has equipotent activity against *K. pneumoniae* compared with ciprofloxacin. The bacterial zones of inhibition values are summarized in Table 2. Antibacterial activity variation of compounds **I–VII** is shown in Fig. 1.

3.2.2. Antifungal activity

Compound **IV** has equipotent activity against *M. audouinii* whereas compound **V** has equipotent activity against *C. albicans* compared with standard clotrimazole. The fungal zones of inhibition values are summarized in Table 3. Antifungal activity variation of compounds **I–VII** is shown in Fig. 2.

3.2.3. Structural–activity relationship

From the results of the antimicrobial activity of synthesized Mannich base derivatives, the following structure–activity relationships can be derived:



Compound **I**

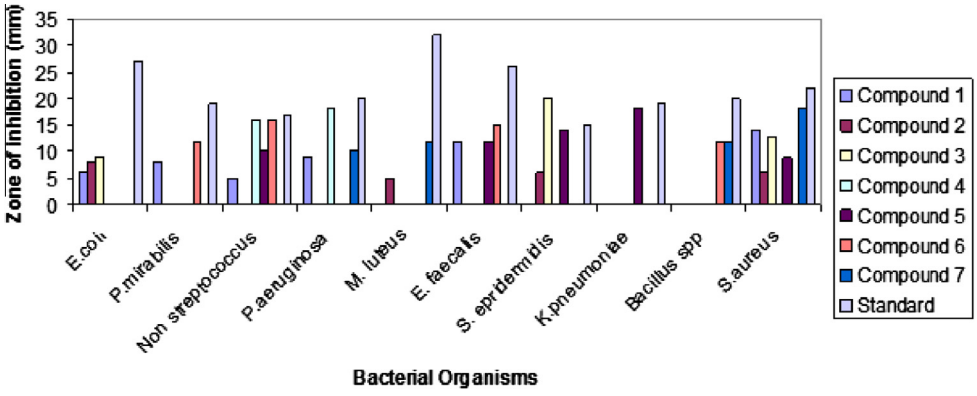


Figure 1 Antibacterial activity of compounds **I–VII**.

Table 3 Antifungal activity of compounds **I–VII**.

Test organisms	Compound **I**	Compound **II**	Compound **III**	Compound **IV**	Compound **V**	Compound **VI**	Compound **VII**	Clotrimazole
A. niger	7	–	10	–	12	–	–	22
C. albicans	8	8	6	6	25	8	6	24
C. neoformans	–	7	5	8	10	6	13	25
M. audouinii	–	9	6	25	6	–	12	26

The compounds were used at concentration 100 μg/mL.
Clotrimazole used as the standard.
Zone of inhibition measured at (mm).

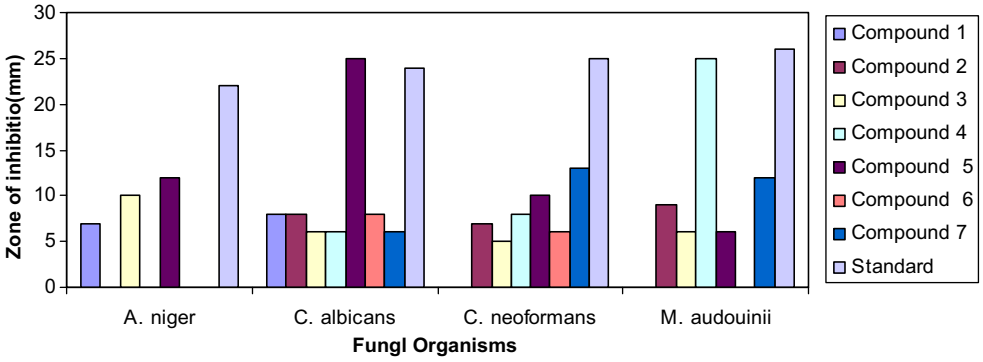
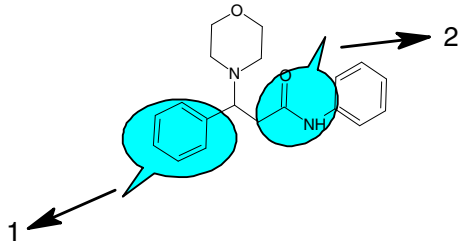


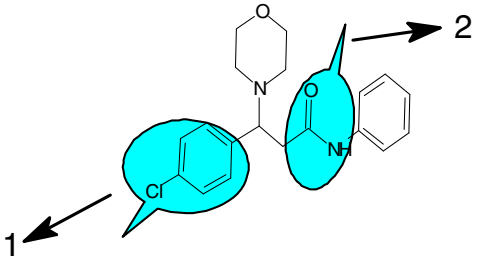
Figure 2 Antifungal activity of compounds **I**–**VII**.

Compound **I** has morpholine N-substituted furyl ring and the amide containing the compound **I** shows a low response with all bacterial and fungal species compared with the standard.



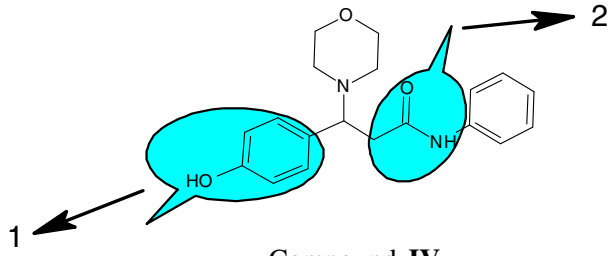
Compound **II**

In general, it was observed that most of the compounds having substituted phenyl rings showed better antimicrobial activity than those with non-substituted phenyl rings. Compound **II** has phenyl(1) and amide groups(2), compound **II** shows a low response with all bacterial and fungal species.



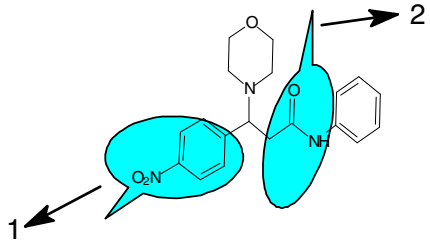
Compound **III**

Compound **III** has chlorobenzene(1) and amide group(2), compound **III** shows a significant antibacterial activity against *S. epridermidis* compared with standard Ciprofloxacin. Phenyl substituted rings having 4-Cl as the electron withdrawing group have no significance of activity in the fungal strain.



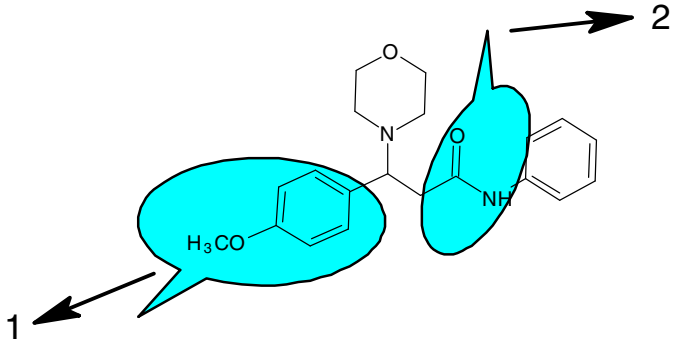
Compound **IV**

Compound **IV** has 4-OH substituted phenyl(1) and amide groups(2), compound **IV** shows a very low activity against the antibacterial strain compared with standard Ciprofloxacin whereas 4-OH as an electron withdrawing group has an equipotent activity against *M. audouinii* compared with standard Clotrimazole.



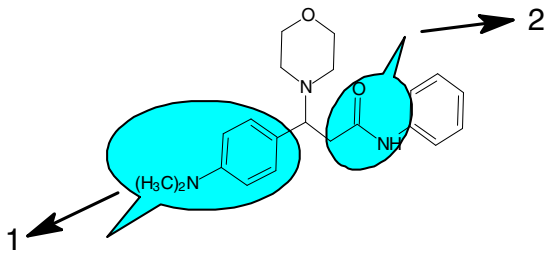
Compound **V**

In compound **V** the presence of the electron donating 4-NO_2 group shows an equipotent activity against *K. pneumoniae* and equipotent activity against *C. albicans* compared with the standard.



Compound **VI**

Compound **VI** has 4-OCH_3 substituted phenyl ring(1) and amide group(2), compound **VI** shows an equipotent activity against *Non hemolytic streptococcus* and antibacterial and very low activity against the antifungal strain compared with standard Ciprofloxacin.



Compound **VII**

Compound **VII** has dimethylnitrobenzyl(1) and amide group(2), compound **VII** shows low response with all bacterial and fungal species but equipotent activity against *S. epridermidis* compared with standard Ciprofloxacin.

4. Conclusion

A new series of Mannich base derivatives **I–VII** were synthesized and screened for antimicrobial activity. Among these, compound **III** has high antibacterial activity against *S. epridermidis* compared with standard Ciprofloxacin, which can be beneficial for further studies. This synthesized compound could be extended to analyse its various pharmacological activities.

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