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Microwave Synthesis and Antimicrobial Evaluation of Mannich Bases of 6-Benzoyl-2(3*H*)-benzothiazolone

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In this study, new eight Mannich base of 6-benzoyl-2(3*H*)-benzothiazolone derivatives have been synthesized in microwave irradiation. Structures of the synthesized compounds have been elucidated by IR and ^{1}H NMR spectral data and their elemental analyses. The *in vitro* antimicrobial activity of the compounds was determined against some gram positive, gram negative bacteria, fungi and their drug-resistant isolates in comparison with reference drugs. All the compounds were also screened for their antimycobacterial activity against *Mycobacterium tuberculosis* H37RV by using the microplate alamar blue assay (MABA) method.

Key Words: 6-Benzoyl-2(3*H*)-benzothiazolone, Mannich base, Antimicrobial activity, Antitubercular activity.

INTRODUCTION

Among infectious diseases, bacterial, fungal and tuberculosis infectious diseases have increased dramatically in recent years. In spite of many significant advanced in infectious therapy, the widespread use and misuse of antibiotics has caused the emergence of bacterial resistance to many chemotherapeutic agents, which is a serious threat to public health. For the treatment of these intractable infections, a new antiinfective agent is needed.

Literature survey reveals 2(3*H*)-benzothiazolone derivatives, which belong to an important group of heterocyclic compounds, have been studied as an extensive subject in the recent years. 2(3*H*)-Benzothiazolone rings have got enormous attentions because of their significant biological activities such as antimicrobial[1-4], antifungal[2-5], anticonvulsant[6,7], antiinflammatory and analgesic[8-17], antiviral[4], antioxidant[18], relaxant[19] and lipid-lowering[20].

In early studies, several 2(3*H*)-benzothiazolone derivatives were also investigated for their antimicrobial, antifungal and antimycobacterial activities. Among them, substituted [(2-oxobenzothiazolin-3-yl)acetyl]-3-thiosemicarbazide derivatives were

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tested against gram positive and gram negative microorganism. Some of derivatives were found active against gram negative bacteria with equal activity (MIC: 0.3 M) of ampicillin trihydrate against *P. aeruginosa*[1].

Simsek and co-workers synthesized 3-(aroylmethyl)-2-benzothiazolone derivatives and investigated antibacterial and antifungal activities. It was reported that antimicrobial activities (MIC: 50-100 µg/mL) of the compounds were more substantial than those of antifungal activities (MIC: 100 µg/mL)[3].

Furthermore, Altas and co-workers studied 3-N,N-disubstituted aminomethyl-2-benzothiazolone derivatives as antibacterial and antifungal agents. It was reported that antimicrobial activities of the compounds were more significant than those of antifungal activities[2]. In addition, many literatures have shown that Mannich bases posses potent biological activities such as antibacterial[21-23], antifungal[21-23], anti-mycobacterial[24-26] and antiviral[24,26] activities. Also N-Mannich bases have been as potentially useful prodrug candidates for imides, amides, amines and urea derivatives. In particularly, Mannich base derivatives and N-4-substituted piperazine containing derivatives were biologically active[27,28].

We designed and synthesized new series of 6-benzoyl-2(3*H*)-benzothiazolone Mannich bases and N-4-substituted piperazine derivatives and their *in vitro* activities against bacteria, fungi and their clinic isolates and *M. tuberculosis* were investigated.

EXPERIMENTAL

All the chemicals used for the synthesis of the compounds were purchased from Aldrich Chemicals, Merck AG and Acros Chemicals. Melting points of the compounds were recorded on an electrothermal-9200 digital melting points apparatus and are uncorrected. The IR spectra of the compounds were recorded on Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) spectrometer as KBr discs. The ^{1}H NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA), in DMSO-d_6. Elemental analysis were performed on Leco 932 CHNS instrument (St. Joseph, MI, USA) and were found within ± 0.4 % of the theoretical values. Microwave irradiation synthesis of the compounds was conducted on Milestone MicroSYNTH (Microwave Labstation for synthesis) microwave apparatus.

Synthesis of 2(3*H*)-benzothiazolone under microwave irradiation: 2(3*H*)-Benzothiazolone was prepared according to the reported method[29].

Synthesis of 6-benzoyl-2(3*H*)-benzothiazolone under microwave irradiation: The 2(3*H*)-benzothiazolone (**1**) (0.02 mol) and benzoic acid (0.04 mol) in polyphosphoric acid (200 mL) were heated in a stirred microwave oven for 15 min at 140 °C (300 W). After completion of the reaction (monitored by TLC using toluene:methanol, 4.5:0.5), the reaction mixture was poured into ice-water and the precipitate was filtered, washed with water, dried and crystallized with ethanol-water. The structures of the products were confirmed by IR, ^{1}H NMR and comparison with conventional sample prepared according to literature method[12].

General procedure for the synthesis of Mannich bases under microwave irradiation: The 6-benzoyl-2(3*H*)-benzothiazolone (**2**) (0.004 mol) was dissolved in a mixture of methanol. Then formaldehyde (37 %, 2 mL) and appropriate secondary amine (0.0044 mol) were added to this solution. The reaction mixture was heated in a stirred microwave oven for 10 or 15 min at 73 °C (350 W). After completion of the reaction (monitored by TLC using toluene:methanol, 9:1) and kept overnight at room temperature. The resulting solid was collected by filtration, washed with cold methanol and crystallized in appropriate solvents.

Microbiological studies

Test materials: Compounds were dissolved in dimethyl sulphoxide (Merck) at a final concentration of 4096 µg/mL and sterilized by filtration using 0.22 µm Millipore filters and used as the stock solutions. The stock solutions of the agents were diluted within liquid media.

Isolates: *Klebsiella pneumoniae* [has extended spectrum β-lactamase (ESβL) enzyme], *Pseudomonas aeruginosa* (resistant to amoxicilin clavulonat), *Escherichia coli* (has ESβL enzyme), *Bacillus subtilis* (resistant to ceftriaxon), *Staphylococcus aureus* [resistant to methicillin (MRSA)].

Standard strains: *P. aeruginosa* ATCC (American type culture collection) 25853, *E. coli* ATCC 25922, *K. pneumoniae* RSKK (Refik saydam hifzisihha center of hygiene culture collection) 574, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231, *Mycobacterium tuberculosis* H37RV ATCC 27294.

Micro dilution method: Standard strains of *P. aeruginosa* ATCC 25853, *E. coli* ATCC 25922, *K. pneumoniae* RSKK 574, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231 and clinical isolates of these microorganisms were included in the study. Resistance in clinical isolates was determined by broth micro dilution method according to the guidelines of clinical and laboratory standards institute (CLSI)[30].

Standard powders of rifampicin, ampicillin, gentamycin sulfate, ofloxacin and amphotericin B were obtained from the manufacturers. Stock solutions were dissolved in dimethyl sulphoxide (ofloxacin), methanol (rifampicin), pH 8 phosphate-buffered saline (PBS) (ampicillin trihydrate) and distilled water (gentamycin sulfate and amphotericin B). All bacterial isolates were subcultured in Mueller Hinton Agar (MHA; Merck) plates and incubated overnight at 37 °C. The solutions of the newly synthesized compounds and standard drugs were prepared and diluted at 4096, 2048, 1024, 512,… 0.0625 µg/mL concentrations in the wells of microplates within the liquid media. Bacterial susceptibility testing was performed according to the guidelines of CLSI M100-S16[31]. The bacterial suspensions used for inoculation were prepared at 10^5 CFU/mL by diluting fresh cultures at MacFarland 0.5 density (10^7 CFU/mL). Suspensions of the bacteria at 10^5 CFU/mL concentration were inoculated to the two-fold diluted solution of the compounds. There were 10^4 CFU/mL bacteria in the wells after inoculations. Mueller Hinton Broth (MHB; Merck) was used for diluting the bacterial suspension and for two-fold dilution of the compound.

Dimethyl sulphoxide (80 %) and ethanol (20 %), methanol, PBS, pure microorganisms and pure media were used as control wells. A 10 µL bacteria inoculum was added to each well of the microdilution trays. The trays were incubated at 37 °C and minimum inhibitory concentration (MIC) endpoints were read after 24 h of incubation. All organisms were tested in triplicate in each run of the experiments. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and MICs were reported.

Candida were subcultured in sabouraud dextrose agar (SDA; Merck) plates and incubated at 35 °C for 24-48 h. Susceptibility testing was performed in RPMI-1640 medium with L-glutamine (Sigma) buffered with MOPS (pH 7) (Sigma) and culture suspensions were prepared through the guideline of CLSI M27-A[32]. The yeast suspensions used for inoculation were prepared at 10^4 CFU/mL by diluting fresh cultures at Mac-Farland 0.5 density (10^6 CFU/mL). Suspensions of the yeast at 10^4 CFU/mL concentration were inoculated to the two-fold diluted solution of the compounds. There were 10^3 CFU/mL yeasts in the wells after inoculations. A 10 µL yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35 °C and MIC end points were read after 48 h of incubation. All organisms were tested in triplicate in each run of the experiments. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and MICs were reported.

Microplate alamar blue assay (MABA): *Mycobacterium tuberculosis* H37RV ATCC 27294 were subcultured on Middlebrook 7H11 agar (Becton Dickinson). Culture suspensions were prepared in 0.04 % (v/v) tween 80-0.2 % bovine serum albumin (Sigma) at MacFarland 1 density. Suspensions were then diluted 1:25 in 7H9GC broth 4.7 g of Middlebrook 7H9 broth base (Difco), 20 mL of 10 % (vol/vol) glycerol, 1 g of Bacto Casitone (Difco), 880 mL of distilled water, 100 mL of oleic acid, albumin, dextrose and catalase (Sigma).

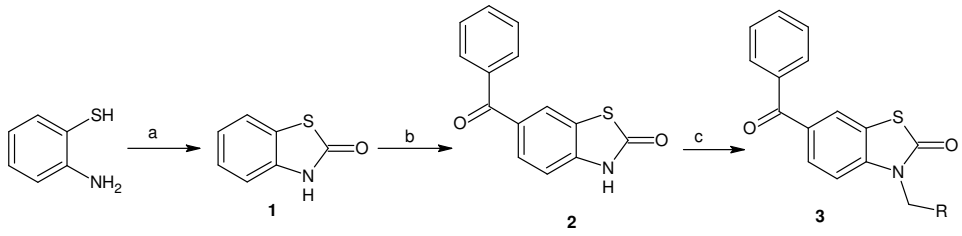
Compounds were dissolved in dimethyl sulphoxide at a final concentration of 4096 µg/mL and sterilized by filtration using 0.22 µm syringe filters (millipore) and used as the stock solutions. The stock solutions of the agents were diluted within liquid media. Stock solutions of EMB (Sigma) was prepared in deionized water. The solution of the newly synthesized compounds and standard drugs were prepared and diluted at 4096, 2048, 1024, 512,… 0.0625 µg/mL concentrations in the wells of microplates within the liquid media.

200 µL of sterile deionized water was added to the outer-perimeter wells to minimize evaporation of the medium in the test wells during incubation. A 100 µL of 7H9GC broth was added to the wells in rows B-G in columns 3-11. A 100 µL of stock solutions were added to the wells in rows B-G in columns 2 and 3 by using a multichannel pipette. 100 µL was transferred from column 3 to column 4 and the contents of the wells were mixed. Serial two-fold dilutions were made through column 10.

100 µL of *M. tuberculosis* inoculum was added to the wells in rows B-G in columns 2-11 by using a multichannel pipette. The wells in column 11 are growth controls. The plates were sealed with parafilm and were incubated at 37 °C for 5 days. 50 µL of a freshly prepared 1:1 mixture of 10X alamar blue (AbD Serotec) reagent and 10 % Tween 80 was added to well B11. The plates were incubated at 37 °C for 24 h. B11 turned pink and the reagent mixture was added to all wells in the microplate. The microplates were resealed with parafilm and were incubated for 24 h at 37 °C and the colours of all wells were recorded. A blue colour in the well was recorded as no growth and a pink colour was scored as growth. The MIC was defined as the lowest drug concentration which prevented a colour change from blue to pink[33].

RESULTS AND DISCUSSION

The synthetic route of the title compounds is illustrated in **Scheme-I**. Syntheses of the compound was started by obtaining 2(3*H*)-benzothiazolone **1** from 2-aminothiophenol under microwave irradiation[29]. Acylation of 2(3*H*)-benzothiazolone with benzoic acid derivative was carried out in polyphosphoric acid under microwave irradiation. 2(3*H*)-Benzothiazolone and 6-benzoyl-2(3*H*)-benzothiazolone were previously prepared in our laboratory with conventional method[12]. The condensation reaction to yield the 2(3*H*)-benzothiazolone and the 6-benzoyl-2(3*H*)-benzothiazolone was found to be selective by microwave irradiation technique with good yield in short time[12].



a: Urea, fusion, MW

b: benzoic acide, PPA, MW

c: Methanol or Isopropanol, formaldehyde, sec. amine, MW

Scheme-I: Synthetic route for the compounds

The final compounds **3a-3i** were prepared from 6-benzoyl-2(3*H*)-benzothiazolone, secondary amine derivatives and formaldehyde according to the Mannich reaction in 41-98 % yield under microwave irradiation. Compound **3h** was previously prepared by Petrov with conventional method[13]. The structures of the product were confirmed by IR, ^{1}H NMR and comparison with conventional sample prepared according to literature method. The Mannich bases of 6-benzoyl-2(3*H*)-benzothiazolone were obtained in very short time with high yield. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) and elemental analyses (Table-1) and the structures were confirmed by spectral data.

TABLE-1
PHYSICOCHEMICAL DATA OF THE COMPOUNDS **3a-i**

Comp.	R	Crystall solvent	Yield (%)	m.p. (ºC)	Elemental analysis calcd./found
3a	Morpholine	Ethanol	61	128-130	C 64.39/63.92, H 5.12/5.59 N 7.90/8.21
3b	Phenyl piperazine	Butanol	53	160-161	C 69.91/69.99, H 5.40/5.50 N 9.78/9.29
3c	2-Fluoro phenyl piperazine	Ethanol	64	150-152	C 67.10/66.93, H 4.95/5.23 N 9.39/9.10
3d	4-Fluoro phenyl piperazine	Butanol	93	158-160	C 67.10/66.91, H 4.95/5.21 N 9.39/9.60
3e	3-Chloro phenyl piperazine	Butanol	88	140-145	C 64.72/64.31, H4.78/4.41 N 9.06/9.07
3f	4-Chloro phenyl piperazine	Methanol	41	149-150	C 64.72/64.40, H 4.78/4.60 N 9.06/9.35
3g	2,3-Dimethylphenyl piperazine	Butanol	86	147-149	C 70.87/70.44, H 5.95/5.69 9.18/9.25
3h[13]	2-Methoxy phenyl piperazine	Methanol	93	154-156	C 67.95/68.04, H5.48/5.50 N 9.14/9.09
3i	2-Pyridyl piperazine	Butanol	98	155-156	C 66.95/66.56, H 5.15/5.16 N 13.01/12.60

In the IR spectra of the compounds, the lactam and ketone stretching bands were determined at 1697-1679 and 1682-1648 cm^{-1}. In the ^1H NMR spectrum, methylene protons at the 3-position in the 6-benzoyl-2(3*H*)-benzothiazolone were observed at 4.80 ppm as a singlet. Than, H^3, H^5 and H^2, H^6 protons of the piperazine ring were observed at 2.9-3.1 ppm and at 2.5-2.7 ppm, respectively. The results were shown in Table-2.

Biological activity: The synthesized compounds were tested for their *in vitro* antibacterial activity against gram positive; *S. aureus*, methicillin-resistant *S. aureus* (MRSA, clinical isolate), *B. subtilis*, *B. subtilis* (clinical isolate), gram negative; *E. coli* and *E. coli* producing extended spectrum β-lactamase (ESBL, clinical isolate), *P. aeruginosa*, *P. aeruginosa* (clinical isolate) and *K. pneumoniae*, *K. pneumoniae* (clinical isolate) and yeast-like fungi such as *C. albicans* by using broth microdilution method. Ampicillin and gentamicin were used as standard antimicrobial agents and amphotericin B was used as standard antifungal agent. The synthesized compounds were also tested *in vitro* for antimycobacterial activity against *M. tuberculosis* by using MABA method. Ethambutol was used as standard antimycobacterial agent. The biological activity results of the compounds were shown in Table-3.

Generally, compounds **3a, 3d-f** showed moderate to good activity against gram positive including *S. aureus* (MIC: 128 µg/mL) but were less active than reference drugs. It was found that, the same compounds had activity against clinical *S. aureus*

isolate (MIC: 256 μg/mL) was less active than *S. aureus*. In contrast, all the synthesized compounds did not show significant activity against gram negative bacteria (MICs > 256 μg/mL).

TABLE-2
IR AND ^{1}H-NMR SPECTRAL DATA OF THE COMPOUNDS **3a-i**

Comp.	IR ν_{max} (cm^{-1})	^{1}H-NMR δ (DMSO-d_6)
3a	1697, 1682	8.14 (1H, d, H^7), 7.76-7.55 (7H, m, H^4, H^7, benzoyl H), 4.75 (2H, s, CH_2), 3.55 (4H, t, morpholin $H^{2,6}$), 2.58 (4H, t, morpholine $H^{3,5}$)
3b	1699, 1684	8.14 (d, 1H, H^7), 7.77-7.74 (m, 2H, H^4, H^5), 7.70-7.64 (m, 3H, benzoyl $H^{2,4,6}$), 7.57 (t, 2H, benzoyl $H^{3,5}$), 7.18 (t, 2H, phenyl $H^{3,5}$), 6.91 (d, 2H, phenyl-$H^{2,6}$), 6.76 (t, 1H, phenyl H^4), 4.84 (s, 2H, CH_2), 3.11 (t, 4H, piperazine $H^{3,5}$), 2.75 (t, 4H, piperazine $H^{2,6}$).
3c	1684, 1651	8.15 (d, 1H, H^7), 7.78-7.73 (m, 2H, H^4, H^5), 7.70-7.64 (m, 3H, benzoyl $H^{2,4,6}$), 7.57 (t, 2H, benzoyl $H^{3,5}$), 7.13-7.06 (d, 2H, phenyl $H^{3,5}$), 7.03-6.95 (m, 2H, phenyl $H^{4,6}$), 4.85 (s, 2H,CH_2), 2.99 (t, 4H, piperazine $H^{3,5}$), 2.77 (t, 4H, piperazine $H^{2,6}$).
3d	1683, 1668	8.14 (d, 1H, H^7), 7.77-7.73 (m, 2H, H^4, H^5),7.70-7.64 (m, 3H, benzoyl $H^{2,4,6}$), 7.57 (t, 2H, benzoyl $H^{3,5}$), 7.05-7.00 (m, 2H, phenyl $H^{3,5}$), 6.94-6.90 (m, 2H, phenyl $H^{2,6}$), 4.84 (s, 2H, CH_2), 3.05 (t, 4H, piperazine $H^{3,5}$), 2.75 (t, 4H, piperazine $H^{2,6}$).
3e	1684, 1651	8.14 (d, 1H, H^7), 7.77-7.74 (m, 2H, H^4, H^5), 7.70-7.64 (m, 3H, benzoyl $H^{2,4,6}$), 7.57 (t, 2H, benzoyl $H^{3,5}$), 7.18 (t, 1H, phenyl H^5), 6.92 (t, 1H, phenyl H^2), 6.87 (dd, 1H, phenyl H^6), 6.77 (dd, 1H, phenyl H^4), 4.84 (s, 2H, CH_2), 3.16 (t, 4H, piperazine $H^{3,5}$), 2.73 (t, 4H, piperazine $H^{2,6}$).
3f	1682, 1651	8.14 (d, 1H, H^7), 7.77-7.74 (m, 2H, H^4, H^5), 7.69-7.64 (m, 3H, benzoyl $H^{2,4,6}$), 7.57 (t, 2H, benzoyl $H^{3,5}$), 7.20 (d, 2H, phenyl $H^{3,5}$), 6.92 (d, 2H, phenyl $H^{2,6}$), 4.84 (s, 2H, CH_2), 3.11 (t, 4H, piperazine $H^{3,5}$), 2.74 (t, 4H, piperazine $H^{2,6}$).
3g	1679, 1655	8.15 (d, 1H, H^7), 7.78-7.74 (m, 2H, H^4, H^5), 7.70-7.64 (m, 3H, benzoyl $H^{2,4,6}$), 7.59 (t, 2H, benzoyl $H^{3,5}$), 7.01 (t, 1H, phenyl H^5), 6.90-6.84 (m, 2H, phenyl $H^{4,6}$), 4.85 (s, 2H, CH_2), 2.77 (s, 4H, piperazine $H^{3,5}$), 2.19 (s, 2H, piperazine $H^{2(6)}$), 2.17 (s, 3H, CH_3), 2.14 (s, 2H, piperazine $H^{6(2)}$), 2.10 (s, 3H, CH_3)
3h	1684, 1648	8.15 (d, 1H, H^7), 7.78-7.64(m, 2H, H^4, H^5), 7.68-7.64 (m, 3H, benzoyl $H^{2,4,6}$), 7.57 (t, 2H, benzoyl $H^{3,5}$), 6.94-6.91 (m, 2H, phenyl $H^{3,5}$), 6.86-6.84 (m, 2H, phenyl $H^{4,6}$), 4.85 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3), 2.93 (s, 4H, piperazine $H^{3,5}$), 2.75 (s, 4H, piperazine $H^{2,6}$)
3i	1684, 1651	8.15 (d, 1H, H^7), 7.78-7.64(m, 2H, H^4, H^5), 7.68-7.64 (m, 3H, benzoyl $H^{2,4,6}$), 7.57 (t, 2H, benzoyl $H^{3,5}$), 6.94-6.91 (m, 2H, phenyl $H^{3,5}$), 6.86-6.84 (m, 2H, phenyl $H^{4,6}$), 4.85 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3), 2.93 (s, 4H, piperazine $H^{3,5}$), 2.75 (s, 4H, piperazine $H^{2,6}$)

While compound **3d**, exhibited the most potent inhibitory activity against clinical *P. aeruginosa* isolate (MIC: 256 μg/mL) and compound **3e** showed the most inhibitory activity against *K. pneumoniae* (MIC: 256 μg/mL), which was equal to compound **2**. Among the all compounds, compound **2** exhibited the most potent inhibitory activity against *E. coli* and *P. aeruginosa*.

TABLE-3
ANTIMICROBIAL AND ANTIMYCOBACTERIAL ACTIVITY RESULTS (MICs, μg/mL) OF THE SYNTHESIZED COMPOUNDS WITH THE STANDARD DRUGS

Compound	A	B	C	D	E	F	G	H	I	J	K	L
2	512	512	256	256	256	512	256	256	256	512	256	512
3a	512	256	128	256	512	512	512	512	512	512	256	64
3b	512	512	512	256	512	512	512	512	512	512	2048	64
3c	512	256	256	256	512	512	512	512	512	512	256	128
3d	1024	256	128	256	512	512	512	256	512	512	256	128
3e	1024	256	128	256	512	512	512	512	256	512	256	128
3f	512	512	128	256	512	512	512	512	512	512	256	512
3g	512	512	512	256	512	512	512	512	512	512	256	256
3h	512	512	512	256	512	512	512	512	512	512	256	64
3i	512	512	512	256	512	512	512	512	512	512	256	64
Ampicillin	2	8	0.5	2	8	512	2048	2048	2	32	–	–
Gentamycin	0.25	0.125	0.5	16	1	32	1	2	0.25	0.5	–	–
Amphotericin B	–	–	–	–	–	–	–	–	–	–	0.5	–
Etambutol	–	–	–	–	–	–	–	–	–	–	–	4

A: *B. subtilis* ATCC 6633, B:, *B. subtilis* isolate, C: *S. aureus* ATCC 25923, D: *S. aureus* isolate, E: *E. coli* ATCC 25922, F: *E. coli* isolate, G: *P. aeruginosa* ATCC 25853, H: *P. aeruginosa* isolate, I: *K. pneumoniae* RSKK 574, J: *K. pneumoniae* isolate, K: *C. albicans* ATCC 10231, L: *M. tuberculosis* H37RV ATCC 27294.

All tested compounds had respectable *in vitro* activity against *C. albicans* (MIC: 256 μg/mL), but were less active than reference drug, with the exception of compound **3b** (MIC: 2048 μg/mL). Although **3a, b, h** and **i**, showed MIC values 64 μg/mL against *M. tuberculosis* other compounds possess a comparable or better activity with respect to references drug. Their activities were 4-fold more than that of compound **2** (MIC: 512 μg/mL).

The obtained antibacterial activity results indicate that among the compounds, compound **2**, which did not carry any substitution on the third position was found potent than that bearing piperazinyl residue on this position and some of the title compounds the antibacterial potency especially against gram positives and *S. aureus*. Among the all compounds, the piperazine portion appeared that presence of electron withdrawig substituents such as fluoro and chloro in the *para* or *meta* position of the phenyl nucleus provide more active compounds (**3d-f**) compared with other substituted derivatives.

Antibacterial and antimycobacterial activities of compound **3a** were found higher with MIC values of 128 and 64 μg/mL, respectively than the compounds carrying morpholinyl moiety in the 3 position.

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