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Synthesis and Biological Activity of Some 3-[(4-Amino-5-thioxo-1,2,4-triazol-3-yl)methyl]benzothiazol-2(3*H*)-one Derivatives

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Fourteen new 3-[(4-amino-5-thioxo-1,2,4-triazol-3-yl)methyl]benzothiazol-2(3*H*)-one derivatives have been synthesized. The structures of these compounds were confirmed by IR, ^{1}H NMR, mass spectrum and elemental analysis. The synthesized compounds were evaluated for their antibacterial activity against various gram-positive and gram-negative strains of bacteria and their clinical isolates, for their antifungal activity against *Candida albicans* and *C. krusei* and for their antimycobacterial activity against *M. tuberculosis* H37Rv and its clinical isolate. Some of the compounds showed promising activity.

Key Words: Benzothiazol-2(3*H*)-one, 4-Amino-5-thioxo-1,2,4-triazol, Antimicrobial, Antitubercular.

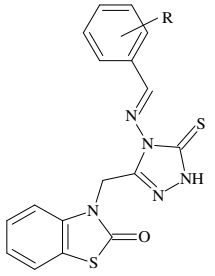
INTRODUCTION

The development of resistance to current antimicrobial and antimycobacterial therapy endorses the search for more effective agents. In addition, primary and opportunistic microbial infections continue to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants). Several reviews have appeared, which illustrate the problems encountered by today's infectious disease clinicians[1-3].

As known, the easily gained resistance is the main problem encountered in developing safe and efficient antimicrobial and antimycobacterial agents. Therefore, there is a need to develop new, potent, fast-acting antimicrobial and antimycobacterial drugs with low toxicity[4,5]. For this reason, it is critical to discover new drugs acting with different mechanism from those drugs which are used in current therapy[6,7]. There has been considerable interest in the chemistry of benzothiazol-2(3*H*)-one ring. This heterocyclic core shows a broad spectrum of biological activity such as antibacterial[8], antifungal[8], antiviral[8], anticonvulsant[9], antiinflammatory[10,11] and analgesic[12-15] activities. In addition, the 1,2,4-triazole nucleus and their heterocyclic derivatives represent an interesting class of compounds which possess a wide range of biological activities, such as anthelmintic[16], antitubercular[17], antiviral[18], antimicrobial[18-21] and anticancer[21] properties. Furthermore, Schiff bases derived from various heterocyclic were reported to possess some biological activities[22-26].

Recently, the use of microwave irradiation (MWI) in accelerating organic reactions is rapidly increasing because it induces short reaction times and improves economic as well as environmental and operational aspects. In our studies, a wide range of organic reactions have been achieved using microwave irradiation[26,27].

In the present study, a number of new Schiff bases (Fig. 1) of 4-amino-5-thioxo-1,2,4-triazole derivatives were prepared by using the biologically active benzothiazol-2(3*H*)-one moiety as starting material utilizing microwave irradiation in order to be able to develop more effective antimicrobial derivatives.



R: H, F, Cl, Br, CF_3, $C(CH_3)_3$, CH_3, OCH_3

Fig. 1.

EXPERIMENTAL

Chemicals and all the solvents, used in this study, were purchased locally from Aldrich, (Germany), Merck (Germany) and Acros (Germany) Chemical. Synthesis of benzothiazol-

TABLE-1
PHYSICO-CHEMICAL DATA OF THE COMPOUNDS (**5a-5m**)

Comp.	R	Cry. sol.	Yield (%)	m.p. (ºC)	m.f.	HR MS (ESI+)	Elemental analysis (cal./found)
5a	H	Acetone-water	74	223-224	$C_{17}H_{13}N_5OS_2$	368.0637	C: 55.57 (55.53); H:3.57 (3.66); N: 19.06 (19.15
5b	4-F	Acetone-ethanol	70	227-228	$C_{17}H_{12}N_5OS_2F$	386.0546	C: 52.97 (52.83); H: 3.14 (3.23); N: 18.17 (18.55)
5c	4-Cl	Acetone-ethanol	63	228-229	$C_{17}H_{12}N_5OS_2Cl$	402.0251	C:50.81 (51.00); H: 3.01 (3.06); N: 17.43 (17.31)
5d	4-Br	Acetone-ethanol	89	238-239	$C_{17}H_{12}N_5OS_2Br$	447.9742	C: 45.75 (46.00); H: 2.71 (2.66); N: 15.69 (15.62)
5e	4-CF_3	Acetone-ethanol	87	245-246	$C_{18}H_{12}N_5OS_2F_3$	436.0525	C: 49.65 (49.83); H: 2.78 (2.80); N: 16.08 (16.12)
5g	4-$C(CH_3)_3$	Acetone-ethanol	81	248-249	$C_{21}H_{21}N_5OS_2$	424.1279	C: 59.55 (59.85); H: 5.00 (4.94); N: 16.53 (16.45)
5h	4-OCH_3	Acetone-ethanol	79	221-222	$C_{18}H_{15}N_5O_2S_2$	398.0745	C: 54.39 (54.74); H: 3.80 (3.89); N: 17.62 (17.59)
5i	2-F	Acetic acid	80	219-220	$C_{17}H_{12}N_5OS_2F$	386.0540	C: 52.97 (53.05); H: 3.14 (3.01; N: 18.17 (18.18)
5j	2-Cl	Acetone	77	235-236	$C_{17}H_{12}N_5OS_2Cl$	402.0253	C: 50.81 (50.85; H: 3.01 (2.97); N: 17.43 (17.44)
5k	2-Br	Acetone	89	219-220	$C_{17}H_{12}N_5OS_2Br$	447.9743	C: 45.75 (45.78); H: 2.71 (2.73); N: 15.69 (15.60)
5m	2- OCH_3	Acetone-ethanol	92	115-116	$C_{18}H_{15}N_5O_2S_2$	398.0746	C: 54.39 (54.56); H: 3.80 (3.64); N: 17.62 (17.51)

2(3*H*)-one (**1**)[28], ethyl(benzothiazol-2(3*H*)-one-3-yl)acetate (**2**)[29], (benzothiazol-2(3*H*)-one-3-yl)acetic acid (**3**)[29] were synthesized according to the published procedures.

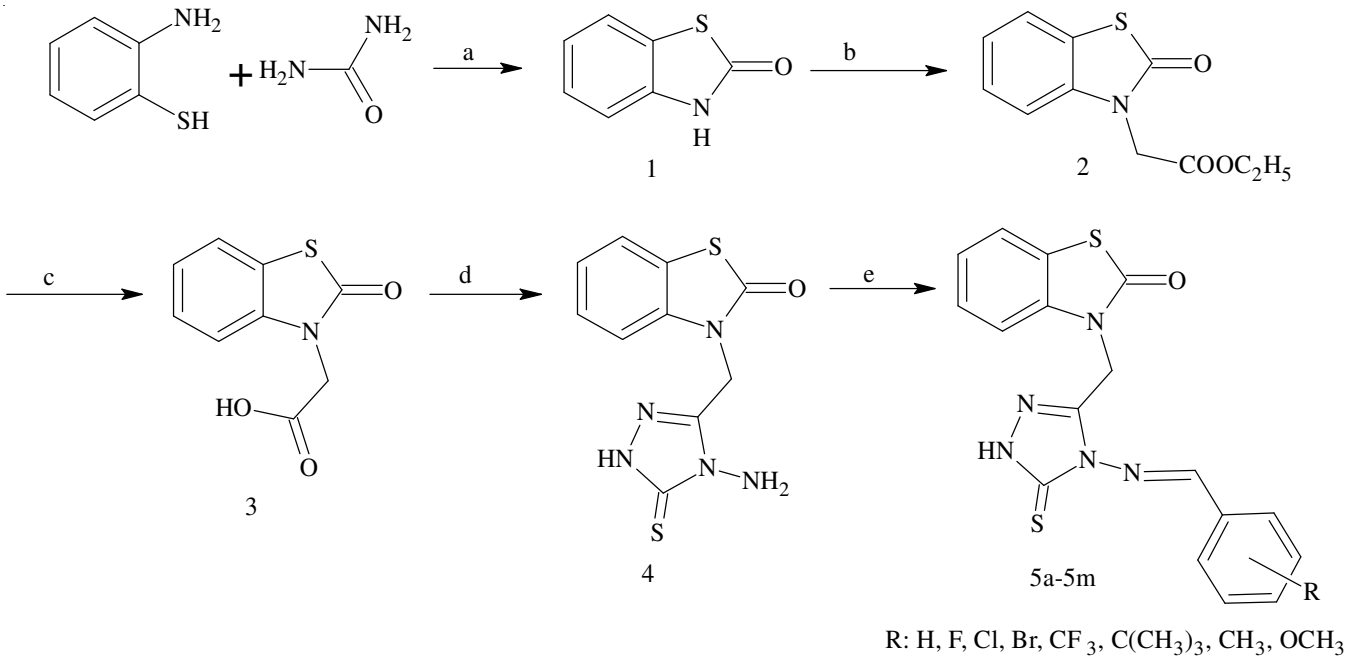
Melting points of the compounds were recorded on an Electrothermal-9200 digital melting points apparatus and are uncorrected. Microwave reaction was carried out in MicroSYNTH Microwave Labstation at 1600 W (2 magnetrons 800W × 2). (Milestone S.R.L. Italy). The ^{1}H NMR spectra was recorded in DMSO-d_6 on Bruker 400 MHz NMR spectrometer. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane. FTIR spectra of the surface layer of grafted membranes were measured with a Perkin-Elmer 400 (USA) ATR attachment (32 scans, wavenumber 4000-650 cm^{-1}) and analyzed using the Spectrum v2.0 software. The mass spectra were obtained on a waters ZQ micromass TOF-MS spectrometer (Waters Corporation, Milford, MA, USA) using the ESI(+) method.

Synthesis of 3-[(4-Amino-5-thioxo-1,2,4-triazol-3-yl)-methyl]benzothiazol-2(3*H*)-one (4): [2(3*H*)-benzo-thiazolon-3-yl]acetic acid (0.01 mol) and thiocarbohydrazide (0.01 mol) fused at 160-170 ºC in an oil bath for 10 min. The fused mass thus obtained was recrystallized from *N,N*-dimethyl formamide-water. Yield 80 %, m.p. 222-223 ºC, ^{1}H-NMR (DMSO-d_6) δ; 13,64 (1H, s, NH), 7.69 (1H, m, H7), 7.33 (1H, m, H4), 7.28 (1H, m, H6), 7.22 (1H, m, H5), 5,66 (2H, s, NH_2), 5.24 (2H, s, CH_2); IR ($\nu_{max,}$ cm^{-1}): 3291, 3109, 1633, 1592, 1309; Anal. calcd. (found) (%) for: $C_{10}H_9N_5OS_2$: C: 43.00 (43.06), H: 3.25 (3.24), N: 25.07 (24.89).

3-[(4-{[Substituted phenylmethylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]benzothiazol-2(3*H*)-one (5a-5m): To a suspension of *o*/*p*-substituted benzaldehyde (0.0015 mol) in glasial acetic acid (3 mL) was added 0.0015 mol of the [(4-amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-benzothiazol-2(3*H*)-one. The reaction mixture was taken in round bottom flask placed in a microwave reactor and irradiated for 15 or 25 min at 125 ºC (400 W). After completion of the reaction (monitored by TLC using toluene: methanol, 9:1) and kept overnight at room temperature. After precipitated by filtration, washed with water, dried and crystallized from a suitable solvent (Table-1).

Microbiological studies

Microdilution method: Standard strains of *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *E. coli* ATCC 35218, *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, clinical isolates of these microorganisms and *C. albicans* ATCC 10231, *C. krusei* ATCC 6258 were included in the study. Resistance in clinical isolates was determined by disc diffusion method according to the guidelines of clinical and laboratory standards institute (CLSI)[30]. Standard powders of ampicillin trihydrate, gentamycin sulfate, amphotericin B and fluconazole were obtained from the manufacturers. Stock solutions were dissolved in 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 ºC. The solutions of the newly synthesized compounds and standard drugs were prepared and diluted at 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-S18[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 concentrations 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 (DMSO), phosphate buffered saline, 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



a: fusion, MW; b: BrCH $_2$COOC$_2$H$_5$, K$_2$CO$_3$, acetone, MW; c: HCl, reflux; d: CS(NHNH$_2$)$_2$, fusion;
e: *o/p*-substitued benzaldehyde, CH$_3$COOH, MW

Scheme-I: Synthesis of 3-[(*o/p*-substituted phenylmethylidene]amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-benzothiazol-2(3*H*)-one (**5a-m**)

were incubated at 37 ºC 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 minimum inhibitory concentrations were reported. Candida were subcultured in sabouraud dextrose agar (SDA; Merck) plates and incubated at 35 ºC 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-A3[32]. Yeast suspensions were prepared according to McFarland 0.5 density and a working suspension was made by a 1:100 dilution followed by a 1:20 dilution of the stock suspension (2.5×10^3 CFU/mL). A 10 µL yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35 ºC and minimum inhibitory concentration endpoints 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 minimum inhibitory concentrations were reported.

Microplate alamar blue assay: 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 dimethylsulphoxide (DMSO; Merck) 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) were prepared in deionized water. The solution of the newly synthesized compounds and standard drugs were prepared and diluted at 2048, 1024, 512,… 0.0625 µg/mL concentrations in the wells of microplates within the liquid media.

The plates were sealed with parafilm and were incubated at 37 ºC for 5 days. Fifty microliters of a freshly prepared 1:1 mixture of 10X alamar blue (AbD Serotec) reagent and 10 % tween 80 was added to the control well. The plates were incubated at 37 ºC for 24 h. Control well 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 ºC 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 minimum inhibitory concentration was defined as the lowest drug concentration which prevented a colour change from blue to pink[33].

RESULTS AND DISCUSSION

In this present work, we reported the preparation of 15 new series of 3-[(4-substituted phenylmethylidene]amino-5-thioxo-1,2,4-triazol-3-yl)methyl]benzothiazol-2(3*H*)-one by using a microwave technique. **Scheme-I** illustrates the way used for the preparation of target compounds. We report that, the condensation of 2-aminothiophenol with urea results in the rapid formation of benzothiazol-2(3*H*)-one in high yield and enhanced reaction rate when subjected to microwave irradiation under solvent-free condition[28]. This reaction needs a long reaction time and a solvent under conventional heating.

Benzothiazol-2(3*H*)-one (**1**) was reacted with ethyl bromoacetate to obtain ethyl(benzothiazol-2(3*H*)-one-3-yl)

TABLE-2
IR AND ^{1}H NMR SPECTRAL DATA OF THE COMPOUNDS **4**, **5a-5m**

Comp.	IR ν_{max} (cm^{-1})	^{1}H NMR δ (DMSO-d_6)
4	3291, 3109, 1633, 1592, 1309	13,64 (1H, s, NH), 7.69 (1H, m, H7), 7.33 (1H, m, H4), 7.28 (1H, m, H6), 7.22 (1H, m, H5), 5,66 (2H, s, NH_2), 5.24 (2H, s, CH_2)
5a	3058,1685, 1591, 1275	14.04 (1H, s, NH), 9.99 (1H, s, =CH), 7.86 (2H, d, phenyl-H2,6), 7.68 (1H, d, H7), 7.62 (1H, t, phenyl-H4), 7.54 (2H, t, phenyl-H3,5), 7.38 (1H, d, H4), 7.34 (1H, t, H6), 7.21 (1H, t, H5), 5.39 (2H, s, CH_2)
5b	3042,1657, 1596, 1263	14.04 (1H, s, NH), 9.96 (1H, s, =CH), 7.90 (2H, m, phenyl-H2,6), 7.68 (1H, d, H7), 7.42-7.32 (mH, m, phenyl-H3,5, H4, H6), 7.23 (1H, t, H5), 5.39 (2H, s, CH_2)
5c	3050, 1666, 1591, 1263	13.70 (1H, s, NH), 9.99 (1H, s, =CH), 7.90 (2H, d, phenyl-H2, 6), 7.68 (1H, d, H7), 7.62 (2H, d, phenyl-H3,5), 7.37-7.34 (2H, m, H4, H6), 7.23 (1H, t, H5), 5.40 (2H, s, CH_2)
5d	3040, 1666, 1586, 1254	14.06 (1H, s, NH), 10.04 (1H, s, =CH), 7.82 (2H, d, phenyl-H2,6), 7.75 (2H, d, phenyl-H3, 5), 7.68 (1H, d, H7), 7.39-7.32 (2H, m, H4, H6), 7.23 (1H, t, H5), 5.04 (2H, s, CH_2)
5e	3070, 1651, 1592, 1267	14.11 (1H, s, NH), 10.26 (1H, s, =CH), 8.11 (2H, d, phenyl-H2, 6), 7.91 (2H, d, phenyl-H3, 5), 7.68 (1H, d, H7), 7.38 (1H, d, H4), 7.34 (1H, t, H6), 7.22 (1H, t, H5), 5.43 (2H, s, CH_2)
5g	3070, 1653, 1595, 1269	14.01 (1H, s, NH), 9.89 (1H, s, =CH), 7.81 (2H, d, H7, H4), 7.68 (1H, d, H6), 7.55 (2H, d, phenyl-H3, 5), 7.37-7.35 (2H, m, phenyl-H2, 6), 7.21 (1H, t, H5), 5.37 (2H, s, CH_2), 1.33 (9H, s, CH_3)
5h	3060, 1673, 1601, 1255	13.65 (1H, s, NH), 9.68 (1H, s, =CH), 7.82 (2H, d, phenyl-H2, 6), 7.67 (1H, d, H7), 7.38-7.32 (2H, m, H4, H6), 7.23 (1H, t, H5), 7.09 (2H, d, phenyl-H3, 5), 5.37 (2H, s, CH_2), 3.86 (3H, s, OCH_3)
5i	3040, 1657, 1590, 1256	14.01 (1H, s, NH), 10.42 (1H, s, =CH), 7.96 (1H, t, phenyl-4, 7.59 (2H, d, H7, H4), 7.30-7.23 (4H, m, phenyl-H3, 5, 6, H6), 7.12 (1H, t, H5), 5.32 (2H, s, CH_2)
5j	3040, 1702, 1589, 1275	14.11 (1H, s, NH), 10.81 (1H, s, =CH), 8.13 (1H, d, H7), 7.70 (1H, d, H4), 7.68-7.62 (2H, m, H6, phenyl-H6), 7.52-7.48 (1H, m, H5), 7.39 (1H, d, phenyl-H3), 7.35 (1H, t, phenyl-H5), 7.22 (1H, t, phenyl-H4), 5.43 (2H, s, CH_2)
5k	3064, 1667, 1583, 1249	14.01 (1H, s, NH), 10.70 (1H, s, =CH), 8.05-8.03 (1H, m, phenyl-H3), 7.73-7.70 (1H, m, phenyl-H5), 7.60 (1H, d, H7), 7.45-7.43 (2H, m, phenyl-H4, 6), 7.31 (1H, d, H4), 7.27 (1H, t, H6), 7.13 (1H, t, H5), 5.34 (2H, s, CH_2)
5m	3052, 1668, 1591, 1249	14.39 (1H, s, NH), 10.31 (1H, s, =CH), 7.94 (1H, dd, H7), 7.67(1H, d, H4), 7.60 (1H, t, H6), 7.39-7.32 (2H, m, phenyl-H4, 6), 7.23-7.18 (2H, m, phenyl-H3, 5), 7.07 (1H, t, H5), 5.37 (2H, s, CH_2), 3.89 (3H, s, OCH_3)

acetate (**2**) catalyzed by potassium carbonate on exposure to microwave irradiation in the presence. Benzothiazol-2(3*H*)-one (**1**), ethyl(benzothiazol-2(3*H*)-one-3-yl) acetate (**2**) and (benzothiazol-2(3*H*)-one-3-yl)acetic acid (**3**) were accomplished according to the previously reported procedures[29]. 3-[(4-Amino-5-thioxo-1,2,4-triazol-3-yl)methyl]benzothiazol-2(3*H*)-one (**4**) was easily prepared from the reaction between (benzothiazol-2(3*H*)-one-3-yl) acetic acid and thiocarbohydrazide in an oil-bath. 3-[(*o/p*-substitutedphenylmethylidene] amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-benzothiazol-2(3*H*)-one (**5**) derivatives thus obtained was reacted with *o/p*-substituebenzaldehyde derivatives in acetic acid under microwave irradiation. Most preparations of Schiff bases were reacted in the ethanol solvent, using an acid as the catalyst. But in this experiment, 3-[(4-amino-5-thioxo-1,2,4-triazol-3-yl)methyl] benzothiazol-2(3*H*)-one was only slightly dissolved in ethanol. Here in, the acetic acid played a role not only as a solvent but also as a catalyst[34]. The significant advantages of these procedures are operational simplicity, short reaction time, pure products and good yields.

The structure of the compounds was elucidated by IR, ^{1}H NMR, mass spectral data and elemental analysis (Tables 1 and 2).

In the IR spectra of all the compounds C=N and C=C bands were observed at *ca.* 1601-1430 cm^{-1} region and benzothiazol-2(3*H*)-ones have C=O stretching bands at 1702-653 cm^{-1} region. According to the IR spectroscopic data of the compounds **5a-m** which have 3-[(4-substitued phenylmethylidene]amino-5-thioxo-1,2,4-triazol-3-yl)methyl]- benzothiazol-2(3*H*)-one structure, the observation of C=S stretching bands at 1275-1249 cm^{-1} and the absence of an absorption at about 2600-2550 cm^{-1} region cited for SH group, have proved that these compounds were in the thionic form.

In the ^{1}H NMR spectra of compounds (**5a-m**) that are taken in DMSO-d_6 (Table-2), NH proton of the Schiff base was seen as singlet at about 14.11-13.95 ppm. The signal due to benzothiazol-2(3*H*)-one-CH_2- methylene protons, present in all compounds, appeared at 5.37-5.43 ppm, as singlet. The -N=CH proton of compounds **5a-m** appeared at 9.74-10.26 ppm as singlet. All the other aromatic and aliphatic protons were observed at the expected regions. Mass spectra (MS (TOF-MS)) of compounds showed M+1 peaks, in agreement with their molecular formula.

The synthesized compounds were tested for their *in vitro* antibacterial activity against some gram positive bacteria; *S. aureus* ATCC 29213, methicillin-resistant *S. aureus* (MRSA, clinical isolate), *E. faecalis* ATCC 29212, *E. faecalis* (resistant to vancomycin, clinical isolate), some gram negative bacteria; *E. coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* isolate, which has an extended spectrum beta lactamase enzyme (ESβL), *P. aeruginosa* ATCC 27853, *P. aeruginosa* (resistant to gentamycin, clinical isolate) and yeast-like fungi; *C. albicans* ATCC 10231 and *C. krusei* ATCC 6258 by using broth microdilution method. Ampicillin and gentamycin were used as standard antimicrobial agents and amphotericin B and fluconazole were used as standard antifungal agents (Table-3). The synthesized compounds were also tested *in vitro* for antimycobacterial activity against *M. tuberculosis* H37RV ATCC 27294, *M. tuberculosis* (clinical isolate) by using microplate alamar blue assay method. Ethambuthol was used as standard antimycobacterial agent. The biological activity results of the compounds were shown in Table-3. Minimum inhibitory concentrations were recorded as the minimum concentration of compound, which inhibits the growth of tested microorganisms.

As shown in Table-3, the synthesized compounds exhibited a broad spectrum of activity with minimum inhibitory concentration values 64-256 μg/mL against both gram-positive and gram-negative bacteria. Generally, synthesized compounds were more active against gram-positive bacteria rather than gram-negative bacteria. Among the gram-positive bacteria

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

Comp.	*E. coli* ATCC 25922	*E.coli* ATCC 35218	*E.coli* isolate	*P. aeruginosa* ATCC 27853	*P.aeruginosa* isolate	*S. aureus* ATCC 29213	*S.aureus* isolate	*E. faecalis* ATCC 29212	*E.faecalis* isolate	*C. albicans* ATCC 10231	*C.krusei* ATCC 6258	*M. tuberculosis* H37RV ATCC 27294	*M. tuberculosis isolate*
4	512	512	512	256	256	512	256	512	512	128	256	256	256
5a	512	512	512	256	256	64	256	256	256	64	256	128	256
5b	256	256	256	256	256	256	256	128	128	128	128	128	256
5c	512	512	512	256	256	512	256	512	512	64	256	256	256
5d	512	512	512	256	256	512	256	256	512	128	128	128	256
5e	512	512	512	128	256	256	128	128	256	128	256	256	128
5f	512	512	512	256	256	512	256	512	512	64	256	128	256
5g	512	512	512	128	256	64	128	128	512	128	256	256	128
5h	512	512	512	256	256	512	256	128	512	64	256	128	256
5i	512	512	512	256	256	512	256	256	512	256	256	128	256
5j	512	512	512	256	256	512	256	256	512	256	256	256	256
5k	512	512	512	256	256	512	256	256	512	256	256	256	256
5l	128	256	256	128	256	128	256	256	128	64	256	256	128
5m	512	512	512	256	256	512	256	512	512	256	128	256	128
Ampicillin	2	-	>1024	-	-	0.5	-	0.5	0.5	-	-	-	-
Gentamycin	0.25	-	512	1	64	0.5	128	8	8	-	-	-	-
Amphotericin B	-	-	-	-	-	-	-	-	-	<0.03	0.5	-	-
Fluconazole	-	-	-	-	-	-	-	-	-	0.0625	32	-	-
Ethambutol	-	-	-	-	-	-	-	-	-	-	-	4	1

tested, *S. aureus* showed relative high sensitivity towards the title compounds. Compounds **5a** and **5g** gave the best inhibitory activity against *S. aureus* ATCC 29213 with a minimum inhibitory concentration value of 64 µg/mL, but having less activity than the tested control drugs. Compound **5b**, **5e**, **5g** and **5h** showed more potent inhibitory effect against *E. faecalis* ATCC 29212 with minimum inhibitory concentration value of 128 µg/mL than the other tested compounds.

With respect to antifungal activity of the synthesized compounds, all compounds displayed antifungal activity against both *C. albicans* and *C. krusei* with a minimum inhibitory concentration value of 64-128 µg/mL. In contrast, all the synthesized compounds did not show significant activity against gram negative bacteria (minimum inhibitory concentrations > 256 µg/mL). Although compounds **5a**, **5c**, **5f**, **5h** and **5l** showed minimum inhibitory concentration value 64 µg/mL against *C. albicans* other compounds possess better activity but they were less active than reference drug and compound **4**. On the otherhand, compounds **5b**, **5d**, **5e** and **5g** were as effective as compound **4** but were less active than reference drug. The compound (**5c**) substituted with a chloro atom has a higher biological activity than those with other halogen atoms. Compounds **5b**, **5d** and **5m** gave slightly inhibitory activity against *C. krusei* with a minimum inhibitory concentration value of 128 µg/mL. Compounds **5l** was found to be effective against both gram positive (*S. aureus*) and gram negative (*P. aeruginosa*) bacteria with a minimum inhibitory concentration value of 128 µg/mL. In the same way, compounds **5e** and **5g** were showed minimum inhibitory concentration value 128 µg/mL against both types of bacteria *P. aeruginosa* and *E. faecalis*. Moreover, compounds **5b** and **5l** were found to be more active against *E. coli* isolate with minimum inhibitory concentration value of 256 µg/mL than the other compounds, even they were more potent than the standard drug, gentamycin.

As a result, when their antimicrobial activity is compared; the *p*-substituted schiff base derivatives are more active than the *o*-substituted Schiff base derivatives. The minimum inhibitory concentration values are generally within the range of 64-256 µg/mL, most often between 128-512 µg/mL against all evaluated strains.

The antimycobacterial assessment revealed that the Schiff base derivatives possess only a moderate or slight activity. However, compounds **5a**, **5b**, **5d**, **5f** and **5i** were more effective than compound **4**.

These compounds are characterized by the presence of a substituent in the *para* or *ortho* position of the phenyl ring but their activity does not depend from electronic effects, since electron-donating and electron-withdrawing substituents produce the same level of activity. However, a few *para* substituted derivatives were active against *C. albicans*.

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