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SCHIFF BASES OF 3-[(4-AMINO-5-THIOXO-1,2,4-TRIAZOLE-3-YL) METHYL]-2(3H)-BENZOXAZOLONE DERIVATIVES: SYNTHESIS AND BIOLOGICAL ACTIVITY

Solen URLU CICEKLI,[a] Tijen ONKOL,[a]* Selda OZGEN[b] and M. Fethi SAHIN[a]

[a] Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 06330-Ankara, Turkey,
Fax: +90 312 223 5018; Tel: +90 312 202 3233
[b] Gazi University, Faculty of Pharmacy, Department of Microbiology, 06330-Ankara, Turkey,
Fax: +90 312 223 5018; Tel: +90 312 202 3262

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New Schiff base derivatives of 3-[(4-amino-5-thioxo-1,2,4-triazole-3-yl)methyl]-2(3*H*)-benzoxazolone with aromatic aldehydes have been synthesized under microwave irradiation. Structures of the synthesized compounds were confirmed by IR, ^{1}H-NMR and elemental analysis. Variable, but modest antimicrobial and antitubercular activity against the investigated strains of bacteria and fungi were observed. Among the derivatives obtained, 4-bromophenylmethylidene derivative **5j** revealed significant antibacterial activity against *P. aeruginosa*.

INTRODUCTION

1,2,4-Triazoles and their derivatives play important an role in medicinal, agricultural and industrial fields. In medicine, they can be used as antimicrobial,[1, 2] antitubercular,[3] anti-inflammatory[4] and analgesics.[5] There are numerous papers reporting the method of synthesizing 1,2,4-triazole Schiff bases and their diverse biological activities such as antioxidant,[6] antitumor,[7] antimicrobial[8-10] and antitubercular.[3,11]

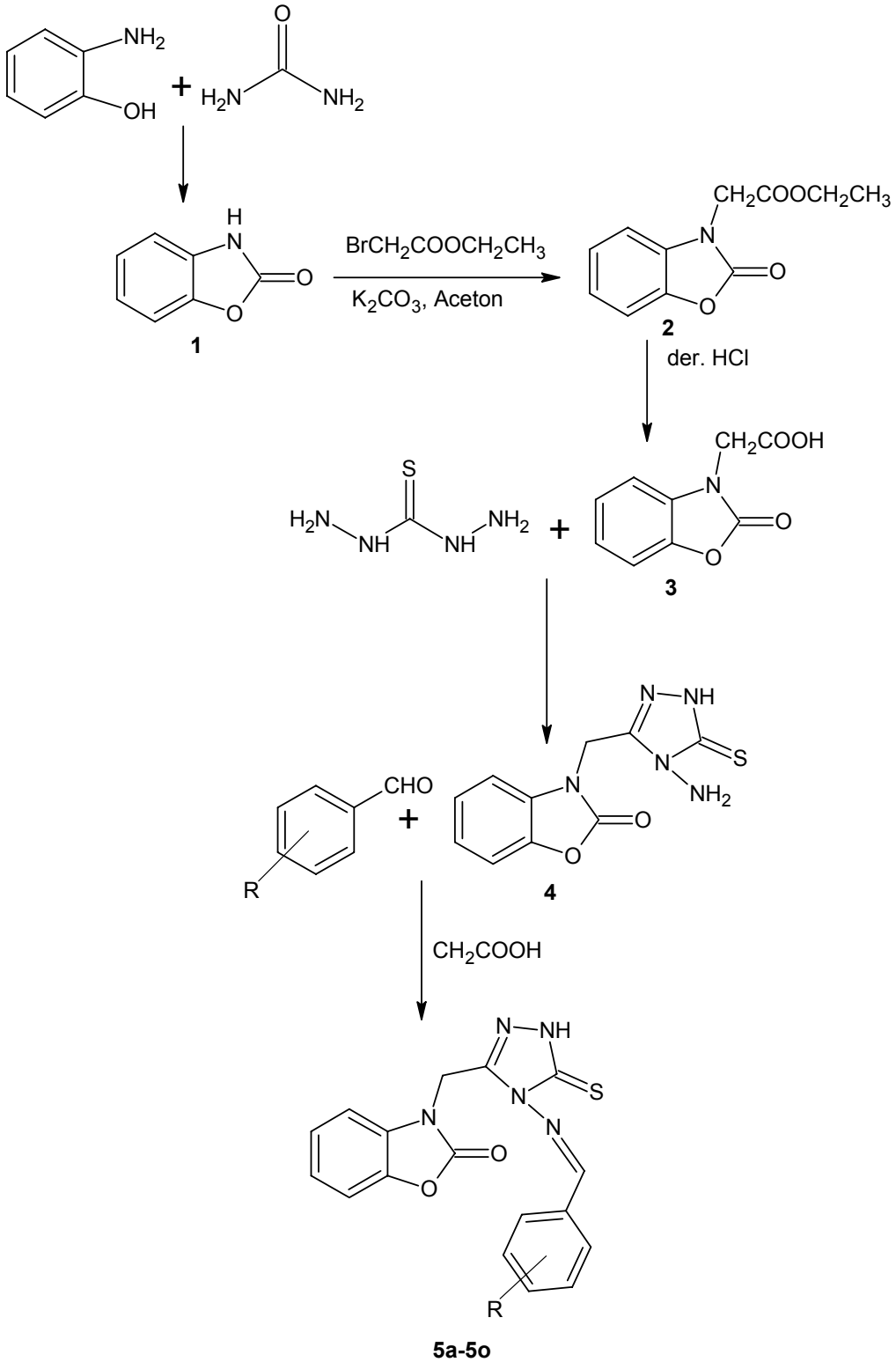
The use of microwave irradiation (MWI) in organic synthesis has become increasingly popular within the pharmaceutical and academic areas due to its potential to accelerate the organic reactions and hence to improve the economic, environmental and operational aspects. Microwave irradiation in organic reactions offer several advantages over conventional homogeneous and heterogeneous reactions with respect to rate enhancement, higher yields, greater selectivity and ease of manipulation.[12, 13]

Starting from the known fact that 1*H*-1,2,4-triazole compounds have antimicrobial property, we synthesized new Shiff base derivatives of 3-[(4-amino-5-thioxo-1,2,4-triazole-3-yl)methyl]-2(3*H*)-benzoxazolone and tested them concerning the possible biological activity.

RESULTS AND DISCUSSION

A series of 3-[(4-substitutedphenyl-methylidene]amino-5-thioxo-1,2,4-triazol-3-yl) methyl]-2(3H)-benzoxazolone have been carried out by using microwave technique as shown in Scheme 1. Condensation of 2-aminophenol with urea resulted in rapid formation of 2(3H)-benzoxazolone **1** in high yield with enhanced reaction rate, when subjected to microwave irradiation under solvent-free condition.[14, 15]

* Corresponding author: tijen@gazi.edu.tr



Scheme 1

2(3H)-benzoxazolone **1** was reacted with ethyl bromoacetate in the presence of potassium carbonate in microwave oven to obtain ethyl-(2(3H)-benzoxazolone-3-yl)acetate **2**. Synthesis of 2(3H)-benzoxazolone **1**, ethyl-(2(3H)-benzoxazolone-3-yl)acetate **2** and (2(3H)-benzoxazolone-3-yl)acetic acid **3** was accomplished according to the previously reported procedures.[16] 3-[(4-amino-5-thioxo-1,2,4-

triazol-3-yl)methyl]-2(3H)-benzoxazolone **4** was easily prepared from the reaction of (2(3H)-benzoxazolone-3-yl)acetic acid **3** and thiocarbohydrazide in oil-bath. 3-[(4-Amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)-benzoxazolone **4** was reacted with *o*/*p*-substituebenzaldehyde derivatives in acetic acid under microwave irradiation to give 3-[(*o*/*p*-substitutedphenylmethylidene]amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)-benzoxazolone **5a-5o** derivatives. Generally, Schiff bases are obtained in ethanol with an acid catalyst, but in our experiment 3-[(4-Amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2-benzoxazolone **4** was dissolved in acetic acid, that acted both as solvent and as catalyst.[17] Advantages of microwave irradiation such as high yield, short reaction time, pure product and easy work up prompted us to synthesize compounds under microwave irradiation.

The structures of the synthesized compounds were elucidated by IR, ^{1}H NMR spectra and elemental analysis.

In the IR spectra of all compounds, C=N and C=C bands were observed at about 1631-1462 cm^{-1} region and C=O stretching bands of 2(3H)-benzoxazolone were observed at 1782-1742 cm^{-1} region. IR spectroscopic data of the **5a-m** compounds, which have 3-[(4-substituephenyl-methylidene]amino-5-thioxo-1,2,4-triazol-3-yl) methyl]-2(3H)-benzoxazolone **4** structure, proved that these compounds were in the thionic form, as a result of the observation of C=S stretching bands at 1283-1233 cm^{-1} and the absence of an absorption at about 2600-2550 cm^{-1} region cited for SH group.

In the ^{1}H NMR spectra of compounds (**5a-m**) which were recorded in dimethylsulphoxide (DMSO)-d_6, NH proton of the Schiff base was seen as singlet at about 14.10-13.93 ppm. The signals due to 2(3H)-benzoxazolone methylene protons present in all compounds, appeared at 5.29-5.18 ppm, as singlet. The -N=CH proton of compounds **5a-m** appeared at 10.83-9.77 ppm as singlet. All other aromatic and aliphatic protons were observed at the expected regions.

The synthesized compounds were tested for their antibacterial activity against gram positive bacteria; *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* (MRSA, clinical isolate), *Enterococcus faecalis* ATCC 29212, *E. faecalis* (resistant to vancomycin, clinical isolate), gram negative bacteria; *Escherichia coli* ATCC 35218 producing extended spectrum β-lactamase and *E. coli* clinical isolate (ESBL), *Pseudomonas aeruginosa* ATCC 27853, *P. aeruginosa* (resistant to gentamicin, clinical isolate) and yeast-like fungi; *Candida albicans* ATCC 10231 and *Candida krusei* ATCC 6258 by using broth microdilution method. Ampicillin and gentamicin were used as standard antimicrobial agents and amphotericin B and fluconazole were used as standard antifungal agents (Table 2). The synthesized compounds were also tested *in vitro* for antimycobacterial activity against *Mycobacterium tuberculosis* H37RV ATCC 27294*, M. tuberculosis* (clinical isolate) by using MABA method. Ethambuthol was used as standard antimycobacterial agent. The results concerning the biological activity of the compounds are shown in Table 3. MICs were recorded as the minimum concentration of compounds which inhibit the growth of tested microorganisms.

Some synthesized compounds showed good to moderate activity with MIC value in the range of 32-128 μg/mL. Particularly, compounds **5e** and **5h** showed good activity (MIC value 128 μg/mL) against *E. coli* and compound **4** showed significant activity (MIC value 32 μg/mL) against *E. coli*. Moreover, **5e** and **5h** displayed comparable activity (MIC value 128 μg/mL) against *E. faecalis* and *E.coli* relative to the compound **4**.

For activity against *P. aeruginosa*, compounds **5j** yielded more promiosing activity, in comparison to other compounds synthesized, with MIC 64 μg/mL. All compounds showed weak or no activity against *S. aureus*. The results of antibacterial activity suggested that compounds with 2-hydroxy, 4-fluoro, and 4-bromo groups on the aromatic ring showed enhanced antibacterial activity among all the synthesized compounds.

Similar to antibacterial activity, the antifungal activity of the present compounds was not comparable to that of the standard drug Fluconazole due to the concentration variation. With respect to antifungal activity of the synthesized compounds, all compounds displayed antifungal activity against both *C. albicans* and *C. krusei* with MIC values of 128-512 μg/mL. Compounds **5e** and **5j** were as effective as compound **4** but were less active than the reference drug. Compounds **5c, 5g, 5n** and **5m** exhibited slightly inhibitory activity against *C. krusei* with MIC value of 128 μg/mL.

Table 1

Physico-chemical data of synthesis compounds (**5a-5o**)

Comp.	R	Crys. Sol.	Mp (^{o}C)	Yield (%)	Formula	Elemental Anal. Cal /Found.
4	-	EtOH-DMF	209	74	$C_{10}H_9N_5O_2S$	C:45.62 / 45.99 H: 3.45 / 3.76 N: 26.60 / 26.65
5a	**H**	Acetone-H_2O	225	27	$C_{17}H_{13}N_5O_2S$	C: 58.11 / 57.81 H: 3.73 / 4.15 N: 19.93 / 19.67
5b	**2-F**	EtOH-Acetone	232-34	59	$C_{17}H_{12}FN_5O_2S$	C: 55.28 / 55.09 H: 3.27 / 3.25 N:18.96 /19.05
5c	**2-Cl**	EtOH- DMF	231-33	36	$C_{17}H_{12}ClN_5O_2S$	C: 52.92 / 52.80 H: 3.13 / 3.16 N: 18.15 / 18.17
5d	**2-Br**	EtOH-Acetone	235	38	$C_{17}H_{12}BrN_5O_2S$	C: 47.45 / 47.13 H: 2.81 / 2.82 N:16.28 / 16.34
5e	**2-OH**	EtOH-Acetone	226	45	$C_{17}H_{13}N_5O_3S$	C: 55.58 / 55.32 H: 3.57 / 3.68 N: 19.06 / 19.01
5f	**2-CH_3**	EtOH-Acetone	221-22	58	$C_{18}H_{15}N_5O_2S$	C: 59.16 / 59.38 H: 4.14 / 3.95 N: 19.17 / 19.15
5g	**2-OCH_3**	EtOH-Acetone	237	55	$C_{18}H_{15}N_5O_3S$	C: 56.68 / 56.90 H: 3.96 / 3.99 N: 18.36 / 18.35
5h	**4-F**	2-Propanol	227-28	41	$C_{17}H_{12}FN_5O_2S$	C: 55.28 / 55.16 H: 3.27 / 3.50 N: 18.96 / 18.89
5i	**4-Cl**	EtOH	244-45	55	$C_{17}H_{12}ClN_5O_2S$	C: 52.92 / 52.73 H: 3.13/ 3.16 N: 18.15 / 18.22
5j	**4-Br**	EtOH- DMF	248-49	77	$C_{17}H_{12}BrN_5O_2S$	C: 47.45 /47.42 H: 2.81 / 2.89 N: 16.28 / 16.36
5k	**4-OH**	2-Propanol	247	45	$C_{17}H_{13}N_5O_3S$	C: 55.58 / 55.57 H: 3.57 /3.68 N: 19.06 / 19.03
5l	**4-CH_3**	EtOH-Acetone	231	62	$C_{18}H_{15}N_5O_2S$	C: 59.16 / 59.44 H: 4.14 / 4.10 N: 19.17 / 19.17
5m	**4-OCH_3**	EtOH- DMF	219-20	50	$C_{18}H_{15}N_5O_3S$	C : 56.68 / 56.58 H: 3.96 / 3.99 N: 18.36 / 18.42
5n	**4-CF_3**	EtOH-Acetone	243	39	$C_{18}H_{12}F_3N_5O_2S$	C : 51.55 / 51.55 H: 2.88 / 2.80 N: 16.70 / 16.67
5o	**4-$C(CH_3)_3$**	EtOH-Acetone	226-27	74	$C_{21}H_{21}N_5O_2S$	C: 61.90 / 61.99 H: 5.19 / 5.12 N: 17.19 / 17.16

Table 2

Antimicrobial activity results (MICs, µg/mL) of the synthesized compounds with the standard drugs

Comp.	A	B	C	D	E	F	G	H	I	J
4	256	256	128	256	32	128	256	128	128	256
5a	512	256	256	512	512	256	256	128	256	256
5b	512	256	512	512	512	512	256	256	256	256
5c	512	256	512	512	512	512	256	256	256	128

Table 2 (continued)

5d	512	256	256	512	512	512	256	256	256	256
5e	512	128	512	512	128	512	256	256	128	256
5f	512	256	512	512	256	512	256	256	256	256
5g	512	256	256	512	512	512	256	256	256	128
5h	512	256	128	512	128	512	256	256	256	256
5i	512	256	256	256	256	512	256	256	256	256
5j	512	128	512	256	512	512	64	256	128	256
5k	512	256	512	512	512	512	256	256	256	256
5l	512	256	256	512	512	512	256	256	256	256
5m	512	256	256	512	512	512	256	256	256	256
5n	512	256	256	512	256	512	256	256	256	128
5o	512	256	512	512	512	512	256	256	256	128
Ampicillin	0.5	-	0.5	0.5	-	>1024	-	-	-	-
Gentamicin	0.5	128	8	8	-	512	1	64	-	-
AmphotericinB	-	-	-	-	-	-	-	-	<0.03	0.5
Fluconazole	-	-	-	-	-	-	-	-	0.0625	32

A: *S. aureus* ATCC 29213, B: *S.aureus* isolated, C: *E. faecalis* ATCC 29212, D: *E.faecalis* isolated, E: *E.coli* ATCC 35218, F: *E.coli* isolated, G: *P. aeruginosa* ATCC 27853, H: *P.aeruginosa* isolated, I: *C. albicans* ATCC 10231, J: *C.krusei* ATCC 6258

Table 3

Antimycobacterial activity results (MICs, μg/mL)
of the synthesized compounds with the standard drugs

Comp.	*Mycobacterium tuberculosis* H37RV ATCC 27294	*Mycobacterium tuberculosis* isolated
4	128	512
5a	256	512
5b	256	512
5c	256	512
5d	128	512
5e	512	256
5f	512	256
5g	512	256
5h	256	512
5i	512	512
5j	512	512
5k	512	256
5l	512	512
5m	512	512
5n	512	256
5o	512	256
Etambuthole	4	1

The synthesized compounds showed moderate activity against mycobacteria with MIC values ranging from 128 to 512 μg/mL. Compounds **4** and **5d** showed better activity against *M. tuberculosis* H37Rv than the other compounds (MICs 128 μg/mL). The results of antimycobacterial activity showed that compounds with a halogen group on the aromatic ring showed good antimycobacterial activity among all the synthesized compounds.

MATERIALS AND METHODS

All chemicals and solvents, used in this study, were purchased from Aldrich, (Germany), Merck (Germany) and Acros (Germany) Chemical. Melting points of the compounds were recorded on an Electrothermal-9200 digital melting points apparatus and are uncorrected. Microwave reactions were carried out in MicroSYNTH Microwave Labstation at 1600 W (2 magnetrons 800Wx2). (Milestone S.r.l. Italy). ^{1}H-NMR spectra of compounds were recorded in DMSO-d_6 on Bruker 400 MHz NMR spectrometer. Chemical shifts were 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.

2(3H)-Benzoxazolone **(1)**

2(3H)-benzoxazolone was prepared under microwave irradiation as previously reported. [14,15]

Ethyl (2(3H)-benzoxazolone-3-yl)acetate **(2)**

The mixture of 2(3H)-benzoxazolone (5 mmol), potassium carbonate (5.75 mmol) and ethyl bromoacetate (5.75 mmol) in 50 mL acetone was prepared in round bottom flasks, placed in a microwave oven and irradiated (350 W, 65°C) for 15 min. After completion of reaction (TLC), 100 mL iced water was added to the cooled (0-10°C) reaction mixture. After stirring for 1 h, the crude was filtered, washed with water, dried, and crystallized from ethanol. Structures of the products were confirmed by IR, ^{1}H-NMR spectra and the obtained result was compared with conventional sample's result prepared according to literature methods. [16]

(2(3H)-Benzoxazolone-3-yl)acetic acid **(3)**

Ethyl (2(3H)-benzoxazolone-3-yl)acetate (0.01mol) was refluxed in hydrochloric acid (50 mL) for 2 hours. The reaction mixture was cooled, and the precipitate was collected by filtration, washed with water, dried, and crystallized from water. [16]

3-[(4-amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)-benzoxazolone **(4)**

(2(3H)-benzoxazolone-3-yl)acetic acid (0.01 mol) and thiocarbohydrazide (0.01 mol) were fused at 160-170°C in an oil bath for 30 min. N,N-dimethylformamide (DMF) was added on the crude and crude was heated until it solves. Subsequently water was added to the reaction mixture and the precipate was filtered, dried and recrystallized from DMF-ethanol.

Yield, 74%, m.p 209°C. IR ν max/cm^{-1} 3278, 3179,1782, 1483, 1243. ^{1}H NMR (DMSO) δ13.63 (1H, s, NH), 7.36 (1H, d, H7), 7.22-7.13 (3H, m, H4, H5, H6), 5.62 (2H,s, CH_2), 5.11 (2H, s, NH_2).

General procedure for *3-[(4-{[phenylmethylidene] amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)-benzoxazolone* **(5a-5m)**

To a suspension of *o*/*p*-substituted benzaldehyde (0.0022 mol) in glacial acetic acid (3 mL), 0.002 mol [(4-amino-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3H)-benzoxazolone was added. The reaction mixture was placed in microwave oven and irradiated for minutes changing between 15-30 min at 125 °C (300 W). After completion of the reaction by monitoring with TLC, the reaction mixture was kept overnight at room temperature. The precipitate was collected by filtration, washed with water, dried, and crystallized from appropriate solvent. (Table 1)

3-[(4-{[Phenylmethylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5a)

Yield, 27%, m.p 225°C. IR ν max/cm^{-1}, 3230, 1776, 1484, 1236. ^{1}H NMR (DMSO-d_6) δ 14.10 (1H, s, NH), 10.01 (1H, s, =CH), 7.85 (2H, d, Ar-H), 7.61 (1H, t, Ar-H), 7.54 (2H, t, Ar-H), 7.36 (1H, d, H7), 7.28 (1H, d, H4), 7.18 (1H, t, H6), 7.16 (1H, t, H5), 5.30 (2H, s, CH_2).

3-[(4-{[(2-Fluorophenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5b)

Yield, 59 %, m.p 232-234°C. IR ν max/cm^{-1}, 3230, 1751, 1484, 1233. ^{1}H NMR (DMSO-d_6) δ 13.93 (1H, s, NH), 10.42 (1H,s, =CH), 7.94 (1H, T, Ar-H), 7.59-7.57 (1H, m, Ar-H), 7.33-7.25 (3H, m Ar-H, H7), 7.19 (1H, d, H4), 7.11 (1H, t, H6), 7.06 (1H, t, H5), 5.21 (2H, s, CH_2).

3-[(4-{[(2-Chlorophenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5c)

Yield, 36 %, m.p 231-33°C. IR ν max/cm^{-1}, 3238, 1748, 1482, 1242. ^{1}H NMR (DMSO-d_6) δ 14.16

(1H, s, NH), 10.83 (1H, s, =CH), 8.13 (H, d, Ar-H), 7.64-7.43 (3H, m, Ar-H), 7.37 (1H, d, H7), 7.29 (1H, d, H4), 7.21-7.16 (2H, m, H6, H5), 5.33 (2H, s, CH_2).

3-[(4-{[(2-Bromophenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5d)
Yield, 38 %, m.p 235 ^oC. IR ν max/cm^{-1}, 3248, 1744, 1480, 1269. ^1H NMR (DMSO-d_6) δ 13.93 (1H, s, NH), 10.72 (1H, s, =CH), 8.04-8.01 (1H, m, Ar-H), 7.71-7.69 (1H, m, Ar-H), 7.44-7.42 (2H, m, Ar-H), 7.28 (1H, d, H7), 7.20 (1H, d, H4), 7.11 (1H, t, H6), 7.06 (1H, t, H5), 5.24 (2H, s, CH_2).

3-[(4-{[(2-Hydroxyphenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5e)
Yield, 45 %, m.p 226 ^oC. IR ν max/cm^{-1}, 3102, 1777, 1485, 1264. ^1H NMR (DMSO-d_6) δ 13.93 (1H, s, NH), 10.32 (1H, s, OH), 10.12 (1H, s, =CH), 7.72 (1H, d, Ar-H), 7.33 (1H, t, Ar-H), 7.26 (1H, d, H7), 7.19 (1H, d, H4), 7.12-7.03 (2H, m, H6, H5), 6.88 (1H, d, Ar-H), 6.81 (1H, t, Ar-H), 5.18 (2H, s, CH_2).

3-[(4-{[(2-Methylphenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5f)
Yield, 58 %, m.p 221-222 ^oC. IR ν max/cm^{-1}, 3186, 1772, 1484, 1268. ^1H NMR (DMSO-d_6) δ 14.16 (1H, s, NH), 10.28 (1H, s, =CH), 7.84 (1H, d, Ar-H), 7.39 (1H, t, Ar-H), 7.29-7.18 (4H, m, Ar-H, H7, H4), 7.12(1H, t, H6), 7.06 (1H, t, H5), 5.21 (2H, s, CH_2), 2.40 (3H, s, CH_3).

3-[(4-{[(2-Methoxyphenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5g)
Yield, 55 %, m.p 237 ^oC. IR ν max/cm^{-1}, 3233, 1750, 1485,1245. ^1H NMR (DMSO-d_6) δ 13.93 (1H, s, NH), 10.25 (1H,s, =CH), 7.82 (1H, d, Ar-H),7.50 (1H, t, Ar-H), 7.28 (1H, d,H7), 7.17 (1H, d, H4), 7.12-7.04 (3H, m, Ar-H, H5, H6), 6.96 (1H, t, Ar-H), 5.18 (2H, s, CH_2), 3.78 (3H, s, OCH_3)

3-[(4-{[(4-Fluorophenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5h)
Yield, 41 %, m.p 227-228 ^oC. IR ν max/cm^{-1}, 3232, 1753, 1480, 1267. ^1H NMR (DMSO-d_6) δ 14.04 (1H, s, NH), 9.98 (1H, s, =CH), 7.95-7.91 (2H, m, Ar-H), 7.41-7.35 (3H, m, Ar-H, H7), 7.27 (1H, d, H4), 7.20 (1H, t, H6), 7.16 (1H, t, H5), 5.29 (2H, s, CH_2)

3-[(4-{[(4-Chlorophenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5i)
Yield, 55 %, m.p 244-245 ^oC. IR ν max/cm^{-1}, 3298, 1744, 1484, 1241. ^1H NMR (DMSO-d_6) δ 14.06 (1H, s, NH), 10.06 (1H, s, =CH)), 7.88 (2H, d, Ar-H), 7.61 (2H, d, Ar-H), 7.36 (1H, d, H7), 7.27 (1H, d, H4), 7.20 (1H, t, H6), 7.17 (1H, t, H5), 5.30 (2H, s, CH_2)

3-[(4-{[(4-Bromophenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5j)
Yield, 75 %, m.p 248-249 ^oC. IR ν max/cm^{-1}, 3258, 1759, 1462, 1265. ^1H NMR (DMSO-d_6) δ 14.06 (1H, s, NH), 10.06 (1H,s, =CH), 7.77 (4H, dd, Ar-H), 7.36 (1H, d, H7), 7.27 (1H, d, H4), 7.19 (1H, t, H6), 7.15 (1H, t, H5), 5.30 (2H, s, CH_2)

3-[(4-{[(4-Hydroxyphenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5k)
Yield, 45 %, m.p 247 ^oC. IR ν max/cm^{-1}, 3236, 1747, 1484, 1283. ^1H NMR (DMSO-d_6) δ 14.07 (1H, s, NH), 10.41 (1H, s, OH), 9.64 (1H, s, =CH), 7.66 (2H, d, Ar-H), 7.35 (1H, d, H7), 7.26 (1H, d, H4), 7.19 (1H, t, H6), 7.14 (1H, t, H5), 6.89 (2H, d, Ar-H), 5.25 (2H, s, CH_2)

3-[(4-{[(4-Methylphenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5l)
Yield, 62 %, m.p 231 ^oC. IR ν max/cm^{-1}, 3265, 1754, 1462, 1238. ^1H NMR (DMSO-d_6) δ 14.07 (1H,s, N), 9.92 (1H,s, =CH), 7.74 (2H, d, Ar-H), 7.37-7.33(3H, m, Ar-H, H7), 7.26 (1H, d, H4), 7.19 (1H, t, H6), 7.17 (1H, t, H5), 5.28 (2H,s, CH_2), 2.39 (3H,s, CH_3)

3-[(4-{[(4-Methoxyphenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5m)
Yield, 50 %, m.p 219-220 ^oC. IR ν max/cm^{-1}, 3189, 1756, 1482, 1253. ^1H NMR (DMSO-d_6) δ 14.07 (1H,s, NH), 9.77 (1H, s, =CH), 7.79 (2H, d, Ar-H), 7.36 (1H, d, H7), 7.26 (1H, d, H4), 7.19 (1H, t, H6), 7.15 (1H, t, H5), 7.08 (2H, d, Ar-H), 5.27 (2H,s, CH_2), 3.86 (3H,s, OCH_3)

3-[(4-{[(4-Trifluoromethylphenyl)methylidene]amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5n)
Yield, 39 %, m.p 243 ^oC. IR ν max/cm^{-1}, 3276, 1742, 1462, 1239. ^1H NMR (DMSO-d_6) δ 14.07 (1H, s, NH), 10.19 (1H,s, =CH), 7.99 (2H, d, Ar-H), 7.81 (2H, d, Ar-H), 7.27 (1H, d, H7), 7.20 (1H,

d, H4), 7.11 (1H, t, H6), 7.07 (1H, t, H5), 5.23 (2H, s, CH_2)

3-[(4-{[(4-Trifluoromethylphenyl)methylidene] amino}-5-thioxo-1,2,4-triazol-3-yl)methyl]-2(3*H*)-benzoxazolone. (5o)

Yield, 74 %, m.p 226-227 ^oC. IR ν max/cm^{-1}, 3289, 1751, 1484, 1239. ^1H NMR (DMSO-d_6) δ 14.07 (1H, s, NH), 9.91 (1H, s, =CH), 7.78 (2H, d, Ar-H), 7.56 (2H, d, Ar-H), 7.36 (1H, d, H7), 7.26 (1H, d, H4), 7.20 (1H, t, H6), 7.17 (1H, t, H5), 5.27 (2H, s, CH_2), 1.32 (9H,s, CH_3)

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).[18] Standard powders of ampicillin trihydrate, gentamicin 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 (gentamicin 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 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.[19] 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. Dimethylsulphoxide (DMSO), phosphate buffer saline (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 3-morpholinopropane-1-sulfonic acid (MOPS) (pH 7) (Sigma) and culture suspensions were prepared through the guideline of CLSI M27-A3.[20] 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 ($2.5x10^3$ CFU/ml). A 10 μL yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35°C and MIC 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 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) between 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 4096, 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 °C for 5 days. Fifty microliters of a freshly prepared 1:1 mixture of 10X alamar blue (AbD Serotec) reagent and 10 % between 80 was added to the control well. The plates were incubated at 37°C 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°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.[21]

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