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Original article

Synthesis and antimicrobial activities of some new 1-(5-phenylamino-[1,3,4]thiadiazol-2-yl)methyl-5-oxo-[1,2,4]triazole and 1-(4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-5-oxo-[1,2,4]triazole derivatives

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Abstract

Acetic acid ethyl esters containing 5-oxo-[1,2,4]triazole ring **(2)** were synthesized by the condensation of compounds **1a–f** with ethyl bromoacetate in basic media. The reaction of compounds **2a–f** with hydrazine hydrate led to the formation of acid hydrazides **(3a–f)**. The treatment of compounds **3** with two divers aromatic aldehydes resulted in the formation of arylidene hydrazides as *cis–trans* conformers **(4a,c,e,f, 5a,e,f)**. The thiosemicarbazide derivatives **(6a,c,d,f)** were afforded by the reaction of corresponding compounds **3** with phenylisothiocyanate. The treatment of compounds **6a,c,d,f** with sulfuric acidic caused the conversion of side-chain of compounds **6a,c,d,f** into 1,3,4-thiadiazol ring; thus, compounds **7a,c,d,f** were obtained. On the other hand, the cyclization of compounds **6a,c,d,f** in the presence of 2 N NaOH resulted in the formation of compounds **8a,c,d,f** containing two [1,2,4]triazole rings which are linked to each other via a methylene bridge. Compounds **4a, f, 5a, 7a, d, f, 8a** and **d** have shown antimicrobial activity against one or more microorganism, but no antifungal activity has been observed against yeast like fungi. Also inhibitory effect on mycelial growth by compounds **4e, 7d** and **8f** has been observed. Compounds **4c** and **5f** were found to possess antitumor active towards breast cancer.

Keywords: Antimicrobial activity; Triazoles; Antitumor activity

1. Introduction

During the last few decades, a considerable attention has been devoted to the synthesis of [1,2,4]triazole derivatives possessing such diverse pharmacological properties as antimicrobial [1–4], antiinflammatory [5], analgesic [6], antitumorial [7], antihypertensive [8], anticonvulsant and antiviral activities [9]. In addition, [1,3,4]thiadiazoles exhibit divers biological activities possibly due to the presence of =N–C–S moiety [10]. Moreover, some biheterocyclic compounds incorporating [1,3,4]thiadiazole or [1,2,4]triazole ring have been produced as antimicrobial agents [11–14].

Some of azole derivatives used as common antibiotics such as Amphotericin B posses a toxic effect on humans as well as their antimicrobial effects [15]. Beside this, although there are antimicrobial agents having different structures are frequently used in treatment of microbial infections, there is increasing resistance to these drugs [15]. To overcome the development of drug resistance it is crucial to synthesize a new class of antibiotics possessing different chemical properties from those of used commonly. 5-Oxo-[1,2,4]triazole ring containing a side chain that has thiosemicarbazide structure is an ideal heterocyclic for this purpose.

The cyclization of the compounds having thiosemicarbazide structure has shown to be an excellent strategy for the synthesis of [1,2,4]triazole, [1,3,4]thiadiazole, [1,3,4]oxadiazole and (or) [1,3,4]triazine derivatives; for instance, the cyclization of *N,N′*-disubstituted thiosemicarbazides resulted in the formation of [1,3,4]thiadiazoles in acidic media [5,16]. On the other hand, the same thiosemicarbazides un-

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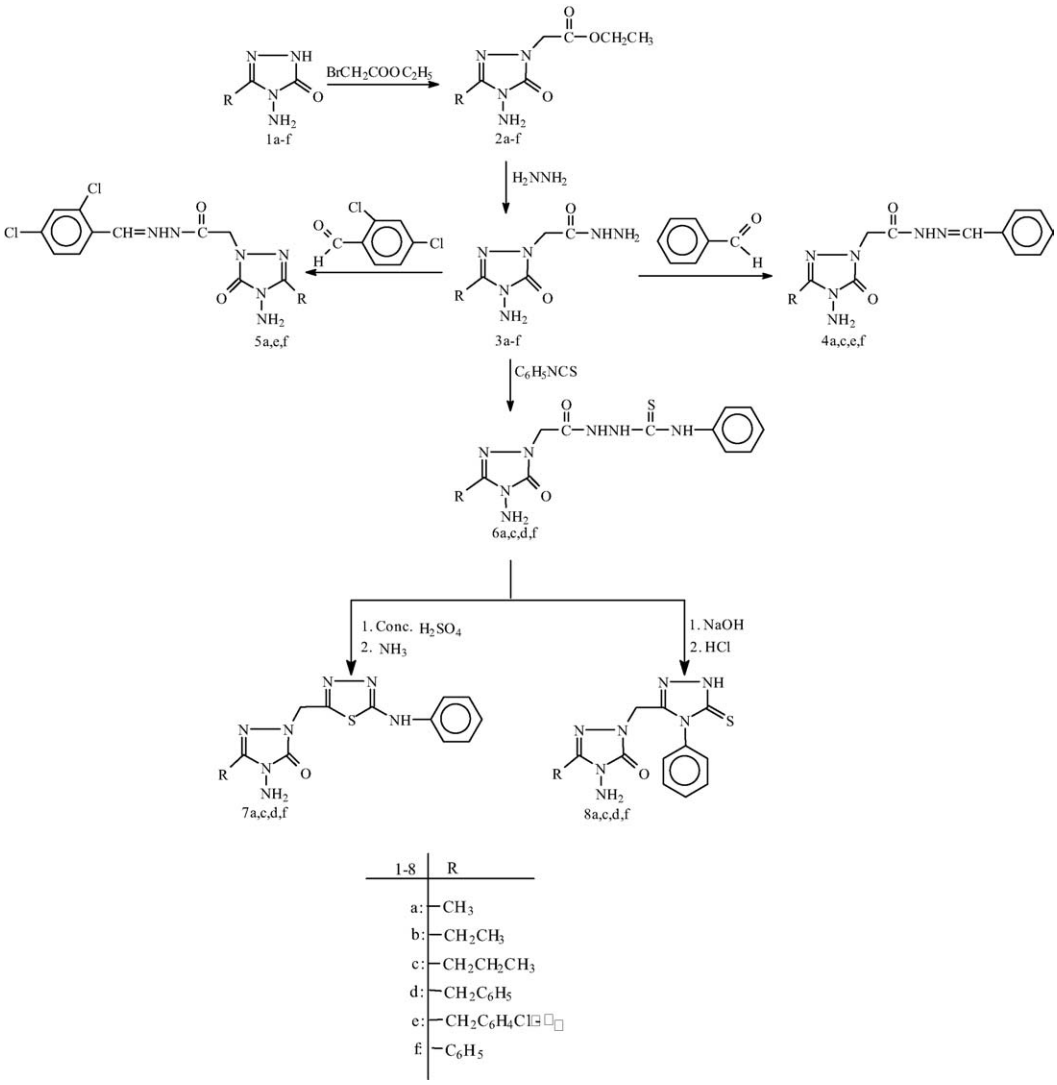


Scheme 1. Synthetic pathway for the preparation of compounds **2–8**.

derwent a cyclization to yield 1,2,4-triazole derivatives in the presence of NaOH, [16–18].

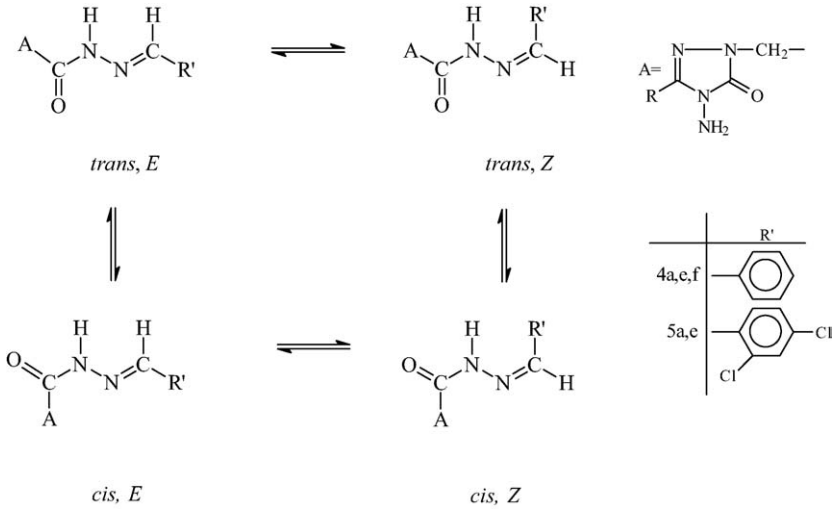
In recent years, various antitumor drugs have been developed for the treatment of cancer. Among these, some [1,2,4]triazole derivatives incorporating Shiff Base structure were synthesized as antitumor agents in our laboratory [7]. However, cancer is still a major health problem because of the insufficiency of the conventional methods.

In view of these facts, the aim of this present study is to obtain [1,2,4]triazole derivatives incorporating Shiff Base structure that possess antitumor activity and the synthesis of [1,2,4]triazole derivatives involving another [1,2,4]triazole or [1,3,4]thiadiazole ring, which is linked to 5-oxo-[1,2,4]triazole ring via a methylene group (Scheme 1) as antibacterial agents.

2. Chemistry

In the first part of the study, compounds **2a–f** were synthesized via the nucleophylic attack of N-1 on 5-oxo-[1,2,4]triazole ring to bromine-bearing C atom of ethyl bromoacetate. Compounds **2a, b** and **f** were prepared as precursors of some biological active 5-oxo-[1,2,4]triazole derivatives previously [19]. Compounds **2c–e** were obtained for the first time in this present study. Compounds **3a–f** were produced from the reaction of compounds **2a–f** with hydrazine hydrate. Although the acid hydrazides contain two amino groups in their structures, only the hydrazide–NH_2 reacted with aldehydes in ethanol leading to the formation of compounds **4a,c,e,f** and **5a,e,f**.

So far, the compounds containing imine bond have been synthesized intensively with various reasons, the major of which is their biological activities [7,20–22]. In addition, the compounds having arylidene–hydrazide structure may exist as *E/Z* geometrical isomers about –C=N double bond and *cis–trans* amide conformers (Scheme 2) [19,22–24]. According to the literature [22,24], the compounds containing imine bond are present in higher percentage in dimethyl-D_6 sulfoxide solution in the form of geometrical *E* isomer about –C=N double bond. The *Z* isomer can be stabilized in less polar



Scheme 2. *E/Z* geometrical isomers and *cis–trans* conformers of compounds **4a,c,d,f** and **5a,e,f**.

solvents by an intramolecular hydrogen bond. In the present study, the spectral data were obtained in dimethyl-D_6 sulfoxide solution and no signal belonging to *Z* isomer was observed. On the other hand, the *cis–trans* conformers of *E* isomer were present in the dimethyl-D_6 sulfoxide solution of compounds **4a,c,e,f 4** and **5a,e,f**.

The key intermediate thiosemicarbazides **(6a,c,d,f)** were obtained from the reaction of compounds **3a,c,d,f** with phenyl isothiocyanate in reasonable good yields.

Compounds **7a,c,d,f** were prepared by the treatment of thiosemicarbazides with concentrated sulfuric acid and the structures of these compounds confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectral data (for compound **7f**), and elemental analyses.

Compounds **8a,c,d,f** were afforded by three different ways, all of which contained thiosemicarbazides **(6a,c,d,f)** as intermediates. According to the literature [13–15] it is expected that the treatment of thiosemicarbazides with aqueous NaOH resulted in a cyclization leading to the formation of 5-thioxo-[1,2,4]triazole ring. Beside this method, in the present study compounds **8a,c,d,f** were prepared by heating of the corresponding compounds **6** in an oil bath upon 130 °C for 2 h. In the third method, compound **8f** was synthesized by refluxing of **6f** in ethanol for 8 h. The yields of these three methods are very close to each other. These results show that the effect of heating on thiosemicarbazides **(6a,c,d,f)** causes a cyclization resulting in the formation of 5-thioxo-[1,2,4]triazole ring, but not [1,3,4]thiadiazole ring.

It is interesting to note that compounds **8a,c,d,f** are present in solid state in –C=S form as indicated by their IR spectra (absence of absorption in the region of 2500–2660 cm^{-1} for –SH stretching and presence of two absorption maxima at 1342–1320 cm^{-1} characteristic of –C=S group in these type of compounds [11]). The –C=S form is present also in dimethyl sulfoxide by suggested NMR spectral data.

3. Results and discussion

In the ^{1}H NMR spectra of compounds **2c–f** additional signals derived from ester group were observed at 4.52 (–NCH_2), 4.10 ppm (–$O\underline{CH_2}CH_3$) and 1.09–1.17 ppm (–$OCH_2\underline{CH_3}$) ppm integrating for two proton, two proton and three proton, respectively. In the ^{13}C NMR spectra of these compounds, the signals belonging to the same groups were recorded at 46.43, 60.93, and 13.86–14.95 ppm, respectively.

The ^{1}H NMR spectra of compounds **3a–f** displayed no signals belonging to OCH_2CH_3 group; instead, new signals derived from hydrazide structure appeared between 4.28–4.46 ppm (–$NH\underline{NH_2}$) and 9.18–9.29 ppm (–$\underline{NH}NH_2$) integrating for two proton and one proton, respectively (controlled by changing with D_2O). In addition, the signals derived from –NH_2 group at position-4 on 5-oxo-[1,2,4]triazole ring resonated between 5.24 and 5.52 ppm integrating for two proton (exchangeable with D_2O). The IR spectra of acid hydrazides **(3a–f)** showed an additional peak at 1651–1682 cm^{-1} due to exocyclic-carbonyl function derived from hydrazide structure beside the endocyclic-carbonyl peak at position-5 of [1,2,4]triazole ring.

In the ^{1}H NMR and ^{13}C NMR spectra of compounds **4a,c,e,f** the signals belonging to benzylidene group were observed at aromatic region while the signals belonging to –$NH\underline{NH_2}$ disappeared. The ^{1}H NMR spectra of compounds **5a,e,f** displayed three different singlets due to 2,4-dichlorobenzylidene group between 7.50 and 8.55 ppm each integrating for one proton. In the ^{1}H NMR spectra of compounds **4a,c,e,f** and **5a,e,f** two sets of signals each belonging to the –NH group of *cis* and *trans* conformers were observed between 11.16–11.92 and 11.72–11.97 ppm; the ratio of *trans–cis* in each case were calculated by using NMR data. The upfield lines of –NH protons were assigned to *cis* conformer of the amide structure and downfield lines of the protons of the same group to *trans*- conformer of the amide structure [21]. Moreover, the ^{1}H NMR spectra of compound **4e** showed additional two singlets derived from –N=CH group at 8.01 and 8.21 each representing *cis* and *trans* conformers; the ^{13}C signals of the same conformers appeared at 144.15 and 144.20 ppm. In addition, the ^{1}H NMR spectra of compound **4f** displayed two singlets belonging to –NH_2

Table 1
Screening for antimicrobial activity of the selected compounds

Compound No.	Solvent	Microorganisms and inhibition zone (mm)									
		Ec	Pa	Yp	Kp	Ef	Sa	Bs	Ca	Ct	Cg
4a	DMSO	+++	++	–	+++	–	–	+++	–	–	–
4e		+	–	–	+	–	++	+	–	+	–
4f		–	+	–	+++	–	–	–	–	–	–
5a		+++	++	–	+++	–	–	++	–	–	–
5e		–	–	–	–	–	–	–	–	–	–
7a		+++	+++	–	+++	–	++	+++	–	–	–
7d	Ethanol	++	+	+	+++	+	++	++	–	–	–
7f	DMSO	–	+	–	++	–	–	–	–	+	–
8a		+	–	–	–	–	++	+	–	–	–
8c		–	–	–	–	–	–	–	+	+	–
8d		–	–	–	–	–	++	+	–	–	–
8f		–	–	–	–	–	–	–	–	–	–
With ethanol		–	–	+	+	–	++	+	–	–	–
With DMSO		–	–	–	–	–	–	–	–	–	–
Ceftazidime		+++	+++	+++	+++	+++	+++	+++			
Triflucan									+++	+++	+++

Results were interpreted in terms of the diameter of the inhibition zone: (–): <5.5 mm; (+): 5.5–10 mm; (++): 11–16 mm; (+++): ≥17 mm.
Ec: *Escherichia coli* ATCC 35218, Pa: *Pseudomonas aeruginosa* ATCC 10145, Yp: *Yersinia pseudotuberculosis* ATCC 911, Kp: *Klebsiella pneumoniae* ATCC 13883, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Bs: *Bacillus subtilis* ATCC 6633, Ca: *Candida albicans* ATCC 60193, Ct: *Candida tropicalis* ATCC 13803, Cg: *Candida glabrata*

group at 5.58 and 5.61 ppm. In the ^{13}C NMR spectra of compounds **4a,c,e,f** and **5a,e,f** –NCH_2, triazole-C-3 and triazole-C-5 signals were recorded as two sets between 46.14–46.72 and 46.17–46.57 ppm (–NCH_2), 144.25–144.77 and 144.28–146.94 ppm (triazole-C-3), 153.03–154.46 and 153.44–163.25 ppm (triazole-C-5) as the result of formation of *cis* and *trans* conformers. Furthermore, in the ^{13}C NMR spectra of compounds **5e** and **f**, the signals belonging to exocyclic-C=O group derived from each *cis* and *trans* conformers were recorded at 168.22 and 168.30 ppm. The ^{13}C NMR values of triazole-C-3 and triazole-C-5 are consistent with literature [7,25]. Besides, compound **5a** gave a molecular ion peak consistent with its formulae.

The elemental analyses of compounds **6a,c,d,f** are consistent with the assigned structures. The IR spectra of compounds **6a,c,d,f** showed an –C=S absorption between 1309 and 1341 cm^{-1}; the ^{13}C signal of this group were observed at 180.37–181.03 ppm. In the ^{1}H NMR spectra of these compounds, the –NH_2 signal derived from hydrazide structure disappeared while the signal belonging to –NH_2 group on 1,2,4-triazole ring was observed between 5.30 and 5.36 ppm. In addition, the ^{1}H NMR spectra of compounds **6a,c,d,f** displayed three singlets due to three different –NH groups around 9.60, 9.70 and 10.30 ppm (exchangeable with D_2O) each integrating for one proton. The aromatic protons derived from phenyl group resonated between 7.18 and 7.54 ppm.

In the ^{1}H NMR spectra of compounds **7a,c,d,f** and **8a,c,d,f** the presence of only one signal for –NH group integrating for one proton (exchangeable with D_2O) confirmed the cyclization at the side chain of triazole ring in compounds **6a,c,d,f**. In the ^{13}C NMR spectra of compounds **7a,c,d,f** no signal due to –C=S group were observed, while the signal derived from this group have resonated between 168.20 and 171.96 ppm in the spectra of compounds **8a,c,d,f**. Beside these, the melting points highly increased when compounds **6** were converted to corresponding products (**7** and **8**). Elemental analyses are consistent with the assigned structures for compounds **7a,c,d,f** and **8a,c,d,f**. The mass spectra of the cyclization products **(7f, 8a,c,d,f)** showed molecular ion peaks which were consistent with their molecular formulae.

Compounds **4a, 5a, 7a,d,f, 8a** and **d** showed antibacterial activity against one and/or more test microorganisms (Table 1). Compounds **4a, 5a** and **7a**, which contain a methyl group on position 3 of 5-oxo-[1,2,4]triazole or [1,3,4]thiadiazol-2-yl-5-oxo-[1,2,4]triazole ring were found to be effective on *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 10145, *K. pneumonia* ATCC 13883 and *Bacillus subtilis* ATCC 6633. No antimicrobial activity was observed on the yeast like fungi (Table 1). The compounds including a phenyl group on position 3 of 5-oxo-[1,2,4]triazole ring **(4f)** or [1,3,4]thiadiazol-2-yl-5-oxo-[1,2,4]triazole ring **(7f)** showed activity against *Klebsiella pneumoniae* ATCC 13883 and lower activity against *P. aeruginosa* ATCC 10145. Compounds **7d** which has a benzyl group on position 3 and consists of [1,3,4]thiadiazol-2-yl-5-oxo-[1,2,4]triazole ring exhibited activity towards *K. pneumoniae* ATCC 13883, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 25923 and *B. subtilis* ATCC 6633, while the compound having a benzyl group at position 3 of 5-thioxo-[1,2,4]triazol-2-yl-5-oxo-[1,2,4]triazole ring **(8d)** was found

Table 2
Effects of test compounds on mould growth (mm)

Compound no.	*Penicillum* spp.	*Aspergillus* spp.
4a	20	17
4e	11	7
4f	ND	ND
5a	16	12
5e	ND	ND
7a	16	10
7d	12	8
7f	20	13
8a	18	12
8c	18	13
8d	20	11
8f	10	9
Triflucan	17	9
Untreated control	20	17

ND: Not detected.

to be effective only against *S. aureus* ATCC 25923. Compound **4e**, which has a *p*-chlorobenzyl group on position 3 showed moderate activity against *S. aureus* ATCC 25923 and lower activity against *B. subtilis* ATCC 6633, while the other compound **(5e)** including *p*-chlorobenzyl group on position 3 exhibited no activity. Among the compounds containing a phenyl group at position 3, compounds **4f** and **7f** showed activity on *K. pneumoniae* ATCC 13883, while **8f** incorporating both 5-oxo- and 5-thioxo-[1,2,4]triazole rings exhibited no activity on test microorganisms. Compound **8c** having an *n*-propyl group on position 3 of 5-thioxo-[1,2,4]triazol-3-yl-5-oxo-[1,2,4]triazole structure showed only lower activity against *Candida albicans* ATCC 60193 and *Candida tropicalis* ATCC 13803. Inhibitory effect on mycelial growth has been especially observed by compounds **4e, 7d** and **8f** (Table 2).

According to the data listed in Tables 3 and 4, compounds **4c** and **5f** both have potentials to reduce the growth of MCF7 cell line. Especially, compound **5f** having a GI_{50} value of 1.87 μM seems to be effective towards breast cancer cell lines. It has been reported that compounds having triazole moieties such as vorozole, letrozole and anastrozole appear to be very effective aromatase inhibitors in terms for preventing breast cancer [26–28]. It is known that [1,2,4]triazole moieties interact strongly with the heme iron and aromatic substituents in the active site of aromatase [29]. It can be speculated that the tested Shiff Bases **(4c, 5f)** possessing a [1,2,4]triazole ring inhibit the MCF7 cell line by possibly interacting with the aromatase. Although both the tested compounds contain such a 1,2,4-triazole ring, the reason for **5f** being most effective against MCF7 cell line might be due to a phenyl group at position 3 of the [1,2,4]triazole ring

Table 4
The GI_{50} values of compounds **4c** and **5f** towards several tumor cell line [a] (μM)

Panel/cell line	**4c**	**5f**
Leukemia		
CCRF-CEM	86.2	14.6
MOLT-4	53.2	>50.0
RPMI-8226	45.2	″
SR	43.7	″
Non-small cell lung cancer		
A549/ATCC	51.8	>50.0
EKVX	>50.0	″
HOP-62	95.8	27.4
HOP-92	b	25.5
NCI-H226	-	24.9
NCI-H23	23.4	>50.0
NCI-H322M	25.5	13.1
NCI-H460	34.1	21.8
NCI-H522	-	35.9
Colon cancer		
HCC-2998	21.1	>50.0
HCT-116	b	19.3
Breast cancer		
MCF7	43.3	1.87
NCI/ADR-RES	24.9	43.1
MDA-MB-231/ATCC	33.6	26.6
MDA-MB-435	56.9	>50.0
CNS cancer		
SF-268	b	34.1
SF-295	77.5	30.6
U251	34.3	18.7
Melanoma		
LOX IMIV	51.4	>50.0
MALME-3M	65.7	22.3
SK-MEL-2	b	45.3
UACC-62	92.3	49.7
Ovarian cancer		
IGROVI	53.2	13.0
OVCAR-3	23.8	>50.0
OVCAR-4	31.0	22.4
OVCAR-5	30.7	>50.0
OVCAR-8	49.9	>50.0
SK-OV-3	56.3	11.8
Renal cancer		
786-0	56.4	>50.0
Achn	23.7	19.6
Cakı-1	23.7	20.0
Sn12c	22.9	49.2
Tk10	25.5	29.7
UO-31	22.5	25.5
Prostate cancer		
PC-3	54.0	27.7

[a] GI_{50}: Molar concentration for 50% growth inhibition.
[b] The GI_{50}value ≥100 μM.

Table 3
Antitumor screening data for the selected Shiff Bases

Compound no.	Number assigned by NCI	Growth percentage of tumor cell		
		MCF7	NCI-H460	SF-268
4c	D-729281	5	96	94
5f	D-729282	1	86	76

seems to fit to the active site of aromatase. This proposal has to be elucidated in detail with [1,2,4]triazole derivatives having aromatic substituents. Finally, our present data suggest that compound **5f** should be a potent therapeutic agent for the treatment of breast cancer.

4. Experimental

4.1. Chemistry

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian-Mercury 200 MHz spectrometer. The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1600 series FTIR spectrometer. Mass spectra were obtained at a Quattro LC-MS (70 eV) Instrument. Combustion analysis was performed on a Carlo Erba 1106 elemental analyzer. All the chemicals were obtained from Fluka Chemie AG Buchs (Switzerland). Compounds **1a–f, 2a, b** and **f** were synthesized by a published method [19,30]. Melting points and IR data of known compounds are consistent with the ones reported previously [19,30].

*4.1.1. General method for the synthesis of compounds **2***

The corresponding 3-alkyl-4-amino-5-oxo-4,5-dihydro-[1,2,4]triazole **(1)** (0.01 mol) was refluxed with equivalent amount of natrium in absolute ethanol for 2 h. Then, ethyl bromoacetate (0.01 mol) was added and refluxed for an additional 5 h. After evaporating at 35–40 °C under reduced pressure, a solid appeared. This was recrystallized from an appropriate solvent to afford the desired compound.

*4.1.1.1. 4-Amino-5-oxo-3-*n*-propyl-4,5-dihydro-[1,2,4]triazol-1-yl-acetic acid ethyl ester **(2c)**.* Recrystallization from ethyl acetate/petroleum ether (1:2), (yield: 62.30%), m.p. 88–89 °C. Analysis (Calc/found %): for $C_9H_{16}O_3N_4$ C: 47.35/47.91, H: 7.06/7.37, N: 24.54/24.32; IR (KBr) (ν, cm^{-1}), 3314–3215 (–NH_2), 1751 (ester –C=O), 1708 (triazole –C=O), 1591 (–C=N), 1216 (–C–O); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.11 (t, J = 5.8 Hz, –$CH_2CH_2\underline{CH_3}$), 1.18 (t, J = 6.5 Hz, –$OCH_2\underline{CH_3}$), 1.65–1.78 (m, –$CH_2\underline{CH_2}CH_3$), 2.45 (q, J = 6.5 Hz, –$\underline{CH_2}CH_2CH_3$), 4.10 (q, J = 5.8 Hz, –$O\underline{CH_2}CH_3$), 4.54 (s, –NCH_2), 5.23 (s, –NH_2); ^{13}C NMR (DMSO-d_6) δ (ppm) 13.82 (–$CH_2CH_2\underline{CH_3}$), 14.74 (–$OCH_2\underline{CH_3}$), 19.01 (–$CH_2\underline{CH_2}CH_3$), 27.13 (–$\underline{CH_2}CH_2CH_3$), 46.54 (NCH_2), 61.17 (–$O\underline{CH_2}CH_3$), 146.54 (triazole-C-3), 151.85 (triazole-C-5), 167.25 (exocyclic-C=O).

*4.1.1.2. 4-Amino-3-benzyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl-acetic acid ethyl ester **(2d)**.* Recrystallization from ethyl acetate/petroleum ether (1:2), (yield: 62.30%), m.p. 105–106 °C. Analysis (Calc/found %): for $C_{13}H_{16}O_3N_4$ C: 56.51/56.45, H: 5.84/5.87, N: 20.28/19.98; IR (KBr) (ν, cm^{-1}), 3330–3212 (–NH_2), 1747 (ester –C=O), 1700 (triazole –C=O), 1578 (–C=N), 1226 (–C–O); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.09 (t, J = 6.0 Hz, –$OCH_2\underline{CH_3}$), 3.89 (s, benzyl–CH_2), 4.13 (q, J = 6.0 Hz, –$O\underline{CH_2}CH_3$), 4.52 (s, –NCH_2), 5.38 (s, –NH_2), 7.10–7.25 (m, 5H, ar-H); ^{13}C NMR (DMSO-d_6) δ (ppm) 14.95 (–$OCH_2\underline{CH_3}$), 30.14 (benzyl–CH_2), 46.43 (NCH_2), 60.93 (–$O\underline{CH_2}CH_3$), ar C: [126.42 (CH), 128.26 (4CH), 135.92 (C)], 145.92 (triazole-C-3), 151.15 (triazole-C-5), 167.85 (exocyclic-C=O).

*4.1.1.3. 4-Amino-3-*p*-chlorobenzyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl-acetic acid ethyl ester **(2e)**.* Recrystallization from ethanol–water (1:2), (yield: 55.30%), m.p. 156–157 °C. Analysis (Calc/found %): for $C_{13}H_{15}O_3N_4Cl$ C: 50.25/51.03, H: 4.86/4.89, N: 18.03/17.98; IR (KBr) (ν, cm^{-1}), 3390–3225 (–NH_2), 1736 (ester –C=O), 1714 (triazole –C=O), 1582 (–C=N), 1233 (–C–O); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.17 (t, J = 6.0 Hz, –$OCH_2\underline{CH_3}$), 3.90 (s, benzyl–CH_2), 4.10 (q, J = 6.0 Hz, –$O\underline{CH_2}CH_3$), 4.52 (s, –NCH_2), 5.17 (s, NH_2), 7.28 (d, J = 8.0 Hz, 2H, ar-H), 7.30 (d, J = 8.0 Hz, 2H, ar H); ^{13}C NMR (DMSO-d_6) δ (ppm) 13.86 (–$OCH_2\underline{CH_3}$), 29.55 (benzyl–CH_2), 46.43 (NCH_2), 60.94 (–$O\underline{CH_2}CH_3$), ar C: [128.22 (2CH), 130.53 (2CH), 131.10 (C), 134.63 (C)], 146.91 (triazole-C-3), 153.10 (triazole-C-5), 167.85 (exocyclic-C=O).

*4.1.2. General method for the synthesis of compounds **3***

A solution of the corresponding compound **2** (0.01 mol) in *n*-butanol was refluxed with hydrazine hydrate (0.025 mol) for 4 h. After cooling to room temperature, a white solid appeared. This was recrystallized from an appropriate solvent to afford the desired product.

*4.1.2.1. 4-Amino-3-methyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl-acetic acid hydrazide **(3a)**.* Recrystallization from ethanol (yield: 67.12%), m.p. 190 °C. Analysis (Calc/found %): for $C_5H_{10}O_2N_6$ C: 32.25/32.55, H: 5.41/5.36, N: 45.14/44.79.18; IR (KBr) (ν, cm^{-1}), 3357–3211 (NH + $2NH_2$), 1716 (triazole –C=O), 1682 (hydrazide –C=O), 1500 (–C=N); ^{1}H NMR (DMSO-d_6) δ 2.09 (s, –CH_3), 4.27 (s, –NCH_2), 4.32 (s, –$NH\underline{NH_2}$), 5.24 (–NH_2), 9.18 (s, –$\underline{NH}NH_2$); ^{13}C NMR (DMSO-d_6) δ 16.11 (–CH_3), 51.34 (–NCH_2), 150.55 (triazole C-3), 158.72 (triazole C-5), 171.58 (exocyclic-C=O).

*4.1.2.2. 4-Amino-3-ethyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl-acetic acid hydrazide **(3b)**.* Recrystallization from ethanol (yield: 68.21%), m.p. 170–171 °C. Analysis (Calc/found %): for $C_6H_{12}O_2N_6$ C: 35.99/36.25, H: 6.04/6.09, N: 41.98/41.15; IR (KBr) (ν, cm^{-1}), 3284–3173 (NH + $2NH_2$), 1705 (triazole –C=O), 1666 (hydrazide –C=O), 1547 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.29 (t, J = 5.8 Hz, –$CH_2\underline{CH_3}$), 2.47 (q, J = 5.8 Hz, –$\underline{CH_2}CH_3$), 4.20 (s, –NCH_2), 4.28 (s, –$NH\underline{NH_2}$), 5.24 (s, –NH_2), 9.19 (s, –$\underline{NH}NH_2$); ^{13}C NMR (DMSO-d_6) δ (ppm) 10.19 (–$CH_2\underline{CH_3}$), 18.22

(–$\underline{CH_2}CH_3$), 38.75 (–NCH_2), 149.52 (triazole-C-3), 154.10 (triazole-C-5), 166.25 (exocyclic-C=O).

*4.1.2.3. 4-Amino-3-*n*-propyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl-acetic acid hydrazide* ***(3c)***. Recrystallization from ethanol (yield: 68.21%), m.p. 144–145 °C. Analysis (Calc/found %): for $C_7H_{14}O_2N_6$ C: 39.24/39.25, H: 6.59/6.18, N: 39.23/39.35; IR (KBr) (ν, cm^{-1}), 3286–3164 (NH + $2NH_2$), 1717 (triazole –C=O), 1653 (hydrazide –C=O), 1547 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.04 (t, J = 6.0 Hz, –$CH_2CH_2\underline{CH_3}$), 1.60–1.72 (m, –$CH_2\underline{CH_2}CH_3$,), 2.40 (q, J = 6.0 Hz, –$\underline{CH_2}CH_2CH_3$), 4.33 (s, –NCH_2), 4.41 (s, –$NH\underline{NH_2}$), 5.26 (s, –NH_2), 9.32 (s, –$\underline{NH}NH_2$); ^{13}C NMR (DMSO-d_6) δ (ppm) 13.43 (–$CH_2CH_2\underline{CH_3}$), 18.81 (–$CH_2\underline{CH_2}CH_3$), 26.23 (–$\underline{CH_2}CH_2CH_3$), 46.19 (–NCH_2), 147.52 (triazole-C-3), 153.95 (triazole-C-5), 165.93 (exocyclic-C=O).

4.1.2.4. 4-Amino-3-benzyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl-acetic acid hydrazide ***(3d)***. Recrystallization from ethanol (yield: 78.29%), m.p. 196–196.5 °C. Analysis (Calc/found %): for $C_{11}H_{14}O_2N_6$ C: 50.37/50.27, H: 5.38/5.23, N: 32.05/32.15; IR (KBr) (ν, cm^{-1}), 3302–3173 (NH + $2NH_2$), 1714 (triazole –C=O), 1651 (hydrazide –C=O), 1548 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 3.87 (s, benzyl–CH_2), 4.21 (s, –NCH_2), 4.27 (s, –$NH\underline{NH_2}$), 5.29 (s, –NH_2), 7.10–7.32 (m, 5H, ar-H), 9.20 (s, –$\underline{NH}NH_2$); ^{13}C NMR (DMSO-d_6) δ (ppm) 30.21 (benzyl–$\underline{CH_2}$), 46.26 (–NCH_2), ar C: [126.50 (CH), 128.51 (2CH), 128.89 (2CH), 136.01 (C)], 147.00 (triazole-C-3), 153.46 (triazole-C-5), 166.09 (exocyclic-C=O).

*4.1.2.5. 4-Amino-3-*p*-chlorobenzyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl-acetic acid hydrazide* ***(3e)***. Recrystallization from ethanol (yield: 72.58%), m.p. 208–209 °C. Analysis (Calc/found %): for $C_{11}H_{13}O_2N_6Cl$ C: 44.52/44.97, H: 4.41/4.47, N: 28.32/28.20; IR (KBr) (ν, cm^{-1}), 3303–3167 (NH + $2NH_2$), 1726 (triazole –C=O), 1658 (hydrazide –C=O), 1569 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 3.87 (s, benzyl–CH_2), 4.22(s, –NCH_2), 4.33 (s, –$NH\underline{NH_2}$), 5.28 (s, –NH_2), 7.31 (d, J =8.0 Hz, 2H, ar-H), 7.38 (d, J =8.0 Hz, 2H, ar H,), 9.20 (s, –$\underline{NH}NH_2$); ^{13}C NMR (DMSO-d_6) δ (ppm) 29.60 (benzyl–$\underline{CH_2}$), 46.10 (–NCH_2), ar C: [128.19 (2CH), 130.58 (2CH), 131.10 (C), 134.74 (C)], 146.10 (triazole-C-3), 153.22 (triazole-C-5), 165.92 (exocyclic-C=O).

4.1.2.6. 4-Amino-3-phenyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl-acetic acid hydrazide ***(3f)***. Recrystallization from ethanol (yield: 72.58%), m.p. 202–203 °C. Analysis (Calc/found %): for $C_{10}H_{12}O_2N_6$ C: 48.38/48.94, H: 4.87/4.87, N: 33.85/33.10; IR (KBr) (ν, cm^{-1}), 3296–3245 (NH + $2NH_2$), 1704 (triazole –C=O), 1658 (hydrazide –C=O), 1542 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.37 (s, –NCH_2), 4.46 (s, –$NH\underline{NH_2}$), 5.52 (s, –NH_2), 7.40–7.58 (m, 3H, ar-H), 7.60–8.10 (m, 2H, ar H), 9.29 (s, –$\underline{NH}NH_2$); ^{13}C NMR (DMSO-d_6) δ (ppm) 46.61 (–NCH_2), ar C: [127.46 (C), 128.16 (2CH), 128.26 (2CH), 128.57 (CH)], 144.30 (triazole-C-3), 153.92 (triazole-C-5), 165.78 (exocyclic-C=O).

4.1.3. General method for the synthesis of compounds ***4*** *and* ***5***

A solution of the corresponding compound **3** (0.01 mol) in ethanol was refluxed with appropriate aldehyde (0.01 mol) for 3 h. After cooling to room temperature, a white solid appeared. This crude product was recrystallized from an appropriate solvent to afford the desired product.

4.1.3.1. 4-Amino-3-methyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl acetic acid benzylidene-hydrazide ***(4a)***. Recrystallization from dimethyl sulfoxide–water (1:1) (yield: 87.41%), m.p. 246–247 °C the ratio of *trans–cis*: 60/40. Analysis (Calc/found %): for $C_{12}H_{14}O_2N_6$ C: 52.54/52.81, H: 5.12/5.17, N: 30.64/30.19; IR (KBr) (ν, cm^{-1}), 3330–3225 (NH + NH_2), 1722 (triazole C=O), 1694 (hydrazide –C=O), 1594 (–C=N), 1410 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 2.23 (s, –CH_3), 4.92 (s, –NCH_2), 5.44 (s, –NH_2), 7.54–7.60 (m, 3H, ar-H), 7.70–7.85 (m, 2H, ar H), 8.10 (s, –N=CH), 11.76 and 11.80 (s, NH, *cis* and *trans* conformers); ^{13}C NMR (DMSO-d_6) δ (ppm) 10.55 (–CH_3), 46.14 and 46.17 (–NCH_2, *cis* and *trans* conformers); ar C: [126.82 (2CH), 127.13 (CH), 129.88 (2CH), 130.07 (C)], 144.16 (N=CH), 144.77 and 144.80 (triazole-C-3, *cis* and *trans* conformers), 154.41 and 153.43 (triazole-C-5, *cis* and *trans* conformers), 168.05 (exocyclic-C=O).

*4.1.3.2. 4-Amino-3-*p*-chlorobenzyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl acetic acid benzylidene-hydrazide* ***(4c)***. Recrystallization from dimethyl sulfoxide–water (1:1) (yield: 83.38%), m.p. 174–175 °C the ratio of *trans–cis*: 60/40. Analysis (Calc/found %): for $C_{14}H_{18}O_2N_6$ C: 55.61/55.81, H: 6.00/6.17, N: 27.80/27.59; IR (KBr) (ν, cm^{-1}), 3330–3219 (NH + NH_2), 1720 (triazole C=O), 1694 (hydrazide C=O), 1599 (C=N), 1443 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.17 (t, J = 6.0 Hz, –$CH_2CH_2\underline{CH_3}$), 1.65–1.72 (m, –$CH_2\underline{CH_2}CH_3$,), 2.44 (q, J = 6.0 Hz, –$\underline{CH_2}CH_2CH_3$), 4.94 (s, –NCH_2), 5.40 (s, –NH_2), 7.50–7.62 (m, 3H, ar-H), 7.71–7.78 (m, 2H, ar H), 8.10 (s, –N=CH), 11.70 and 11.78 (s, NH, *cis* and *trans* conformers); ^{13}C NMR (DMSO-d_6) δ (ppm) 13.67 (–$CH_2CH_2\underline{CH_3}$), 19.03 (–$CH_2\underline{CH_2}CH_3$), 26.21 (–$\underline{CH_2}CH_2CH_3$), 46.14 and 46.18 (–NCH_2, *cis* and *trans* conformers); ar C: [126.91 (2CH), 127.22 (CH), 129.79 (2CH), 130.13 (C)], 144.24 (–N=CH), 144.78 and 144.82 (triazole-C-3, *cis* and *trans* conformers), 154.46 and 153.48 (triazole-C-5, *cis* and *trans* conformers), 168.02 (exocyclic-C=O).

*4.1.3.3. 4-Amino-3-*p*-chlorobenzyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl acetic acid benzylidene-hydrazide* ***(4e)***. Recrystallization from dimethyl sulfoxide–water (1:1) (yield: 88.59%), m.p. 246–247 °C; the ratio of *trans–cis*: 60/40. Analysis (Calc/found %): for $C_{18}H_{17}O_2N_6Cl$ C:

56.18/56.41, H: 4.45/4.47, N: 21.83/21.69; IR (KBr) (ν, cm^{-1}), 3447–3204 (NH + NH_2), 1719 (triazole C=O), 1676 (hydrazide –C=O), 1575 (–C=N), 1489 (–C=N); ^1H NMR (DMSO-d_6) δ (ppm) 3.92 (s, benzyl–CH_2), 4.86 (s, –NCH_2), 5.38 (s, –NH_2), 7.20–7.35 (m, 5H, ar-H), 7.38 (d, J = 8.0 Hz, 2H, ar H), 7.42 (d, J = 8.0 Hz, 2H, ar H), 8.01 and 8.21 (s, –N=CH, *cis* and *trans* conformers), 11.68 and 11.72 (s, –NH, *cis* and *trans* conformers); ^{13}C NMR (DMSO-d_6) δ (ppm) 29.64 (benzyl–CH_2), 46.43 and 46.50 (–NCH_2, *cis* and *trans* conformer), ar C: [127.09 (CH), 128.49 (2CH), 128.96 (2CH), 130.15 (CH), 130.84 (2CH), 131.50 (C), 134.05 (C), 135.05 (C), 138.21 (CH)], 144.15 and 144.20 (–N=CH, *cis* and *trans* conformers), 146.41 and 146.85 (triazole-C-3, *cis* and *trans* conformers), 153.61 and 153.63 (triazole-C-5, *cis* and *trans* conformers), 168.02 (exocyclic-C=O).

4.1.3.4. 4-Amino-3-phenyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl acetic acid benzylidene-hydrazide ***(4f)***. Recrystallization from dimethyl sulfoxide–water (1:1) (yield: 85.59%), m.p. 263–264 °C; the ratio of *trans–cis*: 62/38. Analysis (Calc/found %): for $C_{17}H_{16}O_2N_6$ C: 60.70/60.43, H: 4.80/4.87, N: 24.99/24.64; IR (KBr) (ν, cm^{-1}), 3317–3109 (NH + NH_2), 1722 (triazole –C=O), 1692 (hydrazide –C=O), 1623 (–C=N), 1544 (–C=N); ^1H NMR (DMSO-d_6) δ (ppm) 5.00 (s, –NCH_2), 5.58 and 5.61 (s, –NH_2, *cis* and *trans* conformers), 7.40–7.55 (m, 5H, ar-H), 7.73 (bs, 2H, ar H), 8.05 (bs, 3H, ar H), 8.22 (s, –N=CH), 11.70 and 11.74 (s, –NH, *cis* and *trans* conformer); ^{13}C NMR (DMSO-d_6) δ (ppm) 46.72 and 46.80 (–NCH_2, *cis* and *trans* conformer), ar C: [126.58 (C), 126.92 (2CH), 127.11 (2CH), 127.46 (3CH), 128.35 (2CH), 128.78 (CH), 133.82 (C)], 144.16 (N=CH), 144.25 and 144.28 (triazol-C-3, *cis* and *trans* conformer), 153.03 and 153.05 (triazole-C-5, *cis* and *trans* conformer), 167.92 (exocyclic-C=O).

4.1.3.5. 4-Amino-3-methyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl acetic acid 2,4-dichloro-benzylidene-hydrazide ***(5a)***. Recrystallization from dimethyl sulfoxide–water (1:1) (yield: 74.39%), m.p. 251–252 °C; the ratio of *trans–cis*: 61/39. Analysis (Calc/found %): for $C_{12}H_{12}O_2N_6Cl_2$ C: 41.99/42.47, H: 3.52/3.63, N: 24.48/24.55; IR (KBr) (ν, cm^{-1}), 3382–3240 (NH + NH_2), 1717 (triazole C=O), 1691 (hydrazide –C=O), 1587 (–C=N), 1465 (–C=N); ^1H NMR (DMSO-d_6) δ (ppm) 2.33 (s, –CH_3), 4.85 (s, –NCH_2), 5.34 (s, –NH_2), 7.50 (d, J = 6.0 Hz, 1H, ar-H), 7.71 (s, 1H, ar H), 8.08 (d, J = 6.0 Hz, 1H, ar H), 8.55 (s, –N=CH), 11.90 and 11.95 (s, –NH, *cis* and *trans* conformers); ^{13}C NMR (DMSO-d_6) δ (ppm) 10.60 (–CH_3), 46.19 and 46.25 (–NCH_2, *cis* and *trans* conformers), ar C: [128.16 (CH), 129.60 (CH), 130.19 (C), 133.57 (C), 134.19 (C), 139.50 (CH)], 144.62 (–N=CH), 144.89 and 146.94 (triazole-C-3, *cis* and *trans* conformers), 153.80 and 155.84 (triazole-C-5 *cis* and *trans* conformers), 168.32 (exocyclic-C=O); (EI MS, 70 eV) *m/z* (%) 343 (M^+,6), 341 (13), 339 (25), 325 (21), 255 (19), 111 (46), 96 (49), 95 (70), 80 (100), 64 (38), 62 (56).

*4.1.3.6. 4-Amino-3-*p*-chlorobenzyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl acetic acid 2,4-dichloro-benzylidenehydrazide* ***(5e)***. Recrystallization from dimethyl sulfoxide–water (1:1) (yield: 80.26%), m.p. 266–267 °C; the ratio of *trans–cis*: 61/39. Analysis (Calc/found %): for $C_{18}H_{15}O_2N_6Cl_3$ C: 47.65/48.10, H: 3.33/3.57, N: 18.52/18.47; IR (KBr) (ν, cm^{-1}), 3342–3325 (NH + NH_2), 1702 (triazole –C=O), 1699 (hydrazide –C=O), 1587 (–C=N), 1489 (–C=N); ^1H NMR (DMSO-d_6) δ (ppm) 3.95 (s, benzyl–CH_2), 4.95 (s, –NCH_2), 5.40 (s, –NH_2), 7.30 (d, J = 6.0 Hz, 2H, ar-H), 7.38 (s, 2H, ar H), 7.68 (bs, 1H, ar H), 8.10 (bs, 1H, ar H), 8.38 (bs, 1H, ar H), 8.55 (s, –N=CH), 11.90 and 11.95 (s, –NH, *cis* and *trans* conformer), ^{13}C NMR (DMSO-d_6) δ (ppm) 29.66 (benzyl–CH_2), 46.50 and 46.55 (–NCH_2, *cis* and *trans* conformer), ar C: [127.87 (CH), 128.27 (3CH), 129.26 (CH), 130.19 (C), 130.62 (2CH), 131.28 (C), 133.60 (C), 134.81 (C), 134.94 (C)], 138.94 (–N=CH), 146.57 and 146.83 (triazole-C-3, *cis* and *trans* conformers), 153.61 and 163.22 (triazole-C-5, *cis* and *trans* conformers), 168.22 and 168.35 (exocyclic-C=O, *cis* and *trans* conformers).

4.1.3.7. 4-Amino-5-oxo-3-phenyl-4,5-dihydro-[1,2,4]triazol-1-yl acetic acid 2,4-dichloro-benzylidene-hydrazide ***(5f)***. Recrystallization from dimethyl sulfoxide–water (1:1) (yield: 80.38%), m.p. 272–273 °C; the ratio of *trans–cis*: 60/40. Analysis (Calc/found %): for $C_{17}H_{14}O_2N_6Cl_2$ C: 50.39/50.61, H: 3.48/3.57, N: 20.74/20.47; IR (KBr) (ν, cm^{-1}), 3343–3319 (NH + NH_2), 1701 (triazole –C=O), 1696 (hydrazide –C=O), 1587 (–C=N), 1492 (–C=N); ^1H NMR (DMSO-d_6) δ (ppm) 4.98 (s, –NCH_2), 5.34 (s, –NH_2), 7.39 (m, 3H, ar-H), 7.46 (m, 2H, ar H), 7.62 (bs, 1H, ar H), 8.21 (bs, 1H, ar H), 8.41 (bs, 1H, ar H), 8.58 (s, –N=CH), 11.92 and 11.97 (s, –NH, *cis* and *trans* conformer), ^{13}C NMR (DMSO-d_6) δ (ppm) 46.51 and 46.57 (–NCH_2, *cis* and *trans* conformer), ar C: [127.94 (CH), 128.36 (3CH), 129.63 (CH), 130.38 (C), 130.64 (2CH), 131.28 (C), 133.66 (C), 134.82 (C), 134.94 (C)], 138.94 (–N=CH), 146.57 and 146.80 (triazole-C-3, *cis* and *trans* conformers), 154.21 and 163.25 (triazole-C-5, *cis* and *trans* conformers), 168.22 and 168.30 (exocyclic-C=O, *cis* and *trans* conformers).

4.1.4. General method for the synthesis of thiosemicarbazides ***(6)***

A mixture of corresponding acid hydrazide **(3)** (0.01 mol) and phenyl isothiocyanate (0.015 mol) was refluxed in ethanol for 2 h. The solution was cooled and a white solid appeared. This was filtered and recrystallized from an appropriate solvent to afford the desired product.

4.1.4.1. 1-(4-Amino-3-methyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl)acetyl-4-phenyl thio-semicarbazide ***(6a)***. Recrystallization from ethanol (yield: 82.56%), m.p. 179–180 °C. Analysis (Calc/found %): for $C_{12}H_{15}O_2N_7S$ C: 44.85/44.50, H: 4.70/4.77, N: 30.51/30.57; IR (KBr) (ν, cm^{-1}), 3375–3298 (3NH + NH_2), 1717 (triazole –C=O), 1696 (exocyclic-C=O),

1551 (–C=N), 1309 (–C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 2.10 (s, –CH_3), 4.40 (s, –NCH_2), 5.31 (s, –NH_2), 7.18–7.21 (m, 1H, ar-H), 7.31–7.44 (m, 4H, ar H), 9.66 (s, –NH), 9.75 (s, –NH), 10.30 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 10.57 (–CH_3), 46.44 (–NCH_2), ar C: [125.25 (CH), 128.04 (4CH), 138.37 (C)], 145.15 (triazole-C-3), 153.30 (triazole-C-5), 166.56 (exocyclic-C=O), 180.55 (–C=S).

*4.1.4.2. 1-(3-*n*-Propyl-4-amino-4,5-dihydro-1H-1,2,4-triazol-5-on-1yl)acetyl-4-phenyl thio-semicarbazide* ***(6c)***. Recrystallization from isobutyl acetate (yield: 66.48%), m.p. 140–141 °C. Analysis (Calc/found %): for $C_{14}H_{19}O_2N_7S$ C: 48.12/48.19, H: 5.48/5.91, N: 28.06/28.00; IR (KBr) (ν, cm^{-1}), 3389–3310 (3NH + NH_2), 1717 (triazole –C=O), 1696 (exocyclic-C=O), 1549 (–C=N), 1309 (–C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.08 (t, J = 6.0 Hz, –$CH_2CH_2\underline{CH_3}$), 1.62–1.71 (m, –$CH_2\underline{CH_2}CH_3$,), 2.42 (q, J = 6.0 Hz, –$\underline{CH_2}CH_2CH_3$), 4.41 (s, –NCH_2), 5.30 (s, –NH_2), 7.19–7.24 (m, 2H, ar-H), 7.30–7.42 (m, 3H, ar H), 9.66 (s, –NH), 9.72 (s, –NH), 10.38 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 13.49 (–$CH_2CH_2\underline{CH_3}$), 18.78 (–$CH_2\underline{CH_2}CH_3$), 26.57 (–$\underline{CH_2}CH_2CH_3$), 46.44 (–NCH_2), ar C: [125.20 (2CH), 128.94 (2CH), 129.10 (CH), 137.97 (C)], 145.15 (triazole-C-3), 153.33 (triazole-C-5), 167.05 (exocyclic-C=O), 180.37 (–C=S).

4.1.4.3. 1-(4-Amino-3-benzyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl)acetyl-4-phenyl thio-semicarbazide ***(6d)***. Recrystallization from ethanol (yield: 68.14%), m.p. 110–111 °C. Analysis (Calc/found %): for $C_{18}H_{19}O_2N_7S$ C: 54.39/55.06, H: 4.81/4.97, N: 24.66/24.45; IR (KBr) (ν, cm^{-1}), 3389–3308 (3NH + NH_2), 1715 (triazole –C=O), 1695 (exocyclic-C=O), 1557 (–C=N), 1315 (–C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 3.84 (s, benzyl–CH_2), 4.41 (s, –NCH_2), 5.36 (s, –NH_2), 7.10–7.28 (m, 5H, ar-H), 7.32–7.40 (m, 3H, ar H), 7.49–7.54 (m, 2H, ar-H), 9.46 (s, –NH), 9.95 (s, –NH), 10.76 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 30.47 (benzyl–CH_2), 46.48 (–NCH_2), ar C: [125.35 (2CH), 126.58 (2CH), 126.94 (CH), 128.79 (CH), 128.94 (2CH), 129.10 (2CH), 133.25 (C), 137.97 (C)], 147.15 (triazole-C-3), 153.33 (triazole-C-5), 166.75 (exocyclic-C=O), 180.12 (–C=S).

4.1.4.4. 1-(4-Amino-3-phenyl-5-oxo-4,5-dihydro-[1,2,4]triazol-1-yl)acetyl-4-phenyl thio-sem-carbazide ***(6f)***. Recrystallization from ethanol (yield: 76.54%), m.p. 215–216 °C. Analysis (Calc/found %): for $C_{17}H_{17}O_2N_7S$ C: 53.25/53.92, H: 4.46/4.69, N: 25.57/25.39; IR (KBr) (ν, cm^{-1}), 3295–3208 (3NH + NH_2), 1705 (triazole –C=O), 1680 (exocyclic-C=O), 1548 (–C=N), 1341(–C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.34 (s, –NCH_2), 5.35 (s, –NH_2), 7.42–7.58 (m, 5H, ar-H), 7.72 (bs, 3H, ar H), 8.02–8.18 (bs, 2H, ar-H), 9.38 (s, –NH), 9.96 (s, –NH), 11.26 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 46.78 (–NCH_2), ar C: [126.58 (C), 126.94 (2CH), 127.13 (2CH), 127.61 (3CH), 128.35 (2CH), 128.74 (CH), 133.85 (C)], 148.07 (triazole-C-3), 154.39 (triazole-C-5), 167.45 (exocyclic-C=O), 181.03 (–C=S).

4.1.5. General method for the synthesis of compounds ***7***

A mixture of the corresponding thiosemicarbazide **(6)** (0.01 mol) in cold concentrated sulfuric acid (28 ml) was stirred for 10 min. Then, the mixture was allowed to room temperature. After stirring for an additional 30 min, the resulting solution was poured into ice-cold water and made alkaline to pH 8 with ammonia. The precipitated product was filtered and recrystallized from an appropriate solvent to afford the desired product.

4.1.5.1. 1-(5-Phenylamino-1,3,4-thiadiazol-2-yl)methyl-4-amino-3-methyl-5-oxo-4,5-dihydro-[1,2,4]triazole ***(7a)***. Recrystallization from ethanol (yield: 78.34%), m.p. 227–228 °C. Analysis (Calc/found %): $C_{12}H_{13}ON_7S$ C: 47.51/48.03.92, H: 4.31/4.29, N: 32.32/32.19; IR (KBr) (ν, cm^{-1}), 3316–3140 (NH + NH_2), 1699(–C=O), 1573 (–C=N), 1498 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 2.12 (s, –CH_3), 5.11 (s, –NCH_2), 5.34 (s, –NH_2), 7.00 (d, J = 8 Hz, 2H, ar-H), 7.30 (t, J = 6.4 Hz, 1H, ar H), 7.60 (t, J_1= 8 Hz, J_2= 6.4 Hz, 2H, ar H), 10.37 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 10.56 (–CH_3), 43.60 (–NCH_2), ar C: [117.29 (2CH), 121.86 (CH), 129.00 (2CH), 140.34 (C)], 145.88 (triazole-C-3), 152.49 (triazole-C-5), 154.46 (thiadiazol-C-2), 165.27 (thiadiazol-C-5).

*4.1.5.2. 1-(5-Phenylamino-1,3,4-thiadiazol-2-yl)methyl-4-amino-5-oxo-3-*n*-propyl-4,5-dihydro-[1,2,4]triazole* ***(7c)***. Recrystallization from ethanol (yield: 86.63%), m.p. 165–166 °C. Analysis (Calc/found %): $C_{14}H_{17}ON_7S$ C: 50.74/50.18, H: 5.17/5.35, N: 29.58/28.81; IR (KBr) (ν, cm^{-1}), 3316–3125 (NH + NH_2), 1695(–C=O), 1581 (–C=N), 1496 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 1.06 (t, J = 6.0 Hz, –$CH_2CH_2\underline{CH_3}$), 1.61–1.70 (m, J = 6.0 Hz, –$CH_2\underline{CH_2}CH_3$), 2.44 (t, J = 6.0 Hz, –$\underline{CH_2}CH_2CH_3$), 4.92 (s, –NCH_2), 5.36 (s, –NH_2), 7.11 (bs, 2H, ar-H), 7.26 (bs, 3H, ar H), 10.68 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 13.92 (–$CH_2CH_2\underline{CH_3}$), 19.04 (–$CH_2\underline{CH_2}CH_3$), 31.07 (–$\underline{CH_2}CH_2CH_3$), 45.13 (–NCH_2), ar C: [121.01 (2CH), 121.75 (CH), 128.13 (2CH), 133.35 (C)], 146.11 (triazole-C-3), 152.87 (triazole-C-5), 154.11 (thiadizole-C-2), 165.19 (thiadizole-C-5).

4.1.5.3. 1-(5-Phenylamino-1,3,4-thiadiazol-2-yl)methyl-4-amino-3-benzyl-5-oxo-4,5-dihydro-[1,2,4]triazole ***(7d)***. Recrystallization from ethanol (yield: 80.25%), m.p. 155–186 °C. Analysis (Calc/found %): $C_{18}H_{17}N_7S$ C: 56.97/57.44, H: 4.51/4.79, N: 25.84/26.17; IR (KBr) (ν, cm^{-1}), 3286–3200 (NH + NH_2), 1712(–C=O), 1599 (–C=N), 1550 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 3.81 (s, benzyl–CH_2), 5.05 (s, –NCH_2), 5.29 (s, –NH_2), 6.91 (t, J = 6.0 Hz, 1H, ar H), 7.19–7.30 (m, 7H, ar H), 7.49 (d, J = 8.0 Hz, 2H, ar-H), 10.29 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm), 30 21 (benzyl–CH_2), 43.81 (–NCH_2), ar C: [117.32 (2CH), 121.91 (CH), 126.58 (CH), 128.34 (2CH), 128.64 (2CH), 129.02 (2CH), 135.59 (C), 140.37 (C)], 147.80 (triazole-C-3), 152.62 (triazole-C-5), 154.44 (thiadizole-C-2), 165.31 (thiadizole-C-5).

*4.1.5.4. 1-(5-Phenylamino-1,3,4-thiadiazol-2-yl)methyl-4-amino-3-phenyl-5-oxo-4,5-dihydro-[1,2,4]triazole **(7f)***. Recrystallization from ethanol–water (1:1) (yield: 87.52%), m.p. 222–223 °C. Analysis (Calc/found %): $C_{17}H_{15}ON_7S$ C: 55.87/56.05, H: 4.13/4.19, N: 26.83/26.76; IR (KBr) (*ν*, cm^{-1}), 3318–3266 (NH + NH_2), 1713(–C=O), 1556 (–C=N), 1508 (–C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 5.28 (s, –NCH_2), 5.60 (s, –NH_2), 7.01 (t, *J* = 6.0 Hz, 1H, ar H), 7.33 (t, *J* = 8.0 Hz, 2H, ar H), 7.51–7.60 (m, 5H, ar-H), 7.89 (bs, 2H, ar H), 10.38 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm), 44.01 (–NCH_2), ar C: [117.30 (2CH), 120.88 (CH), 126.21 (C),127.53 (2CH), 128.28 (2CH), 128.99 (2CH), 130.06 (CH), 140.31 (C)], 144.40 (triazole-C-3), 153.00 (triazole-C-5), 154.13 (thiadizole-C-2), 165.33 (thiadizole-C-5); (EI MS, 70 eV) *m/z* (%) 365 (M^+,56), 359 (25), 290 (18), 266.61 (28), 250 (18), 249 (100), 190 (44), 177 (34), 100 (44), 90 (44), 79 (48), 74 (38), 61 (20), 58.29 (80).

*4.1.6. General method for the synthesis of compounds **8***

4.1.6.1. Method A. A solution of corresponding thiosemicarbazide **(6)** (0.01 mol) in 2 N NaOH were refluxed for 3 h. The resulting solution was cooled to room temperature and acidified to pH 3–4 with 37% HCl. The precipitate formed was filtered, washed with water and recrystallized from an appropriate solvent to afford the desired compound.

4.1.6.2. Method B. The corresponding thiosemicarbazide **(6)** was heated in an oil bath at 130 °C for 2 h. The white solid formed recrystallized from an appropriate solvent to afford the compounds **8**.

*4.1.6.3. Method C (only for **8f**).* A mixture of corresponding acid hydrazide **(3f)** (0.01 mol) and phenyl isothiocyanate (0.015 mol) in ethanol was refluxed for 8 h. The solution was cooled and a white solid appeared. This was filtered and recrystallized from dimethyl sulfoxide–water (1:1) to afford the desired product. (Yield: 72.68%).

*4.1.6.1. 1-(4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-4-amino-3-methyl-5-oxo-4,5-dihydro-[1,2,4]triazole **(8a)***. Recrystallization from dimethyl sulfoxide (yield: 76.39%), m.p. 240–241 °C. Analysis (Calc/found %): $C_{12}H_{13}ON_7S$ C: 47.51/48.03.92, H: 4.31/4.29, N: 32.32/32.19; IR (KBr) (*ν*, cm^{-1}), 3317–3223 (NH + NH_2), 1698 (–C=O), 1602 (–C=N), 1573 (–C=N), 1343 (–C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 2.00 (s, –CH_3), 4.78 (s, –NCH_2), 5.05 (s, –NH_2), 7.20–7.30 (m, 2H, ar-H), 7.40–7.50 (m, 3H, ar H), 13.95 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 10.36 (–CH_3), 46.78 (–NCH_2), ar C: [127.51 (2CH), 129.06 (2CH), 129.38 (CH), 132.71 (C)], 145.49 (5-thioxo-triazole-C-2), 147.68 (triazole-C-3), 152.11 (triazole-C-5), 168.20 (5-thioxo-triazole-C-5); (EI MS, 70 eV) *m/z* (%) 303 (M^+14), 249 (100), 205 (66), 102 (20), 101 (37), 79 (41), 60 (22).

*4.1.6.2. 1-(4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-4-amino-5-oxo-3-*n*-propyl-4,5-dihydro-[1,2,4]triazole **(8c)***. Recrystallization from ethanol–ethyl acetate (1:1) (yield: 78.20%), m.p. 215–216 °C. Analysis (Calc/found %): $C_{14}H_{17}ON_7S$ C: 50.74/50.56, H: 5.17/5.48, N: 29.58/29.32; IR (KBr) (*ν*, cm^{-1}), 3304–3117 (NH + NH_2), 1695(–C=O), 1573 (–C=N), 1491 (–C=N), 1362 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 0.93 (t, *J* = 5.9 Hz, –$CH_2CH_2\underline{CH_3}$), 1.53 (hex, *J* = 5.9 Hz, –$CH_2\underline{CH_2}CH_3$), 2.37 (t, *J* = 5.9 Hz, –$\underline{CH_2}CH_2CH_3$), 4.83 (s, –NCH_2), 5.07 (s, –NH_2), 7.47 (bs, 2H, ar-H), 7.51 (bs, 3H, ar H), 12.97 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 17.16 (–$CH_2CH_2\underline{CH_3}$), 22.53 (–$CH_2\underline{CH_2}CH_3$), 29.81 (–$\underline{CH_2}CH_2CH_3$), 44.03 (–NCH_2), ar C: [131.22 (2CH), 132.80 (2CH), 133.12 (CH), 136.48 (C)], 151.37 (triazole-C-3), 151.87 (5-thioxo-triazole-C-3), 156.03 (triazol-C-5), 171.96 (5-thioxo-triazol-C-5); (EI MS, 70 eV) *m/z* (%) 331 (M^+ 56), 254 (17), 249 (19), 215 (14), 176 (13), 143 (14), 79 (100), 64 (19), 63 (16).

*4.1.6.3. 1-(4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-4-amino-3-benzyl5-oxo-4,5-dihydro-[1,2,4]triazole **(8d)***. Recrystallization from dimethyl sulfoxide–water (1:1) (yield: 81.12%), m.p. 294–295 °C. Analysis (Calc/found %): $C_{18}H_{17}ON_7S$ C: 56.97/57.28, H: 4.51/4.83, N: 25.84/26.03; IR (KBr) (*ν*, cm^{-1}), 3357–3173 (NH + NH_2), 1694(–C=O), 1577 (–C=N), 1473 (–C=N), 1384 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 3.78 (s, benzyl–CH_2), 4.81 (s, –NCH_2), 5.10 (s, –NH_2), 7.23–7.29 (m, 4H, ar-H), 7.39–7.43 (m, 6H, ar H), 13.95 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 30.09 (benzyl–CH_2), 40.36 (–NCH_2), ar C: [126.36 (CH), 127.54 (2CH), 128.37 (2CH), 128.73 (2CH), 129.09 (2CH), 129.47 (CH), 132.71 (C), 135.61 (C)], 147.46 (triazole-C-3), 147.59 (5-thioxo-triazole-C-3), 152.29 (triazol-C-5), 168.29 (5-thioxo-triazol-C-5); (EI MS, 70 eV) *m/z* (%) 379 (M^+ 38), 249 (100), 246 (14), 228 (14), 135 (24), 108 (31), 100 (34), 88 (35), 86 (53), 79 (44), 63 (30).

*4.1.6.4 1-. (4-phenyl-5-thioxo-[1,2,4]triazol-3-yl)methyl-4-amino-5-oxo-3-phenyl-4,5-dihydro-[1,2,4]triazole **(8f)***. Recrystallization from dimethyl sulfoxide–water (1:1) (yield: 81.12%), m.p. 292–293 °C. Analysis (Calc/found %): $C_{17}H_{15}ON_7S$ C: 55.87/55.89, H: 4.13/4.29, N: 26.83/26.39; IR (KBr) (*ν*, cm^{-1}), 3344–3145 (NH + NH_2), 1721(–C=O), 1587 (–C=N), 1497 (–C=N), 1320 (C=S); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.97 (s, –NCH_2), 5.34 (s, –NH_2), 7.34 (bs, 2H, ar-H), 7.48 (bs, 6H, ar H), 7.83 (bs, 2H, ar H), 14.00 (s, –NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 40.77 (–NCH_2), ar C: [126. (C), 127.56 (4CH), 128.25 (2CH), 129.15 (2CH), 129.47 (CH), 129.99 (CH), 132.80 (C)], 145.07 (triazole-C-3), 147.51 (5-thioxo-triazole-C-3), 152.61 (triazole-C-5), 168.28 (5-thioxo-triazole-C-5); (EI MS, 70 eV) *m/z* (%) 365 (M^+ 39), 249 (21), 177 (13), 79 (38), 64 (80), 63 (100).

4.2. Biological activity studies

4.2.1. Antimicrobial activity

All test microorganisms except for *Penicillium* spp. and *Aspergillus* spp. were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and are as follows; *E. coli* ATCC 35218, *P. aeruginosa* ATCC 10145, *Yersinia pseudotuberculosis* ATCC 911, *K. pneumoniae* ATCC 13883, *Enterococcus faecalis* ATCC 29212, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *C. albicans* ATCC 60193, *C. tropicalis* ATCC 13803, *Candida glabrata. Penicillum* spp. and *Aspergillus* spp. were isolated from soil. All the newly synthesized compounds except **4c** and **5f** were weighed and dissolved in dimethylsulphoxide (DMSO) and 95% ethanol to prepare extract stock solution of 1 mg/ml.

4.2.1.1. Agar well diffusion method. Simple susceptibility screening test using agar-well diffusion method [31] as adapted earlier [32] was used. Each microorganism was suspended in Brain Heart Infusion (BHI) (Difco, Detroit, MI) broth and diluted to 10^6 colony forming unit (cfu) per ml. They were "flood-inoculated" onto the surface of BHI agar and Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) and then dried. For *C. albicans, C. tropicalis, C. glabrata, Penicillum* spp. and *Aspergillus* spp., SDA were used. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 250–5000 μg/50 μl of the chemical substances were delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ceftazidime (Fortum) (10 μg) and Triflucan (5 μg) were standard drugs. Ethanol and dimethylsulphoxide were used as solved control. Results were interpreted in terms of the diameter of the inhibition zone: (–): <5.5 mm; (+): 5.5–10 mm; (++): 11–16 mm; (+++): ≥17 mm. The results are shown in Table 1.

4.2.1.2. Mycelial growth inhibition test. Mycelial growth inhibition test were carried out by the agar diffusion method [32–34]. Five-millimeter mycelial agar disc were placed on PDA plates containing test compounds. Final concentration in the medium was adjusted to 1000 μg/ml. The plates were incubated at 25 °C for 3 days and diameters of the mycelium colonies were then measured to examine the effects of the chemicals on fungal growth. The results are shown in Table 1.

4.2.2. Antitumor screening studies

The screening experiments were performed by the Developmental Therapeutic Program of the National Cancer Institute (NCI), Bethesda MD, USA. Among Shiff Bases obtained in this study, compounds **4c** and **5f** were selected by the NCI for screening towards three human tumor cell line, breast Cancer (MCF7), non-small cell lung cancer (NCI-H460) and CNS (SF-268). Each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration and culture incubated for 48 h. End-point determinations were made with alamar blue. The screening results are summarized in Table 3. Results for each test agents are reported as the percent of growth of the treated cells when compared to the untreated control cells. Compounds (**4c** and **5f**) which reduce the growth any one of the cell lines to approximately 32% or less are passed on for evaluation in the full panel of 60 cell lines derived from human solid tumors (brain, breast, colon, leukemia, lung, melanoma, ovarian and renal) over a 5-log dose range. Compound **4c** and **5f** was tested at a minimum of five concentrations at 10-fold dilutions. A 48 h continuous drug exposure protocol was used and a sulforhodamin B (SRB) protein assay was used to estimate cell viability or growth. The screening results of compounds **4c** and **5f** towards several tumor cell line are presented in Table 4 as GI_{50} values. As seen in Table 3, compounds **4c** and **5f** are effective towards breast cancer (MCF7).

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