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Original article

Synthesis and biological evaluation of thiazoline derivatives as new antimicrobial and anticancer agents

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ABSTRACT

N′-(3,4-Diarylthiazol-2(3*H*)-ylidene)-2-(arylthio)acetohydrazides were synthesized and evaluated for their antimicrobial activity and cytotoxicity against NIH/3T3 cells. Compound **22** bearing 1-phenyl-1*H*-tetrazole and *p*-chlorophenyl moieties was found to be the most promising antibacterial agent against *Pseudomonas aeruginosa*, whereas compound **23** bearing 1-phenyl-1*H*-tetrazole and *p*-bromophenyl moieties was the most promising antifungal agent against *Candida albicans*. The most effective derivatives were also evaluated for their cytotoxicity against C6 glioma cells. The results indicated that compound **17** bearing 1-phenyl-1*H*-tetrazole and nonsubstituted phenyl moieties (IC_{50} = 8.3 ± 2.6 μg/mL) was more effective than cisplatin (IC_{50} = 13.7 ± 1.2 μg/mL) against C6 glioma cells. Compound **17** also exhibited DNA synthesis inhibitory activity on C6 cells. Furthermore, compound **17** showed low toxicity to NIH/3T3 cells (IC_{50} = 416.7 ± 28.9 μg/mL).

1. Introduction

Infectious diseases caused by bacteria and fungi have emerged as important causes of morbidity and mortality worldwide due to their ability to thwart therapeutic regimens by rapidly evolving resistance to antimicrobial agents [1–6].

Eukaryotic pathogens such as fungi pose a particular therapeutic challenge since they share a close evolutionary relationship with their human hosts, limiting the number of drug targets that can be exploited to selectively kill the pathogen [6]. The treatment of fungal infections, particularly those caused by drug-resistant fungal pathogens is often complicated by high toxicity, low tolerability, or narrow spectrum of activity [3–7].

In the last few decades, the greatly increased incidence of life-threatening bacterial and fungal infections has resulted in a corresponding increase in demand for new effective antimicrobial agents which inhibit the growth of pathogens or kill them and have no or least toxicity to host cells [1–8].

Thiazoles are found in many biologically active compounds, including natural products and pharmaceutical agents [9,10]. The reduced forms of thiazoles, thiazolines, have attracted a great deal of interest as privileged scaffolds due to their synthetic and biological importance. Compounds bearing thiazoline moiety have been reported to exhibit a wide spectrum of biological effects including antimicrobial activity [11–18]. In addition, hydrazides–hydrazones have received considerable attention as important pharmacophores in medicinal chemistry [19,20]. Isoniazid, which possesses a hydrazide moiety in its molecular structure, is the frontline drug employed in the treatment of tuberculosis [21]. Nifuroxazide, which is a nitrofuran antibacterial agent bearing a hydrazone moiety, is widely used as an intestinal antiseptic. Many studies have also confirmed that hydrazone derivatives of isoniazid and other hydrazides exhibit significant antimicrobial activity [19–26].

1-Substituted-1*H*-tetrazole-5-thiol and 5-methyl-1,3,4-thiadiazole-2-thiol have gained great importance in the synthesis of pharmacologically active drugs. Some synthetic β-lactam antibiotics possess 5-thio-1-methyl-1*H*-tetrazole (MTT) or thio-linked thiadiazole as the side chain [10,27].

Triazole antifungal agents, play a leading role in the treatment of systemic fungal infections due to their broad spectrum and improved safety profile. Fluconazole, itraconazole, voriconazole, and posaconazole are widely used antifungal drugs bearing triazole ring for the treatment of systemic fungal infections [28,29].

Medicinal chemists have also carried out considerable research for novel antimicrobial agents bearing a pyrimidine moiety.

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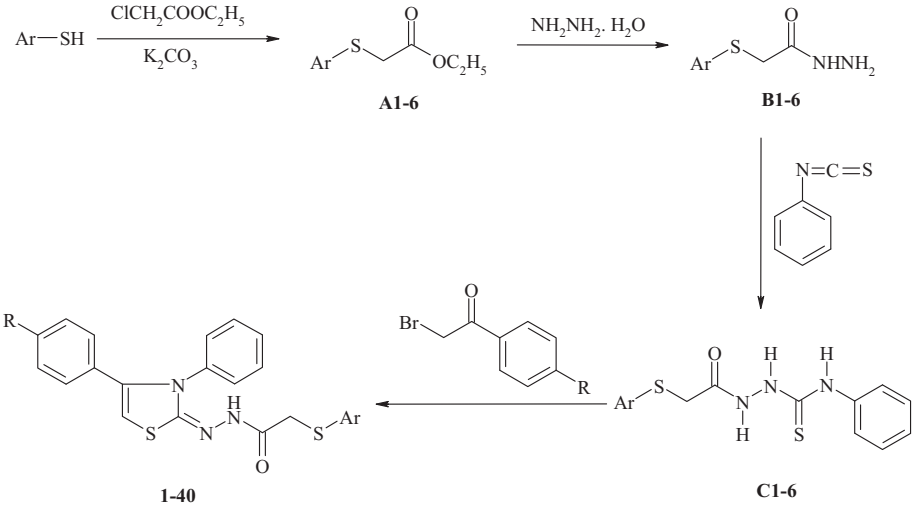

Scheme 1. The synthetic route for the preparation of the target compounds (**1**–**40**).

Trimethoprim and sulfadiazine are chemotherapeutic drugs containing a pyrimidine moiety currently used in the treatment of infectious diseases [10,30].

On the basis of these findings, herein we reported the synthesis of a series of thiazoline derivatives bearing a hydrazone moiety and focused on their potential antimicrobial effects and cytotoxicity. Thio-linked triazole, tetrazole, thiadiazole and pyrimidine were preferred as the side chain while designing the molecules. Furthermore, all compounds were evaluated for their cytotoxic effects against NIH/3T3 cell lines. The most effective antimicrobial derivatives were also

Table 1
Some properties of the compounds (**1**–**40**).

Compound	R	Ar	Yield (%)	M.p. (°C)	Molecular formula	Molecular weight
1	H	4-Methyl-4*H*-1,2,4-triazol-3-yl	70	123–126	$C_{20}H_{18}N_6OS_2$	422
2	NO_2	4-Methyl-4*H*-1,2,4-triazol-3-yl	80	100–103	$C_{20}H_{17}N_7O_3S_2$	467
3	CH_3	4-Methyl-4*H*-1,2,4-triazol-3-yl	70	109–112	$C_{21}H_{20}N_6OS_2$	436
4	OCH_3	4-Methyl-4*H*-1,2,4-triazol-3-yl	71	148–151	$C_{21}H_{20}N_6O_2S_2$	452
5	F	4-Methyl-4*H*-1,2,4-triazol-3-yl	72	167–169	$C_{20}H_{17}FN_6OS_2$	440
6	Cl	4-Methyl-4*H*-1,2,4-triazol-3-yl	75	260–262	$C_{20}H_{17}ClN_6OS_2$	456
7	Br	4-Methyl-4*H*-1,2,4-triazol-3-yl	76	260–263	$C_{20}H_{17}BrN_6OS_2$	501
8	CN	4-Methyl-4*H*-1,2,4-triazol-3-yl	81	246–247	$C_{21}H_{17}N_7OS_2$	447
9	H	1-Methyl-1*H*-tetrazol-5-yl	75	202–206	$C_{19}H_{17}N_7OS_2$	423
10	NO_2	1-Methyl-1*H*-tetrazol-5-yl	80	78–80	$C_{19}H_{16}N_8O_3S_2$	468
11	CH_3	1-Methyl-1*H*-tetrazol-5-yl	74	224–225	$C_{20}H_{19}N_7OS_2$	437
12	OCH_3	1-Methyl-1*H*-tetrazol-5-yl	72	124–125	$C_{20}H_{19}N_7O_2S_2$	453
13	F	1-Methyl-1*H*-tetrazol-5-yl	75	218–219	$C_{19}H_{16}FN_7OS_2$	441
14	Cl	1-Methyl-1*H*-tetrazol-5-yl	77	223–227	$C_{19}H_{16}ClN_7OS_2$	457
15	Br	1-Methyl-1*H*-tetrazol-5-yl	78	229–230	$C_{19}H_{16}BrN_7OS_2$	502
16	CN	1-Methyl-1*H*-tetrazol-5-yl	81	244–245	$C_{20}H_{16}N_8OS_2$	448
17	H	1-Phenyl-1*H*-tetrazol-5-yl	78	71–74	$C_{24}H_{19}N_7OS_2$	485
18	NO_2	1-Phenyl-1*H*-tetrazol-5-yl	85	235–237	$C_{24}H_{18}N_8O_3S_2$	530
19	CH_3	1-Phenyl-1*H*-tetrazol-5-yl	75	232–233	$C_{25}H_{21}N_7OS_2$	499
20	OCH_3	1-Phenyl-1*H*-tetrazol-5-yl	73	230–231	$C_{25}H_{21}N_7O_2S_2$	515
21	F	1-Phenyl-1*H*-tetrazol-5-yl	75	237	$C_{24}H_{18}FN_7OS_2$	503
22	Cl	1-Phenyl-1*H*-tetrazol-5-yl	83	246–248	$C_{24}H_{18}ClN_7OS_2$	519
23	Br	1-Phenyl-1*H*-tetrazol-5-yl	84	242–243	$C_{24}H_{18}BrN_7OS_2$	564
24	CN	1-Phenyl-1*H*-tetrazol-5-yl	86	233–234	$C_{25}H_{18}N_8OS_2$	510
25	H	5-Methyl-1,3,4-thiadiazol-2-yl	79	156–160	$C_{20}H_{17}N_5OS_3$	439
26	NO_2	5-Methyl-1,3,4-thiadiazol-2-yl	88	250–252	$C_{20}H_{16}N_6O_3S_3$	484
27	CH_3	5-Methyl-1,3,4-thiadiazol-2-yl	76	240–241	$C_{21}H_{19}N_5OS_3$	453
28	OCH_3	5-Methyl-1,3,4-thiadiazol-2-yl	75	248–249	$C_{21}H_{19}N_5O_2S_3$	469
29	F	5-Methyl-1,3,4-thiadiazol-2-yl	78	228–231	$C_{20}H_{16}FN_5OS_3$	457
30	Cl	5-Methyl-1,3,4-thiadiazol-2-yl	85	244–245	$C_{20}H_{16}ClN_5OS_3$	473
31	Br	5-Methyl-1,3,4-thiadiazol-2-yl	86	237–239	$C_{20}H_{16}BrN_5OS_3$	518
32	CN	5-Methyl-1,3,4-thiadiazol-2-yl	89	254–255	$C_{21}H_{16}N_6OS_3$	464
33	H	Pyrimidin-2-yl	80	77–78	$C_{21}H_{17}N_5OS_2$	419
34	NO_2	Pyrimidin-2-yl	89	260–263	$C_{21}H_{16}N_6O_3S_2$	464
35	CH_3	Pyrimidin-2-yl	77	84–87	$C_{22}H_{19}N_5OS_2$	433
36	OCH_3	Pyrimidin-2-yl	75	218–219	$C_{22}H_{19}N_5O_2S_2$	449
37	F	Pyrimidin-2-yl	79	241–242	$C_{21}H_{16}FN_5OS_2$	437
38	Cl	Pyrimidin-2-yl	87	247–248	$C_{21}H_{16}ClN_5OS_2$	453
39	Br	Pyrimidin-2-yl	88	245–246	$C_{21}H_{16}BrN_5OS_2$	498
40	CN	Pyrimidin-2-yl	90	256–258	$C_{22}H_{16}N_6OS_2$	444

investigated for their cytotoxicity against C6 rat glioma cells. Among these derivatives, the most effective anticancer compounds were evaluated for their DNA synthesis inhibitory activity.

2. Chemistry

The synthesis of the thiazoline derivatives (**1**–**40**) was carried out according to the steps shown in Scheme 1. In the initial step, ethyl 2-[(aryl)thio]acetate derivatives (**A1**–**6**) were synthesized via the reaction of thiols with ethyl chloroacetate in the presence of potassium carbonate. The treatment of the ester derivatives (**A1**–**6**) with hydrazine hydrate afforded 2-[(aryl)thio]acetohydrazides (**B1**–**6**) [31–34]. 1-(Arylthioacetyl)-4-phenyl thiosemicarbazides (**C1**–**6**) were obtained by the reaction of the hydrazides (**B1**–**6**) with phenyl isothiocyanate [34–36]. In the last step, the ring closure reaction of the thiosemicarbazide derivatives (**C1**–**6**) with 2-bromoacetophenone/4′-substituted-2-bromoacetophenone gave the target compounds (**1**–**40**) [18,36]. Some properties of the compounds were given in Table 1.

3. Results and discussion

The structures of new compounds were confirmed by FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analysis.

In the IR spectra of **C-2** and **C-5**, the C=O stretching vibrations gave rise to a band at 1700–1650 cm^{-1}. The stretching bands for N–H group were observed in the region 3400–3200 cm^{-1}. The stretching bands for aromatic and aliphatic C–H groups occurred at 3100–3000 cm^{-1} and 2980–2900 cm^{-1}, respectively. C=N, C=C stretching and N–H bending vibrations were observed in the region 1600–1450 cm^{-1}.

In the IR spectra of compounds **1**–**40**, the compounds had a strong, characteristic band in the region 1750–1650 cm^{-1} due to the C=O stretching vibration. The stretching bands for N–H group occurred at 3500–3200 cm^{-1}. The aromatic and aliphatic C–H stretching vibrations gave rise to bands at 3120–3000 cm^{-1} and 2990–2700 cm^{-1}, respectively. C=N, C=C stretching and N–H bending vibrations were observed in the region 1620–1450 cm^{-1}. In the IR spectra of the compounds bearing cyano substituent, the stretching bands for C≡N group occurred at 2250–2200 cm^{-1}.

In the ^{1}H NMR spectra of **C-2** and **C-5**, the signal due to the thiosemicarbazide protons appeared in the region 9.5–10.5 ppm. The signal due to the S–CH_2 protons was observed in the region 3.99–4.11 ppm.

In the ^{1}H NMR spectra of compounds **1**–**40**, the signal due to the hydrazone proton appeared in the region 10–13 ppm. The signal due to the S–CH_2 protons was observed in the region 3.86–4.37 ppm. In the ^{1}H NMR spectra of some compounds, N–H and S–CH_2 protons gave rise to two singlet peaks in accordance with the presence of the *E* and *Z* isomers [37,38]. The signal due to the thiazoline proton was observed in the region 6.5–7.3 ppm. Other aromatic and aliphatic protons were observed at expected regions.

In the ^{13}C NMR spectra of all compounds, the signal due to the S–CH_2 carbon was observed in the region 32–36 ppm.

In the ^{13}C NMR spectra of **C-2** and **C-5**, the signal due to the C=S carbon was observed at 181.68 and 181.52 ppm, respectively. The signal due to the C=O carbon was observed at 167–168 ppm.

In the ^{13}C NMR spectra of compounds **1**–**40**, the signal due to the C=O carbon was observed at 160–180 ppm. Other aromatic and aliphatic carbons were observed at expected regions.

The mass spectral data were also consistent with the assigned structures. All compounds gave satisfactory elemental analysis.

Compounds **1**–**40** were tested *in vitro* against pathogenic bacteria and fungi strains. As shown in Tables 2 and 3, the compounds exhibited more significant antimicrobial activity against fungi than bacteria.

Among bacteria species, *Pseudomonas aeruginosa* was the most susceptible bacterium to compounds **5**, **22** and **40**. These compounds and streptomycin exhibited the same level of antibacterial activity against *P. aeruginosa* with a MIC value of 125 μg/mL. Among these derivatives, compound **22** bearing 1-phenyl-1*H*-tetrazole and *p*-chlorophenyl moieties can be identified as the most promising antibacterial agent against *P. aeruginosa* due to its inhibitory effect on *P. aeruginosa* and low toxicity to NIH/3T3 cells (IC_{50} = 313.3 ± 15.3 μg/mL).

Compounds **3** and **23** were more effective than ketoconazole, whereas other derivatives and ketoconazole exhibited the same level of antifungal activity against *Candida albicans*. Compounds **3** and **23** exhibited the inhibitory activity against *C. albicans* with a MIC value of 125 μg/mL, whereas ketoconazole exhibited the inhibitory activity with a MIC value of 250 μg/mL. In addition, IC_{50} values of compounds **3** and **23** were determined as 130.0 ± 20.0 μg/mL and 433.3 ± 28.9 μg/mL, respectively. According to MTT assay, compound **23** bearing 1-phenyl-1*H*-tetrazole and *p*-bromophenyl moieties seems to be the most promising anticandidal agent owing to its inhibitory effect on *C. albicans* and low toxicity to NIH/3T3 cells.

Compounds **1**–**40** exhibited the inhibitory activity against *Aspergillus parasiticus* with a MIC value of 250 μg/mL, whilst

Table 2
Antibacterial activity of the compounds (**1**–**40**) as MIC values (μg/mL).

Compound	A	B	C	D	E	F
1	250	250	250	250	250	250
2	250	250	250	250	125	125
3	250	250	125	250	125	250
4	250	250	250	250	250	250
5	125	125	125	125	250	250
6	125	250	125	250	125	125
7	250	250	250	250	250	250
8	250	250	250	250	250	250
9	250	250	250	250	250	250
10	250	250	250	250	250	500
11	250	250	250	250	250	500
12	250	250	500	250	250	250
13	250	250	125	250	250	250
14	500	250	250	250	250	250
15	250	250	250	250	125	250
16	250	250	250	250	250	500
17	250	250	250	250	250	500
18	250	250	250	250	500	250
19	250	250	250	250	250	250
20	250	250	250	250	125	500
21	250	250	250	250	250	500
22	250	250	125	125	125	250
23	250	250	250	250	250	250
24	500	250	250	250	250	250
25	500	250	250	250	250	500
26	500	250	250	250	250	250
27	500	250	250	250	250	500
28	250	250	250	250	250	250
29	250	250	250	250	250	250
30	250	250	250	250	250	250
31	500	250	250	250	250	500
32	250	250	250	250	250	250
33	250	250	250	250	250	500
34	250	250	250	250	250	250
35	250	250	250	250	250	500
36	250	250	250	250	250	250
37	500	250	250	250	250	500
38	500	250	250	250	250	500
39	500	250	250	250	250	250
40	250	250	250	125	250	250
Streptomycin	31.25	7.81	31.25	125	15.625	15.625

A: *S. aureus* (NRRL B-767), **B**: *L. monocytogenes* (ATCC-7644), **C**: *E. coli* (ATCC-25922), **D**: *P. aeruginosa* (ATCC-254992), **E**: *M. luteus* (NRLL B-4375), **F**: *B. subtilis* (NRS-744).

Table 3
Antifungal activity of the compounds (**1**–**40**) as MIC values (μg/mL).

Compound	A	B	C	D	E	F	G	H
1	250	250	125	250	250	250	250	250
2	250	250	250	250	250	250	250	250
3	250	250	125	250	250	250	250	125
4	250	250	250	250	250	250	250	250
5	250	250	250	250	250	250	250	250
6	250	250	125	250	250	250	250	250
7	250	250	125	250	250	250	250	250
8	250	250	125	250	250	250	250	250
9	250	250	125	250	250	250	250	250
10	250	250	125	250	250	250	250	250
11	250	250	125	250	250	250	125	250
12	250	250	250	250	250	250	250	250
13	250	250	250	250	250	250	250	250
14	250	250	250	250	250	250	250	250
15	250	250	125	250	250	250	125	250
16	250	250	125	250	250	250	250	250
17	250	250	125	250	250	250	125	250
18	250	250	125	250	250	250	250	250
19	250	250	125	250	250	250	125	250
20	250	250	125	250	250	250	125	250
21	250	250	125	250	250	250	125	250
22	250	250	125	250	250	250	250	250
23	250	250	125	250	250	250	250	125
24	250	250	250	250	250	250	250	250
25	250	250	125	250	250	250	250	250
26	250	250	250	250	250	250	250	250
27	250	250	250	250	250	250	250	250
28	250	250	125	250	250	250	250	250
29	250	250	125	250	250	250	250	250
30	250	250	125	250	250	250	250	250
31	250	250	125	250	250	250	250	250
32	250	250	125	250	250	250	250	250
33	250	250	250	250	250	250	250	250
34	250	250	250	250	250	250	250	250
35	250	250	250	250	250	250	250	250
36	250	250	250	250	250	250	250	250
37	250	250	250	250	250	250	250	250
38	250	250	250	250	250	250	250	250
39	250	250	250	250	250	250	250	250
40	250	250	250	250	250	250	250	250
Ketoconazole	125	7.8	250	250	500	500	500	250

A: *A. parasiticus* (NRRL 465), **B**: *P. chrysogenum* (NRRL 1951), **C**: *T. harzianum* (NRRL 20565), **D**: *A. ochraceus* (NRRL 3174), **E**: *F. solani* (NRRL-13414) **F**: *F. moniliforme* (NRLL 1866), **G**: *F. culmorum* (wild culture), **H**: *C. albicans* (ATCC-22019).

ketoconazole exhibited the inhibitory activity with a MIC value of 125 μg/mL. Compounds **1**–**40** and ketoconazole also showed the same level of antifungal activity against *Aspergillus ochraceus* with a MIC value of 250 μg/mL.

Compounds **1**–**40** were more effective than ketoconazole against *Fusarium solani* and *Fusarium moniliforme*. These compounds exhibited the inhibitory activity against *F. solani* and *F. moniliforme* with a MIC value of 250 μg/mL, whereas ketoconazole exhibited the inhibitory activity with a MIC value of 500 μg/mL. The microbiological results demonstrated that the antifungal effects of the compounds on *A. parasiticus*, *A. ochraceus*, *F. solani* and *F. moniliforme* did not depend on the substituents.

Compounds **1**–**40** were found to be more potent than ketoconazole against *Fusarium culmorum*. Compounds **11**, **15**, **17**, **19**, **20** and **21** showed the antifungal activity with a MIC value of 125 μg/mL, whereas other derivatives showed the antifungal activity against *F. culmorum* with a MIC value of 250 μg/mL.

Compounds **2**, **4**, **5**, **12**, **13**, **14**, **24**, **26**, **27**, **33**, **34**, **35**, **36**, **37**, **38**, **39**, **40** and ketoconazole exhibited the inhibitory activity against *Trichoderma harzianum* with a MIC value of 250 μg/mL, other derivatives exhibited the inhibitory activity with a MIC value of 125 μg/mL.

The most effective derivatives were also evaluated for their cytotoxicity against C6 rat glioma cells. The viability and IC_{50} values of compounds **1**, **3**, **8**, **9**, **10**, **11**, **16**, **17**, **19**, **21**, **22**, **23**, **24**, **25**, **27**, **28**, **29**, **30**, **35**, **36**, **37**, **38**, **39** against C6 cells for 24 h were presented in Tables 4 and 5, respectively. Compound **17** bearing 1-phenyl-1*H*-tetrazole and nonsubstituted phenyl moieties can be identified as the most promising anticancer agent against C6 glioma cell lines due to its inhibitory effect on C6 cells and low toxicity to NIH/3T3 cells (IC_{50} = 416.7 ± 28.9 μg/mL). Compound **17** exhibited its inhibitory effect on C6 cells with an IC_{50} value of 8.3 ± 2.6 μg/mL, whereas cisplatin showed its anticancer activity with an IC_{50} value of 13.7 ± 1.2 μg/mL.

The immunostaining procedure was carried out with specific anti-BrdU antibodies in the S-phase of the cell cycle. DNA synthesis inhibitory activity of compounds **16**, **17**, **19**, **21**, **23**, **27**, **28**, **29** and **30** which exhibited significant cytotoxic activity in MTT assay on C6 cell lines is presented in Fig. 1. DNA synthesis inhibition activity of the compounds was evaluated for 24 h. C6 cell lines were incubated with three different concentrations of the compounds that were determined according to their IC_{50} values. Cisplatin was used as a positive control. The tested compounds showed inhibitory activity on dose dependent manner. These results show that the compounds tested in this assay cause DNA synthesis inhibition. Among these compounds, the most potent inhibitors of DNA synthesis against C6 cell lines were found as compounds **16**, **17** and **19**. DNA synthesis inhibition caused by the concentration of IC_{50} for compounds **16**, **17** and **19** tested in C6 cell line were similar. Although compounds **16**, **17** and **19** had similar inhibitory effects on DNA synthesis activity, compound **17** was the best among them due to low IC_{50} value against C6 glioma cells (Table 5). On the other hand, DNA synthesis inhibition caused by the concentration of IC_{50} for compounds **21**, **23**, **27**, **28**, **29** and **30** were similar.

All compounds were also evaluated for their cytotoxic effects against NIH/3T3 cell lines. The MTT assay indicated that compounds **32** and **40** possessed the highest cytotoxicity (IC_{50} < 3.9 μg/mL), whereas compounds **19**, **35** and **37** exhibited the lowest cytotoxicity (IC_{50} > 500 μg/mL) against NIH/3T3 cells among the compounds (Table 5).

4. Conclusion

In the present paper, we described the synthesis of thiazoline derivatives bearing a hydrazone moiety, which were tested *in vitro* against pathogenic bacteria and fungi.

The microbiological results revealed that the compounds exhibited more significant antimicrobial activity against fungi than bacteria.

Compound **22** bearing 1-phenyl-1*H*-tetrazole and *p*-chlorophenyl moieties can be identified as the most promising antibacterial agent against *P. aeruginosa* due to its inhibitory effect on *P. aeruginosa* and low toxicity to NIH3T3 cells (IC_{50} = 313.3 ± 15.3 μg/mL), whereas compound **23** bearing 1-phenyl-1*H*-tetrazole and *p*-bromophenyl moieties was found to be the most promising anticandidal agent owing to its inhibitory effect on *C. albicans* and low toxicity to NIH3T3 cells (IC_{50} = 433.3 ± 28.9 μg/mL). Furthermore, all compounds exhibited significant antifungal activity against *T. harzianum*, *A. ochraceus*, *F. solani*, *F. moniliforme*, *F. culmorum*.

The most effective derivatives were also evaluated for their cytotoxicity against C6 rat glioma cells. Compound **17** bearing 1-phenyl-1*H*-tetrazole and nonsubstituted phenyl moieties was the most promising anticancer agent against C6 glioma cell lines with an IC_{50} value of 8.3 ± 2.6 μg/mL when compared with cisplatin (IC_{50} = 13.7 ± 1.2 μg/mL). Compound **17** also exhibited low toxicity to NIH/3T3 cells with an IC_{50} value of 416.7 ± 28.9 μg/mL. The results also revealed that compounds **16**, **17** and **19** were the most potent inhibitors of DNA synthesis against C6 cell lines.

Table 4
The viability of compounds **1**, **3**, **8**, **9**, **10**, **11**, **16**, **17**, **19**, **21**, **22**, **23**, **24**, **25**, **27**, **28**, **29**, **30**, **35**, **36**, **37**, **38**, **39** against C6 cell lines.

Compounds	Concentration (μg/mL)[a]					
	6	12	25	50	100	200
1	90.0 ± 12.0	79.6 ± 2.4	77.3 ± 3.6	62.4 ± 13.4	38.6 ± 8.8	35.5 ± 3.7
3	74.6 ± 8.3	72.0 ± 11.9	70.7 ± 6.5	53.8 ± 18.9	39.6 ± 7.6	27.8 ± 3.4
8	70.2 + 1.6	62.6 ± 0.6	58.8 ± 2.3	58.8 ± 3.9	55.2 ± 5.4	52.6 ± 2.7
9	85.2 ± 3.4	80.7 ± 5.6	64.2 ± 7.9	56.2 ± 1.0	38.8 ± 8.3	26.8 ± 2.2
10	80.4 ± 4.6	76.2 ± 1.5	69.3 ± 8.1	69.2 ± 1.8	64.1 ± 4.9	58.6 ± 5.0
11	88.0 ± 9.3	68.8 ± 4.7	60.7 ± 6.5	44.3 ± 3.7	39.7 ± 5.7	39.7 ± 6.5
16	63.5 + 11.1	53.9 ± 4.4	43.9 ± 6.2	35.6 ± 4.6	30.9 ± 3.6	28.6 ± 4.3
17	52.1 ± 1.9	47.1 ± 1.8	42.5 ± 6.3	36.7 ± 9.1	35.8 ± 5.9	22.6 ± 0.9
19	66.8 ± 7.3	55.1 ± 7.8	38.8 ± 3.1	29.5 ± 3.4	28.5 ± 4.6	22.2 ± 3.9
21	74.9 ± 0.4	60.0 ± 8.2	49.3 ± 6.5	43.7 ± 4.5	35.8 ± 3.5	30.6 ± 4.3
22	77.4 ± 13.0	69.3 ± 12.3	62.3 ± 12.4	60.0 ± 6.8	54.3 ± 3.4	52.4 ± 2.6
23	61.8 ± 10.3	58.6 ± 8.7	47.9 ± 4.1	32.0 ± 4.9	24.3 ± 2.4	20.4 ± 2.1
24	78.8 + 17.1	74.1 ± 15.3	64.2 ± 5.9	61.1 ± 6.3	59.8 ± 5.9	54.0 ± 2.4
25	72.4 ± 2.9	72.5 ± 4.6	65.9 ± 4.8	63.9 ± 1.7	61.8 ± 4.8	59.2 ± 1.9
27	65.9 ± 6.4	58.3 ± 5.3	50.1 ± 3.7	40.6 ± 5.2	31.5 ± 11.1	13.3 ± 2.8
28	57.9 ± 8.4	53.6 ± 1.9	42.6 ± 9.1	31.5 ± 3.3	25.9 ± 5.1	22.2 ± 6.2
29	74.3 ± 18.1	73.7 ± 16.8	50.1 ± 16.5	37.4 ± 7.1	34.9 ± 11.1	26.5 ± 6.3
30	82.8 ± 10.0	76.6 ± 9.8	53.3 ± 15.1	37.9 ± 3.4	34.1 ± 5.2	29.0 ± 5.1
35	93.2 ± 20.7	83.0 ± 24.1	82.7 ± 10.7	64.4 ± 2.3	45.9 ± 4.4	43.7 + 9.7
36	89.6 + 2.1	78.4 + 8.1	74.0 + 8.4	66.5 + 10.7	51.5 + 8.1	37.6 + 6.6
37	84.0 + 16.7	77.4 + 13.7	63.9 + 3.3	55.5 + 1.4	53.4 + 0.4	32.2 + 6.2
38	95.9 + 19.8	91.6 + 11.9	91.1 + 24.6	61.8 + 2.4	45.5 + 6.6	39.7 + 9.4
39	76.4 + 26.0	72.1 + 11.9	72.3 + 23.6	69.0 + 15.7	67.6 + 14.9	63.2 + 15.1
Cisplatin	57.3 + 2.9	51.7 + 2.8	31.2 + 8.8	27.2 + 4.1	17.3 + 2.5	16.2 + 2.4

[a] Viability % was determined relative to controls.

In the view of the current study, further research can be carried out on the development of new effective antimicrobial and anticancer agents by the modification of these compounds.

5. Experimental

5.1. Chemistry

All reagents were purchased from commercial suppliers and were used without further purification. Melting points were determined on an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected. IR spectra were recorded on Shimadzu 8400 FT-IR spectrophotometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra were recorded on a Bruker 300 MHz and 400 MHz spectrometer (Bruker, Billerica, USA), respectively. ^{13}C NMR spectra were recorded on a Bruker 100 MHz spectrometer (Bruker, Billerica, USA). Mass spectra were recorded on an Agilent LC-MSD-Trap-SL Mass spectrometer (Agilent, Minnesota, USA). Elemental analyses were performed on a Perkin–Elmer EAL 240 elemental analyser (Perkin–Elmer, Norwalk, USA).

5.1.1. General procedure for the synthesis of the compounds

*5.1.1.1. Ethyl 2-[(aryl)thio]acetate derivatives (**A1**–**6**).* A mixture of thiol (0.2 mol) and ethyl chloroacetate (0.2 mol) in the presence of potassium carbonate (0.2 mol) in acetone (60 mL) was refluxed for 24 h. The reaction mixture was cooled, filtered and the crude product was solved in water and then extracted with diethyl ether [31–34].

*5.1.1.2. 2-[(Aryl)thio]acetohydrazide derivatives (**B1**–**6**).* A mixture of the ester (**A**) (0.1 mol) and hydrazine hydrate (0.2 mol) in ethanol (40 mL) was stirred at room temperature for 10 h and then filtered [31–34].

*5.1.1.3. 1-(Arylthioacetyl)-4-phenyl thiosemicarbazide derivatives (**C1**–**6**).* A mixture of the hydrazide (**B**) (0.05 mol) and phenyl isothiocyanate (0.05 mol) in ethanol (30 mL) was refluxed for 1 h and then filtered [34–36].

*5.1.1.3.1. 1-[(1-Methyl-1H-tetrazol-5-yl)thioacetyl]-4-phenyl thiosemicarbazide (**C-2**).* M.p.: 168–169 °C.

IR (KBr) ν_{max} (cm^{-1}): 3296.12, 3236.33, 3215.11 (N–H stretching), 3056.96 (Aromatic C–H stretching), 2975.96, 2937.38 (Aliphatic C–H stretching), 1685.67 (C=O stretching), 1593.09, 1548.73, 1537.16, 1502.44, 1471.59 (C=N, C=C stretching and N–H bending), 1446.51 (C–H bending), 1269.07 (C–N stretching), 1207.36, 1153.35 (Aromatic C–H in plane bending), 752.19, 703.97 (Aromatic C–H out of plane bending).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.93 (3H, s, CH_3), 4.11 (2H, s, S–CH_2), 7.13 (1H, t, J_1 = 7.6 Hz, J_2 = 6.8 Hz, phenyl H_4), 7.30 (2H, t, J_1 = 8 Hz, J_2 = 7.6 Hz, phenyl H_3, H_5), 7.43 (2H, d, J = 7.6 Hz, phenyl H_2, H_6), 9.59 (1H, s, N–H), 9.72 (1H, s, N–H), 10.40 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 34.48 (CH_3), 36.00 (CH_2), 126.01 (2CH), 128.82 (3CH), 139.50 (C), 154.02 (C), 167.11 (C), 181.68 (C).

MS (ESI)(m/z): (M^+ − 1) 322.

For $C_{11}H_{13}N_7OS_2$ calculated: C, 40.85; H, 4.05; N, 30.32; Found: C, 40.84; H, 4.06; N, 30.32.

*5.1.1.3.2. 1-[(Pyrimidin-2-yl)thioacetyl]-4-phenyl thiosemicarbazide (**C-5**).* M.p.: 176–181 °C.

IR (KBr) ν_{max} (cm^{-1}): 3307.69, 3261.40 (N–H stretching), 3103.25 (Aromatic C–H stretching), 2933.53 (Aliphatic C–H stretching), 1681.81 (C=O stretching), 1596.95, 1508.23, 1450.37 (C=N, C=C stretching and N–H bending), 1379.01 (C–H bending), 1317.29, 1274.86 (C–N stretching), 1213.14, 1159.14 (Aromatic C–H in plane bending), 636.47 (C–S stretching).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.99 (2H, s, S–CH_2), 7.14 (1H, t, J_1 = 7.6 Hz, J_2 = 6.8 Hz, phenyl H_4), 7.18 (1H, t, J_1, J_2 = 4.8 Hz, pyrimidine H_4), 7.31 (2H, t, J_1 = 8.4 Hz, J_2 = 7.2 Hz, phenyl H_3, H_5), 7.39 ve 7.48 (2H, 2d, J_1, J_2 = 7.6 Hz, phenyl H_2, H_6), 8.58 (2H, d, J = 4.8 Hz, pyrimidine), 9.55 (1H, s, N–H), 9.70 (1H, s, N–H), 10.26 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 33.64 (CH_2), 118.10 (CH), 125.92 (2CH), 126.53 (CH), 128.81 (2CH), 139.66 (C), 158.48 (2CH), 168.13 (C), 170.87 (C), 181.52 (C).

MS (ESI)(m/z): (M^+ − 1) 318.

Table 5
IC_{50} values of the compounds against NIH/3T3 and C6 cell lines.

Compound	IC_{50} value (μg/mL)	
	NIH/3T3 cell lines	C6 cell lines
1	216.7 ± 15.3	68.3 ± 13.1
2	133.3 ± 11.5	nt
3	130.0 ± 20.0	56.0 ± 10.4
4	166.7 ± 5.8	nt
5	65.0 ± 17.3	nt
6	51.7 ± 20.8	nt
7	58.3 ± 36.8	nt
8	153.3 ± 5.7	>200
9	173.3 ± 20.8	67.7 ± 8.8
10	166.7 ± 28.8	>200
11	243.3 ± 40.4	36.3 ± 4.9
12	45.0 ± 5.0	nt
13	113.3 ± 52.0	nt
14	40.0 ± 13.2	nt
15	19.3 ± 0.6	nt
16	213.3 ± 5.7	23.3 ± 6.2
17	416.7 ± 28.9	8.3 ± 2.6
18	14.3 ± 4.0	nt
19	>500	14.7 ± 3.4
20	11.7 ± 1.5	nt
21	206.7 ± 15.3	24.3 ± 6.3
22	313.3 ± 15.3	>200
23	433.3 ± 28.9	21.0 ± 3.7
24	453.3 ± 53.3	>200
25	196.7 ± 5.8	>200
26	176.7 ± 86.6	nt
27	373.3 ± 30.5	25.3 ± 6.1
28	300.0 ± 50.0	18.7 ± 2.9
29	383.3 ± 28.9	27.3 ± 10.5
30	253.3 ± 25.2	28.0 ± 8.6
31	55.0 ± 5.0	nt
32	<3.9	nt
33	106.7 ± 30.5	nt
34	193.3 ± 11.5	nt
35	>500	85.0 ± 10.8
36	450 ± 50	101.7 ± 16.5
37	>500	110.0 ± 8.2
38	396.7 ± 5.8	83.3 ± 12.5
39	273.3 ± 20.8	>200
40	<3.9	nt
Cisplatin	nt	13.7 ± 1.2

nt: not tested.

For $C_{13}H_{13}N_5OS_2$ calculated: C, 48.88; H, 4.10; N, 21.93; Found: C, 48.85; H, 4.10; N, 21.95.

*5.1.1.4. N′-(3,4-Diarylthiazol-2(3H)-ylidene)-2-(arylthio)acetohydrazide derivatives (**1**–**40**).* A mixture of the thiosemicarbazide (**C**) (2 mmol) and 2-bromoacetophenone/4′-substituted-2-bromoacetophenone (2 mmol) in ethanol (15 mL) was refluxed for 5 h and then filtered [18,36].

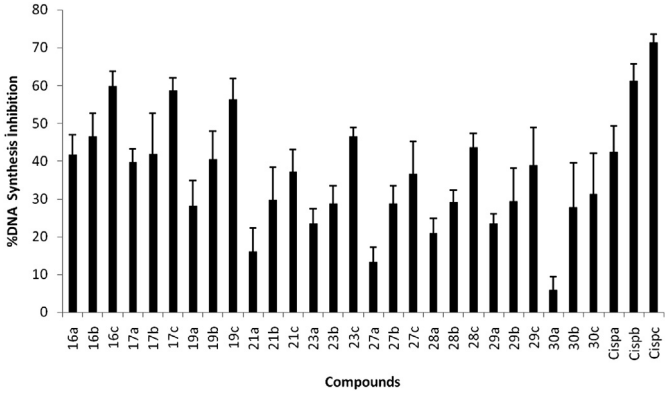

Fig. 1. DNA synthesis inhibitory activity of compounds **16**, **17**, **19**, **21**, **23**, **27**, **28**, **29**, **30** and cisplatin on C6 cells.

*5.1.1.4.1. N′-[(3,4-Diphenylthiazol-2(3H)-ylidene)]-2-[(4-methyl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (**1**).* IR (KBr) ν_{max} (cm^{-1}): 2927.82 (Aliphatic C–H stretching), 1677.23 (C=O stretching), 1572.17, 1511.84, 1488.62 (C=N, C=C stretching and N–H bending), 1442.44, 1356.41 (C–H bending), 1299.82 (C–N stretching), 1198.50, 1155.96, 1092.80, 1072.12, 1011.25 (Aromatic C–H in plane bending), 917.04, 830.77, 760.55, 691.98 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.60 (3H, s, CH_3), 3.86 (2H, s, S–CH_2), 6.54 (1H, s, thiazoline), 7.13–7.42 (10H, m, phenyl), 8.59 (1H, s, triazole), 10.36 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 31.61 (CH_3), 36.28 (CH_2), 99.85 (CH), 128.46 (CH), 128.85 (2CH), 128.94 (CH), 129.17 (CH), 129.23 (CH), 129.29 (2CH), 129.54 (2CH), 131.31 (C), 138.02 (C), 140.21 (CH), 146.90 (C), 149.43 (2C), 167.90 (C).

MS (ESI)(m/z): ($M^+ - 1$) 421.

For $C_{20}H_{18}N_6OS_2$ calculated: C, 56.85; H, 4.29; N, 19.89; Found: C, 56.85; H, 4.30; N, 19.90.

*5.1.1.4.2. N′-[(4-Nitrophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(4-methyl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (**2**).* IR (KBr) ν_{max} (cm^{-1}): 3105.32 (Aromatic C–H stretching), 1688.90 (C=O stretching), 1601.78, 1587.64, 1574.24, 1556.46, 1488.71, 1454.19 (C=N, C=C stretching and N–H bending), 1445.83, 1341.62 (C–H bending), 1318.20, 1300.36, 1287.44 (C–N stretching), 1195.46, 1173.07, 1158.41, 1107.02, 1075.43, 1028.28, 1002.02 (Aromatic C–H in plane bending), 921.02, 852.97, 765.46, 753.34, 691.94 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.46 and 3.56 (3H, 2s, CH_3), 4.06 (2H, s, S–CH_2), 6.70–8.54 (11H, m, thiazoline, phenyl, 4-nitrophenyl, triazole), 11.30 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 31.40 (CH_3), 35.32 (CH_2), 107.11 (CH), 121.51 (CH), 124.13 (CH), 124.19 (CH), 124.44 (2CH), 128.97 (CH), 129.07 (CH), 129.68 (CH), 129.72 (CH), 137.59 (C), 138.56 (C), 143.37 (CH), 146.84 (C), 147.57 (C), 147.90 (C), 150.68 (C), 168.04 (C).

MS (ESI)(m/z): ($M^+ - 1$) 466.

For $C_{20}H_{17}N_7O_3S_2$ calculated: C, 51.38; H, 3.67; N, 20.97; Found: C, 51.40; H, 3.68; N, 20.97.

*5.1.1.4.3. N′-[(4-Methylphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(4-methyl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (**3**).* IR (KBr) ν_{max} (cm^{-1}): 2920.48 (Aliphatic C–H stretching), 1676.49 (C=O stretching), 1598.24, 1557.20, 1497.37, 1451.91 (C=N, C=C stretching and N–H bending), 1354.97 (C–H bending), 1286.79 (C–N stretching), 1183.91, 1111.26, 1065.51, 1017.97 (Aromatic C–H in plane bending), 967.30, 812.24, 783.89, 754.20, 693.09 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.31 (3H, s, CH_3), 3.53 (3H, s, CH_3, triazole), 4.02 (2H, s, S–CH_2), 7.04 (1H, s, thiazoline), 7.23 (2H, d, $J = 8$ Hz, 4-methylphenyl), 7.35–7.53 (7H, m, phenyl, 4-methylphenyl), 8.85 (1H, s, triazole), 10.35 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 21.61 (CH_3), 32.07 (CH_3), 35.48 (CH_2), 101.82 (CH), 124.17 (CH), 124.87 (2CH), 128.44 (C), 129.01 (2CH), 130.01 (2CH), 130.95 (2CH), 139.73 (C), 140.69 (C), 141.10 (CH), 146.83 (C), 149.79 (2C), 167.68 (C).

MS (ESI)(m/z): ($M^+ + 1$) 437.

For $C_{21}H_{20}N_6OS_2$ calculated: C, 57.78; H, 4.62; N, 19.25; Found: C, 57.79; H, 4.60; N, 19.25.

*5.1.1.4.4. N′-[(4-Methoxyphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(4-methyl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (**4**).* IR (KBr) ν_{max} (cm^{-1}): 3355.05 (N–H stretching), 3054.18 (Aromatic C–H stretching), 2871.63 (Aliphatic C–H stretching), 1705.44 (C=O stretching), 1603.12, 1561.21, 1510.66, 1492.71, 1466.63 (C=N, C=C

stretching and N–H bending), 1438.95, 1416.76, 1346.04 (C–H bending), 1300.05, 1258.52, 1230.96 (C–N stretching), 1197.99 (C–O stretching), 1181.13, 1107.16, 1077.25, 1051.47, 1036.95, 1023.50 (Aromatic C–H in plane bending), 961.96, 878.09, 846.37, 832.55, 819.84, 774.79, 766.03, 744.95, 714.93, 694.16, 656.22 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.57 (3H, s, CH_3), 3.82 (3H, s, OCH_3), 4.01 (2H, s, S–CH_2), 6.95 (1H, s, thiazoline), 7.02 (2H, d, J = 9 Hz, 4-methoxyphenyl), 7.38–7.57 (7H, m, phenyl, 4-methoxyphenyl), 8.75 (1H, s, triazole), 12.08 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 31.95 (CH_3), 35.35 (CH_2), 56.07 (CH_3), 100.77 (CH), 114.91 (2CH), 119.98 (CH), 124.11 (C), 128.32 (2CH), 130.72 (2CH), 130.96 (2CH), 140.06 (C), 140.94 (CH), 146.90 (C), 149.70 (2C), 161.25 (C), 167.75 (C).

MS (ESI)(m/z): (M^+ + 1) 453.

For $C_{21}H_{20}N_6O_2S_2$ calculated: C, 55.73; H, 4.45; N, 18.57; Found: C, 55.75; H, 4.45; N, 18.58.

*5.1.1.4.5. N′-[(4-Fluorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(4-methyl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (**5**).* IR (KBr) ν_{max} (cm^{-1}): 3421.07 (N–H stretching), 3061.60 (Aromatic C–H stretching), 2890.79 (Aliphatic C–H stretching), 1704.58 (C=O stretching), 1600.44, 1584.15, 1567.31, 1505.80, 1496.56, 1467.04 (C=N, C=C stretching and N–H bending), 1410.18, 1380.91, 1344.37 (C–H bending), 1311.88, 1276.96, 1232.00 (C–N stretching), 1200.92, 1162.99, 1103.17, 1028.52, 1013.09 (Aromatic C–H in plane bending), 962.40, 832.02, 780.42, 763.32, 747.72, 712.36, 689.84, 654.90 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.59 and 3.68 (3H, 2s, CH_3), 4.03 (2H, d, J = 6 Hz, S–CH_2), 7.05–7.57 (10H, m, thiazoline, phenyl, 4-fluorophenyl), 8.84 and 9.02 (1H, 2s, triazole), 12.10 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 32.28 (CH_3), 35.47 (CH_2), 102.79 (CH), 116.49 (CH), 116.71 (CH), 124.11 (CH), 124.14 (CH), 124.23 (CH), 128.59 (C), 131.02 (2CH), 131.80 (CH), 131.89 (CH), 139.54 (C), 140.02 (CH), 146.74 (C), 150.14 (C), 150.99 (C), 162.50 (C), 167.65 (C).

MS (ESI)(m/z): (M^+ + 1) 441.

For $C_{20}H_{17}FN_6OS_2$ calculated: C, 54.53; H, 3.89; N, 19.08; Found: C, 54.54; H, 3.90; N, 19.08.

*5.1.1.4.6. N′-[(4-Chlorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(4-methyl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (**6**).* IR (KBr) ν_{max} (cm^{-1}): 3037.87 (Aromatic C–H stretching), 2851.33 (Aliphatic C–H stretching), 1718.10 (C=O stretching), 1585.11, 1553.42, 1505.64, 1487.76, 1453.15 (C=N, C=C stretching and N–H bending), 1422.87, 1395.31, 1376.38 (C–H bending), 1310.58 (C–N stretching), 1190.98, 1164.76, 1110.75, 1090.99, 1051.16, 1013.89 (Aromatic C–H in plane bending), 945.69, 963.72, 895.74, 866.25, 829.02, 820.88, 783.59, 760.19, 710.09, 696.14, 684.73 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.71 and 3.72 (3H, 2s, CH_3), 3.87 and 4.00 (2H, 2s, S–CH_2), 7.17–7.41 (10H, m, thiazoline, phenyl, 4-chlorophenyl), 9.04 (1H, s, triazole), 11.80 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 32.95 (CH_3), 35.39 (CH_2), 106.05 (CH), 128.37 (2CH), 129.03 (CH), 129.09 (2CH), 130.69 (C), 130.91 (2CH), 131.77 (2CH), 134.99 (2C), 141.07 (CH), 146.33 (C), 151.57 (2C), 166.05 (C).

MS (ESI)(m/z): (M^+ + 1) 457.

For $C_{20}H_{17}ClN_6OS_2$ calculated: C, 52.57; H, 3.75; N, 18.39; Found: C, 52.58; H, 3.75; N, 18.40.

*5.1.1.4.7. N′-[(4-Bromophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(4-methyl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (**7**).* IR (KBr) ν_{max} (cm^{-1}): 3108.56, 3035.01 (Aromatic C–H stretching), 2850.52, 2734.33 (Aliphatic C–H stretching), 1717.73 (C=O stretching), 1580.57, 1553.70, 1505.47, 1485.83, 1452.55 (C=N, C=C stretching and N–H bending), 1422.99, 1393.45, 1351.23, 1374.05 (C–H bending), 1310.04, 1225.98 (C–N stretching), 1190.48, 1165.12, 1109.10, 1070.32, 1050.98, 1009.23 (Aromatic C–H in plane bending), 963.01, 945.29, 895.41, 866.11, 827.64, 818.18, 782.74, 760.36, 724.31, 714.07, 693.63, 682.24 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.72 and 3.73 (3H, 2s, CH_3), 4.01 and 4.10 (2H, 2s, S–CH_2), 7.14–7.51 (10H, m, thiazoline, phenyl, 4-bromophenyl), 9.29 and 9.38 (1H, 2s, triazole), 11.14 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 32.76 (CH_3), 35.42 (CH_2), 105.53 (CH), 123.66 (C), 128.85 (CH), 129.03 (2CH), 129.40 (2CH), 129.77 (C), 130.61 (2CH), 131.85 (CH), 131.99 (CH), 135.19 (C), 140.95 (CH), 146.39 (C), 151.29 (2C), 165.81 (C).

MS (ESI)(m/z): (M^+ + 1) 501.

For $C_{20}H_{17}BrN_6OS_2$ calculated: C, 47.91; H, 3.42; N, 16.76; Found: C, 47.90; H, 3.40; N, 16.75.

*5.1.1.4.8. N′-[(4-Cyanophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(4-methyl-4H-1,2,4-triazol-3-yl)thio]acetohydrazide (**8**).* IR (KBr) ν_{max} (cm^{-1}): 3433.06 (N–H stretching), 3037.68 (Aromatic C–H stretching), 2908.45 (Aliphatic C–H stretching), 2229.56 (C≡N stretching), 1716.53 (C=O stretching), 1598.88, 1488.94 (C=N, C=C stretching and N–H bending), 1317.29 (C–N stretching), 1110.92 (Aromatic C–H in plane bending), 833.19 (Aromatic C–H out of plane bending).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.76 (3H, s, CH_3), 4.06 and 4.15 (2H, 2s, S–CH_2), 7.28–7.52 (8H, m, thiazoline, phenyl, 4-cyanophenyl), 7.76–7.81 (2H, m, 4-cyanophenyl), 9.52 and 9.56 (1H, 2s, triazole), 11.15 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 32.29 (CH_3), 34.72 (CH_2), 106.14 (CH), 111.64 (C), 118.01 (C), 128.19 (CH), 128.54 (2CH), 129.01 (2CH), 129.73 (CH), 129.79 (CH), 131.05 (2CH), 132.08 (C), 136.55 (C), 139.40 (CH), 145.35 (C), 145.44 (C), 151.05 (C), 164.69 (C).

MS (ESI)(m/z): (M^+ + 1) 448.

For $C_{21}H_{17}N_7OS_2$ calculated: C, 56.36; H, 3.83; N, 21.91; Found: C, 56.37; H, 3.83; N, 21.90.

*5.1.1.4.9. N′-[(3,4-Diphenylthiazol-2(3H)-ylidene)]-2-[(1-methyl-1H-tetrazol-5-yl)thio]acetohydrazide (**9**).* IR (KBr) ν_{max} (cm^{-1}): 3053.11 (Aromatic C–H stretching), 2860.16 (Aliphatic C–H stretching), 1723.44 (C=O stretching), 1595.18, 1573.85, 1557.58, 1514.48, 1458.36 (C=N, C=C stretching and N–H bending), 1445.50, 1397.08, 1368.05 (C–H bending), 1310.69, 1227.67 (C–N stretching), 1210.93, 1194.17, 1179.66, 1124.94, 1076.71, 1019.72, 1003.86 (Aromatic C–H in plane bending), 963.44, 936.93, 917.06, 896.68, 835.43, 811.36, 776.09, 745.91, 691.14 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.84 and 3.95 (3H, 2s, CH_3), 4.20 and 4.23 (2H, 2s, S–CH_2), 7.17 (1H, s, thiazoline), 7.39–7.57 (10H, m, phenyl), 11.38 and 12.41 (1H, 2s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 34.44 (CH_3), 35.75 (CH_2), 103.23 (CH), 124.57 (CH), 127.38 (2CH), 128.97 (CH), 129.06 (CH), 129.17 (CH), 129.26 (2CH), 129.41 (2CH), 130.99 (C), 131.10 (C), 141.21 (2C), 153.37 (C), 167.12 (C).

MS (ESI)(m/z): (M^+ + 1) 424.

For $C_{19}H_{17}N_7OS_2$ calculated: C, 53.88; H, 4.05; N, 23.15; Found: C, 53.90; H, 4.03; N, 23.15.

*5.1.1.4.10. N′-[(4-Nitrophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-methyl-1H-tetrazol-5-yl)thio]acetohydrazide (**10**).* IR (KBr) ν_{max} (cm^{-1}): 3197.61 (N–H stretching), 1721.69 (C=O stretching), 1589.15, 1552.80, 1516.69, 1489.05, 1455.35 (C=N, C=C stretching and N–H bending), 1408.01, 1387.21, 1345.52 (C–H bending), 1288.86 (C–N stretching), 1172.14, 1108.09, 1075.04, 1014.94 (Aromatic C–H in plane bending), 967.57, 915.36, 853.61, 758.06, 738.42, 697.12 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.87 (3H, s, CH_3), 4.08 (2H, s, S–CH_2), 6.76–8.25 (10H, m, thiazoline, phenyl, 4-nitrophenyl), 11.81 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 34.34 (CH_3), 35.69 (CH_2), 104.06 (CH), 121.84 (CH), 122.21 (CH), 123.66 (CH), 124.13 (CH), 124.38 (CH), 128.84 (CH), 128.93 (CH), 129.13 (CH), 129.25 (CH), 137.55 (C), 138.34 (C), 147.57 (C), 148.03 (C), 148.16 (C), 153.24 (C), 167.37 (C).

MS (ESI)(m/z): ($M^+ + 1$) 469.

For $C_{19}H_{16}N_8O_3S_2$ calculated: C, 48.71; H, 3.44; N, 23.92; Found: C, 48.70; H, 3.45; N, 23.90.

*5.1.1.4.11. N′-[(4-Methylphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-methyl-1H-tetrazol-5-yl)thio]acetohydrazide (**11**).* IR (KBr) ν_{max} (cm^{-1}): 3033.02 (Aromatic C–H stretching), 2861.21 (Aliphatic C–H stretching), 1715.69 (C=O stretching), 1604.28, 1570.13, 1509.78, 1497.50, 1457.38 (C=N, C=C stretching and N–H bending), 1387.32, 1359.70 (C–H bending), 1298.45, 1281.47, 1261.97, 1246.99, 1230.14 (C–N stretching), 1192.95, 1171.02, 1127.83, 1077.32, 1018.13, 1005.46 (Aromatic C–H in plane bending), 959.96, 883.71, 842.61, 827.41, 812.77, 794.83, 782.15, 772.95, 753.42, 736.62, 698.75 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.36 (3H, s, CH_3), 3.89 (3H, s, CH_3, tetrazole), 4.24 and 4.32 (2H, 2s, S–CH_2), 7.10 (1H, s, thiazoline), 7.25 (2H, d, $J = 8.4$ Hz, aromatic), 7.37 (2H, d, $J = 7.6$ Hz, aromatic), 7.43–7.48 (3H, m, aromatic), 7.57–7.61 (2H, m, aromatic), 12.34 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 21.64 (CH_3), 34.39 (CH_3), 35.70 (CH_2), 102.11 (CH), 124.46 (CH), 124.69 (2CH), 128.85 (C), 129.07 (2CH), 129.97 (2CH), 131.09 (2CH), 138.96 (C), 140.76 (2C), 141.30 (C), 153.35 (C), 167.12 (C).

MS (ESI)(m/z): ($M^+ + 1$) 438.

For $C_{20}H_{19}N_7OS_2$ calculated: C, 54.90; H, 4.38; N, 22.41; Found: C, 54.89; H, 4.35; N, 22.42.

*5.1.1.4.12. N′-[(4-Methoxyphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-methyl-1H-tetrazol-5-yl)thio]acetohydrazide (**12**).* IR (KBr) ν_{max} (cm^{-1}): 3360.96 (N–H stretching), 2713.08 (Aliphatic C–H stretching), 1699.11 (C=O stretching), 1601.61, 1573.64, 1557.76, 1508.14, 1456.13 (C=N, C=C stretching and N–H bending), 1410.34 (C–H bending), 1299.85, 1256.06 (C–N stretching), 1204.33 (C–O stretching), 1179.66, 1135.14, 1115.76, 1023.98 (Aromatic C–H in plane bending), 962.51, 839.78, 816.69, 766.97, 720.80, 697.73 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.69 and 3.81 (3H, 2s, OCH_3), 3.90 and 3.98 (3H, 2s, CH_3), 4.12–4.22 (2H, m, S–CH_2), 6.65–7.60 (10H, m, thiazoline, phenyl, 4-methoxyphenyl), 11.81 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 34.34 (CH_3), 35.58 (CH_2), 55.96 (CH_3), 99.90 (CH), 114.74 (2CH), 123.36 (CH), 129.20 (C), 130.09 (2CH), 130.35 (2CH), 130.70 (2CH), 130.86 (C), 140.50 (2C), 153.38 (C), 160.96 (C), 167.17 (C).

MS (ESI)(m/z): ($M^+ + 1$) 454.

For $C_{20}H_{19}N_7O_2S_2$ calculated: C, 52.96; H, 4.22; N, 21.62; Found: C, 52.95; H, 4.20; N, 21.63.

*5.1.1.4.13. N′-[(4-Fluorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-methyl-1H-tetrazol-5-yl)thio]acetohydrazide (**13**).* IR (KBr) ν_{max} (cm^{-1}): 3503.26 (N–H stretching), 3116.58 (Aromatic C–H stretching), 1715.79 (C=O stretching), 1602.24, 1566.64, 1496.42, 1455.49 (C=N, C=C stretching and N–H bending), 1407.64, 1371.82 (C–H bending), 1278.53, 1228.28 (C–N stretching), 1173.37, 1160.55, 1101.28, 1075.68, 1025.17, 1010.42 (Aromatic C–H in plane bending), 963.54, 889.11, 855.21, 822.06, 792.38, 777.12, 757.78, 745.09, 703.94, 684.82, 656.09 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.91 (3H, s, CH_3), 4.19 and 4.24 (2H, 2s, S–CH_2), 6.99 (1H, s, thiazoline), 7.25–7.57 (9H, m, phenyl, 4-fluorophenyl), 12.04 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 34.40 (CH_3), 35.60 (CH_2), 102.50 (CH), 116.63 (2CH), 124.01 (CH), 124.40 (2CH), 128.81 (C), 131.09 (2CH), 131.79 (CH), 131.89 (CH), 140.19 (3C), 153.37 (C), 162.52 (C), 167.17 (C).

MS (ESI)(m/z): ($M^+ + 1$) 442.

For $C_{19}H_{16}FN_7OS_2$ calculated: C, 51.69; H, 3.65; N, 22.21; Found: C, 51.70; H, 3.65; N, 22.20.

*5.1.1.4.14. N′-[(4-Chlorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-methyl-1H-tetrazol-5-yl)thio]acetohydrazide (**14**).* IR (KBr) ν_{max} (cm^{-1}): 2879.95 (Aliphatic C–H stretching), 1715.30 (C=O stretching), 1571.56, 1458.25 (C=N, C=C stretching and N–H bending), 1092.79, 1011.35 (Aromatic C–H in plane bending), 752.01 (Aromatic C–H out of plane bending).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.91 (3H, s, CH_3), 4.23 (2H, d, $J = 12$ Hz, S–CH_2), 7.03 (1H, s, thiazoline), 7.33–7.56 (9H, m, phenyl, 4-chlorophenyl), 12.19 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 34.39 (CH_3), 35.49 (CH_2), 102.40 (CH), 124.11 (2CH), 126.63 (CH), 128.34 (2CH), 129.51 (C), 130.94 (2CH), 131.03 (2CH), 135.72 (2C), 139.82 (2C), 153.41 (C), 167.25 (C).

MS (ESI)(m/z): ($M^+ + 1$) 458.

For $C_{19}H_{16}ClN_7OS_2$ calculated: C, 49.83; H, 3.52; N, 21.41; Found: C, 49.82; H, 3.50; N, 21.42.

*5.1.1.4.15. N′-[(4-Bromophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-methyl-1H-tetrazol-5-yl)thio]acetohydrazide (**15**).* IR (KBr) ν_{max} (cm^{-1}): 3049.57 (Aromatic C–H stretching), 2878.02 (Aliphatic C–H stretching), 1710.14 (C=O stretching), 1609.00, 1600.29, 1571.18, 1488.78, 1463.73 (C=N, C=C stretching and N–H bending), 1398.02 (C–H bending), 1286.25, 1230.88 (C–N stretching), 1195.72, 1170.19, 1115.87, 1071.96, 1027.90, 1004.77 (Aromatic C–H in plane bending), 958.17, 915.24, 887.94, 827.97, 805.46, 748.09, 713.05, 700.50, 690.56 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.91 (3H, s, CH_3), 4.24 (2H, d, $J = 9$ Hz, S–CH_2), 7.03 (1H, s, thiazoline), 7.33–7.66 (9H, m, phenyl, 4-bromophenyl), 12.05 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 34.41 (CH_3), 35.56 (CH_2), 103.04 (CH), 124.30 (C), 124.59 (CH), 126.79 (2CH), 128.65 (2CH), 131.06 (C), 131.16 (2CH), 132.43 (2CH), 139.96 (3C), 153.40 (C), 167.22 (C).

MS (ESI)(m/z): ($M^+ + 1$) 503.

For $C_{19}H_{16}BrN_7OS_2$ calculated: C, 45.42; H, 3.21; N, 19.52; Found: C, 45.42; H, 3.21; N, 19.52.

*5.1.1.4.16. N′-[(4-Cyanophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-methyl-1H-tetrazol-5-yl)thio]acetohydrazide (**16**).* IR (KBr) ν_{max} (cm^{-1}): 3429.20 (N–H stretching), 3058.89 (Aromatic C–H stretching), 2894.95 (Aliphatic C–H stretching), 2227.63 (C≡N stretching), 1720.39 (C=O stretching), 1604.66, 1571.88, 1477.37 (C=N, C=C stretching and N–H bending), 1176.50 (Aromatic C–H in plane bending), 856.34 (Aromatic C–H out of plane bending), 667.32 (C–S stretching).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.91 (3H, s, CH_3), 4.22 (2H, d, $J = 16$ Hz, S–CH_2), 6.96–7.11 (1H, thiazoline), 7.28–8.04 (9H, m, phenyl, 4-cyanophenyl), 12.02 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 33.56 (CH_3), 34.56 (CH_2), 110.88 (CH), 112.20 (C), 118.25 (C), 122.67 (CH), 128.77 (2CH), 128.93 (2CH), 130.08 (2CH), 131.44 (CH), 131.80 (CH), 132.44 (C), 138.22 (3C), 152.55 (C), 166.47 (C).

MS (ESI)(m/z): ($M^+ + 1$) 449.

For $C_{20}H_{16}N_8OS_2$ calculated: C, 53.56; H, 3.60; N, 24.98; Found: C, 53.55; H, 3.63; N, 24.98.

*5.1.1.4.17. N′-[(3,4-Diphenylthiazol-2(3H)-ylidene)]-2-[(1-phenyl-1H-tetrazol-5-yl)thio]acetohydrazide (**17**).* IR (KBr) ν_{max} (cm^{-1}): 3108.98 (Aromatic C–H stretching), 1671.06 (C=O stretching), 1616.69, 1578.50, 1551.16, 1497.07 (C=N, C=C stretching and N–H bending), 1445.30, 1386.11, 1357.86 (C–H bending), 1300.61, 1242.75 (C–N stretching), 1155.47, 1090.59, 1074.06, 1014.15 (Aromatic C–H in plane bending), 966.93, 916.68, 830.89, 758.47, 691.94 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 4.20–4.29 (2H, m, S–CH_2), 6.97–7.71 (16H, m, phenyl, thiazoline), 10.46 and 11.34 (1H, 2s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 35.30 (CH_2), 102.50 (CH), 121.93 (CH), 125.05 (2CH), 125.16 (2CH), 128.30 (CH), 128.94 (2CH), 129.16 (2CH), 129.27 (CH), 129.63 (CH), 129.81 (CH), 130.26 (2CH), 130.74 (C), 133.62 (C), 139.66 (C), 140.40 (2C), 154.03 (C), 167.04 (C).

MS (ESI)(m/z): ($M^+ + 1$) 486.

For $C_{24}H_{19}N_7OS_2$ calculated: C, 59.36; H, 3.94; N, 20.19; Found: C, 59.35; H, 3.95; N, 20.21.

*5.1.1.4.18. N′-[(4-Nitrophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-phenyl-1H-tetrazol-5-yl)thio]acetohydrazide (**18**).* IR (KBr) ν_{max} (cm^{-1}): 3051.47 (Aromatic C–H stretching), 2998.42, 2865.67, 2713.80 (Aliphatic C–H stretching), 1706.42 (C=O stretching), 1601.19, 1583.64, 1567.32, 1518.32, 1493.90, 1459.06 (C=N, C=C stretching and N–H bending), 1432.77, 1405.65, 1385.86, 1340.41 (C–H bending), 1281.46, 1236.60 (C–N stretching), 1205.51, 1179.21, 1116.79, 1107.17, 1077.76, 1059.36, 1026.94, 1012.84, 1003.01 (Aromatic C–H in plane bending), 986.22, 964.12, 921.26, 887.34, 868.17, 850.76, 840.40, 807.86, 761.05, 738.39, 710.65, 683.77, 656.04 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 4.32 (2H, s, S–CH_2), 7.13–7.68 (15H, m, thiazoline, phenyl, 4-nitrophenyl), 12.04 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 35.41 (CH_2), 104.37 (CH), 123.96 (CH), 124.39 (2CH), 124.88 (2CH), 130.43 (2CH), 130.78 (2CH), 130.97 (2CH), 131.40 (2CH), 133.51 (CH), 133.93 (C), 138.99 (2C), 148.67 (3C), 153.86 (C), 166.92 (C).

MS (ESI)(m/z): ($M^+ + 1$) 531.

For $C_{24}H_{18}N_8O_3S_2$ calculated: C, 54.33; H, 3.42; N, 21.12; Found: C, 54.35; H, 3.40; N, 21.11.

*5.1.1.4.19. N′-[(4-Methylphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-phenyl-1H-tetrazol-5-yl)thio]acetohydrazide (**19**).* IR (KBr) ν_{max} (cm^{-1}): 3054.94 (Aromatic C–H stretching), 2865.96 (Aliphatic C–H stretching), 1719.31 (C=O stretching), 1604.11, 1595.64, 1566.99, 1511.36, 1497.15, 1458.35 (C=N, C=C stretching and N–H bending), 1430.63, 1400.19, 1388.33, 1372.96 (C–H bending), 1335.31, 1304.67, 1276.09, 1241.89 (C–N stretching), 1198.38, 1126.98, 1091.18, 1075.17, 1059.47, 1028.44, 1012.88, 1004.22 (Aromatic C–H in plane bending), 977.13, 959.48, 917.70, 881.47, 842.12, 813.89, 793.92, 744.85, 756.30, 693.44 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 2.30 (3H, s, CH_3), 4.36 (2H, d, J = 9 Hz, S–CH_2), 6.98 (1H, s, thiazoline), 7.18–7.68 (14H, m, phenyl, 4-methylphenyl), 12.16 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 21.64 (CH_3), 35.59 (CH_2), 102.08 (CH), 124.46 (CH), 124.73 (2CH), 124.98 (2CH), 128.81 (C), 129.10 (CH), 129.93 (2CH), 130.85 (2CH), 131.09 (2CH), 131.46 (2CH), 133.55 (2C), 140.69 (C), 141.30 (2C), 153.92 (C), 166.87 (C).

MS (ESI)(m/z): ($M^+ + 1$) 500.

For $C_{25}H_{21}N_7OS_2$ calculated: C, 60.10; H, 4.24; N, 19.62; Found: C, 60.12; H, 4.25; N, 19.63.

*5.1.1.4.20. N′-[(4-Methoxyphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-phenyl-1H-tetrazol-5-yl)thio]acetohydrazide (**20**).* IR (KBr) ν_{max} (cm^{-1}): 3047.43, 3003.90 (Aromatic C–H stretching), 2839.26, 2755.74 (Aliphatic C–H stretching), 1712.55 (C=O stretching), 1599.73, 1575.50, 1561.78, 1497.12, 1457.11 (C=N, C=C stretching and N–H bending), 1442.55, 1427.94, 1403.56, 1385.67 (C–H bending), 1317.02, 1280.76, 1249.12, 1239.06 (C–N stretching), 1203.67 (C–O stretching), 1181.70, 1155.57, 1110.39, 1079.63, 1063.49, 1041.70, 1024.21 (Aromatic C–H in plane bending), 986.34, 964.04, 916.01, 889.10, 836.98, 812.86, 782.06, 761.19, 744.75, 716.73, 699.26, 685.00 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.78 (3H, s, OCH_3), 4.36 (2H, d, J = 9 Hz, S–CH_2), 6.92–6.96 (3H, m, thiazoline, 4-methoxyphenyl), 7.36–7.70 (12H, m, phenyl, 4-methoxyphenyl), 12.14 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 35.53 (CH_2), 55.94 (CH_3, OCH_3), 101.40 (CH), 114.78 (2CH), 119.68 (CH), 121.80 (C), 124.43 (2CH), 124.98 (2CH), 130.81 (3CH), 131.07 (2CH), 131.46 (2CH), 133.50 (C), 141.17 (3C), 153.95 (C), 161.23 (C), 166.88 (C).

MS (ESI)(m/z): ($M^+ + 1$) 516.

For $C_{25}H_{21}N_7O_2S_2$ calculated: C, 58.24; H, 4.11; N, 19.02; Found: C, 58.25; H, 4.13; N, 19.01.

*5.1.1.4.21. N′-[(4-Fluorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-phenyl-1H-tetrazol-5-yl)thio]acetohydrazide (**21**).* IR (KBr) ν_{max} (cm^{-1}): 3042.79 (Aromatic C–H stretching), 2878.76, 2789.99 (Aliphatic C–H stretching), 1718.32 (C=O stretching), 1601.64, 1580.79, 1563.79, 1495.43, 1459.73 (C=N, C=C stretching and N–H bending), 1433.58, 1410.86, 1392.56 (C–H bending), 1302.22, 1278.20, 1229.76 (C–N stretching), 1197.61, 1161.24, 1126.60, 1090.12, 1072.59, 1055.48, 1022.55, 1011.93 (Aromatic C–H in plane bending), 972.60, 960.57, 919.97, 910.78, 881.99, 834.64, 824.13, 799.66, 756.33, 694.23, 685.64 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 4.35 (2H, d, J = 9 Hz, S–CH_2), 7.00 (1H, s, thiazoline), 7.22–7.68 (14H, m, phenyl, 4-fluorophenyl), 12.12 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 34.70 (CH_2), 101.58 (CH), 115.57 (CH), 115.79 (CH), 123.36 (CH), 123.48 (2CH), 124.21 (2CH), 127.74 (C), 130.04 (2CH), 130.26 (CH), 130.67 (2CH), 130.98 (CH), 131.07 (CH), 132.74 (C), 139.35 (3C), 153.15 (C), 161.68 (C), 166.14 (C).

MS (ESI)(m/z): ($M^+ + 1$) 504.

For $C_{24}H_{18}FN_7OS_2$ calculated: C, 57.24; H, 3.60; N, 19.47; Found: C, 57.23; H, 3.59; N, 19.46.

*5.1.1.4.22. N′-[(4-Chlorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-phenyl-1H-tetrazol-5-yl)thio]acetohydrazide (**22**).* IR (KBr) ν_{max} (cm^{-1}): 3041.64 (Aromatic C–H stretching), 2873.45 (Aliphatic C–H stretching), 1715.75 (C=O stretching), 1611.51, 1595.09, 1570.70, 1557.71, 1489.96, 1458.35 (C=N, C=C stretching and N–H bending), 1427.60, 1402.09, 1390.57 (C–H bending), 1300.05, 1278.84, 1241.02 (C–N stretching), 1198.92, 1125.86, 1090.33, 1073.86, 1055.84, 1025.05, 1010.90 (Aromatic C–H in plane bending), 975.60, 959.49, 913.82, 882.48, 847.42, 830.06, 808.10, 778.74, 751.28, 709.97, 691.25, 683.47 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 4.35 (2H, d, J = 6 Hz, S–CH_2), 7.03 (1H, s, thiazoline), 7.33–7.70 (14H, m, phenyl, 4-chlorophenyl), 12.09 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 35.41 (CH_2), 102.00 (CH), 124.99 (2CH), 126.77 (3CH), 129.45 (2CH), 130.82 (C), 130.88 (2CH), 130.96 (3CH), 131.45 (2CH), 133.54 (2C), 135.63 (C), 139.73 (2C), 153.96 (C), 167.01 (C).

MS (ESI)(m/z): ($M^+ + 1$) 520.

For $C_{24}H_{18}ClN_7OS_2$ calculated: C, 55.43; H, 3.49; N, 18.85; Found: C, 55.42; H, 3.51; N, 18.84.

*5.1.1.4.23. N′-[(4-Bromophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-phenyl-1H-tetrazol-5-yl)thio]acetohydrazide (**23**).* IR (KBr) ν_{max} (cm^{-1}): 3053.59 (Aromatic C–H stretching), 2873.39

(Aliphatic C–H stretching), 1715.31 (C=O stretching), 1611.83, 1595.08, 1570.56, 1495.96, 1457.97 (C=N, C=C stretching and N–H bending), 1399.60 (C–H bending), 1299.59, 1279.05, 1247.77 (C–N stretching), 1199.44, 1124.69, 1087.91, 1073.75, 1056.14, 1023.65, 1007.92 (Aromatic C–H in plane bending), 976.79, 958.57, 912.47, 882.81, 846.65, 828.60, 807.02, 751.10, 711.60, 699.15, 689.58, 682.91 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 4.35 (2H, d, $J = 3$ Hz, S–CH_2), 6.99 (1H, s, thiazoline), 7.32–7.70 (14H, m, phenyl, 4-bromophenyl), 12.02 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 35.51 (CH_2), 103.00 (CH), 124.20 (C), 124.56 (CH), 125.00 (2CH), 125.07 (2CH), 126.90 (2CH), 129.01 (CH), 130.83 (2CH), 131.02 (C), 131.15 (2CH), 131.46 (2CH), 132.40 (C), 133.53 (C), 139.95 (2C), 153.97 (C), 166.99 (C).

MS (ESI)(m/z): ($M^+ + 2$) 566.

For $C_{24}H_{18}BrN_7OS_2$ calculated: C, 51.07; H, 3.21; N, 17.37; Found: C, 51.05; H, 3.20; N, 17.36.

*5.1.1.4.24. N′-[(4-Cyanophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(1-phenyl-1H-tetrazol-5-yl)thio]acetohydrazide (**24**).* IR (KBr) ν_{max} (cm^{-1}): 3438.84 (N–H stretching), 3053.11 (Aromatic C–H stretching), 2864.09, 2752.23 (Aliphatic C–H stretching), 2227.63 (C≡N stretching), 1708.81 (C=O stretching), 1602.74, 1577.66, 1496.66 (C=N, C=C stretching and N–H bending), 1406.01 (C–H bending), 1284.50 (C–N stretching), 1116.71 (Aromatic C–H in plane bending), 854.41, 759.90, 694.33 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 4.42 (2H, d, $J = 10.4$ Hz, S–CH_2), 7.28 (1H, s, thiazoline), 7.40–7.43 (3H, m, phenyl), 7.55–7.59 (5H, m, phenyl), 7.64–7.73 (4H, m, phenyl, 4-cyanophenyl), 7.90 (2H, d, $J = 8.4$ Hz, 4-cyanophenyl), 12.37 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 34.73 (CH_2), 103.48 (CH), 112.52 (C), 118.21 (C), 123.31 (CH), 124.18 (2CH), 127.58 (2CH), 129.11 (2CH), 129.70 (CH), 130.04 (2CH), 130.22 (2CH), 130.66 (2CH), 132.44 (C), 132.74 (C), 138.60 (3C), 153.11 (C), 166.14 (C).

MS (ESI)(m/z): ($M^+ + 1$) 511.

For $C_{25}H_{18}N_8OS_2$ calculated: C, 58.81; H, 3.55; N, 21.95; Found: C, 58.82; H, 3.52; N, 21.97.

*5.1.1.4.25. N′-[(3,4-Diphenylthiazol-2(3H)-ylidene)]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide (**25**) [36].* IR (KBr) ν_{max} (cm^{-1}): 3089.35 (Aromatic C–H stretching), 2867.03 (Aliphatic C–H stretching), 1706.41 (C=O stretching), 1601.98, 1581.41, 1518.32, 1490.97 (C=N, C=C stretching and N–H bending), 1443.60, 1385.33, 1342.49 (C–H bending), 1282.24, 1236.05 (C–N stretching), 1195.45, 1153.90, 1116.47, 1076.69, 1026.93, 1012.08 (Aromatic C–H in plane bending), 959.19, 921.19, 888.11, 868.06, 850.63, 807.40, 761.50, 738.33, 690.85 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 2.61 and 2.68 (3H, 2s, CH_3), 4.05–4.12 (2H, m, S–CH_2), 6.96–7.43 (11H, m, thiazoline, phenyl), 10.36 and 11.29 (1H, 2s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 15.96 (CH_3), 36.09 (CH_2), 102.84 (CH), 124.51 (3CH), 127.54 (CH), 129.29 (2CH), 129.43 (2CH), 130.89 (2CH), 131.09 (C), 138.88 (C), 141.29 (3C), 164.45 (C), 166.52 and 167.14 (C).

MS (ESI)(m/z): ($M^+ + 1$) 440.

For $C_{20}H_{17}N_5OS_3$ calculated: C, 54.65; H, 3.90; N, 15.93; Found: C, 54.66; H, 3.92; N, 15.90.

*5.1.1.4.26. N′-[(4-Nitrophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide (**26**).* IR (KBr) ν_{max} (cm^{-1}): 3068.23 (Aromatic C–H stretching), 2902.74 (Aliphatic C–H stretching), 1725.01 (C=O stretching), 1603.82, 1566.58, 1513.90, 1495.24, 1475.36, 1459.37 (C=N, C=C stretching and N–H bending), 1409.26, 1381.52, 1346.75 (C–H bending), 1291.94, 1232.17 (C–N stretching), 1191.79, 1165.64, 1121.52, 1108.97, 1069.62, 1022.03, 1013.07 (Aromatic C–H in plane bending), 954.42, 909.14, 888.78, 865.71, 852.39, 837.60, 810.92, 778.23, 765.70, 740.97, 708.49, 691.18 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 2.59 (3H, s, CH_3), 4.21 (2H, d, $J = 9$ Hz, S–CH_2), 7.14 (1H, s, thiazoline), 7.27–7.54 (5H, m, phenyl), 7.74 (2H, d, $J = 9$ Hz, 4-nitrophenyl), 8.24 (2H, d, $J = 9$ Hz, 4-nitrophenyl), 11.99 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 15.71 (CH_3), 35.48 (CH_2), 102.80 (CH), 123.63 (CH), 124.39 (2CH), 127.64 (2CH), 130.22 (2CH), 130.91 (2CH), 134.21 (C), 138.87 (2C), 148.51 (3C), 164.15 (C), 166.26 and 167.35 (C).

MS (ESI)(m/z): ($M^+ + 1$) 485.

For $C_{20}H_{16}N_6O_3S_3$ calculated: C, 49.57; H, 3.33; N, 17.34; Found: C, 49.55; H, 3.34; N, 17.36.

*5.1.1.4.27. N′-[(4-Methylphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide (**27**).* IR (KBr) ν_{max} (cm^{-1}): 3037.33 (Aromatic C–H stretching), 2890.43 (Aliphatic C–H stretching), 1706.73 (C=O stretching), 1602.97, 1557.64, 1509.59, 1480.58, 1455.68 (C=N, C=C stretching and N–H bending), 1427.62, 1370.00 (C–H bending), 1259.28, 1229.94 (C–N stretching), 1214.55, 1186.58, 1073.63, 1046.71, 1030.98, 1017.64, 1002.56 (Aromatic C–H in plane bending), 955.19, 880.59, 839.81, 819.18, 808.22, 781.67, 759.38, 739.61, 694.39 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 2.34 (3H, s, CH_3), 2.66 (3H, s, CH_3, thiadiazole), 4.23 (2H, s, S–CH_2), 6.98 (1H, s, thiazoline), 7.22–7.59 (9H, m, phenyl, 4-methylphenyl), 12.11 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 15.91 (CH_3), 21.65 (CH_3), 35.93 (CH_2), 102.05 (CH), 124.48 (3CH), 124.72 (C), 128.84 (2CH), 129.11 (2CH), 129.96 (2CH), 131.08 (C), 140.66 (C), 141.37 (3C), 164.47 (C), 166.42 and 167.15 (C).

MS (ESI)(m/z): ($M^+ + 1$) 454.

For $C_{21}H_{19}N_5OS_3$ calculated: C, 55.60; H, 4.22; N, 15.44; Found: C, 55.61; H, 4.20; N, 15.45.

*5.1.1.4.28. N′-[(4-Methoxyphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide (**28**).* IR (KBr) ν_{max} (cm^{-1}): 2998.70, 2901.02 (Aliphatic C–H stretching), 1717.46 (C=O stretching), 1602.24, 1560.36, 1509.10, 1489.85, 1455.92 (C=N, C=C stretching and N–H bending), 1424.03, 1397.60, 1372.00, 1354.23 (C–H bending), 1294.48, 1251.05, 1226.20 (C–N stretching), 1182.55, 1133.69, 1064.62, 1035.52, 1020.08 (Aromatic C–H in plane bending), 955.01, 884.79, 852.09, 838.10, 822.02, 783.55, 762.97, 743.91, 695.88 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 2.65 (3H, s, CH_3), 3.80 (3H, s, OCH_3), 4.24 (2H, s, S–CH_2), 6.96–6.99 (3H, m, thiazoline, 4-methoxyphenyl), 7.39–7.59 (7H, m, phenyl, 4-methoxyphenyl), 12.13 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 15.84 (CH_3), 36.04 (CH_2), 56.04 (CH_3), 101.66 (CH), 114.85 (2CH), 119.71 (CH), 124.55 (C), 128.93 (2CH), 130.88 (2CH), 131.10 (2CH), 138.79 (C), 141.30 (3C), 161.31 (C), 164.49 (C), 166.49 and 167.15 (C).

MS (ESI)(m/z): ($M^+ + 1$) 470.

For $C_{21}H_{19}N_5O_2S_3$ calculated: C, 53.71; H, 4.08; N, 14.91; Found : C, 53.70; H, 4.10; N, 14.92.

*5.1.1.4.29. N′-[(4-Fluorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide (**29**).* IR (KBr) ν_{max} (cm^{-1}): 3058.84 (Aromatic C–H stretching), 2899.54 (Aliphatic C–H stretching), 1708.38 (C=O stretching), 1601.57, 1582.91, 1563.21, 1506.61, 1493.66, 1450.70 (C=N, C=C stretching and N–H bending), 1384.77, 1351.30 (C–H bending), 1225.19 (C–N stretching), 1185.93, 1163.54, 1103.92, 1070.29, 1026.05 (Aromatic C–H in plane bending), 883.45, 856.84, 821.97, 791.26, 777.42, 746.95, 694.88 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 2.65 (3H, s, CH_3), 4.22 (2H, s, S–CH_2), 7.00 (1H, s, thiazoline), 7.24–7.57 (9H, m, phenyl, 4-fluorophenyl), 12.04 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 15.83 (CH_3), 35.89 (CH_2), 102.82 (CH), 116.36 (CH), 116.58 (CH), 121.75 (CH), 124.39 (2CH), 128.75 (C), 130.48 (2CH), 131.06 (CH), 131.91 (CH), 137.88 (C), 140.28 (3C), 162.50 (C), 164.29 (C), 166.44 and 167.21 (C).

MS (ESI)(m/z): ($M^+ + 1$) 458.

For $C_{20}H_{16}FN_5OS_3$ calculated: C, 52.50; H, 3.52; N, 15.31; Found: C, 52.52; H, 3.51; N, 15.29.

*5.1.1.4.30. N′-[(4-Chlorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide (**30**).* IR (KBr) ν_{max} (cm^{-1}): 3468.63 (N–H stretching), 3056.87 (Aromatic C–H stretching), 2962.51, 2918.10 (Aliphatic C–H stretching), 1708.68 (C=O stretching), 1600.34, 1567.72, 1557.93, 1489.71, 1451.18 (C=N, C=C stretching and N–H bending), 1428.07, 1406.25, 1383.78, 1350.71 (C–H bending), 1225.28 (C–N stretching), 1186.78, 1155.33, 1087.85, 1070.70, 1052.86, 1026.74, 1010.63 (Aromatic C–H in plane bending), 949.91, 918.06, 883.88, 854.58, 832.85, 806.38, 779.61, 759.95, 749.89, 707.23, 694.01 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 2.67 (3H, s, CH_3), 4.23 (2H, s, S–CH_2), 7.03 (1H, s, thiazoline), 7.34–7.57 (9H, m, phenyl, 4-chlorophenyl), 12.04 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 15.89 (CH_3), 35.69 (CH_2), 102.81 (CH), 121.53 (2CH), 124.07 (CH), 126.51 (2CH), 128.43 (C), 129.46 (2CH), 130.42 (2CH), 131.02 (C), 135.65 (C), 139.90 (3C), 164.29 (C), 166.41 and 167.25 (C).

MS (ESI)(m/z): ($M^+ + 1$) 474.

For $C_{20}H_{16}ClN_5OS_3$ calculated: C, 50.68; H, 3.40; N, 14.77; Found: C, 50.69; H, 3.42; N, 14.76.

*5.1.1.4.31. N′-[(4-Bromophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide (**31**).* IR (KBr) ν_{max} (cm^{-1}): 3061.03 (Aromatic C–H stretching), 2882.43, 2750.05 (Aliphatic C–H stretching), 1716.93 (C=O stretching), 1597.84, 1587.35, 1571.34, 1555.38, 1508.37, 1482.05, 1453.65 (C=N, C=C stretching and N–H bending), 1421.97, 1396.34, 1379.30 (C–H bending), 1260.27 (C–N stretching), 1217.93, 1186.32, 1127.90, 1073.22, 1060.82, 1031.15, 1007.78 (Aromatic C–H in plane bending), 970.35, 957.25, 925.19, 878.29, 852.92, 830.73, 810.64, 772.15, 755.12, 701.88, 670.49 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 2.68 (3H, s, CH_3), 4.24 (2H, s, S–CH_2), 7.05 (1H, s, thiazoline), 7.34–7.64 (9H, m, phenyl, 4-bromophenyl), 12.04 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 16.06 (CH_3), 35.89 (CH_2), 103.31 (CH), 124.43 (C), 124.64 (CH), 126.73 (2CH), 128.81 (2CH), 131.09 (C), 131.25 (2CH), 132.19 (2CH), 132.43 (C), 140.15 (3C), 164.34 (C), 166.44 and 167.28 (C).

MS (ESI)(m/z): ($M^+ + 1$) 519.

For $C_{20}H_{16}BrN_5OS_3$ calculated: C, 46.33; H, 3.11; N, 13.51; Found: C, 46.34; H, 3.12; N, 13.50.

*5.1.1.4.32. N′-[(4-Cyanophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)thio]acetohydrazide (**32**).* IR (KBr) ν_{max} (cm^{-1}): 3444.63 (N–H stretching), 3045.39 (Aromatic C–H stretching), 2902.67 (Aliphatic C–H stretching), 2223.77 (C≡N stretching), 1724.24 (C=O stretching), 1610.45, 1569.95, 1477.37 (C=N, C=C stretching and N–H bending), 1382.87 (C–H bending), 1292.22 (C–N stretching), 1191.93, 1122.49 (Aromatic C–H in plane bending), 833.19, 757.97 (Aromatic C–H out of plane bending).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 2.69 (3H, s, CH_3), 4.29 (2H, d, J = 5.2 Hz, S–CH_2), 7.27 (1H, s, thiazoline), 7.42 (3H, d, J = 7.2 Hz, phenyl), 7.57 (2H, t, J_1 = 7.6 Hz, J_2 = 8 Hz, phenyl), 7.70 (2H, d, J = 8.4 Hz, 4-cyanophenyl), 7.88–7.90 (2H, d, J = 8 Hz, 4-cyanophenyl), 11.51 and 12.28 (1H, 2s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 15.13 (CH_3), 34.92 (CH_2), 103.47 (CH), 112.37 (C), 118.24 (C), 120.80 (CH), 123.32 (2CH), 127.61 (2CH), 129.09 (2CH), 130.23 (2CH), 132.44 (C), 138.66 (4C), 163.40 (C), 165.57 and 166.50 (C).

MS (ESI)(m/z): ($M^+ + 1$) 465.

For $C_{21}H_{16}N_6OS_3$ calculated: C, 54.29; H, 3.47; N, 18.09; Found: C, 54.32; H, 3.45; N, 18.11.

*5.1.1.4.33. N′-[(3,4-Diphenylthiazol-2(3H)-ylidene)]-2-[(pyrimidin-2-yl)thio]acetohydrazide (**33**).* IR (KBr) ν_{max} (cm^{-1}): 3116.21 (Aromatic C–H stretching), 2927.91 (Aliphatic C–H stretching), 1673.26 (C=O stretching), 1618.49, 1583.35, 1550.63, 1489.09 (C=N, C=C stretching and N–H bending), 1445.64, 1379.64 (C–H bending), 1304.73, 1239.08 (C–N stretching), 1172.91, 1072.89, 1014.78 (Aromatic C–H in plane bending), 958.97, 925.10, 881.62, 829.55, 806.50, 766.78, 747.03, 727.95, 693.79 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.91 and 3.94 (2H, 2s, S–CH_2), 6.98–7.44 (12H, m, thiazoline, phenyl, pyrimidine), 8.48 (1H, d, J = 6 Hz, pyrimidine), 8.66 (1H, d, J = 3 Hz, pyrimidine), 10.30 and 11.16 (1H, 2s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 33.03 (CH_2), 101.00 (CH), 118.09 (CH), 121.61 (CH), 121.88 (2CH), 128.28 (CH), 128.94 (CH), 128.99 (CH), 129.26 (2CH), 129.63 (CH), 129.68 (CH), 130.25 (C), 139.95 (C), 140.38 (2C), 158.34 (CH), 158.50 (CH), 168.16 (C), 170.20 (C).

MS (ESI)(m/z): ($M^+ + 1$) 420.

For $C_{21}H_{17}N_5OS_2$ calculated: C, 60.12; H, 4.08; N, 16.69; Found: C, 60.10; H, 4.11; N, 16.71.

*5.1.1.4.34. N′-[(4-Nitrophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(pyrimidin-2-yl)thio]acetohydrazide (**34**).* IR (KBr) ν_{max} (cm^{-1}): 3099.76, 3048.66 (Aromatic C–H stretching), 2916.81 (Aliphatic C–H stretching), 1720.72 (C=O stretching), 1602.77, 1588.89, 1571.48, 1552.18, 1513.61, 1491.23, 1455.61 (C=N, C=C stretching and N–H bending), 1445.73, 1351.48 (C–H bending), 1318.33, 1287.98, 1253.72 (C–N stretching), 1195.34, 1173.10, 1158.16, 1116.35, 1075.68, 1026.26, 1002.42 (Aromatic C–H in plane bending), 920.62, 866.11, 852.66, 810.07, 764.91, 739.15, 691.88 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.93 and 4.08 (2H, 2d, J_1, J_2 = 15 Hz, S–CH_2), 7.11–7.56 (7H, m, thiazoline, phenyl, pyrimidine), 7.70 (2H, d, J = 9 Hz, 4-nitrophenyl), 8.08 (2H, d, J = 9 Hz, 4-nitrophenyl), 8.45 (2H, d, J = 6 Hz, pyrimidine), 11.77 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 33.20 (CH_2), 104.77 (CH), 118.10 (CH), 124.06 (3CH), 124.14 (2CH), 128.48 (2CH), 130.51 (CH), 131.08 (CH), 133.97 (C), 139.24 (C), 148.47 (3C), 158.36 (2CH), 168.27 (C), 169.83 (C).

MS (ESI)(m/z): ($M^+ + 1$) 465.

For $C_{21}H_{16}N_6O_3S_2$ calculated: C, 54.30; H, 3.47; N, 18.09; Found: C, 54.32; H, 3.46; N, 18.11.

*5.1.1.4.35. N′-[(4-Methylphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(pyrimidin-2-yl)thio]acetohydrazide (**35**).* IR (KBr) ν_{max} (cm^{-1}): 3178.95 (N–H stretching), 1678.05 (C=O stretching), 1609.67, 1584.42, 1553.13, 1489.35 (C=N, C=C stretching and N–H bending), 1446.33, 1380.68 (C–H bending), 1307.59, 1235.57 (C–N stretching), 1183.19, 1070.10, 1027.89, 1015.99 (Aromatic C–H in plane bending), 956.22, 883.18, 811.97, 765.62, 746.57, 695.04 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 2.30 (3H, s, CH_3), 3.93 (2H, s, S–CH_2), 6.34 (1H, phenyl), 6.97–7.39 (10H, m, thiazoline, phenyl, 4-methylphenyl, pyrimidine), 8.49 (2H, d, J = 3 Hz, pyrimidine), 11.09 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 21.55 (CH_3), 33.02 (CH_2), 95.00 (CH), 118.06 (CH), 121.96 (3CH), 128.21 (C), 129.55 (2CH), 129.69 (2CH), 130.29 (2CH), 139.24 (2C), 140.05 (2C), 158.31 (2CH), 168.15 (C), 170.18 (C).

MS (ESI)(m/z): ($M^+ + 1$) 434.

For $C_{22}H_{19}N_5OS_2$ calculated: C, 60.95; H, 4.42; N, 16.15; Found: C, 60.95; H, 4.40; N, 16.16.

*5.1.1.4.36. N′-[(4-Methoxyphenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(pyrimidin-2-yl)thio]acetohydrazide (**36**).* IR (KBr) ν_{max} (cm^{-1}): 3458.23 (N–H stretching), 3090.59, 3061.82, 3006.60 (Aromatic C–H stretching), 2906.63 (Aliphatic C–H asymmetric stretching), 2839.64 (Aliphatic C–H symmetric stretching), 1707.44 (C=O stretching), 1603.05, 1571.51, 1548.99, 1509.43, 1495.36, 1455.61 (C=N, C=C stretching and N–H bending), 1426.26, 1381.49, 1363.53 (C–H bending), 1309.03, 1296.15, 1249.38, 1228.42 (C–N stretching), 1204.89 (C–O stretching), 1180.18, 1147.29, 1070.66, 1049.92, 1037.73, 1070.66, 1021.86 (Aromatic C–H in plane bending), 919.16, 884.81, 847.85, 831.51, 814.28, 781.39, 756.20, 736.26, 718.95, 693.25 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.77 (3H, s, OCH_3), 4.01 and 4.13 (2H, 2d, J_1, J_2 = 15 Hz, S–CH_2), 6.81–6.84 (3H, m, phenyl, 4-methoxyphenyl), 6.89 (1H, s, thiazoline), 7.18–7.58 (7H, m, phenyl, 4-methoxyphenyl, pyrimidine), 8.49 (2H, d, J = 6 Hz, pyrimidine), 11.97 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 33.29 (CH_2), 55.97 (CH_3), 102.00 (CH), 114.52 (2CH), 118.18 (2CH), 119.62 (C), 124.61 (2CH), 130.81 (2CH), 131.12 (2CH), 138.45 (C), 141.57 (2C), 158.37 (2CH), 161.08 (C), 168.07 (C), 169.83 (C).

MS (ESI)(m/z): ($M^+ + 1$) 450.

For $C_{22}H_{19}N_5O_2S_2$ calculated: C, 58.78; H, 4.26; N, 15.58; Found: C, 58.80; H, 4.25; N, 15.58.

*5.1.1.4.37. N′-[(4-Fluorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(pyrimidin-2-yl)thio]acetohydrazide (**37**).* IR (KBr) ν_{max} (cm^{-1}): 3439.55, 3214.83 (N–H stretching), 3111.10 (Aromatic C–H stretching), 2897.96 (Aliphatic C–H stretching), 1710.06 (C=O stretching), 1605.03, 1582.01, 1565.56, 1548.99, 1507.25, 1496.78, 1468.84 (C=N, C=C stretching and N–H bending), 1449.85, 1413.21, 1379.79, 1364.77 (C–H bending), 1252.01, 1232.50 (C–N stretching), 1204.74, 1190.26, 1165.02, 1147.37, 1106.85, 1067.77, 1051.30, 1024.36, 1012.48 (Aromatic C–H in plane bending), 908.86, 879.03, 843.44, 831.39, 808.43, 792.62, 772.88, 754.16, 745.95, 707.60 (Aromatic C–H out of plane bending).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.96 and 4.09 (2H, 2d, J_1 = 15 Hz, J_2 = 18 Hz, S–CH_2), 7.01 (1H, s, thiazoline), 7.09–7.59 (10H, m, phenyl, 4-fluorophenyl, pyrimidine), 8.52 (2H, d, J = 3 Hz, pyrimidine), 11.79 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 33.20 (CH_2), 102.88 (CH), 116.08 (CH), 116.30 (CH), 118.24 (CH), 123.99 (CH), 124.49 (2CH), 128.91 (C), 131.12 (2CH), 131.81 (CH), 131.89 (CH), 140.52 (3C), 158.42 (2CH), 162.31 (C), 168.13 (C), 169.79 (C).

MS (ESI)(m/z): ($M^+ + 1$) 438.

For $C_{21}H_{16}FN_5OS_2$ calculated: C, 57.65; H, 3.69; N, 16.01; Found: C, 57.65; H, 3.71; N, 16.02.

*5.1.1.4.38. N′-[(4-Chlorophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(pyrimidin-2-yl)thio]acetohydrazide (**38**).* IR (KBr) ν_{max} (cm^{-1}): 3446.68, 3221.62 (N–H stretching), 3106.47 (Aromatic C–H stretching), 2896.97 (Aliphatic C–H stretching), 1712.54 (C=O stretching), 1608.42, 1571.18, 1551.32, 1492.20, 1470.47, 1450.21 (C=N, C=C stretching and N–H bending), 1422.93, 1406.31, 1378.72, 1365.82 (C–H bending), 1266.95, 1240.30 (C–N stretching), 1186.87, 1166.73, 1143.08, 1094.72, 1071.21, 1053.18, 1024.40, 1011.91 (Aromatic C–H in plane bending), 907.41, 878.07, 832.54, 810.88, 784.21, 772.79, 755.25, 744.55, 712.70, 692.71 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.95 and 4.09 (2H, 2d, J_1, J_2 = 15 Hz, S–CH_2), 7.03 (1H, s, thiazoline), 7.19–7.58 (10H, m, phenyl, 4-chlorophenyl, pyrimidine), 8.50 (2H, d, J = 3 Hz, pyrimidine), 11.78 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 32.26 (CH_2), 103.32 (CH), 118.23 (CH), 124.46 (2CH), 126.37 (CH), 128.90 (2CH), 129.18 (C), 131.05 (2CH), 131.11 (2CH), 135.67 (C), 138.96 (C), 140.34 (2C), 158.34 (2CH), 168.14 (C), 169.75 (C).

MS (ESI)(m/z): ($M^+ + 1$) 454.

For $C_{21}H_{16}ClN_5OS_2$ calculated: C, 55.56; H, 3.55; N, 15.43; Found: C, 55.55; H, 3.55; N, 15.44.

*5.1.1.4.39. N′-[(4-Bromophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(pyrimidin-2-yl)thio]acetohydrazide (**39**).* IR (KBr) ν_{max} (cm^{-1}): 3448.17, 3222.00 (N–H stretching), 3104.75 (Aromatic C–H stretching), 2896.91 (Aliphatic C–H stretching), 1711.55 (C=O stretching), 1606.93, 1586.30, 1570.01, 1552.24, 1494.72, 1470.70, 1450.75 (C=N, C=C stretching and N–H bending), 1423.41, 1402.49, 1378.75 (C–H bending), 1267.03, 1232.97 (C–N stretching), 1183.85, 1165.79, 1141.66, 1074.38, 1053.64, 1024.00, 1008.74 (Aromatic C–H in plane bending), 906.46, 877.71, 823.54, 811.65, 783.10, 772.48, 754.67, 744.71, 702.92, 688.55 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (300 MHz) (DMSO-d_6) δ (ppm): 3.95 and 4.09 (2H, 2d, J_1, J_2 = 15 Hz, S–CH_2), 7.02 (1H, s, thiazoline), 7.20–7.58 (10H, m, phenyl, 4-bromophenyl, pyrimidine), 8.51 (2H, d, J = 3 Hz, pyrimidine), 11.76 (1H, s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 33.27 (CH_2), 103.19 (CH), 118.26 (CH), 124.45 (C), 124.52 (CH), 126.80 (2CH), 128.86 (2CH), 131.12 (C), 131.22 (2CH), 132.11 (2CH), 140.40 (3C), 158.37 (2CH), 168.17 (C), 169.77 (C).

MS (ESI)(m/z): ($M^+ + 1$) 499.

For $C_{21}H_{16}BrN_5OS_2$ calculated: C, 50.60; H, 3.24; N, 14.05; Found: C, 50.60; H, 3.25; N, 14.02.

*5.1.1.4.40. N′-[(4-Cyanophenyl-3-phenylthiazol-2(3H)-ylidene)]-2-[(pyrimidin-2-yl)thio]acetohydrazide (**40**).* IR (KBr) ν_{max} (cm^{-1}): 3419.56 (N–H stretching), 3085.89 (Aromatic C–H stretching), 2912.31, 2827.45 (Aliphatic C–H stretching), 2229.56 (C≡N stretching), 1720.39 (C=O stretching), 1604.66, 1562.23, 1548.73, 1477.37 (C=N, C=C stretching and N–H bending), 1390.58 (C–H bending), 1255.57 (C–N stretching), 1182.28, 1122.49, 1033.77 (Aromatic C–H in plane bending), 802.33, 765.69, 628.75 (Aromatic C–H out of plane bending and C–S stretching).

^{1}H NMR (400 MHz) (DMSO-d_6) δ (ppm): 3.99 and 4.16 (2H, 2d, J_1, J_2 = 11.7 Hz, S–CH_2), 7.23 (1H, t, J_1 = 5.2 Hz, J_2 = 4.4 Hz, pyrimidine), 7.27 (1H, s, thiazoline), 7.41–7.45 (3H, m, phenyl), 7.58 (2H, t, J_1 = 7.2 Hz, J_2 = 8.4 Hz, phenyl), 7.66 (2H, d, J = 8 Hz, 4-cyanophenyl), 7.76 (2H, d, J = 8.4 Hz, 4-cyanophenyl), 8.52 (2H, d, J = 4.8 Hz, pyrimidine), 11.29 and 11.99 (1H, 2s, N–H).

^{13}C NMR (100 MHz) (DMSO-d_6) δ (ppm): 32.43 (CH_2), 103.61 (CH), 112.37 (C), 117.46 (CH), 118.06 (C), 120.84 (CH), 123.42 (2CH), 127.78 (2CH), 129.16 (2CH), 130.28 (2CH), 132.16 (C), 138.87 (3C), 157.62 (2CH), 167.41 (C), 169.03 (C).

MS (ESI)(m/z): ($M^+ + 1$) 445.

For $C_{22}H_{16}N_6OS_2$ calculated: C, 59.44; H, 3.63; N, 18.91; Found: C, 59.43; H, 3.61; N, 18.90.

5.2. Microbiology

5.2.1. Antibacterial and anticandidal activity

The antimicrobial activities of compounds **1**–**40** were tested using the microbroth dilution method [39]. Tested microorganism strains were *Micrococcus luteus* (NRLL B-4375), *Bacillus subtilis* (NRS-744), *P. aeruginosa* (ATCC-254992), *Staphylococcus aureus* (NRRL B-767), *Escherichia coli* (ATCC-25922), *Listeria monocytogenes* (ATCC-7644) *C. albicans* (ATCC-22019). Microbroth dilution-susceptibility assay was used for antimicrobial evaluation of the compounds. Stock solutions of the samples were prepared in dimethyl sulfoxide. Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.0039 mg/mL in micro-test tubes that

were transferred to 96-well microtiter plates. Overnight-grown bacterial and *C. albicans* suspensions in double-strength Mueller-Hinton broth were standardized to 10^8 CFU/mL using McFarland No: 0.5 standard solutions. Hundred microliter of each microorganism suspension was then added into the wells. The last well-chain without a microorganism was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 37 °C for 18–24 h, antimicrobial activity was detected by spraying of 0.5% TTC (triphenyl tetrazolium chloride, Merck) aqueous solution. MIC was defined as the lowest concentration of compounds that inhibited visible growth, as indicated by the TTC staining.

5.2.2. Antifungal activity

The antifungal activities of compounds **1**–**40** were tested using the microbroth dilution method with some modifications [39,40]. Tested fungal strains were *A. parasiticus* (NRRL-465), *A. ochraceus* (NRRL 3174) *Penicillium chrysogenum* (NRRL 1951), *Trichoderma harzianum* (NRRL 20565), *F. solani* (NRRL-13414), *F. culmorum* (wild culture) and *F. moniliforme* (NRRL 1866). Microbroth dilution-susceptibility assay was used for antimicrobial evaluation of the compounds. Stock solutions of the samples were prepared in dimethyl sulfoxide. Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.0039 mg/mL in micro-test tubes that were transferred to 96-well microtiter plates. Fungal strains grown on PDA at 25 °C for 5 suspensions in double-strength Potato Dextrose Broth (PDB) were standardized to 10^5 spores/mL. Hundred microliter of each spore suspension was then added into the wells. The last well-chain without a fungus was used as a negative control. Sterile distilled water and the medium served as a positive growth control. After incubation at 25 °C for 48–72 h, antifungal activity was detected by investigation of mycelia growing under stereo microscope.

Streptomycin was used as standard antibacterial agent, whereas ketoconazole was used as an antifungal agent.

5.3. Biochemistry

5.3.1. Cell culture and drug treatment

C6 rat glioma cells and NIH/3T3 mouse embryonic fibroblast cells were obtained from the American Type Culture Collection (ATCC, USA). The cells were incubated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Life Technologies, UK), 100 IU/mL penicillin (Gibco, Paisley, Scotland) and 100 mg/mL streptomycin (Gibco) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Exponentially growing cells were plated at 2×10^4 cells/mL into 96-well microtiter tissue culture plates (Nunc, Denmark) and incubated for 24 h before the addition of the drugs (the optimum cell number for cytotoxicity assays was determined in preliminary experiments). Stock solutions of compounds were prepared in dimethyl sulfoxide (DMSO; Sigma–Aldrich, Poole, UK) and further dilutions were made with fresh culture medium (the concentration of DMSO in the final culture medium was <0.1% which had no effect on the cell viability).

5.3.2. MTT assay

The level of cellular MTT (Sigma) reduction was quantified as previously described in the literature with small modifications [41,42].

After 24 h of preincubation, the compounds and cisplatin (positive control) were added to give final concentration in the range 3.9–500 μg/mL for NIH/3T3 cells and 1.5–200 μg/mL for C6 glioma cells and the cells were incubated for 24 h. At the end of this period, MTT was added to a final concentration of 0.5 mg/mL and the cells were incubated for 4 h at 37 °C. After the medium was removed, the formazan crystals formed by MTT metabolism were solubilized by addition of 200 μL DMSO to each well and absorbance was read at 540 nm with a microtitre plate spectrophotometer (Bio-Tek plate reader). Each concentration was repeated in three wells and IC_{50} values were defined as the drug concentrations that reduced absorbance to 50% of control values.

5.3.3. Analysis of DNA synthesis

Analysis of DNA synthesis was measured by BrdU Cell Proliferation ELISA (colorimetric) kit (Roche). This immunostaining procedure is based on the use of specific anti-BrdU antibodies for measuring the incorporation of bromodeoxyuridine (BrdU) into nuclear DNA in place of thymidine during the S-phase of the cell cycle [43]. Hence, this method provides a colorimetric measurement for DNA synthesis inhibition ratio of the carcinogenic cells. After cells were seeded into 96 well flat-bottomed microtiter plates at a density of 2×10^3, various doses of compounds which were chosen after MTT test (compounds **16**, **17**, **19**, **21**, **23**, **27**, **28**, **29**, **30**) and cisplatin were administrated into C6 tumor cells. Microtiter plates were incubated at 37 °C in a 5% CO_2/95% air humidified atmosphere for 24 h. The cells were labeled with 10 μL BrdU solution for 2 h and then fixed. Anti-BrdU-POD (100 μL) was added and incubated for 90 min. After washing steps with 1xPBS, cells were incubated with substrate. Absorbance of the samples was measured with an ELX808-IU Bio-Tek apparatus at 450 nm. For each compound dose, triplicate wells were used.

5.3.4. Statistical analyses

Statistical analyses were evaluated by Statistical Package for the Social Science (SPSS) for Windows 15.0. Data was expressed as Mean ± SD. Comparisons were performed by one way ANOVA test for normally distributed continuous variables and Tukey test was used for post hoc analyses of group differences.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.12.060.

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