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Article

Synthesis and Antitumor Evaluation of New Heterocycles Derived from 3-Methyl-2-benzothiazolinone Hydrazone

Nabila M. Ibrahim,[a] Hisham A. A. Yosef,[a] Ewies F. Ewies,[a] Mohamed R. H. Mahran,[,a] Mamdouh M. Ali[b] and Abeer E. Mahmoud[b]*

[a]*Department of Organometallic and Organometalloid Chemistry and* [b]*Biochemistry Department, National Research Centre, El-Buhouth St, 12622 Dokki, Cairo, Egypt*

3-Methyl-2-benzothiazolinone hydrazone condensed with some selected aldehydes to give the corresponding Schiff bases. Cyclizing the obtained Schiff base derivatives with thioglycolic acid afforded the respective thiazolylideneaminothiazolidenones. Condensation of 3-methyl-2-benzothiazolinone hydrazone with ethyl cyanoacetate yielded a mixture of the bishydrazine and the cyanoacetohydrazide derivatives. Meanwhile, its condensation with ethyl acetoacetate produced a mixture of the monoketone and the β-diketone derivatives. Besides, the reaction of the starting hydrazone with malononitrile afforded a mixture of the cyanomethylylidene and cyanomethyl hydrazone derivatives beside the bishydrazine derivative. Elementary and spectroscopic measurements were in good accord with the structures postulated for the new compounds. The antitumor activities of certain selected new compounds were screened, *in vitro*, against a panel of four (liver, HepG2; breast, MCF-7; lung, A549; and colon; HCT116) human solid tumor cell lines. The cells that showed better activity were HepG2 and MCF-7. Structure-activity relationship (SAR) was also discussed.

Keywords: 3-methyl-2-benzothiazolinone hydrazone, aldehydes, Schiff bases, active methylenes, antitumor activity

Introduction

Benzothiazoles are interesting heterocycles which are ubiquitous in nature and show antiviral,[1] antibacterial,[2] antimicrobial[3] and antifungal activities.[4] They are also useful as anti-allergic,[5] antidiabetic,[6] antitumor,[7] anti-inflamatory,[8] anthelemintic,[9] and anti-HIV agents.[10] The wealth of interest in the chemistry of benzothiazoles is also due to their industrial importance[11] and for being important elements and pharmacophores for designing several biological activities.[10,12-14] Many benzothiazole derivatives have been synthetically and biologically evaluated through reacting 2-aminobenzothiazoles with various reagents.[15,16] Limited attention, on the other hand, has been paid to making use of 2-hydrazonobenzothiazoles for similar purposes.[17,18] In context to our interest in this realm,[19] the present work has been endeavored aiming at designing and synthesizing new compounds bearing the benzothiazole scaffold in their molecules to be evaluated for their anticancer activities. This has been achieved through reacting 3-methyl-2-benzothiazolinone hydrazone **1** with certain carbonyl reagents and active methylenes.

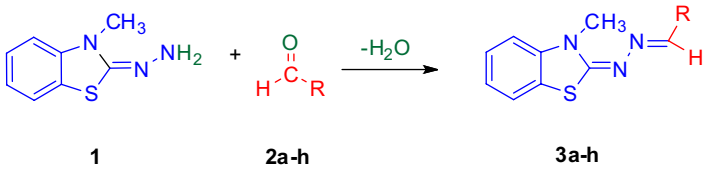
*e-mail: mohamed_rh_mahran@yahoo.com

Results and Discussion

Chemistry

It has been found that hydrazone **1** condenses with aldehydes **2a**-**h** in absolute ethanol at the reflux temperature in the presence of a few drops of piperidine to give the respective Schiff bases **3a**-**h** in high percentage yields (Scheme1). The assigned structures were attested by compatible analytical and spectroscopic measurements.

For example, correct elementary analyses and molecular weight determination (MS) for 4-(((3-methylbenzo[*d*]thiazol-2(3*H*)-ylidene)hydrazono)methyl)benzonitrile **3f** corresponded to $C_{16}H_{12}N_4S$ (MS *m/z* 292, 48%). Its infrared (IR) spectrum (KBr) showed absorption bands at ν/cm^{-1} 3084 (aromatic C–H), 2921 (aliphatic C–H), 2219 (C≡N), 1651 (C=N) and 1566 (aromatic C=C). The ^{1}H nuclear magnetic resonance (NMR) of **3f** (500 MHz, DMSO-d_6) disclosed the presence of signals at δ 3.64 (s, 3H, NCH_3),



1 **2a-h** **3a-h**

2,3 **a**, R = C_6H_5
b, R = 4-Cl-C_6H_4
c, R = 4-(piperidin-1-yl)-C_6H_4
d, R = 4-O_2N-C_6H_4
e, R = 2-thienyl
f, R = 4-CN-C_6H_4
g, R = 2-ferrocenyl
h, R = 3,4-$(HO)_2$-C_6H_3

Scheme 1. Synthesis of Schiff bases **3a**-**h**.

7.12 (t, 1H, *J* 7.8 Hz, Ar*H*, benzothiazole ring), 7.34 (d, 1H, *J* 8.1 Hz, Ar*H*, benzothiazole ring), 7.62 (t, 1H, *J* 8.1 Hz, Ar*H*, benzothiazole ring), 7.88 (d, 1H, *J* 7.8 Hz, Ar*H*, benzothiazole ring), 7.91 (d, 2H, *J* 8.1 Hz, AB system, Ar*H*, benzene ring), 8.40 (d, 2H, *J* 8.1 Hz, AB system, Ar*H*, benzene ring), 8.43 (s, 1H, N=C*H*).

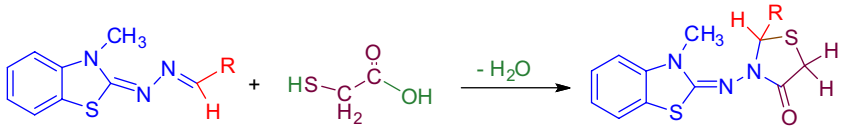
Schiff bases **3a**-**e** were cyclized upon treatment with thioglycolic acid to yield the respective thiazolylideneaminothiazolidinones **4a**-**e** (Scheme 2).

Correct elementary analyses and molecular weight determination for 2-(4-chlorophenyl) -3-(3-methylbenzo[d] thiazol-2(3*H*)-ylideneamino)thiazolidin-4-one **4b** taken as a representative example, corresponded to $C_{17}H_{14}ClN_3OS_2$ (MS *m/z* 375, 100%). Its IR spectrum (KBr) revealed the presence of bands at ν/cm^{-1} 3081 (aromatic C–H), 2923 (aliphatic C–H), 1688 (C=O), at 1608, 1555 for the C=N, aromatic C=C groups, respectively and 740 (Cl–C, aromatic). The ^{1}H NMR spectrum of **4b** (500 Hz, DSMO-d_6) showed signals at δ 3.36 (s, 3 H, CH_3), 5.87 (s, 1 H, NC*H*–S). Protons of the methylene group (2H) appeared as two doublets (each with *J* 16.3 Hz) at δ 3.71 and δ 3.87 ppm, indicating that they are magnetically not equivalent. The four protons of the benzothiazole ring gave signals at 7.08 (t, *J* 7.6 Hz), 7.18 (d, *J* 7.6 Hz), 7.23 (t, *J* 7.6 Hz) and at 7.55 (d, *J* 7.6 Hz), while the two doublets due to the 1,4-disubstituted benzene ring appeared at δ 7.43 (2H, *J* 8.6 Hz) and δ 7.51 (2H, *J* 8.6 Hz).

Reaction of hydrazone **1** with ethyl cyanoacetate was conducted in absolute ethanol to give a mixture of two products, which were separated by column chromatography (Scheme 3).

The first product was proved to be 1,2-bis(3-methylbenzo[*d*]thiazol-2(3*H*)-ylidene)hydrazine **5** by comparing its melting point (mp) and IR spectra with a reference sample.[19] An ORTEP overview of compound **5** is represented in Figure 1.[19]

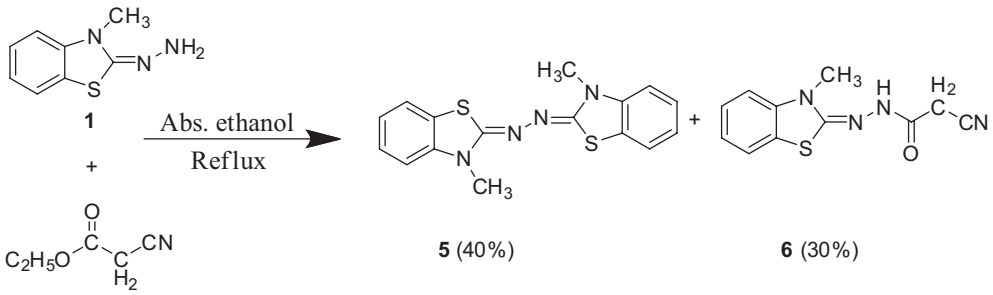
The second product was formulated as 2-cyano-*N*'-(3-methylbenzo[*d*]thiazol-2(3*H*)-ylidene)acetohydrazide **6** for the following reasons: (*i*) correct elementary analyses and molecular weight determination for compound **6** corresponded to $C_{11}H_{10}N_4OS$ (MS *m/z* 246, M$^+$, 52%); (*ii*) its IR spectrum (KBr) showed strong absorption bands at ν/cm^{-1} 3428 (N–H), 3151 (aromatic C–H), 2981, 2862 (aliphatic C–H), 2257 (C≡N), 1667 (C=O), 1603 (C=N) and 1578 (aromatic C=C); (*iii*) the ^{1}H NMR spectrum of **6** (500 MHz, DMSO-d_6) disclosed the presence of signals at δ 3.67(s, 3H, NCH_3), 3.83 (d, 2H, *J* 7.4 Hz, W-conformation, NH–C(O)–CH_2), 7.04 (t, 1H, *J* 8.1 Hz,



3a-e **4a-e**

3,4 **a**, R = C_6H_5
b, R = 4-Cl-C_6H_4
c, R= 4-(piperidin-1-yl)-C_6H_4
d, R = 4-NO_2-C_6H_4
e, R = 2-thienyl

Scheme 2. Cycloaddtion reaction of thioglycolic acid with Schiff bases **3a**-**e**.



Scheme 3. Condensation of hydrazone **1** with ethyl cyanoacetate.

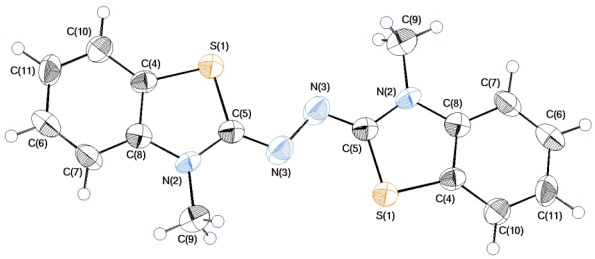


Figure 1. ORTEP overview of compound **5**.

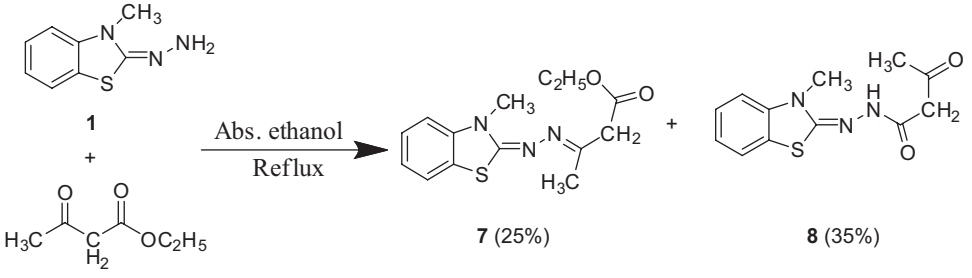
Ar*H*), 7.19 (d, 1H, *J* 8.1 Hz, Ar*H*), 7.31 (t, 1H, *J* 7.8 Hz, Ar*H*), 7.56 (d, 1H, *J* 7.8 Hz, Ar*H*) and 10.78 (brs, 1H, N*H*, D_2O exchangeable); (*iv*) the ^{13}C NMR spectrum of compound **6** (125 MHz, DMSO-d_6) showed signals at δ 24.6 (CH_2CN), 31.0 (N-CH_3), 110.0 ($C{\equiv}N$). The fused carbon atoms (3C) gave signals at δ 158.7 (S–*C*=N), 141.3 (*C-4*, benzothiazole ring) and 117.0 (*C-9*, benzothiazole ring). The aromatic *C*H signals (4C) appeared at δ 127.3, 122.9, 122.1 and 121.8. The signal present at δ 164.1 is attributed to the carbonyl carbon atom.

Condensation of hydrazone **1** with ethyl acetoacetate proceeded also in absolute ethanol to give a mixture of two products, which were separated by column chromatography. The first (25%) was obtained in yellow crystals and formulated as ethyl 3-(3-methylbenzo[*d*] thiazol-2(3*H*)-ylidene)hydrazono)butanoate **7** (Scheme 4).

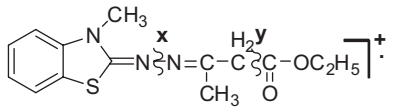
Correct elementary analyses and molecular weight determination for compound **7** corresponded to $C_{14}H_{17}N_3O_2S$ (MS *m/z* 291, M^+, 100%). Its IR spectrum (KBr) showed strong absorption bands at ν/cm^{-1} 2922, 2857 (aliphatic C–H), 1732 (C=O, ester), 1633 (C=N) and 1582 (aromatic C=C). Its ^1H NMR spectrum (500 MHz, DMSO-d_6) showed signals at δ 1.18 (t, 3H, *J* 7.6 Hz, ethoxy-CH_3), 2.03 (s, 3H, CH_3–C=N), 3.43 (s, 2H, N=C–CH_2–C=O), 3.48 (s, 3H, NCH_3) and 4.08 (q, 2H, *J* 7.6 Hz, ethoxy-CH_2). The four protons of benzothiazole ring gave signals at 7.02 (t, 1H, *J* 8.6 Hz,), 7.20 (d, 1H, *J* 8.6 Hz), 7.26 (t, 1H, *J* 7.6 Hz,), 7.51 (d, 1H, *J* 7.6 Hz). The mass spectrum of compound **7** shows the molecular ion peak at *m/z* 291 (100%), which is also the base peak denoting its relative stability upon electron bombardment. Ejection of ethanol molecule from M^+ yields the radical cation **a** at *m/z* 245 (52%), which can lose CH_3 radical to give cation **b** at *m/z* 230 (21%). Cleavage of M^+ at axis x affords cation **c** at *m/z* 163 (24%). Meanwhile, the prominent ion peak present at *m/z* 218 (cation **d**, 10%) can arise via cleavage of M^+ at axis y (Scheme 5).

The second product (35%) was yielded in golden crystals and formulated as *N*'-(3-methylbenzo[*d*]thiazol-2(3*H*)-ylidene)-3-oxobutanehydrazide **8** for the following reasons: (*i*) its elementary analyses and molecular weight determination (MS) corresponded to $C_{12}H_{13}N_3O_2S$ (*m/z* 263, M^+, < 5%); (*ii*) the IR spectrum of **8** (KBr) showed strong absorption bands at ν/cm^{-1} 3430 (N–H), 2923, 2855 (aliphatic C–H), 1730 (C=O), 1677 (C=O) and at 1587, 1525 (C=N and aromatic C=C, respectively); (*iii*) its ^1H NMR spectrum (500 MHz, DMSO-d_6) showed signals at δ 2.46 (s, 2H, CH_2), 3.79 (s, 3H, NCH_3), 3.83 (s, 3H, OCH_3), 6.03 (brs, 1H, N*H*, D_2O exchangeable), 6.95-7.90 (m, 4H, benzothiazole ring).

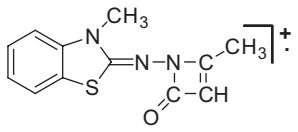
Condensation of hydrazone **1** with malononitrile proceeded in absolute ethanol at the reflux temperature



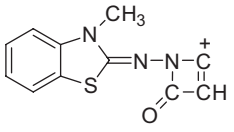
Scheme 4. Condensation of hydrazone **1** with ethyl acetoacetate.



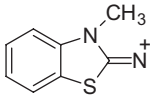
7, M^+, $C_{14}H_{17}N_3O_2S$,
m/z 291 (100%)



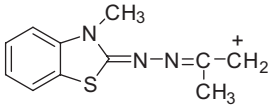
a, $C_{12}H_{11}N_3OS$
m/z 245 (52%)



b, $C_{11}H_8N_3OS$
m/z 230 (21%)



c, $C_8H_7N_2S$
m/z 163 (24%)



d, $C_{11}H_{12}N_3S$
m/z 218 (10%)

Scheme 5. The fragmentation pattern of compound **7.**

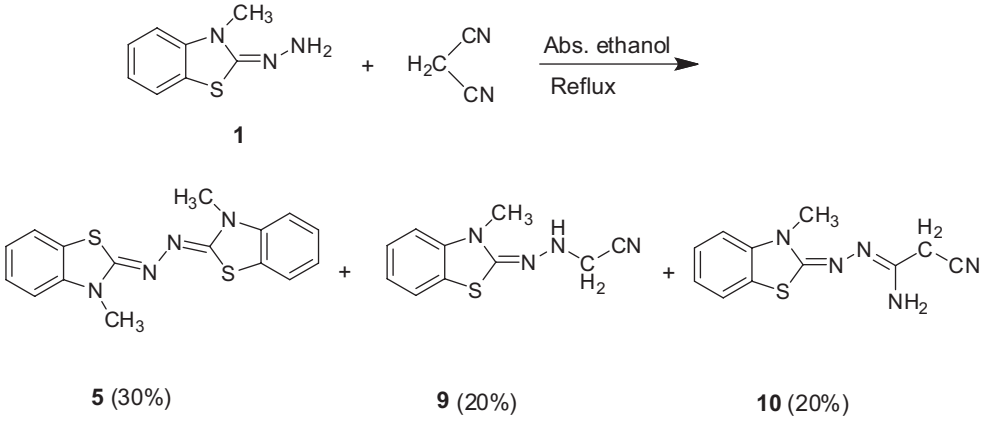
to give a mixture of three products which were separated by column chromatography (Scheme 6). The first (30%) was proved to be compound **5** by comparing its mp and IR spectra with those of a reference sample.[19]

The second product (20%, pale brown crystals) was formulated as 2-(2-(3-methylbenzo[*d*]thiazol-2(3*H*)-ylidene)hydrazinyl)acetonitrile **9** for the following reasons: (*i*) its elementary analyses and molecular weight determination (MS) corresponded to $C_{10}H_{10}N_4S$ (*m/z* 218, M^+, < 5%); (*ii*) the IR spectrum of **9** revealed strong absorption bands at ν/cm^{-1} 3267 (N–H), 2919, 2849 (aliphatic C–H), 2202 (C≡N), 1628 (C=N) and at 1577, 1523 (aromatic C=C); (*iii*) its ^{1}H NMR spectrum (500 MHz, DMSO-d_6) showed signals at δ 3.68 (s, 3H, NCH_3) and 3.90 (s, 2 H, CH_2). Protons of the benzothiazole ring (4H) gave a multiplte signal at a range of 6.99-7.52. The NH_2 group protons appeared as D_2O exchangeable broad signal at δ 8.58 ppm.

The third compound (20%, dark brown crystals) was formulated as 2-cyano-*N*'-(3-methylbenzo[*d*]thiazol-2(3*H*)-ylidene)acetohydrazonamide **10** (Scheme 6). Its elementary analyses and molecular weight determination corresponded to $C_{11}H_{11}N_5S$ (MS *m/z* 245, 72%). The IR spectrum of compound **10** (KBr) revealed strong absorption bands at ν/cm^{-1} 3476, 3370 (NH_2), 3051 (aromatic C–H), 2917 (aliphatic C–H), 2251 (C≡N), 1656, 1617 (C=N) and 1565 (aromatic C=C). Its ^{1}H NMR spectrum (500 MHz, DMSO-d_6) showed signals at δ 3.44 (s, 3H, NCH_3), 4.32 (s, 2H, CH_2), 6.32 (brs, 2H, NH_2, D_2O exchangeable), 6.96 (t, 1H, *J* 7.6 Hz, benzothiazole ring), 7.09 (d, 1H, *J* 7.6 Hz, benzothiazole ring), 7.22 (t, 1H, *J* 7.6 Hz, benzothiazole ring), 7.47 (d, 1H, *J* 7.6 Hz, benzothiazole ring). The ^{13}C NMR spectrum of compound **10** (125 MHz, DMSO-d_6) showed signals at δ 22.4 (*C*H_2CN), 30.9 (N–*C*H_3) and 109.4 (*C*N). The fused carbon atoms (3C) gave signals at δ 159.0 (S–*C*=N), 141.7 (*C*-4, benzothiazole ring) and 117.4 (*C*-9, benzothiazole ring). The aromatic *C*H signals (4C) appeared at δ 126.6, 123.9, 122.6 and 121.1. The signal present at δ 148.2 ppm is attributed to N=*C*–NH_2 carbon atom.

Biological evaluation

Conventional chemotherapy, although directed toward certain macromolecules or enzymes, typically does not discriminate effectively between rapidly dividing normal cells (e.g., bone marrow and gastrointestinal tract) and tumor cells, thus leading to several toxic side effects. Tumor responses from cytotoxic chemotherapy are usually partial, brief and unpredictable. In contrast, targeted therapies interfere with molecular targets that have a role in tumor growth or progression. These targets are usually located in tumor cells, although some like the antiangiogenic agents may target other cells such as endothelial cells.[20] Thus,



1

5 (30%) **9** (20%) **10** (20%)

Scheme 6. Reaction of malononitrile with hydrazone **1**.

targeted therapies have a high specificity toward tumor cells, providing a broader therapeutic window with less toxicity. They are also often useful in combination with cytotoxic chemotherapy or radiation to produce additive or synergistic anticancer activity since their toxicity profiles often do not overlap with traditional cytotoxic chemotherapy. Thus, targeted therapies represent a new and promising approach to cancer therapy, leading to beneficial clinical effects. There are multiple types of available targeted therapies, including monoclonal antibodies, inhibitors of tyrosine kinases and antisense inhibitors of growth factor receptors.[20]

Poly(ADP-ribose)polymerase-1 (PARP-1) is a chromatin-bound nuclear enzyme engaged in the detection and repair of DNA damage.[21] During poly(ADP) ribosylation, PARP-1 consumes nicotinamide adenine dinucleotide to synthesize poly(ADP)-ribose either on itself or on an array of nuclear target proteins such as histones, topoisomerases, DNA polymerases and DNA ligases.[22] This results in high negatively charged nuclear proteins that subsequently unwind and repair the damaged DNA through the base excision repair (BER) pathway. Therefore, inhibition of PARP sensitizes tumor cells to cytotoxic drugs that induce DNA damage, which would normally be repaired through the BER pathway. Undeniably PARP inhibition has been shown to sensitize tumors to DNA alkylating agents (e.g., temozolomide) and topoisomerase I poisons (e.g., irinotecan).[23] Moreover, some PARP inhibitors have been shown to display single agent activity for tumors lacking BRCA1- or BRCA2-dependent DNA double-stranded repair mechanisms.[24] Thus, development of PARP inhibitors is an appealing research area offering innovative cancer treatment opportunities.

In the same direction and in continuing effort to find more potent and selective anticancer compounds, herein we designed and synthesized a series of benzothiazole compounds for studying their antiproliferative activities against 4 different human cancer cell lines including liver HepG2, breast MCF-7, lung A549 and colon HCT116 cancer cell lines as well as investigating their mechanism of action on poly(ADP-ribose)polymerase-1 inhibition.

Cell lines and culturing

Anticancer activity screening for the tested compounds utilizing 4 different human tumor cell lines including liver HepG2, breast MCF-7, lung A549 and colon HCT116 cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). The tumor cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}) at 37 °C in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50×10^6 were grown in a 25 cm^2 flask in 5 mL of complete culture medium.

In vitro cytotoxicity assay

The antiproliferative activity was measured *in vitro* using the sulfo-rhodamine-B stain (SRB) assay according to the previously reported standard procedure.[25] Cells were inoculated in 96-well microtiter plate (10^4 cells $well^{-1}$) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in dimethyl sulfoxide (DMSO) at 1 mg mL^{-1} immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentrations of tested compounds (0-50 μg mL^{-1}) and doxorubicin (DOX) were added to the cells. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained for 30 min with 0.4% (m/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an enzyme-linked immunosorbent assay (ELISA) reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1. The results were compared to the antiproliferative effects of the reference control DOX.[26]

PARP-1 inhibitors comprise a promising new class of compounds for the treatment of cancer. The activity of PARP-1 in the lysate of hepatic HepG2 and breast MCF-7 cancer cells treated with the prepared compounds was measured using ELISA kit purchased from Glory Science Co., Ltd (Del Rio, TX78840, USA).

Statistical analysis

The results are reported as mean ± standard error (S.E.) for at least three time experiments. Statistical differences were analyzed according to one way ANOVA test followed by student's *t*-test wherein the differences were considered to be significant at $p < 0.05$.

Antiproliferative activity of the tested compounds

The antiproliferative activities were expressed by median growth inhibitory concentration IC_{50} (μg mL^{-1}).

Table 1. The *in vitro* cytotoxicity activity of the tested compounds expressed as IC_{50} values in 4 different human cancer cell lines[a,b]

Compound	IC_{50} / (μg mL^{-1})			
	HepG2	MCF-7	A549	HCT116
1	43.00 ± 4.80	40.60 ± 5.10	N.A.	N.A.
3a	41.20 ± 4.30	N.A.	N.A.	N.A.
3b	N.A.	36.10 ± 0.60	N.A.	N.A.
3c	N.A.	38.60 ± 4.00	N.A.	N.A.
3d	6.20 ± 0.73	8.10 ± 0.90	N.A.	N.A.
3e	4.30 ± 0.40	5.30 ± 0.60	N.A.	N.A
3f	N.A.	17.30 ± 3.00	N.A.	N.A.
3g	7.00 ± 0.80	7.90 ± 0.86	N.A.	N.A.
4a	39.90 ± 4.70	N.A.	N.A.	N.A.
4b	34.00 ± 3.50	N.A.	N.A.	N.A.
4c	21.00 ± 2.30	24.25 ± 3.90	N.A.	N.A.
4d	7.00 ± 0.80	4.90 ± 0.60	N.A.	N.A.
4e	5.80 ± 0.66	5.00 ± 0.55	N.A.	N.A.
DMSO	N.A.	N.A.	N.A.	N.A.
DOX	4.20 ± 0.44	4.80 ± 0.50	5.30 ± 0.60	6.00 ± 0.65

[a]IC_{50} dose of the compounds which reduces survival to 50%; [b]data were expressed as average of three independent experiments; N.A.: no activity.

As shown in Table 1 and Figure 2, the antiproliferative activity of the synthetic compounds was evaluated against human hepatocellular carcinoma HepG2, breast adenocarcinoma MCF-7, human lung cancer A549 and colon cancer HCT116 cell lines using SRB assay, in comparison with DOX as a reference drug. The results revealed that all compounds did not exert any activity against human lung A549 and colon HCT116 cancer cell lines. Many of the tested compounds showed remarkable anticancer activity against liver HepG2 cancer cell lines. While compounds **3b**, **3c** and **3f** had no influence on the cancer cells, compounds **3d**, **3e**, **3g**, **4d**, and **4e** were found to be potent anticancer agents having IC_{50} values near to the standard drug (IC_{50} 7.00 ± 0.80, 6.20 ± 0.73, 4.30 ± 0.40, 7.00 ± 0.80 and 5.80 ± 0.66 μg mL^{-1}, respectively *versus* 4.20 ± 0.44 μg mL^{-1} for DOX). The rest of the tested compounds revealed slight to moderate activity. In the same sense, evaluation of the anticancer effect of the tested compounds against human breast MCF-7 cancer cell lines revealed that although compounds **3a**, **4a** and **4b** had no effect on the MCF-7 cancer cell, compounds **3d**, **3e**, **3g**, **4d**, and **4e** showed anticancer activity close to that of the standard drug (IC_{50} values 8.10 ± 0.90, 5.30 ± 0.60, 7.90 ± 0.86, 4.90 ± 0.60 and 5.00 ± 0.55 μg mL^{-1}, respectively, *versus* 4.80 ± 0.50 μg mL^{-1} for DOX). The rest of the tested compounds revealed slight activity. From the forgoing result, the synthesized compounds especially **3d**, **3e**, **3g**, **4d**, and **4e** showed cytotoxicity and growth inhibitory activity on both breast and liver cancer cell lines.

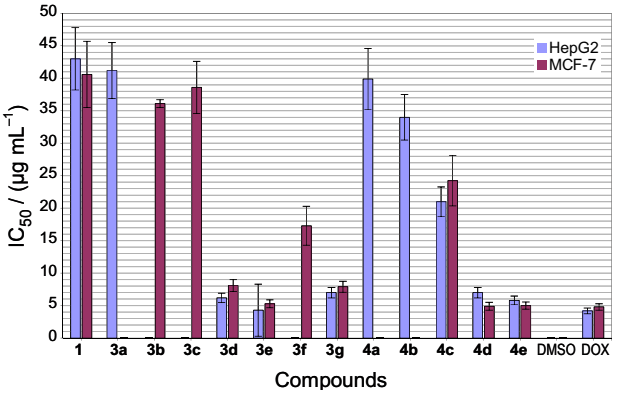


Figure 2. The *in vitro* cytotoxicity activity of the tested compounds expressed as IC_{50} values against HepG2 and MCF-7 human cancer cell lines.

From Table 1 and Figure 2, it is clear that: (*i*) the cytotoxic activity of the tested compounds against liver carcinoma (HepG2) decreases in the order: **3e > 4e > 3d > 3g = 4d > 4c > 4b > 4a > 3a > 1**; (*ii*) the cytotoxic activity of the tested compounds against breast carcinoma (MCF-7) decreases in the order: **4d > 4e > 3e > 3g > 3d > 3f > 4c > 3b > 3c > 1**.

Poly(ADP-ribose)polymerase-1 (PARP-1) activity

To identify the mechanism of action responsible for the cytotoxicity of the prepared compounds **3d**, **3e**, **3g**, **4d**, and **4e**, the activity of PARP-1 expressed in the two cell lines (hepatic HepG2 and breast MCF-7 cancer cell lines) were estimated quantitatively and the results were calculated as percentage of the control cancer cells as shown in Figure 3.

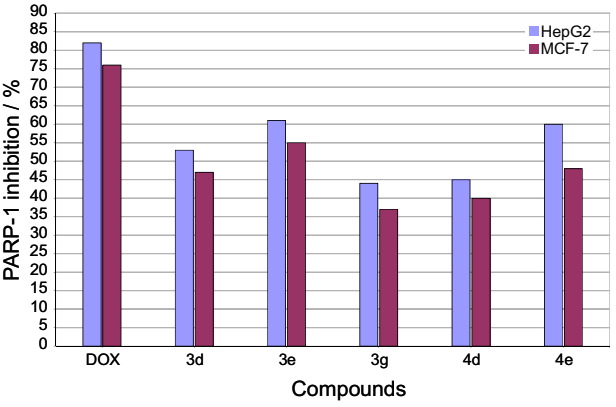


Figure 3. The effect of the prepared compounds on inhibition of PARP-1 enzyme activity in HepG2 and MCF-7 cells. The data were compared with the control cancer cells.

In this work, the activity of PARP-1 in the lysate of hepatic HepG2 and breast MCF-7 cancer cells treated with the prepared compounds and DOX as a known inhibitor was measured and the data were calculated as percentage of inhibition as compared to the control untreated cancer cells. Meanwhile, treatment of hepatic HepG2 and breast MCF-7 cancer cells with DOX resulted in 82% and 76% inhibition, respectively as compared with control cancer cells. The treatment with compounds **3d**, **3e**, **3g**, **4d** and **4e** resulted in 53%, 61%, 44%, 45% and 60% inhibition, respectively in hepatic HepG2 cells. Similarly, the treatment of breast MCF-7 cells with the compounds resulted in inhibition of the activity of PARP-1 by 47%, 55%, 37%, 40% and 48%, respectively (Figure 3).

In summation, these findings suggest that there are correlation between the cytotoxicity of the tested compounds and inhibition of the PARP-1 activity. The tested compounds exert anti-carcinogenic activity in hepatic HepG2 and breast MCF-7 cancer cell lines through down regulation of the activity of PARP-1 enzyme, which may reduce the cell proliferation and result in significant growth inhibition.

Conclusions

The present study reports on simple and efficient approaches for the synthesis of new benzothiazolinone derivatives. 3-Methyl-2-benzothiazolinone hydrazone **1** was successfully utilized as a starting material for implementing this goal. Some of the new products recorded pronounced *in vitro* antitumor activities when tested against liver (HepG2) and breast (MCF-7) human solid tumor cell lines. The most promising result against liver carcinoma was recorded by compound **3e** (IC_{50} value of 4.30 ± 0.40 μg mL^{-1}) which is the closest in value to that recorded by the reference drug (DOX, IC_{50} value of 4.20 ± 0.44 μg mL^{-1}). Meanwhile, compounds **4d** (IC_{50} 4.90 ± 0.60 μg mL^{-1}), **4e** (IC_{50} 5.00 ± 0.55 μg mL^{-1}) and **3e** (IC_{50} 5.30 ± 0.60 μg mL^{-1}) showed the highest activity compared to the reference drug (DOX, IC_{50} 4.80 ± 0.50 μg mL^{-1}) when tested against breast carcinoma. The marked cytotoxic and growth inhibitory activity of compound **3e** against both liver and breast carcinoma may be correlated with the presence of the thienyl moiety in its molecule, which is known to promote the biological activity.[27] On the other hand, hydrazone **1** showed the least appreciable cytotoxic activity amongst the tested compounds. However, it was successfully used to prepare new products (e.g., **3e**, **4d**, and **4e**) of promise as antitumor agents; particularly against liver and breast carcinoma. These results, supplement to the well known activity of many benzothiazole derivatives in reducing the incidence of various cancer types.[7]

Experimental

General

Solvents were purified and dried according to the usual procedures. 2-Hydrazono-3-methyl-2,3-dihydrobenzo[*d*] thiazole **1** was prepared according to a known procedure.[28] The reacting aldehydes were purified directly before use by distillation and/or recrystallization. They are commercially available except for 4-piperdine-1-ylbenzaldehyde, **2c**, which was prepared by reacting 4-fluorobenzaldehyde with piperidine in DMSO in the presence of K_2CO_3.[29] The reactions were monitored (thin layer chromatography, TLC) and the purity of the isolated products were controlled by using silica gel with fluorescent indicator F_{254} coated on aluminum sheets of layer thickness 0.2 mm [Fluka]. Column chromatography was performed on silica gel, grain size 0.063-0.2 mm (Merck). Melting points were recorded on Electrothermal melting point apparatus and were uncorrected. The IR spectra were measured in KBr pellets using a JASCO FT/IR-300E Fourier Transform Infrared Spectrophotometer and reported in cm^{-1}. NMR spectra were recorded on a Joel-500 MHz spectrometer (^{1}H NMR at 500 MHz and ^{13}C NMR at 125 MHz) in deuterated dimethylsulphoxide (DMSO-d_6). The proton chemical shifts are reported in δ (ppm) downfield from tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Finnigan SSQ 7000 Spectrometer at 70 eV (Electron Impact). Analytical data were obtained at the analytical laboratory of the National Research Centre (or in the Microanalytical unit, Cairo University, Giza, Egypt). Satisfactory elemental analyses were gained for the new products. The antitumor activity was carried out at the Department of Biochemistry at the National Research Centre (NRC). Fetal bovine serum (FBS) and L-glutamine were obtained from Gibco Invitrogen Company (Scotland, UK). DMEM medium was provided from Cambrex (New Jersey, USA). DMSO, DOX, penicillin, streptomycin and sulfo-rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Human poly(ADP-ribose) polymerase 1 (PARP-1) activity ELISA kit was purchased from Glory Science Co., Ltd (Del Rio, TX78840, USA).

Chemistry

General procedure for the synthesis of Schiff bases **3a-h**

A mixture of 3-methyl-2-benzothiazolinone hydrazone **1** (1.79 g, 0.01 mol) and the appropriate aldehyde **2a-h** (0.01 mol) was refluxed in absolute ethanol (20 mL) for

4-6 h in the presence of a few drops of piperidine. After cooling the reaction mixture to room temperature, the formed solid was collected and recrystallized from ethanol to give the new Schiff base derivatives **3a**-**h**.

2-(Benzylidenehydrazono)-3-methyl-2,3-dihydrobenzo[d] thiazole (**3a**)

Pale yellow crystals; yield 2.22 g, 80%; mp 163-165 °C; IR (KBr) ν/cm^{-1} 3049, 3022 (C–H, aromatic), 2932 (C–H, aliphatic), 1620 (C=N), 1577 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.55 (s, 3H, N–CH_3), 7.07 (t, 1H, *J* 7.6 Hz, Ar–*H*, benzothiazole ring), 7.25 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.32 (t, 1H, *J* 6.9 Hz, Ar*H*, benzene ring), 7.39 (t, 2H, *J* 6.9 Hz, Ar–*H*, benzene ring), 7.41 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.57 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.72 (d, 2H, *J* 8.0 Hz, Ar*H*, benzene ring), 8.35 (s, 1H, N=C*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 30.0 (N–*C*H$_3$), 110.6, 122.8, 123.9, 127.0, 127.6, 128.9, 129.2, 130.2 (aromatic *C*–H), 122.3 (*C*-9, benzothiazole ring), 135.4 (CH=*C*–, benzene ring), 141.3 (*C*-4, benzothiazole ring), 152.7 (N=*C*H–Ph), 167.1 (S–C=N); MS (EI, 70 eV) *m/z* (%) 267 (100) [M$^+$]; anal. calcd.: $C_{15}H_{13}N_3S$ (267.35) C 67.39%, H 4.90%, N 15.72%, S 11.99%; found: C 67.28%, H 4.91%, N 15.77%, S 12.03%.

2-((4-Chlorobenzylidene)hydrazono)-3-methyl-2,3-dihydrobenzo[d]thiazole (**3b**)

Colorless crystals; yield 2.25 g, 75%; mp 179-180 °C; IR (KBr) ν_{max}/cm^{-1} 3151 (C–H, aromatic), 2928 (C–H, aliphatic), 1621 (C=N), 1590, 1572 (C=C, aromatic), 740 (Cl–C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.60 (s, 3H, NCH_3), 7.07 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.26 (d, 1H, *J* 6.9 Hz, Ar*H*, benzothiazole ring), 7.31 (t, 1H, *J* 6.9 Hz, Ar*H*, benzothiazole ring), 7.47 (d, 2H, *J* 8.4 Hz, AB system, Ar*H*, benzene ring), 7.58 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.72 (d, 2H, *J* 8.4 Hz, AB system, Ar*H*, benzene ring), 8.35 (s, 1H, N=C*H*); MS (EI, 70 eV) *m/z* (%) 301 (65) based on ^{35}Cl and 303 (28) based on ^{37}Cl; anal. calcd.: $C_{15}H_{12}ClN_3S$ (301.79) C 59.70%, H 4.01%, Cl 11.75%, N 13.92%, S 10.62%; found: C 59.83%, H 3.98%, Cl 11.70%, N 13.88%, S 10.66%.

3-Methyl-2-((4-(piperidin-1-yl)benzylidene)hydrazono)-2,3-dihydrobenzo[d]-thiazole (**3c**)

Colorless crystals; yield 2.62 g, 75%; mp 157-159 °C; IR (KBr) ν_{max}/cm^{-1} 3070 (C–H, aromatic), 2926, 2849 (C–H, aliphatic), 1612 (C=N), 1568 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.52-1.54 (m, 6H, piperidinyl ring), 3.22-3.30 (m, 4H, piperidinyl ring), 3.49 (s, 3H, NCH_3), 6.93 (d, 2H, *J* 8.6Hz, AB system, Ar*H*, benzene ring), 7.03 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.22 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.27 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.50 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.52 (d, 2H, *J* 8.6 Hz, AB system, Ar*H*, benzene ring), 8.22 (s,1H, N=C*H*); MS (EI, 70 eV) *m/z* (%) 352 (10) [M$^+$+2]; anal. calcd.: $C_{20}H_{22}N_4S$ (350.48) C 68.54%; H 6.33%, N 15.99%, S 9.15%; found: C 68.44%, H 6.36%, N 15.94%, S 9.12%.

3-Methyl-2-[(4-nitrobenzylidene)hydrazinylidene]-2,3-dihydro-1,3-benzothiazole (**3d**)

Red crystals; yield 2.18 g, 70%; mp 247-248 °C; IR (KBr) ν_{max}/cm^{-1} 3062 (C–H, aromatic), 2923 (C–H, aliphatic), 1611 (C=N), 1590 (C=C, aromatic), 1511,1325 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.58 (s, 3H, N–CH_3), 7.12 (t, 1H, *J* 7.8 Hz, Ar*H*, benzothiazole ring), 7.34 (m, 2H, Ar*H*, benzothiazole ring), 7.64 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.95 (d, 2H, *J* 8.4 Hz, AB system, Ar*H*, nitrobenzene ring), 8.27 (d, 2H, *J* 8.4 Hz, AB system, Ar*H*, nitrobenzene ring), 8.48 (s, 1H, N=C*H*); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.2 (N–*C*H$_3$), 111.3, 124.1, 124.2, 126.8, 128.6, 133.1 (aromatic *C*–H), 122.6 (*C*-9, benzothiazole ring), 139.4 (N–CH=*C*–, benzene ring), 141.6 (*C*-4, benzothiazole ring), 146.2 (N=*C*H–Ph), 147.9 (*C*–NO_2, benzene ring), 165.2 (S–*C*=N); MS (EI, 70 eV) *m/z* (%) 312 (100) [M$^+$]; anal. calcd.: $C_{15}H_{12}N_4O_2S$ (312.35) C 57.68%, H 3.87%, N 17.94%, S 10.27%; found: C 57.80%, H 3.83%, N 17.89%, S 10.30%.

3-Methyl-2-((thiophen-2-ylmethylene)hydrazono)-2,3-dihydrobenzo[d]thiazole (**3e**)

Buff crystals; yield 1.91 g, 70%; mp 185-187 °C; IR (KBr) ν_{max}/cm^{-1} 3050 (C–H, aromatic), 2924 (C–H, aliphatic), 1610 (C=N), 1558 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.69 (s, 3H, NCH_3), 7.01 (t, 1H, *J* 7.6 Hz, thiophen-*H*), 7.13 (t, 1H, *J* 9.2 Hz, Ar*H*, benzothiazole ring), 7.33 (m, 2H, Ar*H*, benzothiazole ring), 7.51 (d, 1H, *J* 9.2 Hz, Ar*H*, benzothiazole ring), 7.61 (d, 1H, *J* 7.6 Hz, thiophene-*H*), 7.77 (d, 1H, *J* 4.3 Hz, thiophene-*H*), 8.02 (s, 1H, N=C*H*); MS (EI, 70 eV) *m/z* (%) 273 (100) [M$^+$]; anal. calcd.: $C_{13}H_{11}N_3S_2$ (273.38) C 57.12%, H 4.06%, N 15.37%, S 23.46%; found: C 57.02%, H 4.05%, N 15.32%, S 23.49 %.

4-(((3-Methylbenzo[d]thiazol-2(3H)-ylidene)hydrazono) methyl)benzonitrile (**3f**)

Yellow crystals; yield 2.17 g, 70%; mp 215-216 °C; IR (KBr) ν_{max}/cm^{-1} 3084 (C–H, aromatic), 2921 (C–H, aliphatic), 2219 (C≡N), 1615 (C=N), 1566 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.64 (s, 3H, NCH_3), 7.12 (t, 1H, *J* 7.8 Hz, Ar*H,* benzothiazole ring), 7.34 (d, 1H, *J* 8.1 Hz, Ar*H*, benzothiazole ring), 7.62 (t, 1H, *J* 8.1 Hz,

Ar*H*, benzothiazole ring), 7.88 (d, 1H, *J* 7.8 Hz, Ar*H*, benzothiazole ring), 7.91 (d, 2H, *J* 8.1 Hz, AB system, Ar*H*, benzene ring), 8.40 (d, 2H, *J* 8.1 Hz, AB system, Ar*H*, benzene ring), 8.43 (s, 1H, N=C*H*); MS (EI, 70 eV) *m/z* (%) 292 (48) [M$^+$]; anal. calcd.: $C_{16}H_{12}N_4S$ (292.36) C 65.73%, H 4.14%, N 19.16%, S 10.97%; found: C 65.59%, H 4.17%, N 19.18%, S 11.01%.

2-((2-Ferrocenyl)hydrazono)-3-methyl-2,3-dihydrobenzo[d]thiazole (**3g**)

Orange crystals; yield 2.81 g, 75%; mp 161-162 °C; IR (KBr) ν_{max}/cm^{-1} 3082 (C–H, aromatic), 2927 (C–H, aliphatic), 1611 (C=N), 1590, 1555 (C=C, aromatic and ferrocenyl); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.47 (s, 3H, CH_3), 4.17 (s, 5H, ferrocenyl-*H*), 4.04 (s, 2H, ferrocenyl-*H*), 4.61 (s, 2H, ferrocenyl-*H*), 7.03 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.20 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.27 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.53 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 8.15 (s, 1H, N=C*H*); MS (EI, 70 eV) *m/z* (%) 378 (80) [M$^+$+3]; anal. calcd.: $C_{19}H_{17}FeN_3S$ (375.27) C 60.81%, H 4.57%, Fe 14.88%, N 11.20%, S 8.54%; found: C 60.93%, H 4.53%, N 11.17%, S 8.50%.

4-(((3-Methylbenzo[d]thiazol-2(3H)-ylidene)hydrazono)methyl)benzene-1,2-diol (**3h**)

Dark brown crystals; yield 2.09 g, 70%; mp 255-257 °C; IR (KBr) ν_{max}/cm^{-1} 3468 (O–H), 3080 (C–H, aromatic), 2929 (C–H, aliphatic), 1623 (C=N), 1602 (C=C, olefinic), 1563 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.49 (s, 1H, NCH_3), 6.74 (d, 1H, *J* 8.4 Hz, Ar*H*, benzene ring), 6.94 (d, 1H, *J* 8.4 Hz, Ar*H*, benzene ring), 7.04 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.21 (s, 1H, Ar*H*, benzene ring), 7.22 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.28 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.55 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 8.17 (s, 1H, N=C*H*), 9.16 and 9.34 (s, 2H, 2O*H*, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6) δ 30.0 (N–CH_3), 110.3, 113.5, 116.0, 121.0, 122.0, 122.7 (aryl CH, 7C), 121.0 (*C*-9, benzothiazole ring), 126.9 (CH=*C*–, benzene ring), 141.45 (*C*-4, benzothiazole ring), 146.0 (*C*–OH, benzene ring), 148.3 (*C*–OH, benzene ring), 153.33 (N=*C*H–Ph), 165.3 (S–*C*=N); MS (EI, 70 eV) *m/z* (%) 300 (72) [M$^+$+1]; anal. calcd.: $C_{15}H_{13}N_3O_2S$ (299.35) C 60.18%, H 4.38%, N 14.04%, S 10.71%; found: C 60.27%, H 4.33%, N 13.98%, S 10.68%.

General procedure for the reaction of Schiff base derivatives **3a**-**e** with thioglycolic acid

Thioglycolic acid (1.4 g, 1.1 mL, 0.015 mol) was added dropwise to a solution of the appropriate Schiff base derivative **3a**-**e** (0.01 mol) in dry benzene (50 mL) at room temperature. After refluxing the reaction mixture for 6-8 h, the volatile materials were removed under reduced pressure. The residual substance was collected and chromatographed on silica gel to give the corresponding 4-thiazolidinone derivatives **4a**-**e** as colorless crystals.

3-(3-Methylbenzo[d]thiazol-2(3H)-ylideneamino)-2-phenylthiazolidin-4-one (**4a**)

Eluent: petroleum ether (60-80 °C)/acetone (85/15, v/v); colorless crystals; yield 2.55 g, 75%; mp 262-263 °C; IR (KBr) ν_{max}/cm^{-1} 3061 (C–H, aromatic), 2924 (C–H, aliphatic), 1687 (C=O), 1628 (C=C, aromatic), 1571 (C=N); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.31 (s, 3H, NCH_3), 3.71, 3.83 (2d, 2H, each with *J* 16.0 Hz, –O=C–CH_2–S), 5.86 (s, 1H, NC*H*–S), 7.20-7.40 (m, 9H, aromatic); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.0 (S–*C*H$_2$CO), 32.0 (N–CH_3), 63.9 (Ph–*C*H–S), 110.7 (C-9, benzothiazole ring), 129.2 (Ph–*C*–CH–S), 128.9, 127.9, 127.2, 122.7, 122.5 (aromatic CH, 9C), 140.8 (C-4, benzothiazole ring), 166.9 (S–C=N), 167.8 (C=O); MS (EI, 70 eV) *m/z* (%) 341 (10) [M$^+$]; anal. calcd.: $C_{17}H_{15}N_3OS_2$ (341.45) C 59.80%, H 4.43%, N 12.31%, S 18.78%; found: C 59.77%, H 4.45%, N 12.82%, S 18.73%.

2-(4-Chlorophenyl)-3-(3-methylbenzo[d]thiazol-2(3H)-ylideneamino)-thiazolidin-4-one (**4b**)

Eluent: petroleum ether (60-80 °C)/acetone (90/10, v/v); colorless crystals; yield 2.81 g, 75%; mp 194-196 °C; IR (KBr) ν_{max}/cm^{-1} 3081 (C–H, aromatic), 2923 (C–H, aliphatic), 1688 (C=O), 1608 (C=N), 1555 (C=C, aromatic), 740 (Cl–C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.36 (s, 3H, NCH_3), 3.71, 3.87 (2d, 2H, each with *J* 16.3 Hz, –O=C–CH_2–S), 5.88 (s, 1H, NC*H*–S), 7.08 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.18 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.23 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.43 (d, 2H, *J* 8.6 Hz, AB system, Ar*H*, benzene ring), 7.51 (d, 2H, *J* 8.6 Hz, AB system, Ar*H*, benzene ring), 7.55 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring); MS (EI, 70 eV) *m/z* (%) 375 (100) based on ^{35}Cl and 377 (46) based on ^{37}Cl; anal. calcd.: $C_{17}H_{14}ClN_3OS_2$ (375.90) C 54.32%, H 3.75%, Cl 9.43%, N 11.18%, S 17.06%; found: C 54.44%, H 3.72%, Cl 9.40%, N 11.15%, S 17.11%.

3-(3-Methylbenzo[d]thiazol-2(3H)-ylideneamino)-2-(4-(piperidin-1-yl)-phenyl)thiazolidin-4-one (**4c**)

Eluent: petroleum ether (60-80 °C)/acetone (75/25, v/v); colorless crystals; yield 2.33 g, 55%; mp 202-203 °C; IR (KBr) ν_{max}/cm^{-1} 3033 (C–H, aromatic), 2930 (C–H, aliphatic, asymmetric), 2869 (C–H, aliphatic,

symmetric), 1692 (C=O), 1607 (C=N), 1562 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.45-1.51 (m, 6H, piperidinyl ring), 3.07-3.09 (m, 4H, piperidinyl ring), 3.32 (s, 3H, NCH_3), 3.68, 3.78 (2d, 2H, each with *J* 16.0 Hz, –O=C–CH_2–S), 5.76 (s, 1H, NC*H*–S), 6.80 (d, 2H, *J* 8.1 Hz, AB system, Ar*H*, benzene ring), 7.04 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.15 (d, 1H, *J* 6.7 Hz, Ar*H*, benzothiazole ring), 7.25 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.27 (d, 2H, *J* 8.1 Hz, AB system, Ar*H*, benzene ring), 7.53 (d, 1H, *J* 6.7 Hz, Ar*H*, benzothiazole ring); MS (EI, 70 eV) *m/z* (%) 425 (83) [M$^+$+1]; anal. calcd.: $C_{22}H_{24}N_4OS_2$ (424.58) C 62.23%, H 5.70%, N 13.20%, S 15.10%; found: C 62.14%, H 5.73%, N 13.16%, S 15.11%.

3-(3-Methylbenzo[d]thiazol-2(3H)-ylideneamino)-2-(4-nitrophenyl)- thiazolidin-4-one (**4d**)

Eluent: petroleum ether (60-80 °C)/acetone (85/15, v/v); colorless crystals; yield 2.31 g, 60%; mp 140-141 °C; IR (KBr) ν_{max}/cm^{-1} 3080 (C–H, aromatic), 2932 (C–H, aliphatic), 1703 (C=O), 1619 (C=N), 1590 (C=C, aromatic), 1401, 1378 (NO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.38 (s, 3H, NCH_3), 3.72, 3.80 (2d, 2H, each with *J* 15.6 Hz, –O=C–CH_2–S), 5.78 (s, 1H, NC*H*–S), 7.07 (t, 1H, *J* 7.7 Hz, Ar*H*, benzothiazole ring), 7.34 (m, 3H, Ar*H*, benzothiazole and nitrobenzene rings), 7.64 (d, 1H, *J* 7.7 Hz, Ar*H*, benzothiazole ring), 7.88 (d, 1H, *J* 7.7 Hz, Ar*H*, benzothiazole ring), 8.17 (d, 2H, *J* 8.0 Hz, Ar*H*, nitrobenzene ring); MS (EI, 70 eV) *m/z* (%) 386 (< 5) [M$^+$]; anal. calcd.: $C_{17}H_{14}N_4O_3S_2$ (386.45) C 52.84%, H 3.65%, N 14.50%, S 16.59%; found: C 2.71%, H 3.68%, N 14.54%, S 16.62%.

3-(3-Methylbenzo[d]thiazol-2(3H)-ylideneamino)-2-(thiophen-2-yl)thiazolidin-4-one (**4e**)

Eluent: petroleum ether (60-80 °C)/acetone (90/10, v/v); colorless crystals; yield 1.90 g, yield 55%; mp 122-123 °C; IR (KBr) ν_{max}/cm^{-1} 3046 (C–H, aromatic), 2931 (C–H, aliphatic), 1710 (C=O), 1628 (C=N), 1580 (C=C, aromatic); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.53 (s, 3H, NCH_3), 3.51, 3.65 (2d, 2H, each with *J* 14.4 Hz, –O=C–CH_2–S), 5.68 (s, 1H, NC*H*–S), 6.95-7.31 (m, 7H, Ar*H*, benzothiazole and thiophene rings); MS (EI, 70 eV) *m/z* (%) 164 (5) [M$^+$-$C_7H_5S_2N$]; anal. calcd.: $C_{15}H_{13}N_3OS_3$ (347.48) C 51.85%, H 3.77%, N 12.09%, S 27.68%; found: C 52.02%, H 3.74%, N 12.04%, S 27.72%.

Reaction of 2-hydrazono-3-methyl-2,3-dihydrobenzo[d] thiazole **1** with ethyl cyanoacetate

To a solution of compound **1** (1.79 g, 0.01 mol) in ethanol (50 mL), ethyl cyanoacetate (1.69 g, 1.6 mL, 0.015 mol) was added at room temperature and the reaction mixture was refluxed for 5 h. The volatile materials were removed under reduced pressure and the residual substance was collected and chromatographed on silica gel to give compounds **5** and **6**.

1,2-Bis(3-methylbenzo[d]thiazol-2(3H)-ylidene)hydrazine (**5**)

Eluent: petroleum ether (60-80 °C)/acetone (95/5, v/v); pale yellow crystals; yield 1.30 g, yield 40%; mp 259-261 °C (lit. 266 °C);[19] IR (KBr) ν_{max}/cm^{-1} 3049, 3009 (C–H, aromatic), 2923 (C–H, aliphatic, asymmetric), 2854 (C–H, aliphatic, symmetric), 1606 (C=N), 1571 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.67 (s, 6H, 2NCH_3), 6.97 (t, 2H, *J* 8.4 Hz, Ar*H*, benzothiazole ring), 7.10 (d, 2H, *J* 8.4 Hz, Ar*H*, benzothia-zole ring), 7.23 (t, 2H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.48 (d, 2H, *J* 7.6 Hz, Ar*H*, benzothiazole ring); MS (EI, 70 eV) *m/z* (%) 326 (100) [M$^+$].

2-Cyano-*N*'-(3-methylbenzo[d]thiazol-2(3H)-ylidene) acetohydrazide (**6**)

Eluent: petroleum ether (60-80 °C)/acetone (50/50, v/v); yellowish brown crystals; yield 0.74 g, 30%; mp 222-224 °C; IR (KBr) ν_{max}/cm^{-1} 3428 (N–NH), 3151 (C–H, aromatic), 2981 (C–H, aliphatic, asymmetric), 2862 (C–H, aliphatic, symmetric), 2257 (C≡N), 1667 (C=O), 1603 (C=N), 1578 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.67 (s, 3H, NCH_3), 3.83 (d, 2H, ^4J 7.4 Hz, W-conformation, NH–C(O)–CH_2–CN), 7.04 (t, 1H, *J* 8.1 Hz, Ar*H*, benzothiazole ring), 7.19 (d, 1H, *J* 8.1 Hz, Ar*H*, benzothiazole ring), 7.31 (t, 1H, *J* 7.8 Hz, Ar*H,* benzothiazole ring)*,* 7.56 (d, 1H, *J* 7.8 Hz, Ar*H*, benzothiazole ring), 10.48 (brs, 1 H, N*H*, D_2O exchangeable); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.6 (*C*H_2CN), 31.1 (N–*C*H_3), 110.0 (*C*≡N), 117.0 (C-9, benzo-thiazole ring),127.3, 122.9, 122.1, 121.8 (aromatic *C*, 4C), 141.3 (*C*-4, benzothiazole ring), 158.7 (S–*C*=N), 164.1 (*C*=O); MS (EI, 70 eV) *m/z* (%) 246 (52) [M$^+$]; anal. calcd.: $C_{11}H_{10}N_4OS$ (246.29) C 53.65%, H 4.09%, N 22.75%, S 13.02%; found: C 53.79%, H 4.01%, N 22.69%, S 13.06%.

Reaction of hydrazone **1** with ethyl acetoacetate

A mixture of compound **1** (1.79 g, 0.01 mol) and ethyl acetoacetate (1.95 g, 1.9 mL, 0.015 mol) was refluxed in absolute ethanol (20 mL) for 6 h. The volatile materials were removed from the reaction mixture under reduced pressure and the residual substance was chromatographed on silica gel to give compounds **7** and **8**, respectively.

Ethyl3-(2-(3-methylbenzo[d]thiazol-2(3H)-ylidene) hydrazinyl)but-2-enoate (**7**)

Eluent: petroleum ether (60-80 °C)/acetone (90/10, v/v); yellow crystals; yield 0.72 g, 25%; mp 128-130 °C; IR (KBr) ν_{max}/cm^{-1} 3432 (N–H), 2922 (C–H aliphatic, asymmetric), 2857 (C–H aliphatic, symmetric), 1732 (ester, C=O), 1633 (C=N), 1582 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (t, 3H, *J* 7.6 Hz, ethoxy-CH_3), 2.03 (s, 3H, CH_3–C=N), 3.43 (s, 2H, N=C–CH_2–C=O), 3.48 (s, 3H, NCH_3), 4.08 (q, 2H, *J* 7.6 Hz, ethoxy-CH_2), 7.02 (t, 1H, *J* 8.6 Hz, Ar*H*, benzothiazole ring), 7.20 (d, 1H, *J* 8.6 Hz, Ar*H*, benzothiazole ring), 7.26 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.51 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring); MS (EI, 70 eV) *m/z* (%) 291 (100) [M$^+$]; anal. calcd.: $C_{14}H_{17}N_3O_2S$ (291.37) C 57.71%, H 5.88%, N 14.42%, S 11.00%; found: C 57.83%, H 5.84%, N 14.38%, S 10.97%.

N'-(3-Methylbenzo[d]thiazol-2(3H)-ylidene)-3-oxobutanehydrazide (**8**)

Eluent: petroleum ether (60-80 °C)/acetone (85/15, v/v); golden yellow crystals; yield 0.92 g, 35%; mp 199-202 °C; IR (KBr) ν_{max}/cm^{-1} 3430 (N–H), 2923 (C–H aliphatic, asymmetric), 2855 (C–H aliphatic, symmetric), 1730, 1677 (C=O), 1587 (C=N), 1525 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.46 (s, 2H, CH_2), 3.79 (s, 3H, NCH_3), 3.83 (s, 3H, OCH_3), 6.03 (brs, 1H, N*H*, D_2O exchangeable), 6.95-7.90 (m, 4H, benzothiazole protons); MS (EI, 70 eV) *m/z* (%) 263 (< 5) [M$^+$]; anal. calcd.: $C_{12}H_{13}N_3O_2S$ (263.32) C 54.74%, H 4.98%, N 15.96%, S 12.18%; found: C 54.47%, H 4.58%, N 15.75%, S 12.22%.

Reaction of hydrazone **1** with malononitrile

A mixture of compound **1** (1.79 g, 0.01 mol) and malononitrile (1 g, 0.015 mol) was refluxed in absolute ethanol (20 mL) for 6 h. The volatile materials were removed under reduced pressure and the residual substance was collected and chromatographed on silica gel to give compounds **5**, **9** and **10**.

1,2-Bis(3-methylbenzo[d]thiazol-2(3H)-ylidene)hydrazine (**5**)

Eluent: petroleum ether (60-80 °C)/ acetone (95/5, v/v); pale yellow crystals; 1.11 g, yield 40%; mp 259-261 °C (lit. 266 °C).[19]

2-(2-(3-Methylbenzo[d]thiazol-2(3H)-ylidene)hydrazinyl) acetonitrile (**9**)

Eluent: petroleum ether (60-80 °C)/acetone (80/20, v/v); pale brown crystals; yield 0.43 g, 20%; mp 175-177 °C; IR (KBr) ν_{max}/cm^{-1} 3267 (N–H), 3064 (C–H, aromatic), 2919 (C–H aliphatic, asymmetric), 2849 (C–H aliphatic, symmetric), 2202 (C≡N), 1628 (C=N), 1577, 1523 (C=C, aromatic); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.68 (s, 3H, NCH_3), 3.90 (s, 2H, CH_2), 6.99-7.52 (m, 4H, benzothiazole ring protons), 8.58 (brs, 2H, NH_2, D_2O exchangeable); MS (EI, 70 eV) *m/z* (%) 218 (2) [M$^+$]; anal. calcd.: $C_{10}H_{10}N_4S$ (218.28) C 55.02%, H 4.62%, N 25.67%, S 14.69%; found: C 55.11%, H 4.59%, N 25.61%, S 14.72%.

2-Cyano-*N*'-(3-methylbenzo[d]thiazol-2(3H)-ylidene) acetohydrazonamide (**10**)

Eluent: petroleum ether (60-80 °C)/acetone (80/20, v/v); dark brown crystals; yield 0.49 g, 20%; mp 140-141 °C; IR (KBr) ν_{max}/cm^{-1} 3476, 3370 (NH$_2$), 3051 (C–H, aromatic), 2917 (C–H, aliphatic), 2251 (C≡N), 1656, 1617 (C=N), 1565 (aromatic C=C); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.44 (s, 3H, NCH_3), 4.32 (s, 2H, CH_2), 6.32 (brs, 2H, NH_2, D_2O exchangeable), 6.96 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.09 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.22 (t, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring), 7.47 (d, 1H, *J* 7.6 Hz, Ar*H*, benzothiazole ring); ^{13}C NMR (125 MHz, DMSO-d_6) δ 22.5 (*C*H$_2$CN), 30.9 (N–CH_3), 109.5 (*C*N), 117.4 (*C*-9, benzothiazole ring),126.6, 123.9, 122.7, 121.2 (aromatic *C*, 4C), 141.7 (*C*-4, benzothiazole ring), 148.3 (N=*C*–NH$_2$), 159.0 (S–*C*=N); MS (EI, 70 eV) *m/z* (%) 245 (72) [M$^+$]; anal. calcd.: $C_{11}H_{11}N_5S$ (245.30) C 53.86%, H 4.52%, N 28.55%, S 13.07%; found: C 53.98%, H 4.49%, N 28.59%, S 13.05%.

Supplementary Information

IR, ^{1}H NMR, ^{13}C NMR and MS spectra of new products are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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