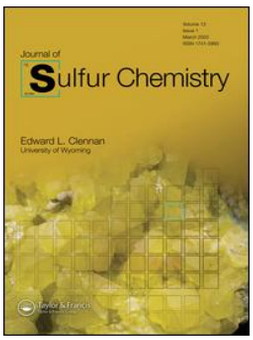


Journal of Sulfur Chemistry

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/gsrp20

Design, Synthesis and Antiproliferative Activity of Novel Heterocycles from 6-Iodo-2-phenyl-4*H*-benzo[*d*][1,3]thiazine-4-thione

Marwa S. Salem , Selima A. M. Al-Mabrook & Maher A. E. M. El-Hashash

To cite this article: Marwa S. Salem , Selima A. M. Al-Mabrook & Maher A. E. M. El-Hashash (2020): Design, Synthesis and Antiproliferative Activity of Novel Heterocycles from 6-Iodo-2-phenyl-4*H*-benzo[*d*][1,3]thiazine-4-thione, Journal of Sulfur Chemistry, DOI: 10.1080/17415993.2020.1847287

To link to this article: https://doi.org/10.1080/17415993.2020.1847287

JOURNAL OF SULFUR CHEMISTRY
https://doi.org/10.1080/17415993.2020.1847287

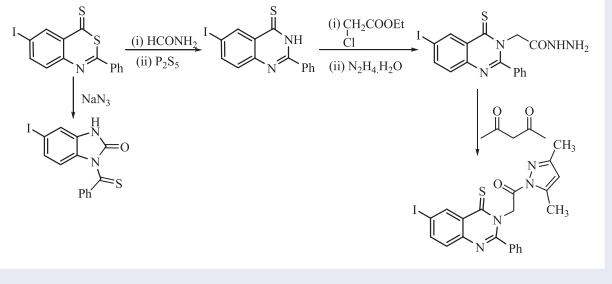
Design, Synthesis and Antiproliferative Activity of Novel Heterocycles from 6-Iodo-2-phenyl-4*H*-benzo[*d*][1,3]thiazine-4-thione

Marwa S. Salem [a], Selima A. M. Al-Mabrook[a,b] and Maher A. E. M. El-Hashash [a]

[a]Chemistry Department, Faculty of Science, Ain Shams University, Abbasiya, Egypt; [b]Faculty of science, Alasmarya Islamic University, Zliten, Libya

ABSTRACT

The present work is dedicated to utilize the reactivity of 6-iodo-2-phenyl-4*H*-benzo[*d*][1,3]thiazine-4-thione to motivate new different heterocyclic systems namely, quinazoline-4(3*H*)-thione,benzimidazol-2(3*H*)-one, pyrazole and thiadiazole derivatives which have been structurally characterized with spectroscopic data such as IR, ^{1}HNMR and mass spectra. The synthesized compounds were evaluated for the anticancer activity against HepG-2 and MCF-7 cell lines. 1-(3,5-Dimethyl-1*H*-pyrazol-1-yl)-2-(6-iodo-2-phenyl-4-thioxoquinazolin-3(4*H*)-yl)ethanone and ethyl 3-(2-(2-(6-iodo-2-phenyl-4-thioxoquinazolin-3(4*H*)-yl)acetyl)hydrazono)butanoate showed the highest cytotoxic activities against the two cell lines comparable to that of the reference compound doxorubicin. Most of the synthesized compounds also exhibited good cytotoxic activity.



ARTICLE HISTORY
Received 5 May 2020
Accepted 27 October 2020

KEYWORDS
Acetohydrazide; antitumor; oxadiazole; pyrazole; benzimidazolone

1. Introduction

Nitrogen-containing heterocyclic compounds are very important in drug design. Thus, the motivation for this present study was the known widespread application of benzo-fused *N*-heterocycles. Furthermore, benzothiazinethione derivatives are considered as an attractive

CONTACT Marwa S. Salem marwa_k@sci.asu.edu.eg Chemistry Department, Faculty of Science, Ain Shams University, Abbasiya, Cairo 11566, Egypt

Supplemental data for this article can be accessed here. https://doi.org/10.1080/17415993.2020.1847287

target for medicinal chemists, because they are the scaffold of several potent heterocyclic systems antimycobacterial properties and antitubercular agent [1–3].

Quinazolinones are mostly derived from microorganisms and seldom from plants [4]. Natural quinazolinones have been reported to exhibit various pharmacological activities, such as, anti-inflammatory [5], anticancer [6–9] antifungal [10], anticonvulsant, antihyperlipidemic [11], antimalarial [12] and antimicrobial [13] activities. A series of some substituted quinazoline-4-thione derivatives have been exhibited antimycobacterial, photosynthesis-inhibiting, and antialgal activity [14]. Benzimidazoles are considered as potential bioactive heterocyclic aromatic compounds with a variety of biological activities like anti-inflammatory [15], antiparasitic [16], antimalarial [17], antimycobacterial [18], antineoplastic [19], antiviral [20], antihypertensive [21] and anticonvulsant [22] activities.

It has been noted that pyrazole derivatives represent a major pharmacophore with various biological properties, and some pyrazole-containing derivatives have already been used for therapeutic purposes such as anti-diabetic [23], anti-viral [24,25], anticonvulsant, analgesic and anti-inflammatory [26,27].

Based on our results and on literature data, the present study aims at synthesis of new molecular hybridization between quinazolinone and thiadiazole and pyrazolo moieties in an attempt to obtain new effective compounds with antitumor activity.

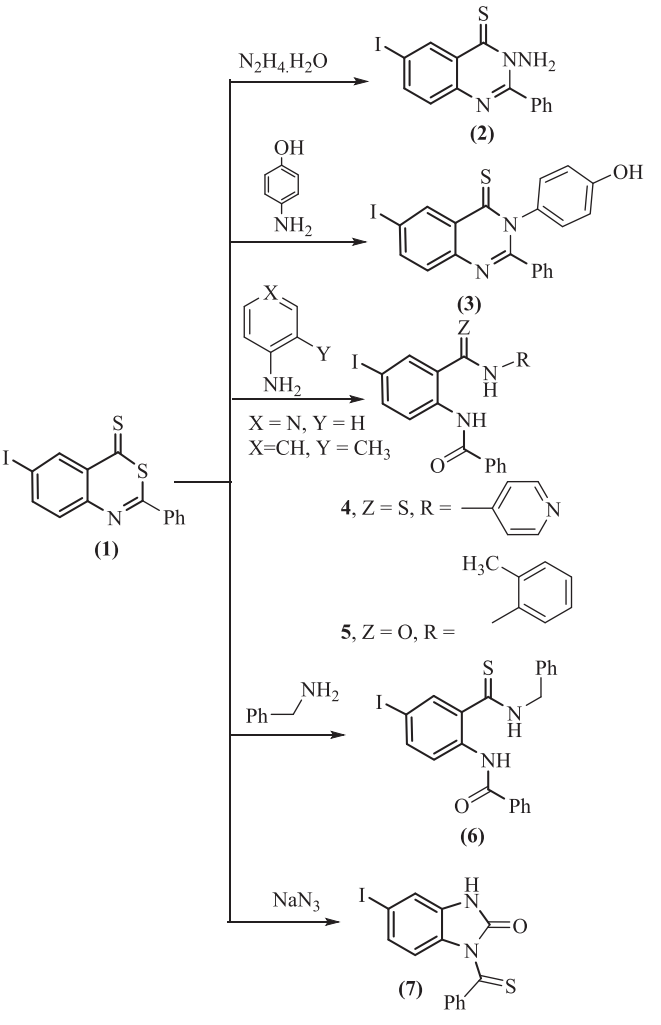
2. Results and discussion

2.1. Chemistry

In continuation of our previous work [8,9,13,28–37] in heterocyclic synthesis, 6-iodo-2-phenyl-4*H*-benzo[*d*][1,3]thiazine-4-thione **(1)** was prepared *via* reaction of the *O*-analogue [35] of compound **1** with phosphorous pentasulfide [38] in dry toluene. The IR spectrum of **1** displayed a band at 1199 cm^{-1} corresponding to C = S and lacked the stretching band for C = O group. The reactivity of benzothiazinethione derivative **1** encouraged us to a robust approach for the synthesis of diverse novel heterocyclic compounds. Hydrazinolysis of benzothiazinethione **1** with hydrazine hydrate afforded 3-amino-6-iodo-2-phenylquinazoline-4(3*H*)-thione **(2)**. IR spectrum showed characteristic bands at ν 3386, 3293 and 1218 cm^{-1} assignable to NH_2 and C = S stretching frequencies, respectively. Also, the ^{1}H NMR spectrum revealed signal at δ 6.77 ppm for NH_2 exchangeable with D_2O.

The reactivity of benzothiazinethione derivative **1** has been studied towards different amines namely, 4-hydroxyaniline, 4-aminopyridine, o-toluidine, and /or benzylamine to give 2-phenylquinazoline-4(3*H*)-thione **(3)** and benzamide derivatives **4–6**, respectively (cf. Scheme 1). The spectral properties of the new products **3–6** agree with their proposed structures.

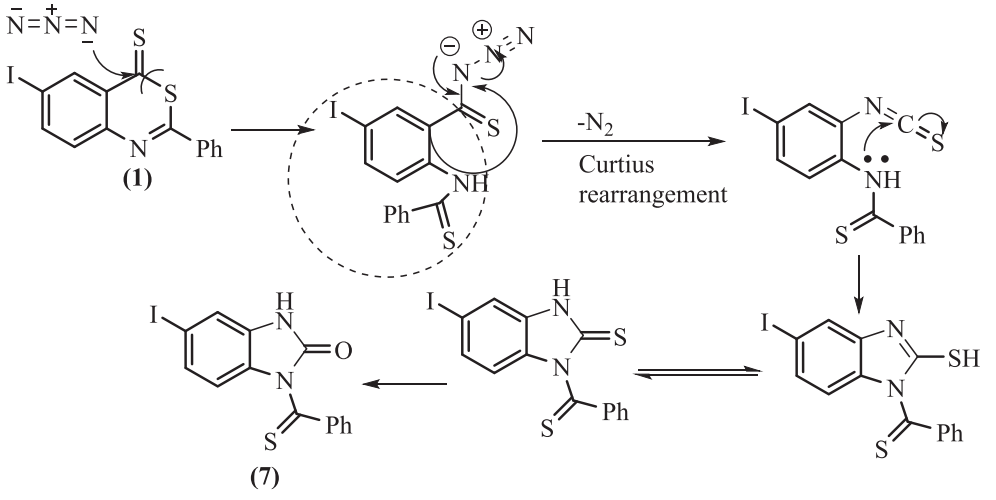
Benzothiazinethione derivative **1** reacted with sodium azide in boiling glacial acetic acid to afford benzimidazolone derivative **7**. According to our point of view [13], the formation of compound **7** proceeded *via* attack of azide ion at C-4, then Curtius rearrangement took place *via* loss of nitrogen molecule and migration of the aryl-group from the thiocarbonyl carbon to the closest nitrogen in a concerted mechanism to give the isothiocyanate, which attacked by NH group to afford benzimidazolone **7** (cf. Scheme 2).



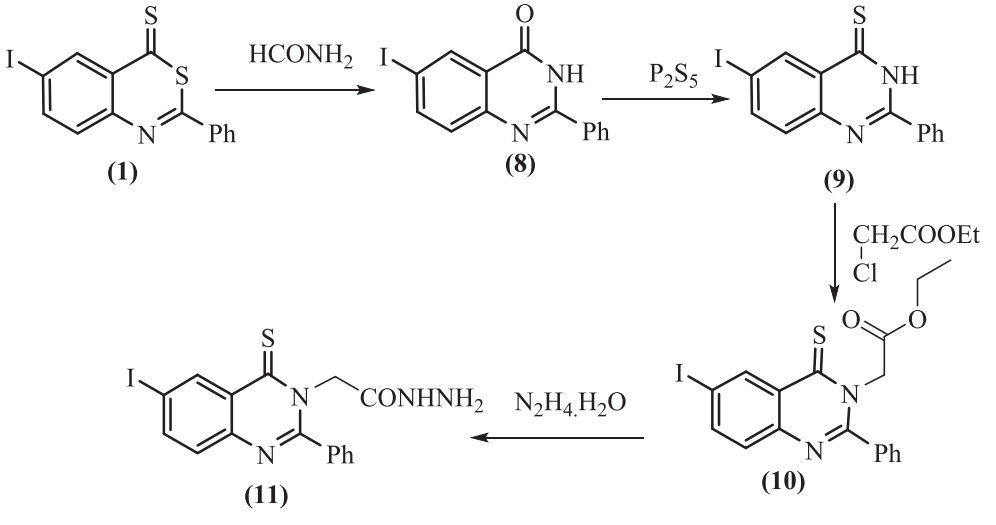
Scheme 1. Reactivity of benzothiazinethione derivative 1 towards nitrogen nucleophiles.

Quinazolinone derivative **8** was prepared through the reaction of the benzothiazinethione **1** with formamide. The latter product has been synthesized in one of our recent publication [7] *via* reaction of *O*-analogues of benzothiazinethione **1** with formamide. Thiation of quinazolinone derivative **8** with phosphorous pentasulfide in dry toluene gave the corresponding thiated derivative **9** which reacted with ethyl chloroacetate to afford ethyl 2-(6-iodo-2-phenyl-4-thioxoquinazolin-3(4*H*)-yl)acetate **10**. Hydrazinolysis of compound **10** gave the corresponding acetohydrazide derivative **11**. The existence of ν NH_2 and NH at 3436, 3220 cm^{-1} in the IR spectrum of compound **11** confirmed the suggested structure. Strong support of structure **11** was forthcoming from ^{1}H NMR spectrum which showed the disappearance of the triplet and quartet signals attributed to the ethyl protons of its precursor **10** (cf. Scheme 3).

The behavior of quinazolinylacetohydrazide derivative **11** towards carbon electrophiles has been investigated with a view to obtain some interesting quinazolinone derivatives.

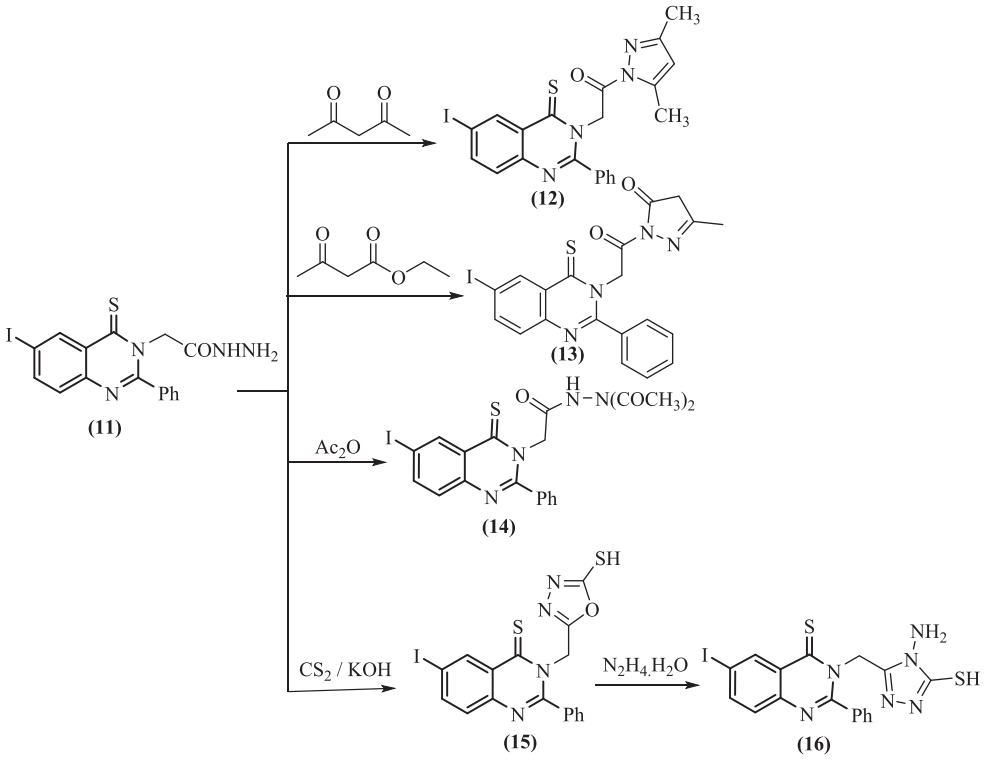


Scheme 2. Reaction mechanism of benzothiazinethione derivative 1 with sodium azide.



Scheme 3. Synthesis of quinazolinylacetohydrazide derivative 11.

Interaction of acetohydrazide derivative **11** with acetylacetone afforded 1-(3,5-dimethyl-1*H*-pyrazol-1-yl)-2-(6-iodo-2-phenyl-4-thioxoquinazolin-3(4*H*)-yl)ethanone (**12**). The IR, ^{1}H NMR spectra of the quinazolinone derivative **12** are devoid of any signals for NH_2 group and appearance of signals at 6.99 and 4.21 ppm attributable to CH pyrazole ring and CH_2 protons in ^{1}H NMR spectrum. On the other hand, reaction of acetohydrazide derivative **11** with ethyl acetoacetate gave 2-(2-(6-iodo-2-phenyl-4-thioxoquinazolin-3(4*H*)-yl)acetyl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one **(13)**. IR spectrum confirmed the suggested structure *via* appearance of $\nu_{C=O}$ at 1724 and 1694 cm^{-1}. Acetylation of acetohydrazide derivative **11** furnished the corresponding diacetyl derivative **14**, which was stablished *via* spectroscopic data. IR showed $\nu_{NH,}$ $\nu_{C=O}$ imide, $\nu_{C=O}$ amide,



Scheme 4. Reaction of quinazolinylacetohydrazide derivative 11 with carbon electrophiles.

3169, 1735 and 1679 cm^{-1}, respectively.^{1}H NMR exhibited singlet signal at δ 10.40 ppm for NH, exchangeable with D_2O and 2.60 ppm singlet signal for $2CH_3$. Reaction of acetohydazide derivative **11** with carbon disulfide in alcoholic potassium hydroxide solution gave 6-iodo-3-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-2-phenylquinazoline-4(3*H*)-thione **(15)**, which confirmed chemically *via* reaction of compound **15** with hydrazine hydrate to afford 3-((4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)methyl)-6-iodo-2-phenylquinazoline-4(3*H*)-thione **(16)** (Scheme 4).

2.2. Cytotoxicity and antitumor evaluation

Anti-proliferative activity of most of the synthesized compounds were screened *in vitro* against two human cancer cell lines, namely, hepatocellular carcinoma (HePG-2) and mammary gland breast cancer (MCF-7) using MTT colorimetric assay. *In-vitro* cytotoxicity evaluation using viability assay was performed using doxorubicin as a reference cytotoxic compound. Growth inhibitory concentration (IC_{50}) values has been measured (Table 1).

The obtained results revealed that compound **12** was the most potent derivatives against the two cell lines as active as doxorubicin, which showed the percentage viability IC_{50} at 6.39 and 5.46 μg/ml for HePG-2 and MCF-7, respectively. This activity of compound **12** is due to the presence of pyrazole moieties which hybrid with thioxoquinazoline. Compound

Table 1. Cytotoxic activity of some compounds against human tumor cells.

	In vitro Cytotoxicity IC_{50} (μg/ml)•	
Compounds	HePG2	MCF-7
DOX	4.50 ± 0.2	4.17 ± 0.2
1	35.86 ± 2.3	47.62 ± 2.8
2	58.42 ± 2.9	42.85 ± 2.3
3	50.10 ± 2.5	54.17 ± 2.8
4	75.69 ± 3.7	35.37 ± 2.0
5	80.90 ± 4.7	85.80 ± 4.9
6	37.14 ± 2.5	52.39 ± 3.3
7	43.82 ± 2.4	53.47 ± 2.6
8	86.96 ± 3.9	82.26 ± 4.1
10	11.68 ± 1.2	26.35 ± 1.8
12	6.39 ± 0.7	5.46 ± 0.6
13	7.07 ± 0.6	8.80 ± 0.9
14	53.42 ± 2.6	40.02 ± 2.2
15	26.58 ± 1.9	15.08 ± 1.3
16	21.83 ± 1.5	12.32 ± 1.1

•**IC_{50} (μg/ml)**: 1–10 (very strong). 11–20 (strong). 21–50 (moderate). 51–100 (weak) and above 100 (non-cytotoxic).
•**DOX**: Doxorubicin.

13 displayed high cytotoxic activities against HePG-2 ($IC_{50} = 7.07 \pm 0.6$) and MCF-7 ($IC_{50} = 8.80 \pm 0.9$). The reactivity of **13** may be attributed to its chemical structure dihydropyrazolone present on one side of thioxoquinazoline ring without any steric hindrances facilitate bonding with the receptors.

Compounds **15** and **16** have a strong activity towards MCF-7 cell line ($IC_{50} = 15.08 \pm 1.3$ and 12.32 ± 1.1, respectively). Compound **16** is more reactive than **15** because the presence of triazol and quinazolinethione moieties in a single molecular framework.

On the other hand, a compound **10** has a strong activity towards HePG-2 cell line ($IC_{50} = 11.68 \pm 1.2$). Compounds **1**, **6**, and **7** revealed a moderate activity toward the two

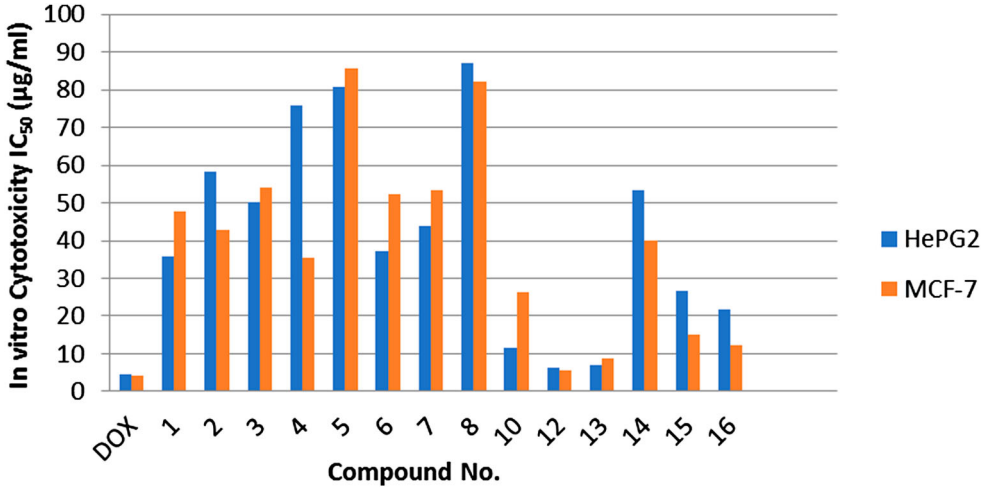


Figure 1. Cytotoxicity of synthetic compounds.

cell lines. However, other compounds exhibited weak activity toward HePG-2 and MCF-7 (Figure 1).

3. Experimental

Potassium bromide disks technique on a Pye Unicam SP-3-300 infrared spectrophotometer was used to record the IR spectra. All melting points were determined using a Gallenkamp melting point apparatus and were not corrected. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometers at 70 eV. ^{1}H-NMR experiments were performed at 300 and 400 MHz on a Varian Mercury VX-300 NMR spectrometer using TMS as internal standard in deuterated chloroform or dimethyl sulfoxide chemical shifts are quoted as δ. All spectral measurements were carried out at Central laboratory of Ain-Shams University and Main Defense Chemical Laboratory, Egypt. Biological activity was screened in Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. All the new compounds afforded satisfactory results in elemental analyses. The purity of the synthesized compounds was checked by TLC.

3.1. Synthesis

6-Iodo-2-phenyl-4H-benzo[d][1,3]thiazine-4-thione **(1)**

A solution of benzoxazinone (5 mmol, 1.75 g), and phosphorus pentasulfide (5 mmol, 1.1 g) in dry toluene (20 ml) was heated at reflux temperature for 2 h. then filtered while hot. The mixture was left to cool at room temperature and the solid product was filtered off, dried and recrystallized from DMF to give benzothiazinethione **1** as yellow crystals, mp190–193°C, yield 44%. FT-IR (KBr, cm^{-1}): 3047 ν_{CH} aromatic, 1199 $\nu_{C=S}$, 1611 $\nu_{C=N.}$ ^{1}H NMR (400 MHz, DMSO-d_6): 8.91 (s, 1H, C5-H), 8.33 (d, 1H, C7-H, J = 12 Hz), 8.09 (d, 1H, C8-H, J = 8 Hz), 7.70–7.58 (m, 5H, Ar-H). ^{13}C NMR (100 MHz, DMSO–d$_6$) δ (ppm): 209.5, 163.7, 148.3, 145.5, 142.0, 135.2, 134.1, 133.8, 133.4, 129.9, 127.3 and 97.6. MS (m/z (%)): 381 ($M^{+\bullet}$, 18.07), 305 (46.20), 254 (41.66), 178 (100.00). Anal. Calcd for $C_{14}H_8INS_2$ (381.25): C, 44.11; H, 2.12; N, 3.67; S, 16.82. Found: C, 43.87; H, 2.01; N, 3.54; S, 16.70.

3-Amino-6-iodo-2-phenylquinazoline-4(3H)-thione ***(2)***

A mixture of benzothiazinethione **1** (2.5 mmol, 1 g) and hydrazine hydrate (2.5 mmol, 0.125 ml) in absolute ethanol (30 ml) was heated at reflux temperature for 10 h. left to cool. The crude solid product that deposited after cooling was collected by filtration, dried and recrystallized from acetic acid to afford quinazolinthione **2** as yellow crystals, mp 155–156°C, yield 60%. FT-IR (KBr, cm^{-1}): 3386, 3293 $\nu_{NH,}$ 3059 ν_{CH} aromatic, 1676 $\nu_{C=N}$, 1218 $\nu_{C=S.}$ ^{1}H NMR (400 MHz, DMSO-d_6): 8.53-7.42 (m, 8H, Ar-H), 6.76 (s, 2H, NH_2, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO–d$_6$) δ (ppm): 179.23, 157.07, 143.13, 140.98, 138.73, 129.86, 129.71, 129.08, 128.15, 127.09,123.80, and 93.73. MS (m/z (%)): 363 ($[M\text{-}NH_2]^{+\bullet}$, 27.53), 270 (81.85), 204 (63.86), 111 (45.89), 106 (47.25), 69 (100.00). Anal. Calcd for $C_{14}H_{10}IN_3S$ (379.22): C, 44.34; H, 2.66; N, 11.08; S, 8.45. Found: C, 44.10; H, 2.43; N, 10.87; S, 8.28.

Reaction of 6-iodo-2-phenyl-4H-benzo[d][1,3]thiazine-4-thione ***(1)*** *with substituted amines namely 4-hydroxy aniline, 4-aminopyridine, o-toluidine, and /or benzyl amine.*

3.1.1. General procedure

A mixture of benzothiazinethione **1** (2.5 mmol, 1 g) and substituted amines namely 4-hydroxy aniline, 4-aminopyridine, o-toluidine, and /or benzyl amine (0.01 mmol) in ethanol (20 ml) was heated at reflux temperature for 5–10 h, left to cool the solid product was collected, dried and recrystallized to afford 2-phenylquinazolinthione **3** and benzamide derivatives **4–6**.

3-(4-Hydroxyphenyl)-6-iodo-2-phenylquinazoline-4(3H)-thione ***(3)*** recrystallized from methanol (red crystals), mp 275–279°C, yield 55%. FT-IR (KBr, cm^{-1}): 3425 $\nu_{OH,}$ 3056 ν_{CH} aromatic, 1223 $\nu_{C=S}$. ^{1}H NMR (400 MHz, DMSO-d_6): 9.58 (s, 1H, OH, D_2O exchangeable), 8.20-8.13, 7.56-7.51 (2 m, 2H, Ar-H), 7.36-7.33 (m, 2H, Ar-H), 7.25-7.19 (m, 4H, Ar-H),7.04, 6.60 (2 d, 4H, phenol moiety, $J = 8$ Hz). ^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 188.63, 157.38, 156.11, 143.61, 142.14, 139.18, 136.44, 135.26, 133.82, 130.68, 130.53,129.25, 129.15, 127.80, 115.71 and 94.66. MS (m/z (%)): 456 ($M^{\bullet+}$, 100.00), 330 (74.81), 204 (60.08), 105 (50.83), 77 (46.71), 65 (21.26). Anal. Calcd for $C_{20}H_{13}IN_2OS$ (456.30): C, 52.65; H, 2.87; N, 6.14; S, 7.03. Found: C, 52.51; H, 2.70; N, 5.97; S, 6.98.

N-(4-Iodo-2-(pyridin-4-ylcarbamothioyl)phenyl)benzamide ***(4)*** recrystallized from ethanol (red crystals), mp 150–152°C, yield 43%. FT-IR (KBr, cm^{-1}): 3384, 3183 ν_{NH}, 1670 $\nu_{C=O}$ amide, 1199 $\nu_{C=S.}$ ^{1}H NMR (400 MHz, DMSO-d_6): 8.25 (d, 2H, pyridine-H, $J = 8$ Hz), 8.21-8.05 (m, 3H, Ar-H), 8.00 (s, 1H, NHCO, D_2O exchangeable), 7.68-7.96 (m, 5H, Ar-H), 7.06 (s, 1H, NHCS, D_2O exchangeable), 6.61 (d, 2H, pyridine-H, $J = 4$ Hz). ^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 208.85, 157.72, 145.48, 144.60, 135.20, 134.12, 133.82, 133.43, 129.92, 129.01,127.86, 127.56, 127.25, 109.21 and 97.33. MS (m/z (%)): 460 ($[M+1]^{\bullet+}$, 3.69), 406 (30.65), 301 (57.69), 238 (83.59), 226 (78.34), 143 (26.18), 58 (83.11), 44 (100.00). Anal. Calcd for $C_{19}H_{14}IN_3OS$ (459.31): C, 49.69; H, 3.07; N, 9.15; S, 6.98. Found: C, 49.75; H, 2.94; N, 8.97; S, 6.76.

2-Benzamido-5-iodo-N-(o-tolyl)benzamide ***(5)*** recrystallized from dioxane (red crystals), mp 144–146°C, yield 60%. FT-IR (KBr, cm^{-1}): 3284, 3250 $\nu_{NH,}$ 3062 ν_{CH} aromatic, 2955ν_{CH} aliphatic, 1652 $\nu_{C=O}$. ^{1}H NMR (400 MHz, DMSO-d_6): 11.69, 10.52 (2s, 2H, 2NHCO, D_2O exchangeable), 8.30-7.15 (m, 12H, Ar-H), 2.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 166.23, 165.01, 140.99, 138.85, 137.29, 136.10, 134.68, 134.01, 132.61, 129.50,129.38, 127.46, 125.13, 123.66, 121.75, 87.51 and 20.98.MS (m/z (%)): 456 ($M^{\bullet+}$, 1.26), 438 (1.10), 349 (6.35), 254 (22.95), 211 (4.09), 127 (3.19), 106 (100.00), 77 (26.99). Anal. Calcd for $C_{21}H_{17}IN_2O_2$ (456.28): C, 55.28; H, 3.76; N, 6.14. Found: C, 55.12; H, 3.63; N, 6.01.

N-(2-(Benzylcarbamothioyl)-4-iodophenyl)benzamide ***(6)*** recrystallized from light petroleum ether (80–100°C) (yellow crystals), mp 108–109°C, yield 65%. FT-IR (KBr, cm^{-1}): 3305, 3256 $\nu_{NH,}$ 3060 ν_{CH} aromatic, 2949 ν_{CH} aliphatic, 1679 $\nu_{C=O}$ amide, 1232 $\nu_{C=S.}$ ^{1}H NMR (400 MHz, DMSO-d_6): 11.48 (s, 1H, CONH-, D_2O exchangeable), 8.35 (s, 1H, CH_2NH-, D_2O exchangeable), 8.33-6.86 (m, 13H, Ar-H), 3.87 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 187.06, 167.17, 142.93, 138.85, 140.19, 139.02, 134.53, 132.82, 129.45, 128.78,128.61, 128.28, 127.55, 126.45, 123.42, 119.85, 87.43 and 53.35. MS (m/z (%)): 471 ($[M-1]^{\bullet+}$, 1.42), 452 (22.57), 363 (20.17), 299 (34.55), 149 (100.00). Anal. Calcd for $C_{21}H_{17}IN_2OS$ (472.34): C, 53.40; H, 3.63; N, 5.93; S, 6.79. Found: C, 53.31; H, 3.47; N, 5.78; S, 6.62.

*5-Iodo-1-(phenylcarbonothioyl)-1H-benzo[d]imidazol-2(3H)-one **(7)***

A mixture of benzothiazinethione **1** (2.5 mmol, 1 g) and sodium azide (2.5 mmol, 0.2 g) in glacial acetic acid (20 ml) was heated at reflux temperature for 8 h. After cooling, the reaction mixture was poured onto ice/cold water and the formed solid was filtered dried and recrystallized from toluene to give benzo[d]imidazolone **7,** as yellow crystals, mp 250–251°C, yield 40%. FT-IR (KBr, cm^{-1}): 3114, $\nu_{NH,}$ 3058 ν_{CH} aromatic, 1702 $\nu_{C=O,}$ 1201 $\nu_{C=S}$. ^{1}H NMR (400 MHz, DMSO-d_6): 12.37 (s, 1H, NH, D_2O exchangeable), 8.50 (d, 1H, C4-H, $J = 8$ Hz), 8.28 (d, 1H, C7-H, $J = 4$ Hz), 7.95-7.92, 7.65-7.55 (2 m, 6H, Ar-H). ^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 196.80, 169.03, 165.13, 142.52, 141.09, 139.64, 134.79, 132.72, 129.43, 127.90,122.41and 86.57.MS (m/z (%)): 380 ($M^{+\bullet}$, not observed), 298 (1.26), 126 (4.95), 105 (86.72), 77 (100.00), 51 (50.57). Anal. Calcd for $C_{14}H_9IN_2OS$ (380.20): C, 44.23; H, 2.39; N, 7.37; S, 8.43. Found: C, 44.15; H, 2.24; N, 7.21; S, 8.23.

*2-(6-Iodo-2-phenylquinazolin-4-ylthio)acetohydrazide2-(6-iodo-2-phenyl-4-thioxoquin azolin-3(4H)yl)acetohydrazide **(11)***

A mixture of compound **10** (2.5 mmol, 1 g) and hydrazine hydrate (2.5 mmol, 0.125 ml) in absolute ethanol (30 ml) was heated at reflux temperature for 10 h, left to cool, the crude solid product that deposited after cooling was collected by filtration, dried and recrystallized from DMF to give acetohydrazide derivative **11** as red crystals, mp > 300°C, yield 40%. FT-IR (KBr, cm^{-1}): 3436, 3220 $\nu_{NH2,NH,}$ 3054 ν_{CH} aromatic, 2922 ν_{CH} aliphatic, 1665 $\nu_{C=O}$, 1140 $\nu_{C=S}$.^{1}H NMR (400 MHz, DMSO-d_6): 11.57 (s, 1H, NH, D_2O exchangeable), 8.80 (s, 2H, NH_2, D_2O exchangeable), 8.50-7.01 (m, 8H, Ar-H), 4.37 (s, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 186.92, 158.39, 147.67, 147.99, 143.65, 137.82, 137.63, 131.83, 130.42, 129.11,128.61, 123.41, 94.12 and 61.60. MS (m/z (%)): 436 ($M^{+\bullet}$, 0.71), 377 (12.65), 296 (13.67), 250 (12.68), 103 (37.69), 83 (34.03), 77 (77.12), 73 (99.15), 57 (71.95), 43 (100.00). Anal. Calcd for $C_{16}H_{13}IN_4OS$ (436.27): C, 44.05; H, 3.00; N, 12.84; S, 7.35. Found: C, 43.94; H, 2.85; N, 12.63; S, 7.12.

*1-(3,5-Dimethyl-1H-pyrazol-1-yl)-2-(6-iodo-2-phenyl-4-thioxoquinazolin-3(4H)-yl)eth anone **(12)***

A mixture of acetohydrazide derivative **11** (2.5 mmol, 1 g) with acetylacetone (2.5 mmol, 0.25 ml) in ethanol (20 ml) was heated at reflux temperature for 15 h, left to cool, the crude solid product that deposited after cooling was collected by filtration, dried and recrystallized from benzene to give quinazolinethione derivative **12** as red crystals, mp 174–175°C, yield 40%. FT-IR (KBr, cm^{-1}): 3060 ν_{CH} aromatic, 2979 ν_{CH} aliphatic, 1678 $\nu_{C=O}$ amide, 1610 $\nu_{C=N,}$ 1117$\nu_{C=S}$.^{1}H NMR (300 MHz, $CDCl_3$): 8.64-7.35 (m, 8H, Ar-H), 6.99 (s, 1H, pyrazol-H), 4.21(s, 2H, CH_2CO), 3.49 (s, 6H, $2CH_3$). MS (m/z (%)): 500 ($M^{+\bullet}$, 0.00), 474 (0.03), 468 (0.04), 349 (55.25), 310 (65.31), 296 (59.69), 272 (100.00), 245 (11.94), 216 (22.39), 145 (47.68), 105 (73.77), 77 (53.06). Anal. Calcd for $C_{21}H_{17}IN_4OS$ (500.36): C, 50.41; H, 3.42; N, 11.20; S, 6.41. Found: C, 50.27; H, 3.28; N, 11.11; S, 6.25.

*2-(2-(6-Iodo-2-phenyl-4-thioxoquinazolin-3(4H)-yl)acetyl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one **(13)***

A mixture of acetohydrazide derivative **11** (2.5 mmol, 1 g) with ethyl acetoacetate (2.5 mmol, 0.32 ml) in ethanol (20 ml) was heated at reflux temperature for 15 h, left to cool, the crude solid product that deposited after cooling was collected by filtration, dried and recrystallized from ethanol to give **13** as red crystals, mp 85–87°C, yield 60%. FT-IR (KBr, cm^{-1}): 3073 ν_{CH} aromatic, 2978 ν_{CH} aliphatic, 1724, 1694 $\nu_{C=O}$, 1608 $\nu_{C=N,}$ 1220

$\nu_{C=S}$.^{1}H NMR (300 MHz, DMSO-d_6): 8.62-7.47 (m, 8H, Ar-H), 4.19 (s, 2H, -NCH_2CO), 3.41 (s, 2H, CH_2-CO), 1.90 (s, 3H, CH_3).^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 187.57, 179.80, 168.87, 159.95, 148.46, 146.64, 143.06, 140.26, 138.45, 134.57, 129.85, 129.08, 128.75, 127.64, 124.89, 121.37, 92.21, 51.25, 40.59 and 17.90. MS (m/z (%)): 502 ($M^{+\bullet}$, 0.00), 347 (40.32), 272 (99.90), 245 (17.31), 145 (19.84), 90 (24.87), 63 (12.95). Anal. Calcd for $C_{20}H_{15}IN_4O_2S$ (502.33): C, 47.82; H, 3.01; N, 11.15; S, 6.38. Found: C, 47.65; H, 3.21; N, 11.01; S, 6.23.

N',N'-Diacetyl-2-(6-iodo-2-phenyl-4-thioxoquinazolin-3(4H)-yl)acetohydrazide ***(14)***

A mixture of acetohydrazide derivative **11** (2.5 mmol, 1 g) in acetic anhydride (10 ml) was heated at reflux temperature for 2 h, then the mixture was poured into ice, the crude solid product that deposited was collected by filtration, dried and recrystallized from benzene to give **14** as black crystals, mp 65–66°C, yield 40%. FT-IR (KBr, cm^{-1}): 3169 $\nu_{NH,}$ 3060 ν_{CH} aromatic, 2954 ν_{CH} aliphatic, 1735 $\nu_{C=O}$ imide, 1679 $\nu_{C=O}$ amide, 1608 $\nu_{C=N,}$ 1220 $\nu_{C=S}$.^{1}H NMR (300 MHz, $CDCl_3$): 12.29 (s, 1H, NH, D_2O exchangeable), 8.65-7.35 (m, 8H, Ar-H), 4.63 (s, 2H, CH_2CO), 2.60 (s, 6H, $2CH_3$). ^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 172.40, 169.63, 168.21, 165.07, 159.48, 143.42, 131.77, 130.62, 129.36, 129.15, 128.82, 128.69, 128.54, 128.32, 127.69, 122.39, 93.18, 61.92, 22.39 and 21.04. MS (m/z (%)): 520 ($M^{+\bullet}$, 0.00), 434 (4.62), 386 (44.75), 349 (50.51), 324 (99.92), 105 (54.63), 77 (46.02). Anal. Calcd for $C_{20}H_{17}IN_4O_3S$ (520.35): C, 46.17; H, 3.29; N, 10.77; S, 6.16. Found: C, 46.01; H, 3.12; N, 10.58; S, 6.01.

6-Iodo-3-((5-mercapto-1,3,4-oxadiazol-2-yl)methyl)-2-phenylquinazoline-4(3H)-thione ***(15)***

To a solution of acetohydrazide derivative **11** (5 mmol, 2 g) in alcoholic potassium hydroxide (5 mmol, 0.28 g) carbon disulfide (2 ml) was added then heated in water bath for 15 h. The mixture was poured onto cold diluted HCl, the crude solid product that deposited was collected by filtration, washed with water, dried and recrystallized from dioxane to give **15** as yellow crystals, mp 230–231°C, yield 60%. FT-IR (KBr, cm^{-1}): 3052 ν_{CH} aromatic, 2954 ν_{CH} aliphatic, 1613 $\nu_{C=N,}$ 1266 $\nu_{C=S}$. ^{1}H NMR (400 MHz, DMSO-d_6): 12.68 (s, 1H, SH, D_2O exchangeable), 8.40-8.09, 7.60-7.46 (2 m, 8H, Ar-H), 3.55 (s, 2H, CH_2CO).^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 179.72, 161.44, 156.37, 134.65, 132.96, 132.15, 130.88, 130.40, 129.08, 128.92, 128.56, 128.28, 95.46 and 47.58. MS (m/z (%)): 478 ($M^{+\bullet}$, 0.00), 372 (4.45), 348 (33.92), 272 (2.94), 254 (99.93), 127 (58.05), 105 (18.27). Anal. Calcd for $C_{17}H_{11}IN_4OS_2$ (478.33): C, 42.69; H, 2.32; N, 11.71; S, 13.41. Found: C, 42.51; H, 2.24; N, 11.53; S, 13.27.

3-((4-Amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-6-iodo-2-phenylquinazoline-4 (3H)-thione ***(16)***

A mixture of **15** (2.5 mmol 1.3 g) with hydrazine hydrate (2.5 mmol, 0.125 ml) in absolute ethanol (20 ml) was heated at reflux temperature for 10 h, left to cool, the crude solid product that deposited after cooling was collected by filtration, dried and recrystallized from DMF to give **16** as yellow crystals, mp 270–271°C, yield 40%. FT-IR (KBr, cm^{-1}): 3356, 3280 $\nu_{NH2,}$ 3163 $\nu_{NH,}$ 3061 ν_{CH} aromatic, 2918 ν_{CH} aliphatic, 1618 $\nu_{C=N,}$ 1225 $\nu_{C=S}$.^{1}H NMR (400 MHz, DMSO-d_6): 13.63 (s, 1H, SH, D_2O exchangeable), 8.47-7.47 (m, 8H, Ar-H), 5.64 (s, 2H, NH_2, D_2O exchangeable), 5.24 (s, 2H, CH_2).^{13}C NMR (100 MHz, DMSO–d_6) δ (ppm): 167.35, 161.38, 159.12, 153.38, 149.16, 148.47, 148.37, 147.98, 143.46,

134.74, 134.60, 129.91, 129.08, 128.28, 123.63, 92.88 and 41.06. MS (m/z (%)): 492 ($M^{+\bullet}$, 0.00), 348 (48.41), 272 (55.44), 254 (99.90), 245 (31.55), 127 (45.67), 90 (17.81). Anal. Calcd for $C_{17}H_{13}IN_6S_2$ (492.36): C, 41.47; H, 2.66; N, 17.07; S, 13.02. Found: C, 41.23; H, 2.48; N, 16.97; S, 12.89.

4. Cytotoxicity and antitumor evaluation

Procedure MTT assay [31].

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Marwa S. Salem http://orcid.org/0000-0001-7237-6451
Maher A. E. M. El-Hashash http://orcid.org/0000-0003-2550-8479

References

[1] Gao C, Ye TH, Wang NY, et al. Synthesis and structure–activity relationships evaluation of benzothiazinone derivatives as potential anti-tubercular agents. Bioorg Med Chem Lett. 2013;23:4919–4922.
[2] Majewski MW, Tiwaria R, Miller PA, et al. Design, syntheses, and anti-tuberculosis activities of conjugates of piperazino-1,3-benzothiazin-4-ones (pBTZs) with 2,7dimethylimidazo[1,2-a]pyridine-3-carboxylic acids and 7phenylacetyl cephalosporins. Bioorg Med Chem Lett. 2016 Apr 15;26(8):2068–2071.
[3] Peng CT, Gao C, Wang NY, et al. Synthesis and antitubercular evaluation of 4-carbonyl piperazine substituted 1,3-benzothiazin-4-one derivatives. Bioorg Med Chem Lett. 2015 Apr 1;25(7):1373–1376.
[4] He D, Wang M, Zhao S, et al. Pharmaceutical prospects of naturally occurring quinazolinone and its derivatives. Fitoterapia. 2017;119:136–149.
[5] Patel MB, Kumar SP, Valand NN, et al. Synthesis and biological evaluation of cationic fullerene quinazolinone conjugates and their binding mode with modeled Mycobacterium tuberculosis hypoxanthine-guanine phosphoribosyltransferase enzyme. J Mol Model. 2013;19(8):3201–3217.
[6] Kubo K, Shimizu T, Ohyama S, et al. Novel potent orally active selective VEGFR-2 tyrosine kinase inhibitors: synthesis, structure-activity relationships, and antitumor activities of N-phenyl-N'-4-(4-quinolyloxy)phenylureas. J Med Chem. 2005;48(5):1359–1366.
[7] El-Hashash MAM, Salem MS, Al-Mabrook SAM. Synthesis and anticancer activity of novel quinazolinone and benzamide derivatives. Res Chem Intermed. 2018;44(4):2545–2559. DOI:10.1007/s11164-017-3245-4
[8] Marzouk MI, Shaker SA, Farghaly T A, et al. Synthesis of some novel quinazolinone derivatives with anticipated biological activity. J Heterocyclic Chem. 2017;54:3331–3341.
[9] Radwan TM, El-Hashash MA, Wasfy AAF, et al. Synthesis and characteristics of metastable 2-benzyl-4*H*-3,1-benzoxazin-4-one as anticancer agent and its Comparison with other heterocyclic compounds. Chemistry Select. 2019;4:14056–14062.
[10] Song F, Ren B, Yu K, et al. Quinazolin-4-one coupled with pyrrolidin-2-iminium alkaloids from marine-derived fungus Penicillium aurantiogriseum. Mar Drugs. 2012;10(6):1297–1306.
[11] Khodarahmi G, Jafari E, Hakimelahi G, et al. Synthesis of some new quinazolinone derivatives and evaluation of their antimicrobial activities. Iran J Pharm Res. 2012;11(3):789–797.

[12] Patil A, Barge M, Rashinkar G, et al. Aqueous hydrotrope: an efficient and reusable medium for a green one-pot, diversity-oriented synthesis of quinazolinone derivatives. Mol Divers. 2015;19(3):435–445.
[13] El-Hashash MA, El-Naggar AM, El-Bordany EA, et al. 6-Iodo-2-isopropyl-4H-3,1-benzoxazin-4-one as building block in heterocyclic synthesis. Synth Commun. 2016;46(24):2009–2021.
[14] Kubicová L, Ustr M, Kráľová K, et al. Synthesis and biological evaluation of quinazoline-4-thiones. Molecules. 2003;8:756–769.
[15] El-Feky SA, Thabet HK, Ubeid MT. Synthesis, molecular modeling and anti-inflammatory screening of novel fluorinated quinoline incorporated benzimidazole derivatives using the Pfitzinger reaction. J Fluorine Chem. 2014;161:87–94.
[16] Andrzejewskaa M, Yepez-Mulia L, Tapia A, et al. Synthesis, and antiprotozoal and antibacterial activities of S-substituted 4,6-dibromo- and 4,6-dichloro-2-mercaptobenzimidazoles. Eur J Pharm Sci. 2004;21:323–329.
[17] Camacho J, Barazarte A, Gamboa N, et al. Synthesis and biological evaluation of benzimidazole-5-carbohydrazide derivatives as antimalarial, cytotoxic and antitubercular agents. Bioorg Med Chem. 2011;19:2023–2029.
[18] Gong Y, Karakaya SS, Guo X, et al. Benzimidazole-based compounds kill Mycobacterium tuberculosis. Eur J Med Chem. 2014;75:336–353.
[19] Abonia R, Cortes E, Insuasty B, et al. Synthesis of novel 1,2,5-trisubstituted benzimidazoles as potential antitumor agents. Eur J Med Chem. 2011;46:4062–4070.
[20] Fonseca T, Gigante B, Marques MM, et al. Synthesis and antiviral evaluation of benzimidazoles, quinoxalines and indoles from dehydroabietic acid. Bioorg Med Chem. 2004;12:103–112.
[21] Kaur N, Kaur A, Bansal Y, et al. Design, synthesis, and evaluation of 5-sulfamoyl benzimidazole derivatives as novel angiotensin II receptor antagonists. Bioorg Med Chem. 2008;16:10210–10215.
[22] Falco JL, Pique M, Gonzalez M, et al. Synthesis, pharmacology and molecular modeling of N-substituted 2-phenyl-indoles and benzimidazoles as potent GABAA agonists. Eur J Med Chem. 2006;41:985–990.
[23] Futatsugi K, Mascitti V, Guimarães CR, et al. From partial to full agonism: Identification of a novel 2,4,5,6-tetrahydropyrrolo[3,4-c]pyrazole as a full agonist of the human GPR119 receptor. Bioorg Med Chem Lett. 2013;23:194–197.
[24] Jia H, Bai F, Liu N, et al. Design, synthesis and evaluation of pyrazole derivatives as non-nucleoside hepatitis B virus inhibitors. Eur J Med Chem. 2016;123:202–210.
[25] Liu GN, Luo RH, ZhouY ZXJ, et al. Synthesis and anti-HIV-1 activity evaluation for novel 3a,6a-dihydro-1H-pyrrolo[3,4-c]pyrazole-4,6-dione derivatives. Molecules. 2016;21:1198–1209.
[26] Hassan GS, Abou-Seri SM, Kamel G, et al. Celecoxib analogs bearing benzofuran moiety as cyclooxygenase-2 inhibitors: design, synthesis and evaluation as potential anti-inflammatory agents. Eur J Med Chem. 2014;76:482–493.
[27] Karrouchi K, Chemlal L, Doudach L, et al. Synthesis, anti-inflammatory and antioxidant activities of some new pyrazole derivatives. J Pharm Res. 2014;8:1171–1177.
[28] Madkour HMF, El-Hashash MAM, Salem MS, et al. Synthesis, antileishmanial and cytotoxicity activities of fused and nonfused tetrahydroquinoline derivatives. Res Chem Intermed. 2018;44:3349–3364.
[29] Radwan TM, El-Hashash MAM, Wasfy AAF, et al. Antitumor, cytotoxic, and antioxidant evaluation of six heterocyclic compounds containing different heterocycle moieties. J Heterocyclic Chem. 2020;57(3):1111–1122.
[30] Madkour HMF, El-Hashash MAM, Salem MS, et al. Design, synthesis, and in vitro antileishmanial and antitumor activities of new tetrahydroquinolines. J Heterocyclic Chem. 2018;55:391–401.
[31] Khlosy TA, Salem MS, Ali AT, et al. Synthesis and cytotoxic activity against human tumor cells of heterocyclic systems derived from 2-thioxo-1,2-dihydro-4*H*-3,1-benzothazin-4-one. J Heterocyclic Chem. 2020;57:60–68.

[32] Salem MS, El-Helw E AE, Derbala HAY. Development of promising chromones-pyrazoles based anticancer agents. Russ J Bioorg Chem. 2020;46(1):77–84.
[33] Salem MS, Hussein RA, El-Sayed WM. Substitution at phenyl rings of chalcone and schiff base moieties accounts for their antiproliferative activity. Anticancer Agents Med Chem. 2019;19(5):620–626.
[34] El-Helw EAE, Derbala HAY, El-Shahawi MM, et al. Synthesis and *In Vitro* antitumor activity of novel chromenones bearing benzothiazole moiety. Russ J Bioorg Chem. 2019;45(1):42–53.
[35] El-Hashash MA, Assy MG, Aly A, et al. Behavior of 6-Iodobenzoxazinone towards some nitrogen nucleophiles and evaluation of 4(3H) – quinazolinones derivatives as potential antimicrobial agents. Nat Sci. 2016;14(5):76–84.
[36] Mohamed AMM, Ismail MF, Madkour HMF, et al. Straightforward synthesis of 2-chloro-N-(5-(cyanomethyl)-1,3,4-thiadiazol-2-yl)benzamide as a precursor for synthesis of novel heterocyclic compounds with insecticidal activity. Synth Commun. 2020. DOI:10.1080/00397911.2020.1802652.
[37] Salem MS, Al-Mabrook SAM, El-Hashash MAM. Synthesis and antiproliferative evaluation of some novel quinazolin-4(3H)-one derivatives, J Heterocyclic Chem. 2020. in press.
[38] Ozturk T, Ertas E, Mert O. A berzellus reagent; P_4S_{10} in organic syntheses. Chem Rev. 2010;110:3419–3478.