Received: 2 October 2019 | Revised: 8 November 2019 | Accepted: 19 November 2019

DOI: 10.1002/jhet.3847



WILEY

ARTICLE

# Antitumor, cytotoxic, and antioxidant evaluation of six heterocyclic compounds containing different heterocycle moieties

**Tasneem Mokhtar Radwan**[1] | **Maher Abdel-Aziz El-Hashash**[2] | **Ashraf Abdel-Hamid Farouk Wasfy**[1] | **Suzan Abdelhalim Abdallah**[3]

[1]Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

[2]Chemistry Department, Faculty of Science, Ain-Shams University, Cairo, Egypt

[3]Botany and Microbiology Department, Faculty of Science, Benha University, Benha, Egypt

**Correspondence**
Tasneem Mokhtar Radwan, Chemistry Department, Faculty of Science, Benha University, Benha, Qalyubia, Egypt.
Email: tasneemmokhatar@gmail.com

**Abstract**

Heterocyclic compounds with different heterocycle moieties, namely benzoxazinone, benzimidazole, quinazolinone, and benzofuranone heterocyclic rings, were synthesized, characterized, and evaluated for their anticancer activity against human hepatocellular carcinoma cell line (HepG2) using sulforhodamine B (SRB) and dimethylthiazol-diphenyltetrazolium bromide (MTT) assays. Also, their cytotoxic activities were tested against human epithelioid carcinoma (Hela) cell line in comparison with normal cell, amniotic epithelial (WISH) cell line, as an *in vitro* toxicity estimation model. The results showed clearly that 2-(2-benzyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide **4** is the most potent antioxidant and anticancer agents. Although, 3-amino-2-benzylquinazolin-4(3*H*)-one **5** is less potent anticancer agent against Hela but it is more safe against normal cell (WISH).

## 1 | INTRODUCTION

Cancer is moving to be a significant health problem in the world.[1–3] According to a World Health Organization (WHO) report, cancer is the second leading cause of death globally and is responsible for an estimated 9.6 million deaths in 2018.[4] Globally, about one in six deaths is due to cancer.[4] The mortality rate of patients is 782 000 deaths for liver cancer.[4] Cervical cancer is the fourth most frequent cancer in women with an estimated 570 000 new cases in 2018 representing 6.6% of all female cancers. Approximately 90% of deaths from cervical cancer occurred in low- and middle-income countries.[5]

For the *in vitro* chemosensitivity testing of tumor cell lines, several rapid calorimetric assays have been described. The tetrazolium (MTT) test was the most commonly used in latest years.[6] The colorimetric test for MTT (dimethylthiazol-diphenyltetrazolium bromide) is based on mitochondrial uptake and succinate dehydrogenase reduction of soluble, yellow, MTT tetrazolium salt to an insoluble blue MTT formazan product.[7] In contrast, colorimetric assay for the sulforhodamine B (SRB) is based on the ability of the SRB dye to bind basic amino acid residues on proteins.[7]

Several of the present treatments have problems with toxicity and drug-resistance, these encouraged researchers to explore effective new treatments for cancer disease.[8] Consequently, synthesis of new bioactive compounds such as heterocyclic compounds is an important aim for many researchers. Heterocyclic compounds have an active role as antibacterial, antiviral, antifungal, anti-inflammatory, and antitumor drugs.[9] For example, benzoxazinone derivatives are biologically interesting and they are now receiving considerable attention due to their applications in medicine use for antifungal, antiviral, antibacterial, antimalarial, antipyretic, anticancer, and anti-HIV (human immunodeficiency virus) activities.[10] Moreover, 4*H*-3,1-benzoxazin-4-one derivatives are shown to be inhibitors of α-chymotrypsin[11] and rhomboid proteases.[12] They consist of six-membered ring containing oxygen and

nitrogen atoms.[10] Benzoxazinone derivatives showed a variety of pharmacological activities, such as antifungal, antiviral, antibacterial, antimalarial, antipyretic, anticancer, and anti-HIV activities.[13–19] On the other hand, a benzimidazole compound consists of the fusion of benzene and imidazole. They have been reported to have potential antitumor activity.[20] While quinazolinone derivatives are containing two nitrogen atoms in a six-membered ring fused with a benzene ring. They reveal a variety of biological effects, such as antibacterial and antitumor activities.[21] Moreover, benzofuranone derivatives exhibited anticancer activity.[22] It composed of a benzene ring fused to furanone.

The search for a bioactive compound for treating cancer stimulates us to turn our interest in the synthesis of different heterocyclic compounds to select the best heterocyclic nucleus for treating different types of cancer. Depending upon their biological activity, different six heterocyclic compounds, represented four heterocyclic classes (2-benzyl-3,1-benzoxazin-4-one (**1**) represented benzoxazinone class, *N*-(2-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-2-phenyl acetamide (**2**) represented benzimidazole class, ethyl 2-(2-benzyl-4-oxoquinazolin-3(4*H*)-yl)acetate (**3**), 2-(2-benzyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (**4**), 3-amino-2-benzylquinazolin-4(3*H*)-one (**5**) represented quinazolinone class and 2-benzyl-3-((3-oxoisobenzofuran-1(3*H*)-ylidene)amino)quinazolin-4(3*H*)-one (**6**) referred to benzofuranone and quinazolinone classes as shown in Figure 1), have been designed, synthesized, and characterized. Hence, we selected those compounds to be evaluated for their anticancer effect against human hepatocellular carcinoma cell line (HepG2) using SRB and tetrazolium (MTT) assays.

It has been noted that compound (**1**) which has been synthesized in our study was synthesized before by M.E. Diener *et al.* during conduction of this study. In addition, they did not evaluate its potential activity as anticancer activity against any cancerogenic cell lines.[23] Here, this study is the first to evaluate the potential implications of this compound in several cancerogenic cell lines.

Heterocyclic compounds with different heterocycles moieties, namely benzoxazinone, benzimidazole, quinazolinone, and benzofuranone heterocyclic rings, were synthesized, characterized, and evaluated for their anticancer activity against human HepG2 using two different methods, SRB and tetrazolium (MTT) assays. Additionally, DNA binding affinity of these compounds was investigated as a potential mechanism for the anticancer activity against a human HepG2 using DNA/methyl green assay. Moreover, the obtained compounds were evaluated for their *in vitro* cytotoxic activities against human epithelioid carcinoma (Hela) cell line in comparison with normal cell, amniotic epithelial (WISH) cell line, as an *in vitro* model for toxicity assessment. Furthermore, synthesized compounds were screened for their antioxidant activities.

## 2 | RESULTS AND DISCUSSION

### 2.1 | Chemistry

In this paper, we used 2-benzyl-3,1-benzoxazin-4-one (**1**) to synthesize novel benzimidazole, quinazolinone, and benzofuranone compounds. In the present study,

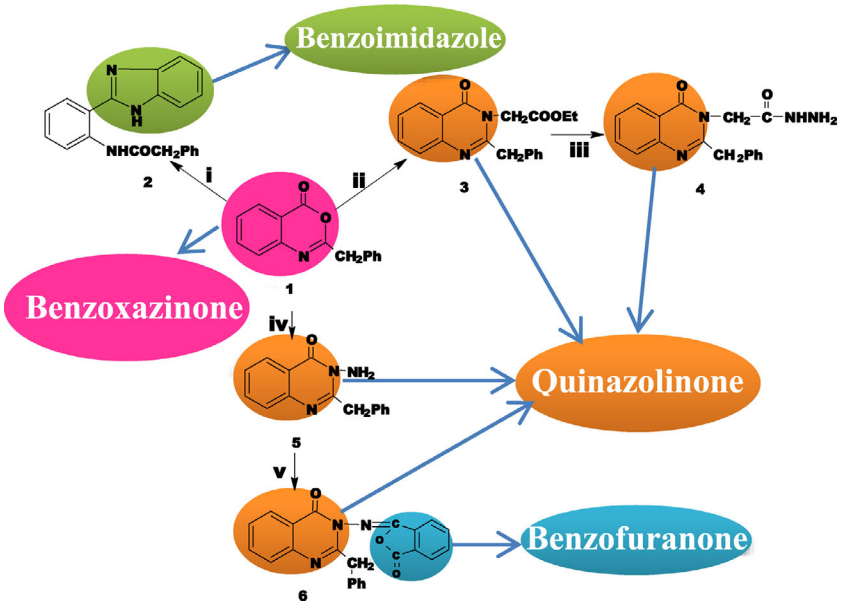



**FIGURE 1** This design illustrates the different heterocyclic compounds with different moieties, namely benzoxazinone, benzimidazole, quinazolinone, and benzofuranone heterocyclic rings. (i) *o*-Phenylenediamine, ethanol. (ii) Ethyl glycinate hydrochloride, ethanol. (iii) Hydrazine hydrate, ethanol. (iv) Hydrazine hydrate, ethanol. (v) Phthalic anhydride, ethanol

2-benzyl-4*H*-3,1-benzoxazin-4-one (so-called metastable benzoxazinone) was obtained via interaction of phenylacetyl chloride with anthranilic acid in pyridine and yielded 2-phenylacetylamino benzoic acid followed by ring closure in acetic anhydride to afford the desired product benzoxazinone (**1**) in high yield (Scheme 1).

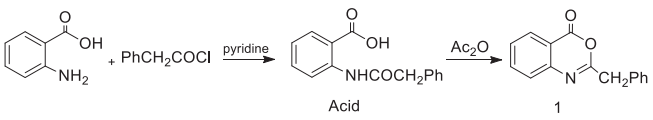
The structure of compound (**1**) was confirmed by its $^1$H NMR spectrum when run DMSO-$d_6$ shows the following signals ppm at $\delta$ 4 (2H, s, $CH_2Ph$), 7.3 to 8.5 (9H, m, ArH) and devoid any band for a hydroxyl group and its IR spectrum exhibits absorption at 1760 cm$^{-1}$ for $\upsilon_{C=O}$ of $\delta$-lactone and lacked any band for $\upsilon_{NH}$ conforming the formation of oxazinone ring. Its structure confirmed by its $^{13}$C NMR analysis in DMSO-$d_6$ shows the following signals ppm: 169.536 (2C), 161.085, 159.149, 145.890, 136.839, 134.702 (2C), 131.049, 129.523 (2C), 128.607 (2C), 127.892 (2C), 126.976 (2C), 119.765, 116.503, 44.698, and 40.425.

While, $\delta$ C signals of 40 169 were indicated for open structure (**S**) of compound (**1**) as indicated in Scheme 2.

It is well known that benzoxazinone derivatives are opened by time, so we prepare benzoxazinone compound (**1**) and divided it into different lots for physical analysis (IR, mass, $^1$H NMR, and $^{13}$C NMR) and biological assays. These lots were stored under control condition (desiccator) until use. Compound (**1**) was not immediately analyzed in NMR instrument because it was analyzed in other university (at NMR unit, Faculty of Science, Mansoura University). However, it not affected their potential as indicated because we confirmed chemical structure of compound (**1**) using IR spectra (IR spectrum exhibits absorption at 1760 cm$^{-1}$ for $\upsilon_{C=O}$ of $\delta$-lactone and lacked any band for $\upsilon_{NH}$ conforming the formation of oxazinone ring) before performing anticancer assays.

The mechanism of inactivation of enzyme, as one way to treat cancer disease by benzoxazinone is depended upon the nucleophilic reaction of benzoxazinone with enzyme hydroxyl group at the active site of the enzyme, which leads to ring opening and formation of an acylated enzyme (inactive form). The inhibitory activity of benzoxazinone may be based on the electrophilicity of the lactone carbonyl group which may form a hydrogen bond with enzyme as indicated in Scheme 3.

While, the open structure (**S**) of compound (**1**) cannot react with enzyme and it cannot form a hydrogen bond with enzyme as indicated in Scheme 4.



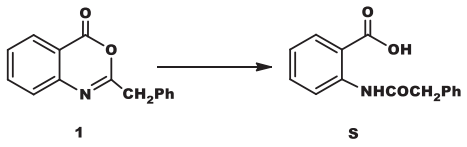

**SCHEME 1** Synthesis of benzoxazinone compound (**1**)

The enzyme will not be functioning if the oxazinone ring opens. Thus, the acid has no effect on the resulting activity. Moreover, oxazine derivatives are shown to be antioxidant and anticancer agents.[24]

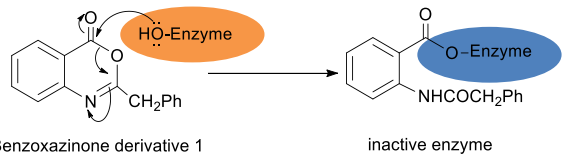
The reaction mechanism for synthesis compound (**1**) possibly takes place via the following mechanism (Figure 2):

Synthesis of benzimidazole, quinazolinone, and benzofuranone compounds (*N*-(2-(1*H*-benzo[d]imidazol-2-yl)phenyl)-2-phenyl acetamide (**2**) represented benzimidazole class, ethyl 2-(2-benzyl-4-oxoquinazolin-3(4*H*)-yl) acetate (**3**), 2-(2-benzyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (**4**), 3-amino-2-benzylquinazolin-4(3*H*)-one (**5**) represented quinazolinone class and 2-benzyl-3-((3-oxoisobenzofuran-1(3*H*)-ylidene)amino) quinazolin-4(3*H*)-one (**6**) represented to benzofuranone and quinazolinone classes), has been achieved following an individual steps procedure starting from 2-benzyl-3,-1-benzoxazin-4-one (**1**). When a benzoxazinone compound (**1**) was subjected to react with nitrogen nucleophile namely *o*-phenylenediamine in boiling ethanol afforded benzimidazole compound *N*-(2-(1*H*-benzo[*d*]imidazol-2-yl)phenyl)-2-phenyl acetamide (**2**) (Scheme 5).

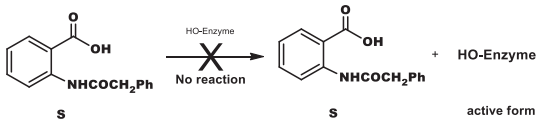
The structure of compound (**2**) was inferred by its $^1$H NMR spectrum when run DMSO-$d_6$ shows the following signals ppm at $\delta$ 3.4 (2H, s, $CH_2Ph$), 7.1 to 8.5 (9H, m, ArH), and 11.1 (1H, s, NH). In IR spectra, strong absorption peaks at 1674, 3283, 3208 cm$^{-1}$ attributable to $\upsilon_{C=O}$ and $\upsilon_{NH}$ nonbonded and bonded respectively indicates the formation of the imidazole ring. Its structure confirmed by its $^{13}$C NMR analysis in DMSO-$d_6$ shows the following signals ppm: 169.612 (2C), 140.797, 134.855 (2C), 131.096, 129.551, 128.626, 127.033, 122.760, 119.860, and 44.698. EIMS shows *m/e* at 327 ($M^{\bullet+}$).




**SCHEME 2** Hydrolysis of benzoxazinone compound (**1**)




**SCHEME 3** Mechanism of the inactivation of the enzyme by benzoxazinone




**SCHEME 4** The impossibility of a chemical reaction between the acid and the enzyme

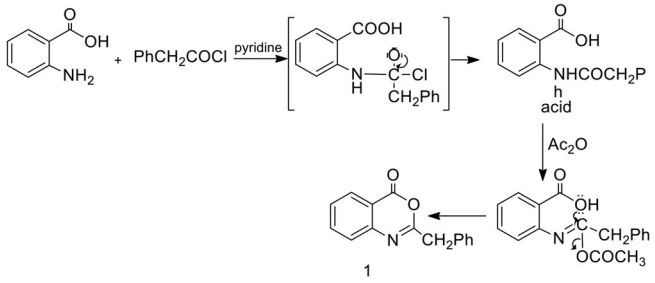
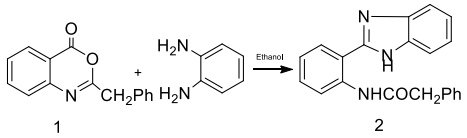
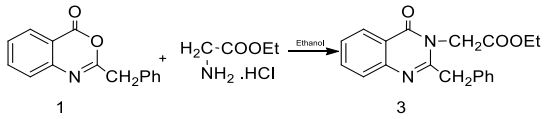



**FIGURE 2** Mechanism of synthesis of benzoxazinone



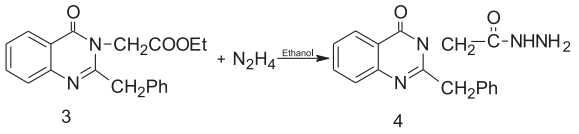

**SCHEME 5** Synthesis of benzimidazole compound (**2**)




**SCHEME 6** Synthesis of ester (**3**)

Quinazolinone derivative ethyl 2-(2-benzyl-4-oxoquinazolin-3(4*H*)-yl)acetate (**3**) was obtained via unambiguous route via the reaction of benzoxazinone (**1**) with ethyl glycinate hydrochloride in refluxing ethanol (Scheme 6).

The structure of compound (**3**) was confirmed by its $^1$H NMR spectrum when run DMSO-$d_6$ shows the following signals ppm at $\delta$ 3.4 (2H, s, $CH_2$Ph), 3.9 (3H, t, $CH_3$ (ethyl)), 4.1 (2H, q, $CH_2$ (ethyl)), 4.8 (2H, s, $CH_2$), 7.1 to 8.5 (9H, m, ArH) and absence of NH proton peak. Moreover, its IR spectrum revealed strong absorption bands at 1672, 1752 cm$^{-1}$ attributable to $\upsilon_{C=O}$ of two carbonyl groups and devoid any band for $\upsilon_{NH}$ conforming the formation of ester. Its structure confirmed by its $^{13}$C NMR analysis in DMSO-$d_6$ shows the following signals ppm: 169.593 (2C), 167.685 (2C), 161.343, 155.524, 140.787, 134.902 (3C), 131.087, 129.551, 129.551, 128.769 (3C), 127.1 (2C), 126.213, 122.741, 119.832 (2C), 61.600 (2C), 45.519, 44.708, 40.988, and 14.014 (2C, these two signals confirm the formation of ester).




**SCHEME 7** Synthesis of hydrazide derivative (**4**)

On the other hand, when the ester (**3**) was allowed to react with hydrazine hydrate in boiling ethanol yielded the hydrazide derivative (2-(2-benzyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide) (**4**) (Scheme 7).

The structure of compound (**4**) was confirmed by its $^1$H NMR spectrum when run DMSO-$d_6$ shows the following signals ppm at $\delta$ 3.4 (2H, s, $CH_2$Ph), 5 (2H, s, $CH_2$), 7.2 to 8.8 (10H, m, ArH), and 9.3 (1H, s, NH). Its IR spectrum revealed strong absorption bands at 1669 cm$^{-1}$ ($\upsilon_{C=O}$), 3170, 3323 cm$^{-1}$ due to $\upsilon_{NH}$ indicating the conversion of ester into hydrazide. Its structure confirmed by its $^{13}$C NMR analysis in DMSO-$d_6$ shows the following signals ppm: 180.934, 166.131, 161.409, 155.992, 146.901, 135.541, 134.654, 128.779 (2C), 127.005, 126.833 (2C), 119.870, 44.403, 41.017 and disappearance of two signals at 14.014. This confirmed the formation of hydrazide.

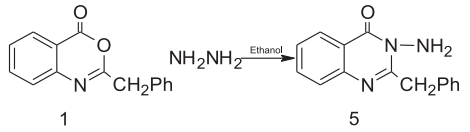
On other hand, the interaction of benzoxazinone compound (**1**) with hydrazine hydrate in boiling ethanol afforded 3-amino-2-benzylquinazolin-4(3*H*)-one (**5**) (Scheme 8).

The structure of compound (**5**) was confirmed by its $^1$H NMR spectrum when run DMSO-$d_6$ shows the following signals ppm at $\delta$ 3.7 (2H, s, $CH_2$Ph), 7.1 to 8.5 (9H, m, ArH), and 11.2 (2H, t, $NH_2$) indicating the formation of quinazolinone and its IR spectrum exhibits strong absorption bands at 1672, 3198, and 3315 cm$^{-1}$ attributable to $\upsilon_{C=O}$ and $\upsilon_{NH}$ bonding and nonbonding respectively. Its structure confirmed by its $^{13}$C NMR analysis in DMSO-$d_6$ shows the following signals ppm: 169.603 (2C), 140.797, 134.845 (2C), 131.087, 129.551, 128.617 (2C), 127.024, 122.760, 119.851, 116.407, and 44.689.

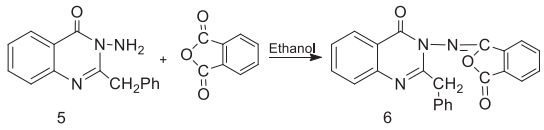
EIMS of compound (**5**) showed peaks at *m/e* 251($M^{\bullet+}$), 146 (M-PhCH$_2$, $NH_2$)$^{\bullet+}$, 119 (M-PhCH$_2$, $NH_2$, HCN)$^{\bullet+}$, 91(M-PhCH$_2$, $NH_2$, HCN, CO)$^{\bullet+}$, and 65.

The interaction of compound (**5**) with phthalic anhydride gave the lactone derivative (2-benzyl-3-((3-oxoisobenzofuran-1(3*H*)-ylidene)amino)quinazolin-4(3*H*)-one)(**6**) (Scheme 9).

IR spectrum of compound (**6**) revealed strong absorption bands at 1660, 1747 cm$^{-1}$ due to $\upsilon_{max}$ of two carbonyl groups confirming the formation of the lactone ring. EIMS of compound (**6**) showed *m/e* at 381 ($M^{\bullet+}$). When spectrum of compound (6) run in DMSO, the $^1$H NMR exhibits signals at $\delta$ 3.7 (s, 2H, benzylic proton) and 7.1 to 8.5 (m, 13H, ArH).




**SCHEME 8** Synthesis of quinazolinone derivative (**5**)




**SCHEME 9** Synthesis of benzofuranone derivative (**6**)

**Quinazolinone fused with Benzofuranone (6) > Benzoxazinone (1) > Benzoimidazole (2) > Quinazolinone (ester) (3)> Quinazolinone (5)> Quinazolinone (hydrazide) (4).**

**FIGURE 3** Illustrated the order of investigated compounds, represented heterocyclic classes, according to their activity against human hepatocellular carcinoma cell line (HepG2)

## 2.2 | Anticancer activity

### 2.2.1 | Anticancer activity against human HepG2

The synthesized compounds were screened for their anticancer activity against human HepG2 using two different methods, SRB and tetrazolium (MTT) assays. In the SRB assay, the dye binds to basic amino acids of cellular proteins and cell density determination refers to total protein mass.[25] While, tetrazolium (MTT) technique depended upon mitochondrial dehydrogenase enzyme in which these enzymes can reduce the tetrazolium dye to its insoluble formazan with a purple color.[26]

First, we are screening six compounds against human HepG2 using SRB assay at two concentrations 10 μm and 100 μm. The obtained results revealed that six investigated compounds exhibited different degrees of activity.

At 10 μm, all compounds had slight effects on HepG2. However, the activities of different tested heterocyclic compounds against HepG2 cell line can be summarized in Figure 3.

From these results, we can conclude that compound (**6**) with benzofuranone moiety has the best activity among other tested compounds. This may be due to furanone ring which contain carbonyl group besides oxygen atom.

Also, the same behavior, slight activities of tested compounds against HepG2 cell line, has been indicated at 100 μm. So, the activities of the tested compounds

**Benzoxazinone (1) > Quinazolinone (hydrazide) (4) > Quinazolinone (ester) (3) > Quinazolinone fused with Benzofuranone (6) > Benzoimidazole (2) > Quinazolinone (5)**

**FIGURE 4** Illustrated the order of investigated compounds, represented heterocyclic classes, according to their activity against human hepatocellular carcinoma cell line (HepG2)

against HepG2 cell line at 100 μm can be summarized in Figure 4.

At 100 μm, the arrangement of tested compound toward the same cell line was different than at 10 μm (Figure 5). This different may be due to the increasing the concentration of compounds. Hence, the highest anticancer activity was displayed by benzoxazinone compound (**1**) which showed cancer cell viability (76.24%). Moreover, all remaining compounds exhibited weak inhibitory activity. By comparing the activity of compounds reported in this study to their structures, the structure-activity relationship can be predicted. Benzoxazinone compound (**1**), possess oxygen atom in addition to a nitrogen atom, is the most potent compound toward HepG2 cell line. This activity may be related to the electronegativity of the oxygen in comparison to the nitrogen atom and the ability to be only a hydrogen bond acceptor. Dependence on all previous data, the mechanism of kill cancer cells can be predicted.

The mechanism of inactivation of enzyme, as one way to treat cancer disease, for example, seine proteinase[27] (such as α-chymotrypsin enzyme,[11] neutrophil elastase,[28] or rhomboid proteases[12]), by benzoxazinone is depended upon the nucleophilic reaction of benzoxazinone with enzyme hydroxyl group at the active site of the enzyme,[11] which leads to ring opening and formation of an acylated enzyme (inactive form).[29] The inhibitory activity of benzoxazinone may be based on the electrophilicity of the lactone carbonyl group which may form a hydrogen bond with enzyme[11] (Scheme 3).

When oxygen atom of benzoxazinone moiety is replaced with the nitrogen atom to yield quinazolinone, the activity decreased as shown in Scheme 10, because the electronegativity of oxygen is greater than nitrogen.

We conclude from the above that benzoxazinone compound (**1**) was the best compound among tested compounds having slight effect toward human HepG2.

This fact stimulates us to focus on the benzoxazinone compound. So, we tested various concentrations of 2-benzyl-3,1-benzoxazin-4-one ranging from 0.01, 0.1, 1, 10, 100 μg/mL against HepG2 using SRB to evaluate $IC_{50}$ (the inhibition concentration where the response is reduced by half) (Table 1).

Using sigmablot program version 6, the half maximal inhibitory concentration ($IC_{50}$) value was calculated and the standard curve was drawn as shown in Figure 6.

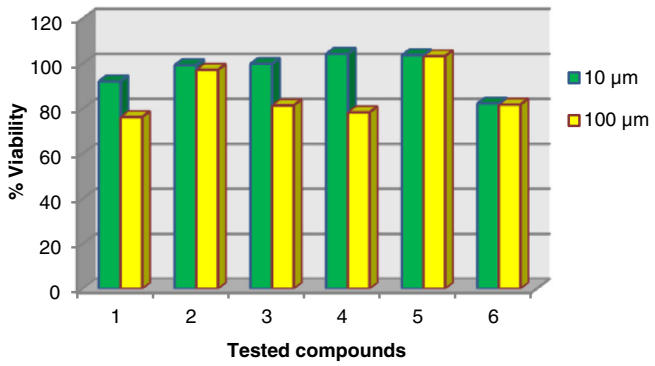
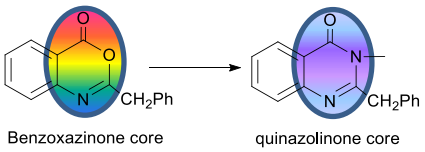



**FIGURE 5** Comparison between in vitro activities of investigated compounds against HepG2 at two concentrations 10 µm and 100 µm




**SCHEME 10** Synthesis of quinazolinone moiety

**TABLE 1** *In vitro* anticancer assay for benzoxazinone compound (**1**) against HepG2 at different concentrations

| Compound | Concentration | % Viability[a] | |
|---|---|---|---|
| | | Mean | SD[b] |
| 1 | 0 | 100.00 | 0 |
| | 0.01 | 94.89 | 2.880709 |
| | 0.1 | 86.76 | 1.501332 |
| | 1 | 81.85 | 0.66099 |
| | 10 | 75.77 | 0.880606 |
| | 100 | 62.36 | 0.27677 |

[a]The % viability: percentage of cancer cells is alive.
[b]SD is standard deviation.

We found that compound (**1**) has slight antiproliferative activity against hepatocellular carcinoma cell line. From this value, we conclude that this compound had not anticancer activity against HepG2 using SRB assay.

This stimulated us to evaluate the cytotoxicity effect for these tested compounds against HepG2 using tetrazolium (MTT) assay (Table 2). Doxorubicin was used as a positive control.

These results can be summarized in Figure 7.

The obtained results (Table 2, Figure 7) revealed that six investigated compounds exhibited different degrees of activity against HepG2 using MTT assay beginning from

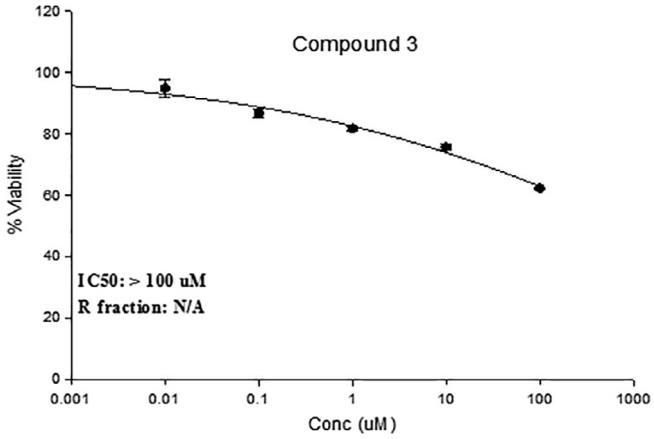



**FIGURE 6** Spectrum of the antiproliferative activity of the compound (**1**) against HepG2 via sulforhodamine B (SRB) assay

**TABLE 2** *In vitro* cytotoxic activity of tested compounds against hepatocellular carcinoma cell line (HepG2) using MTT assay

| Compound | $IC_{50}$ (µM)[a] |
|---|---|
| Acid[b] | 64.02 ± 3.6 |
| 1 | 51.94 ± 3.2 |
| 2 | 17.08 ± 1.4 |
| 3 | 42.33 ± 3.0 |
| 4 | 10.71 ± 0.9 |
| 5 | 26.83 ± 2.1 |
| 6 | 34.52 ± 2.5 |
| DOX[c] | 4.50 ± 0.2 |

[a]$IC_{50}$ (µM): (mean ± SD), 1 to 10 (very strong), 11 to 20 (strong), 21 to 50 (moderate), 51 to 100 (weak), and above 100 (noncytotoxic).
[b]Acid: 2-(2-phenylacetamido)benzoic acid.
[c]DOX: doxorubicin.

very strong to weak. On the contrast to SRB assay, compound (**4**), quinazolinone hydrazide derivative, was found to be the most potent compound against HepG2 cell line. This activity may be due to the presence of hydrazide moiety. On the other hand, the lowest potent compound was benzoxazinone compound (**1**). However, compound (**1**), possess oxazinone ring, was more potent than acid (2-(2-phenylacetamido)benzoic acid) owing opened ring. It proved that the activity of compound (**1**) was arising from the presence of oxazinone ring.

In tetrazolium (MTT) assay, the order of investigated compounds according to their activity against human HepG2 has been different from these results have been obtained in SRB assay. These differences can be due to the differences between mechanisms of the two methods, SRB and tetrazolium (MTT) assays. In the SRB assay, the dye binds to basic amino acids of cellular proteins.[25]

**Quinazolinone (hydrazide) (4)> Benzoimidazole (2)> Quinazolinone (5) > Quinazolinone fused with Benzofuranone (6)> Quinazolinone (ester) (3) > Benzoxazinone (1)> Acid.**

**FIGURE 7** Illustrated the order of investigated compounds, represented heterocyclic classes, according to their activity against human hepatocellular carcinoma cell line (HepG2) using MTT assay

While, tetrazolium (MTT) technique depended upon mitochondrial dehydrogenase enzyme in which these enzymes can reduce the tetrazolium dye to its insoluble formazan with a purple color.[26] This can be appearing clarify when G. Zheng *et al*. found that isonicotinic acid hydrazide, owing hydrazide moiety, cause human hepatoblastoma cell line (HepG2), healthy cell, injury by mitochondrial DNA damage.[30] In addition to, MTT is based on mitochondrial uptake.[7] From all the previous reasoning, it can be easily explained the difference between the obtained results from two assays. In tetrazolium (MTT) assay, compound (**4**), owing hydrazide moiety, could be damaging mitochondrial DNA of human HepG2, cancerogenic cell line. To elicit this prediction, DNA/methyl green assay was performed. Methyl green reversibly binds to DNA, and the colored complex is stable at neutral pH, whereas free methyl green fades at this pH value. DNA-binding active compounds displace DNA from its methyl green complex. The displacement was determined by a spectrophotometric assay as a decrease in the absorbance at 630 nm. The obtained results were shown in Table 3.

The results of the binding DNA test were in line with cytotoxicity studies, using tetrazolium (MTT) assay, where the most potent compound, compound (**4**), quinazolinone (hydrazide), showed the best DNA binding. Also, the lowest potent compound, compound (**1**), benzoxazinone compound, showed the worst DNA binding and the same arrangement of tested compounds, in tetrazolium (MTT) cytotoxicity assay, was noticed. There is only one exception that acid showed DNA binding better than benzoxazinone compound (**1**). So, other studies will be performed to elicit the underlying mechanism of the activity benzoxazinone compound (**1**) toward HepG2 cell line. These results can be summarized in Figure 8.

### 2.2.2 | Anticancer activity against human epithelioid carcinoma (Hela)

Moreover, tested compounds were further tested against human epithelioid carcinoma (Hela) cell line using tetrazolium (MTT) assay. The obtained results were indicated in Table 4.

**TABLE 3** DNA/methyl green colorimetric assay of the DNA-binding compounds using MTT assay

| Compound | $IC_{50}$ (μM)[a] |
|---|---|
| Acid[b] | 74.89 ± 3.2 |
| 1 | 81.47 ± 3.5 |
| 2 | 33.96 ± 1.8 |
| 3 | 49.42 ± 2.5 |
| 4 | 28.35 ± 1.5 |
| 5 | 40.06 ± 2.1 |
| 6 | 42.81 ± 2.3 |
| DOX[c] | 31.27 ± 1.8 |

[a]$IC_{50}$ values represent the concentration (mean ± SD, $n$ = 3-5 separate determinations) required for a 50% decrease in the initial absorbance of the DNA/methyl green solution.
[b]Acid: 2-(2-phenylacetamido)benzoic acid.
[c]DOX: doxorubicin.

**Quinazolinone (hydrazide) (4) > Benzoimidazole (2)> Quinazolinone (5)> Quinazolinone fused with Benzofuranone (6)> Quinazolinone (ester) (3) > Acid > Benzoxazinone (1).**

**FIGURE 8** Illustrated the order of investigated compounds, represented heterocyclic classes, according to their DNA binding affinity

From the obtained results, compound (**4**), hydrazide derivative, has been showed the most potent compound against Hela cell line. Furthermore, compound (**1**), benzoxazinone compound, has been showed the lowest potent compound. Likewise, arrangement of tested compounds according to their activity toward Hela cell line was the same to their arrangement for the previous cytotoxicity studies, HepG2 cell line. These results can be summarized in Figure 9.

### 2.2.3 | Anticancer activity against human amnion (WISH)

To further investigate their toxicity of these tested compounds on normal human, the cytotoxicity effect of the tested compounds was performed against human amnion (WISH) cell line using tetrazolium (MTT) assay. This cell line has been selected as normal human cell of human epithelioid carcinoma (Hela) cell line. This selection is aiming to study toxicity of tested compounds upon uterus cells which represented by WISH cell line. The obtained results were indicated in Table 5.

From the above data, compounds **5** (quinazolinone) and **6** (quinazolinone fused with benzofuranone) were less toxic toward WISH cell line. So, they are more safety

**TABLE 4** *In vitro* cytotoxic activity of tested compounds against human epithelioid carcinoma (Hela) cell line using MTT assay

| Compound | $IC_{50}$ (μM)[a] |
|---|---|
| Acid[b] | 58.60 ± 3.4 |
| 1 | 62.91 ± 3.6 |
| 2 | 13.90 ± 1.2 |
| 3 | 31.04 ± 2.2 |
| 4 | 8.14 ± 0.7 |
| 5 | 19.78 ± 1.6 |
| 6 | 22.16 ± 1.8 |
| DOX[c] | 5.57 ± 0.4 |

[a]$IC_{50}$ (μM): (mean ± SD), 1 to 10 (very strong), 11 to 20 (strong), 21 to 50 (moderate), 51 to 100 (weak), and above 100 (noncytotoxic).
[b]Acid: 2-(2-phenylacetamido)benzoic acid.
[c]DOX: doxorubicin.

**Quinazolinone (hydrazide) > Benzoimidazole > Quinazolinone > Quinazolinone fused with Benzofuranone> Quinazolinone (ester) > Acid > Benzoxazinone.**

**FIGURE 9** Illustrated the order of investigated compounds, represented heterocyclic classes, according to their activity against human epithelioid carcinoma (Hela) cell line using MTT assay

**TABLE 5** *In vitro* toxicity activity of tested compounds against human amnion (WISH) cell line using MTT assay

| Compound | $IC_{50}$ (μM)[a] |
|---|---|
| Acid[b] | 24.90 ± 1.9 |
| 1 | 30.26 ± 2.3 |
| 2 | 29.15 ± 2.3 |
| 3 | 19.17 ± 1.7 |
| 4 | 22.48 ± 1.9 |
| 5 | 48.35 ± 3.2 |
| 6 | 51.26 ± 3.5 |

[a]$IC_{50}$ (μM): (mean ± SD), the highest value indicate that the lowest toxic compounds because it expresses the cell viability of normal, healthy, cell line.
[b]Acid: 2-(2-phenylacetamido)benzoic acid.

than other tested compounds. While, compound (**3**) was more active against WISH cell line. These results can be summarized in Figure 10.

Figure 11 shows the effect of the tested compounds on the cancerogenic cell, human epithelioid carcinoma (Hela), and compared them to their effect on the healthy cell line, human amnion (WISH).

From the competitive studies, compound **5**, quinazolinone derivative, was found the best bioactive compound among tested compounds because it was less toxic upon healthy cell line (WISH) than other tested

**Quinazolinone fused with Benzofuranone (6)> Quinazolinone (5) > Benzoxazinone (1)> Benzoimidazole (2)> Acid> Quinazolinone (hydrazide) (4) > Quinazolinone (ester) (3).**

**FIGURE 10** Illustrated the order of investigated compounds, represented heterocyclic classes, according to their activity against human amnion (WISH) cell line using MTT assay

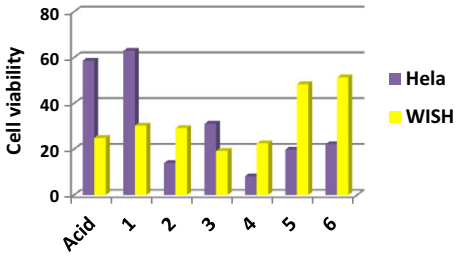



**FIGURE 11** Comparative toxicity of investigated compounds against cancerogenic cell line (Hela) and healthy cell line (WISH)

compounds, except compound **6** (quinazolinone fused with benzofuranone) which is the lowest toxic compound. But, compound **5** was more potent against cancerogenic cell line (Hela) than compound **6.**

## 2.3 | Antioxidant activity screening assay; ABTS method

The antioxidant activities of tested compounds were evaluated. The obtained results were compared with ascorbic acid as antioxidant compounds (Table 6).

The results showed clearly that, compound **4**, quinazolinone (hydrazide), had the best activity, while compound **1**, benzoxazinone compound, exhibited the lowest activity. Introduction hydrazide moiety may be enhancing the antioxidant property of compound **4**. Likewise, arrangement of tested compounds according to their antioxidant activities the same to their arrangement for the previous cytotoxicity studies, human epithelioid carcinoma (Hela) and human hepatocellular carcinoma (HepG2) cell lines. These results can be summarized in Figure 12.

Ethyl 2-(2-benzyl-4-oxoquinazolin-3(4*H*)-yl)acetate (**3**), 2-(2-benzyl-4-oxoquinazolin-3(4*H*)-yl)acetohydrazide (**4**), 3-amino-2-benzylquinazolin-4(3*H*)-one (**5**) represented quinazolinone class and 2-benzyl-3-((3-oxoisobenzofuran-1(3*H*)-ylidene)amino) quinazolin-4(3*H*)-one (**6**) are derivatives for quinazolinone (all contain, quinazolinone moiety). Compound (**4**) has three hydrogen atoms available for formation of a hydrogen bond with nitrogenous bases of DNA, while compound (**5**) has only two hydrogen atoms. While, compounds (**3**) and (**6**) have no hydrogen atoms available

**TABLE 6** Antioxidant activity assay (ABTS) of tested compounds

| Compound | Absorbance of samples | % Inhibition |
|---|---|---|
| Control of ABTS[a] | 0.518 | 0 |
| Ascorbic-acid | 0.058 | 88.8 |
| Acid[b] | 0.421 | 18.7 |
| 1 | 0.442 | 14.7 |
| 2 | 0.337 | 34.9 |
| 3 | 0.392 | 24.3 |
| 4 | 0.254 | 51.0 |
| 5 | 0.358 | 30.9 |
| 6 | 0.368 | 28.9 |

[a]ABTS: 2,2′-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid).
[b]Acid: 2-(2-phenylacetamido)benzoic acid.

**Quinazolinone (hydrazide) (4) > Benzoimidazole (2) > Quinazolinone (5) > Quinazolinone fused with Benzofuranone (6)> Quinazolinone (ester) (3) > Acid > Benzoxazinone (1).**

**FIGURE 12** Illustrated the order of investigated compounds, represented heterocyclic classes, according to their antioxidant activities

for hydrogen bond formation. Compound (**6**) might be react with nitrogen bases of DNA via acylation more than compound (**3**), thus the order of reactivity **4** > **5** > **6** > **3**. This agreed well with the results obtained in Tables 2–4, and 6. Reactivity of compound (**2**) might be due to the presence of benzimidazole moiety and compound (**1**) could be due to oxazinone moiety.

## 3 | CONCLUSIONS

In conclusion, in vitro, 2-(2-benzyl-4-oxoquinazolin-3 (4*H*)-yl)acetohydrazide (**4**) is the most potent antioxidant and anticancer agents against human epithelioid carcinoma (Hela). Moreover, it showed anticancer activity against human HepG2 in which it was proved using DNA binding affinity. Although, 3-amino-2-benzylquinazolin-4(3*H*)-one (**5**) is less potent anticancer agent against (Hela) than compound (**4**) but it is more safe against normal cell, human amnion (WISH). In spite of compound 2-benzyl-3-((3-oxoisobenzofuran-1(3*H*)-ylidene) amino)quinazolin-4(3*H*)-one (**6**) is the most safe, but compound (**5**) was more potent against cancerogenic cell lines, Hela and HepG2. So, compound (**5**), quinazolinone derivative, was the best compound among other tested compound.

A further in-depth study to elucidate the underline mechanism (s) involved in such promising activities against tested cell lines will be performed in order to develop an appropriate efficient strategic treatment for patients having hepatic and/or uterus cancers. In the future, we will focus upon compounds belong to the quinazolinone class according to their promising activities against different cell lines.

## 4 | EXPERIMENTAL

### 4.1 | Chemistry

#### 4.1.1 | General

Melting points were determined on an electric melting point apparatus and are uncorrected. FTIR spectra (KBr disk) were performed in the microanalytical center, Cairo University, Egypt. The infrared spectra were recorded with FTIR-4100 spectrophotometer product of Jasco, Japan. The spectra were recorded from 4000 $cm^{-1}$ to 450 $cm^{-1}$. $^{13}C$ NMR spectra were determined on JNM-ECA II 500 MHz at NMR unit, Mansoura University. $^{1}H$ NMR spectra were determined on Bruker Avance (III) 400 MHz at Center for Drug Discovery research and development, Ain Shams University, while $^{1}H$ NMR spectrum of compound (**1**) was determined on JNM-ECA II 500 MHz at NMR unit, Mansoura University. The chemical shifts are in ppm. The solvent was DMSO. We used dimethyl sulfoxide (DMSO) as solvent for NMR analysis because we used it for anticancer assays. The splitting patterns (multiplicities) in $^{1}H$ NMR were designated as singlet (s), doublet (d), triplet (t), quarter (q), and multiplet (m). Mass spectra were recorded using GC MS—Qp-2010 Plus Shimadzu at microanalytical center, Cairo University, Egypt. Mass spectra were recorded at 70 ev.

#### 4.1.2 | Synthesis tested compound 1 to 6

*Synthesis of 2-(2-phenylacetamido)benzoic acid*

To a solution of anthranilic acid (36.46 mmol, 5 g) in pyridine (10 mL), phenylacetyl chloride (54.67 mmol, 7.23 mL) was added gradually with continuous stirring. After the addition complete, the reaction mixture was stirred in an ice bath for two hours and then poured upon hydrochloric acid containing crushed ice. The solid product that deposited was collected by filtration and washed with water, dried and recrystallized from ethanol to give 2-phenylacetylamino benzoic acid as beige crystals, m. p. 166 to 168°C, yield 91.43%.

*Synthesis of 2-benzyl-3,1-benzoxazin-4-one (**1**)*

A solution of 2-phenylacetylamino benzoic acid (7.83 mmol, 2 g) in acetic anhydride (1.5 mL) was heated

under reflux in a water bath for one hour. The reaction mixture was left until solidify to give benzoxazinone (**1**).

Benzoxazinone compound (**1**) has been prepared and divided it into different lots for physical analysis (IR, mass, $^{1}$H NMR and $^{13}$C NMR) and biological assays. These lots were stored under control condition (desiccator) until use.

Yield 95.76%, yellow crystals, m.p. 152°C, IR (KBr), υ/cm$^{-1}$: 1760 (C=O) of δ-lactone, and lacked any band for $\upsilon_{NH}$. $^{1}$H NMR (500 MHz): $\delta = 4$ (s, 2H, $CH_2$Ph), 7.3 to 8.5 (m, 9H, ArH) and devoid any band for a hydroxyl group. $^{13}$C NMR (500 MHz): $\delta = 169.536$(2C), 161.085, 159.149, 145.890, 136.839, 134.702 (2C), 131.049, 129.523 (2C), 128.607 (2C), 127.892 (2C), 126.976 (2C), 119.765, 116.503, 44.698, 40.425.

*Synthesis of N-(2-(1H-benzo[d]imidazol-2-yl)phenyl)-2-phenyl acetamide (**2**)*

*O*-Phenylenediamine (12.65 mmol, 1.3674 g) was added to a solution of benzoxazinone (**1**) (8.43 mmol, 2 g) in ethanol (30 mL). The reaction mixture was heated under reflux for three hours, left to cool. The solid product that deposited was collected by filtration, dried and recrystallized from benzene to give pure product (**2**).

Yield 48.80%, grey crystals, m.p. 158 to 160°C, IR (KBr), υ/cm$^{-1}$: 1674 (C=O), 3283 (nonbonded NH), 3208 (bonded NH). $^{1}$H NMR (400 MHz): $\delta = 3.4$ (s, 2H, $CH_2$Ph), 7.1 to 8.5 (m, 9H, ArH), 11.1 (s, 1H, NH). $^{13}$C NMR (500 MHz): $\delta = 169.612$ (2C), 140.797, 134.855 (2C), 131.096, 129.551, 128.626, 127.033, 122.760, 119.860, 44.698. HRMS (ESI): *m/z* calcd for $C_{21}H_{17}N_3O$: 327.38 $[M + H]^+$; found: 327.00.

*Synthesis of ethyl 2-(2-benzyl-4-oxoquinazolin-3(4H)-yl) acetate (**3**)*

A mixture of benzoxazinone (**1**) (8.43 mmol, 2 g) and glycine ethyl ester hydrochloride (16.94 mmol, 2.3558 g) in ethanol/anhydrous sodium acetate mixture (30 mL/0.5 g) was refluxed for three hours and then left to cool. The solid product that deposited was collected by filtration, dried and recrystallized from ethanol to give the pure product (**3**).

Yield 65.28%, yellow crystals, m.p. 94 to 96°C, IR (KBr), υ/cm$^{-1}$: 1672 (C=O), 1752 (C=O (ester)) and devoid any band for υNH. $^{1}$H NMR (400MHz): $\delta = 3.4$ (s, 2H, $CH_2$Ph), 3.9 (t, 3H, $CH_3$ (ethyl)), 4.1 (q, 2H, $CH_2$ (ethyl)), 4.8 (s, 2H, $CH_2$), 7.1 to 8.5 (m, 9H, ArH). $^{13}$C NMR (500 MHz): $\delta = 169.593$ (2C), 167.685 (2C), 161.343, 155.524, 140.787, 134.902 (3C), 131.087, 129.551, 129.551, 128.769 (3C), 127.1 (2C), 126.213, 122.741, 119.832 (2C), 61.600 (2C), 45.519, 44.708, 40.988, 14.014 (2C).

*Synthesis of 2-(2-benzyl-4-oxoquinazolin-3(4H)-yl) acetohydrazide (**4**)*

A mixture of ester (**3**) (6.21 mmol, 2 g) and hydrazine hydrate (63.91 mmol, 3.1 mL) in ethanol (30 mL) was heated under reflux for eight hours, left to cool. The solid product that deposited was collected by filtration, dried and recrystallized from ethanol to give the pure product (**4**).

White crystals, m.p. 260 to 262°C, IR (KBr), υ/cm$^{-1}$: 1669 (C=O), 3170 (NH), 3323 (NH). $^{1}$H NMR (400 MHz): $\delta = 3.4$ (s, 2H, CH2Ph), 5 (s, 2H, CH2), 7.2 to 8.8 (m, 9H, ArH), 9.3 (s, 1H, NH). $^{13}$C NMR (500 MHz): $\delta = 180.934$, 166.131, 161.409, 155.992, 146.901, 135.541, 134.654, 128.779 (2C), 127.005, 126.833 (2C), 119.870, 44.403, 41.017.

*Synthesis of 3-amino-2-benzylquinazolin-4(3H)-one (**5**)*

A mixture of benzoxazinone (**1**) (8.47 mmol, 2 g) and hydrazine hydrate (12.99 mmol, 0.63 mL) in ethanol (30 mL) was heated under reflux for three hours, left to cool. The solid product that deposited was collected by filtration, dried and recrystallized from ethanol to give the pure product (**5**).

Yield 50.52%, white crystals, m.p. 160 to 162°C, IR (KBr), υ/cm$^{-1}$: 1672 (C=O), 3198 (NH), 3315 (NH). $^{1}$H NMR (400 MHz): $\delta = 3.7$ (s, 2H, $CH_2$Ph), 7.1 to 8.5 (m, 9H, ArH), 11.2 (t, 2H, NH2). $^{13}$C NMR (500 MHz): $\delta = 169.603$ (2C), 140.797, 134.845 (2C), 131.087, 129.551, 128.617 (2C), 127.024, 122.760, 119.851, 116.407, 44.689. HRMS (ESI): calcd for $C_{15}H_{13}N_3O$: 251.28 $[M + H]^+$; found: 251.10.

*Synthesis of 2-benzyl-3-((3-oxoisobenzofuran-1(3H)-ylidene) amino)quinazolin-4(3H)-one (**6**)*

A mixture of quinazolinone derivative (**5**) (4.78 mmol, 1.2 g) and phthalic anhydride (7.16 mmol, 1.06 g) in ethanol (30 mL) was heated under reflux for three hours, left to cool. The solid product that deposited was collected by filtration, dried and recrystallized from ethanol to give the pure compound (**6**).

Yield 39.09%, white crystals, m.p. 162 to 164°C, IR (KBr), υ/cm$^{-1}$: 1660 (C=O), 1747 (C=O). $^{1}$H NMR (400 MHz): $\delta = 3.7$(s, 2H, benzylic proton), 7.1 to 8.5 (m, 13H, ArH). HRMS (ESI): calcd for $C_{23}H_{15}N_3O_3$: 381.38 $[M + H]^+$; found: 381.35.

## 4.2 | Anticancer activity

### 4.2.1 | For SRB assay

*Cell line*

Hepatocellular carcinoma (HepG2) cell lines were obtained from Nawah Scientific Inc. (Mokatam, Cairo,

Egypt). Cells were maintained in DMEM (Dulbecco's Modified Eagle Medium) media supplemented with 100 mg/mL of streptomycin, 100 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5% (vol/vol) $CO_2$ atmosphere at 37°C.

*Cytotoxicity assay*

Cytotoxicity assays were performed at Nawah Scientific Inc. (Mokatam, Cairo, Egypt). Cell viability was assessed by SRB assay. Aliquots of 100 μL cell suspension ($5 \times 10^3$ cells) were in 96-well plates and incubated in complete media for 24 hours. Lung cancer cells were treated with another aliquot of 100 μL media containing six compounds at two concentrations 10 μm and 100 μm for quick screening while tested compound (**1**) at various concentrations ranging from 0.01, 0.1, 1, 10, and 100 μg/mL for routine analysis. Tested compounds were completely soluble in DMSO for anticancer assay. After 72 hours of drug exposure, cells were fixed by replacing media with 150 μL of 10% trichloroacetic acid (TCA) (act as acidic medium) and incubated at 4°C for one hour. TCA solution was removed, and the cells were washed five times with distilled water. Aliquots of 70 μL SRB solution (0.4% wt/vol) were added and incubated in a dark place at room temperature for 10 minutes. Plates were washed three times with 1% acetic acid and allowed to air-dry overnight. Then, 150 μL of TRIS (10 mM) was added to dissolve protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH FLUOstar Omega microplate reader (Ortenberg, Germany). The same assay was performed for compound (**1**) toward HepG2 at different concentrations ranging from 0.01, 0.1, 1, 10, and 100 μg/mL for routine analysis.

### 4.2.2 | For MTT assay

*Cell line*

Hepatocellular carcinoma (HePG-2), Epithelioid carcinoma (Hela), and Human amnion (WISH). The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

*Chemical reagents*

The reagents were Roswell Park Memorial Institute (RPMI-1640) medium, MTT and DMSO (Sigma co., St. Louis, Missouri) and Fetal Bovine serum (GIBCO, UK). Doxorubicin was used as a standard anticancer drug for comparison.

*Cytotoxicity assay*

Cytotoxicity assays were performed at Faculty of Pharmacy, Mansoura University, Mansoura, Egypt. In order to test the inhibitory effects of compounds upon cell growth using the MTT test, cell lines mentioned above were used. The conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells is the basis of the colorimetric assay. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics were added 100 units/mL penicillin and 100 μg/mL streptomycin at 37°C in a 5% $CO_2$ incubator. The cells were seeded in a 96-well plate at a density of $1.0 \times 10^4$ cells/well at 37°C for 48 hours under 5% $CO_2$. After incubation, the cells were treated with different concentrations of compounds and incubated for 24 hours. After 24 hours, of drug treatment, 20 μL of MTT solution at 5 mg/mL was added and incubated for 4 hours. Tested compounds were completely soluble in DMSO for anticancer assay. DMSO in volume of 100 μL is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) × 100.[31,32]

## 4.3 | Colorimetric assay for compounds that bind DNA

One hundred mL of 0.05 M Tris-HCl buffer (pH 7.5) mixing with 7.5 mM $MgSO_4$ was for suspending DNA methyl green (20 mg); using a magnetic stirrer, the mixture was stirred at 37°C for 24 hours. 10, 100, 1000 mg of samples have been dissolved in the ethanol, under vacuum solvent was removed, and 200 μL of the DNA/methyl green solution were added to each tube. Samples in the dark were incubated at ambient temperature. The samples were finally absorbed at 642 to 645 nm after 24 hours. Readings for original absorption have been fixed and normalized in the untreated standard.[33]

## 4.4 | Antioxidant activity screening assay using ABTS method

Antioxidant activity determinations were evaluated from the bleaching of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) derived radical cations. The reaction of ABTS (60 mL) with $MnO_2$ (3 mL, 25 mg/mL) in phosphate buffer solution (10 mM, pH 7, 5 mL) was performed for synthesize ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] for obtaining radical cation. Centrifugation and filtration of the solution did after shaking it for a few minutes. The resulting green-blue solution (ABTS radical solution) of was absorbed

and recorded at $\lambda_{max}$ 734 nm to obtain A control. The addition of (20 mL of 1 mg/mL) solution of the tested sample in spectroscopic grade MeOH/buffer (1:1, vol/vol) to the ABTS solution was for recording absorbance (A test).[34] The inhibition has been determined according to the following:

$$\%\text{Inhibition} = \frac{\text{A(control)} - \text{A(test)}}{\text{A(control)}} \times 100,$$

where % Inhibition is the absorption reduction.[34]

A standard antioxidant, ascorbic acid (20 mL, 2 mM) solution, was used as positive control. Without ABTS, solvent was used for running blank sample.[34]

## ORCID

*Tasneem Mokhtar Radwan* https://orcid.org/0000-0002-9274-9988

## REFERENCES AND NOTES

[1] K. Paul, A. Sharma, V. Luxami, *Bioorg Med Chem Lett* **2014**, *24*, 624.
[2] A. A. Abdel-Aziz, A. S. El-Azab, H. I. El-Subbagh, A. M. Al-Obaid, A. M. Alanazi, M. A. Al-Omar, *Bioorg Med Chem Lett* **2012**, *22*, 2008.
[3] E. Taherian, G. Khodarahmi, M. R. Khajouei, F. Hassanzadeh, N. Dana, *Res Pharm Sci* **2019**, *14*, 247.
[4] World Health Organization, **2018**. World Health Organization report "Cancer", https://www.who.int/en/news-room/fact-sheets/detail/cancer (accessed: 12 September 2018).
[5] World Health Organization, World Health Organization report entitled "Cervical cancer", https://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/
[6] Y. P. Keepers, P. E. Pizao, G. J. Peters, J. V. Ark-Otte, B. Winograd, H. M. Pinedo, *Eur J Cancer* **1991**, *27*, 897.
[7] L. Vajrabhaya, S. Korsuwannawong, *J Anal Sci Technol* **2018**, *9*, 15. https://doi.org/10.1186/s40543-018-0146-0.
[8] W. Kemnitzer, J. Kuemmerle, S. Jiang, H. Zhang, N. Sirisoma, S. Kasibhatla, C. Crogan-Grundy, B. Tseng, J. Drewe, S. X. Cai, *Bioorg Med Chem Lett* **2008**, *18*, 6259.
[9] P. Martins, J. Jesus, S. Santos, L. R. Raposo, C. Roma-Rodrigues, P. V. Baptista, A. R. Fernandes, *Molecules* **2015**, *20*, 16852.
[10] Golec, B.; Nawara, K.; Thummel, R. P.; Waluk, J. *Photochem Photobiol Sci*. **2019**, *18*, 2225. DOI: 10.1039/c8pp00587g. [Epub ahead of print]
[11] B. P. Marasini, F. Rahim, S. Perveen, A. Karim, K. M. Khan, M. I. Atta-ur-Rahman Choudhary, *Bioorg Med Chem Lett* **2017**, *70*, 210.
[12] T. S. Reddy, H. Kulhari, V. G. Reddy, V. Bansal, A. Kamal, R. Shukla, *Eur J Med Chem* **2015**, *101*, 790.
[13] J. C. Powers, J. L. Asgian, Ö. D. Ekici, K. E. James, *Chem Rev* **2002**, *102*, 4639.
[14] A. Krantz, R. W. Spencer, T. F. Tam, T. J. Liak, L. J. Copp, E. M. Thomas, S. P. Rafferty, *J Med Chem* **1990**, *33*, 464.
[15] P. W. Hsieh, F. R. Chang, C. H. Chang, P. W. Cheng, L. C. Chiang, F. L. Zeng, K. H. Lin, Y. C. Wu, *Bioorg Med Chem Lett* **2004**, *14*, 4751.
[16] A. M. F. Eissa, R. El-Sayed, *J. Heterocycl Chem* **2006**, *43*, 1161.
[17] R. L. Jarvest, M. J. Parratt, C. M. Debouck, J. G. Gorniak, L. J. Jennings, H. T. Serafinowska, J. E. Strickler, *Bioorg Med Chem Lett* **1996**, *6*, 2463.
[18] O. M. O. Habib, H. M. Hassan, A. El-Meka, *Am J Org Chem* **2012**, *2*, 45.
[19] V. H. Palvidis, P. J. Perry, *Synth Commun* **1994**, *24*, 533.
[20] J. Yang, M. Barniol-Xicota, M. T. N. Nguyen, A. Ticha, K. Strisovsky, S. H. L. Verhelst, *Bioorg Med Chem Lett* **2018**, *28*, 1423.
[21] H. Mostafavi, M. R. Islami, H. Khabazzadeh, M. Khaleghi, *ChemistrySelect* **2019**, *4*, 3169.
[22] R. Doonaboyina, A. Mittal, S. B. Gummadi, *Int J Res Pharm Sci* **2019**, *10*, 730.
[23] M. E. Diener, A. J. Metrano, S. Kusano, J. Scott, S. J. Miller, *J Am Chem Soc* **2015**, *137*, 12369.
[24] D. S. Zinad, A. Mahal, R. K. Mohapatra, A. K. Sarangi, M. R. F. Pratama, *Chem Biol Drug Des* **2019**, *00*, 1. https://doi.org/10.1111/cbdd.13633.
[25] Kuete, V.; Karaosmanoğlu, O.; Sivas, H. *Medicinal Spices and Vegetables from Africa*, Vol. 19 (Eds.: Victor Kuete), Elsevier, Netherlands, 1st edition, **2017**, Ch. 10, pages 271.
[26] L. Vajrabhaya, S. Korsuwannawong, *Anal Sci Technol* **2018**, *9*, 1.
[27] R. W. Spencer, L. J. Copp, B. Bonaventura, T. F. Tam, T. J. Liak, R. J. Billedeau, A. Krantz, *Biochem Biophys Res Commun* **1986**, *140*, 928.
[28] M. Gautier, A. J. P. Alix, J. Sapi, W. Hornebeck, E. Bourguet, *Anticancer Agents Med Chem* **2012**, *12*, 565.
[29] M. I. Marzouk, T. A. Farghaly, M. A. El-Hashash, S. A. Shaker, S. M. Hussein, *Heterocycles* **2015**, *91*, 1399.
[30] G. Zheng, M. Wang, Q. Ren, T. Han, Y. Li, S. Sun, X. Li, F. Feng, *Exp Ther Med* **2019**, *17*, 4289.
[31] T. Mosmann, *J Immunol Methods* **1983**, *65*, 55.
[32] F. Denizot, R. Lang, *J Immunol Methods* **1986**, *22*, 271.
[33] N. Burres, A. Frigo, R. Rasmussen, J. McAlpine, *J Nat Prod* **1992**, *55*, 1582.
[34] M. A. Metwally, M. A. Gouda, A. N. Harmal, A. M. Khalil, *Eur J Med* **2012**, *56*, 254.

**How to cite this article:** Radwan TM, El-Hashash MA-A, Wasfy AA-HF, Abdallah SA. Antitumor, cytotoxic, and antioxidant evaluation of six heterocyclic compounds containing different heterocycle moieties. *J Heterocycl Chem*. 2020;1–12. https://doi.org/10.1002/jhet.3847