Synthesis, Antiproliferative, and Antioxidant Evaluation of 2-Pentylquinazolin-4(3H)-one(thione) Derivatives with DFT Study

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Abstract: The current study was chiefly designed to examine the antiproliferative and antioxidant activities of some novel quinazolinone(thione) derivatives 6–14. The present work focused on two main points; firstly, comparing between quinazolinone and quinazolinthione derivatives. Whereas, antiproliferative (against two cell lines namely, HepG2 and MCF-7) and antioxidant (by two methods; ABTS and DPPH) activities of the investigated compounds, the best quinazolinthione derivatives were 6 and 14, which exhibited excellent potencies comparable to quinazolinone derivatives 5 and 9, respectively. Secondly, we compared the activity of four series of Schiff bases which included the quinazolinone moiety (11a–d). In addition, the antiproliferative and antioxidant activities of the compounds with various aryl aldehyde hydrazone derivatives (11a–d) analogs were studied. The compounds exhibited potency that increased with increasing electron donating group in p-position (OH > OMe > Cl) due to extended conjugated systems. Noteworthy, most of antiproliferative and antioxidant activities results for the tested compounds are consistent with the DFT calculations.

Keywords: quinazolin-4(3H)-one; quinazolin-4(3H)-thione; Schiff base; antiproliferative activity; antioxidant activity; DFT study

1. Introduction

Cancer is the second leading cause of death globally, and the contribution of cancer disease to the overall mortality rate is increasing. Economically, the total annual cost of cancer in 2010 was estimated at approximately US$ 1.16 trillion [1]. So that, more rational design, synthesis, and evaluation of new compounds as anticancer, with higher efficiency is considered as urgent mission in the medicinal chemistry field.

Quinazoline and quinazolinone derivatives are considered as tremendous targets for the medicinal chemists, due to the fact that they are the scaffold of different potent anticancer drugs, such as Gefitinib (trade name Iressa®), Erlotinib (trade name Tarceva®) [2–4], Methaqualone [5], Afloqualone (as anticonvulsant activity) [6,7], Chloroqualone (as antitussive), and Diproqualone (as sedative-hypnotic agents) [8] (Figure 1).
Dictyophora indusiata acetic anhydride to give 2-pentyl-4H-benzo[\(d\)][1,3]oxazin-4-one 4 \[39,40\] (Scheme 1). Figure 1. Some structures of synthetic drugs scaffold quinazoline and quinazolinone derivatives.

In the last decades and until now, various compounds including quinazolinone moiety conspicuously exhibited broad spectrum in numerous pharmacological activities such as antitumour [9–14], anticonvulsants [15], antiproliferative [16], anti-inflammatory [17], antihypertensive [18], antifungal [19], antibacterial, antioxidant [20], antimicrobial [21], anti-allergic [22], antimalarial [23], antileishmanial [24], and treatment of Alzheimer’s disease (AD) [25].

Generally, the natural products are considered as one of the most interesting sources of biologically active compounds. Among them, naturally occurring quinazolin-4(3H)-one derivatives, which can be isolated from various plants and microorganisms such as Luotonin A (sources; \(Peganum nigellastrum\)) [26], 2-(heptan-3-yl)quinazolinone (sources; \(Bacillus cereus\)) [27], Dictyoquinazol A (sources; \(Dictyophora indusiata\)) [28], and Echinozolinone (sources; \(Echinops echinatus\)) [29] (Figure 2).

Quinazolinones have been synthesized by different methodologies [28,30–37], in the present study, the conventional methodology to construct novel quinazolinone compounds has been adopted, followed by the study of the antiproliferative activity, antioxidant activity, and DFT calculations for the synthesized compounds.

2. Results and Discussion

2.1. Chemistry

In this interesting work, curing of anthranilic acid 1 with hexanoyl chloride 2 in dry pyridine afforded the corresponding N-hexanoyl derivative 3 [38], which was cyclized by heating in distilled acetic anhydride to give 2-pentyl-4H-benzo[\(d\)][1,3]oxazin-4-one 4 [39,40] (Scheme 1).

Figure 2. Some structures of naturally occurring quinazolin-4(3H)-one derivatives.
Benzoxadinone derivative 4 was utilized in situ as a precursor to construct new quinazolinone derivatives. For instance, reaction of benzoxadinone derivative 4 with formamide afforded 2-pentylquinazolin-4(3H)-one 5 [41] (Scheme 2). The $^1$H NMR spectrum of 5 exhibited a singlet peak at 12.13 ppm exchangeable with D$_2$O corresponding to NH proton, two doublet and two triplet peaks in the aromatic region at 8.05–7.42 ppm corresponding to four aromatic protons, and four characteristic peaks upfield at 2.56–0.84 ppm for n-pentyl protons.

Afterwards, sulfuration of 2-pentylquinazolin-4(3H)-one 5 by utilizing of phosphorus pentasulfide in dry toluene afforded 2-pentylquinazoline-4(3H)-thione 6 (Scheme 2). The formation of compound 6 was unambiguously elaborated by the presence of intense band at 1236 cm$^{-1}$ corresponding to OCC=S and the absence of the stretching band of OCC=O in the IR spectrum. On the other hand, the incorporation of β-ν-glucose pentaacetate with quinazolinone derivative 5 at the nitrogen atom of the later awarded N-(β-ν-glucopyranosyl-2,3,4,6-tetraacetate)-2-pentyl quinazolin-4(3H)-one 7 (Scheme 2), via attacking of the lone pair of nitrogen atom of quinazolinone derivative 5 at the anemic carbon (C$_1$) of β-ν-glucose pentaacetate, followed by ring opening and then ring closure with expulsion of acetate as a leaving group.

The chemical structure of compound 7 was explained by the IR spectrum, whereas it showed a band at 1746 cm$^{-1}$ compatible with νC=O of the acetate groups and lacked the absorption band for the NH group. Moreover, this structure was also interpreted by the $^1$H-NMR spectrum which revealed seven signals at 5.92–3.51 ppm and four singlet signals at 1.97–1.91 ppm all of them corresponding to the protons of β-ν-glucopyranosyl-2,3,4,6-tetraacetate moiety.

Curing of the benzoxadinone derivative 4 with ethanolamine under reflux for 3 h afforded 3-(2-hydroxyethyl)-2-pentylquinazolin-4(3H)-one 8 as the sole product. The IR spectrum of compound 8 showed a broad band at 3395 cm$^{-1}$ corresponding to OH functionality. Furthermore, the $^1$H-NMR spectrum appreciably emerged a triplet peak at 4.95 ppm exchangeable with D$_2$O corresponding to OH proton, triplet, and quartet peaks at 4.11 and 3.65 ppm, respectively, compatible with ethyl protons of 2-hydroxyethyl moiety. As well, the $^{13}$C-NMR spectrum exhibited two peaks at 58.8 and 46.1 ppm corresponding to the two carbons of 2-hydroxyethyl moiety.

3-Amino-2-pentylquinazolin-4(3H)-one 9 was commenced by refluxing of compound 4 with hydrazine monohydrate in absolute ethanol for 4 h (Scheme 2). The formation of compound 9 was confirmed by spectroscopic and elemental data. In particular, the $^1$H-NMR spectrum of compound 9 manifested a singlet signal commutable in D$_2$O at 5.70 ppm corresponding to NH$_2$ protons.

Scheme 2. Synthetic route to compounds 5–9.
Reaction of 3-amino-2-pentyquinazolin-4(3H)-one 9 with various aldehydes 10a–d gave Schiff bases 11a–d as the sole product in each case (Scheme 3). The $^1$H-NMR spectra of compounds 11a–d exhibited the appearance of a singlet signal in the region between 8.81–8.69 ppm compatible with methine proton of N=CH group.

The thiazolidin-4-one moiety 12 was constructed by the reaction of Schiff base 11a with methyl thioglycolate in absolute ethanol including a small amount of piperidine as a catalyst for 3 h (Scheme 3). The prospective structure 12 is in keeping with its spectral and elemental analyses.

Additionally, the nucleophilicity of the amino group of compound 9 was also estimated by fusion of it with 4,5,6,7-tetrachloroisobenzofuran-1,3-dione in oil bath for an hour and that afforded phthalimido derivative 13 in an excellent yield (Scheme 3). The foreseeable structure of compound 13 was elucidated by their spectral data and elemental analyses. Obviously, its IR spectrum showed stretching absorption bands at 1788, and 1746 cm$^{-1}$ corresponding to the carbonyl groups of phthalimido moiety and at 1707 cm$^{-1}$ corresponding to carbonyl group of the quinazolinoine moiety. The $^1$H-NMR spectrum exhibited four peaks for four aromatic protons and another four peaks for n-pentyl protons. Furthermore, its $^{13}$C-NMR spectrum emerged variant peaks, all of them fit with the proposed structure.

Eventually, the thione derivative 14 was obtained via sulfuration of compound 9 by utilizing phosphorus pentasulfide as the above pervious method (Scheme 3). The structure of 14 was unequivocally explained via the existence of a peak in the $^{13}$C NMR spectrum at 182.1 ppm compatible with the carbon of the thione functional group.

Scheme 3. Synthetic route to compounds 11–14.
2.2. Biological Evaluation

2.2.1. Antiproliferative Screening

Twelve compounds possessing quinazolinone(thione) moieties 5–14 along with compound 3 were screened against two cell lines, namely hepatocellular carcinoma (HepG2) and mammary gland (MCF-7) in vitro by utilizing MTT assay [42,43]. The latter assay is a colorimetric test based on the change of the yellow MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells and the Doxorubicin (DOX) was used as a standard reference.

The results listed in Table 1 and illustrated in Figure 3, demonstrate that compounds 6 and 11d have a very strong efficacy against HePG2 cell line with IC$_{50}$ values at 5.20 ± 0.5 and 7.63 ± 0.6 µM, respectively. Meanwhile, compounds 6, 11d, and 14 have a very strong efficacy against the MCF-7 cell line with IC$_{50}$ values at 6.88 ± 0.4, 8.60 ± 0.7, and 10.78 ± 0.9 µM, respectively. Compounds 11b, 11c have a strong efficacy against both cell lines with IC$_{50}$ values in the range (12.54 ± 1.1–19.68 ± 1.6 µM). For the HePG2 cell line, compounds 5, 9, 11a, and 14 have a moderate efficacy with IC$_{50}$ values in the range (23.75 ± 1.9–41.92 ± 2.8 µM). Where, for the MCF-7 cell line, compounds 3, 5, 9, and 11a have a moderate efficacy with IC$_{50}$ in the range (21.98 ± 1.8–47.53 ± 2.9 µM). Ultimately, the remaining compounds in both cases have weak efficacies with IC$_{50}$ values > 50 µM.

Table 1. Cytotoxic efficacy of thirteen compounds against hepatocellular carcinoma (HePG2) and mammary gland (MCF-7) cell lines.

| Compounds | HePG2 (µM) | MCF-7 (µM) |
|-----------|------------|------------|
| DOX       | 4.50 ± 0.3 | 4.17 ± 0.2 |
| 3         | 52.01 ± 3.3 | 47.53 ± 2.9 |
| 5         | 23.75 ± 1.9 | 21.98 ± 1.8 |
| 6         | 5.20 ± 0.5 | 6.88 ± 0.4 |
| 7         | 66.84 ± 4.0 | 54.23 ± 3.2 |
| 8         | 85.55 ± 4.5 | 70.86 ± 4.1 |
| 9         | 33.27 ± 2.5 | 28.69 ± 2.2 |
| 11a       | 41.92 ± 2.8 | 33.39 ± 2.5 |
| 11b       | 17.27 ± 1.4 | 19.68 ± 1.6 |
| 11c       | 12.54 ± 1.1 | 14.10 ± 1.3 |
| 11d       | 7.63 ± 0.6 | 8.60 ± 0.7 |
| 12        | 76.14 ± 4.3 | 59.15 ± 3.8 |
| 13        | 60.34 ± 3.8 | 56.28 ± 3.5 |
| 14        | 27.39 ± 2.3 | 10.78 ± 0.9 |
Figure 3. Cytotoxic efficacy of thirteen compounds against HePG2 and MCF-7 cell lines.

Through our screening of the antiproliferative efficacy of the synthesized compounds, it was determined that the average of relative viability of cells (%) with different concentrations such as 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 μM against two cell lines (HePG2 and MCF-7) as shown in Figures 4 and 5.

Figure 4. Average of relative viability of HePG-2 cell line (%) with different concentrations.
with variable potencies according to the following sequence: 3,5-(OMe)-4-OH-C₆H₄ 11d > 3-OH-4-(OMe)-C₆H₄ 11c > 4-(OMe)-C₆H₄ 11b > 4-Cl-C₆H₄ 11a, whereas, the OH group in p-position is more electron donating group than the OMe group and Cl atom (i.e., the delocalization of n-π electrons in the above sequence).

4. Construction of the thiazolidinone ring in compound 12 decreased the antiproliferative activity comparable with the hydrazone derivative 11a, due to decreasing of the delocalization of n-π electrons after replacement of the C=N group (electron attracting group) by the thiazolidinone ring.

2.2.2. Antioxidant Activity Screening

One of the aims of this work is the screening of all synthesized compounds for antioxidant activity using two different methods, namely ABTS [2,2’-azino-bis(3-ethyl benzothiazoline-6-sulfonic acid)] and DPPH assays. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay based on electron-transfer that produces a violet solution in ethanol. This free radical is stable at ambient temperature and reduced in the presence of an antioxidant molecule, leading to colorless ethanol solution. After investigation of these results as listed in Table 2 and Figure 6, it was realized that, compounds 6 and 11d have promising activity through using ABTS assay. Meanwhile, in the case of using DPPH assay, compounds 6, 11d and 14 have also very high activity. Ascorbic acid was used as a reference through the antioxidant activity screening.

The results depicted in Table 2 and Figure 6 demonstrated that, DPPH assay findings are very approximately related to those of ABTS assay with only one exception, compound 14 has an excellent antioxidant activity against DPPH (IC₅₀ = 26.87 ± 0.23 μM) than that of the ABTS method (IC₅₀ = 71.42 ± 0.52 μM). Noteworthy, all the screened compounds in the case of the DPPH method exhibited IC₅₀...
smaller than the corresponding ones of the same compounds in the case of the ABTS method, and it
proposed that these compounds are more promising scavengers of the DPPH radical than those of the
ABTS radical.

By comparing the antioxidant efficacy of the thirteen synthesized compounds in this study to their
chemical structures, it was concluded that the following structure antioxidant activity relationship’s
(SAR’s) is hypothesized:

1. The presence of C=S enhanced antioxidant activity than the presence of C=O, as shown in
   compounds 6 and 14 comparable with compounds 5 and 9, respectively.

2. The hydrazone derivatives (11a-d) analogs have variable potencies according to the following
   sequence: 3,5-(OMe)2-4-OH-C6H2 11d > 3-OH-4-(OMe)-C6H3 11c > 4-(OMe)-C6H4 11b > 4-Cl-C6H4
   11a, whereas, OH group in p-position is a more electron donating group (has more conjugated system)
   than OMe group and Cl atom.

3. In compound 12, replacement of C=N group by the thiazolidinone ring decreased the antioxidant
   activity comparable with 11a, because of the lack of the conjugated system.

Table 2. Antioxidant activities of all synthesized compounds by using 2,2’-azino-bis(3-ethyl
benzothiazoline-6-sulfonic acid (ABTS) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) methods.

| Compounds | ABTS IC50 (µM) | DPPH IC50 (µM) |
|-----------|----------------|----------------|
| Ascorbic acid | 31.26 ± 0.20 | 16.83 ± 0.15 |
| 3 | 93.24 ± 0.70 | 52.14 ± 0.65 |
| 5 | 65.13 ± 0.46 | 41.16 ± 0.42 |
| 6 | 35.48 ± 0.23 | 19.25 ± 0.18 |
| 7 | 114.63 ± 0.73 | 59.43 ± 0.68 |
| 8 | 160.92 ± 0.94 | 92.36 ± 0.76 |
| 9 | 77.38 ± 0.56 | 46.23 ± 0.48 |
| 11a | 89.10 ± 0.64 | 50.12 ± 0.54 |
| 11b | 58.03 ± 0.41 | 38.47 ± 0.37 |
| 11c | 52.87 ± 0.36 | 32.69 ± 0.29 |
| 11d | 46.25 ± 0.28 | 22.91 ± 0.21 |
| 12 | 154.51 ± 0.85 | 73.03 ± 0.72 |
| 13 | 131.95 ± 0.77 | 65.58 ± 0.66 |
| 14 | 71.42 ± 0.52 | 26.87 ± 0.23 |

Figure 6. Antioxidant activities of all synthesized compounds by using ABTS and DPPH methods.
Previous reports of structurally similar compounds (in quinazoline ring) but with different substituents have demonstrated different results in antiproliferative and antioxidant activities from our results [9,33,44].

2.3. Density Functional Theory

According to the frontier molecular orbital (FMO) theory, the highest occupied molecular orbital (HOMO) acts as an electron-donor and the lowest unoccupied molecular orbital (LUMO) acts as an electron-acceptor [45]. Meanwhile, both play remarkable roles in the electronic studies by using quantum chemical calculations and they are also of significant importance in modern biochemistry and molecular biology [46]. A molecule is considered as a softer and has an excellent chemical reactivity when it has a smaller energy gap. Meanwhile, a molecule is considered to have a higher chemical hardness and assumed to have good stability when it has a larger energy gap [47–51].

The quantum chemical calculations were implemented by the density functional theory (DFT) method by using the Gaussian(R) 09 program at the B3LYP level in conjunction with 6-31G(d,p) basis set and computed parameters are summarized in Table 3.

By computing and using the energy gap \( \Delta E = E_{\text{LUMO}} - E_{\text{HOMO}} \) and dipole moment values beside another quantum chemical parameters such as ionization energy \( (I = -E_{\text{HOMO}}) \), electron affinity \( (A = -E_{\text{LUMO}}) \) [52], chemical hardness \( (\eta = 1/\mu) \) [53], and binding energy, we can rationally explicate the relation between the chemical structure and the antiproliferative activity (SAR’s). Whereas, the energy gap of compound 6 \( (\Delta E = 3.98 \text{ eV}) \) is smaller than that corresponding of compound 5 \( (\Delta E = 4.85 \text{ eV}) \) and also compound 6 has a higher chemical softness value \( (\mu = 0.50 \text{ eV}^{-1}) \) than that of compound 5 \( (\mu = 0.41 \text{ eV}^{-1}) \). These results are matching with the results of the antiproliferative screening whereas, compound 6 has a higher potency comparable with compound 5 for both cell lines (HepG2 and MCF-7) as shown in Table 1 and Figure 3. Similarly, compound 14 has a smaller energy gap and a higher chemical softness than that corresponding of compound 9 as shown in Table 1 and Figure 3. Notably, the dipole moment values of compounds 6 \( (\mu = 3.4641 \text{ D}) \) and 14 \( (\mu = 1.852 \text{ D}) \) are higher than that of compounds 5 \( (\mu = 3.2867 \text{ D}) \) and 9 \( (\mu = 1.764 \text{ D}) \), respectively.

On the other hand, compounds 11a-d possess antiproliferative activity in the following order 11d > 11c > 11b > 11a, meanwhile, the energy gaps of these compounds increase in the following order 11a \( (\Delta E = 2.99 \text{ eV}) \) < 11d \( (\Delta E = 3.05 \text{ eV}) \) < 11c \( (\Delta E = 3.10 \text{ eV}) \) < 11b \( (\Delta E = 3.13 \text{ eV}) \). The lower of the antiproliferative activity of compound 11a may be explained by values of the dipole moment whereas; the dipole moment of compound 11a is smaller than that of compounds 11b-d as shown in Table 3.

### Table 3. Quantum chemical parameters of the selected compounds with Density Functional Theory (DFT) at B3LYP/6-31G (d,p) basis set.

| Comp. No. | 5    | 6    | 9    | 14   | 11a  | 11b  | 11c  | 11d  |
|-----------|------|------|------|------|------|------|------|------|
| \( E_{\text{HOMO}} \) (eV) | -5.9432 | -5.6352 | -5.9405 | -4.7724 | -5.7277 | -5.6363 | -5.6717 | -5.6602 |
| \( E_{\text{LUMO}} \) (eV) | -1.0917 | -1.6515 | -1.1298 | -1.6836 | -2.7301 | -2.4983 | -2.5709 | -2.6009 |
| \( \Delta E \) Energy gap (eV) | 4.8515 | 3.9387 | 4.8107 | 3.0888 | 2.9976 | 3.3130 | 3.1008 | 3.0593 |
| \( I \) Ionization energy (eV) | 5.9432 | 5.6352 | 5.9405 | 4.7724 | 5.7277 | 5.6363 | 5.6717 | 5.6602 |
| \( A \) Electron affinity (eV) | 1.0917 | 1.6515 | 1.1298 | 1.6836 | 2.7301 | 2.4983 | 2.5709 | 2.6009 |
| \( \eta \) Chemical hardness (eV) | 2.4258 | 1.9919 | 2.4054 | 1.5444 | 1.4988 | 1.5690 | 1.5504 | 1.52969 |
| \( S \) Chemical softness (eV\(^{-1}\)) | 0.4122 | 0.5020 | 0.4157 | 0.6475 | 0.6672 | 0.6374 | 0.6450 | 0.6537 |
| \( E_f \) Binding energy (kcal/mol) | -689.79 | -1012.73 | -745.11 | -1068.05 | -1473.81 | -1128.73 | -1203.94 | -1318.44 |
| \( \mu \) Dipole moment (D) | 3.2867 | 3.4641 | 1.764 | 1.852 | 3.2722 | 4.4472 | 4.1944 | 6.232 |

The distributions of the HOMO and LUMO orbitals of the selected compounds are computed at the same level of the DFT theory and are provided in Figures 7 and 8. The results manifested that possible reactive sites exist as shown below:
1. The HOMO of compounds 5 and 9 are nearly similar and the distribution of orbitals are mainly situated on the quinazolinone moiety, also, the LUMO of these compounds are situated on the same moiety.

2. The HOMO of compounds 6 and 14 are nearly similar and the distribution of orbitals are mainly situated on C=S, while, the LUMO of these compounds are mainly situated on the quinazolinthione moiety.

3. The HOMO of compounds 11a–d are nearly similar and the distribution of orbitals are mainly situated on the quinazolinonone moiety, meanwhile, the LUMO of these compounds are mainly situated on the aryl aldehyde hydrazone system.

![Figure 7. Schematic representation of HOMO and LUMO coefficient distribution of compounds 5, 6, 9, and 14.](image)

![Figure 8. Schematic representation of highest occupied molecular orbital (HOMO) and least unoccupied molecular orbital (LUMO) coefficient distribution of compounds 11a–d.](image)

### 3. Materials and Methods

#### 3.1. Chemistry

The melting point is uncorrected and was measured on a Stuart SMP 30 advanced digital electric melting point apparatus (Cole-Parmer, Staffordshire, UK). All reactions were monitored by TLC (Kieselgel 60 F254, Merck, Munchen, Germany) and spots were visualized using UV (254 nm). In the region (400–4000 cm⁻¹), the IR spectrum was measured in the KBr phase by using the Nicolet iS10 FT-IR spectrometer (Shimadzu Corporation, Kyoto, Japan). The ¹H-NMR (at 400 MHz) and ¹³C-NMR (at 100 MHz) spectra were performed at chemical warfare labs, Egypt, with a Varian Gemini spectrometer (Metrohim, California, United States) in DMSO-d₆ as a solvent by using tetramethylsilane (TMS) as a reference. Perkin-Elmer 2400 CHN elemental analyzer (Waltham, MA, USA) was used to
record CHN elemental analysis at the Faculty of Science, Cairo University, Egypt. The mass spectrum was measured on Shimadzu GC-MS QP1000EX apparatus (Shimadzu Corporation, Kyoto, Japan) at the central analytical lab, Ain Shams University, Cairo, Egypt.

3.1.1. 2-Hexanamidobenoic Acid 3

Hexanoyl chloride 2 (1.39 mL, 0.01 mol) was added dropwise to anthranilic acid 1 (1.37 g, 0.01 mol) dissolved in dry pyridine (20 mL) at ambient temperature with stirring. The stirring was continued for an hour, and then the resulting emulsion was acidified with cold 10% HCl solution. The white solid which separated was collected by filtration and then recrystallized from benzene to give 3 [38] as white crystals; m.p.: 92–95 °C (Lit. m.p.: 93–95 °C) [38], yield: 92%. IR (KBr, cm⁻¹): 3426–2463 (br) (OH), 3206 (NH), 2959, 2934, 2861 (CH₃₈); 13.51 (brs, 1H, OH, exchangeable with D₂O), 11.09 (s, 1H, NH, exchangeable with D₂O), 8.48 (d, 1H, Ar-H, H₈, J = 8.8 Hz), 7.95 (d, 1H, Ar-H, H₈, J = 8.8 Hz), 7.55 (t, 1H, Ar-H, H₈, J = 7.8 Hz, J = 8.0 Hz), 7.11 (t, 1H, Ar-H, H₈, J = 7.4 Hz, J = 7.8 Hz), 2.35 (t, 2H, COCH₂, J = 7.2 Hz, J = 7.6 Hz), 1.60 (quintet, 2H, COCH₂CH₂, J = 7.2 Hz, J = 7.6 Hz), 1.31–1.26 (m, 4H, 4H, CH₃CH₂CH₂), 0.85 (t, 3H, CH₃, J = 6.8 Hz, J = 7.2 Hz), MS m/z (%): 235 (M⁺; 29.4). Anal. Calcd. for C₁₃H₁₇NO₃ (235.28): C, 66.36; H, 7.28; N, 5.95. Found: C, 66.36; H, 7.28; N, 5.95.

3.1.2. 2-Pentyl-4H-benzo[d][1,3]oxazin-4-one 4

A suspension of 2-hexanamidobenoic acid 3 (2.35 g, 0.01 mol) in freshly distilled acetic anhydride (10 mL) was heated in water bath for an hour followed by a concentration of the mixture in vacuo and used in situ [39,40].

3.1.3. 2-Pentylquinazolin-4(3H)-one 5

A solution of benzoaxazinone 4 (2.17 g, 0.01 mol) in formamide (15 mL) was refluxed for 7 h. After cooling, the reaction mixture was poured onto ice cold water, the obtained solid was filtered off, dried, and recrystallized from petroleum ether 60–80 °C to give 5 [41] as white crystals; m.p.: 142–144 °C (Lit. m.p.: 153–154 °C) [41], yield: 92%. IR (KBr, cm⁻¹): 3181 (NH), 2958, 2928, 2860 (CH₃₈), 1680 (C=O), 1614 (C=N or C=C). 1H-NMR (400 MHz, DMSO-d₆) δ (ppm): 12.13 (s, 1H, NH, exchangeable with D₂O), 8.05 (d, 1H, Ar-H, H₈, J = 8.0 Hz), 7.74 (t, 1H, Ar-H, H₈, J = 7.6 Hz, J = 7.8 Hz), 7.56 (d, 1H, Ar-H, H₈, J = 7.8 Hz), 7.42 (t, 1H, Ar-H, H₈, J = 7.6 Hz), 2.56 (t, 2H, N=CH₂, J = 7.6 Hz, J = 8.0 Hz), 1.70 (quintet, 2H, N=CH₂CH₂, J = 7.6 Hz, J = 7.2 Hz), 1.30–1.26 (m, 4H, CH₃CH₂CH₂), 0.84 (t, 3H, CH₃, J = 6.8 Hz, J = 7.2 Hz). 13C-NMR (100 MHz, DMSO-d₆) δ (ppm): 162.2, 157.9, 149.4, 134.7, 127.2, 126.3, 126.1, 121.2, 34.9, 31.1, 26.9, 22.2, 14.2. MS m/z (%): 216 (M⁺; 26.3). Anal. Calcd. for C₁₃H₁₆N₂O (216.28): C, 72.19; H, 7.46; N, 12.95. Found: C, 72.26; H, 7.49; N, 12.86.

3.1.4. 2-Pentylquinazoline-4(3H)-thione 6

To a solution of quinazolinone 5 (2.16 g, 0.01 mol) in dry toluene (30 mL), P₂S₅ (2.22 g, 0.01 mol) was added. The reaction mixture was refluxed for 1 h, and then filtered off. The obtained filtrate was evaporated under reduced pressure, the formed solid was collected by filtration, dried, and recrystallized from ethanol to give 6 as light brown crystals; m.p.: 101–103 °C, yield: 76%. IR (KBr, cm⁻¹): 3184, 3141 (NH), 2967, 2935, 2852 (CH₃₈), 1618 (C=N), 1604 (C=C), 1236 (C=S). 1H-NMR (400 MHz, DMSO-d₆) δ (ppm): 13.69 (s, 1H, NH, exchangeable with D₂O), 8.52 (d, 1H, Ar-H, H₈, J = 8.4 Hz), 7.83 (t, 1H, Ar-H, H₈, J = 7.6 Hz), 7.63 (d, 1H, Ar-H, H₈, J = 8.4 Hz), 7.52 (d, 1H, Ar-H, H₈, J = 7.4 Hz, J = 7.8 Hz), 2.70 (t, 2H, N=CH₂, J = 7.6 Hz, J = 8.0 Hz), 1.72 (quintet, 2H, N=CH₂CH₂, J = 7.6 Hz, J = 7.2 Hz), 1.31–1.26 (m, 4H, CH₃CH₂CH₂), 0.85 (t, 3H, CH₃, J = 6.8 Hz). MS m/z (%): 232 (M⁺; 16.7). Anal. Calcd. for C₁₃H₁₆N₂S (232.35): C, 67.20; H, 6.94; N, 12.06; S, 13.80. Found: C, 67.31; H, 7.03; N, 12.01; S, 13.85.
3.1.5. N-(β-D-Glucopyranosyl-2,3,4,6-tetraacetate)-2-pentylquinazolin-4(3H)-one 7

Quinazolinone 5 (2.16 g, 0.01 mol) was refluxed with β-D-glucose pentaacetate (3.90 g, 0.01 mol) in absolute ethanol (50 mL) for 3 h. The solid obtained after slow evaporation of the resulting solution was collected and recrystallized from ethanol to give 7 as white crystals; m.p.: 135–137 °C, yield: 62%. IR (KBr, cm⁻¹): 2955, 2924, 2854 (CH₃, CH₂), 1746 (C=O, ester), 1678 (C=O, quinazolinone), 1613 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 8.05 (d, 1H, Ar-H, H₆, J = 8.0 Hz), 7.74 (t, 1H, Ar-H, H₄, J = 8.4 Hz, J = 6.8 Hz), 7.56 (d, 1H, Ar-H, H₄, J = 8.4 Hz), 7.43 (t, 1H, Ar-H, H₆, J = 8.0 Hz, J = 7.2 Hz), 5.92 (d, 1H, C₁-H, J = 8.4 Hz), 5.39 (t, 1H, C₂-H, J = 9.6 Hz), 4.93 (t, 1H, C₃-H, J = 9.6 Hz), 4.90 (t, 1H, C₄-H, J = 8.4 Hz, J = 10.0 Hz), 4.14, 412 (d, d, 1H, C₆-H, J = 10.4 Hz, J = 5.6 Hz), 3.97 (d, 1H, C₅-H, J = 10.4 Hz), 3.51 (m, 1H, C₃-H), 2.56 (t, 2H, N=CCH₂, J = 8.0 Hz, J = 7.6 Hz), 1.978 (s, 3H, CH₃), 1.973 (s, 3H, CH₃), 1.961 (s, 3H, CH₃), 1.917 (s, 3H, CH₃), 1.69 (q, 2H, N=CCH₂CH₂, J = 7.6 Hz, J = 6.8 Hz), 1.31–1.26 (m, 4H, CH₃CH₂CH₂), 0.84 (t, 3H, CH₃, J = 6.8 Hz, J = 7.2 Hz). MS m/z (%): 546 (M⁺; 32.4). Anal. Calcd. for C₂₇H₂₄N₂O₁₀ (546.57): C, 59.33; H, 6.27; N, 5.13. Found: C, 59.18; H, 6.21; N, 5.08.

3.1.6. 3-(2-Hydroxyethyl)-2-pentylquinazolin-4(3H)-one 8

A solution of benzoxazinone 4 (2.17 g, 0.01 mol) in ethanolamine (15 mL) was heated under reflux for 3 h. The reaction mixture was poured onto ice cold water, the obtained solid was filtered off, dried, and then recrystallized from ethanol to give 8 as white crystals; m.p.: 84–85 °C, yield: 47%. IR (KBr, cm⁻¹): 3395 (OH), 2953, 2931, 2872 (CH₃, CH₂), 1648 (C=O), 1611 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 8.07 (d, 1H, Ar-H, H₆, J = 8.0 Hz), 7.75 (t, 1H, Ar-H, H₄, J = 7.8 Hz, J = 7.6 Hz), 7.57 (d, 1H, Ar-H, H₄, J = 8.0 Hz), 7.44 (t, 1H, Ar-H, H₆, J = 7.6 Hz, J = 7.2 Hz), 4.95 (t, 1H, OH, exchangeable with D₂O, J = 5.6 Hz), 4.11 (t, 2H, CH₂CH₂OH, J = 5.6 Hz, J = 6.0 Hz), 3.65 (q, 2H, CH₂OH, J = 6.0 Hz, J = 5.6 Hz), 2.93 (t, 2H, N=CCH₂, J = 7.6 Hz, J = 8.0 Hz), 1.75 (q, 2H, N=CCH₂CH₂, J = 7.6 Hz, J = 7.6 Hz), 1.40–1.32 (m, 4H, CH₃CH₂CH₂), 0.88 (t, 3H, CH₃, J = 7.2 Hz). ¹³C-NMR (100 MHz, DMSO-d₆) δ (ppm): 161.7, 158.4, 147.4, 134.6, 127.1, 126.5, 126.4, 120.4, 58.8, 46.1, 34.6, 31.3, 26.3, 24.4, 14.3. MS m/z (%): 260 (M⁺; 41.2). Anal. Calcd. for C₁₅H₂₀N₂O₂ (260.34): C, 69.20; H, 7.74; N, 10.76. Found: C, 69.17; H, 7.68; N, 10.81.

3.1.7. 3-Amino-2-pentylquinazolin-4(3H)-one 9

A mixture of benzoxazinone 4 (2.17 g, 0.01 mol) and hydrazine hydrate (1.5 mL) in absolute ethanol (20 mL) was refluxed for 3 h. The mixture was poured onto ice cold water, the formed solid was filtered off, and recrystallized from ethanol to give 9 as buff crystals; m.p.: 58–60 °C, yield: 43%. IR (KBr, cm⁻¹): 3306, 3263 (NH₂), 2954, 2931, 2910, 2856 (CH₃, CH₂), 1673 (C=O), 1630 (C=N). ¹H NMR (400 MHz, DMSO-d₆) δ (ppm): 8.08 (d, 1H, Ar-H, H₆, J = 7.8 Hz), 7.57 (t, 1H, Ar-H, H₄, J = 7.4 Hz, J = 7.6 Hz), 7.59 (d, 1H, Ar-H, H₄, J = 7.6 Hz, 7.45 (t, 1H, Ar-H, H₆, J = 7.2 Hz, J = 7.6 Hz), 5.70 (s, 2H, NH₂, exchangeable with D₂O), 2.90 (t, 2H, N=CCH₂, J = 7.2 Hz, J = 8.0 Hz), 1.74 (q, 2H, N=CCH₂CH₂, J = 7.6 Hz, J = 7.2 Hz), 1.37–1.30 (m, 4H, CH₃CH₂CH₂), 0.87 (t, 3H, CH₃, J = 6.4 Hz, J = 7.2 Hz). ¹³C-NMR (400 MHz, DMSO-d₆) δ (ppm): 160.9, 158.8, 147.0, 134.3, 127.2, 126.39, 126.31, 120.2, 34.0, 31.4, 26.0, 22.3, 14.3. MS m/z (%): 231 (M⁺; 41.1). Anal. Calcd. for C₁₃H₁₂N₃O (231.30): C, 67.51; H, 7.41; N, 18.17. Found: C, 67.39; H, 7.34; N, 18.24.

3.1.8. General Procedure for Synthesis of 11a-d

A mixture of compound 9 (2.31 g, 0.01 mol) and the appropriate aldehydes 10a-d (0.01 mol) in absolute ethanol (30 mL) was refluxed for 4–6 h. The reaction mixture was evaporated under reduced pressure; the obtained residue was collected and recrystallized from the proper solvent to give the corresponding benzylidene derivatives 11a-d, respectively.
3-((4-Chlorobenzylidene)amino)-2-pentylquinazolin-4(3H)-one 11a

Yellow crystals; m.p.: 176–178 °C (ethanol), yield: 72%. IR (KBr, cm⁻¹): 2946, 2866 (CH₃(aryl)), 1667 (C=O), 1624 (C=N). ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 8.69 (s, 1H, N=CH), 7.95 (d, 1H, Ar-H, Hₓ, J = 7.8 Hz), 7.88 (d, 2H, Ar-H, Hₓ + Hₜ, J = 8.8 Hz), 7.66 (t, 1H, Ar-H, Hₓ, J = 8.4 Hz), 7.57 (d, 3H, Ar-H, Hₓ + Hₜ), 7.46 (t, 1H, Ar-H, Hₓ, J = 8.4 Hz), 2.34 (2H, N=CH₂, J = 7.6 Hz), 1.60 (quintet, 2H, N=CH₂, J = 7.2 Hz), 1.31–1.26 (m, 4H, CH₂CH₂CH₂H₂), 0.85 (t, 3H, CH₃, J = 6.8 Hz). MS m/z (%): 353 (M⁺; 4₀). Anal. Calcd. for C₂₃H₂₂ClN₃O (353.88): C, 76.79; H, 5.70; Cl, 10.02; N, 11.88. Found: C, 76.78; H, 5.62; Cl, 9.89; N, 11.79.

3-((4-Methoxybenzylidene)amino)-2-pentylquinazolin-4(3H)-one 11b

White crystals; m.p.: 83–84 °C (petroleum ether 60–80 °C), yield: 64%. IR (KBr, cm⁻¹): 2946, 2912, 2882, 2843 (CH₃(aryl)), 1669 (C=O), 1606 (C=N or C=C). ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 8.81 (s, 1H, N=CH), 8.12 (d, 1H, Ar-H, Hₓ, J = 7.8 Hz), 7.89 (d, 2H, Ar-H, Hₓ + Hₜ, J = 8.8 Hz), 7.80 (t, 1H, Ar-H, Hₓ, J = 8.2, Hz, Ar-H, Hₓ, J = 7.4 Hz), 7.65 (d, 1H, Ar-H, Hₓ, J = 8.0 Hz), 7.50 (t, 1H, Ar-H, Hₓ, J = 7.6, Hz, J = 7.4 Hz), 7.12 (d, 2H, Ar-H, Hₓ + Hₜ, J = 8.8 Hz), 3.85 (s, 3H, OCH₃), 2.80 (t, 2H, N=CH₂, J = 7.6 Hz, J = 8.0 Hz), 1.71 (quintet, 2H, N=CH₂CH₂J = 7.6 Hz, J = 7.2 Hz), 1.33–1.26 (m, 4H, CH₂CH₂CH₂H₂), 0.81 (t, 3H, CH₃, J = 7.2 Hz). MS m/z (%): 349 (M⁺; 11). Anal. Calcd. for C₂₁H₂₂N₂O₂ (349.43): C, 72.18; H, 6.63; N, 12.03. Found: C, 72.29; H, 6.69; N, 11.88.

3-((3-Hydroxy-4-methoxybenzylidene)amino)-2-pentylquinazolin-4(3H)-one 11c

White crystals; m.p.: 150–152 °C (ethanol), yield: 57%. IR (KBr, cm⁻¹): 3277 (OH), 2956, 2927, 2892, 2863, 2845 (CH₃(aryl)), 1650 (C=O), 1603 (C=N or C=C). ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 9.49 (s, 1H, OH, exchangeable with D₂O), 8.70 (s, 1H, N=CH), 8.12 (d, 1H, Ar-H, Hₓ, J = 8.0 Hz), 7.79 (t, 1H, Ar-H, Hₓ, J = 8.0 Hz, J = 8.4 Hz), 7.65 (d, 1H, Ar-H, Hₓ, J = 7.6 Hz), 7.49 (t, 1H, Ar-H, Hₓ, J = 8.0 Hz, J = 7.2 Hz), 7.42 (d, 1H, Hₓ, Jₓ = 2 Hz), 7.30, 7.28 (d, 1H, Ar-H, Hₓ, J = 8.4 Hz, Jₓ = 2 Hz), 7.07 (d, 1H, Ar-H, Hₓ, J = 8.4 Hz), 3.85 (s, 3H, OCH₃), 2.78 (t, 2H, N=CH₂, J = 7.6 Hz), 1.71 (quintet, 2H, N=CH₂CH₂J = 7.6 Hz, J = 7.2 Hz), 1.31–1.28 (m, 4H, CH₂CH₂CH₂H₂), 0.81 (t, 3H, CH₃, J = 6.8 Hz, J = 7.2 Hz). MS m/z (%): 365 (M⁺; 23.4). Anal. Calcd. for C₂₁H₂₂N₂O₃ (365.43): C, 69.02; H, 6.34; N, 11.50. Found: C, 68.88; H, 6.28; N, 11.62.

3-((4-Hydroxy-3,5-dimethoxybenzylidene)amino)-2-pentylquinazolin-4(3H)-one 11d

White crystals; m.p.: 148–150 °C (benzene), yield: 61%. IR (KBr, cm⁻¹): 3408 (OH), 2952, 2911, 2844 (CH₃(aryl)), 1668 (C=O), 1591 (C=N or C=C). ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 9.36 (brs, 1H, OH, exchangeable with D₂O), 8.72 (s, 1H, N=CH), 8.12 (d, 1H, Ar-H, Hₓ, J = 8.2 Hz), 7.79 (t, 1H, Ar-H, Hₓ, J = 8.0 Hz, J = 7.4 Hz), 7.65 (d, 1H, Ar-H, Hₓ, J = 7.6 Hz), 7.50 (t, 1H, Ar-H, Hₓ, J = 8.0 Hz, J = 7.2 Hz), 7.23 (s, 2H, Hₓ + Hₜ), 3.82 (s, 6H, 2OCH₃), 2.81 (t, 2H, N=CH₂, J = 7.6 Hz, J = 8.0 Hz), 1.72 (quintet, 2H, N=CH₂CH₂J = 7.6 Hz, J = 7.2 Hz), 1.36–1.26 (m, 4H, CH₂CH₂CH₂H₂), 0.81 (t, 3H, CH₃, J = 6.8 Hz, J = 7.2 Hz). ¹³C-NMR (100 MHz, DMSO-d₆) δ (ppm): 169.8, 158.0, 156.5, 148.6 (2), 146.7, 140.8, 134.6, 127.4, 127.0, 126.7, 122.8, 121.3, 106.8 (2), 56.5 (2), 34.5, 31.3, 26.0, 22.2, 14.2. MS m/z (%): 395 (M⁺; 62.1). Anal. Calcd. for C₂₂H₂₅N₃O₄ (395.46): C, 66.82; H, 6.37; N, 10.63. Found: C, 66.95; H, 6.41; N, 10.58.

3.1.9. 2-(4-Chlorophenyl)-3-(4-oxo-2-pentylquinazolin-3(4H)-yl)thiazolidin-4-one 12

A mixture of compound 11a (3.53 g, 0.01 mol) and methyl thioglycolate (0.89 mL, 0.01 mol) in absolute ethanol (30 mL) containing piperidine (0.5 mL) was refluxed for 3 h. The obtained solid after evaporation of the solvent was collected and recrystallized from petroleum ether 60–80 °C to give 12 as pale yellow crystals; m.p.: 78–80 °C, yield: 47%. IR (KBr, cm⁻¹): 2951, 2925, 2868 (CH₃(aryl)), 1736 (C=Othiazolidinone), 1671 (C=Oquinazolinone), 1608 (C=NC or C=C). ¹H-NMR (100 MHz, DMSO-d₆) δ (ppm): 8.13 (d, 1H, Ar-H, Hₓ, J = 8.2 Hz), 7.96 (d, 2H, Ar-H, Hₓ + Hₜ, J = 8.0 Hz), 7.80 (t, 1H, Ar-H,
H, J = 7.6 Hz), 7.65 (d, 1H, Ar-H, H_d, J = 8.0 Hz), 7.64 (d, 2H, Ar-H, H_x + H_y, J = 8.4 Hz), 7.51 (t, 1H, Ar-H, H_b, J = 7.6 Hz, J = 7.8 Hz), 5.70 (s, 1H, SCH), 3.75, 3.67 (d,d, 2H, CH_2thiazolidine), J = 23.6 Hz, J = 23.2 Hz), 2.81 (t, 2H, N=C=CH_2, J = 8.0 Hz, J = 7.6 Hz), 1.71 (quintet, 2H, N=C=CH_2, J = 7.6 Hz, J = 8.0 Hz), 1.35–1.17 (m, 4H, CH_2CH_2CH_2), 0.80 (t, 3H, CH_3, J = 7.2 Hz). MS m/z (%): 427 (M^+; 11.8). Anal. Calcd. for C_{22}H_{22}ClN_3O_2S (427.95): C, 61.75; H, 5.18; Cl, 8.28; N, 9.82; S, 7.49. Found: C, 61.66; H, 5.12; Cl, 8.31; N, 9.75; S, 7.55.

3.1.10. 4,5,6,7-Tetrahydro-2-(4-oxo-2-pentylquinazolin-3(4H)-yl)isoindoline-1,3-dione 13

Compound 9 (2.31 g, 0.01 mol) was fused with 4,5,6,7-tetrahydroisobenzofuran-1,3-dione (2.85 g, 0.01 mol) in oil bath for an hour. The resulting solid was recrystallized from ethanol to give 13 as orange crystals; m.p.: 178–180 °C, yield: 86%. IR (KBr, cm⁻¹): 2943, 2856 (CH_3), 1788, 1746 (C=O, C=O). Anal. Calcd. for C_{16}H_{16}O_4 (247.36): C, 63.12; H, 6.93; N, 16.99; S, 12.96. Found: C, 63.19; H, 6.96; N, 17.11; S, 12.82.

3.1.11. 3-Amino-2-pentylquinazoline-4(3H)-thione 14

A mixture of compound 9 (2.31 g, 0.01 mol) and P_2S_5 (2.22 g, 0.01 mol) in dry toluene (15 mL) was heated under reflux for 4 h. The mixture was filtered off, the filtrate was evaporated under reduced pressure, the obtained solid was collected, dried, and recrystallized from ethanol to give 14 as yellow crystals; m.p.: 57–59 °C, yield: 53%. IR (KBr, cm⁻¹): 3240, 3200 (NH), 2925, 2855 (CH_3), 1591 (C=N or C=C), 1238 (C=S), 1282 (C=S), 962 (C=O), 762 (C=O). Anal. Calcd. for C_{16}H_{16}S (224.32): C, 58.8; H, 4.5; S, 36.7. Found: C, 58.7; H, 4.5; S, 36.6.

3.2. Cytotoxicity and Antiproliferative Evaluation

MTT Assay

The implement of MTT methodology for the antiproliferative screening of quinazolinone derivatives 5–14 along with compound 3 against two cell lines, namely, hepatocellular carcinoma (HepG2) and mammary gland (MCF-7) were obtained from ATCC through the Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. The reference anticancer drug used was Doxorubicin. The MTT assay was carried out at the pharmacology department, Faculty of pharmacy, Mansoura University, Egypt according to the reported literatures [42,43,54]. The cells were cultured in a RPMI-1640 medium with 10% fetal bovine serum, followed by the addition of antibiotics (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 × 10^4 cells/well) at 37 °C for 48 h under 5% CO_2. Treatment of cells with different concentrations of compounds such as 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 µM was carried out and placed in the incubator for 24 h. Then, 20 µL of MTT solution at 5 mg/mL was added and incubated for 4 h. DMSO (100 µL) was added into each well to dissolve the purple formazan formed. At 570 nm absorbance the colorimetric assay was measured and recorded by using a plate reader (BioTek EL x800 Microplate Reader, BioTek Instruments, Inc, Winooski, VT, USA).

Calculation of the relative cell viability (%) = (A of treated samples /A of untreated sample) × 100.
3.3. Antioxidant Assay

3.3.1. Antioxidant Activity Screening Assay

**ABTS Method**

By the bleaching of ABTS derived radical cations, the detections of antioxidant activities were estimated. The radical cation was prepared by the reaction of ABTS \([2,2’-\text{azino-bis(3-ethyl benzothiazoline-6-sulfonic acid)}]\) (60 µL) with MnO\(_2\) (3 mL, 25 mg/mL) in a phosphate buffer solution (10 µM, pH 7.5 mL). The solution was shaken for 3 min, centrifuged, filtered, and recorded at \(\lambda_{\text{max}}\) 734 nm the absorbance \(A_{\text{control}}\) of the resulting ABTS radical solution (green-blue). Upon the addition of the tested sample solution (20 µl) with different concentrations of compounds such as 200, 100, 50, 25, and 12.5 µM in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution, the absorbance \(A_{\text{test}}\) was measured. The decreasing in the absorbance is expressed as % inhibition which was calculated according to the following equation [55]:

\[
\% \text{ Inhibition} = \left[ \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \right] \times 100
\]

where; the reference and standard antioxidant compound in this test is the ascorbic acid solution (20 µL, 2 mM) and the blank sample was performed by the solvent without ABTS.

**DPPH Method**

According to the methodology described by Brand-Williams et al. [56], the measurement of the DPPH radical scavenging activity was implemented. The samples with different concentrations of compounds such as 200, 100, 50, 25, and 12.5 µM were allowed to react with the stable DPPH radical in ethanol solution. Whereas, the reaction mixture consisted of sample (0.5 mL), absolute ethanol (3 mL), and DPPH radical solution (0.3 mL) 0.5 mM in ethanol. DPPH is reduced when it reacts with an antioxidant compound, which can donate hydrogen. The changes in color (from deep violet to light yellow) were recorded [absorbance \((Abs)\)] at \(\lambda_{\text{max}}\) 517 nm after 100 min of reaction using a UV-Vis spectrophotometer (Schimadzu Co., Tokyo, Japan). The blank solution was prepared by mixing ethanol (3.3 mL) and the sample (0.5 mL). Meanwhile, the mixture of ethanol (3.5 mL) and DPPH radical solution (0.3 mL) serve as a positive control.

The scavenging activity percentage \((AA\%)\) was determined according to Mensor et al. [57]:

\[
AA\% = 100 - \left[ \frac{(Abs_{\text{sample}}) - Abs_{\text{blank}}}{Abs_{\text{control}}} \right] \times 100
\]

3.4. Computational Procedures

All theoretical calculations and results of the studied compounds were implemented by utilizing Gaussian(R) 09 D.01 [58] (Semichem Inc., Shawnee Mission, KS, USA) by applying the DFT operation with the hybrid functional B3LYP level [59,60] in conjunction with the 6-31G(d,p) basis set. The visualization of these results was achieved using GaussView 6.0.16 software (Semichem Inc., Shawnee Mission, KS, USA).

4. Conclusions

In conclusion, this work focused on the study of the antiproliferative and antioxidant activities in vitro in addition to the theoretical calculation of the DFT theory of some novel quinazolinone(thione) derivatives 6–14. Two main points were the principal targets; firstly, by comparing the activities of quinazolinone and quinazolinthione derivatives. Secondly, comparing the activities of four series of Schiff bases, that have quinazolinolino moiety. The results of this study imply that the quinazolinthione derivatives 6 and 14 have promising potent antiproliferative activity comparable with quinazolinone derivatives 5 and 9, respectively. According to the DFT study, compounds 6 and 14 have a smaller
energy gap and a higher chemical softness than that of compounds 5 and 9, respectively. Additionally, screening of various aryl aldehyde hydrazone derivatives (11a–d) analogs exhibited that the potency increased with increasing the electron donating group in p-position due to increasing of the conjugated system, and that was supported by the DFT study.

On the other hand, compounds 6 and 11d showed promising antioxidant activity using ABTS assay. While in the DPPH assay, compounds 6, 11d, and 14 have showed potent activities comparable to the ascorbic acid which was used as a reference drug. Noteworthy, the results of both antiproliferative and antioxidant activities for each compound individually are nearly the same.

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**Sample Availability:** Samples of the compounds are available from the authors.

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