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Original article

Antioxidant activities and molecular docking of 2-thioxobenzo[g] quinazoline derivatives

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ABSTRACT

Background: Oxidative stress and related diseases resulting from the overproduction of free radicals can be counteracted by designing and developing novel antioxidative agents that can protect the human body against the damage caused by free radicals.
Methods: The present study evaluated the antioxidant activities of 15 derivatives of 2-thioxobenzo[g] quinazoline using three different assays: 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging, reducing power capability, and ferric reduction antioxidant power.
Results: Some benzoquinazolines had good activity and had the capacity to deplete DPPH and free radicals compared to a positive control butylated hydroxyl toluene (BHT). A docking study identified the possible interactions between binding models and the antioxidant activities of the target compounds.
Conclusions: The active compounds can be used as templates for further development of more potent antioxidative agents.

Introduction

Oxidative processes are fundamental metabolic processes for all living organisms [1], but free radicals produced during chain reactions in the oxidation process are responsible for cellular damage. Imbalances between the outputs of reactive oxygen/nitrogen species (ROS/RNS) and the antioxidant effects of related enzymes result in oxidative stress. This oxidative effect causes damage to tissues and some biomolecules, including lipids, DNA, and proteins, further decreasing their antioxidant protection power. Moreover, oxidative stress plays an important role in human-related diseases, such as cancer, asthma, respiratory diseases, stroke, cardiovascular dysfunctions, atherosclerosis, and diabetes [1–5]. Furthermore, the skin is quite vulnerable to oxidative stress given its persistent exposure to direct ultraviolet (UV), sunlight radiation that can bring about hyperpigmentation and pre-matured aging [6,7]. Thus, there is considerable interdisciplinary research investigating new potent and effective natural and synthetic antioxidants to prevent the causative damages and toxicity accompanied by free radicals.

Melanin is played a critical role in skin protection against induced damage UV/ free radicals and considered the main determinant factor for skin pigments. Tyrosinase is the rate-limiting enzyme in the first two steps of the melanogenesis process. It catalyzes the hydroxylation of L-tyrosine into L-DOPA, and oxidation of L-DOPA to form the respective *O*-dopaquinone [8,9].

Different skin disorders (eg. melasma, age spots, freckles, solar lentigines and hyperpigmentation) resulted from abnormal accumulation of melanin. Therefore, tyrosinase inhibitors are essential to ensure a decrease in the content of melanin and to design and develop new depigmenting compounds useful in pharmaceutical and cosmetic area [8,9]. Antioxidants are widely used to block, delay or enucleate oxidative stress in the human body. They are classified based on their origin as either endogenous or exogenous agents. Antioxidants act by scavenging free radicals, chelating metals and inhibiting pro-oxidant enzymes inhibition [10]. Antioxidants give up electrons to free radicals, thereby neutralizing them [10]. The natural physicochemical and biopharmaceutical characteristics of exogenous antioxidants, including

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how they access target sites, play an essential role in their efficacy against oxidative stress. Hence, much attention has been paid to create synthetic antioxidants to prevent the cellular damage caused by free radicals [11].

The quinazoline ring represents a core unit in the construction of a pharmacologically interesting class of molecules with antioxidant activities [12–17]. In our previous study, we incorporated triazole into quinazoline to create a triazoloquinazoline structure that was directly related to products with important antioxidant characteristics [1]. Benzo[g]quinazolines have been described as antiviral, antimicrobial, and anticancer agents [18–21]. Considering the apparent antioxidant properties of quinazolines and their annulated heterocycles, we aimed to develop novel compounds to combat free radicals and protect the body from the damage caused by them.

The objective of the present study was to evaluate the antioxidant activities of previously synthesized and well-characterized benzoquinazolines **1–15** [21] using *in vitro* assays. We then used molecular docking to fit the proposed benzoquinazolines into the active site of a target enzyme to identify possible interactions between the binding models and their antioxidant activities.

Materials and methods

Chemicals

All chemicals, including 1,1-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), 2,4,6-tri (2-pyridyl)-1,3,5-triazine (TPTZ), ferrous chloride ($FeCl_2$), iron trichloride ($FeCl_3$), potassium ferricyanide $K_3[Fe(CN)_6]$, and Trolox were purchased from Sigma Chemical Company (St. Louis, MO, USA). All chemicals were of analytical grade from Sigma, Merck and Aldrich.

Investigating antioxidant activity

DPPH assay

The free radical scavenging activities of the target benzoquinazolines were determined using the DPPH free radical scavenging assay according to Brand-Williams et al. [22]. A fresh methanolic solution of DPPH (20 μg/mL) was prepared and stored at 10 °C in the dark. Benzoquinazolines were dissolved in 85% methanol: water (v/v). The benzoquinazoline (0.5 mL) was added to freshly prepared methanolic DPPH solution (1.0 mL) and stirred. After reacting for 5 min, discoloration was recorded at 517 nm. The absorbance of the DPPH radicals without antioxidant was also measured as a control, and 95% methanol was used as blank. The absorbances were compared with those of the blank control. The reactions were performed in three replicates and averaged. Antioxidant activity was calculated as follows:

% Antioxidant activity = [(control absorbance − sample absorbance) / control absorbance] × 100%

Reducing power capability assay

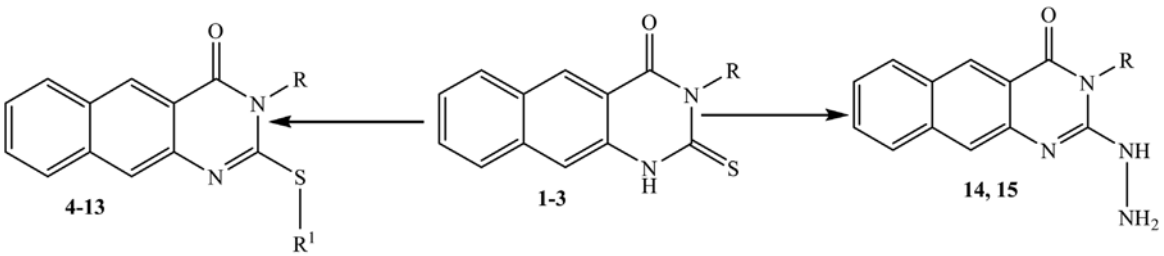
We used the Oyaizu procedure to determine the reducing power of the benzoquinazolines [23]. We prepared $K_3[Fe(CN)_6]$ (1%) and phosphate buffer (0.2 M, pH = 6.6). Benzoquinazoline solution (0.5 mL, 100 μg/mL) was added to a mixture of phosphate buffer (2.5 mL) and $K_3[Fe(CN)_6]$ (2.5 mL). At a temperature of 50 °C, the mixture was incubated for 20 min, then centrifuged at 1000 rpm for 10 min after aliquots of trichloroacetic acid (2.5 mL, 10%) were added. The mixture was allowed to separate into two layers. The solution in the upper layer (2.5 mL) was mixed with 0.1% $FeCl_3$ (0.5 mL) and distilled water (2.5 mL). The absorbance of the prepared mixture was measured at 700 nm. Increments of reducing power were indicated by the absorbance of the reaction mixture.

Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was based on the Benzie and Strain method [24], with some modifications. Stock solutions of acetate buffer (300 mM, pH = 3.6), $FeCl_3.6H_2O$ (20 mM) and TPTZ (10 mM) in HCl (40 mM) were prepared. A freshly mixed solution was prepared by mixing $FeCl_3.6H_2O$ (2.5 mL), acetate buffer (25 mL) and TPTZ solution (2.5 mL). The mixture was warmed at 37 °C. In dark conditions, the FRAP solution (2850 μL) was allowed to react with the benzoquinazoline (150 μL) for 30 min. Readings of the colored product (ferrous tripyridyltriazine complex) were measured at 593 nm. The results are expressed in μM Trolox/100 g dry matter. When the measured FRAP value exceeded the linear range of the standard curve, additional dilution was applied.

Molecular docking

All tested molecules were prepared using MOE software, and the 3D structure of tyrosinase was downloaded from the PDB site (PDB entry 3NM8; www.rcsb.org). Water molecules were removed from the proteins, and hydrogen atoms were added. The ligands were prepared, optimized and thereafter docked. We used the London dG and GBVI/WSA dG functions in the MOE® software to identify receptor binding pockets. Scoring and re-scoring of the docked poses were followed and achieved. Configurations of the target molecules and the receptors were assessed and chosen based on their scores and root mean square deviation values.



1: R = butyl, **2** : R = allyl, **3** : R = phenyl, **4**: R = butyl, R_1= allyl; **5**: R = butyl, R_1=2-morpolinoethyl; **6**: R = butyl, R_1= 2-piperidinoethyl; **7:** R = butyl, R_1= 2-(phthalimido-2-yl)propyl; **8** : R = allyl, R_1= allyl; **9**: R = allyl, R_1= *m*-OMe-benzyl; **10**: R = phenyl, R_1=*m*-CN-benzyl; **11**: R = phenyl, R1= *p*-Cl-benzyl; **12**: R = phenyl, R_1= 2-piperidinoethyl; **13**: R = phenyl, R_1= 2-(phthalimido-2-yl)propyl; **14**: R= butyl, **15**: R= allyl

Scheme 1. Synthetic routes for benzo[g]quinazolines (**1–15**).

Results

The benzo[*g*]quinazolines (**1**–**15**) were characterized by their physicochemical and spectral data in our previous paper (Scheme 1) [21]. In the present study, we used DPPH radical scavenging, reducing power capability and FRAP assays to evaluate the *in vitro* antioxidant activity of the target compounds (Figs. 1–3). The results are represented in Figs. 1–3 and compared with those of BHT and Trolox as standard synthetic antioxidants.

The antioxidant activities (radical scavenging, reducing power capabilities and FRAP activities) of the target benzoquinazolines (**1**–**15**) ranged between weak and high. Their antioxidant activities could be related to their redox properties, which allow them to scavenge-free radicals by acting as reducing agents or hydrogen atom donors. Benzoquinazolines **2, 9** and **10** exhibited relatively high DPPH scavenging activities, though lower than BHT (Fig. 1). Benzoquinazolines **13**–**15** showed moderate DPPH radical scavenging activities unlike compounds **2, 9**, and **10**, whereas compounds **1**, **3**–**8**, **11** and **12** appeared to possess weak scavenging activities relative to the other compounds and BHT (Fig. 1). The reducing capacities of benzoquinazolines **2**, **12** and **15** showed good antioxidant activities, and compounds **1** and **3** exhibited moderate reducing capacities (Fig. 2). However, the reducing power capabilities of the target benzoquinazolines were much lower than those of the standard BHT. Benzoquinazolines **3**, **10**, **11**, and **15** exhibited good activity in relation to Trolox. The FRAP values of compounds **1**, **2** and **12**-**14** ranged between 808 and 965 (μM Trolox/100 g), which were slightly lower than antioxidant activities of compounds **3**, **10**, **11**, and **15.** Meanwhile, compounds **4**–**9** showed the lowest activities (Fig. 3).

Discussion

The antioxidant activities of benzoquinazolines **1**–**15** were correlated with the type and position of the substituents on the benzoquinazoline ring. The high DPPH scavenging activities of compounds **2**, **9,** and **10** compared to those of the other benzoquinazolines may be due to the allyl group in **2** and **9**, although compound **9** was more active. This could be attributed that the stabilization of the resulting oxygen centered radical, which was enhanced by an electron donating group (OCH_3). There were more resonance conjugating structures in compounds with OCH_3 groups than in those without. Thus, through electron donation, the antioxidant targets may quench the DPPH free radicals, leading to absorbances at 517 nm (the developed color and absorbance with slow rate, the potent antioxidant activity). On the other hand, compound **10** was characterized by a phenyl group at position 3 and an electron withdrawing group (CN) on benzyl ring. Thus, the presence of such an electron withdrawing group and electron density on the phenyl ring could positively influence activity and stabilize the ring.

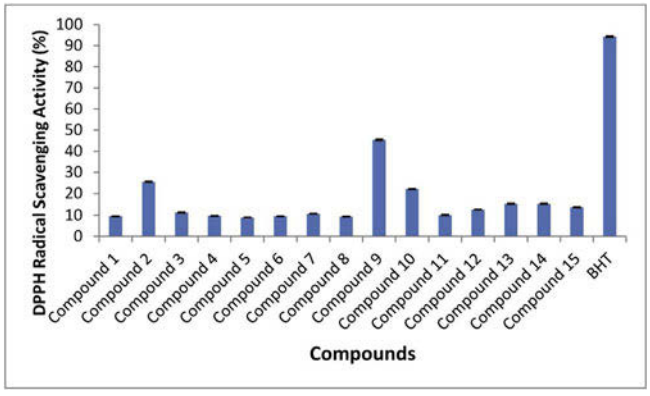


Fig. 1. Radical scavenging activities of compounds **1**–**15** (1 mg/mL) and butylated hydroxyl toluene (BHT) to 1,1-diphenyl-2-picryl hydrazyl (%) (DPPH). Data are means ± standard deviations of triplicate experiments.

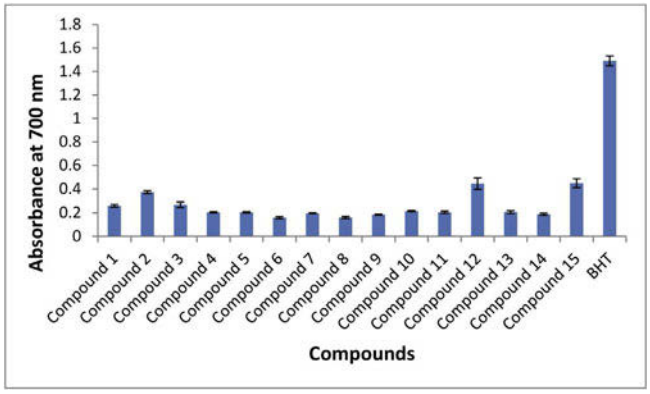


Fig. 2. Reducing power capabilities of compounds **1**–**15** (1 mg/mL) and butylated hydroxyl toluene (BHT). Data are means ± standard deviations of triplicate experiments.

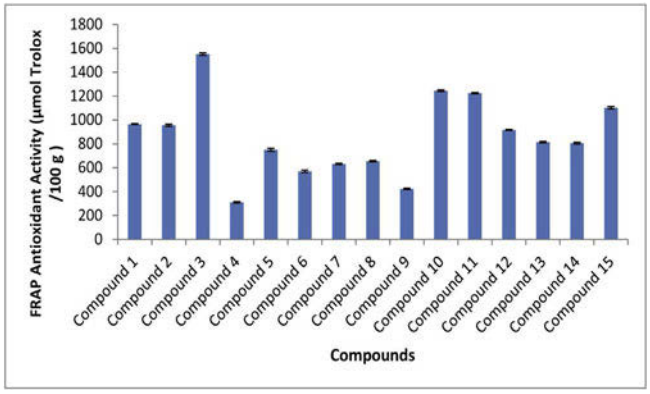


Fig. 3. Ferric reducing antioxidant power (FRAP) activity of compounds **1**–**15** (1 mg/mL). Higher FRAP values indicate higher antioxidant activities. Data are means ± standard deviations of triplicate experiments.

The antioxidant capability captured by the FRAP method depends on the reduction of the ferric ion complex TPTZ. The reduction of Fe^{3+}/ferricyanide complex to Fe^{2+}/ ferrocyanide can be achieved in the presence of antioxidants. Hence, the binding between the ligand and Fe^{2+} creates a very intense navy-blue color. Therefore, the amount of reduced iron can be monitored by measuring the formation of Perl's Prussian blue at 700 nm, and then correlated with the amount of antioxidants [1].

From the *in vitro* activity results, a number of benzoquinazoline derivatives showed enhanced activities. The incorporation of certain functional groups into compounds **1–3** was advantageous to the antioxidant activity. This was also seen in compounds **9**, **10** and **11,** which demonstrated better DPPH scavenging than their parents **1**, **2** and **3,** respectively. Similarly, compound **12** appeared more active than **3** in reducing power capacity profiles. The replacement of a thioxo group in compound **2** by a hydrazine group in compound **15** enhanced the ferric and reducing abilities of the compound, which could be attributed to hydrazine's ability to act as a hydrogen supplying group [1]. The potential antioxidant significance is indicated by its reducing capacity.

Docking poses were analyzed and compared to the co-crystallized standard antioxidant nordihydroguaiaretic acid (NDGA). Docking was performed on crystal structure of tyrosinase

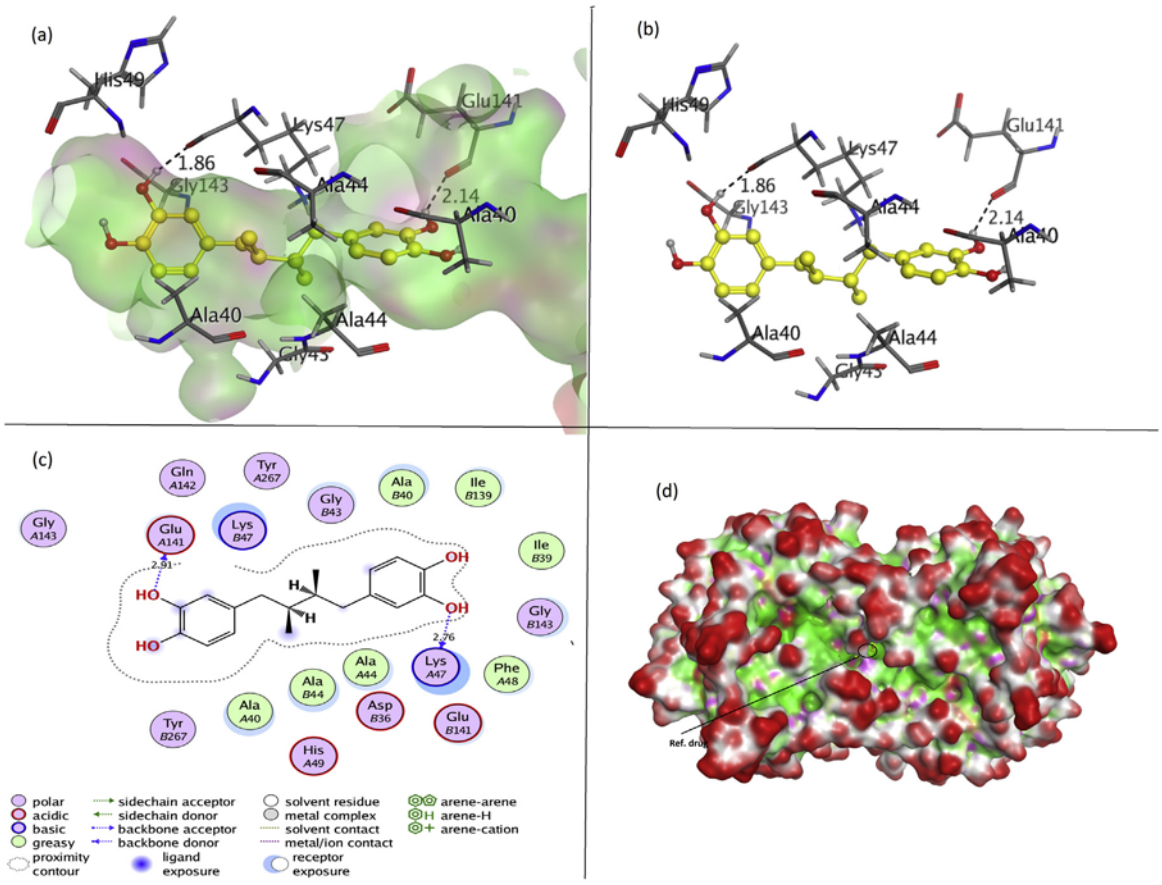


Fig. 4. Displaying the tyrosinase binding site cavity 3d interaction diagram of NDGA with pocket (a) and without pocket (b); Binding mode of NDGA as 2D diagram (c); The interaction of NDGA with the tyrosinase enzyme (PDB code: 3nm8), presented at the tyrosinase binding site cavities by MOE 2015: The binding patterns NDGA in the active site of tyrosinase, in which NDGA is disappeared in stick model with yellow while the surface of tyrosinase is shown in green, red and purple (d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 1
Docking results of compounds (**1–15**) and Nordihydroguaiaretic acid (**NDGA**).

Compounds	Binding affinity	Refined root mean square deviation	Amino acid residues	Atoms of compound	Bond distance (Å)	Types of bond
1	−6.78	0.771	LYS 47 (A)	O 1	3.00	H-acceptor
2	−6.79	0.910	LYS 47 (A)	O 1	3.02	H-acceptor
3	−6.2	1.511	LYS 47 (A)	S 18	3.04	H-acceptor
4	−6.78	1.279	PRO 219 (B)	S 18	4.09	H-donor
5	−7.73	1.689	GLU 141 (A)	S 18	4.08	H-donor
6	−7.89	1.668	LYS 47 (A)	S 18	3.24	H-acceptor
7	−8.15	1.28	GLY 43 (A)	S 18	3.26	H- acceptor
8	−6.39	1.00	PRO 219 (B)	S 13	4.29	H-donor
9	−8.76	1.696	LYS 47 (A)	S 13	3.55	H-acceptor
			ALA 40 (A)	6-ring	3.91	pi-H
			LYS 47 (A)	6-ring	3.50	pi-cation
			ALA 40 (A)	6-ring	4.94	pi-H
10	−7.88	1.208	ALA 40 (A)	S 16	4.12	H-donor
			LYS 47 (A)	6-ring	3.84	pi-cation
11	−7.00	2.073	LYS 47 (A)	O 1	2.86	H-acceptor
12	−7.37	1.107	LYS 47 (A)	S 16	3.12	H-acceptor
13	−7.46	1.735	GLY 43 (A)	O 1	3.44	H-acceptor
			LYS 47 (A)	O 39	3.17	H-acceptor
14	−7.44	1.886	PRO 219 (B)	N 18	2.87	H-donor
			GLU 141 (B)	N 20	3.52	H-acceptor
			LYS 47 (A)	O 1	2.92	H-acceptor
15	−7.10	1.229	PRO 219 (A)	N 13	3.02	H-donor
			GLU 141 (A)	N 15	3.19	H-donor
NDGA	−7.72	2.172	LYS 47 (A)	O 36	2.76	H-donor
			GLU 141 (A)	O 43	3.52	H-donor

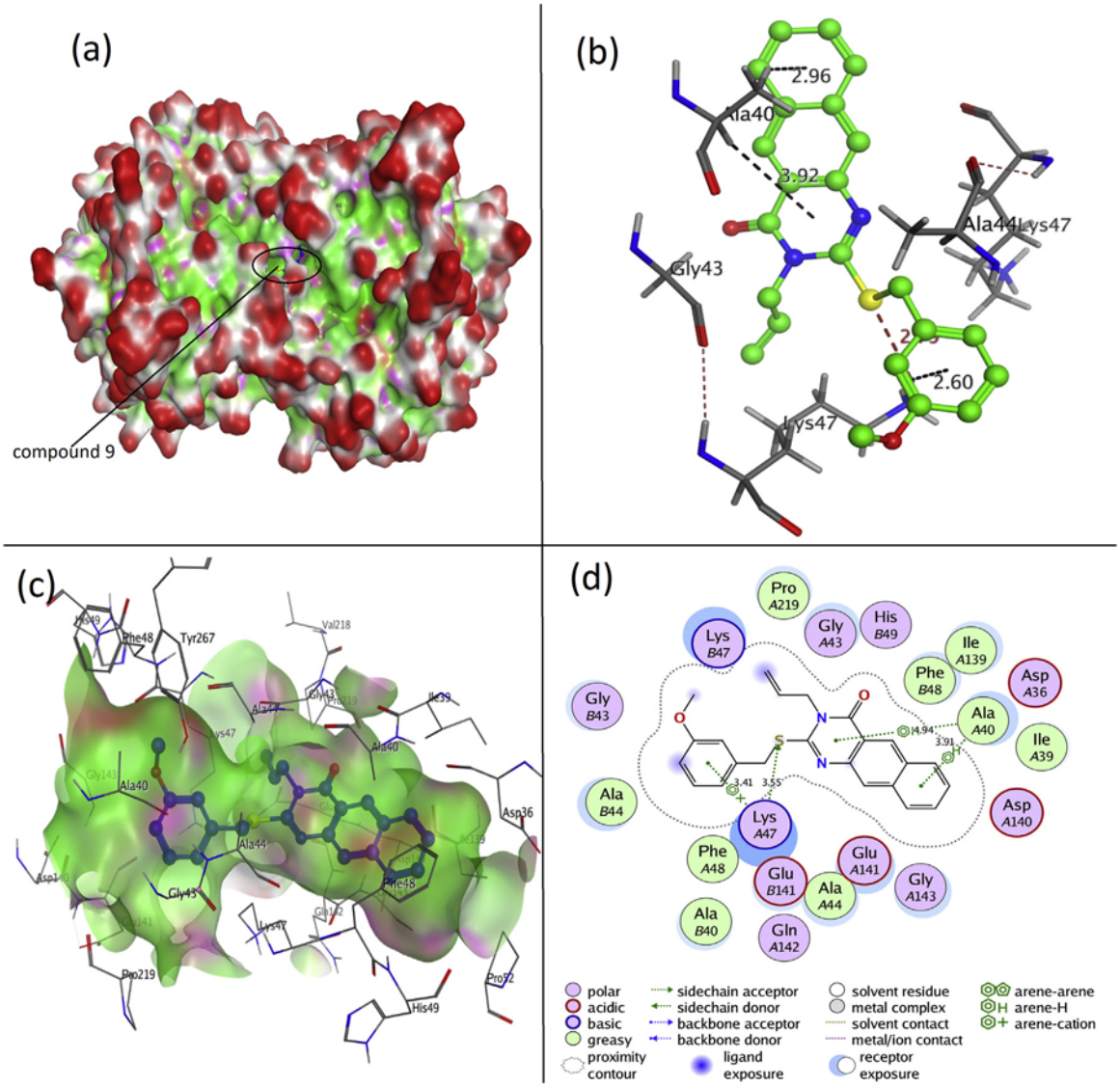


Fig. 5. The interaction of compound **9** with the tyrosinase enzyme (PDB code: 3nm8). Presented at the tyrosinase binding site cavities by MOE 2015: The binding patterns of **9** in the active site of tyrosinase, in which compound **9** is disappeared in stick model with yellow and blue color while the surface of tyrosinase is shown in green, red and purple (a); Displaying the tyrosinase binding site cavity 3d interaction diagram of compound **9** without pocket (b) and with pocket (c); Binding mode of compound **9** as 2D diagram (d) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

from *Bacillus megaterium* (PDB code: 3NM8) using MOE software https://www.rcsb.org/structure/3NM8). Tyrosinase is a widely distributed copper-containing enzyme. However, tyrosinase catalyzes two enzymatic reactions: the hydroxylation of monophenols to diphenols and the oxidation of diphenols to quinones [8,9,25].

The obtained conformations of the tested compounds were analyzed to choose the most plausible poses with similar interactions as reference antioxidants (Fig. 4). The binding energies, root mean square deviations, and the number of closest residues for the target benzoquinazolines are presented in Table 1. Based on the obtained finding, Compound **9** was the most active antioxidant. It had a better dock score (−8.76 kcal/mol) than NDGA (−7.72 kcal/mol), and it formed direct contact residues in the active site, similar to those of NDGA. Moreover, compound **9** formed hydrophobic contact with Lys47A, Lys47B, Glu141A, Ala40A, Gly143A, Ala44B, and Ile139A (Fig. 5). The rational conformation of **9** shows that the location of an *o*-methoxy group was in a favorable position as it became closer to the hydrophobic interactions. In addition, compound **9** formed three strong hydrogen bonds with Ala40A, Gly43A and Lys47A, with distances of 4.4, 4.08 and 3.5 Å, respectively. Compound **10** had a slightly lower dock score (−7.88 kcal/mol) than NDGA (−7.72 kcal/mol). This binding mode of compound **10** showed more activity with the cyanide group in the *meta*- position, which suggests that the cyanide group in the *m*-position is favorable for activity.

Conclusions

Benzoquinazolines exhibited antioxidant activities based on three antioxidant assays. Compounds **3**, **9** and **15** had the largest reducing power capability, DPPH radical scavenging activity, and FRAP, respectively, which can be attributed to the effects of phenyl (electron-rich moiety), methoxy (electron donating group) and hydrazine (supplying hydrogen atoms), respectively. The docking pose of compound **9** revealed that hydrophobic contacts were formed between the lipophilic residues in the pocket, namely with

Lys47A, Lys47B, Glu141A, Ala40A, Gly143A, Ala44B, and Ile139A. These pharmacophoric features clarify the activity of this ligand. The results of the SAR and molecular docking studies will be taken into consideration for future research to design new and more active antioxidant compounds.

Competing interests

The authors declare that they have no competing interests.

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