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Synthesis of new benzimidazole-1,2,3-triazole hybrids as tyrosinase inhibitors

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This paper is dedicated to the memory of our unique teacher in Chemistry and Medicinal Chemistry, Professor Abbas Shafiee.

A novel series of benzimidazole-1,2,3-triazole hybrids containing substituted benzyl moieties were designed, synthesized and evaluated for their inhibitory activity against mushroom tyrosinase. The results indicated that 2-(4-((1-(3,4-dichlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (**6g**) and 2-(4-((1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy)phenyl)-1*H*-benzo[*d*]imidazole (**6h**) exhibited effective inhibitory activity with IC_{50} values of 9.42 and 10.34 μM, respectively, comparable to that of kojic acid as the reference drug (IC_{50} = 9.28 μM). Kinetic study of compound **6g** confirmed mixed-type inhibitory activity towards tyrosinase indicating that it can bind to free enzyme as well as enzyme-substrate complex. Also, molecular docking analysis was performed to determine the binding mode of the most potent compounds (**6g** and **6h**) in the active site of tyrosinase. Consequently, **6g** and **6h** derivatives might serve as promising candidates in cosmetics, medicine or food industry, and development of such compounds may be of an interest.

Key words: Benzimidazole; 1,2,3-Triazole; Tyrosinase inhibitor

Introduction

Benzimidazole and 1,2,3-triazole derivatives are known as the impressive heterocyclic scaffolds owing to their various valuable biological activities. Benzimidazole moiety is a fused heterocyclic aromatic system and found in the structure of vitamin B12. It has also depicted antiviral, anticancer, antibacterial, anticonvulsant, analgesic and anti-inflammatory, anti-diabetic, antifungal, antioxidant, and antihypertensive activities.[1]

Recently, some alkylbenzimidazole and 2-substitutedbenzyl-4(7)-phenyl-1*H*-benzo[*d*]imidazoles have shown inhibitory activity towards tyrosinase.[2][3]

1,2,3-Triazoles have emerged as the versatile pharmacophore since they are able to construct hydrogen bonding and dipole-dipole interactions which have made them very stable to hydrolysis and oxidative/reductive conditions.[4] Moreover, 1,2,3-triazloles have been introduced as the peptide bond isosteres and 1,2-3-triazole-containing analogues of the naturally occurring cyclo-[Pro-Val-Pro-Tyr] have shown potent tyrosinase inhibitory activity.[5] 1,2,3-Triazole moiety is not naturally produced; hence, synthetic molecules have attracted lots of attention and in this regard, copper(I)-catalyzed formation of 1,2,3-triazoles through azides and terminal alkynes has been a powerful tool in drug discovery developments.[6-12]

Tyrosinase (EC 1.14.18.1), a copper containing monooxygenase and also known as polyphenol oxidase (PPO), is widely distributed in mammals, plants, bacteria and fungi. It catalyzes two reactions: hydroxylation of L-tyrosine to 3,4-dihydroxy phenylalanine (L-DOPA) and oxidation of L-DOPA to dopaquinone.[13][14] In humans, dopaquinone is converted to melanin by a series of reactions and tyrosinase plays a crucial role in the synthesis of melanin which can cause to hyperpigmentation disorders such as melasma, seborrheic, etc.[15] In addition, tyrosinase is responsible for fruits and vegetables enzymatic browning due to oxidation of phenolic compounds leading to the reduction of nutritional values.[16] Moreover, tyrosinase is associated with some neurodegenerative disorders such as Parkinson's and Huntington's diseases.[17][18] Therefore, safe and effective tyrosinase inhibitors are essential in the field of clinical medicine, cosmetics, agricultural, and food industries.[13][19]

In this study, we designed and synthesized a wide range of benzimidazole-1,2,3-triazole hybrids. All 18 synthesized compounds were evaluated for their tyrosinase inhibitory activity and the most active derivative was investigated for its inhibition kinetic. Finally, docking analysis was performed to gain better insight into the ligand-receptor interactions of the most potent compounds.

Results and Discussion

Design strategy

The structure of target benzimidazole-1,2,3-triazole hybrids was designed by the molecular hybridization approach based on the structure of some potent tyrosinase inhibitors as reported in the literature (compounds **A**-**D**) (Fig. **1**). 2-(3-Methylbenzyl)-4(7)-phenyl-1*H*-benzo[*d*]imidazole (**A**) demonstrated tyrosinase inhibitory activity with IC_{50} value of 37.86 μM.[20] It has been reported that benzothiazole analogs possessing phenolic substituent (such as **B**) were effective tyrosinase inhibitors which showed higher inhibition than kojic acid at 50 μM.[21] Moreover, it has been described that 1,2-3-triazole-containing analogues of the naturally occurring cyclo-[Pro-Val-Pro-Tyr] (such as **C**) were effective tyrosinase inhibitors (IC_{50} = 0.5 mM).[3] It has been also reported that the kojic acid derivative containing 1,2,4-triazole (**D**) exhibited better tyrosinase inhibitor activity (IC_{50} = 4.50 μM) than kojic acid (IC_{50} = 19.00 μM).[22] Considering these structural features and principles of bioisoterism, we designed a series of tyroinase inhibitors by employing fragment-based drug design strategy.

Chemistry

The synthetic procedure for the preparation of benzimidazole-1,2,3-triazole hybrids **6** is shown in Scheme **1**. The required substrates **3** were prepared through the reaction of 4-(prop-2-yn-1-yloxy)benzaldehyde **1** and *o*-phenylenediamines **2** in the presence of $Na_2S_2O_5$ in

dimethylacetamide (DMA) at 120 °C for 12 h. It should be noted that aldehyde **1** was prepared by the reaction of 4-hydroxybenzaldehyde and propargyl bromide in DMF at 80 °C.[7] The title compounds **6** were synthesized through click reaction described by Kolb et al.[23] For this purpose, various organic azides **5** were prepared by the reaction of different benzyl chlorides/bromides **4** and sodium azide in the presence of triethylamine (NEt_3) in the mixture of H_2O/*t*-BuOH at room temperature. Then, compound **3**, sodium ascorbate, and catalytic amount of $CuSO_4.5H_2O$ (7 mol%) were added to the freshly prepared azides **5** leading to the formation of different benzimidazole-1,2,3-triazole hybrids **6a-r**. The final synthesized derivatives are listed in Table **1**.

Biological activity

All synthesized compounds (**6a**-**r**) and kojic acid (as the positive control) were evaluated using tyrosinase inhibition assay and the IC_{50} values were reported in Table **1**. As it is stated in Table **1**, compounds **6c**, **6d**, **6g**, **6h**, **6i**, **6j**, and **6l** showed inhibitory activity against mushroom tyrosinase (IC_{50}s = 9.42-48.00 µM). Among them, compounds **6g** and **6h** exhibited potent inhibitory activity towards tyrosinase with IC_{50} values of 9.42 and 10.34 µM, respectively, which were comparable to that of kojic acid (IC_{50} = 9.28 µM). However, compounds **6a**, **6b**, **6e**, **6f**, **6k**, **6l-r** demonstrated no anti-tyrosinase activity indicating the fact that the inhibitory activity is dependent on the nature and position of the substitutions on the benzimidazole and arylidene moieties. The most potent compound **6g** contained 3,4-dichloroarylidene group on 1,2,3-triazole ring and lacked substituents on the benzimidazole moiety. Changing the number and position of chlorine atom in compounds **6e** and **6f** deleted anti-tyrosinase activity. In addition, it was found that compounds **6m-p** having methyl group at 6-position of benzimidazole ring and chlorine (chlorines) on the arylidene moiety, showed no inhibitory activity.

The second potent compound **6h** possessing 4-bromoarylidene group connected to1,2,3-triazole ring also lacked substituents on the benzimidazole moiety. Its counterpart, compound **6q** having methyl group at 6-position of benzimidazole ring did not show inhibition activity on tyrosinase enzyme. Introduction of fluorine into aryliden moiety of compounds **6c** and **6d** having 2- and 4-fluorobenzyl group connected to 1,2,3-triazole ring showed moderate inhibitory activity towards tyrosinase with IC_{50} values of 45.22 and 46.89 µM, respectively. However, the better inhibitory activity was obtained by compound **6l** containing 4-fluorobenzyl group connected to 1,2,3-triazole ring and methyl group at 6-position of benzimidazole moiety (IC_{50} = 29.31 µM).

Generally, it can be stated that compounds bearing methyl group at C6 position of benzimidazole core (compounds **6j-n**) showed less inhibitory activity comparing with their counterparts lacking methyl group at the same position (**6a-i**). However, there is an exception, compound **6l** showed anti-tyrosinase activity with IC_{50} value of 29.31 µM, more active than compound **6d**.

Kinetic studies

The enzyme inhibition mode by the most potent derivative **6g** was determined by Lineweaver–Burk plot analysis as shown in Fig. **2**. The Lineweaver–Burk plots (plot of 1/V versus 1/[S]) for the inhibition of tyrosinase by the selected compound **6g** was obtained with several concentrations of the corresponding compound and L-DOPA as the substrate. The Lineweaver-Burk double reciprocal plots yielded a group of lines that intercepted in the second quadrant. It was found that K_m value increased and V_{max} value decreased by the increase of the concentration of **6g** confirming that compound **6g** demonstrated mixed-type inhibition towards tyrosinase. It can inhibit both free enzyme and enzyme-substrate complex.

Molecular docking analysis

The binding models of the compounds **6g** and **6h** with tyrosinase (PDB code: 2Y9X) were represented in Fig. **3** and Fig. **4**, respectively. The results revealed that both compounds were well accumulated in the binding pocket of tyrosinase by hydrogen bond, Pi-H and Pi-Pi interactions. The following interactions could be seen in the case of compound **6g** (Fig. **3**). The benzimidazole core exhibited a hydrogen bond and a Pi-H interaction with Asn81 (distance: 2.08 Å) and His85, respectively. Oxygen of compound **6g** formed another hydrogen bond with His244 (distance: 1.99 Å) and additionally, 3,4-dichlorophenyl ring involved in a Pi-Pi interaction with Val283. It seems that the binding orientation of compound **6h** (Fig. **4**) is different from that of **6g**. Consequently, 1,2,3-triazole ring in compound **6h** involved in two interactions including a hydrogen bond with His244 (distance: 1.72 Å) and a Pi-Pi interaction with His85. Furthermore, benzimidazole core formed a hydrogen bonding interaction with Val283 (distance: 2.40 Å).

Conclusion

In conclusion, hybridization strategy was utilized to prepare and evaluate benzimidazole-1,2,3-triazole hybrids as tyrosinase inhibitors. According to the obtained results, compounds **6g** and **6h** lacking substituents on the imidazole moiety and possessing 3,4-dichlorobenzyl and 4-bromobenzyl on the 1,2,3-triazole ring were the most potent tyrosinase inhibitors respectively with IC_{50} values of 9.42 and 10.34 μM. The inhibitory potential was dependent on the substituents on the arylidene and benzimidazole moieties. Consequently, derivatives bearing hydrogen at C6 position of benzimidazole ring had superior activity over their counterparts possessing methyl substituent at the same position. Also, compounds containing halogen atoms at the *para* position of phenyl ring connected to 1,2,3-triazole core showed good inhibitory activity. The binding patterns of compounds **6g**

and **6h** within the active site of tyrosinase were predicted through docking analysis. Docking results showed that benzimidazole, 1,2,3-triazole and arylidene moieties provided some favorite interactions with the enzyme active site. The key residues forming interactions were Asn81, His244, His85 and Val283. Therefore, compounds **6g** and **6h** could be introduced as the potent tyrosinase inhibitors and might be promising compounds in the field of drug discovery.

Experimental Section

General chemistry

Melting points were measured on a Kofler hot stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker FT-500, using TMS as an internal standard. IR spectra were obtained on a Nicolet Magna FTIR 550 spectrophotometer (KBr disks). Elemental analysis was performed on an Elementar Analysensystem GmbH VarioEL CHNS mode. All chemicals were obtained from Merck and Aldrich and used without further purification.

*General procedure for the synthesis of compounds **6***

A mixture of 4-(prop-2-yn-1-yloxy)benzaldehyde **1** (1 mmol), *o*-phenylenediamine derivative **2** (1 mmol), and sodium metabisulfite (1.1 mmol) in DMA (12 mL) was heated at 120 °C for 12 h. After completion of reaction, the mixture was poured into crushed ice and the precipitate was filtered off, washed with water, and dried. It was used for next step with no further purification. Then, a solution of benzyl chloride/bromide derivative **4** (1.1 mmol), sodium azide (0.9 mmol), and Et_3N (1.3 mmol) in the mixture of water (4 mL)/t-BuOH (4 mL) was stirred at room temperature for 1 h. Subsequently, mixture of compound **3** (1 mmol), sodium ascorbate (0.1 mmol), and $CuSO_4.5H_2O$ (7 mol %) was added to the freshly prepared azide derivative **5** and the reaction mixture was stirred at room temperature for 24 h.

Upon completion of the reaction (checked by TLC), the reaction mixture was diluted with water, poured into crushed ice; the precipitated product was filtered off, and washed with cold water. All products were recrystallized from petroleum ether/ethyl acetate to give pure products **6**.

*2-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole (**6a**)*

Yield: 75%. Mp: 249-250 °C. IR (KBr): 3436, 3135, 3057, 2958, 2876, 1610, 1589 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 5.24 (s, 2H, CH_2), 5.62 (s, 2H, CH_2), 7.17-7.39 (m, 9H, H5, H6, H3′, H5′, H2″, H3″, H4″, H5″, H6″), 7.51 (d, J = 7.5 Hz, 1H, H7), 7.63 (d, J = 7.5 Hz, 1H, H4), 8.12 (d, J = 7.0 Hz, 2H, H2′, H6′), 8.31 (s, 1H, triazole), 12.74 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 52.8, 61.2, 111.0, 115.1, 118.5, 121.4, 122.0, 123.0, 124.7, 127.9, 128.1, 128.7, 135.9, 142.7, 143.9, 159.3. Anal. Calcd for $C_{23}H_{19}N_5O$: C, 72.42; H, 5.02; N, 18.36. Found: C, 72.21; H, 4.88; N, 18.19.

*2-(4-((1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole (**6b**)*

Yield: 78%. Mp>250 °C. IR (KBr): 3340, 3138, 3055, 2920, 2880, 1611, 1584 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 2.27 (s, 3H, CH_3), 5.23 (s, 2H, CH_2), 5.56 (s, 2H, CH_2), 7.17-7.23 (m, 8H, H5, H6, H3′, H5′, H2″, H3″, H5″, H6″), 7.50 (d, J = 7.5 Hz, 1H, H7), 7.61 (d, J = 7.5 Hz, 1H, H4), 8.10 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.25 (s, 1H, triazole), 12.70 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 20.6, 52.6, 61.2, 110.9, 115.1, 118.4, 121.4, 122.0, 123.0, 124.5, 127.9, 129.2, 132.9, 137.4, 142.7, 143.8, 159.3. Anal. Calcd for $C_{24}H_{21}N_5O$: C, 72.89; H, 5.35; N, 17.71. Found: C, 72.62; H, 5.70; N, 17.90.

*2-(4-((1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole (**6c**)*

^{1}H NMR (500 MHz, DMSO-d_6): Yield: 80%. Mp: 234-235°C. IR (KBr): 3418, 3132, 3056, 2963, 2877, 1611, 1495 cm^{-1}. 5.23 (s, 2H, CH_2), 5.69 (s, 2H, CH_2), 7.19-7.27 (m, 6H, H5, H6, H3′, H5′, H5″, H6″), 7.35-7.45 (m, 2H, H3″, H4″), 7.50 (d, J = 7.5 Hz, 1H, H7), 7.61 (d, J = 7.5 Hz, 1H, H4), 8.10 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.29 (s, 1H, triazole), 12.70 (s, 1H,

NH). ^{13}CNMR (125 MHz, DMSO-d_6): 46.9, 61.1, 110.5, 115.0, 115.5 (d, J_{C-F} = 21.2 Hz), 118.5, 121.4, 121.8, 123.0, 124.8 (d, J_{C-F} = 7.5 Hz), 126.3, 127.9, 130.7, 138.2, 142.6, 143.2, 159.3, 161.2 (d, J_{C-F} = 245.0 Hz). Anal. Calcd for $C_{23}H_{18}FN_5O$: C, 69.16; H, 4.54; N, 17.53. Found: C, 69.35; H, 4.35; N, 17.67.

2-(4-((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole ***(6d)***

Yield: 84%. Mp: 236-238 °C. IR (KBr): 3420, 3139, 3067, 2924, 2882, 1609, 1507 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 5.27 (s, 2H, CH_2), 5.82 (s, 2H, CH_2), 7.17-7.23 (m, 6H, H5, H6, H3′, H5′, H3″, H5″), 7.41 (dd, J = 8.2, 5.5 Hz, 2H, H2″, H6″), 7.49 (d, J = 7.0 Hz, 1H, H7), 7.62 (d, J = 7.0 Hz, 1H, H4), 8.11 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.30 (s, 1H, triazole), 12.70 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 52.0, 61.2, 111.0, 115.1, 115.5 (d, J_{C-F} = 21.2 Hz), 118.5, 122.0, 123.0, 124.6, 127.9, 130.2 (d, J_{C-F} = 8.7 Hz), 132.1, 142.8, 143.8, 159.3, 161.5 (d, J_{C-F} = 241.0 Hz). Anal. Calcd for $C_{23}H_{18}FN_5O$: C, 69.16; H, 4.54; N, 17.53. Found: C, 69.31; H, 4.63; N, 17.38.

2-(4-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole ***(6e)***

Yield: 80%. Mp>250 °C. IR (KBr): 3335, 3189, 2984, 2805, 1617, 1589, 1497 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 5.25 (s, 2H, CH_2), 5.37 (s, 2H, CH_2), 7.14-7.26 (m, 4H, H5, H6, H3′, H5′), 7.36-7.42 (m, 2H, H4″, H5″), 7.49 (m, 3H, H4, H7, H6″), 7.62 (d, J = 7.5 Hz, 1H, H3″), 8.11 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.29 (s, 1H, triazole), 12.72 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 50.6, 61.1, 111.0, 115.1, 118.5, 121.4, 122.0, 123.0, 125.1, 127.7, 127.9, 129.6, 130.2, 130.5, 133.1, 142.6, 143.8, 159.3. Anal. Calcd for $C_{23}H_{18}ClN_5O$: C, 66.43; H, 4.36; N, 16.84. Found: C, 66.61; H, 4.51; N, 16.62.

2-(4-((1-(2,3-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole ***(6f)***

Yield: 79%. Mp: 248-250 °C. IR (KBr): 3350, 3190, 3160, 1615, 1589, 1498 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 5.26 (s, 2H, CH_2), 5.79 (s, 2H, CH_2), 7.17-7.19 (m, 3H, H5, H6,

H6″), 7.21 (d, *J* = 9.0 Hz, 2H, H3′, H5′), 7.40 (t, *J* = 8.0 Hz, 1H, H5″), 7.50 (d, *J* = 7.5 Hz, 1H, H7), 7.62 (d, *J* = 7.5 Hz, 1H, H4), 7.66 (dd, *J* = 8.0, 2.0 Hz, 1H, H4″), 8.11 (d, *J* = 9.0 Hz, 2H, H2′, H6′), 8.32 (s, 1H, triazole), 12.72 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 51.1, 61.1, 110.9, 115.1, 118.5, 121.4, 122.0, 123.0, 125.2, 127.9, 128.5, 128.9, 130.5, 130.7, 135.7, 141.8, 142.6, 159.3. Anal. Calcd for $C_{23}H_{17}Cl_2N_5O$: C, 61.34; H, 3.81; N, 15.75. Found: C, 61.54; H, 3.66; N, 15.51.

2-(4-((1-(3,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole ***(6g)***

Yield: 84%. Mp: 230-231 °C. IR (KBr): 3400, 3189, 3150, 2942, 1614, 1581, 1496 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 5.25 (s, 2H, CH_2), 5.65 (s, 2H, CH_2), 7.17-7.18 (m, 4H, H5, H6, H3′, H5′), 7.31 (dd, *J* = 8.2, 2.0 Hz, 1H, H6″), 7.50 (d, *J* = 7.5 Hz, 1H, H7), 7.61-7.67 (m, 3H, H4, H2″, H5″), 8.11 (d, *J* = 8.5 Hz, 2H, H2′, H6′), 8.36 (s, 1H, triazole), 12.72 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 51.4, 61.2, 115.1, 121.7, 121.9, 123.0, 124.9, 127.9, 128.3, 129.2, 129.3, 130.1, 130.9, 131.2, 136.9, 142.9, 143.8, 159.3. Anal. Calcd for $C_{23}H_{17}Cl_2N_5O$: C, 61.34; H, 3.81; N, 15.55. Found: C, 61.11; H, 3.70; N, 15.39.

*2-(4-((1-(4-Bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole (****6h****)*

Yield: 82%. Mp: 239-241 °C. IR (KBr): 3400, 3139, 3061, 2956, 1610, 1496 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 5.24 (s, 2H, CH_2), 5.62 (s, 2H, CH_2), 7.18-7.22 (m, 4H, H5, H6, H3′, H5′), 7.29 (d, *J* = 8.0 Hz, 2H, H2″, H6″), 7.53-7.62 (m, 4H, H4, H7, H3″, H5″), 8.14 (d, *J* = 8.5 Hz, 2H, H2′, H6′), 8.33 (s, 1H, triazole), 12.82 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 52.0, 61.2, 115.1, 121.3, 122.0, 123.8, 123.9, 124.8, 127.8, 130.1, 131.6, 135.3, 140.8, 142.8, 146.8, 159.3. Anal. Calcd for $C_{23}H_{18}BrN_5O$: C, 60.01; H, 3.94; N, 15.21. Found: C, 59.87; H, 4.18; N, 15.40.

*2-(4-((1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole (****6i****)*

Yield: 78%. Mp: 120-122 °C. IR (KBr): 3425, 3142, 3077, 2945, 1608, 1520, 1497, 1346 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 5.27 (s, 2H, CH_2), 5.82 (s, 2H, CH_2), 7.17-7.18 (m, 2H, H5, H6), 7.22 (d, J = 8.5 Hz, 2H, H3′, H5′), 7.54-7.56 (m, 4H, H4, H7, H2″, H6″), 8.12 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.24 (d, J = 8.5 Hz, 2H, H3″, H5″), 8.39 (s, 1H, triazole), 12.73 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 51.9, 61.2, 115.1, 121.7, 121.8, 123.0, 123.8, 125.1, 127.9, 128.0, 129.0, 142.9, 143.3, 147.2, 151.2, 159.3. Anal. Calcd for $C_{23}H_{18}N_6O_3$: C, 64.78; H, 4.25; N, 19.71. Found: C, 64.61; H, 4.41; N, 19.56.

*2-(4-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-methyl-1H-benzo[d]imidazole (**6j**)*

Yield: 77%. Mp: 190-191 °C. IR (KBr): 3235, 3033, 2923, 1611, 1584, 1495 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 2.42 (s, 3H, Me), 5.23 (s, 2H, CH_2), 5.62 (s, 2H, CH_2), 6.99 (d, J = 8.5 Hz, 1H, H4), 7.19 (d, J = 8.5 Hz, 2H, H3′, H5′), 7.32-7.43 (m, 7H, H5, H7, H2″, H3″, H4″, H5″, H6″), 8.08 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.31 (s, 1H, triazole), 12.58 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 21.2, 52.8, 61.2, 115.0, 118.0, 121.8, 123.1, 123.2, 124.7, 125.2, 126.8, 127.8, 127.9, 128.1, 128.7, 130.5, 135.9, 142.7, 150.5, 159.2. Anal. Calcd for $C_{24}H_{21}N_5O$: C, 72.89; H, 5.35; N, 17.71. Found: C, 72.68; H, 5.18; N, 17.56.

*6-Methyl-2-(4-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole (**6k**)*

Yield: 80%. Mp: 171-173 °C. IR (KBr): 3235, 3030, 2921, 2858, 1612, 1584, 1477 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 2.27 (s, 3H, Me), 2.42 (s, 3H, Me), 5.22 (s, 2H, CH_2), 5.56 (s, 2H, CH_2), 7.00 (d, J = 7.5 Hz, 1H, H4), 7.17-7.23 (m, 6H, H3′, H5′, H2″, H3″, H5″, H6″), 7.41-7.55 (m, 2H, H5, H7), 8.08 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.27 (s, 1H, triazole), 12.57 (bs, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 20.6, 21.2, 52.6, 61.2, 110.8, 115.1, 118.0, 123.1, 124.5, 127.8, 127.9, 128.0, 129.2, 131.3, 132.5, 132.9, 137.5, 142.7, 144.0, 151.0, 159.2. Anal. Calcd for $C_{25}H_{23}N_5O$: C, 73.33, H, 5.66; N, 17.10. Found: C, 73.58; H, 5.80; N, 16.88.

*2-(4-((1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-methyl-1H-benzo[d]imidazole (**6l**)*

Yield: 83%. Mp: 183-185 °C. IR (KBr): 3201, 2924, 2871, 1613, 1492 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 2.42 (s, 3H, Me), 5.23 (s, 2H, CH_2), 5.69 (s, 2H, CH_2), 6.99 (d, J = 7.5 Hz, 1H, H4), 7.19 (d, J = 8.0 Hz, 2H, H3′, H5′), 7.23-7.48 (m, 6H, H5, H4, H3″, H4″, H5″, H6″), 8.08 (d, J = 8.0 Hz, 2H, H2′, H6′), 8.30 (s, 1H, triazole), 12.56 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 21.2, 46.9, 61.1, 110.7, 115.0, 115.5 (d, J_{C-F} = 21.2 Hz), 118.1, 122.1, 122.3, 122.4, 122.6, 123.2, 123.9, 124.0, 124.8, 127.2, 127.8, 130.7, 142.6, 144.2, 159.1, 161.0 (d, J_{C-F} = 245.2 Hz). Anal. Calcd for $C_{24}H_{20}FN_5O$: C, 69.72, H, 4.88; N, 16.94. Found: C, 69.58; H, 4.67; N, 17.14.

*2-(4-((1-(2-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-methyl-1H-benzo[d]imidazole (**6m**)*

Yield: 76%. Mp: 227-228 °C. IR (KBr): 3202, 2916, 2855, 1611, 1473, 1447 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 2.42 (s, 3H, Me), 5.27 (s, 2H, CH_2), 5.82 (s, 2H, CH_2), 6.99 (d, J = 7.5 Hz, 1H, H4), 7.19 (d, J = 8.5 Hz, 2H, H3′, H5′), 7.24 (d, J = 7.5 Hz, 1H, H5), 7.35-7.42 (m, 4H, H7, H4″, H5″, H6″), 7.53 (dd, J = 7.0, 1.0 Hz, 1H, H3″), 8.08 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.28 (s, 1H, triazole), 12.55 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 21.2, 50.6, 61.1, 115.1, 120.9, 121.5, 123.2, 123.7, 125.1, 126.6, 127.7, 127.8, 127.9, 129.6, 130.2, 130.5, 130.6, 132.6, 133.1, 142.6, 144.8, 159.1. Anal. Calcd for $C_{24}H_{20}ClN_5O$: C, 67.05, H, 4.69; N, 16.29. Found: C, 66.87; H, 4.80; N, 16.45.

*2-(4-((1-(3-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-methyl-1H-benzo[d]imidazole (**6n**)*

Yield: 80%. Mp: 237-238 °C. IR (KBr): 3225, 2919, 2850, 1612, 1495 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 2.43 (s, 3H, Me), 5.24 (s, 2H, CH_2), 5.65 (s, 2H, CH_2), 6.97-7.01 (m, 2H, H4, H6"), 7.19 (d, J = 8.0 Hz, 2H, H3′, H5′), 7.27-7.49 (m, 5H, H5, H7, H2", H4", H5"), 8.08

(d, J = 8.0 Hz, 2H, H2′, H6′), 8.35 (s, 1H, triazole), 12.57 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 21.2, 52.0, 61.1, 110.7, 115.0, 118.0, 118.2, 120.8, 122.8, 123.2, 123.4, 124.8, 126.6, 127.8, 128.1, 130.6, 131.2, 131.5, 138.3, 141.7, 142.8, 159.3. Anal. Calcd for $C_{24}H_{20}ClN_5O$: C, 67.05, H, 4.69; N, 16.29. Found: C, 67.28; H, 4.84; N, 16.10.

*2-(4-((1-(2,3-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-methyl-1H-benzo[d]imidazole (**6o**)*

Yield: 80%. Mp: 248-250 °C. IR (KBr): 3198, 2918, 2855, 1612, 1583, 1448 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 2.42 (s, 3H, Me), 5.25 (s, 2H, CH_2), 5.79 (s, 2H, CH_2), 6.99 (d, J = 7.5 Hz, 1H, H4), 7.18-7.23 (m, 3H, H3′, H5′, H6″), 7.38-7.44 (m, 3H, H5, H7, H5"), 7.67 (d, J = 8.0 Hz, 1H, H4″), 8.09 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.33 (s, 1H, triazole), 12.59 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 21.2, 51.1, 61.1, 115.1, 118.5, 123.2, 123.3, 125.2, 125.3, 126.2, 127.8, 128.5, 128.9, 130.5, 132.2, 132.3, 133.3, 134.1, 135.8, 142.6, 145.2, 159.1. Anal. Calcd for $C_{24}H_{19}Cl_2N_5O$: C, 62.08, H, 4.12; N, 15.08. Found: C, 62.29; H, 4.40; N, 15.32.

*2-(4-((1-(3,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-methyl-1H-benzo[d]imidazole (**6p**)*

Yield: 83%. Mp: 225-226 °C. IR (KBr): 3201, 3157, 3031, 2919, 1612, 1585 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-d_6): 2.42 (s, 3H, Me), 5.24 (s, 2H, CH_2), 5.65 (s, 2H, CH_2), 6.99 (d, J = 8.0 Hz, 1H, H4), 7.18 (d, J = 8.5 Hz, 2H, H3′, H5′), 7.32 (dd, J = 7.2, 1.5 Hz, 1H, H6″), 7.40-7.66 (m, 4H, H5, H7, H2″, H5″), 8.08 (d, J = 8.5 Hz, 2H, H2′, H6′), 8.36 (s, 1H, triazole), 12.57 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 21.2, 51.4, 61.2, 115.0, 118.1, 123.2, 124.2, 124.9, 125.2, 125.8, 126.1, 126.3, 127.8, 128.3, 130.1, 130.9, 131.2, 136.8, 138.8, 142.9, 148.5, 159.1. Anal. Calcd for $C_{24}H_{19}Cl_2N_5O$: C, 62.08, H, 4.12; N, 15.08. Found: C, 61.87; H, 3.89; N, 14.85.

*2-(4-((1-(4-Bromobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-6-methyl-1H-benzo[d]imidazole (**6q**)*

Yield: 81%. Mp: 208-209 °C. IR (KBr): 3421, 3194, 2983, 2930 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 2.42 (s, 3H, Me), 5.23 (s, 2H, CH_2), 5.61 (s, 2H, CH_2), 6.99 (d, *J* = 7.5 Hz, 1H, H4), 7.18 (d, *J* = 8.5 Hz, 2H, H3′, H5′), 7.28 (d, *J* = 8.5 Hz, 2H, H2″, H6″), 7.39-7.47 (m, 2H, H5, H7), 7.58 (d, *J* = 8.5 Hz, 2H, H3″, H5″), 8.08 (d, *J* = 8.5 Hz, 2H, H2′, H6′), 8.30 (s, 1H, triazole), 12.52 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 21.2, 52.0, 61.2, 110.8, 115.0, 118.1, 121.4, 122.7, 122.8, 123.2, 123.5, 124.7, 127.8, 130.1, 131.6, 133.9, 135.3, 142.8, 143.5, 159.1. Anal. Calcd for $C_{24}H_{20}BrN_5O$: C, 60.77, H, 4.25; N, 14.76. Found: C, 60.91; H, 4.42; N, 14.53.

*6-Methyl-2-(4-((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)-1H-benzo[d]imidazole (**6r**)*

Yield: 85%. Mp: 114-116 °C. IR (KBr): 3201, 3157, 3031, 2919, 1612, 1585 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): 2.42 (s, 3H, Me), 5.26 (s, 2H, CH_2), 5.81 (s, 2H, CH_2), 6.99 (d, *J* = 8.0 Hz, 1H, H4), 7.20 (d, *J* = 8.0 Hz, 2H, H3', H5'), 7.35 (s, 1H, H7), 7.44 (d, *J* = 8.0 Hz, 1H, H5), 7.55 (d, *J* = 8.5 Hz, 2H, H2″, H6″), 8.10 (d, *J* = 8.0 Hz, 2H, H2′, H6′), 8.24 (d, *J* = 8.5 Hz, 2H, H3″, H5″), 8.38 (s, 1H, triazole), 12.60 (s, 1H, NH). ^{13}CNMR (125 MHz, DMSO-d_6): 21.2, 51.9, 61.2, 115.1, 115.2, 118.2, 123.0, 123.2, 123.8, 124.0, 124.8, 125.1, 125.5, 127.8, 129.0, 134.8, 141.0, 142.9, 143.3, 159.2. Anal. Calcd for $C_{24}H_{20}N_6O_3$: C, 65.45, H, 4.58; N, 19.08. Found: C, 65.30; H, 4.37; N, 18.84.

Tyrosinase inhibition assay

Mushroom tyrosinase (EC 1.14.18.1) (Sigma Chemical Co.) inhibitory activity was evaluated using L-DOPA as substrate and formation of dopachrome was observed at 475 nm. All test samples were first dissolved in DMSO at 40 mM and diluted to the required

concentrations. First, 10 μL of tyrosinase (0.5 $mg.mL^{-1}$) was mixed with 160 μL of 50 mM phosphate buffer (pH 6.8) and then 10 μL of the test sample was added to 96-well microplates. After pre-incubation at 28 °C for 20 min, 20 μL of L-DOPA solution (0.5 mM) was added to the each well. DMSO without test compound was used as the control and kojic acid was used as the positive control. Each assay was conducted as three separate replicates. The inhibitory effects of the tested compounds were expressed as the concentrations that inhibited 50% of the enzyme activity (IC_{50}).

Kinetic study

A series of experiments were performed to determine the inhibition kinetics of compound **6g**. The inhibitor concentrations were 10 and 50 μM. Substrate L-DOPA concentrations were 0.000, 0.125, 0.250, 1.000 and 2.000 mM in all kinetic studies. Pre-incubation and measurement time was as the same as mushroom tyrosinase inhibition assay. The tyrosinase inhibition rate was then calculated using Lineweaver-Burk plot analysis. The Michaelis constant (K_m) and maximal velocity (V_{max}) were also calculated by Lineweaver-Burk plots with different concentrations of L-DOPA up to ten minutes after adding L-DOPA during a one minute interval.[24]

Molecular docking study

Molecular docking study was achieved using AutoDock 4.2 and AutoDock Tools 1.5.4 (ADT). The X-ray crystal structure of tyrosinase carrying tropolone as innate ligand in binding site (PDB code: 2Y9X) were obtained from protein data bank (http://www.rcsb.org). Water molecules and tropolone were removed and PDB file of protein was regenerated by adding polar hydrogen and computing Gasteiger charges. Structures of ligands were sketched and optimized (by molecular mechanics, MM+, and semi-empirical, AM1, methods) using

Hyperchem software. The PDBQT files were generated by adding charges and defining the degree of torsions. In order to create the grid parameter file, a grid of 40, 40, and 40 points in x, y, and z directions with 0.375 Å grid spacing was built using AutoGrid. Rigid macromolecule and Lamarckian Genetic Algorithm (LGA), with 100 GA runs was selected to generate the docking parameter file.

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Author Contribution Statement

Mohammad Mahdavi, Arsalan Ashtari, Mahsima Khoshneviszadeh, Sara Ranjbar, and Ameneh Dehghani synthesized compounds, performed biological tests, docking studies, and wrote the article. Tahmineh Akbarzadeh and Bagher Larijani analysed data. Mehdi Khoshneviszadeh and Mina Saeedi designed the experiments and wrote the article.

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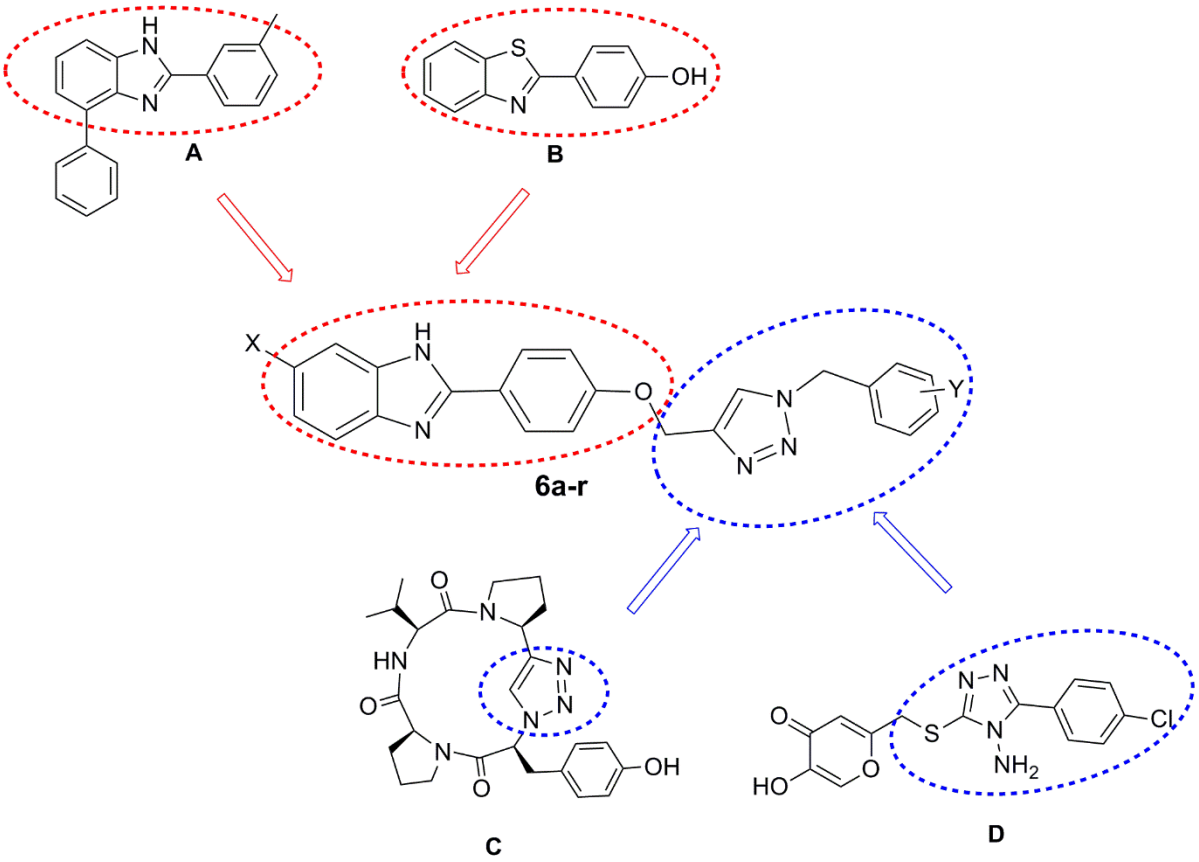
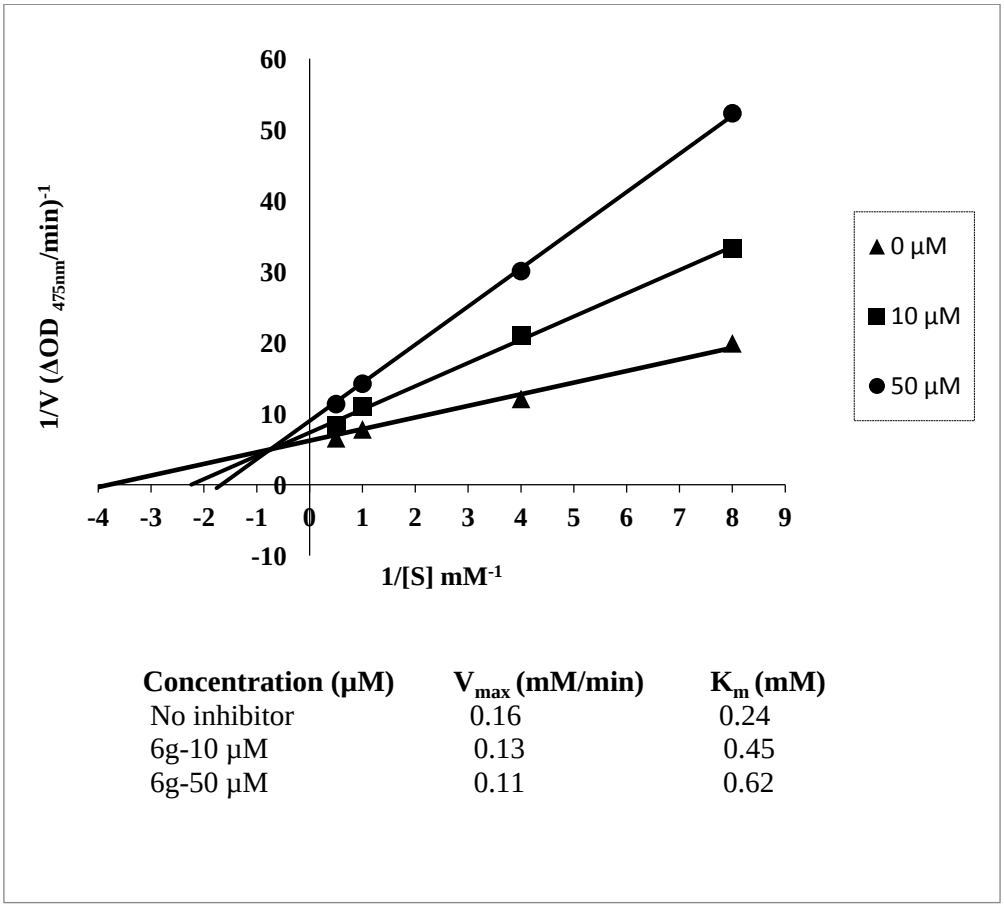


Fig. 1. Design of tyrosinase inhibitors using molecular hybridization approach.



Concentration (µM)	V_{max} (mM/min)	K_m (mM)
No inhibitor	0.16	0.24
6g-10 µM	0.13	0.45
6g-50 µM	0.11	0.62

Fig. 2. Lineweaver−Burk plot of mushroom tyrosinase enzyme inhibition by different concentrations of **6g** in the presence of L-DOPA as a substrate. The reciprocal tyrosinase inhibitory activity was plotted against the reciprocal substrate concentration (double reciprocal plot, n = 3). K_m is the Michaelis-Menten constant and V_{max} is the maximum reaction velocity.

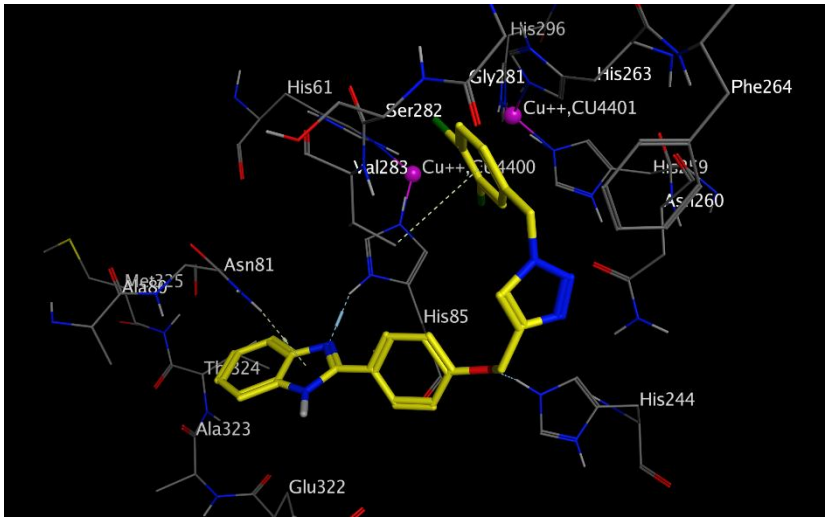


Fig. 3. Interactions and binding mode of compound **6g** into tyrosinase (2Y9X).

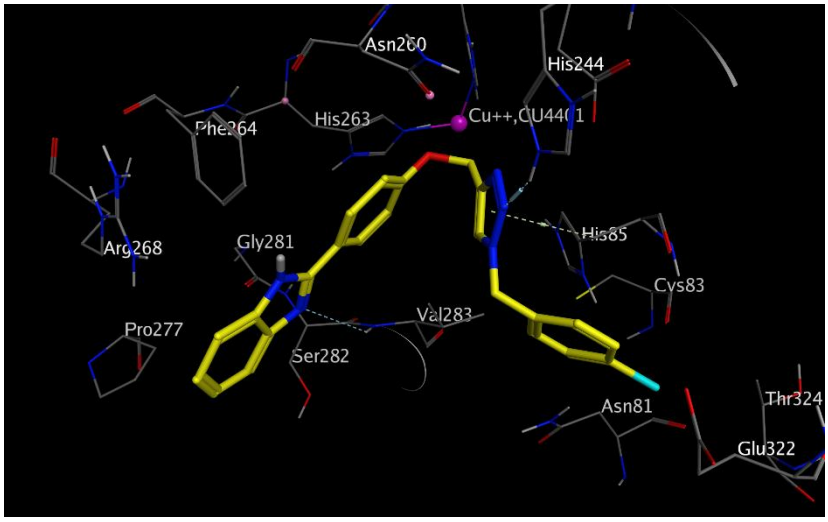
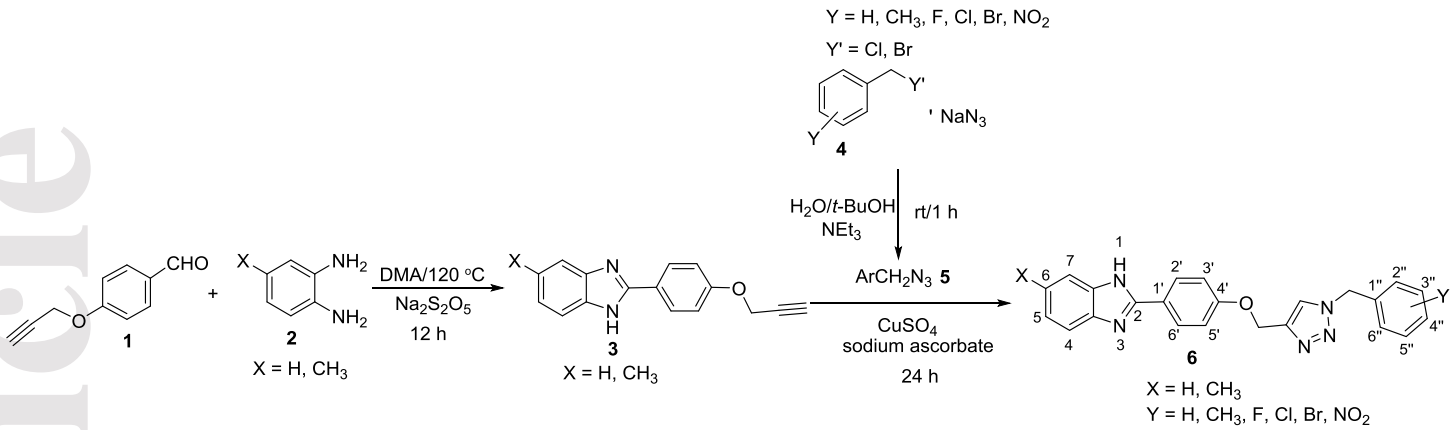


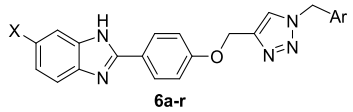
Fig. 4. Interactions and binding mode of compound **6h** into tyrosinase (2Y9X).



Scheme 1. Synthesis of benzimidazole-1,2,3-triazole hybrids **6**.

Table 1

Structure of synthesized compounds **6a-r** and their inhibitory effects on mushroom tyrosinase.



6a-r

Entry	Compound **6**	X	Ar	IC_{50}[a](μM)
1	**6a**	H	Phenyl	>50
2	**6b**	H	4-Methylphenyl	>50
3	**6c**	H	2-Fluorophenyl	45.22 ± 1.15
4	**6d**	H	4-Fluorophenyl	46.89 ± 0.52
5	**6e**	H	2-Chlorophenyl	>50
6	**6f**	H	2,3-Dichorophenyl	>50
7	**6g**	H	3,4-Dichlorophenyl	9.42 ± 0.60
8	**6h**	H	4-Bromophenyl	10.34 ± 1.06
9	**6i**	H	4-Nitrophenyl	48.00 ± 1.05
10	**6j**	CH_3	Phenyl	43.30 ± 0.75
11	**6k**	CH_3	4-Methylphenyl	>50
12	**6l**	CH_3	4-Fluorophenyl	29.31 ± 0.89
13	**6m**	CH_3	2-Chlorophenyl	>50
14	**6n**	CH_3	3-Chlorophenyl	>50
15	**6o**	CH_3	2,3-Dichorophenyl	>50
16	**6p**	CH_3	3,4-Dichorophenyl	>50
17	**6q**	CH_3	4-Bromophenyl	>50

18	**6r**	CH_3	4-Nitrophenyl	>50
20	Kojic acid	-	-	9.28±0.81

[a] 50 % inhibitory concentration (IC_{50}). Values represent means ± SE of four independent experiments