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Synthesis and Biological Evaluation of Di-Substituted Thiotriazole Linked to Quinazolinone as a Tyrosinase Inhibitor

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Abstract

In this study, a series of di-substituted thiotriazole linked to quinazolinone derivatives (6a–p) were synthesized and evaluated for their tyrosinase inhibitory activity. The inhibitory activities of the compounds were measured and compared to the reference inhibitor, kojic acid. Compound 6n (R_1: benzyl, R_2: 4-nitrophenyl) exhibited the most potent inhibition (IC_{50} = 16.26 ± 2.57 µM), surpassing kojic acid (IC_{50} = 23.6 ± 2.56 µM). Molecular docking studies provided further insight into the binding interactions of compound 6n, demonstrating key hydrogen bonding and π-π stacking interactions with important residues such as Val283, Phe264, His244, and Asn81. These findings suggest that the substituted thiotriazole linked to quinazolinone derivatives hold promise as potent tyrosinase inhibitors, with potential therapeutic applications in the treatment of hyperpigmentation disorders and other melanin-related conditions.

Keywords: Molecular docking, Tyrosinase, Thiotriazole, Quinazolinone.

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Introduction

Tyrosinase is a multifunctional copper-containing enzyme that plays a pivotal role in melanogenesis, the biochemical process responsible for melanin production in living organisms. Melanin, a natural pigment, is found in humans' skin, hair, eyes, and other species, providing essential photoprotective functions by absorbing ultraviolet (UV) radiation and scavenging free radicals [1,2]. However, the dysregulation of melanin synthesis can lead to various dermatological conditions such as hyperpigmentation, melasma, and age spots, as well as contribute to the browning of fruits and vegetables in the food industry [3-5].

The catalytic action of tyrosinase involves the oxidation of tyrosine to dopaquinone, which subsequently undergoes a series of chemical transformations to form melanin. While melanin is crucial for protection against UV-induced damage, its overproduction can have undesirable effects, necessitating the development of effective tyrosinase inhibitors to regulate its activity [6].

As a result, potent tyrosinase inhibitors have garnered significant attention in medicinal chemistry due to their applications in treating hyperpigmentation disorders, developing skin-whitening agents, and extending the shelf life of food products [7,8].

Quinazolinones are nitrogen-containing bicyclic compounds that exhibit remarkable biological activities, including enzyme inhibition. Their planar and aromatic structure allows effective π-π stacking with tyrosinase residues, while functional substitutions at specific positions can enhance binding affinity and selectivity [9-11]. These features make quinazolinone-based derivatives attractive candidates for targeting tyrosinase in the treatment of hyperpigmentation disorders and other melanin-related conditions [12,13].

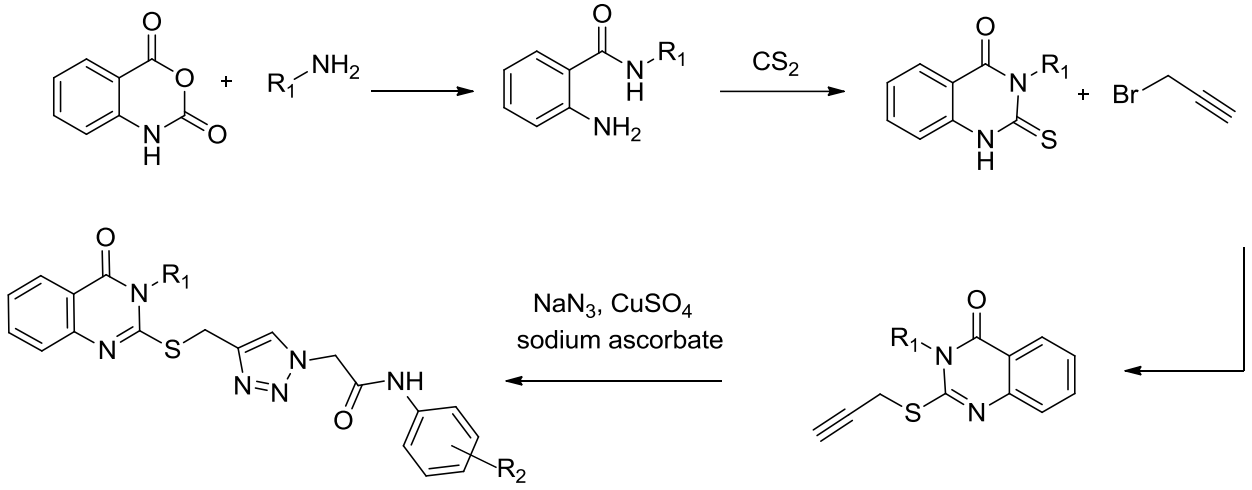
Similarly, thio-triazole derivatives are sulfur-containing five-membered heterocycles with potent antioxidant and enzyme-modulating properties. The sulfur atom in thio-triazoles can interact with the copper ions in tyrosinase's active site through chelation, effectively blocking its catalytic function [14]. Furthermore, thio-triazoles can act as radical scavengers, providing an additional mechanism for mitigating oxidative stress associated with melanogenesis [15].

As a result, in the current study, quinazolinone and thio-triazole moieties, previously identified as tyrosinase inhibitors, were coupled to develop hybrid compounds with improved potency and selectivity. Sixteen derivatives were synthesized, and their tyrosinase inhibitory activities were evaluated to elucidate the structure-activity relationship (SAR).

Experimental

Chemistry

A mixture of isatoic anhydride (2 mmol) and the appropriate amine (2 mmol) was stirred in water (5 mL) at room temperature for 30 minutes, yielding a white precipitate that was filtered and used without further purification. This precipitate (1 mmol) was then reacted with carbon disulfide (1.2 mmol) and KOH (1.5 mmol) in ethanol (5 mL) under reflux for 12 hours. After reaction completion (monitored by TLC), the mixture was poured into cold, acidified water (pH = 5) to obtain 3-R-2-thioxo-2,3-dihydroquinazolin-4(1H)-one, which was filtered, washed, and used for the next step. This compound (2 mmol) was reacted with propargyl bromide (2 mmol) and K_2CO_3 (2.4 mmol) in DMF (10 mL) at room temperature for 8 hours. After completion, water (100 mL) was added, and the precipitate was filtered, dried under reduced pressure, and purified by recrystallization in ethyl acetate. Finally, the pure compound (1 mmol) was reacted with in situ prepared azide derivatives in a mixture of H_2O (5 mL) and t-BuOH (5 mL) containing sodium ascorbate (15 mol%) and $CuSO_4$ (7 mol%) at room temperature for 17–23 hours. The reaction mixture was then poured into crushed ice, and the resulting precipitate was filtered, washed with water, and purified by recrystallization in ethyl acetate.



Scheme 1. Synthesis of **6a-p.**

Tyrosinase assay

The half-maximal inhibitory concentration (IC_{50}) is the most widely used and informative measure of a drug's efficacy. Many drugs are believed to exert their biological effect because of enzyme inhibition. One approach to the understanding of the mechanism of action of such drugs has been to study the effect of drug concentration on the rate of reaction of an isolated enzyme [16]. The mushroom tyrosinase (EC 1.14.18.1) assay was performed using L-Dopa as the substrate, following a modified spectrophotometric method to monitor dopachrome production at 490 nm. Test

compounds and kojic acid were dissolved in DMSO and diluted to the desired concentrations. In a 96-well microplate, 10 µl of each sample was mix0ed with 160 µl of 50 mM phosphate buffer (pH 6.8), followed by the addition of 10 µl of tyrosinase enzyme (600 U mL^{-1}). After a 20-minute pre-incubation at 28 °C, 20 µl of L-Dopa solution (final concentration 0.7 mM) was added. The reaction was incubated for 10 minutes, and absorbance was measured. The inhibitory activity was quantified as IC_{50} values, representing the concentration needed to inhibit 50% of the enzyme activity [17,18].

Molecular docking

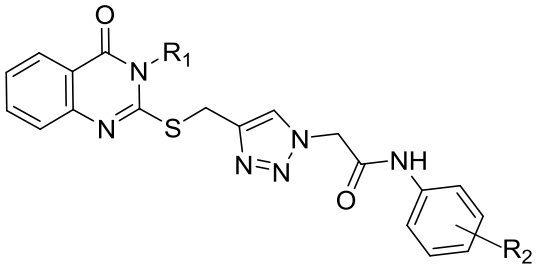
Molecular docking aims to predict the ligand-receptor complex through computer-based methods. The process of docking involves two main steps, which include sampling the ligand and utilizing a scoring function. Sampling algorithms help to identify the most energetically favorable conformations of the ligand within the protein's active site, taking into account their binding mode. These confirmations are then ranked using a scoring function. Molecular docking is widely used in hit identification in drug discovery. It helps in identifying potential drug candidates by predicting the binding affinity of small molecules to a protein or receptor of interest. Docking can be used to screen a large database of small molecules to identify those that can bind to a protein of interest with high affinity [19]. Molecular docking studies for the most potent analog was conducted using Schrödinger Suite's Maestro platform. The X-ray crystallographic structure of tyrosinase (PDB ID: 2Y9X) was obtained from the RCSB Protein Data Bank. Protein preparation involved removing co-crystallized atoms and water molecules, filling missing loops using the Prime tool, and optimizing the structure at pH 7.4 with EPIK and PROPKA to assign H-bonding interactions. Ligands were designed in Chem Draw and prepared as SDF files, optimized with the OPLS3e force field. An induced-fit docking approach was employed, with van der Waals radii set to 0.7 for the receptor and 0.5 for the ligand, to analyze ligand-receptor interactions [20, 21].

Results and discussion

Synthesis

Sharafati et al [22] reported the results of these syntheses data with other method. The melting points of these compounds were completely consistent. The melting points of compounds 6a–p are shown in Table 1.

Table 1. Melting points of **6a-p.**



Compound	R_1	R_2	M.p °C
6a	Isopropyl	2,4-Dimethyl phenyl	195-197
6b	Isopropyl	3-Chloro phenyl	213-214
6c	Isopropyl	4-Bromo phenyl	232-233
6d	Isopropyl	4-Methyl phenyl	208-210
6e	Isopropyl	4-Nitro phenyl	198-200
6f	Isopropyl	Phenyl	213-215
6g	Phenyl	3,5-Dimethyl phenyl	206-208
6h	Phenyl	3-Chloro phenyl	220-223
6i	Phenyl	4-Bromo phenyl	185-187
6j	Phenyl	4-Nitro phenyl	213-232
6k	Phenyl	Phenyl	224-226
6l	Benzyl	3,5-Dimethyl phenyl	219-221
6m	Benzyl	4-Bromo phenyl	198-199
6n	Benzyl	4-Nitro phenyl	211-212
6o	Benzyl	Phenyl	223-225
6p	Benzyl	3-Chloro phenyl	238-239

Tyrosinase inhibitory activity

The tyrosinase inhibitory activity of compounds 6a–p, along with the reference inhibitor kojic acid (IC_{50} = 23.6 ± 2.56 µM), is presented in Table 2. To evaluate SARs, the compounds were categorized into three groups based on the substitution at the R_1 position: isopropyl, phenyl, or benzyl, with varied R_2 substituents.

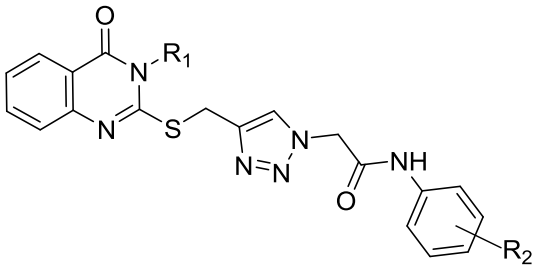
Compounds 6a–f, (R_1 = isopropyl): These compounds displayed IC_{50} values ranging from 20.64 ± 1.79 µM (6a) to 32.68 ± 2.67 µM (6f). The lowest IC_{50} was observed for compound 6a (R_2 = 2,4-dimethyl phenyl), indicating that electron-donating groups at R_2, such as methyl, enhanced the inhibitory potency. Electron-withdrawing groups like 3-chloro (6b) and 4-bromo (6c) also contributed to moderate activity.

Compounds 6g–k (R_1 = phenyl): Compounds in this group exhibited IC_{50} values between 20.44 ± 2.19 µM (6k) to 35.48 ± 4.91 µM (6j). Compound 6k (R_2 = phenyl) demonstrated the highest potency, with an IC_{50} comparable to that of 6a. In contrast, compounds with electron-withdrawing groups such as 4-nitro (6j) showed lower inhibitory activity, possibly due to steric hindrance at the active site.

Compounds 6l–p (R_1 = benzyl): These compounds showed IC_{50} values ranging from 16.26 ± 2.57 µM (6n) to 35.22 ± 1.88 µM (6o). The best activity was observed for compound 6n (R_2 = 4-nitro phenyl), highlighting the favorable impact of electron-withdrawing groups in this subgroup. Compound 6l (R_2 = 3,5-dimethyl phenyl) also showed significant activity, suggesting that the bulkiness and electronic effects of the substituents at R_2 modulate activity.

Overall, it can be indicated that tyrosinase inhibition is influenced by both the electronic nature and steric effects of the R_1 and R_2 substituents. The most potent analog among all derivatives is compound 6n. While electron-donating groups generally enhanced activity in Group 1, electron-withdrawing groups improved potency in Group 3. The phenyl group at R1 provided optimal interactions for compounds in Group 2, as seen in 6k. These findings offer guidance for designing more potent tyrosinase inhibitors by strategically modifying substituents to optimize enzyme binding.

Table 2. Tyrosinase inhibitory activity of **6a-p**



Compound	R_1	R_2	IC_{50} µM
6a	Isopropyl	2,4-Dimethyl phenyl	20.64±1.79
6b	Isopropyl	3-Chloro phenyl	30.97±2.68
6c	Isopropyl	4-Bromo phenyl	24.76±3.19
6d	Isopropyl	4-Methyl phenyl	25.86±4.67
6e	Isopropyl	4-Nitro phenyl	24.77±5.12
6f	Isopropyl	Phenyl	32.68±2.67
6g	Phenyl	3,5-Dimethyl phenyl	34.62±1.67
6h	Phenyl	3-Chloro phenyl	26.71±2.42
6i	Phenyl	4-Bromo phenyl	25.37±1.13

6j	Phenyl	4-Nitro phenyl	35.48±4.91
6k	Phenyl	Phenyl	20.44±2.19
6l	Benzyl	3,5-Dimethyl phenyl	18.20±4.52
6m	Benzyl	4-Bromo phenyl	27.31±1.43
6n	Benzyl	4-Nitro phenyl	16.26±2.57
6o	Benzyl	Phenyl	35.22±1.88
6p	Benzyl	3-Chloro phenyl	29.08±2.24
Kojic acid	-	-	23.6±2.56

Molecular Docking Study

To investigate the binding pose of compound 6n in the tyrosinase active site, a molecular docking study was performed, as shown in Fig. 1. The quinazoline moiety forms an H-bond interaction with Val283 and engages in π-π stacking with Phe264. The amino methyl triazole linker contributes to two H-bonds with Glu322 and His85, along with π-π stacking interactions with His244. Lastly, the terminal 4-nitrophenyl group establishes an H-bond with Asn81, highlighting the key interactions responsible for its inhibitory activity.

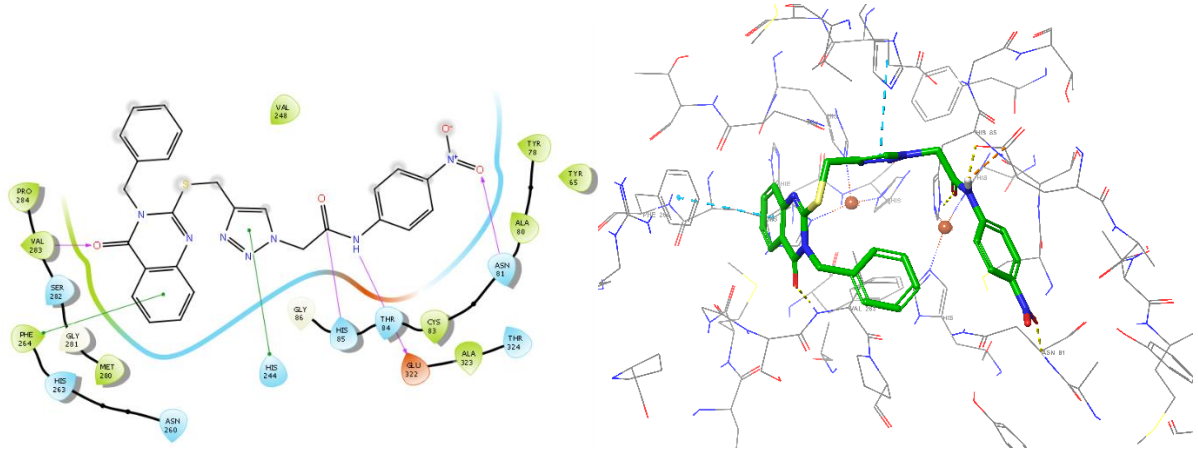


Figure1. 2D and 3D pose of 6l-BChE.

Conclusion

In this study, di-substituted thio-triazole linked to quinazolinone derivatives were synthesized and evaluated as potential tyrosinase inhibitors. A series of 16 compounds were synthesized and assessed for their inhibitory activity against tyrosinase, with compound **6n** (R_1: benzyl, R_2:4-nitrophenyl) showing the most potent inhibition (IC_{50} = 16.26 ± 2.57 µM), outperforming the reference inhibitor kojic acid (IC_{50} = 23.6 ± 2.56 µM). SAR analysis revealed that both the electronic nature and steric effects of substituents at the R_1 and R_2 positions significantly influenced

inhibitory potency. Electron-donating groups at R_2 enhanced activity in compounds with isopropyl and phenyl groups at R_1, while electron-withdrawing groups at R_2 improved potency in compounds with benzyl groups at R_1. Molecular docking studies confirmed the key interactions between compound 6n and the tyrosinase active site, including hydrogen bonding and π-π stacking interactions with critical residues such as Val283, Phe264, His244, and Asn81. These findings provide a foundation for designing tyrosinase inhibitors, with potential applications in treating hyperpigmentation disorders and other melanin-related conditions.

Acknowledgments

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