# TiPS

# Novel heterocyclic hybrid of 2-(aryl)-1H-indene-1,3(2H)-dione targeting tyrosinase: design, biological evaluation and in silico studies

Aida Iraji[1,3], Ali Nemati[2], Hona Hosseinpoor[2], Najmeh Edraki[1], Mahsima Khoshneviszadeh[1], Mahshid Attarroshan[1], Hossein Sadeghpour[2], Mehdi Khoshneviszadeh[1,2,*]

[1]Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Department of Medicinal Chemistry, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
[3]Central Research Laboratory, Shiraz University of Medical Sciences, Shiraz, Iran.

## Abstract

Melanogenesis is a process of melanin synthesize, which is a primary response for the pigmentation of human skin. Tyrosinase is a key enzyme, which catalyzes a rate-limiting step of the melanin formation, natural products have shown potent inhibitors, but some of these possess toxicity. Numerous synthetic inhibitors have been developed in recent years may lead to the potent anti-tyrosinase agents. Therefore its inhibition may be an efficient way for the development of depigmenting agents. A novel series of 2-arylidine-1H-indene-1,3(2H)-dione analogs were designed, synthesized and screened for their in vitro tyrosinase inhibitory activity. **3d** derivative bearing nitrothiophene revealed excellent anti-tyrosinase activity with an $IC_{50}$ value of 3.55 μM comparable to kojic acid as a positive control. **3d** as the most potent inhibitor and **3f** as the least active derivative were subjected to *in silico* evaluations considering the 3D conformations, ΔGb of bindings and interactions within the active site of tyrosinase.

## 1. Introduction

Tyrosinase (TYR) is exploited for a variety of biological and environmental applications. TYR has attracted lots of attention as the main cause of disease resulting from overproduction of melanin, as well as premature skin aging and carcinogenesis (1). There is growing evidence that TYR is also involved in neurodegenerative disorders as well as undesired browning of fruits, vegetables, and crops (2, 3). Therefore, controlling the activity of the enzyme by TYR inhibitors believed to avoid side effects of hyper pigmentary disorders of mammals as well as enzymatic browning of fruits and fungi.

TYR is a highly conserved enzyme present in many species including microbial organisms, plants, and humans (4). Structurally, TYR (EC 1.14.18.1) consists of a binding pocket located between the N-terminal, and the C-terminal domains (5). The catalytic center formed by binuclear type-3 coppers embedded in the binding pocket (known as CuA and CuB) which is coordinated by six histidine residues. TYR catalyzes the orthohydroxylation of monophenol and the subsequent oxidation of the diphenolic product to the resulting quinone (6). The final synthesized product (quinone) considered as a reactive precursor for the synthesis of melanin pigments and downstream signaling happened as a result (7). Over the past few years, various TYR inhibitors discovered from different sources, including synthetic compounds, natural products and even by in silico based screening (1). Several pharmacologically active structural units,

*Corresponding Author*: Mehdi Khoshneviszadeh, Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran & Department of Medicinal Chemistry, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
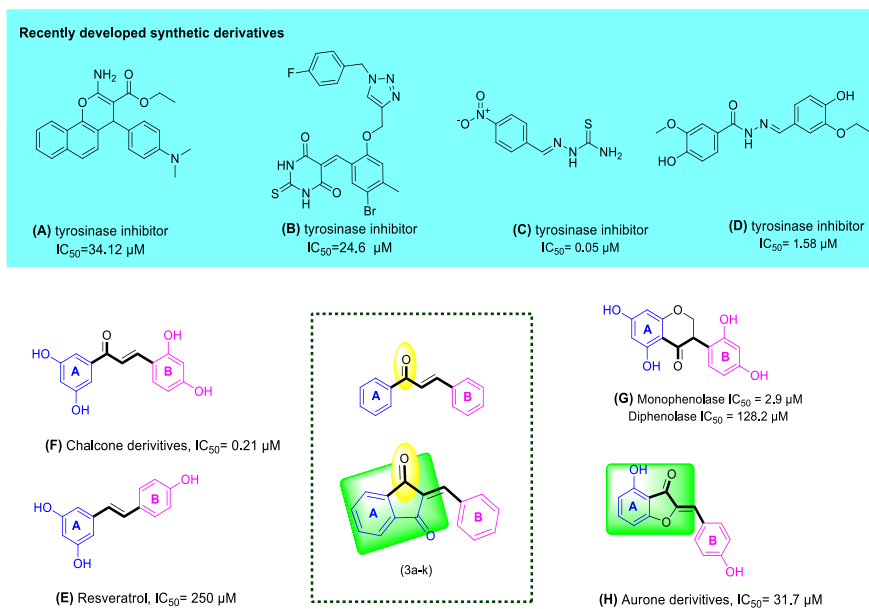Email: M.khoshneviszadeh@gmail.com

Figure 1. Chemical structures of some tyrosine inhibitors and newly designed compounds, green squre is pharmacophore of designed compounds.

including benzochromene (Figure 1, compound A) (8), triazine (9), triazole (Figure 1, compound B) (10), thiosemicarbazone (Figure 1, compound C) (5, 11) and benzylidenehydrazine (Figure 1, compound D) (12) are being explored to identify novel lead anti-TYR molecules.

Indene-1,3(2H)-dione-containing compounds have shown a broad range of biological activities such as anticancer (13, 14), antibacterial(15) , anti-inflammatory (16), anti-β-amyloid aggregation (17) and anti-diabetes activities (18).

Considering the above-mentioned finding, 2-arylidine-1H-indene-1,3(2H)-dione were designed, synthesized and assessed as tyrosinase inhibitors. Moreover, to gain information on the ligand–receptor interactions, a molecular docking analysis was performed.

## 2. Materials and methods

All reagents were reagent grade quality and obtained from Sigma-Aldrich (Prague, Czech Republic). The reaction process was monitored using thin-layer chromatography on the glass-backed silica gel sheets (Silica Gel 60 GF254) and visualized under UV light (254 nm). 1H and 13C NMR spectra were determined by a Bruker FT-300 MHz spectrometer in DMSO-d6. All the chemical shifts were reported as (δ) values (ppm). The infrared (IR) spectra were run as KBr disk on Perkin-Elmer

Spectrum RXI FTIR.

## 2.1 General procedure for the synthesis of 2-(aryl)-1H-indene-1,3(2H)-dione derivatives (3a-k)

Required 2-(Aryl)-1H-indene-1,3(2H)-dione was exactly prepared according to our previous report (19). Briefly, 1H-indene-1,3(2H)-dione (1, 1 mmol) and different aldehydes (2, 2 mmol) was added to 10 mL EtOH in the presence of the catalytic amount of acetic acid. The mixture was refluxed for 24 h. After completion of the reaction (checked by TLC), the solvent was removed and the solid product was recrystallized from ethyl acetate and petroleum ether.

### 2-benzylidene-1H-indene-1,3(2H)-dione (3a)
Yield: 84%. M.p: 145-147 ºC. I.R (KBr, cm-1): CH-aromatic: 3071; C=O: 1694, 1717.1H-NMR (CDCl3, 300 MHz) δH (ppm): 7.40 (s, 1H, H-3'), 7.51 (s, 1H, H-5'), 7.86-8.03 (m, 6H, H-5,6,7,8,2',6'), 8.26 (s, 1H, -C=C-ph), 8.70-8.73 (m, 1H, H-4'). 13C-NMR (CDCl3, 75 MHz) δc (ppm): 123.1, 123.5, 127.1, 127.7, 129.7, 134.5, 135.7, 138.2, 138.5, 138.9, 140.5, 142.4, 188.5, 189.3.

### 2-(4-hydroxybenzylidene)-1H-indene-1,3(2H)-dione (3b)

Yield: 54%. M.p: 235-237 ℃; I.R (KBr, cm-1): OH: 3125; CH-aromatic: 2926; C=O, 1680, 1717. 1H-NMR (CDCl3, 300 MHz) δH (ppm): 6.94 (d, 2H, H-3',5', J=8.7 Hz), 7.74 (s, 1H, -C=C-ph), 7.92 (d, 4H, H-6,7,2',6', J= 2.7 Hz), 8.53 (d, 2H, H-5,8, J= 8.7 Hz), 10.87 (s, 1H, 4'-OH); 13C-NMR (CDCl3, 75 MHz) δc (ppm): 39.7, 39.9, 40.2, 118.4, 123.1, 123.2, 125, 125.6, 135.8, 136, 139.7, 142.1, 146.7, 163.7, 189.4, 190.4.

## 2-(4-bromobenzylidene)-1H-indene-1,3(2H)-dione (3c)

Yield: 77%. m.p.: 170-172 ℃. I.R(KBr, cm-1): CH-aromatic: 3056; C=O: 1691, 1727. 1H-NMR (CDCl3-d6, 300 MHz) δH (ppm): 7.55 (d, 2H, H-3',5', J= 8.4 Hz), 7.71-7.75 (m, 3H, H-2',6', -C=CH-phenyl), 7.91-7.93 (m, 2H, H-6,7), 8.25 (d, 2H, H-5,8, J= 8.4 Hz); 13C-NMR (CDCl3-d6, 75 MHz) δc (ppm): 76.6, 77, 77.3, 77.4, 123.4, 128.3, 129.6, 131.9, 132.1, 135.3, 135.5, 140, 142.5, 145.2, 188.9, 189.9.

## 2-((5-nitrothiophen-2-yl)methylene)-1H-indene-1,3(2H)-dione (3d)

Yield: 72%; M.p: 298-300 ℃. I.R(KBr, cm-1): CH-aromatic: 3090; N=O: 1342; N-O: 1497; C=O: 1687, 1729. 1H-NMR (CDCl3-d6, 300 MHz) δH (ppm): 7.99-8.02 (m, 4H, H-5,6,7,8), 8.09 (s, 1H, -C=CH-th), 8.18 (s, 2H, H-4', H-5')

## 2-(4-(dimethylamino)benzylidene)-1H-indene-1,3(2H)-dione (3e)

Yield: 70 %; M.p: 204-206 ℃; I.R (KBr, cm-1): C-N: 1188; CH-aromatic: 3031; CH-aliphatic: 2855; C=O: 1662; 1722. 1H-NMR (CDCl3-d6, 300 MHz) δH (ppm): 3.14 (s, 6H, NCH3), 6.73 (d, 2H, H-3',5', J= 9.3 Hz), 7.71-7.74 (m, 2H, H-6,7), 7.77 (s, 1H, C=CH-ph), 7.90-7.94 (m, 2H, H-5,8), 8.53 (d, 2H, H-2'-6', J= 8.7 Hz); 13C-NMR (CDCl3-d6, 75 MHz) δc (ppm): 40, 76.6, 77, 77.4, 111.3, 121.9, 122.4, 122.9, 134, 134.3, 135.3, 137.9, 139.8, 142.2, 147.4, 153.9, 189.9, 191.7 .

## 2-(3-nitrobenzylidene)-1H-indene-1,3(2H)-dione (3f)

Yield: 92%. M.p: 243-245 ℃. I.R(KBr, cm-1): N=O: 1351; N-O: 1530; CH-aromatic: 3071; CH-aliphatic: 2925; C=O: 1687, 1732; 1H-NMR

(CDCl3-d6, 300 MHz) δH (ppm): 8.01-8.05 (m, 6H, H-5,6,7,8,5',6'), 8.42 (d, 1H, H-4', J= 4.5 Hz), 8.69-8.70 (m, 1H, -C=C-ph), 9.57-9.58 (m, 1H, H-2').

## 2-(3-ethoxy-4-hydroxybenzylidene)-1H-indene-1,3(2H)-dione (3g)

Yield: 88%. M.p: 240-242 ℃. I.R(KBr, cm-1): OH: 3445; CH-aromatic: 3064; CH-aliphatic: 2854; C=O: 1670, 1708. 1H-NMR (CDCl3-d6, 300 MHz) δH (ppm): 1.44-1.48 (m, 3H, OCH3), 4.25- 4.29 (m, 2H, OCH2CH3), 6.40 (s, 1H, H-5'), 6.93 (d, 1H, H-2', J= 4.2 Hz), 7.53-7.57 (m, 1H, H-6'), 7.69-7.74 (m, 3H, H-6,7, -C=CH-phenyl), 7.89-7.91 (m, 2H, H-8,9), 8.83 (s, 1H, 4'-OH , J=1.2 Hz); 13C-NMR (CDCl3-d6, 75 MHz) δc (ppm): 14.7, 64.8, 114.6, 115.9, 122.9, 123, 126.5, 132, 134.8, 135, 139.9, 142.3, 145.7, 147.8, 151.4.

## 2-(2-methoxybenzylidene)-1H-indene-1,3(2H)-dione (3h)

Yield: 80%. M.p: 168-170 ℃. I.R(KBr, cm-1): CH-aromatic: 3032; CH-aliphatic: 2925; C=O: 1687, 1727. 1H-NMR (CDCl3-d6, 300 MHz) δH (ppm): 3.95 (s, 3H, OCH3), 6.95 (m, 2H, Ar-H), 7.54 (s, 1H, H-3'), 7.78-7.81 (m, 2H, H-6,7), 7.98-8.00 (m, 2H, H-5,9), 8.51 (s, 1H, -C=CH-phenyl), 8.88-8.92 (m, 1H, H-6'). 13C-NMR (CDCl3-d6, 75 MHz) δc (ppm): 55.7, 110.7, 120.4, 122.7, 123.0, 123.2, 133.9, 134.9, 135.2, 135.4,140.1, 141.3, 142.4, 160.5, 189.2, 190.6.

## 2-(2,3,4-trimethoxybenzylidene)-1H-indene-1,3(2H)-dione (3i)

Yield: 91%. M.p: 200-202 ℃. I.R(KBr, cm-1): CH-aromatic: 3004; CH-aliphatic: 2925; C=O: 1682, 1716. 1H-NMR (CDCl3-d6, 300 MHz) δH (ppm): 3.77-3.98 (m, 9H, OCH3), 7.73 (s, 1H, C=CH-phenyl), 7.77-7.79 (m, 2H, H-6,7), 7.92 (s, 4H, H-5',6',5,8); 13C-NMR (CDCl3-d6, 75 MHz) δc (ppm): 56.1, 56.3, 61.1, 111.9, 123.1, 123.2, 127.7, 128.4, 135.1, 135.2, 139.8, 142.4, 143.0, 147.3, 152.8, 189.4, 190.4 .

## 2-(4-hydroxy-3-methoxybenzylidene)-1H-indene-1,3(2H)-dione (3j)

Yield: 84%. M.p: 218-220 ℃. I.R (KBr, cm-1): OH: 3444; CH-aromatic: 3013; CH-aliphatic:

2926; C=O: 1672, 1716. 1H-NMR (CDCl3-d6, 300 MHz) δH (ppm): 4.12 (s, 3H, OCH3), 6.36 (s, 1H, H-5'), 7.03 (d, 1H, H-2', J= 8.4 Hz) 7.81-7.84 (m, 4H, H-5,6,7,8), 7.99-8.01 (m. 2H, H-6', C=CH-phenyl), 8.97 (s, 1H, 4'-OH); 13C-NMR (CDCl3-d6, 75 MHz) δc (ppm): 56.2, 114.6, 115.3, 122.9, 123.1, 126.2, 126.6, 132.2, 134.9, 135.1, 135.2 142.4, 146.4, 147.8, 151.3, 191.2, 193.5 .

*2-(3-hydroxy-4-methoxybenzylidene)-1H-indene-1,3(2H)-dione (3k)*
Yield: 82%. M.p: 218-220 ℃. I.R (KBr, cm-1): OH: 3530; CH-aromatic: 3074; CH-aliphatic: 2919; C=O: 1685, 1721. 1H-NMR (DMSO-d6, 300 MHz) δH (ppm): 3.90 (s, 3H, OCH3), 7.11 (d, 1H, H-5', J= 8.7 Hz), 7.69 (s, 1H, H-2'), 7.92-7.94 (m, 5H, H-5,6,7,8,6'), 8.31 (s, 1H, C=CH-phenyl), 9.53 (s, 1H, 4'-OH); 13C-NMR (DMSO-d6, 75 MHz) δc (ppm): 56.3, 112.2, 120.3, 122.3 123.3, 126.6, 130.1,134.9, 135.2, 136.1, 139.7, 142.2, 146.7, 146.9, 151.7, 189.4, 190.3.

## 2.2 Inhibitory activities against TYR

The *in vitro* anti-TYR activity of all synthesized compounds 3a-k was assayed according to the literature. Briefly, 10 μL of TYR (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 in 96-well microplates. The mixture was incubated at 28 ℃ for 20 min and then 20 μL of L-DOPA solution (0.5 mM) was added to the phosphate buffer. Enzyme activity was checked by detecting dopachrome formation at 475 nm.The IC50 was calculated by CurveExpert v1.3 (12).

## 2.3. Molecular Docking study

To figure out the binding modes of 3d and 3f, the docking simulation was performed. The crystal structure of 2Y9X was retrieved from the PDB, which comprises the Agaricus bisporus tyrosinase having tropolone as the native ligand in the binding site that containing the best conformation of ligand and free binding energy. Target molecule was docked using the Autodock vina 4.2 software. the RMSD value of selfdock was 0.5Å. It should be noted that the binding site of the tyrosinase contains copper ions which were kept in the structure for docking. The selected ligand was

created using Chem3D Ultra software, and energy minimizations were done by the semiempirical MM+ force field. Flexible ligand dockings were accomplished with the grid boxes of 60, 60 and 60 points in the x, y and z directions (20, 21). The best positions of the selected compound in each target protein was chosen by analyzing the interactions between the enzymes and inhibitor. The best-scoring positions, as achieved by the docking score, were then selected and visualized using Discovery Studio Client 2017.

## 3. Results and discussion
### 3.1. Design approach

Several TYR inhibitors from natural origin including resveratrol, flavonoids (22), chalcone, stilbene (23), and polyphenolic compounds, have been discovered and are currently being under investigation. Resveratrol (trans-3,4 ′,5-trihydroxystilbene, Figure 1 compound E) inhibited tyrosinase through the suicide substrate type inhibition. In vivo evaluation of UVB-irradiated dorsal skin of pigs treated with resveratrol confirmed the reduction of hyperpigmentation (24). In the search for new TYR inhibitors, chalcones have been used as a valid scaffold (Figure 1 compound F) with a wide variety of biological activity including potent antioxidant, anti-aging and anti-TYR. The SARs indicated that both ring A and ring B are important for inhibiting the TYR. compound F exhibited 700-fold potent inhibition compared to arbutin (25). Plenty of compounds based on the typical chalcone skeleton were introduced, showed that, with the appropriate substitution, these synthetic derivatives exhibit high TYR inhibitory potency and selectivity.(26).

Also, several groups have reported that heterocyclic flavanone based compounds can form complex with TYR and inactivate the enzyme. By way of illustration, compound G isolated from Camylotropis hirtella showed potent inhibitory activities in monophenolase and diphenolase pathway against tyrosinase (27).

Aurone is a heterocyclic flavonoid-type (Figure 1. compound H) introduced as TYR inhibitor with IC50 values near to that of kojic acid as a reference compound (28, 29). However, among the few synthesized aurone derivatives which
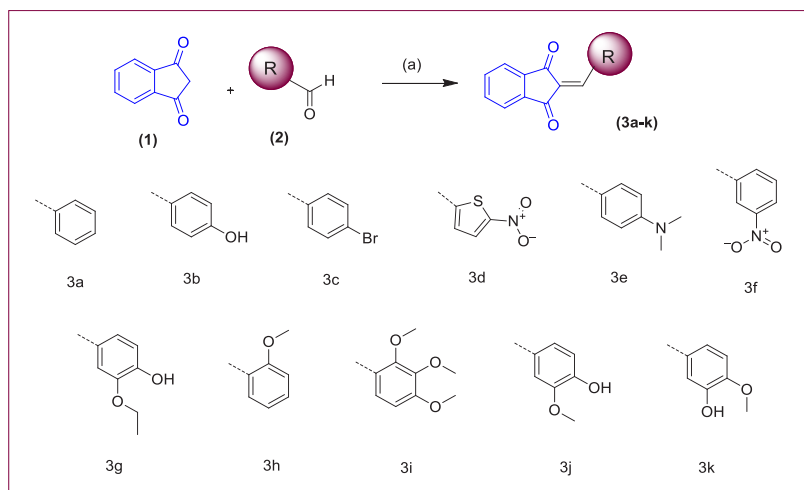
Scheme 1. Synthesis of 2-(Aryl)-1H-indene-1,3(2H)-dione: a) CH₃COOH (cat.), EtOH, reflux.

demonstrated weak to moderate TYR inhibitory activity; those derivatives with more than one hydroxyl group (preferably at para positions) can induce significant tyrosinase inhibition. The most potent aurone in this series induces 75% inhibition at 0.1 mM concentration (30).

Inspired by these results, we reported the design and synthesis of 1,3-indandione derivatives (similar to aurone natural compound) as a new TYR inhibitor linked to different substituted heterocyclic rings. More specifically, regarding the skeleton of chalcone, phenyl ring (B) is replaced with different substituted heterocyclic rings while the A ring is embedded in the aurone structure. Additionally, the structure-activity relationship (SAR), type of inhibition and molecular docking study against TYR were discussed (31).

### 3.2. Chemistry

Synthesis of desired derivatives 3a-k was achieved according to Scheme 1. The reaction of 1H-indene-1,3(2H)-dione (compounds 1) and selected aldehydes (compounds 2) in the presence of a catalytic amount of acetic acid in ethanol under reflux conditions lead to the formation of the corresponding 3a-k derivatives. The structure of all compounds was confirmed using MS, NMR and IR spectroscopy analysis.
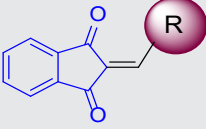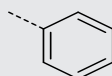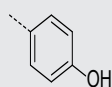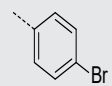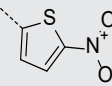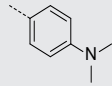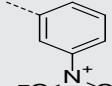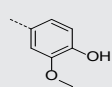
### 3.3. In vitro TYR inhibitory activities

In this research, eleven synthesized derivatives were tested *in vitro* against a mushroom tyrosinase in the presence of L-dopa as a substrate. For each compound, inhibition percent at 50 and 100 µM was determined and reported in Table 1. Synthetic derivatives showed $IC_{50}$ values in the range of 3.55–>100 µM.
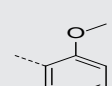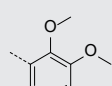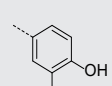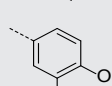
• The unsubstituted compound 4a displayed no TYR inhibitory properties in the whole range of concentrations studied.

• It was found that the presence of halogen (3c, R=4-Bromophenyl) led to the improvement in TYR inhibitory activity.

• Substitutions of OH at the para position of benzyl ring (3b, $IC_{50}$=96.71 µM ) did not significantly improve the TYR inhibition respect to the unsubstituted one (3a).

• Similarly, substitutions of methoxy with strong electron-donating properties at the ortho position of the benzyl ring (3h, $IC_{50}$=95.20µM) did not alter the activity significantly.

• Based on derivatives containing multi substitutions (3g and 3i-k), it was found that the presence of OH motif and MeO simultaneously at one molecule led to good TYR activity; however, the activity was affected by their positions. More specifically, compound 3k having 3-OH and 4-OMe motifs known as second top potent derivatives showed an $IC_{50}$ value of 19.32 µM while the $IC_{50}$ value of the other derivative (3j) containing 3-MeO/4-OH substitutions was calculated as 80.28 µM.

• Comparing our results from the anti-TYR activity of the previous studies with aurones struc-

Table 1. TYR inhibitory activity of 2-(Aryl)-1H-indene-1,3(2H)-dione 3a-k[a].



| Compound | | % Inhibition at 50 µMa | % Inhibition at 100 µMa | IC50 (µM) |
|---|---|---|---|---|
| 3a | | 8.01±2.15 | 30.82±3.26 | >100 |
| 3b | | 52.84±3.11 | 52.84±6.89 | 96.71±5.29 |
| 3c | | 14.60±6.87 | 97.67±5.28 | 82.55±4.86 |
| 3d | | 74.33±1.25 | 96.54±4.25 | 3.55±1.31 |
| 3e | | 8.55±2.36 | 19.03±1.27 | >100 |
| 3f | | 5.24±1.77 | 11.00±2.55 | >100 |
| 3g | | 18.72±2.98 | 24.56±3.64 | >100 |
| 3h | | 20.57±3.13 | 32.99±5.81 | 95.20±8.19 |
| 3i | | 26.32±4.58 | 57.38±6.87 | 89.69±6.55 |
| 3j | | 43.69±2.88 | 52.53±2.12 | 80.28±4.58 |
| 3k | | 60.59±4.96 | 69.75±6.14 | 19.32±2.33 |
| Kojic acid b | | ------- | ------- | 21.57±1.26 |

[a] Data represented in terms of mean±SD

[b] Standard tyrosinase inhibitor

ture it was found that aurones are weak inhibitors, but those derivatives with more than one hydroxyl group are more preferably with an $IC_{50}$ value of 31.1 µM for the most potent compound (30). Similar to our results, 3k compound having 3-OH and 4-OMe motifs were demonstrated good TYR inhibition ($IC_{50}$=19.32 µM).

•	bioisosteric ring replacement of benzyl ring (3a) with thiophene produced the most potent TYR inhibitor (compound 3d, $IC_{50}$=3.55 µM)

Table 2. Docking results of the TYR inhibitors at the TYR binding site.

| Code | ΔG (kcal/mol) | Interactions | Atom of ligand | Amino acid |
|------|--------------|--------------|----------------|------------|
| 3d | -9.7 | H -bond | O of Nitrothiophene | Met280 |
| | | H -bond | O of Nitrothiophene | His263 |
| | | H -bond | O of Nitrothiophene | Ser282 |
| | | H -bond | C=O of 1,3-Indandione | His244 |
| | | H -bond | C=O of 1,3-Indandione | Asn260 |
| | | Pi-sigma | 1,3-Indandione | Val283 |
| | | Pi-sigma | Nitrothiophene | Val283 |
| | | Pi-pi | 1,3-Indandione | His85 |
| | | Pi-pi | Nitrothiophene | His259 |
| | | Pi-pi | Nitrothiophene | Ser282 |
| | | Pi-aryl | Nitrothiophene | Ala286 |
| | | van der Waals | C=O of 1,3-Indandione | His85 |
| 3f | -6.1 | H -bond | O of Nitrobenzyl | Met280 |
| | | van der Waals | O of Nitrobenzyl | His263 |
| | | Pi-sigma | Nitrobenzyl | Val283 |

with 30 times improvement in the inhibitory potency compared to 3a.

## 3.5. Docking study

Binding pocket of TYR consists of two copper ions. One of them named CuA is coordinated to His61, His85, and His94. The second copper ion (CuB) is coordinated to His259, His263, and His296. Interaction with these Cu ions and His residues might improve the inhibitory potencies (32). The docking interaction analysis of 3d and 3f against the tyrosinase enzyme has been done using Autodock vina. Docking results and binding energy of the enzyme-inhibitor complexes are listed in Table 2. Compound 3d as the most potent compound ($IC_{50}$=3.55 µM) generated the binding energy of -9.7 kcal/mol. Analysis of binding interactions (figure 2) illustrated three hydrogen bonds between Met280, His263, Ser282 and O of nitrothiophene and two H-bond interactions with His244, Asn260. In addition, a pi-pi stacked interaction was also observed with His85, His259 and Ser282. Val283 demonstrated two Pi-sigma interactions with 1,3-indandione and Nitrothiophene rings.

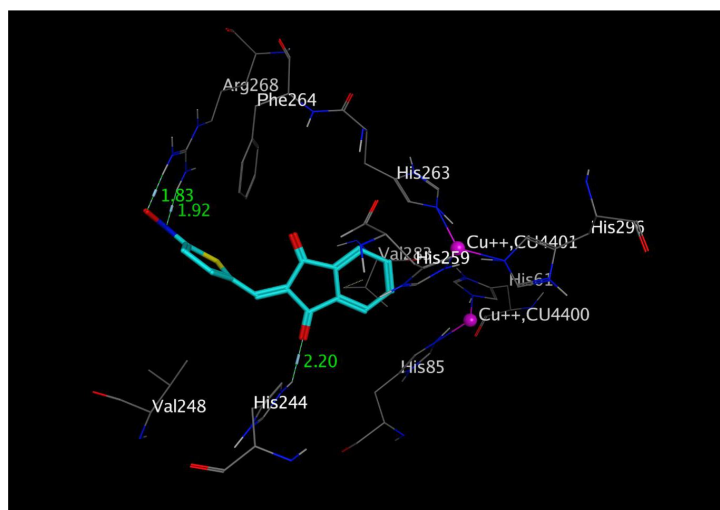On the other hand, the replacement of the



Figure 2. The binding orientation of 3d within the active site of tyrosinase enzyme (PDB: 2Y9X).
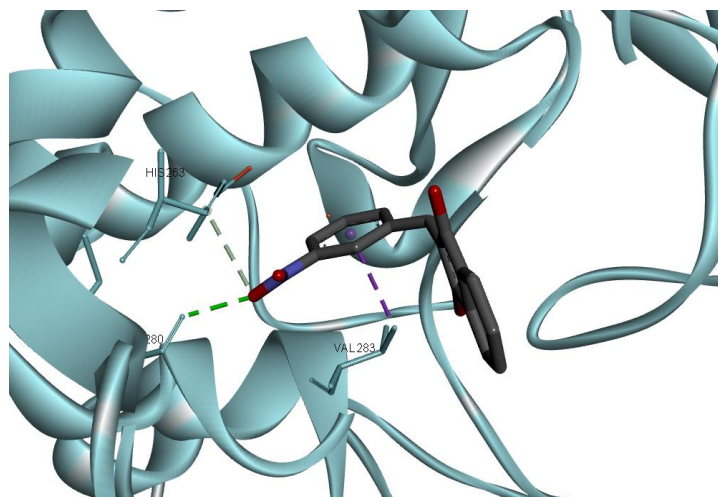
Figure 3. The binding orientation of 3f within the active site of tyrosinase enzyme (PDB: 2Y9X).

nitrothiophene of 3d with nitrobenzene generated 3f which showed the least activity in the enzymatic assay. This substitution got the backbone away from critical His residues in such a way that it was not able to make these critical H-bonding interactions. As illustrated in Figure 3, 3D interaction pattern of 3f showed different orientation in the enzyme active site so that just one hydrogen bond with Met280, and also one van der Waals interaction with His263 and Pi-sigma with Val283 were observed

## 4. Conclusions

TYR, catalyzes the rate-limiting steps of melanogenesis, has been recognized as a therapeutic target to control abnormal melanin synthesis. As a result, we reported the design and synthesis of 1,3-indandione derivatives (similar to aurone natural compound) as a new TYR inhibitor linked to different substituted heterocyclic rings. All synthesized analogs were assessed for their in vitro anti-TYR activity. The TYR inhibitory evaluations revealed the ability of the compounds 3d ($IC_{50}$=3.55 μM) containing nitrothiophene moiety to inhibit TYR was 3 times better than kojic acid

($IC_{50}$=9.28 μM) as the reference drug. Also, 3d illustrated good fitting and favorable binding modes with TYR enzyme in the docking study through the formation of critical hydrogen bonds with the essential Cu ions and His residues.

## Declarations
## Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author upon request. We have presented all data in the form of tables and figures.

## Conflict of Interest

None declared.

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