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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Molecular docking and biological evaluation of hydroxy-substituted (*Z*)-3-benzylideneindolin-2-one chalcones for the lead identification as tyrosinase inhibitors

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Abstract The increased cases of hyperpigmentation and other related dermatological problems in human beings have led to the development of a number of tyrosinase inhibitors. In the present study, we have used a docking algorithm to simulate binding between tyrosinase and hydroxy-substituted (*Z*)-3-benzylideneindolin-2-one chalcones and studied the inhibition of tyrosinase. The results of virtual screening studies indicated that the estimated free energy of binding of all the docked ligands ranged between −8.08 and −4.27 kcal/mol, while their estimated inhibition constants (*Ki*) were found to be between 1.20 and 736.75 μM. Among all the compounds docked, 2,4,6-trihydroxy-substituted chalcone (**11**) showed the lowest estimated free energy of binding followed by dihydroxy and monohydroxy-substituted analogs. In the in vitro tyrosinase inhibition assay, **11** displayed an IC_{50} of 46.26 μM. Moreover, in ADMET study, **11** was found to be safe and non-toxic. The present study suggested that the strategy of predicting tyrosinase inhibition based on hydroxy-substituted (*Z*)-3-benzylideneindolin-2-one chalcones and their orientation would be useful for developing novel potent tyrosinase inhibitors.

Sharad Kumar Suthar, and Vaibhav Aggarwal authors hold an equal contribution.

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Introduction

Polyphenol oxidase commonly known as tyrosinase is a copper-containing monooxygenase, widely distributed in the phylogenetic scale (Gawande *et al*., 2013). It is responsible for melanization in animals, browning reactions in plants, and also plays an important role in cuticle formation in insects (Si *et al*., 2011). Melanogenesis is defined as the process leading to the formation of dark macromolecular pigments, i.e., melanin formation in the skin (Chang, 2009). Melanin is formed by the combination of a number of enzymatically catalyzed chemical reactions. Melanogenesis is considered as a major defense mechanism of human skin against UV light (Iozumi *et al*., 1993). Tyrosinase is a key enzyme in the melanogenic pathway responsible for the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and oxidation of L-DOPA to dopaquinone, and are also the main steps in melanin biosynthesis (Fig. 1) (Lerner *et al*., 1955; Mason,1955). Abnormal production and accumulation of melanin in the skin may lead to serious dermatological problems. Recently, the development of tyrosinase inhibitors as skin whitening agents has become increasingly significant, and research in this field is continuously increasing (Bandgar *et al*., 2012; Ha *et al*., 2011, Ha *et al*., 2012; Kang *et al*., 2012). A large number of tyrosinase inhibitors, such as hydroquinone, kojic acid, arbutin, retinol, linoleic acid, and galangin have already been reported from both synthetic and natural sources (Fisher, 1983; Fujimoto *et al*., 1999; Solano *et al*., 2006).

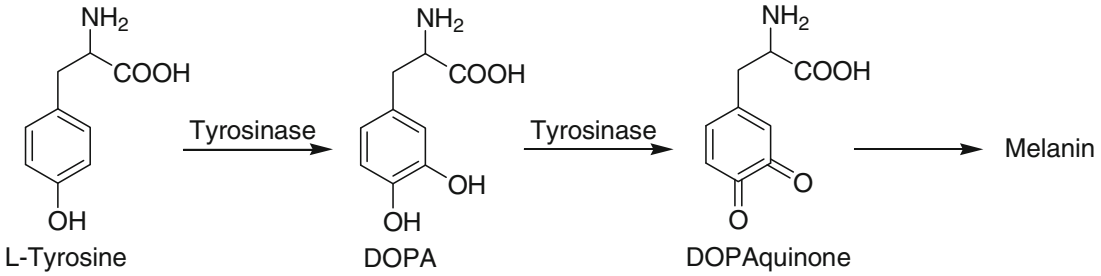


Fig. 1 Biosynthesis of melanin via tyrosinase

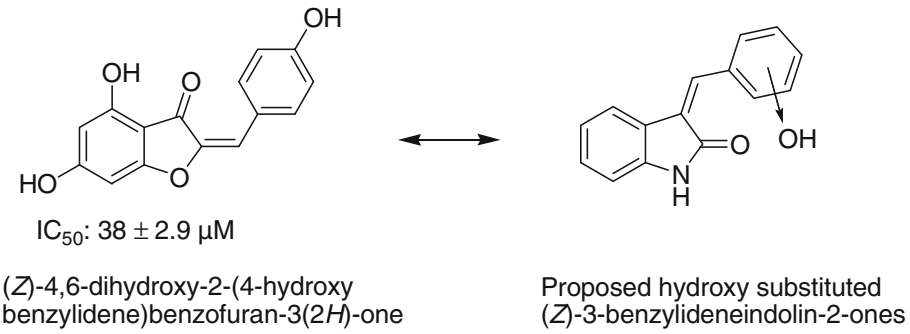


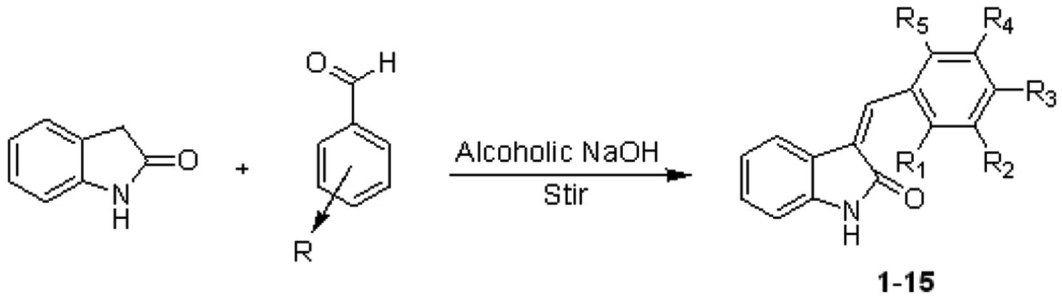
Fig. 2 Pharmacophoric similarity between the lead tyrosinase inhibitor (*Z*)-4,6-dihydroxy-2-(4-hydroxybenzylidene)benzofuran-3(2*H*)-one and proposed hydroxy substituted (*Z*)-3-benzylideneindolin-2-ones

With regard to the indole intermediates in melanin biosynthesis, indole moiety plays a very interesting role in tyrosinase inhibition (Seo *et al.*, 2003). A large number of indole derivatives such as *N*-(*p*-coumaroyl serotonin), *N*-feruloyl serotonin, and *N*-caffeoyl serotonin have been reported to possess tyrosinase inhibitory activity (Roh *et al.*, 2004). Besides these, the tyrosinase inhibitory property of hydroxy-substituted chalcones is also reported. This property of hydroxy-substituted chalcones may be due to their potent antioxidant property that can prevent pigmentation, resulting from auto-oxidative process (Jun *et al.*, 2007; Khatib *et al.*, 2005). Okombi *et al.* (2006) reported the discovery of hydroxy-substituted benzylidenebenzofuran-3(2*H*)-one chalcones as inhibitors of tyrosinase; among them, the most potent compound (*Z*)-4,6-dihydroxy-2-(4-hydroxybenzylidene)benzofuran-3(2*H*)-one showed an IC_{50} of 38 ± 2.9 μM and thus emerged as the highly potent inhibitor of tyrosinase. The pharmacophoric similarity of the lead tyrosinase inhibitor (*Z*)-4,6-dihydroxy-2-(4-hydroxybenzylidene) benzofuran-3(2*H*)-one and our proposed compounds (**1–15**) is shown in Fig. 2. In the novel approaches of drug discovery methods, docking is widely used as a computational method to find out the possible binding modes of a ligand to the active site of the receptor. On the basis of previous literature and current clinical research, we decided to design and dock the hydroxy-substituted (*Z*)-3-benzylideneindolin-2-one chalcones (Fig. 3) in order to find out the possible effects of oxindole moiety and hydroxy groups on tyrosinase inhibition. The lead compound was further synthesized and biologically evaluated for tyrosinase inhibitory potential.

Results and discussion

Before carrying out the virtual screening studies, all the proposed hydroxy-substituted (*Z*)-3-benzylideneindolin-2-one chalcones were assessed manually against the Lipinski's Rule of Five (Lipinski *et al.*, 2001). The Lipinski's Rule of Five states that for chemical compound to be a successful oral drug, it should fall within the following criteria: (i). Number of hydrogen bond donors (sum of OHs and NHs) should be ≤5, (ii). Number of hydrogen bond acceptors (sum of Os and Ns) should be ≤10, (iii). Its molecular weight should be ≤500, (iv). Its CLogP value should be ≤5. The calculation of Lipinski's Rule of Five parameters indicated that none of the proposed compounds showed a violation of the Lipinski's Rule of Five, and all the compounds fell well within the limits of the parameters described by Lipinski *et al.* (2001) (Table 1).

To predict the possible binding interactions between the proposed hydroxy-substituted (*Z*)-3-benzylideneindolin-2-one chalcones and tyrosinase, all the ligands were docked into the active site of tyrosinase. Results of virtual



Compd.	R_1	R_2	R_3	R_4	R_5
1	OH	–	–	–	–
2	–	OH	–	–	–
3	–	–	OH	–	–
4	OH	–	OH	–	–
5	OH	–	–	OH	–
6	**OH**	–	–	–	**OH**
7	–	OH	OH	–	–
8	–	OH	–	OH	–
9	OH	OH	OH	–	–
10	OH	–	**OH**	**OH**	–
11	**OH**	–	**OH**	–	**OH**
12	–	OH	OH	OH	–
13	–	Cl	OH	OH	–
14	Br	Br	OH	OH	–
15	OH	Br	OH	Br	–

Fig. 3 Scheme for the synthesis of hydroxy substituted (*Z*)-3-benzylideneindolin-2-one chalcones (**1**–**15**)

screening studies indicated that the estimated free energy of binding of docked ligands ranged between −8.08 and −4.27 kcal/mol, while their estimated inhibition constant (*K*i) was found to be between 1.20 and 736.75 μM. Further analyses of results showed that trihydroxy-substituted chalcones displayed the highest affinity toward the tyrosinase followed by dihydroxy- and monohydroxy-substituted chalcones, which were in agreement with the fact that the tyrosinase enzyme needs hydroxy group bearing substrates for its activity. Among the most active trihydroxy-substituted chalcones, compound **11** possessing a 2,4,6-trihydroxy group demonstrated the lowest estimated inhibition constant (*K*i) against the tyrosinase enzyme. Another trihydroxy-substituted compound **10** (2,4,5-trihydroxy substituted) also showed the promising affinity toward the tyrosinase. Apart from compounds **10** and **11**, other trihydroxy-substituted compounds **9** and **12** also possessed a fair affinity toward the tyrosinase. The replacement of a trihydroxy group with a dihydroxy on benzylidene ring decreased the affinity for the target enzyme, and this trend was further continued in the monohydroxy-substituted chalcones. Nevertheless, dihydroxy-substituted chalcones were still active, and among them, compound **6** with 2,6 dihydroxy substitution displayed a single digit estimated inhibition constant (*K*i) value. The monohydroxy-substituted chalcones showed less binding attraction for the tyrosinase than dihydroxy-substituted ones. The substitution of an electron withdrawing chlorine atom on benzylidene ring retained the binding affinity; though, when a chlorine atom was replaced with a bromine atom, the attraction for tyrosinase was dramatically decreased, which can be attributed to decreased electronegativity and larger size of bromide atom than chlorine. Detailed results of virtual screening studies are presented in Table 2.

Among all the docked compounds, **11** showed the lowest estimated free energy of binding (−8.08 kcal/mol) and estimated inhibition constant (1.20 μM). The docked structure of compound **11** indicated that the 4-hydroxy

Table 1 Calculation of various descriptors for proposed hydroxy-substituted (*Z*)-3-benzylideneindolin-2-one chalcones according to Lipinski's Rule of Five

Compd.	No. of H-bond donors	No. of H-bond acceptors	CLogP	Molecular weight	Lipinski's violations
1	2	2	2.563	237.25	0
2	2	2	2.563	237.25	0
3	2	2	2.563	237.25	0
4	3	3	1.896	253.25	0
5	3	3	1.896	253.25	0
6	3	3	1.896	253.25	0
7	3	3	1.966	253.25	0
8	3	3	1.896	253.25	0
9	4	4	1.299	269.25	0
10	4	4	1.299	269.25	0
11	4	4	1.229	269.25	0
12	4	4	1.299	269.25	0
13	3	3	2.734	287.70	0
14	3	3	3.732	411.04	0
15	3	3	3.302	411.04	0

Table 2 Molecular docking analysis results of hydroxy-substituted (*Z*)-3-benzylideneindolin-2-one chalcones (**1–15**)

Compd.	Substitution on benzylidene ring	Estimated free energy of binding (kcal/mol)	Final intermolecular energy (kcal/mol)	Final total internal energy (kcal/mol)	Estimated inhibition constant (*Ki*) in μM
1	2-OH	−6.50	−7.10	−0.15	17.15
2	3-OH	−6.74	−7.63	−0.41	11.51
3	4-OH	−5.56	−6.16	+0.30	83.35
4	2,4-diOH	−5.38	−6.28	+0.25	113.01
5	2,5-diOH	−6.17	−7.06	+0.02	30.25
6	**2,6-diOH**	**−7.19**	−8.19	+0.05	**1.64**
7	3,4-diOH	−6.81	−8.00	−0.074	10.23
8	3,5-diOH	−6.02	−7.22	−0.34	38.37
9	2,3,4-triOH	−6.51	−8.00	−0.97	16.87
10	**2,4,5-triOH**	**−6.96**	−8.46	−0.70	**7.85**
11	**2,4,6-triOH**	**−8.08**	−8.67	+0.06	**1.20**
12	3,4,5-triOH	−6.30	−7.79	−0.76	24.21
13	3-Cl-4,5-diOH	−6.51	−7.71	−0.54	16.85
14	2,3-diBr-4,5-diOH	−4.27	−5.47	−0.36	736.75
15	3,5-diBr-2,4-diOH	−4.98	−6.17	+0.29	224.41
16	*Lead	−5.18	−6.37	−0.46	160.16

* Lead: lead compound from the previous study-(*Z*)-4,6-dihydroxy-2-(4-hydroxybenzylidene) benzofuran-3(2*H*)-one (In vitro IC_{50} against tyrosinase: 38 ± 2.9 μM)

The most active compounds or significant results are highlighted in bold

group of **11** was extensively hydrogen bonded to the His-42, His-69, and His-231 residues with bond distances of 2.8, 3.1, and 3.3 Å, respectively (Fig. 4). Another hydroxy group of compound **11**, i.e., 6-hydroxy was also involved in a hydrogen bond interaction with the Asn-205 residue of the tyrosinase. The Asn-205 was further hydrogen bonded with the carbonyl group of oxindole scaffold of **11**. Additionally, Pro-201, Val-207, Val-211, Val-217, Val-218, Met-215, and Phe-227 residues of the tyrosinase displayed van der Waals and hydrophobic interactions with compound **11**, wherein Val-207 and Val-218 were involved in the relatively strong hydrophobic interactions with **11** (Fig. 5).

The compound **6** bearing 2,6-dihydroxy substitution showed the second best estimated free energy of binding (−7.89 kcal/mol) and estimated inhibition constant (1.64 μM). The stereoview of the docked complex

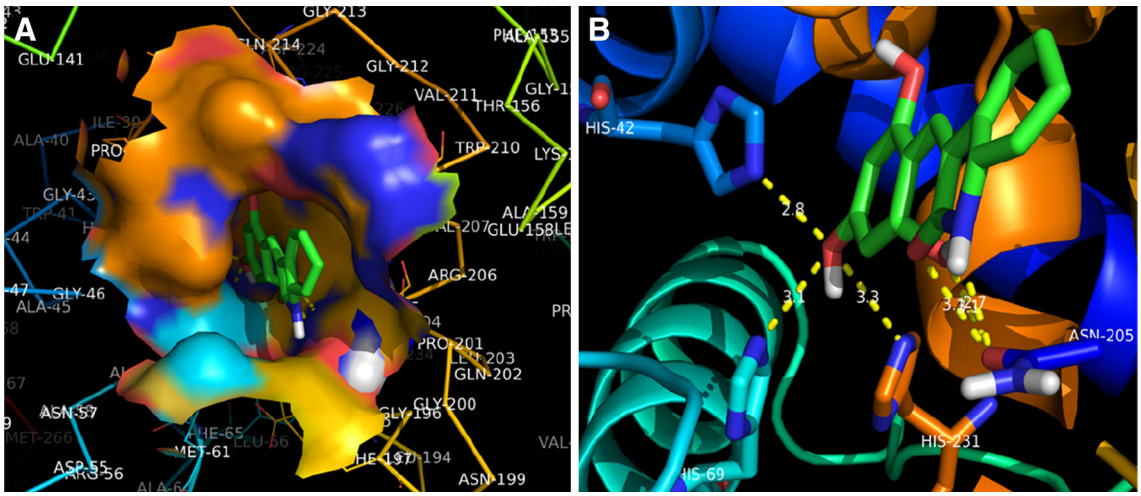


Fig. 4 **a** The binding orientation of compound **11** within the active site of tyrosinase. **b** Molecular docking of **11** within the active site of tyrosinase. The amino acids His-42, His-69, His-231, and Asn-205 involved in the hydrogen bond interactions with compound **11** are highlighted

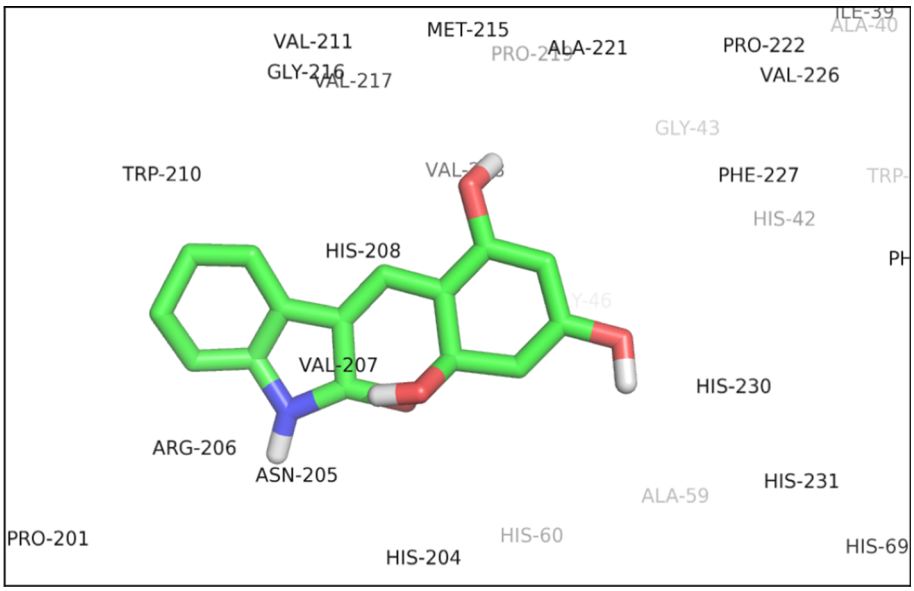


Fig. 5 Docking of compound **11** into the active site of tyrosinase. The residues of tyrosinase present in the vicinity of compound **11** are highlighted

indicated that the compound **6** was extensively hydrogen bonded with the Asn-205, Arg-209, and Met-215 residues of the tyrosinase (Fig. 6). Both NH and CO groups of oxindole moiety were involved in hydrogen bond formations with the residues of the tyrosinase. The NH group displayed hydrogen bonding with Met-215 (1.8 Å), while the CO group was involved in hydrogen bond formations with Asn-205 (3.2 Å) and Arg-209 (3.6 Å) residues. Moreover, 2-hydroxy group of compound **6** interacted with Asn-205 via a hydrogen bond, and it was 2.5 Å away from the Asn-205. Other residues of tyrosinase, viz. Val-217 and Val-218 demonstrated strong hydrophobic interactions with compound **6**, while Pro-201, Pro-219, Pro-222, Phe-197, and Phe-227 exhibited weak hydrophobic and van der Waals contacts with compound **6** (Fig. 7).

The compound **10** bearing 2,4,5-trihydroxy substitution showed the estimated free energy of binding −6.96 kcal/mol and estimated inhibition constant (*Ki*) in a single digit unit (7.85 μM). The stereoview of the docked complex indicated that both 2- and 4-hydroxy groups of compound **10** interacted with the tyrosinase by means of hydrogen bondings (Fig. 8). The 2-hydroxy group of **10** was hydrogen bonded to the His-208 and Met-215 residues with bond distances of 2.7 and 1.8 Å, respectively; while 4-hydroxy group was hydrogen bonded to the His-42 and His-60 residues with bond distances of 3.3 and 2.6 Å,

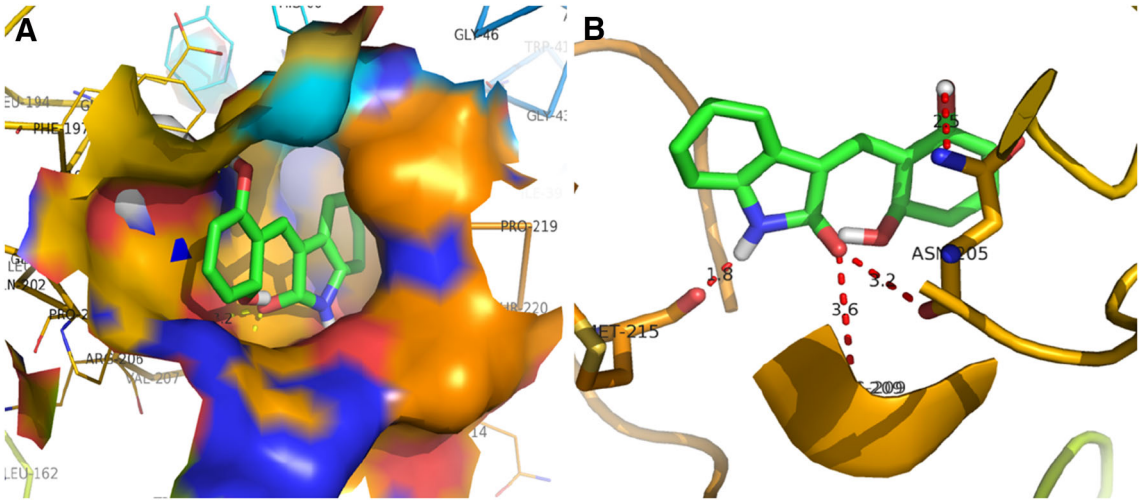


Fig. 6 **a** The 3-dimentional orientation of docked compound **6** into the binding site of tyrosinase. **b** Stereoview of docked compound **6** within the active site of tyrosinase. The amino acids Asn-205, Arg-209, and Met-215 involved in the hydrogen bond interactions with compound **6** are highlighted

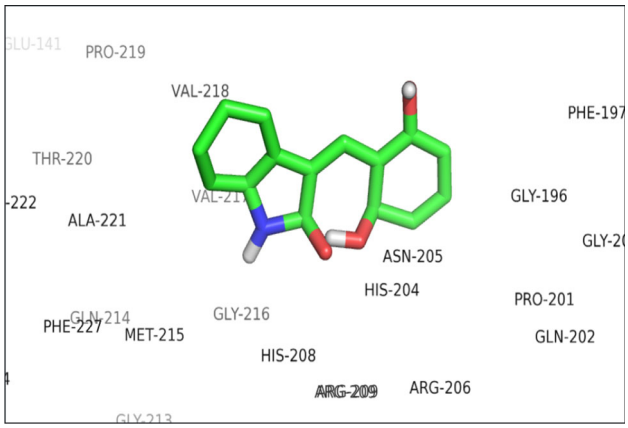


Fig. 7 Molecular docking of compound **6** into the active site of tyrosinase. The residues of tyrosinase present in the vicinity of compound **6** are highlighted

correspondingly. The carbonyl oxygen of oxindole moiety interacted with the Val-218 of tyrosinase via a hydrogen bond (C=O…H–N, 2.8 Å). Apart from aforementioned interactions, compound **10** also exhibited hydrophobic and van der Waals contacts with Phe-197, Val-217, Val-218, Pro-201, and Pro-222 residues of the tyrosinase (Fig. 9).

The comparison in binding mode of ligands with the tyrosinase was also made among the most active compounds **6** (2,6-dihydroxy substituted), **10** (2,4,5-trihydroxy substituted), and **11** (2,4,6-trihydroxy substituted), worst performing compounds **14** (2,3-dibromo-4,5-dihydroxy substituted) and **15** (3,5-dibromo-2,4-dihydroxy substituted), compounds showing average or moderate binding affinity **3** (4-hydroxy substituted) and **4** (2,4-dihydroxy substituted), and a lead compound from the previous study (*Z*)-4,6-dihydroxy-2-(4-hydroxybenzylidene) benzofuran-3(2*H*)-one. It is perceived that hydrogen bonds are critical for the folding of a protein into the biologically optimum conformation, and they are supposed to be strongest when the atoms of the protein involved in bindings are arranged linearly. From the detailed observation of the docked complexes, it was found that all the proposed 15 derivatives of the oxindole were involved in the extensive hydrogen bondings with the tyrosinase, but the most active ligand from the previous study (*Z*)-4,6-dihydroxy-2-(4-hydroxybenzylidene) benzofuran-3(2*H*)-one showed only two hydrogen bonds with the tyrosinase, and it appeared moderately active when compared to the most active derivatives of oxindole.

The attraction of the negatively charged electrons of an atom toward the positively charged nucleus of another atoms leads to van der Waals interactions. The van der Waals interactions are said to be weakest among all the attractions generated between an interacting ligand and the protein; however, a large number of such interactions significantly affect the binding energy, required for a ligand to bind with the target protein. The other interactions, which are caused by hydrophobic or non-polar parts of the ligand or protein, are known as hydrophobic interactions and are one of the major contributors in the binding affinity of ligand to the receptor. From the analyses of the docked complexes, it was evident that all the proposed ligands followed the same pattern and along with the extensive hydrogen bondings, were encircled within the hydrophobic pocket created by Phe-197, Pro-201, Val-207, Val-211,

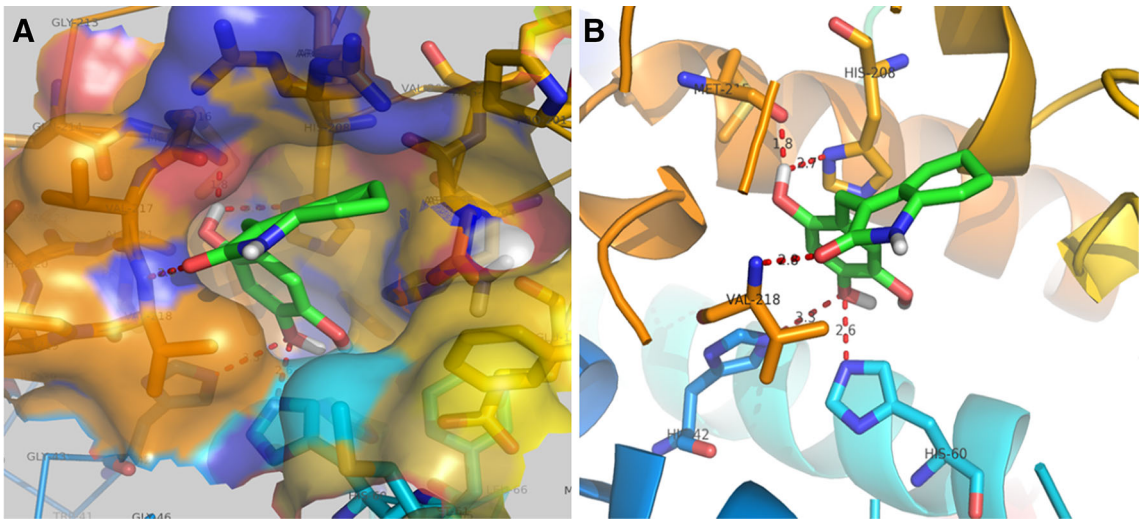


Fig. 8 **a** The binding orientation of compound **10** into the active site of tyrosinase. **b** Molecular docking of compound **10** into the active site of tyrosinase. The amino acids His-42, His-60, His-208, Met-215, and Val-218 involved in the hydrogen bond interactions with compound **10** are highlighted

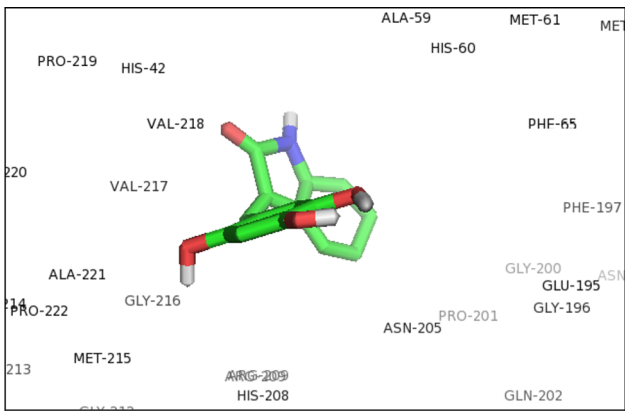


Fig. 9 Docking of compound **10** into the active site of tyrosinase. The residues of tyrosinase present in the vicinity of compound **10** are highlighted

Met-215, Val-217, Val-218, Pro-219, Pro-222, and Phe-227. The striking difference among the most active and moderately or poorly active ligands was the presence and position of the Met-61 residue. It was present in more close proximity to the scaffolds of the ligands showing less affinity for tyrosinase. The ligands substituted with bromine atoms (**14** and **15**) showed the least affinity for tyrosinase; because, the bromine atom is larger in size than chlorine atom and hydroxy group, and this caused weak fitting of the bromo substituted ligands into the hydrophobic pocket created by the earlier discussed amino acids and pushed the ligand scaffolds more close toward the Met-61 residue. The moderately active ligands (**3** and **4**) too were loosely fitted into the hydrophobic cavity and were closer to the Met-61 than the most active ligands (**6**, **10**, and **11**). Moreover, these moderately active ligands were substituted with mono or dihydroxy groups. Accordingly, loose fitting of the ligands into the hydrophobic cavity and proximity with the Met-61 residue decreased their binding affinity for the tyrosinase. The most active compound from the previous study (*Z*)-4,6-dihydroxy-2-(4-hydroxybenzylidene) benzofuran-3(2*H*)-one (IC_{50}: 38 ± 2.9 μM) was also docked into the active site of tyrosinase, and it showed binding affinity comparable or equal to that of moderately active ligands (**3** and **4**). It showed estimated free energy of binding −5.18 kcal/mol (Table 2), while the ligand **4** with a moderate binding affinity demonstrated estimated free energy of binding −5.38 kcal/mol. The molecular docking analyses of the reasonably performing ligands **3** and **4**, worst performing ligands **14** and **15**, and the lead compound from the previous study (*Z*)-4,6-dihydroxy-2-(4-hydroxybenzylidene) benzofuran-3(2*H*)-one are presented in the supplementary data file.

The most active chalcone **11** bearing 2,4,6-trihydroxybenzylidene moiety was further evaluated biologically for tyrosinase inhibitory potential using L-tyrosine as the substrate. Results of biologically assay indicated that **11** displayed significant tyrosinase inhibitory activity with an IC_{50} value of 46.26 μM. Thus, the noteworthy biological activity of the lead compound **11** supported its superior ranking achieved in the in-silico studies. The best performing ligand **11** and the worst performing ligand **14** were further studied and compared for absorption, distribution, metabolism, excretion, and toxicity (ADMET) studies, and results are summarized in Table 3. The ADMET study indicated that the lead compound **11** was far non-toxic and safer than the worst performing compound **14**.

Table 3 Prediction of ADMET profiles of the most active compound (2,4,6-trihydroxy substituted) **11** and the least active compound (2,3-dibromo-4,5-dihydroxy substituted) **14**

Parameter	Compound **11** (Most active)		Compound **14** (Least active)	
Absorption	Result	Probability	Result	Probability
Blood–brain barrier	BBB^+	0.8945	BBB^+	0.8827
Human intestinal absorption	HIA^+	0.9952	HIA^+	0.9971
Caco-2 permeability (LogPapp, cm/s)	**Caco2**$^-$ 1.2044	0.5410	**Caco2**$^+$ 1.2854	0.5187
P-glycoprotein substrate	Non-substrate	0.5580	Non-substrate	0.5225
P-glycoprotein inhibitor	Non-inhibitor	0.9671	Non-inhibitor	0.9618
Renal organic cation transporter	Non-inhibitor	0.8658	Non-inhibitor	0.8764
Distribution and metabolism				
CYP450 2C9 substrate	Non-substrate	0.8185	Non-substrate	0.8702
CYP450 2D6 substrate	Non-substrate	0.7986	Non-substrate	0.8014
CYP450 3A4 substrate	**Non-substrate**	0.5088	**Substrate**	0.5139
CYP450 1A2 inhibitor	Inhibitor	0.8972	Inhibitor	0.9181
CYP450 2C9 inhibitor	Inhibitor	0.5816	Inhibitor	0.7192
CYP450 2D6 inhibitor	Non-inhibitor	0.7507	Non-inhibitor	0.8600
CYP450 2C19 inhibitor	Non-inhibitor	0.8069	Non-inhibitor	0.8285
CYP450 3A4 inhibitor	**Non-inhibitor**	0.5823	**Inhibitor**	0.7805
CYP inhibitory promiscuity	High	0.7545	High	0.8105
Excretion and toxicity				
AMES toxicity	**Non AMES toxic**	0.5401	**AMES toxic**	0.5397
Carcinogens	Non-carcinogens	0.9147	Non-carcinogens	0.9150
Acute oral toxicity	III	0.3908	III	0.4736
Carcinogenicity (three class)	**Non-required**	0.5169	**Danger**	0.4148
Rat acute toxicity (LD_{50}, mol/kg)	**2.7935**	–	**2.6958**	–
Other parameters				
Total polar surface area	**93.546** Å	–	**73.318** Å	–

Acute Oral Toxicity: Category III includes compounds with LD_{50} values greater than 500 mg/kg but less than 5,000 mg/kg. Carcinogenicity (three-class): Carcinogenic compounds with TD_{50} (tumorigenic dose rate 50) $\leq$10 mg/kg body wt/day were assigned as "Danger," those with $TD_{50} > 10$ mg/kg body wt/day were assigned as "Warning," and non-carcinogenic chemicals were assigned as "Non-required." Probability indicates scale between 0 and 1. Parameters indicating difference between the most active and the least compound have been highlighted in *bold* letters

Conclusions

We have employed molecular docking studies to explore the structure–activity relationship of a series of hydroxy-substituted (*Z*)-3-benzylideneindolin-2-one chalcones as novel tyrosinase inhibitors. The docking studies revealed that the substitution of hydroxy groups in (*Z*)-3-benzylideneindolin-2-one chalcones plays a critical role in the drug-receptor interactions and should be kept in order to achieve the maximum potency. Among all the compounds docked, (*Z*)-3-(2,4,6-trihydroxybenzylidene)indolin-2-one (**11**) showed the lowest estimated free energy of binding and estimated inhibition constant, which in the biological assay showed the inhibition of tyrosinase enzyme with a noticeable degree. ADMET study predicted safe and non-toxic profile of **11**. Taken together, the lead compound **11** is a promising drug candidate against tyrosinase, warranting further investigations. Currently, results of this study are being used for the design and synthesis of newer compounds bearing hydroxy-substituted oxindole moiety to achieve superior tyrosinase inhibitory potential.

Experimental

Materials and methods

Oxindole and 2,4,6-trihydroxybenzaldehyde were purchased from Aldrich, India, while chemicals for biological activity were purchased from Sigma Life Science, India. The ^{1}H-NMR spectrum was obtained with a Bruker AVANCE II 400 MHz spectrophotometer (Bruker, Germany), and chemical shifts are reported in parts per million (δ). Tetramethylsilane was used as an internal standard for

NMR recording. The HRMS (ESI–MS) spectrum was recorded with a Bruker, Compact, Qq-TOF LC-MSMS mass spectrometer (Bruker, Germany). The three dimensional structure of target protein tyrosinase was selected from RCSB Protein Data Bank, which contains information about experimentally determined structures of proteins. The downloaded target protein possessed high resolution (2.00 Å), and none of the active site residues was missing. CS ChemDraw ultra 8.0 was used for ligand preparation. AutoDock 4.2 was used for molecular docking studies, while PyMOL 1.6 was employed for the visualization and further analyses of results.

Protein preparation

The crystal structure of tyrosinase from *Bacillus megaterium* was obtained from the Protein Data Bank (PDB entry: 3NM8) (http://www.rcsb.org/pdb/explore/explore.do?structureId=3NM8). The hydrogens were added; water molecules and other hetero atoms were removed. Non-polar hydrogens were merged, and partial charges were added to the protein. Optimization of hydrogen bond network was carried out by reorienting the hydroxy groups. The concluding step in the protein preparation was to refine the structure.

Ligand design

One of the important determinants for a successful docking is the structure of the ligands; thus, pre-analysis of the ligand structures is utmost important. The drugs like properties of the designed ligands were confirmed by manually applying the Lipinski's Rule of Five and the ligands those passed through this filter were only used for the further studies. The two dimensional structures of the designed ligands were prepared by using CS ChemDraw ultra 8.0. These two dimensional structures were converted into three dimensional, and their energy was minimized by the MM2 energy optimization program using Chem3D software. Original states of ionization were retained, and chiralities were determined from the 3D structures.

Ligand docking

AutoDock is the one of the most widely used automated procedures for docking a molecule into the active target site and is being continuously developed. AutoDock allows fully flexible modeling of specific portions of the protein, in a manner similar to that of ligand (Morris *et al*., 2009). AutoDock Tools are executed in the object-oriented programming language Python and are assembled from reusable software components (Morris *et al*., 2009). Therefore, all the commands of Python Molecular Viewer viz. reading and writing files, computing and showing secondary structures, adding or removing hydrogens, calculating charges and molecular surfaces, etc. are present in AutoDockTools (Morris *et al*., 2009). In the present study, AutoDock 4.2 version 4.2.5 was used to dock the designed ligands into the active site of tyrosinase.

Gasteiger partial charges were added to the ligand. Non-polar hydrogens were merged, and rotatable bonds were defined. A grid of 30, 42, and 24 points in x, y, and z directions was built with centers $x = -11$, $y = 12$, and $z = -8$ and grid point spacing of 0.375 Å. The Lamarckian genetic algorithm (LGA) was used to find the conformers with lowest binding energy. The docking task was finally performed by using the following two commands; (i). autogrid4 −p my_docking.gpf −l my_docking.glg, and (ii). autodock4 −p my_docking.dpf −l my_docking.dlg. Results of molecular docking studies were obtained in dlg file in the forms of estimated free energy of binding (kcal/mol) and estimated inhibition constant (Ki in μM) at 298.15 K temperature. The estimated inhibition constant (Ki) was calculated as $K\text{i} = \exp(\Delta G/(R^*T))$; where ΔG is the change in free energy, R is the gas constant, and T is the absolute temperature. The dlg file was then converted into pdb format in two steps by using the following commands in Linux; step-(1). grep '^DOCKED' my_docking.dlg | cut-c9->my_docking.pdbqt and step-(2). cut -c-66 my_docking.pdbqt>my_docking.pdb. The first command converted the dlg file into pdbqt format, which was then converted into pdb format by employing the second command. Above commands for conversion of dlg file to pdb are available at http://autodock.scripps.edu/faqs-help/faq/is-there-a-way-to-save-a-protein–ligand-complex-as-a-pdb-file-in-autodock web page.

Visualization and analyses of docking results

For visualization and analyses of docking results, PyMOL molecular viewer version 1.6 was used, which explored hydrogen, hydrophobic, and van der Waals contacts (The PyMOL Molecular Graphics System, Version 1.6, Schrödinger, LLC).

Synthesis of lead compound (*Z*)-3-(2,4,6-trihydroxybenzylidene)indolin-2-one (11)

Oxindole (133.05 mg, 1 mmol) was stirred in an alcoholic solution (80 %, 25 ml) containing 2 % sodium hydroxide in ice-cold conditions for about 30 min, and then 2,4,6-trihydroxybenzaldehyde (154.12 mg, 1 mmol) was added, and stirring was continued in same conditions for another 2 h (Fig. 3). Reaction mixture was kept in a refrigerator for an overnight, 10 % HCl was added with stirring, and mixture was kept aside in cold conditions till product

precipitate out, which was collected by filtration to yield a compound **11**.

Yield: 74 %; ^{1}H-NMR (400 MHz, mixture of DMSO.d_6 and $CDCl_3$) δ: 9.6519 (s, broad, *4H*, one NH and three OHs), 8.7553 (s, 1H, =CH–), 7.1091–7.1748 (m, *2H*, Ar-H), 6.8765–6.9124 (t, $J = 7.18$ Hz, *1H*, Ar-H), 6.7874–6.8068 (d, $J = 7.76$ Hz, *1H*, Ar-H), 6.0186 (s, *1H*, Ar-H of phenyl ring), 5.8608 (s, *1H*, Ar-H of phenyl ring) ppm; HRMS (ESI–MS) (*m/z*): Calculated for $C_{15}H_{11}NO_4$: 269.0688; Found: 269.0729 (100 %) (M^-).

Tyrosinase inhibition assay

0.9 mmol of L-tyrosine and 13.8 units/ml tyrosinase were prepared in 0.1 mol phosphate buffer of pH 6.8. The assay was performed in a 96-well plate. For this, 10 µl of test compound and 280 µl of L-tyrosine were added in each well as triplicates, followed by addition of 5 µl of tyrosinase. Control wells were occupied with 10 µl of DMSO, 280 µl of L-tyrosine, and 5 µl of tyrosinase. The plate was incubated at 25 °C for 30 min. The extent of dopachrome developed was recorded at 492 nm by employing microtiter plate reader. An interpolation of the dose–response curve provided the IC_{50} value of the compound.

Predicting ADMET profiles of the most active and worst active ligands

For the prediction of absorption, distribution, metabolism, excretion, and toxicity parameters, automated online program admetSAR was used (Cheng *et al*., 2012), while for the calculation of total polar surface area, Molinspiration property engine v2013.09 was used.

Implementation and availability

AutoDock is currently distributed freely under the GNU GPL for all users and can be downloaded from http://autodock.scripps.edu/downloads/autodock-registration/autodock-4-2-download-page/ page, while AutoDockTools, or ADT, is the free GUI for AutoDock, which can be downloaded from http://autodock.scripps.edu/resources/adt page. The admetSAR is available free of charge on http://www.admetexp.org/predict/ web page, while Molinspiration property engine is available free of cost at http://www.molinspiration.com/cgi-bin/properties web page.

Supplementary data

Molecular docking studies of ligands **3**, **4**, **14**, **15**, and (*Z*)-4,6-dihydroxy-2-(4-hydroxybenzylidene) benzofuran-3(2*H*)-one are provided in the supplementary data file.

References

Bandgar BP, Adsul LK, Chavan HV, Shringare SN, Korbad BL, Jalde SS, Lonikar SV, Nile SH, Shirfule AL (2012) Synthesis, biological evaluation, and molecular docking of *N*-{3-[3-(9-methyl-9*H*-carbazol-3-yl)-acryloyl]-phenyl}-benzamide/amide derivatives as xanthine oxidase and tyrosinase inhibitors. Bioorg Med Chem 20:5649–5657

Chang TS (2009) An updated review of tyrosinase inhibitors. Int J Mol Sci 10:2440–2475

Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, Lee PW, Tang Y (2012) admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. J Chem Inf Model 52:3099–3105

Fisher AA (1983) Current contact news. Hydroquinone uses and abnormal reactions. Cutis 31:240–244

Fujimoto N, Onodera H, Mitsumori K, Tamura T, Maruyama S, Ito A (1999) Changes in thyroid function during development of thyroid hyperplasia induced by kojic acid in F344 rats. Carcinogenesis 20:1567–1571

Gawande SS, Warangkar SC, Bandgar BP, Khobragade CN (2013) Synthesis of new heterocyclic hybrids based on pyrazole and thiazolidinone scaffolds as potent inhibitors of tyrosinase. Bioorg Med Chem 21:2772–2777

Ha YM, Kim JA, Park YJ, Park D, Kim JM, Chung KW, Lee EK, Park JY, Lee JY, Lee HJ, Yoon JH, Moon HR, Chung HY (2011) Analogs of 5-(substituted benzylidene)hydantoin as inhibitors of tyrosinase and melanin formation. Biochim Biophys Acta 1810:612–619

Ha YM, Park YJ, Kim JA, Park D, Park JY, Lee HJ, Lee JY, Moon HR, Chung HY (2012) Design and synthesis of 5-(substituted benzylidene)thiazolidine-2,4-dione derivatives as novel tyrosinase inhibitors. Eur J Med Chem 49:245–252

Iozumi K, Hoganson GE, Pennella R, Everett MA, Fuller BB (1993) Role of tyrosinase as the determinant of pigmentation in cultured human melanocytes. J Invest Dermatol 100:806–811

Jun N, Hong G, Jun K (2007) Synthesis and evaluation of 2′,4′,6′-trihydroxychalcones as a new class of tyrosinase inhibitors. Bioorg Med Chem 15:2396–2402

Kang SM, Heo SJ, Kim KN, Lee SH, Yang HM, Kim AD, Jeon YJ (2012) Molecular docking studies of a phlorotannin, dieckol isolated from Ecklonia cava with tyrosinase inhibitory activity. Bioorg Med Chem 20:311–316

Khatib S, Nerya O, Musa R, Shmuel M, Tamir S, Vaya J (2005) Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. J Bioorg Med Chem 13:433–441

Lerner AB, Fitzpatrick TB, Calkins E, Summerson WH (1955) Role of tyrosinase as the determinant of pigmentation in cultured human melanocytes. J Biol Chem 187:793–802

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46:3–26

Mason HS (1955) Comparative biochemistry of the phenolase complex. Adv Enzymol 16:105–184

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ (2009) AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 30:2785–2791

Okombi S, Rival D, Bonnet S, Mariotte AM, Perrier E, Boumendjel A (2006) Discovery of benzylidenebenzofuran-3(2*H*)-one (aurones) as inhibitors of tyrosinase derived from human melanocytes. J Med Chem 49:329–333

Roh JS, Han JY, Kim JH, Hwang JK (2004) Inhibitory effects of active compounds isolated from safflower (*Carthamus tinctorius* L.) seeds for melanogenesis. Biol Pharm Bull 27:1976–1978

Seo SY, Sharma VK, Sharma N (2003) Mushroom tyrosinase: recent prospects. J Agric Food Chem 51:2837–2853

Si Y-X, Yin S-J, Park D, Chung HY, Yan L, Lü Z-R, Zhou H-M, Yang J-M, Qian G-Y, Park Y-D (2011) Tyrosinase inhibition by isophthalic acid: kinetics and computational simulation. J Biol Macromol 48:700–704

Solano F, Briganti S, Picardo M, Ghanem G (2006) Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. Pigment Cell Res 19:550–571