Abstract

The designed bi-heterocyclic sulfonamides were synthesized through a two-step protocol and their structures were ascertained by spectral techniques including IR, $^1$H NMR and $^{13}$C NMR along with CHN analysis. The in vitro inhibitory effects of these sulfonamides were evaluated against tyrosinase and kinetics mechanism was analyzed by Lineweaver–Burk plots. The binding modes of these molecules were ascribed through molecular docking studies. These synthesized bi-heterocyclic molecules were identified as potent inhibitors relative to the standard (kojic acid) and compound 5 inhibited the tyrosinase non-competitively by forming an enzyme-inhibitor complex. The inhibition constant $K_i$ (0.09 µM) for compound 5 was calculated from Dixon plots. Computational results also displayed that all compounds possessed good binding profile against tyrosinase and interacted with core residues of target protein.

Keywords: Bi-heterocycles; 1-Phenylpiperazine; Sulfonamides; Tyrosinase; Chemical kinetics; Computational study

1. Introduction

Sulfonamides find their wide acceptance as the sulfa drugs across the world and these organic compounds have deep impacts on the biological systems owing to their numerous pharmacological activities. They are widely used to cure the bacterial infections. They find their usage as anti-cancer, anti-microbial, antiviral, anti-inflammatory and anti-tumor agents along with carbonic anhydrase inhibitors. The carbonic anhydrase bustles of sulfonamides possess an appreciated treatment for Alzheimer’s disease. Certain other piperazine derivatives have also antimicrobial and antimalarial potentials. Piperidine derivatives have been reported to have anticancer activity. Some sulfonamide based piperidine derivatives have an efficient role as acetylcholinesterase inhibitors. Several other substituted piperidine based derivatives have the potential of inhibition of ureases and α-glucosidases.

Tyrosinase (polyphenol oxidase, PPO, E.C.1.14.18.1) which is a copper-containing metalloenzyme, catalyzes two major reactions in the biosynthesis pathway of melamin pigment: the hydroxylation and oxidation of monophenols to o-quinones (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase acti-
Synthesis of Bi-Heterocyclic Sulfonamides as Tyrosinase Inhibitors

The aim of the present research work was to synthesize new biologically active compounds with low toxicity. Indeed, the current need is to introduce pharmacologically active drugs to help in pharmacy against the increasing enzyme inhibition.

2. Results and Discussion

2.1. Chemistry

In our present research work, four bi-heterocyclic sulfonamides were synthesized in two steps and their synthesis is shown in Scheme 1. In the first step, 4-(bromomethyl)benzenesulfonyl chloride (1) was reacted with various heterocyclic amines, including piperidine (2), morpholine (6), 4-methylpiperidine (9) and 3,5-dimethylpiperidine (12) to obtain respective sulfonamide containing electrophiles, 3, 7, 10 and 13, respectively. In the second step, these newly synthesized electrophiles were coupled with nucleophilic 1-phenylpipерidine (4) to acquire the desired bi-heterocyclic sulfonamides 5, 8, 11 and 14. The structures of these derivatives were affirmed by spectral techniques like IR, $^1$H NMR and $^{13}$C NMR, in addition to the CHN analysis data. The spectral data are given in the experimental section. The successful synthesis of targeted bi-heterocyclic sulfonamides was achieved in good yields through a two-step protocol and the structures of these molecules were confirmed through spectral data of IR, $^1$H NMR and $^{13}$C NMR, along with CHN analysis. For the benefit of the reader, the structural characterization of one compound, 5, is discussed hereby. Its molecular formula, C$_{23}$H$_{29}$N$_3$O$_2$S, was established by CHN analysis and by counting the number of protons in its $^1$H NMR spectrum. Similarly, the counting of number of carbon resonances in its $^{13}$C NMR spectrum also supported this assignment. The salient functional groups in the molecule were identified through absorption bands at 2986 (C–H, str. of aromatic ring), 2905 (–CH$_2$ stretching), 1680 (aromatic C=C stretching), 1382 (S=O), 1115 (C–N–C) cm$^{-1}$. Two ortho-coupled doublets in its $^1$H NMR spectrum at $\delta$ 7.70 (br. d, $J = 7.6$ Hz, 2H, H-3' and H-5'), and 7.60 (br. d, $J = 7.6$ Hz, 2H, H-2' and H-6') are typical for a 4-substituted benzenesulfonyl moiety, while a phenyl ring attached with the piperazine unit was rationalized by three signals in the aromatic region at $\delta$ 7.20 (br. t, $J = 7.2$ Hz, 2H, H-3” and H-5”), 6.92 (br. d, $J = 7.7$ Hz, 2H, H-2” and H-6”) and 6.77 (br. t, $J = 6.9$ Hz, 1H, H-4”). The pseudo-symmetrical 1,4-piperazine unit was corroborated by overall two signals in aliphatic region at $\delta$ 3.14 (br. s) and 2.87 (br. s) (8H, CH-2”, CH-3”, CH-5” and CH-6”), while a peculiar singlet at $\delta$ 3.63 (s, 2H, CH$_2$-7”) was assignable to a methylene connecting the piperazine heterocycle to the aromatic ring. The presence of a 1-piperidinyl moiety was justified by three signals at $\delta$ 3.51–3.46 (m, 4H, CH$_2$-2 and CH$_2$-6), 1.53 (br. s, 4H, CH$_2$-3 and CH$_2$-5) and 1.35 (br. s, 2H, CH$_2$-4). The $^1$H NMR spectrum of this compound is shown in Figures S1 and S2.

The carbon skeleton of this molecule was also fully supported by its $^{13}$C NMR spectrum, shown in the Figure S3. The $^{13}$C NMR spectrum demonstrated overall fifteen carbon resonances due to some symmetrical duplets in the molecule. The 4-substituted benzenesulfonyl group was characterized by two quaternary and two duplet methine signals at $\delta$ 144.16 (C-4’), 134.72 (C-1’), 129.90 (C-2’ and C-6’) and 127.87 (C-3’ and C-5’). The phenyl ring attached to the nitrogen atom of piperazine was obvious by four signals $\delta$ 151.43 (C-1’’), 129.37 (C-3’’ and 5’’), 119.29 (C-4’’ and 115.85 (C-2’’ and C-6’’). The pseudo-symmetrical piperazine heterocycle was corroborated by two signals at $\delta$ 53.05 (C-3’’ and C-5’’) and 47.04 (C-2’’ and C-6’’), while the connecting methylene was rationalized by a signal at $\delta$ 61.72 (C-7’’). The remaining signals $\delta$ 48.67, 48.63 (C-2 and C-6), 25.14 (C-3 and C-5) and 23.32 (C-4) were attributed to a piperidinyl heterocycle in the molecule. Thus, on the basis of the above cumulative evidences, the structure of 5 was confirmed and it was named as 1-phenyl-4-[4-(1-piperidinyl)sulfonyl]benzyl piperazine. The structures of other compounds were verified in a similar pattern. The $^1$H NMR and $^{13}$C NMR of all other compounds are shown in supplementary data (Figures S4–S9).
2. 2. Biological Activities (in vitro)

2. 2. 1. Enzyme Inhibition Activity

The synthesized bi-heterocyclic sulfonamides, 5, 8, 11, 14, were screened against tyrosinase and their in vitro inhibitory activities are presented in Table 1. These molecules exhibited outstanding potentials, which are evident from their lower IC50 (µM) values, relative to standard, kojic acid, having IC50 value of 16.8320 ± 1.1600 µM. Although, the experimental activity is cumulative for the whole molecule, however, nevertheless, a partial structure-activity relationship (SAR) was established by analyzing the effect of varying heterocyclic parts on the inhibitory potential. It needs to be mentioned, that except this variation of heterocyclic moiety, all other parts were the same in these molecules. The general structural parts of the inspected compounds are labeled in Fig. 1.

The comparison of inhibitory potential of 5 (IC50 = 0.0586 ± 0.0033 µM) and 8 (IC50 = 0.4078 ± 0.0151 µM), revealed that the presence of an additional oxygen atom within the heterocyclic ring (4-morpholinyl group) in 8 resulted in a decrease of activity as compared to 5, in which a simple piperidinyl ring was present. The compound 5 was the most active compound in the series as well. It means, the presence of piperidinyl ring is a credible option for the promising activity of such compounds (Fig. 2).

In compound 11, a para-methyl group was present at the piperidinyl ring (4-methyl-1-piperidinyl), while in 14 two methyl groups were present at the meta-positions of this heterocyclic part (Fig. 3). When the inhibitory potential of these two molecules is compared, it was perceived that molecule 14 with two methyl groups in variable heterocyclic part behaved as a slightly better inhibitor. It means when the methyl groups were present in a pseudo-symmetrical manner at 3 and 5 positions (3,5-dimethyl-1-piperidinyl), the compound 14 made some better interactions with the enzyme relative to its mono-methylated analogue 11.

| Compounds | Varying heterocyclic part | Tyrosinase activity IC50 ± SEM (µM) |
|-----------|--------------------------|-------------------------------------|
| 5         | ![image](image1.png)     | 0.0586 ± 0.0033                     |
| 8         | ![image](image2.png)     | 0.4078 ± 0.0151                     |
| 11        | ![image](image3.png)     | 0.1436 ± 0.0056                     |
| 14        | ![image](image4.png)     | 0.0706 ± 0.0013                     |
| Kojic acid|                           | 16.8320 ± 1.1600                    |

SEM = Standard error of the mean; values are expressed in mean ± SEM.

Figure 1. General structural parts of compounds 5, 8, 11, and 14.
Moreover, on a closer look, it was also elucidated that compounds 11 and 14 both have substituted piperidinyl ring and possessed slightly lesser inhibitory potentials as compared to 5 in which an un-substituted piperidinyl ring was present. From this, it was conceivable that the presence of any methyl group in this heterocyclic ring may render some steric repulsions and thus tending to retard the interactions of the compound with the enzyme, although to a minor extent. Hence, the presence of an un-substituted piperidinyl ring was the most suited option for the excellent activity of such molecules.

2.2.2. Kinetic Analysis

To understand the inhibitory mechanism of these bi-heterocyclic sulfonamides on tyrosinase, kinetic study was performed. Based on our IC50 results, the most potent compound 5 was selected to determine the inhibition type and inhibition constant. The kinetic results (Table 2) of the enzyme by the Lineweaver–Burk plot of 1/\( V \) versus 1/[S] in the presence of different inhibitor concentrations gave a series of straight lines (Figure 4a). The result of Lineweaver–Burk plot of 5 showed that this compound intersected within the second quadrant. The analysis showed that \( V_{\text{max}} \) decreased in new increasing doses of inhibitors, on the other hand, \( K_m \) remains the same. This behavior indicated that 5 inhibited the tyrosinase non-competitively to form an enzyme-inhibitor complex. Secondary plot of slope against the concentrations of inhibitor showed enzyme-inhibitor dissociation constant \( (K_i) \) (Figure 4b).

2.2.3. Mushroom Tyrosinase Structural Assessment

Mushroom tyrosinase, a copper containing protein comprises 391 residues. The detail structure analysis of the target protein showed that it consists of 39% α-helices, 14% β-sheets and 46% coils. The Ramachandran plots and values indicate that 95.90% of protein residues are present in the favored region and 100.0% residues lie in the allowed region. The Ramachandran graph values show good accuracy of phi (\( \phi \)) and psi (\( \psi \)) angles among the coordinates of receptor and most of residues are plunged in the acceptable region. The overall protein structure and Ramachandran graph is shown in Figure 5 (A, B).

2.2.4. Computational Docking

2.2.4.1. Glide Energy Evaluation of Synthesized Compounds

Molecular docking examination is the best approach to study the binding conformation of ligands within the

| Concentration (µM) | \( V_{\text{max}} \) (ΔA /Min) | \( K_m \) (mM) | Inhibition Type | \( K_i \) (µM) |
|-------------------|-------------------------------|----------------|----------------|-------------|
| 0.00              | 0.000384                      | 0.2222         |                |             |
| 0.0586            | 7.105673 \times 10^{-5}       | 0.2222         |                |             |
| 0.1172            | 5.438583 \times 10^{-5}       | 0.2222         | Non-Competitive| 0.09        |
| 0.2344            | 3.968313 \times 10^{-5}       | 0.2222         |                |             |

Note: \( V_{\text{max}} \) is the reaction velocity; \( K_m \) is the Michaelis–Menten constant; \( K_i \) is the EI dissociation constant.
active region of target proteins. To predict the conformational positions of synthesized ligands, compounds 5, 8, 11 and 14 were docked against tyrosinase, separately. The generated docked complexes were examined on the basis of glide docking energy values (kcal/mol) and bonding interaction (hydrogen/hydrophobic) pattern. The docking results showed that all the ligands were bound within the active region of the target protein with different conformational poses (Figure 6A). The glide docking energy values fluctuated among all ligands (5, 8, 11 and 14) and exhibited well docking energy values –4.50, –5.65, –4.89 and –5.81 kcal/mol, respectively. The comparative results show that no big energy value differences were observed (Fig. 6B) as the basic skeleton of ligands was similar in all synthetic compounds with the variation of only one heterocyclic moiety.

Figure 4. Lineweaver–Burk plots for inhibition of tyrosinase in the presence of compound 5. (a) Concentrations of 5 were 0.00, 0.0586, 0.1172 and 0.2344 µM, respectively. Substrate L-DOPA concentrations were between 0.0625 and 2 mM, respectively. (b) The inset represents the plot of the slope versus inhibitor 5 concentrations to determine inhibition constant. The lines were drawn using linear least squares fit.

Figure 5. The overall protein structure (A) of tyrosinase and its Ramachandran graph (B).

2.4.2. Ligand-Binding Analysis of Tyrosinase Docked Complexes
It was envisaged that hydrogen bonds and π–π interactions were observed in 8, 11 and 14 docking complexes. In 5–tyrosinase docking no hydrogen bonds were observed, however its binding conformation was quite similar with other ligands docking complexes. In 8–tyrosinase docking complex three hydrogen bonds were observed at Glu322 and His85. The benzene ring form hydrogen bond with Glu322 having bond length 2.61 Å, whereas the oxygen atoms of benzenesulfonyl part were involved in hydrogen bonding against His85 having bond distances 2.67 Å and 1.99 Å, respectively. In 11–tyrosinase docking couple of hydrogen bonds were observed between the benzene ring of ligand and tyrosinase residues His85 and Cys83 with bonds length 2.59 Å and 2.46 Å, respectively. Moreover, the com-
pound 14 also showed two hydrogen bonds against His85 and Cys83 with bond distances 2.61 Å and 2.27 Å, respectively. The 3D and 2D graphical representations of all docking complexes are shown in Figures 7, 8 and 9, respectively.

Figure 6. All the tyrosinase docking complexes (A) and their docking energy values (B).

Figure 7. 3D binding interactions of 5, 8, 11 and 14 against tyrosinase protein.
Figure 8. 2D binding interactions of 5 and 8 against tyrosinase protein.

Figure 9. 2D binding interactions of 11 and 14 against tyrosinase protein.
Moreover, computational results also explored the good binding profiles against target protein. All compounds (5, 8, 11, and 14) exhibited good docking energy values and bound within active region of the target protein. His85 was common in all docking results. The His85 is copper bonded residue which ensures that our ligands bind within the active region of the target protein. Literature data also ensured the importance of these residues in bonding with other tyrosinase inhibitors which strengthen our docking results.22,23 The comparative results showed that compound 5 might be considered as a superb template for the designing of new inhibitors against tyrosinase.

3. Experimental

3.1. General

All the chemicals, along with analytical grade solvents, were purchased from Sigma Aldrich, Alfa Aesar (Germany), or Merck through local suppliers. Pre-coated silica gel Al-plates were used for TLC with ethyl acetate and n-hexane as solvent system. Spots were detected by UV

\[\text{Scheme 1. Outline for the synthesis of 1-phenyl-4-[4-substituted-sulfonyl]benzyl]piperazines (5, 8, 11, 14). Reagents and conditions: (I) 10\% Na}_2\text{CO}_3, \text{pH} 9–10, \text{stirring at room temperature for 3–4 h; (II) dimethylformamide (DMF), LiH, refluxed for 0.5 h, followed by addition of respective electrophile (one in each reaction) and then refluxing further for 4–5 h.} \]
co-320-A spectrophotometer. \(^1\)H NMR spectra (δ, ppm) were recorded at 600 MHz \(^{13}\)C NMR spectra, at 150 MHz) in DMSO-\(d_6\) using Bruker Advance III 600 As-cend spectrometer using BBO probe.

3. 2. Synthesis of 1-[(4-(Bromomethyl)phenyl)sulfonyl]un/substituted-piperidines (3, 10, 13) and 1-[(4-(Bromomethyl)phenyl)sulfonyl]morpholine (7)

The synthesis of 1-[(4-bromomethyl)phenyl)sulfonyl]un/substituted-piperidines (3, 10, 13) and 1-[(4-(bromomethyl)phenyl)sulfonyl]morpholine (7) was carried out by reaction of respective un/substituted-piperidines (2, 9, 12) or morpholine (6) with 4-(bromomethyl)benzenesulfonyl chloride (1) in equimolar quantities (0.001 moles) and shaking manually in 10% aqueous Na\(_2\)CO\(_3\) solution. Solid precipitates were formed after 2–3 h, which were filtered and washed with cold distilled water to obtain the desired electrophiles (3, 7, 10, 13).

3. 3. General Procedure for the Synthesis of 1-Phenyl-4-[(4-substituted-sulfonyl)benzyl]piperazines (5, 8, 11, 14)

1-Phenylpiperazine (0.2 g, 4) was added in DMF (5 mL) contained in a 250 mL round bottom flask at room temperature, added one pinch of LiH and stirred for 30 min. Then the respective electrophile from 3, 7, 10, 13 (one in each reaction) was added in equimolar amount and stirred for 4–5 h. The completion of the reaction was monitored by TLC and after its completion, the reaction mixture was quenched with ice cold water (100 mL). Consequently, the respective derivatives (5, 8, 11, 14) were collected through filtration in purified form.

1-Phenyl-4-[(4-(1-piperidinylsulfonyl)benzyl)piperazine (5)

White crystalline solid; yield: 72%; m.p: 116–117 °C; mol. Formula: C\(_{23}\)H\(_{29}\)N\(_3\)SO\(_2\); mol. weight: 399 g/mol; IR (KBr, ν, cm\(^{-1}\)) 2996 (C-H, str. of aromatic ring), 2905 (d) using Bruker Advance III 600 As-cend spectrometer using BBO probe.

4-[(4-(Phenyl-1-piperazinyl)methyl)phenyl)sulfonyl]morpholine (8)

White crystalline solid; yield: 91%; m.p: 176–177 °C; mol. Formula: C\(_{23}\)H\(_{29}\)N\(_3\)SO\(_2\); mol. weight: 401 g/mol; IR (KBr, ν, cm\(^{-1}\)) 2981 (C-H, str. of aromatic ring), 2901 (d) using Bruker Advance III 600 As-cend spectrometer using BBO probe.

1-[(4-(Methyl-1-piperidinyl)sulfonyl)benzyl]4-phenylpiperazine (11)

White crystalline solid; yield: 76%; m.p: 81–82 °C; mol. Formula: C\(_{23}\)H\(_{29}\)N\(_3\)SO\(_2\); mol. weight: 413 g/mol; IR (KBr, ν, cm\(^{-1}\)) 2983 (C-H, str. of aromatic ring), 2900 (d) using Bruker Advance III 600 As-cend spectrometer using BBO probe.

1-[(4-(3,5-Dimethyl-1-piperidinyl)sulfonyl)benzyl]4-phenylpiperazine (14)

White crystalline solid; yield: 70%; m.p: 110–111 °C; mol. Formula: C\(_{23}\)H\(_{29}\)N\(_3\)SO\(_2\); mol. weight: 427 g/mol; IR (KBr, ν, cm\(^{-1}\)) 2990 (C-H, str. of aromatic ring), 2915 (d) using Bruker Advance III 600 As-cend spectrometer using BBO probe.

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3.4. Biological Activity Assays (in vitro)

3.4.1. Tyrosinase Inhibitory Activity

The inhibition of mushroom tyrosinase was determined by a modification of the dopachrome method using L-DOPA as the substrate.24–27 In detail, 140 µL of phosphate buffer (20 mM, pH 6.8), 20 µL of mushroom tyrosinase (30 U/mL) and 20 µL of the inhibitor solution were placed in the wells of a 96-well microplate. After pre-incubation for 10 minutes at room temperature, 20 µL of L-DOPA (3,4-dihydroxyphenylalanine, Sigma Chemical, USA) (0.85 mM) was added and the assay plate was further incubated at 25 °C for 20 minutes. After incubation time, the absorbance was read at 475 nm and the inhibition percentage calculated in relation to the control. Phosphate buffer and kojic acid were tested under the same conditions as negative and positive control, respectively. The amount of inhibition by the test compounds was expressed as the percentage of concentration necessary to achieve 50% inhibition (IC50). Each concentration was determined in three independent experiments. IC50 values were calculated by nonlinear regression using GraphPad Prism 5.0.

The % inhibition of tyrosinase was calculated as follows:

\[
\text{Inhibition} \% = \frac{B - S}{B} \cdot 100
\]

Here, the B and S are the absorbances for the blank and samples.

3.4.2. Kinetics Assay

On the basis of IC50 results, the most potent molecule, 5, was selected for kinetic analysis. A series of experiments were performed to determine the inhibition kinetics of 5 by following the already reported methods.23,28 The inhibitor concentrations for 5 were 0.00, 0.0586, 0.1172 and 0.2344 µM. Substrate L-DOPA concentrations were between 0.0625 to 2 mM in all kinetic studies. Pre-incubation and measurement time was the same as discussed in the mushroom tyrosinase inhibition assay protocol. Maximal initial velocity was determined from the initial linear portion of absorbance up to five minutes after addition of enzyme at a 30 s interval. The inhibition type of the enzyme was assayed by Lineweaver–Burk plots of the inverse of velocities (1/V) versus the inverse of substrate concentration 1/[L-DOPA] mM–1. The EI dissociation constant Ki was determined by the secondary plot of 1/V versus inhibitors concentrations.

3.4.3. Molecular Docking Methodology

3.4.3.1. Retrieval of Tyrosinase in Protein Preparation Wizard

The mushroom tyrosinase structure was retrieved from Protein Data Bank (PDB) (www.rcsb.org) with PDB ID 2Y9X29 in protein preparation wizard. The selected protein structure of tyrosinase was pre-processed and minimized using default parameters in Maestro interface.

3.4.3.2. Grid Generation and Molecular Docking

Prior to molecular docking, the optimized tyrosinase structure was prepared using the “Protein Preparation Wizard” workflow in Schrödinger Suite. Bond orders were assigned and hydrogen atoms were added to the protein. The structure was then minimized to reach the converged root mean square deviation (RMSD) of 0.30 Å with the OPLS_2005 force field. The active site of the enzyme was defined from the co-crystallized ligands from Protein Data Bank and literature data.30 Furthermore, docking experiment was performed against all synthesized ligands (5, 8, 11 and 14) sketched by 2D sketcher in Maestro and target protein by using Glide docking protocol.31 The predicted binding energies (docking scores) and conformational positions of ligands within active region of protein were also performed using Glide experiment. Throughout the docking simulations, both partial flexibility and full flexibility around the active site residues were performed by Glide/SP/XP and induced fit docking (IFD) approaches32,33

4. Conclusion

A structurally unique series of sulfonamides, hybrid with a piperazine, and heterocyclic secondary amines, was synthesized and recognized with very superb tyrosinase inhibition. It was postulated from the SAR studies that molecules particularly bearing un-substituted or symmetrically methylated piperidinyl moiety, generally inhibited the tyrosinase in an excellent manner. So, it was concluded that molecule 5 in particular, and all these bi-heterocyclic sulfonamides in general, can be utilized as leading medicinal scaffolds for the treatment of melanogenesis.
Acknowledgement
The present study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B03034948).

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Povzetek
S pomočjo dvostopenjskega protokola smo sintetizirali načrtovane bi-heterociklične sulfonamide; njihove strukture smo določili s spektroskopskimi tehnikami, vključno z IR, $^1$H NMR in $^{13}$C NMR ter s CHN analizo. In vitro inhibitorne učinke teh sulfonamidov smo določili na tirozinazi, mehanizem kinetike pa smo analizirali z Lineweaver–Burkovimi grafi. Vezavni model teh molekul je bil določen s pomočjo študij računskega sidranja. Sintetizirane bi-heterociklične molekule so se izkazale kot učinkoviti inhibitorji glede na standard 5-hidroksi-2-(hidroksimetil)-4H-piran-4-on (»kojic acid«); spojina 5 je nekompetitivno inhibirala tirozinazo tako, da je tvorila kompleks encim-inhibitor. Inhibicijsko konstanto $K_i$ (0.09 $\mu$M) za spojino 5 smo izračunali iz Dixonovih grafov. Računski rezultati so pokazali, da vse spojine izkazujejo ugoden vezavni profil za tirozinazo in da interagirajo z aminokislinskimi ostanki v jedru tarčnega proteina.