Anti-tyrosinase Activities and in silico ADME Properties of Fluorine-containing 1,2,4-triazole-5-on Derivatives

Flor içeren 1,2,4-triazol-5-on Türevlerinin Anti-tirosinaz Aktiviteleri ve in silico ADME Özellikleri

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ABSTRACT

In this study, inhibition potentials of fluorine-containing 1,2,4-triazole-5-one derivatives (2a-b, 3a-b, 4a-d, 5a-b, 6a-b, 7a-b, 8a-b, 9a-b) on the activity of clinically significant tyrosinase enzyme were investigated. The IC_{50} values of the compounds were calculated. The type of inhibition and Ki value of the compound showing the best inhibition feature among the compounds were determined. Kinetic experiments were carried out after identifying the optimum reaction conditions for commercially available mushroom tyrosinase and it was defined that the compound with the lowest IC50 value was 8b. 8a-b and 9a-b (IC_{50} values 32.2±0.7 μM; 22.9±0.6 μM; 22.8±0.5 μM; 23.8±0.6 μM, respectively) have been found to have a highly efficient inhibitory property on tyrosinase activity when compared to kojic acid (IC_{50} value 45.7±0.9 μM) as the reference inhibitor compound. The inhibition mechanism for the 8b was stated to be noncompetitive and the K_i was determined as 6.09 μM. In addition, the ADME properties of all compounds were examined and it was defined that each compound has a high potential as a drug candidate molecules. As a result of these, 8a-b and 9a-b can be considered as highly effective and promising inhibitor compounds against tyrosinase activity.

Key Words

ADME properties, anti-tyrosinase activity, triazoles.

ÖZ

Bu çalışmada, flor içeren 1,2,4-triazol-5-on türevlerinin (2a-b, 3a-b, 4a-d, 5a-b, 6a-b, 7a-b, 8a-b ve 9a-b) klinik öneme sahip tirosinaz enziminin aktivitesi üzerine inhibisyon potansiyelleri incelenmiştir. Moleküllerin IC_{50} değerleri belirlenmiştir. Moleküller arasında en iyi inhibisyon özelliği gösteren molekülün inhibisyon türü ve Ki değeri hesaplanmıştır. Ticari olarak temin edilen mantar tirosinaz için optimum reaksiyon şartları belirlendikten sonra, kinetik çalışmalar yapılarak en düşük IC50 değerine sahip molekülün 8b olduğu tespit edilmiştir. Referans inhibitör molekül olarak kullanılan kojik aside (IC_{50}=45,7±0,9 μM) göre 8a-b, 9a-b (IC_{50} değerleri sırasıyla 32,2±0,7 μM; 22,9±0,6 μM; 22,8±0,5 μM; 23,8±0,6 μM) moleküllerinin tirosinaz aktivitesi üzerinde oldukça etkili inbitör özelliğine sahip olduğu tespit edilmiştir. 8b molekülü için inhibisyon türü nonkompetetif olarak belirlenmiş ve K_i değeri 6,09±0,12 μM olarak hesaplanmıştır. Ayrıca tüm moleküllerin ADME özellikleri de incelenmiş olup, her bir molekülün ilaç aday molekülü olarak yüksek bir potansiyele sahip olduğu tespit edilmiştir. Bu sonuçlar neticesinde 8a-b ve 9a-b molekülleri, tirosinaz aktivitesine karşı oldukça etkili ve umut verici inhibitör bileşikler olarak kabul edilebilir.

Anahtar Kelimeler

ADME özellikleri, anti-tirosinaz aktivite, triazoller.

INTRODUCTION

Tyrosinase (EC 1.14.18.1), is a metalloenzyme that involves copper in the central domain and commonly found in microorganisms, animals and plants [1]. It is ubiquitous in organisms and has a multicatalytic function, including hydroxylation of tyrosine to produce L-3,4-dihydroxyphenylalanine (L-DOPA) and oxidation of L-DOPA to produce dopaquinone [2,3].

Melanin acts a major role in preventing the skin from the detrimental impact of ultraviolet (UV) radiation from the sun. Freckles, hyperpigmentation, melasma, age spots, melanoma, post-inflammatory melanoderma induced by UV induction are due to abnormal melanin production [4]. Enzymatic darkening during the harvest of fresh-cut vegetables and fruit, especially in mushrooms, is undesirable and reduces nutritional quality and the commercial value of the product [5]. Tyrosinase is also an enzyme involved in the production of neuromelanin and damaged neurons associated with Parkinson's disease [6,7]. For these reasons, tyrosinase inhibitors have potential practice areas in the food industry, medical and cosmetic products. Electron-rich phenols, α-arbutin, retinoids, caftaric acid, chrysosplenetin, azelaic acid, hydroquinone, resveratrol, phenylethyl resorcinol, valonia tannin, kojic acid, catechins and tropolone are synthetic and natural tyrosinase inhibitors [8,9]. Kojic acid is a common used tyrosinase inhibitör, which is applicated as a plant growth regulator, food preservative, food additive and skin-whitening agent. In addition, it is used as a positive control in studies [10]. Tyrosinase inhibitors used have undesirable side effects such as weak penetrability, low stability, cytotoxicity, insufficient activity, erythema and contact dermatitis to the skin [4,11]. Because of these undesirable side effects, there is a require to improve new tyrosinase inhibitors with high action and safety.

The compounds used in this study were synthesized by Bekircan et al. (2016) and inhibitory effects of these compounds on urease and xanthine oxidase enzymes were investigated [12]. Herein, the ADME properties of these compounds were specified and the inhibition effects on the tyrosinase enzyme activity were examined. After calculate the IC_{50} values, the kinetic mechanism of the compound with the best inhibition effect was studied.

MATERIALS and METHODS

Materials
The solvents and chemicals used in this work were procured from Fluka, Merck and Sigma Aldrich.

Determination of ADME properties
The absorption, distribution, metabolism and excretion (ADME) properties of inhibitor molecules were calculated by the Molinspiration and Molsoft web tool online [13,14].

Detection of tyrosinase activity
Tyrosinase activity was performed with L-tyrosine substrate, using the method described by Espin vd. [15]. Briefly, pH 5.0, 50 mM of Mcilvaine buffer (979 µL), 10 mM stock 3-Methyl-2-benzothiazolinone hydrazone hydrochloride hydrate solution (120 µL), N, N-dimethylformamide (DMF, 24 µL) and L-tyrosine solution (41 µL) were vortexed in the tube. Then, 36 µL of tyrosinase solution was added to this reaction and mixed. Finally, the absorption was measured for 3 minutes at 475 nm.

Optimization of Enzyme Activity

Optimum pH and temperature
To determine the pH value at which the enzyme shows the highest activity, Mcilvaine buffers with concentration of 50 mM and pH values varying between 3.0-7.0 were used. The optimum pH value for mushroom tyrosinase was specified according to the pH-Relative Activity (%) graph drawn using the obtained data.

The optimum temperature value of the mushroom tyrosinase enzyme was defined by making a series of activity determinations varying in the range of 10-50 °C. The optimum temperature for tyrosinase was found according to the Temperature-Relative Activity (%) graph drawn using the data obtained as a result of the activity determinations [16].

The stated optimum temperature and pH values were used as reaction temperature and pH in further studies.

Effect of enzyme concentration on the activity
In this study, the activity was identified as the final enzyme concentration (10-100 µg/mL) in the reaction mixture at a constant substrate concentration, optimum temperature and pH. The effective enzyme concentra-

tion was defined by plotting the activity graph against the enzyme concentration with the results obtained.

Effect of substrate concentration on the activity

To examine the action of substrate amount on mushroom tyrosinase activity, reaction solutions were arranged at substrate concentrations between 0.01 and 1.25 mM. Lineweaver-Burk plot was scratched with the acquired results and V_{max}, K_m were computed [17]. The calculated K_m value was used as the substrate concentration for further studies.

Anti-tyrosinase activity

Stock solutions of compounds used in inhibition studies were prepared in dimethyl sulfoxide (DMSO). Then, solutions of inhibitor compounds were diluted using these stock solutions. Kojic acid was used as the standard inhibitor. Inhibitor solution (12 µL) and 1 mg/mL enzyme solution (36 mL) were mixed and pre-incubated for 10 minutes, at 25°C. pH 5.0, 50 mM Mcilvaine buffer (967 µL), 10 mM MBTH solution (120 µL), DMF (24 µL) and 5.0 mM substrat solution (41 µL) were added. Measurements were carried out at 475 nm, for 3 minutes using a UV/Vis spectrophotometer. The relative enzyme activity (%) was composed opposite the inhibitor concentrations of every organic molecules and the half-maximum inhibitory concentration values (IC_{50}) of each inhibitor compounds were determined.

Inhibition type, K_m, V_{max} and K_i values

To determine the K_m, V_{max}, K_i values and inhibition type of the inhibitor compound with the smallest IC_{50} between the studied inhibitor compounds; the Lineweaver-Burk curve was created by using two distinctive inhibitor concentrations (20 µM and 40 µM) at a substrate concentration ranging from 0.01-1.25 mM [16,18,19].

Statistical analysis

All experiments in this study were applied independently in triplicate. The data obtained are shown as arithmetic means ± standard deviations. Statistical analysis was stated using One-Way ANOVA.

Table 1. ADME properties of compounds.

Entry	% ABS	TPSA (A2)	n-ROTB	MV	MW	miLogP	n-ON acceptors	n-OHNH donors	Nviolations	Drug-LikenessScore
Rule	-	-	-	-	≤500	≤5	≤10	≤5	≤1	
2a	77.2	92.16	6	252.17	294.29	0.98	7	2	0	-0.52
2b	77.2	92.16	6	252.17	294.29	1.02	7	2	0	-0.00
3a	67.3	120.97	4	233.51	280.26	-1.60	8	5	0	-0.08
3b	67.3	120.97	4	233.51	280.26	-1.56	8	5	0	0.47
4a	72.0	107.32	6	321.82	386.36	1.98	8	3	0	0.57
4b	72.0	107.32	6	321.82	386.36	2.02	8	3	0	0.67
4c	72.0	107.32	7	384.19	436.37	2.71	8	3	0	0.15
4d	72.0	107.32	7	384.19	436.37	2.76	8	3	0	0.36
5a	67.9	119.01	8	351.23	433.44	1.02	9	5	0	0.53
5b	67.9	119.01	8	351.23	433.44	1.07	9	5	0	0.73
6a	74.7	99.47	5	332.64	415.43	1.39	8	3	0	0.23
6b	74.7	99.47	5	332.64	415.43	1.43	8	3	0	0.01
7a	75.7	96.57	8	426.91	523.54	4.08	8	2	1	-0.03
7b	75.7	96.57	8	426.91	523.54	4.12	8	2	1	-0.19
8a	71.9	107.68	4	252.50	322.32	-0.37	8	3	0	-0.16
8b	71.9	107.68	4	252.50	322.32	-0.33	8	3	0	0.19
9a	68.3	118.08	9	393.78	511.46	2.82	10	3	1	0.03
9b	68.3	118.08	9	393.78	511.46	2.87	10	3	1	0.23
Kojic acid	84.6	70.67	1	117.43	142.11	-0.89	4	2	0	-1.04

RESULTS and DISCUSSION

Determination of ADME properties

The results of percentage absorption (% ABS), topological polar surface area (TPSA), number of rotatable bonds (n-ROTB), molecular volume (MV), molecular weight (MW), the logarithm of partition coefficient of compound between n-octanol and water (miLog P), number of hydrogen bond acceptors (n-ON acceptors), number of hydrogen bonds donors (n-OHNH donors) of the compounds and kojic acid are shown in Table 1. Eighteen compounds (excluding only the molecular weight characteristic of compounds 7a-b, 9a-9b) were found to meet Lipinski's Five Rules (MW ≤ 500 g/mol; LogP ≤ 5 (or MLogP ≤ 4.15); n-OHNH donors ≤ 5, n-ON acceptors ≤ 10 and (TPSA) Å² < 140 Å²) [20,21]. In addition, the % ABS values of the compounds vary between 67.3% and 75.7%. Also prediction of drug-likeness model score of all synthesized compounds were studied utilizing the tool MolSoft server (Table 1). Compounds with zero or negative value should not be act as drug-like. The maximum drug-likeness score was found to be 0.73 for 5b followed by 4b and 4a with a drug-likeness scores of 0.67 and 0.57, respectively. These scores of 4a, 4b and 5b were observed to be larger than the kojic acid drug creating attention for developing these compounds to act as good candidates.

Anti-tyrosinase activity

Before determining the tyrosinase inhibition effects of the compounds, the reaction conditions of commercially available mushroom tyrosinase were defined as optimum temperature 25°C, optimum pH 5.0, L-tyrosine concentration of 0.17 mM (final concentration) and effective enzyme amount of 30 μg/mL, respectively. Under these determined conditions, the inhibition potentials of 18 compounds on tyrosinase activity were studied and the results were given in Table 2. The IC_{50} of kojic acid used as the reference inhibitor compound was gotten to be 45.7±0.9 μM. 5a (1486.2±44,5 μM) showed the lowest effect on tyrosinase activity. Besides, 3a, 4a-d, 6a and 7a did not inhibit tyrosinase. When the IC_{50} value of kojic acid was compared with the IC_{50} values of the inhibitor compounds, it was stated that 8a-b and 9a-b showed better inhibitory properties. The IC_{50} values of 8a-b and 9a-b were determined as 32.2±0.7 μM, 22.9±0.6 μM, 22.8±0.5 μM and 23.8±0.6 μM, respectively.

Table 2. IC_{50} values of compounds.

Compounds	IC50, μM	Max inhibition	
		%	[I], μM
2a	683.4±15.3	63.8±0.6	1200
2b	562.9±12.1	62.2±0.5	1200
3a	>4530	27.9±0.3	4530
3b	Not detected	Not detected	Not detected
4a	>100	11.0±0.2	100
4b	>100	0	100
4c	>50	4.1±0.2	50
4d	>50	3.4±0.1	50
5a	1486.2±44.5	75.9±0.8	2500
5b	89.9±2.6	63.4±0.6	500
6a	>500	20.1±0.4	500
6b	100±	91.5±0.8	800
7a	>66	39.0±0.3	66
7b	58.8±1.3	68.5±0.5	162
8a	32.2±0.7	89.0±0.8	40
8b	22.9±0.6	91.9±0.6	100
9a	22.8±0.5	93.8±0.5	100
9b	23.8±0.6	92.9±0.7	100
Kojic acid	45.7±0.9	98.5±0.9	100

Determination of inhibition type, K_m, V_{max} and K_i values

When the ADME properties and IC_{50} values between compounds were compared, kinetic studies were carried out using the 8b, which is the most effective in tyrosinase inhibition. Lineweaver–Burk graph was scratched utilization the data obtained from the activity studies performed in the presence and lack of 8b and the kinetic data were calculated (Figure 1, Table 3). It is seen that with increasing concentrations of 8b, the K_m value remains constant, while the V_{max} value decreases. According to these results, 8b has noncompetitive inhibition type against tyrosinase enzyme. K_i value was calculated as 6.09±0.12 µM.

CONCLUSION

In this present study, inhibition potentials of fluorine-containing 1,2,4-triazole-5-on derivatives on tyrosinase enzyme were investigated separately. When the IC_{50} value of kojic acid (45.7±0.9 µM) and the IC_{50} of the compounds were compared, it was stated that 8a-b and 9a-b (IC_{50} values 32.2±0.7 µM, 22.9±0.6 µM, 22.8±0.5 µM, 23.8±0.6 µM respectively) inhibited tyrosinase more effectively (about two times more). According to the results, the K_i value of the 8b was calculated as 6.09 µM and it was determined that this compound had a non-competitive inhibition type. In addition, the ADME screening data and the inhibition study findings support each other.
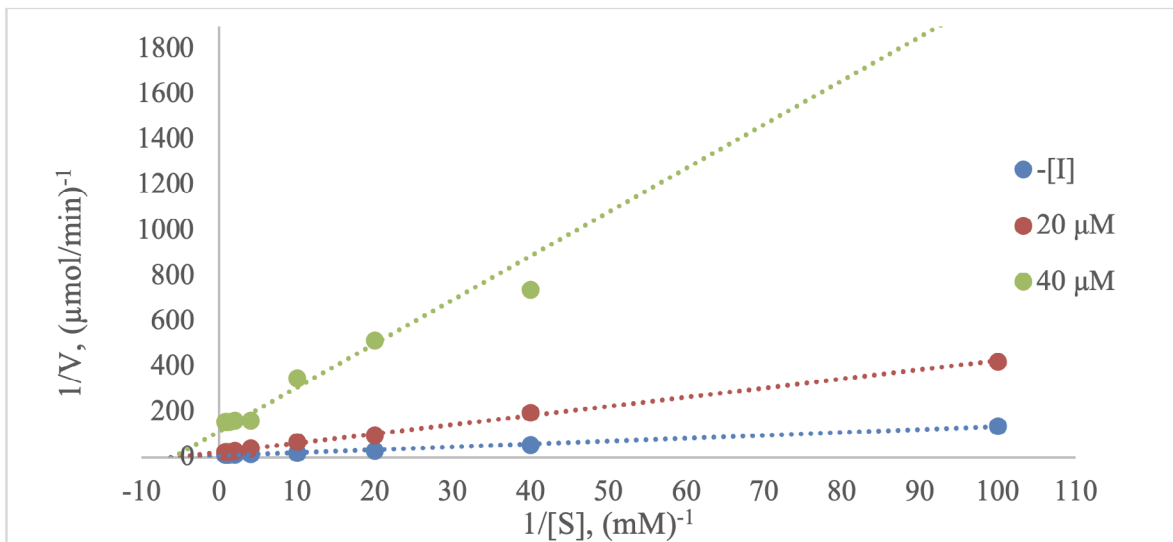
Figure 1. Lineweaver–Burk plots for compounds 8b against tyrosinase.

Table 3. The contrast of the kinetic parameters interested in the tyrosinase in the existence and lack of inhibitor and the inhibition type.

Compound	Inhibitor, (µM)	Km (mM)	Vmax (µmol/min)	Type of inhibition	Ki (µM)
8b	0	0.17	0.13	Noncompetitive	6.09
	8b	0.17	0.04		
	40	0.17	0.01		

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Conflict of Interest

The authors support that this article content has no clash of interest.

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