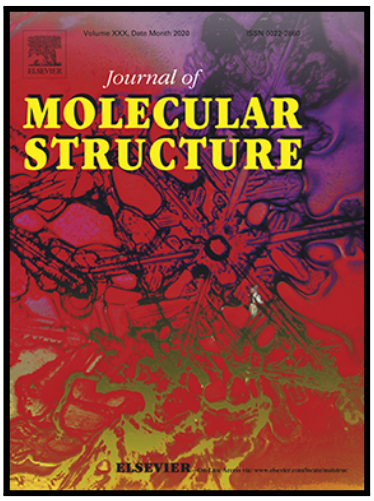


Design, Synthesis, In Vitro Evaluation and Molecular Docking Study of N'-Arylidene imidazo [1,2-a] pyridine -2-Carbohydrazide Derivatives as Novel Tyrosinase Inhibitors

Tahereh Damghani , Saba Hadaegh , Mahsima khoshneviszadeh , Somayeh Pirhadi , Razieh Sabet , Mehdi Khoshneviszadeh , Najmeh Edraki

PII: S0022-2860(20)31201-1
DOI: https://doi.org/10.1016/j.molstruc.2020.128876
Reference: MOLSTR 128876

To appear in: *Journal of Molecular Structure*

Received date: 20 April 2020
Revised date: 8 July 2020
Accepted date: 9 July 2020

Please cite this article as: Tahereh Damghani , Saba Hadaegh , Mahsima khoshneviszadeh , Somayeh Pirhadi , Razieh Sabet , Mehdi Khoshneviszadeh , Najmeh Edraki , Design, Synthesis, In Vitro Evaluation and Molecular Docking Study of N'-Arylidene imidazo [1,2-a] pyridine -2-Carbohydrazide Derivatives as Novel Tyrosinase Inhibitors, *Journal of Molecular Structure* (2020), doi: https://doi.org/10.1016/j.molstruc.2020.128876

Highlights

- A series of imidazo[1,2-a]pyridine 2-carbohudrazide derivatives bearing different arylidene pendantrs were designed and synthesized (10 compounds).
- Compounds bearing 3-nitro (6j) and 4-hydroxy (6g) moieties on the arylidene pendant exhibited the best Tyrosinase inhibitory activity with IC_{50} values of 7.19 and 8.11μM, respectively.
- Compounds 6j, 6h and 6g showed the potential of two critical pi-pi interactions with His263 and Phe264 in the active site of Tyrosinase.

The results indicated that 6j and 6g could be introduced as potent Tyrosinase inhibitors.

Design, Synthesis, In Vitro Evaluation and Molecular Docking Study of N'-Arylidene imidazo [1,2-a] pyridine -2-Carbohydrazide Derivatives as Novel Tyrosinase Inhibitors

Tahereh Damghani[1,2], Saba Hadaegh[1,2], Mahsima khoshneviszadeh[1], Somayeh Pirhadi[1], Razieh Sabet[2], Mehdi Khoshneviszadeh[1,2*], Najmeh Edraki[1*].

1. Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

2. School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

***Corresponding authors:**

Najmeh Edraki, PhrmD, PhD

Medicinal and Natural Products Chemistry Research Center, Shiraz university of Medical Sciences, Shiraz, Iran

Tel: +98 713 230 3872; Fax: +98 713 230 2225

E-mail: edrakin@sums.ac.ir, najmeh_edraki@yahoo.com

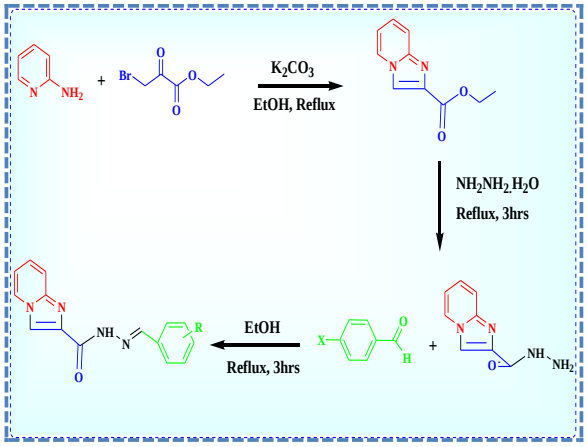
Mehdi Khoshneviszadeh, PhrmD, PhD

Medicinal and Natural Products Chemistry Research Center, Shiraz university of Medical Sciences, Shiraz, Iran

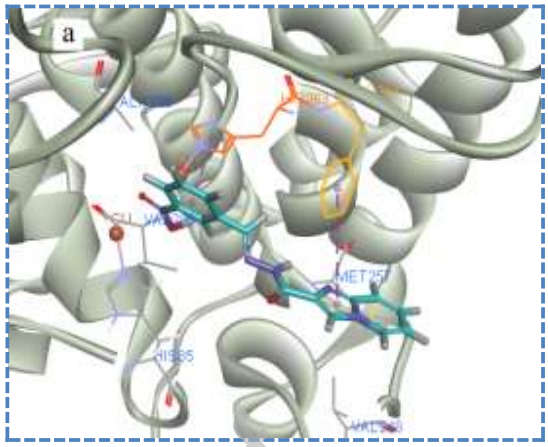
School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

E-mail: m.khoshneviszadeh@gmail.com

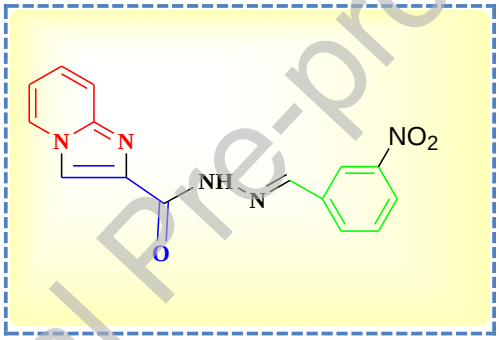
Graphical abstract



Synthesis route of Tyrosinase inhibitors



Molecular docking interaction inside Tyrosinase active site



Compound **6j** with IC_{50}= 7.19 µM

Abstract

A novel series of imidazo[1,2-a]pyridine 2-carbohudrazide derivatives bearing different arylidene pendantrs were designed, synthesized and evaluated for their inhibitory activity against mushroom Tyrosinase. It was found that compounds bearing 3-nitro (6j) and 4-hydroxy (6g) moieties on the arylidene pendant exhibited the best Tyrosinase inhibitory activity with IC_{50} values of 7.19 and 8.11μM, respectively. These results were comparable to that of kojic acid as the reference drug (IC_{50} = 9.64±0.5 μM). Additionally, molecular docking analysis was performed to study the interactions and binding modes of compounds 6j, 6h and 6g which are showing the potential of two critical pi-pi interactions with His263 and Phe264 in the active site of Tyrosinase. The results indicated that 6j and 6g could be introduced as potent Tyrosinase inhibitors that might serve as promising candidates in medicine, cosmetics or food industry.

Keywords: Tyrosinase inhibitor; imidazo [1,2-a]pyridine; docking; Kojic acid

1. Introduction

Melanin, the key pigment primarily responsible for the hair, eyes and skin pigmentation of human, is produced by melanocytes through melanogenesis. In regard to photoprotection, melanogenesis and skin pigmentation are the most significant factors in damage response to ultraviolet radiation from the sun and skin photocarcinogenesis.

The increased melanin synthesis and accumulation of these pigments occur in specific parts of the skin disorders including hyperpigmentation, lentigines, neurodegeneration associated with Parkinson's disease and skin cancer risk[1-3]

Though melanogenesis (the synthesis of melanin) is a very complex process represented by many enzymatic and chemical reactions, the enzymes such as Tyrosinase and other Tyrosinase-related proteins (TYRP1 and TYRP2) have a key role in melanin synthesis [4].

Tyrosinase a multifunctional copper-containing metalloenzyme with dinuclear copper ions, is responsible for the distinct reaction of melanin biosynthesis from tyrosine [5, 6]. Moreover Tyrosinase is the main factor for undesired browning of fruits and vegetables which leads to rapid degradation through the postharvest and handing process [7].

Considering the above mentioned points, controlling the activity of this enzyme by Tyrosinase inhibitors is an important effort for the treatment of hyperpigmentation disorders and fruit enzymatic browning. In addition, many Tyrosinase inhibitors are of great interest in the medical and cosmetic products as well as in food and environmental industries [8-10]. However, due to some limitations such as lack of safety, allergic reactions, low efficacy and poor bioavailability, development of effective and safe Tyrosinase inhibitors is still a field of great interest [11, 12].

Many natural and synthetic products such as simple phenolic and hydrazine-containing compounds can be classified as Tyrosinase inhibitors. for example vanillin [13] and its derivatives [14], hydroquinone [15, 16] and compounds of this type [17, 18] such as resorcinol (or resorcin) [19], 4-n-butylresorcinol [20**],** tropolon and kojic acid have been reported as possible phenolic inhibitors of the Tyrosinase in the scientific literature (Fig. 1).

Imidazo [1,2-a]pyridine have displayed a broad range of biological and pharmacological activities including anti-inflammatory [21], anticancer [22], anti-ulcer [23, 24] and antifungal [25]. Additionally, a number of hydrazides, acylated derivatives of hydrazine have been reported to

possess a number pharmaceutically effects such as antitumoral, anticonvulsant, antioxidant, anti-inflammatory and antimicrobial [26-29]. In this study, in order to the discovery of new Tyrosinase inhibitors, we report the synthesis and biological evaluation of imidazo [1,2-a]pyridine-2-carbohydrazide derivatives bearing various arylidene pendants. Additionally, molecular docking analysis of the synthetic compounds was carried out to find a perception of the ligand-receptor interactions of the compounds in the active site of Tyrosinase enzyme.

2. Materials and methods

2.1. Chemistry

All starting materials, reagents and solvents were purchased from the suppliers (Sigma-Aldrich, Fluka and Merck) and were used without more purification. Reaction progress was monitored by thin layer chromatography (TLC) on MERCK precoated silica gel 60-F254 (0.5 mm) aluminum plates and visualized under UV light (254 nm).

The melting points of title compounds were determined with Thermo Scientific Electrothermal digital apparatus (Thermo Fisher Scientific Inc.). The IR spectra obtained using Perkin-Elmer Spectrum RXI FTIR spectrophotometer (KBr disks). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 Hz) spectra were measured using a Bruker 300 MHz NMR instrument by using DMSO-d6 as solvent and TMS as an internal standard. The chemical shifts (**δ**) are expressed in parts per million (ppm). The MS spectra were recorded using Agilent 7000 triple quadrupole mass spectrometer at an electron impact mode with an ionization voltage of 70 eV.

2.2. Synthesis

2.2.1. Procedure for the synthesis of ethyl imidazo [1,2-a]pyridine-2-carboxylate **(3)**

2-Aminopyridine (5 mmol) was added to 10 ml of ethanol and the mixture was stirred on ice bath. Then ethyl 3-bromo 2-oxopropanoat (7.5 mmol) was added and the mixture was refluxed for about 24 h. After cooling, the solid was filtered and washed with cold diethyl ether and recrystallized from ethanol.

Pale yellow solid; Yield: 90%, mp: 135-142 °C. IR (KBr, cm^{-1}): 3086, 2986, 1729. ^{1}HNMR (300MHz, CD_3COCD_3): δ_H (ppm) 8.41 (d, J = 6 Hz, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 7.43 (d, J = 9 Hz, Ar-H), 7.20(t, J = 6 Hz, 1H, Ar-H), 6.84 (t, J = 6 Hz, 1H, Ar-H), 4.22 (q,

2H,COO$\underline{CH_2}CH_3$), 1.23 (t, J = 9Hz, 3H, COOCH$_2\underline{CH_3}$) MS (EI) m/z (%): 190 (M+, 28), 145 (34), 118 (100), 91 (19), 78 (40).

2.2.2. Procedure for the synthesis of imidazo [1,2-a]pyridine-2-carbohydrazide **(4)**

A mixture of ethyl imidazo [1,2-a]pyridine-2-carboxylate (5 mmol) and hydrazine hydrate (4ml) was refluxed for 3 h. After cooling, the mixture of reaction was washed with cold ethanol and recrystallized from ethanol to give pure imidazo [1,2-a]pyridine-2-carbohydrazide.

White solid; Yield: 25%, mp: 192-198 ºC. IR (KBr, cm^{-1}): 3353, 3187, 3050, 1660. ^{1}HNMR (300MHz, $CDCl_3$): δ_H (ppm) 9.53 (s, 1H, CON$\underline{H}$), 8.58 (d , J = 6 Hz, Ar-H), 8.36 (s, 1H, Ar-H), 7.59 (d, J = 9 Hz, 1H, Ar-H), 7.33 (t, J = 9 Hz, 1H, Ar-H), 6.94 (t, J = 6 Hz, 1H, Ar-H), 4.48 (s, 2H, CONH$\underline{NH_2}$) MS (EI) m/z (%): 176 (M+, 100), 145 (100), 117 (45), 97 (34), 78 (39) .

2.2.3. Procedure for the synthesis of substituted derivative of imidazo [1,2-a]pyridine-2-carboxylic acid benzylidene-hydrazide **(6a-6j)**

Different derivatives of **(6a-6j)** were prepared via the reaction of appropriate imidazo [1,2-a]pyridine-2-carbohydrazide**(4)** (1.2 mmol) with benzaldehyde derivatives **(5a-5j)** (2 mmol) in chloroform/ethanol (9:1). This reaction mixture was stirred under reflux for 24 h. After completion of the reaction confirmed by TLC, the solid salts were separated by filtration and the filtrate was washed with n-hexane and further purified with appropriate solvent.

2.3.1 Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (2-methoxy-benzylidene)-hydrazide **(6a)**

White solid; Yield: 88%, mp: 110-114 ºC, IR (KBr, cm^{-1}): 3290, 3140, 3000, 2835, 2400, 1673, 1381. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.99 (s, 1H, CON$\underline{H}$), 8.92 (s, 1H, Ar-H), 8.62(d, 1H, J=7 Hz, Ar-H), 8.53 (s, 1H, N=C$\underline{H}$), 7.87 (d, 1H, J=7 Hz, Ar-H), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.39 (m, 2H, Ar-H), 7.09 (d, 1H, J=7 Hz, Ar-H), 7.02 (t, 2H, J=7 Hz, Ar-H), 3.86 (s, 3H, OC$\underline{H3}$). ^{13}CNMR (75MHZ):159.04, 158.29, 144.4, 144.08, 139.14, 131.87, 128.16, 127.01, 126.10, 123.19, 121.1, 177.78, 116.23, 113.79, 112.34, 56.22. MS (EI) *m/z* (%): 214 (M+, 9.9), 161 (68.82), 145 (66.6), 118 (100), 78 (42.21).

2.3.2 Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (4-methoxy-benzylidene)-hydrazide **(6b)**

White solid; Yield: 83%, mp: 174-178 ºC, IR (KBr, cm^{-1}): 3405, 3141, 2999, 2838, 2400, 1655, 1443. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm)) 11.83 (s, 1H, C=ONH), 8.63(d, 1H, J=6 Hz, Ar-H), 8.51 (s, 2H, Ar-H and N=CH),7.81 (d,1H, J=9 Hz, Ar-H),7.64 (d, 2H, J=8 Hz, Ar-H), 7.39 (t, 1H, J=8 Hz, Ar-H), 7.00-7.04 (m, 3H, Ar-H),3.80 (s, 3H, OCH_3) .^{13}CNMR (75MHZ):161.23, 158.75, 148.37, 144.38, 139.19, 129.14, 128.18, 127.49, 127.0, 117.72, 116.20, 114.79, 113.83, 55.74. MS (EI) *m/z* (%): 294.1 (M+, 17.88), 161 (69.43), 145 (63.15), 118 (100), 78 (36.82).

2.3.3. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3,4,5-trimethoxy-benzylidene)-hydrazide **(6c)**

White solid; Yield: 27%, mp: 221-225 ºC. IR (KBr, cm^{-1}): 3194, 3121, 2998, 2940, 2400, 1668, 1360, 1380, 1433. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.95 (s, 1H, C=ONH), 8.63 (d, 1H, J=7 Hz, Ar-H), 8.54 (s, 1H, Ar-H),8.51 (s, 1H, N=CH), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.39 (t, 1H, J=7 Hz Ar-H), 6.99-7.05 (m, 3H, Ar-H), 3.85 (s, 6H, $OCH_{3\ 3}$), 3.71(s, 3H, OCH_3). ^{13}CNMR (75MHZ): 158.96, 153.65, 148.34, 144.40, 139.61, 138.97, 130.50, 128.19, 127.15, 117.72, 116.35, 113.88, 104.66, 60.59, 56.45. MS (EI) *m/z* (%): 354.1 (M+, 21.5), 161 (53.75), 145 (59.13), 118 (100), 78 (29.02).

2.3.4. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-hydroxy-4-methoxy-benzylidene)-hydrazide **(6d)**

White solid; Yield: 36%, mp: 260-264 ºC IR (KBr, cm^{-1}): 3583, 3307, 3019, 2964, 2400, 1711. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.74(s, 1H, C=ONH), 9.32 (s, 1H, OH), 8.62 (d, 1H, J=7 Hz, Ar-H), 8.51 (s,1H, N=CH), 8.45(s, 1H, Ar-H), 7.62 (d, 1H, J=9 Hz, Ar-H), 7.38 (t, 1H, J=7Hz, Ar-H), 7.26(S, 1H, Ar-H), 6.88-7.04 (m, 3H, Ar-H), 3.81 (s, 3H, OCH_3). ^{13}CNMR (75MHZ):158.77, 150.17, 148.57, 147.33, 144.39, 139.18, 128.16, 127.83, 127.05, 120.64, 117.71, 116.14, 113.79, 112.83, 112.35, 56.03. MS (EI) *m/z* (%): 310 (M+, 31.57), 161 (63.12), 145 (86.26), 118 (100), 78 (38.92).

2.3.5. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-ethoxy-4-hydroxy-benzylidene)-hydrazide **(6e)**

White solid; Yield: 43%, mp: 139-143 ºC, IR (KBr, cm^{-1}): 3168, 2964, 2853, 2964, 2400, 1655. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.76 (s, 1H, C=ONH), 9.52(s, 1H, Ar-H), 8.62 (d, 1H, J=6 Hz, Ar-H), 8.53 (s, 1H, N=CH), 8.46 (s, 1H, Ar-H), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.39 (t, 1H, J=8 Hz, Ar-H), 7.29(s,1H, Ar-H), 6.84-7.04 (m, 2H, Ar-H), 6.5 (d, 1H, J=7 Hz, Ar-H), 4.06 (t, 2H, J=6 Hz, OCH_2), 1.34 (broad, 3H, CH_3). ^{13}CNMR (75MHZ): 158.70, 149.61, 149.04, 147.64, 144.32, 138.97, 128.19, 127.21, 126.32, 122.55, 117.62, 116.14, 115.96, 113.87, 110.72, 64.32, 15.19. MS (EI) *m/z* (%): 324 (M+, 19), 161 (50), 145 (70), 118 (100), 78 (35).

2.3.6. Synthesis of Acetic acid 4-[(imidazo [1,2-a]pyridine-2-carbonyl)-hydrazonomethyl]-2-methoxy-phenyl ester **(6f)**

White solid; Yield: 40%, mp: 208-212 ºC. IR (KBr, cm^{-1}): 3451, 3228, 3146, 3076, 2400, 1759. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.98 (s, 1H, C=ONH), 8.59-8.55 (m, 3H, Ar-H and N=CH), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.45 (s, 1H, Ar-H), 7.39 (t, 1H, J=7 Hz, Ar-H), 7.26 (d, 1H, J=7 Hz, Ar-H), 7.18 (d, 1H, J=7 Hz, Ar-H), 7.03 (t, 1H, J=7Hz, Ar-H), 3.85 (s, 3H, OCH_3), 2.28 (s, 3H, $O=CH_3O$). ^{13}CNMR (75MHZ): 168.91, 159.07, 151.68, 147.81, 144.42, 141.21, 138.90, 133.87, 128.19, 127.17, 123.76, 120.98, 117.74, 116.39, 113.09, 110.18, 56.31, 20.87. MS (EI) m/z (%): 352 (M+, 8.8), 161 (86.65), 145 (71.11), 118 (100), 78 (38.88).

2.3.7. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (4-hydroxy-benzylidene)-hydrazide **(6g)**

Wihte solid; Yield: 63%, mp: 236-240 ºC. IR (KBr, cm^{-1}): 3243, 3242.7, 2400, 1627

^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.72 (s, 1H, C=ONH), 9.98 (s, 1H, OH), 8.62(d, 1H, J=6Hz, Ar-H), 8.48-8.51(m, 2H, Ar-H and N=CH), 7.63(d, 1H, J=9 Hz, Ar-H), 7.54 (d, 2H, J=9 Hz, Ar-H), 7.39 (t, 1H, J=7 Hz, Ar-H), 7.02 (t, 1H, J=7 Hz, Ar-H), 6.84 (d, 2H, J=7 Hz, , Ar-H). ^{13}CNMR (75MHZ): 159.80, 158.70, 148.80, 144.33, 138.99, 129.32, 128.19, 127.20, 125.91, 117.62, 116.28, 113.86. MS (EI) m/z (%): 280 (M+, 14.7), 161 (73.5), 145 (71.56), 118 (100), 78 (53.9).

2.3.8. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (2,4-dihydroxy-benzylidene)-hydrazide **(6h)**

White solid; Yield: 21%, mp: 300-305 ºC, IR (KBr, cm^{-1}): 3583, 3307, 3019, 2964, 2400, 1711. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 12.15 (s, 1H, C=ONH), 11.67 (s, 1H, OH), 10.02 (s, 1H, OH), 8.54-8.64 (m, 3H, Ar-H and N=CH), 7.65 (d, 1H, J=8 Hz, Ar-H), 7.40 (t, 1H, J=7 Hz, Ar-H), 7.22 (d, 1H, J=7 Hz, Ar-H), 7.03 (s, 1H, Ar-H), 6.35(m, 2H, OH). ^{13}CNMR (75MHZ): 161.10, 160.06, 158.54, 150.30, 144.42, 138.49, 132.13, 128.24, 127.30, 117.63, 116.31, 113.93, 111.01, 108.13, 103.17. MS (EI) *m/z* (%): 296 (M+, 14), 145(99), 118 (80), 78 (79).

2.3.9. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-hydroxy-benzylidene)-hydrazide **(6i)**

White solid; Yield: 48%, mp: 143-145 ºC. IR (KBr, cm^{-1}): 3544, 3148, 3019, 2400, 1644. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.90 (s, 1H, C=ONH), 9.67 (s, 1H, OH), 8.62 (d, 1H, J=6, Ar-H), 8.52-8.54 (m, 2H, Ar-H and N=CH), 7.63 (d, 1H, J=9 Hz, Ar-H), 7.38(t, 1H, J=7 Hz, Ar-H), 7.25 (t, 1H, J=7 Hz, Ar-H), 7.19 (broad, 1H, Ar-H), 7.08 (d, 1H, J=6 Hz, Ar-H), 7.02 (t, 1H, J=6 Hz, Ar-H), 6.84 (d, 1H, J=7Hz, Ar-H). ^{13}CNMR (75MHZ): 158.98, 158.11, 148.59, 144.39, 138.89, 136.22, 130.34, 128.19, 127.19, 119.21, 117.82, 117.69, 116.31, 113.88, 113.14. MS (EI) *m/z* (%): 280 (M+, 9.25), 161 (84.05), 145 (97.12), 118 (100), 78 (41.62).

2.3.10. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-nitro-benzylidene)-hydrazide **(6j)**

Light yellow solid; Yield: 44%, mp: 258-262 ºC. IR (KBr, cm^{-1}): 3446, 3154, 3029, 2400, 2400, 1658. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 12.20 (s, 1H, C=ONH), 8.70 (s, 1H, Ar-H), 8.57-8.62-(m, 2H, Ar-H and N=CH), 8.48 (s, 1H, Ar-H), 8.23 (d, 1H, J=7 Hz, Ar-H), 8.10 (d, 1H, J=6 Hz, Ar-H), 7.73 (t, 1H, J=6 Hz, Ar-H), 7.65 (d, 1H, J=8 Hz, Ar-H), 7.38 (s, 1H, Ar-H), 7.02 (s, 1H, Ar-H). ^{13}CNMR (75MHZ): 159.30, 148.63, 146.01, 144.45, 138.65, 136.77, 133.70, 130.91, 128.19, 127.21, 124.59, 121.25, 117.75, 116.59, 113.92. MS (EI) *m/z* (%): 309 (M+, 9.7), 161 (70.59), 145 (80.43), 118 (100), 78 (49.95).

2.3. Tyrosinase assay

Mushroom Tyrosinase (EC 1.14.18.1) (Sigma Chemical Co.) was tested according to a previous study with slight modifications using LDOPA as substrate [30]. Enzyme activity was checked by detecting dopachrome formation at 475 nm. The stock solutions of all test samples and kojic acid were prepared by dissolving in DMSO at 40mM and then diluted with phosphate buffer (pH=6.8) to the required concentrations. Initially, 10 µL of Tyrosinase (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. After the mixture was pre-incubated at 28 ºC for 20 min, 20 µL of L-DOPA solution (0.5 mM) was added to the phosphate buffer. DMSO in the absence of the test compounds was applied as the control, and kojic acid was used as a positive control. Each concentration was analyzed in three separate replicates. The inhibitory activity of the test compounds was expressed as the concentration that inhibited 50% of the enzyme activity (IC_{50}). The percent inhibition ratio was calculated according to the following equation:

$$\text{Inhibition (\%)} = 100 - (Abs_{control} - Abs_{compound}) / Abs_{control}$$

2.4. Molecular docking study

All compounds were sketched using MarvinSketch 18.20.0 [31] and energy of compounds was optimized using steepest descent algorithm by Open Babel 2.4.0 software [32]. GOLD 2018 docking program [33] was applied to dock all compounds using PDB code of 2Y9X. This structure is a Tyrosinase from Agaricus bisporus having tropolone as the native ligand in the binding site. Two Cu^{2+} metal ions exist in the binding site of Tyrosinase and were included for docking calculations. Protein structure was prepared using Discovery studio client [34] and all hydrogens were added. Binding site of the enzyme for docking was defined automatically by setting 8 Å around the coordinates of the native ligand tropolone. All available scoring functions in GOLD, namely CHEMPLP, ChemScore, ASP, and GoldScore were tried to re-dock tropolone inside the 2Y9X and then RMSD measure was calculated to validate and find the most appropriate scoring function for docking analyses.

3. Results and discussion

3.1. Design strategy

We designed the target compounds bearing imidazo [1,2-a]pyridine-carbohydrazid scaffold based on the structures of some potent Tyrosinase inhibitors reported in the literature [35, 36]. Some benzimidazole derivatives such as 2-(3-Methylbenzyl)-4(7)-phenyl-1*H* benzo[*d*]imidazole (**I**) illustrated Tyrosinase inhibitory activity with IC_{50} value of 37.86 μM [37]. The benzothiazole analogs like compound **(II)** have been reported as potential Tyrosinase inhibitors which demonstrated higher inhibition than kojic acid at 50 μM [38]. Additionally, the hydrazine-containing compounds such as compound **(III)** have been reported as effective Tyrosinase inhibitors (IC_{50}=1.93 μM) which exhibited better Tyrosinase inhibitor activity than kojic acid (Fig. 2) [39]. Based on these findings, herein we designed a series of imidazo [1,2-a]pyridine-2-carbohydrazide derivatives containing various arylidene pendants as tyroinase inhibitors via hybridization of imidazo pyridine core with hydrazine moiety as copper chelator. Furthermore, various phenolic moieties and its derivatives were introduced as a substituted benzylidene pendant to meet the essential requirements of Tyrosinase inhibitors and constructing structure–activity relationships (SARs).

3.2. Synthesis

The synthetic route to target compounds **6a–6j** is represented in Scheme 1. Reaction of 2-aminopyridine **(1)** and ethyl bromopyruvate **(2)** in refluxing ethanol resulted in ethyl imidazo [1,2-a]pyridine-2-carboxylate (**3**). In the next step, reaction of compound **(3)** with hydrazine hydrate under reflux condition afforded imidazo [1,2-a]pyridine-2-carbohydrazide **(4)**. Finally different substituted benzaldehyde derivatives **(5a-j)** were reacted with **2** in chloroform/ethanol (9:1). The resulting mixture was stirred under reflux and completion of the reaction was monitored by TLC to give compounds **6a–6j**.

3.3. Tyrosinase inhibitory activity

The inhibitory effect of all the synthesized derivatives were conducted on mushroom Tyrosinase enzyme by evaluation of their potential to inhibit enzymatic oxidation of L-Dopa. Kojic acid was used as the reference inhibitor according to the literature protocol [30, 40]. The chemical structure of the synthesized compounds and results are presented in Table 1.

The most potent compounds 6j and 6g exhibited considerable inhibitory potential with IC_{50} values of 7.19±2.56 μM and 8.11±1.25 μM, respectively against mushroom Tyrosinase compared to kojic acid with IC_{50} value of 9.64±0.50 μM. Compounds 6c, 6h, 6b, 6f and 6e showed moderate to weak inhibitory activities (IC_{50}s = 11.1-81.9 μM) while the rest of compounds (6a, 6d and 6i) were almost inactive.

Considering the obtained results from the SAR, there is a strong relation between the nature and position of the phenyl ring substitutions and Tyrosinase inhibition that can be shown as follows:

It seems that the presence of an electron donating group such as hydroxy and methoxy at the para position of the phenyl ring enhanced the inhibitory activity (compounds **6g** and **6b**). In addition, introduction of some groups such as nitro into the meta position of the phenyl moiety increased the anti-Tyrosinase potential of synthesized compound (compound **6j**). In contrast, compound **6i** bearing hydroxyl group on meta position of the phenyl ring was completely inactive. Furthermore, it was found that compound **6a** bearing 2-methoxy group on the phenyl ring was completely inactive while compound **6h** bearing 2,4 di hydroxyl residues exhibited the moderate Tyrosinase inhibitory activities. The comparison of substituents on different positions of phenyl ring indicated that compounds **6f**, **6e** and **6d** bearing different substitutes such as 3-methoxy-4-acetoxy, 3-ethoxy-4-hydroxy and 3-hydroxyl-4methoxy showed weak to inactive inhibitory activities; however, increasing the number of electrondonating group as in compound **6c** bearing 3,4,5 tri methoxy improved the activity. Consequently, the anti-Tyrosinase activity of **6j** and **6g** are higher than kojic acid and we hope that the imidazopyridine derivative would overcome some limitations of kojic acid such as formulation stability and skin penetration difficulties [41].

3.4. Molecular docking analysis

Molecular docking analysis was carried out to investigate interactions and binding poses of all synthesized compounds inside the binding site of Tyrosinase enzyme. RMSD value of redocking process of tropolone inside the active site of Tyrosinase with all available scoring functions in the GOLD was calculated in such a way that for ChemScore was 2.00 Å and for other scoring function algorithms was higher than 2.00 Å. Therefore, score values and binding modes of Tyrosinase inhibitors with the enzyme with PDB code 2Y9X was investigated by ChemScore fitness function. ChemScore fitness value of docking of all compounds along with their important interactions with amino acid residues inside the Tyrosinase active site were shown in

Table 1. ChemScore fitness values ranged from 34.73 in compound **6j** to 20.37 in compound **6c**. 3D interaction patterns of two active compounds **6j** and **6g** with IC_{50} values 7.19 and 8.11 µM respectively, and two inactive compounds **6a** and **6i** with IC_{50} values more than 100 µM, were depicted in Fig. 3.

Fig. 3a shows the docking interactions of compound **6j**. Docking score of this compound is 34.73 which is the highest score value among all compounds. Backbone structure of the compound made two pi-pi interactions via its aromatic rings: one pi-pi t-shaped interaction from imidazo [1,2-a]pyridine ring to Phe264 and one pi-pi stacked interaction from nitrobenzene group to His263. These two pi-pi interactions with these residues were among important ones. Other interaction types comprised pi-sigma and pi-alkyl interactions with Val283 and Ala286 respectively and pi-sigma interaction from imidazo [1,2-a]pyridine ring toVal283.

Docking interaction analysis of compound **6g**, Fig. 3b, shows the same pattern of pi-pi interactions with Phe264 and His263 from imidazo [1,2-a]pyridine ring and phenyl ring, respectively. Replacement of 3-nitro with 4-hydroxy group changed the orientation of compound **6g** in such a way that one hydrogen bond was made between carbonyl group and residue Val283. This additional hydrogen bond interaction did not cause any significant difference in activity against Tyrosinase in comparison to compound **6j**. Chemscore fitness value of this compound is a high value of 29.29. Compound **6g** made other interaction types such as a pi-cation from imidazo [1,2-a]pyridine ring to residue Arg268, a pi-alkyl interaction and a pi-sigma interaction between phenol and Ala286, and Val283 respectively that all further strengthened its binding towards Tyrosinase enzyme.

Another moderate to active compound is **6h** with IC_{50} value of 12.6 µM having ChemScore fitness value of 31.53. This compound made two important pi-pi interactions of the backbone with Phe264 and His263. In comparison to compound **6g** bearing 4-hydroxy moiety, compound **6h** has an additional hydroxyl in position 2 that donated a hydrogen bond to Asn260. This hydroxyl also made a metal-acceptor interaction with one Cu^{2+}. Another hydrogen bond interaction was formed between carbonyl group and Val283. A pi-alkyl interaction between Val283 and phenyl ring was also made, see Fig 3c.

In inactive compound **6i**, 3-hydroxy replaced 4-hydroxy in compound **6g**. This substitution got the backbone away from Phe264 in such a way that it was not able to make this critical pi-pi

interaction and just made pi-pi stacked interaction with His263. ChemScore fitness value of **6i** is 30.17 which might be resulted from other not that much important hydrophobic interactions it made like pi-alkyl with Ala286 and pi-sigma with Val248 inside the active site of Tyrosinase, see Fig. 3d.

In overall, inactive compounds, **6a, 6d** and **6i**, lacked one or two critical pi-pi interactions with His263 and Phe264 that resulted from a different orientation of these molecules in the enzyme active site. Presence of an electrondonating group such as hydroxyl in the para position of the phenyl ring makes compound able to form a metal-acceptor interaction with Cu^{+2} ion and also changes the orientation of the molecule to be able to form a hydrogen bond from carbonyl group to residue Val283.

4. **Conclusions**

In conclusion, we developed a series of imidazo [1,2-a]pyridine-2-carbohydrazide derivatives bearing various arylidene pendants as novel Tyrosinase inhibitors. Based on the obtained data, compounds **6j** and **6g** containing 3-nitro and 4-hydroxyl moieties were the most potent compounds against Tyrosinase with respective IC_{50} values of 7.19 μM and 8.11 μM. The results indicated that the inhibitory potential was dependent on the substituted moiety on the phenyl ring. Therefore, derivatives bearing an electrondonating such as hydroxyl and electron-withdrawing group such as nitro at the para and meta position of the phenyl ring exhibited considerable inhibitory activity, respectively. Molecular docking analysis results demonstrated that potent compounds **6g**, **6h** and **6j** had high docking score values and made two critical pi-pi interactions with Phe264 and His263. These results indicate that the compounds can serve as structural outlines and promising leads in order to design and develop novel effective Tyrosinase inhibitors.

Declaration of competing interest

The authors report no conflict of interest.

Credit Author Statement

Najmeh Edraki : principle investigator, supervision of the team in all research steps including data gathering, data analysis, and manuscript preparation/revision

Mehdi Khoshneviszadeh: Supervision of the design, synthetic steps and biological evaluation, manuscript preparation.

Tahereh Damghani: contribution in the data gathering, design and synthesis of compounds, data analysis, preparation of results, writing and revising of this manuscript.

Saba Hadaegh : contribution in the synthesis of compounds.

Mahsima khoshneviszadeh: Contribution in the biological and enzymatic assay..

Somayeh Pirhadi : Supervision and performance of *computational* docking and data analysis of this study.

Razieh Sabet : Supervision of synthetic parts.

Acknowledgment

The authors wish to thank the support of the Vice-Chancellor for Research, Shiraz University of Medical Sciences (Grant Number: 14006). This work was the PharmD thesis of Saba Hadaegh.

References

[1] T.F. Cestari, L.P. Dantas, J.C. Boza, Acquired hyperpigmentations, Anais brasileiros de dermatologia, 89 (2014) 11-25. DOI: https://doi.org/10.1590/abd1806-4841.20142353.

[2] S. Nouveau, D. Agrawal, M. Kohli, F. Bernerd, N. Misra, C.S. Nayak, Skin hyperpigmentation in Indian population: Insights and best practice, Indian journal of dermatology, 61 (2016) 487. DOI: 10.4103/0019-5154.190103.

[3] S. Dogra, R. Sarangal, Pigmentary disorders: An insight, Pigment International, 1 (2014) 5. DOI: 10.4103/2349-5847.135429.

[4] A. Lapedriza, K. Petratou, R.N. Kelsh, Neural crest cells and pigmentation, in: Neural Crest Cells, Elsevier, 2014, pp. 287-311. DOI: https://doi.org/10.1016/B978-0-12-401730-6.00015-6.

[5] A. Garcia-Jimenez, J.A. Teruel-Puche, P.A. Garcia-Ruiz, J. Berna, J.N. Rodríguez-López, J. Tudela, F. Garcia-Canovas, Action of 2, 2′, 4, 4′-tetrahydroxybenzophenone in the biosynthesis pathway of melanin, International journal of biological macromolecules, 98 (2017) 622-629. DOI: https://doi.org/10.1016/j.ijbiomac.2017.02.032.

[6] C.S. Nunes, K. Vogel, Tyrosinases—physiology, pathophysiology, and applications, in: Enzymes in Human and Animal Nutrition, Elsevier, 2018, pp. 403-412. DOI: https://doi.org/10.1016/B978-0-12-805419-2.00020-4.

[7] O. Nerya, R. Ben-Arie, T. Luzzatto, R. Musa, S. Khativ, J. Vaya, Prevention of Agaricus bisporus postharvest browning with tyrosinase inhibitors, Postharvest Biology and Technology, 39 (2006) 272-277. DOI: https://doi.org/10.1016/j.postharvbio.2005.11.001.

[8] T. Pillaiyar, M. Manickam, V. Namasivayam, Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors, Journal of enzyme inhibition and medicinal chemistry, 32 (2017) 403-425. DOI: https://doi.org/10.1080/14756366.2016.1256882.

[9] S.Y. Lee, N. Baek, T.-g. Nam, Natural, semisynthetic and synthetic tyrosinase inhibitors, Journal of Enzyme Inhibition and Medicinal Chemistry, 31 (2016) 1-13. DOI: https://doi.org/10.3109/14756366.2015.1004058.

[10] M. Loizzo, R. Tundis, F. Menichini, Natural and synthetic tyrosinase inhibitors as antibrowning agents: an update, Comprehensive Reviews in Food Science and Food Safety, 11 (2012) 378-398. DOI: https://doi.org/10.1111/j.1541-4337.2012.00191.

[11] T.-S. Chang, An updated review of tyrosinase inhibitors, International journal of molecular sciences, 10 (2009) 2440-2475. DOI: https://doi.org/10.3390/ijms10062440.

[12] F.A. Larik, A. Saeed, P.A. Channar, U. Muqadar, Q. Abbas, M. Hassan, S.-Y. Seo, M. Bolte, Design, synthesis, kinetic mechanism and molecular docking studies of novel 1-pentanoyl-3-arylthioureas as inhibitors of mushroom tyrosinase and free radical scavengers, European journal of medicinal chemistry, 141 (2017) 273-281. DOI: https://doi.org/10.1016/j.ejmech.2017.09.059.

[13] M.H.K. Nguyen, H.X. Nguyen, M.T.T. Nguyen, N.T. Nguyen, Phenolic constituents from the heartwood of Artocapus altilis and their tyrosinase inhibitory activity, Natural product communications, 7 (2012) 1934578X1200700214. DOI: https://doi.org/10.1177/1934578X1200700214.

[14] Z. Ashraf, M. Rafiq, S.-Y. Seo, M.M. Babar, Synthesis, kinetic mechanism and docking studies of vanillin derivatives as inhibitors of mushroom tyrosinase, Bioorganic & medicinal chemistry, 23 (2015) 5870-5880. DOI: https://doi.org/10.1016/j.bmc.2015.06.068.

[15] K. Sakuma, M. Ogawa, K. Sugibayashi, K.-i. Yamada, K. Yamamoto, Relationship between tyrosinase inhibitory action and oxidation-reduction potential of cosmetic whitening ingredients and phenol derivatives, Archives of pharmacal research, 22 (1999) 335-339. DOI: https://doi.org/10.1007/BF02979054.

[16] A. Hashimoto, M. Ichihashi, Y. Mishima, The mechanism of depigmentation by hydroquinone: a study on suppression and recovery processes of tyrosinase activity in the pigment cells in vivo and in vitro, Nihon Hifuka Gakkai zasshi. The Japanese journal of dermatology, 94 (1984) 797.

[17] Y.-R. Chen, R.Y.-Y. Chiou, T.-Y. Lin, C.-P. Huang, W.-C. Tang, S.-T. Chen, S.-B. Lin, Identification of an Alkylhydroquinone from Rhus succedanea as an Inhibitor of Tyrosinase and Melanogenesis, Journal of agricultural and food chemistry, 57 (2009) 2200-2205. DOI: https://doi.org/10.1021/jf802617a.

[18] A. Sasaki, Y. Yamano, S. Sugimoto, H. Otsuka, K. Matsunami, T. Shinzato, Phenolic compounds from the leaves of Breynia officinalis and their tyrosinase and melanogenesis inhibitory activities, Journal of natural medicines, 72 (2018) 381-389. DOI: https://doi.org/10.1007/s11418-017-1148-8.

[19] K. Tasaka, C. Kamei, S. Nakano, Y. Takeuchi, M. Yamato, Effects of certain resorcinol derivatives on the tyrosinase activity and the growth of melanoma cells, Methods and findings in experimental and clinical pharmacology, 20 (1998) 99-110. DOI: 10.1358/mf.1998.20.2.485637.

[20] L. Kolbe, T. Mann, W. Gerwat, J. Batzer, S. Ahlheit, C. Scherner, H. Wenck, F. Stäb, 4-n-butylresorcinol, a highly effective tyrosinase inhibitor for the topical treatment of hyperpigmentation, Journal of the European Academy of Dermatology and Venereology, 27 (2013) 19-23. DOI: https://doi.org/10.1111/jdv.12051.

[21] F. Awouters, J. Vermeire, F. Smeyers, P. Vermote, R. Van Beek, C.J. Niemegeers, Oral antiallergic activity in ascaris hypersensitive dogs: a study of known antihistamines and of the new compounds ramastine (R 57 959) and levocabastine (R 50 547), Drug Development Research, 8 (1986) 95-102. DOI: https://doi.org/10.1002/ddr.430080112.

[22] H. Huang, X. Ji, X. Tang, M. Zhang, X. Li, H. Jiang, Conversion of pyridine to imidazo [1, 2-a] pyridines by copper-catalyzed aerobic dehydrogenative cyclization with oxime esters, Organic letters, 15 (2013) 6254-6257. DOI: https://doi.org/10.1021/ol403105p.

[23] M. Bollini, J.J. Casal, D.E. Alvarez, L. Boiani, M. González, H. Cerecetto, A.M. Bruno, New potent imidazoisoquinolinone derivatives as anti-Trypanosoma cruzi agents: Biological evaluation and structure–activity relationships, Bioorganic & medicinal chemistry, 17 (2009) 1437-1444. DOI: https://doi.org/10.1016/j.bmc.2009.01.011.

[24] J.J. Kaminski, C. Puchalski, D.M. Solomon, R.K. Rizvi, D.J. Conn, A.J. Elliott, R.G. Lovey, H. Guzik, P. Chiu, Antiulcer agents. 4. Conformational considerations and the antiulcer activity of substituted imidazo [1, 2-a] pyridines and related analogs, Journal of medicinal chemistry, 32 (1989) 1686-1700. DOI: https://doi.org/10.1021/jm00128a005.

[25] Y. Rival, G. Grassy, A. Taudou, R. Ecalle, Antifungal activity in vitro of some imidazo [1, 2-a] pyrimidine derivatives, European journal of medicinal chemistry, 26 (1991) 13-18. DOI: https://doi.org/10.1016/0223-5234(91)90208-5.

[26] M. Abdel-Aziz, H.M. Abdel-Rahman, Synthesis and anti-mycobacterial evaluation of some pyrazine-2-carboxylic acid hydrazide derivatives, European journal of medicinal chemistry, 45 (2010) 3384-3388. DOI: https://doi.org/10.1016/j.ejmech.2010.04.025.

[27] A. Kajal, S. Bala, S. Kamboj, V. Saini, Synthesis, characterization, and computational studies on phthalic anhydride-based benzylidene-hydrazide derivatives as novel, potential anti-inflammatory agents, Medicinal Chemistry Research, 23 (2014) 2676-2689. DOI: https://doi.org/10.1007/s00044-013-0848-1.

[28] R. Narang, B. Narasimhan, S. Sharma, A review on biological activities and chemical synthesis of hydrazide derivatives, Current medicinal chemistry, 19 (2012) 569-612. DOI: https://doi.org/10.2174/092986712798918789.

[29] R. Sreenivasulu, K.T. Reddy, P. Sujitha, C.G. Kumar, R.R. Raju, Synthesis, antiproliferative and apoptosis induction potential activities of novel bis (indolyl) hydrazide-hydrazone derivatives, Bioorganic & medicinal chemistry, 27 (2019) 1043-1055. DOI: https://doi.org/10.1016/j.bmc.2019.02.002.

[30] Y.M. Ha, S.W. Chung, S. Song, H. Lee, H. Suh, H.Y. Chung, 4-(6-Hydroxy-2-naphthyl)-1, 3-bezendiol: a potent, new tyrosinase inhibitor, Biological and Pharmaceutical Bulletin, 30 (2007) 1711-1715. DOI: https://doi.org/10.1248/bpb.30.1711.

[31] http://www.chemaxon.com.

[32] N.M. O'Boyle, M. Banck, C.A. James, C. Morley, T. Vandermeersch, G.R. Hutchison, Open Babel: An open chemical toolbox, Journal of cheminformatics, 3 (2011) 33. DOI: https://doi.org/10.1186/1758-2946-3-33.

[33] G. Jones, P. Willett, R.C. Glen, A.R. Leach, R. Taylor, Development and validation of a genetic algorithm for flexible docking, Journal of molecular biology, 267 (1997) 727-748. DOI: https://doi.org/10.1006/jmbi.1996.0897.

[34] D.S. BIOVIA, Discovery studio visualizer, Release 2017, San Diego: Dassault Systèmes, 2016, to be found under http://accelrys. com/products/collaborative-science/biovia-discovery-studio/visualization-download. php (accessed Dec 12, 2017), (2017).

[35] S. Zolghadri, A. Bahrami, M.T. Hassan Khan, J. Munoz-Munoz, F. Garcia-Molina, F. Garcia-Canovas, A.A. Saboury, A comprehensive review on tyrosinase inhibitors, Journal of enzyme inhibition and medicinal chemistry, 34 (2019) 279-309. DOI: https://doi.org/10.1080/14756366.2018.1545767.

[36] J.P. Germanas, S. Wang, A. Miner, W. Hao, J.M. Ready, Discovery of small-molecule inhibitors of tyrosinase, Bioorganic & medicinal chemistry letters, 17 (2007) 6871-6875. DOI: https://doi.org/10.1016/j.bmcl.2007.10.014.

[37] İ.S. Doğan, A. Özel, Z. Birinci, B. Barut, H.E. Sellitepe, B. Kahveci, Synthesis of Some Novel 2-substitutedbenzyl-(4) 7-phenyl-1H-benzo [d] imidazoles in Mild Conditions as Potent Anti-tyrosinase and Antioxidant Agents, Archiv der Pharmazie, 349 (2016) 881-888. DOI: https://doi.org/10.1002/ardp.201600224.

[38] H.Y. Chung, M.H. Park, Y.M. Ha, Y.K. Han, J.Y. Park, Y.J. Park, J.A. Kim, J.Y. Lee, Y.M. Song, H.R. Moon, Compound having skin-whitening, anti-oxidizing and PPAR activities and medical use therefor, in, Google Patents, 2014.

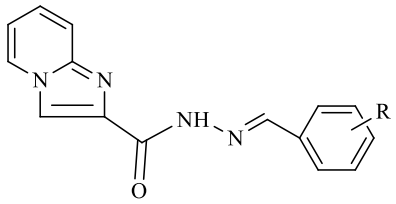
[39] W. Yi, R.-H. Cao, Z.-Y. Chen, L. Yu, L. Ma, H.-C. Song, Design, synthesis and biological evaluation of hydroxy-or methoxy-substituted phenylmethylenethiosemicarbazones as tyrosinase inhibitors, Chemical and Pharmaceutical Bulletin, 57 (2009) 1273-1277. DOI: https://doi.org/10.1248/cpb.57.1273.

[40] S. Son, H. Kim, H.Y. Yun, S. Ullah, S.J. Kim, Y.-J. Kim, M.-S. Kim, J.-W. Yoo, P. Chun, H.R. Moon, (E)-2-Cyano-3-(substituted phenyl) acrylamide analogs as potent inhibitors of tyrosinase: A linear β-phenyl-α, β-unsaturated carbonyl scaffold, Bioorganic & medicinal chemistry, 23 (2015) 7728-7734. DOI: https://doi.org/10.1016/j.bmc.2015.11.015.

[41] J. Hermanns, C. Pierard-Franchimont, G. Pierard, Skin colour assessment in safety testing of cosmetics. An overview, International journal of cosmetic science, 22 (2000) 67-71. DOI: 10.1046/j.1467-2494.2000.00021.x .

Table 1

Chemical structure, Tyrosinase inhibitory effects and molecular docking studies of synthesized compounds **6a-6j**.



compounds	R	Mw	ChemScore fitness value	Binding interactions with Tyrosinase active site	Yields*	IC_{50}(µm)±SE**
6a	2- methoxy	294	22.62	Hydrogen bonds: Val283	88%	> 100
6b	4- methoxy	294	23.93	Hydrogen bonds: Val283 Pi-pi interactions: His263,	83%	14.3±3.11
6c	3,4,5-trimethoxy	354	20.37	Hydrogen bonds: Arg268 Pi-pi interactions: Phe264	27%	11.10±1.55
6d	3-hydroxy-4-methoxy	310	28.47	Hydrogen bonds: Asn260, Val283	36%	> 100
6e	3-ethoxy-4-hydroxy	324	27.29	Pi-pi interactions: His263, Metal acceptor interaction: 1 Cu^{+2}	43%	81.9±9.75
6f	3-methoxy-4-acetoxy	352	28.49	Metal acceptor interaction: 2 Cu^{+2}	40%	41.70±2.40
6g	4-hydroxy	280	29.29	Hydrogen bonds: Ala283 Pi-pi interactions: His263, Phe264	63%	8.11±1.25
6h	2,4-dihydroxy	296	31.53	Hydrogen bonds: Asn260, Val283 Pi-pi interactions: His263, Phe264 Metal acceptor interaction: 1 Cu	21%	12.60±3.67
6i	3-hydroxy	280	30.17	Pi-pi interactions: His263	48%	> 100
6j	3-nitro	309	34.73	Pi-pi interactions: His263, Phe264	44%	7.19±2.56
Kojic Acid* **	-	-				9.64±0.50

*Reported yields are based on the yield of final step of synthesis.

** Values represent means ± SE of 3 independent experiments.*** Kojic Acid was used as a positive control.

Highlights

- A series of imidazo[1,2-a]pyridine 2-carbohudrazide derivatives bearing different arylidene pendantrs were designed and synthesized (10 compounds).
- Compounds bearing 3-nitro (6j) and 4-hydroxy (6g) moieties on the arylidene pendant exhibited the best Tyrosinase inhibitory activity with IC_{50} values of 7.19 and 8.11μM, respectively.
- Compounds 6j, 6h and 6g showed the potential of two critical pi-pi interactions with His263 and Phe264 in the active site of Tyrosinase.

The results indicated that 6j and 6g could be introduced as potent Tyrosinase inhibitors.

Design, Synthesis, In Vitro Evaluation and Molecular Docking Study of N'-Arylidene imidazo [1,2-a] pyridine -2-Carbohydrazide Derivatives as Novel Tyrosinase Inhibitors

Tahereh Damghani[1,2], Saba Hadaegh[1,2], Mahsima khoshneviszadeh[1], Somayeh Pirhadi[1], Razieh Sabet[2], Mehdi Khoshneviszadeh[1,2*], Najmeh Edraki[1*].

1. Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

2. School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

[*]**Corresponding authors:**

Najmeh Edraki, PhrmD, PhD

Medicinal and Natural Products Chemistry Research Center, Shiraz university of Medical Sciences, Shiraz, Iran

Tel: +98 713 230 3872; Fax: +98 713 230 2225

E-mail: edrakin@sums.ac.ir, najmeh_edraki@yahoo.com

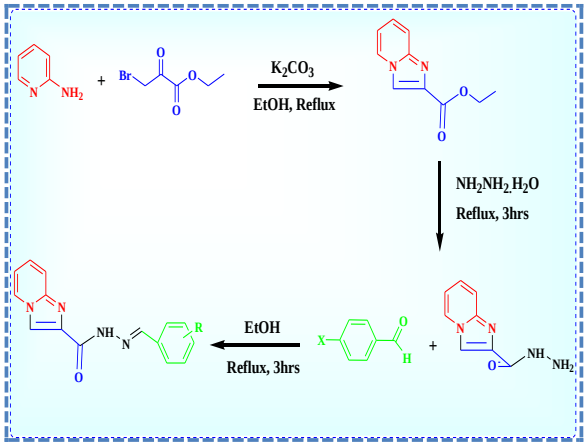
Mehdi Khoshneviszadeh, PhrmD, PhD

Medicinal and Natural Products Chemistry Research Center, Shiraz university of Medical Sciences, Shiraz, Iran

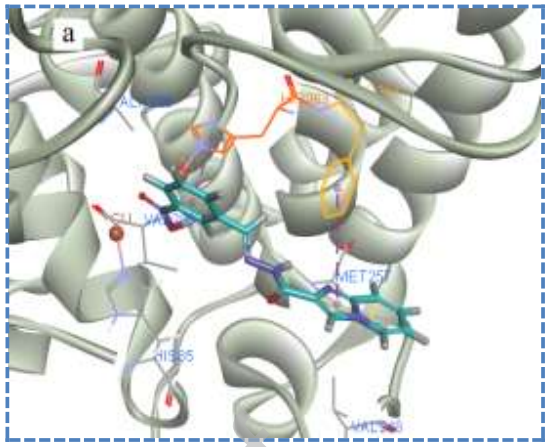
School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

E-mail: m.khoshneviszadeh@gmail.com

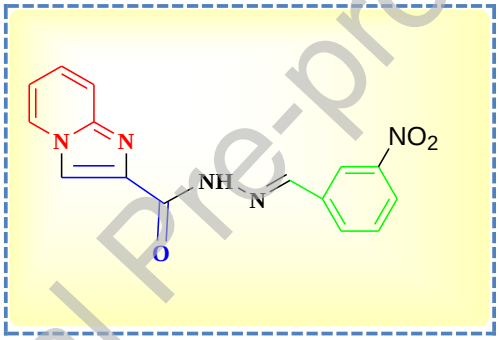
Graphical abstract



Synthesis route of Tyrosinase inhibitors



Molecular docking interaction inside Tyrosinase active site



Compound **6j** with IC_{50}= 7.19 µM

Abstract

A novel series of imidazo[1,2-a]pyridine 2-carbohudrazide derivatives bearing different arylidene pendantrs were designed, synthesized and evaluated for their inhibitory activity against mushroom Tyrosinase. It was found that compounds bearing 3-nitro (6j) and 4-hydroxy (6g) moieties on the arylidene pendant exhibited the best Tyrosinase inhibitory activity with IC_{50} values of 7.19 and 8.11μM, respectively. These results were comparable to that of kojic acid as the reference drug (IC_{50} = 9.64±0.5 μM). Additionally, molecular docking analysis was performed to study the interactions and binding modes of compounds 6j, 6h and 6g which are showing the potential of two critical pi-pi interactions with His263 and Phe264 in the active site of Tyrosinase. The results indicated that 6j and 6g could be introduced as potent Tyrosinase inhibitors that might serve as promising candidates in medicine, cosmetics or food industry.

Keywords: Tyrosinase inhibitor; imidazo [1,2-a]pyridine; docking; Kojic acid

3. Introduction

Melanin, the key pigment primarily responsible for the hair, eyes and skin pigmentation of human, is produced by melanocytes through melanogenesis. In regard to photoprotection, melanogenesis and skin pigmentation are the most significant factors in damage response to ultraviolet radiation from the sun and skin photocarcinogenesis.

The increased melanin synthesis and accumulation of these pigments occur in specific parts of the skin disorders including hyperpigmentation, lentigines, neurodegeneration associated with Parkinson's disease and skin cancer risk[1-3]

Though melanogenesis (the synthesis of melanin) is a very complex process represented by many enzymatic and chemical reactions, the enzymes such as Tyrosinase and other Tyrosinase-related proteins (TYRP1 and TYRP2) have a key role in melanin synthesis [4].

Tyrosinase a multifunctional copper-containing metalloenzyme with dinuclear copper ions, is responsible for the distinct reaction of melanin biosynthesis from tyrosine [5, 6]. Moreover Tyrosinase is the main factor for undesired browning of fruits and vegetables which leads to rapid degradation through the postharvest and handing process [7].

Considering the above mentioned points, controlling the activity of this enzyme by Tyrosinase inhibitors is an important effort for the treatment of hyperpigmentation disorders and fruit enzymatic browning. In addition, many Tyrosinase inhibitors are of great interest in the medical and cosmetic products as well as in food and environmental industries [8-10]. However, due to some limitations such as lack of safety, allergic reactions, low efficacy and poor bioavailability, development of effective and safe Tyrosinase inhibitors is still a field of great interest [11, 12].

Many natural and synthetic products such as simple phenolic and hydrazine-containing compounds can be classified as Tyrosinase inhibitors. for example vanillin [13] and its derivatives [14], hydroquinone [15, 16] and compounds of this type [17, 18] such as resorcinol (or resorcin) [19], 4-n-butylresorcinol [20**],** tropolon and kojic acid have been reported as possible phenolic inhibitors of the Tyrosinase in the scientific literature (Fig. 1).

Imidazo [1,2-a]pyridine have displayed a broad range of biological and pharmacological activities including anti-inflammatory [21], anticancer [22], anti-ulcer [23, 24] and antifungal [25]. Additionally, a number of hydrazides, acylated derivatives of hydrazine have been reported to

possess a number pharmaceutically effects such as antitumoral, anticonvulsant, antioxidant, anti-inflammatory and antimicrobial [26-29]. In this study, in order to the discovery of new Tyrosinase inhibitors, we report the synthesis and biological evaluation of imidazo [1,2-a]pyridine-2-carbohydrazide derivatives bearing various arylidene pendants. Additionally, molecular docking analysis of the synthetic compounds was carried out to find a perception of the ligand-receptor interactions of the compounds in the active site of Tyrosinase enzyme.

4. Materials and methods

2.1. Chemistry

All starting materials, reagents and solvents were purchased from the suppliers (Sigma-Aldrich, Fluka and Merck) and were used without more purification. Reaction progress was monitored by thin layer chromatography (TLC) on MERCK precoated silica gel 60-F254 (0.5 mm) aluminum plates and visualized under UV light (254 nm).

The melting points of title compounds were determined with Thermo Scientific Electrothermal digital apparatus (Thermo Fisher Scientific Inc.). The IR spectra obtained using Perkin-Elmer Spectrum RXI FTIR spectrophotometer (KBr disks). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 Hz) spectra were measured using a Bruker 300 MHz NMR instrument by using DMSO-d6 as solvent and TMS as an internal standard. The chemical shifts (**δ**) are expressed in parts per million (ppm). The MS spectra were recorded using Agilent 7000 triple quadrupole mass spectrometer at an electron impact mode with an ionization voltage of 70 eV.

2.2. Synthesis

2.2.1. Procedure for the synthesis of ethyl imidazo [1,2-a]pyridine-2-carboxylate **(3)**

2-Aminopyridine (5 mmol) was added to 10 ml of ethanol and the mixture was stirred on ice bath. Then ethyl 3-bromo 2-oxopropanoat (7.5 mmol) was added and the mixture was refluxed for about 24 h. After cooling, the solid was filtered and washed with cold diethyl ether and recrystallized from ethanol.

Pale yellow solid; Yield: 90%, mp: 135-142 $^{\circ}$C. IR (KBr, cm^{-1}): 3086, 2986, 1729. ^{1}HNMR (300MHz, CD_3COCD_3): δ_H (ppm) 8.41 (d, J = 6 Hz, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 7.43 (d, J = 9 Hz, Ar-H), 7.20(t, J = 6 Hz, 1H, Ar-H), 6.84 (t, J = 6 Hz, 1H, Ar-H), 4.22 (q,

2H,COO$\underline{CH_2}CH_3$), 1.23 (t, J = 9Hz, 3H, COOCH$_2\underline{CH_3}$) MS (EI) m/z (%): 190 (M+, 28), 145 (34), 118 (100), 91 (19), 78 (40).

2.2.2. Procedure for the synthesis of imidazo [1,2-a]pyridine-2-carbohydrazide **(4)**

A mixture of ethyl imidazo [1,2-a]pyridine-2-carboxylate (5 mmol) and hydrazine hydrate (4ml) was refluxed for 3 h. After cooling, the mixture of reaction was washed with cold ethanol and recrystallized from ethanol to give pure imidazo [1,2-a]pyridine-2-carbohydrazide.

White solid; Yield: 25%, mp: 192-198 ºC. IR (KBr, cm^{-1}): 3353, 3187, 3050, 1660. ^{1}HNMR (300MHz, $CDCl_3$): δ_H (ppm) 9.53 (s, 1H, CON$\underline{H}$), 8.58 (d , J = 6 Hz, Ar-H), 8.36 (s, 1H, Ar-H), 7.59 (d, J = 9 Hz, 1H, Ar-H), 7.33 (t, J = 9 Hz, 1H, Ar-H), 6.94 (t, J = 6 Hz, 1H, Ar-H), 4.48 (s, 2H, CONH$\underline{NH_2}$) MS (EI) m/z (%): 176 (M+, 100), 145 (100), 117 (45), 97 (34), 78 (39) .

2.2.3. Procedure for the synthesis of substituted derivative of imidazo [1,2-a]pyridine-2-carboxylic acid benzylidene-hydrazide **(6a-6j)**

Different derivatives of **(6a-6j)** were prepared via the reaction of appropriate imidazo [1,2-a]pyridine-2-carbohydrazide**(4)** (1.2 mmol) with benzaldehyde derivatives **(5a-5j)** (2 mmol) in chloroform/ethanol (9:1). This reaction mixture was stirred under reflux for 24 h. After completion of the reaction confirmed by TLC, the solid salts were separated by filtration and the filtrate was washed with n-hexane and further purified with appropriate solvent.

2.3.1 Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (2-methoxy-benzylidene)-hydrazide **(6a)**

White solid; Yield: 88%, mp: 110-114 ºC, IR (KBr, cm^{-1}): 3290, 3140, 3000, 2835, 2400, 1673, 1381. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.99 (s, 1H, CON$\underline{H}$), 8.92 (s, 1H, Ar-H), 8.62(d, 1H, J=7 Hz, Ar-H), 8.53 (s, 1H, N=C$\underline{H}$), 7.87 (d, 1H, J=7 Hz, Ar-H), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.39 (m, 2H, Ar-H), 7.09 (d, 1H, J=7 Hz, Ar-H), 7.02 (t, 2H, J=7 Hz, Ar-H), 3.86 (s, 3H, OC$\underline{H3}$). ^{13}CNMR (75MHZ):159.04, 158.29, 144.4, 144.08, 139.14, 131.87, 128.16, 127.01, 126.10, 123.19, 121.1, 177.78, 116.23, 113.79, 112.34, 56.22. MS (EI) *m/z* (%): 214 (M+, 9.9), 161 (68.82), 145 (66.6), 118 (100), 78 (42.21).

2.3.2 Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (4-methoxy-benzylidene)-hydrazide **(6b)**

White solid; Yield: 83%, mp: 174-178 ºC, IR (KBr, cm^{-1}): 3405, 3141, 2999, 2838, 2400, 1655, 1443. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm)) 11.83 (s, 1H, C=ONH), 8.63(d, 1H, J=6 Hz, Ar-H), 8.51 (s, 2H, Ar-H and N=CH),7.81 (d,1H, J=9 Hz, Ar-H),7.64 (d, 2H, J=8 Hz, Ar-H), 7.39 (t, 1H, J=8 Hz, Ar-H), 7.00-7.04 (m, 3H, Ar-H),3.80 (s, 3H, OCH_3) .^{13}CNMR (75MHZ):161.23, 158.75, 148.37, 144.38, 139.19, 129.14, 128.18, 127.49, 127.0, 117.72, 116.20, 114.79, 113.83, 55.74. MS (EI) *m/z* (%): 294.1 (M+, 17.88), 161 (69.43), 145 (63.15), 118 (100), 78 (36.82).

2.3.3. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3,4,5-trimethoxy-benzylidene)-hydrazide **(6c)**

White solid; Yield: 27%, mp: 221-225 ºC. IR (KBr, cm^{-1}): 3194, 3121, 2998, 2940, 2400, 1668, 1360, 1380, 1433. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.95 (s, 1H, C=ONH), 8.63 (d, 1H, J=7 Hz, Ar-H), 8.54 (s, 1H, Ar-H),8.51 (s, 1H, N=CH), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.39 (t, 1H, J=7 Hz Ar-H), 6.99-7.05 (m, 3H, Ar-H), 3.85 (s, 6H, $OCH_{3\ 3}$), 3.71(s, 3H, OCH_3). ^{13}CNMR (75MHZ): 158.96, 153.65, 148.34, 144.40, 139.61, 138.97, 130.50, 128.19, 127.15, 117.72, 116.35, 113.88, 104.66, 60.59, 56.45. MS (EI) *m/z* (%): 354.1 (M+, 21.5), 161 (53.75), 145 (59.13), 118 (100), 78 (29.02).

2.3.4. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-hydroxy-4-methoxy-benzylidene)-hydrazide **(6d)**

White solid; Yield: 36%, mp: 260-264 ºC IR (KBr, cm^{-1}): 3583, 3307, 3019, 2964, 2400, 1711. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.74(s, 1H, C=ONH), 9.32 (s, 1H, OH), 8.62 (d, 1H, J=7 Hz, Ar-H), 8.51 (s,1H, N=CH), 8.45(s, 1H, Ar-H), 7.62 (d, 1H, J=9 Hz, Ar-H), 7.38 (t, 1H, J=7Hz, Ar-H), 7.26(S, 1H, Ar-H), 6.88-7.04 (m, 3H, Ar-H), 3.81 (s, 3H, OCH_3). ^{13}CNMR (75MHZ):158.77, 150.17, 148.57, 147.33, 144.39, 139.18, 128.16, 127.83, 127.05, 120.64, 117.71, 116.14, 113.79, 112.83, 112.35, 56.03. MS (EI) *m/z* (%): 310 (M+, 31.57), 161 (63.12), 145 (86.26), 118 (100), 78 (38.92).

2.3.5. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-ethoxy-4-hydroxy-benzylidene)-hydrazide **(6e)**

White solid; Yield: 43%, mp: 139-143 ºC, IR (KBr, cm^{-1}): 3168, 2964, 2853, 2964, 2400, 1655. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.76 (s, 1H, C=ONH), 9.52(s, 1H, Ar-H), 8.62 (d, 1H, J=6 Hz, Ar-H), 8.53 (s, 1H, N=CH), 8.46 (s, 1H, Ar-H), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.39 (t, 1H, J=8 Hz, Ar-H), 7.29(s,1H, Ar-H), 6.84-7.04 (m, 2H, Ar-H), 6.5 (d, 1H, J=7 Hz, Ar-H), 4.06 (t, 2H, J=6 Hz, OCH_2), 1.34 (broad, 3H, CH_3). ^{13}CNMR (75MHZ): 158.70, 149.61, 149.04, 147.64, 144.32, 138.97, 128.19, 127.21, 126.32, 122.55, 117.62, 116.14, 115.96, 113.87, 110.72, 64.32, 15.19. MS (EI) *m/z* (%): 324 (M+, 19), 161 (50), 145 (70), 118 (100), 78 (35).

2.3.6. Synthesis of Acetic acid 4-[(imidazo [1,2-a]pyridine-2-carbonyl)-hydrazonomethyl]-2-methoxy-phenyl ester **(6f)**

White solid; Yield: 40%, mp: 208-212 ºC. IR (KBr, cm^{-1}): 3451, 3228, 3146, 3076, 2400, 1759. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.98 (s, 1H, C=ONH), 8.59-8.55 (m, 3H, Ar-H and N=CH), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.45 (s, 1H, Ar-H), 7.39 (t, 1H, J=7 Hz, Ar-H), 7.26 (d, 1H, J=7 Hz, Ar-H), 7.18 (d, 1H, J=7 Hz, Ar-H), 7.03 (t, 1H, J=7Hz, Ar-H), 3.85 (s, 3H, OCH_3), 2.28 (s, 3H, $O=CH_3O$). ^{13}CNMR (75MHZ): 168.91, 159.07, 151.68, 147.81, 144.42, 141.21, 138.90, 133.87, 128.19, 127.17, 123.76, 120.98, 117.74, 116.39, 113.09, 110.18, 56.31, 20.87. MS (EI) m/z (%): 352 (M+, 8.8), 161 (86.65), 145 (71.11), 118 (100), 78 (38.88).

2.3.7. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (4-hydroxy-benzylidene)-hydrazide **(6g)**

Wihte solid; Yield: 63%, mp: 236-240 ºC. IR (KBr, cm^{-1}): 3243, 3242.7, 2400, 1627

^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.72 (s, 1H, C=ONH), 9.98 (s, 1H, OH), 8.62(d, 1H, J=6Hz, Ar-H), 8.48-8.51(m, 2H, Ar-H and N=CH), 7.63(d, 1H, J=9 Hz, Ar-H), 7.54 (d, 2H, J=9 Hz, Ar-H), 7.39 (t, 1H, J=7 Hz, Ar-H), 7.02 (t, 1H, J=7 Hz, Ar-H), 6.84 (d, 2H, J=7 Hz, , Ar-H). ^{13}CNMR (75MHZ): 159.80, 158.70, 148.80, 144.33, 138.99, 129.32, 128.19, 127.20, 125.91, 117.62, 116.28, 113.86. MS (EI) m/z (%): 280 (M+, 14.7), 161 (73.5), 145 (71.56), 118 (100), 78 (53.9).

2.3.8. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (2,4-dihydroxy-benzylidene)-hydrazide **(6h)**

White solid; Yield: 21%, mp: 300-305 ºC, IR (KBr, cm^{-1}): 3583, 3307, 3019, 2964, 2400, 1711. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 12.15 (s, 1H, C=ONH), 11.67 (s, 1H, OH), 10.02 (s, 1H, OH), 8.54-8.64 (m, 3H, Ar-H and N=CH), 7.65 (d, 1H, J=8 Hz, Ar-H), 7.40 (t, 1H, J=7 Hz, Ar-H), 7.22 (d, 1H, J=7 Hz, Ar-H), 7.03 (s, 1H, Ar-H), 6.35(m, 2H, OH). ^{13}CNMR (75MHZ): 161.10, 160.06, 158.54, 150.30, 144.42, 138.49, 132.13, 128.24, 127.30, 117.63, 116.31, 113.93, 111.01, 108.13, 103.17. MS (EI) *m/z* (%): 296 (M+, 14), 145(99), 118 (80), 78 (79).

2.3.9. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-hydroxy-benzylidene)-hydrazide **(6i)**

White solid; Yield: 48%, mp: 143-145 ºC. IR (KBr, cm^{-1}): 3544, 3148, 3019, 2400, 1644. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.90 (s, 1H, C=ONH), 9.67 (s, 1H, OH), 8.62 (d, 1H, J=6, Ar-H), 8.52-8.54 (m, 2H, Ar-H and N=CH), 7.63 (d, 1H, J=9 Hz, Ar-H), 7.38(t, 1H, J=7 Hz, Ar-H), 7.25 (t, 1H, J=7 Hz, Ar-H), 7.19 (broad, 1H, Ar-H), 7.08 (d, 1H, J=6 Hz, Ar-H), 7.02 (t, 1H, J=6 Hz, Ar-H), 6.84 (d, 1H, J=7Hz, Ar-H). ^{13}CNMR (75MHZ): 158.98, 158.11, 148.59, 144.39, 138.89, 136.22, 130.34, 128.19, 127.19, 119.21, 117.82, 117.69, 116.31, 113.88, 113.14. MS (EI) *m/z* (%): 280 (M+, 9.25), 161 (84.05), 145 (97.12), 118 (100), 78 (41.62).

2.3.10. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-nitro-benzylidene)-hydrazide **(6j)**

Light yellow solid; Yield: 44%, mp: 258-262 ºC. IR (KBr, cm^{-1}): 3446, 3154, 3029, 2400, 2400, 1658. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 12.20 (s, 1H, C=ONH), 8.70 (s, 1H, Ar-H), 8.57-8.62-(m, 2H, Ar-H and N=CH), 8.48 (s, 1H, Ar-H), 8.23 (d, 1H, J=7 Hz, Ar-H), 8.10 (d, 1H, J=6 Hz, Ar-H), 7.73 (t, 1H, J=6 Hz, Ar-H), 7.65 (d, 1H, J=8 Hz, Ar-H), 7.38 (s, 1H, Ar-H), 7.02 (s, 1H, Ar-H). ^{13}CNMR (75MHZ): 159.30, 148.63, 146.01, 144.45, 138.65, 136.77, 133.70, 130.91, 128.19, 127.21, 124.59, 121.25, 117.75, 116.59, 113.92. MS (EI) *m/z* (%): 309 (M+, 9.7), 161 (70.59), 145 (80.43), 118 (100), 78 (49.95).

2.5. Tyrosinase assay

Mushroom Tyrosinase (EC 1.14.18.1) (Sigma Chemical Co.) was tested according to a previous study with slight modifications using LDOPA as substrate [30]. Enzyme activity was checked by detecting dopachrome formation at 475 nm. The stock solutions of all test samples and kojic acid were prepared by dissolving in DMSO at 40mM and then diluted with phosphate buffer (pH=6.8) to the required concentrations. Initially, 10 µL of Tyrosinase (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. After the mixture was pre-incubated at 28 ºC for 20 min, 20 µL of L-DOPA solution (0.5 mM) was added to the phosphate buffer. DMSO in the absence of the test compounds was applied as the control, and kojic acid was used as a positive control. Each concentration was analyzed in three separate replicates. The inhibitory activity of the test compounds was expressed as the concentration that inhibited 50% of the enzyme activity (IC_{50}). The percent inhibition ratio was calculated according to the following equation:

Inhibition (%) = 100 - ($Abs_{control}$ - $Abs_{compound}$) / $Abs_{control}$

2.6. Molecular docking study

All compounds were sketched using MarvinSketch 18.20.0 [31] and energy of compounds was optimized using steepest descent algorithm by Open Babel 2.4.0 software [32]. GOLD 2018 docking program [33] was applied to dock all compounds using PDB code of 2Y9X. This structure is a Tyrosinase from Agaricus bisporus having tropolone as the native ligand in the binding site. Two Cu^{2+} metal ions exist in the binding site of Tyrosinase and were included for docking calculations. Protein structure was prepared using Discovery studio client [34] and all hydrogens were added. Binding site of the enzyme for docking was defined automatically by setting 8 Å around the coordinates of the native ligand tropolone. All available scoring functions in GOLD, namely CHEMPLP, ChemScore, ASP, and GoldScore were tried to re-dock tropolone inside the 2Y9X and then RMSD measure was calculated to validate and find the most appropriate scoring function for docking analyses.

3. Results and discussion

3.1. Design strategy

We designed the target compounds bearing imidazo [1,2-a]pyridine-carbohydrazid scaffold based on the structures of some potent Tyrosinase inhibitors reported in the literature [35, 36]. Some benzimidazole derivatives such as 2-(3-Methylbenzyl)-4(7)-phenyl-1*H* benzo[*d*]imidazole (**I**) illustrated Tyrosinase inhibitory activity with IC_{50} value of 37.86 μM [37]. The benzothiazole analogs like compound **(II)** have been reported as potential Tyrosinase inhibitors which demonstrated higher inhibition than kojic acid at 50 μM [38]. Additionally, the hydrazine-containing compounds such as compound **(III)** have been reported as effective Tyrosinase inhibitors (IC_{50}=1.93 μM) which exhibited better Tyrosinase inhibitor activity than kojic acid (Fig. 2) [39]. Based on these findings, herein we designed a series of imidazo [1,2-a]pyridine-2-carbohydrazide derivatives containing various arylidene pendants as tyroinase inhibitors via hybridization of imidazo pyridine core with hydrazine moiety as copper chelator. Furthermore, various phenolic moieties and its derivatives were introduced as a substituted benzylidene pendant to meet the essential requirements of Tyrosinase inhibitors and constructing structure–activity relationships (SARs).

3.2. Synthesis

The synthetic route to target compounds **6a–6j** is represented in Scheme 1. Reaction of 2-aminopyridine **(1)** and ethyl bromopyruvate **(2)** in refluxing ethanol resulted in ethyl imidazo [1,2-a]pyridine-2-carboxylate (**3**). In the next step, reaction of compound **(3)** with hydrazine hydrate under reflux condition afforded imidazo [1,2-a]pyridine-2-carbohydrazide **(4)**. Finally different substituted benzaldehyde derivatives **(5a-j)** were reacted with **2** in chloroform/ethanol (9:1). The resulting mixture was stirred under reflux and completion of the reaction was monitored by TLC to give compounds **6a–6j**.

3.3. Tyrosinase inhibitory activity

The inhibitory effect of all the synthesized derivatives were conducted on mushroom Tyrosinase enzyme by evaluation of their potential to inhibit enzymatic oxidation of L-Dopa. Kojic acid was used as the reference inhibitor according to the literature protocol [30, 40]. The chemical structure of the synthesized compounds and results are presented in Table 1.

The most potent compounds 6j and 6g exhibited considerable inhibitory potential with IC_{50} values of 7.19±2.56 μM and 8.11±1.25 μM, respectively against mushroom Tyrosinase compared to kojic acid with IC_{50} value of 9.64±0.50 μM. Compounds 6c, 6h, 6b, 6f and 6e showed moderate to weak inhibitory activities (IC_{50}s = 11.1-81.9 μM) while the rest of compounds (6a, 6d and 6i) were almost inactive.

Considering the obtained results from the SAR, there is a strong relation between the nature and position of the phenyl ring substitutions and Tyrosinase inhibition that can be shown as follows:

It seems that the presence of an electron donating group such as hydroxy and methoxy at the para position of the phenyl ring enhanced the inhibitory activity (compounds **6g** and **6b**). In addition, introduction of some groups such as nitro into the meta position of the phenyl moiety increased the anti-Tyrosinase potential of synthesized compound (compound **6j**). In contrast, compound **6i** bearing hydroxyl group on meta position of the phenyl ring was completely inactive. Furthermore, it was found that compound **6a** bearing 2-methoxy group on the phenyl ring was completely inactive while compound **6h** bearing 2,4 di hydroxyl residues exhibited the moderate Tyrosinase inhibitory activities. The comparison of substituents on different positions of phenyl ring indicated that compounds **6f**, **6e** and **6d** bearing different substitutes such as 3-methoxy-4-acetoxy, 3-ethoxy-4-hydroxy and 3-hydroxyl-4methoxy showed weak to inactive inhibitory activities; however, increasing the number of electrondonating group as in compound **6c** bearing 3,4,5 tri methoxy improved the activity. Consequently, the anti-Tyrosinase activity of **6j** and **6g** are higher than kojic acid and we hope that the imidazopyridine derivative would overcome some limitations of kojic acid such as formulation stability and skin penetration difficulties [41].

3.4. Molecular docking analysis

Molecular docking analysis was carried out to investigate interactions and binding poses of all synthesized compounds inside the binding site of Tyrosinase enzyme. RMSD value of redocking process of tropolone inside the active site of Tyrosinase with all available scoring functions in the GOLD was calculated in such a way that for ChemScore was 2.00 Å and for other scoring function algorithms was higher than 2.00 Å. Therefore, score values and binding modes of Tyrosinase inhibitors with the enzyme with PDB code 2Y9X was investigated by ChemScore fitness function. ChemScore fitness value of docking of all compounds along with their important interactions with amino acid residues inside the Tyrosinase active site were shown in

Table 1. ChemScore fitness values ranged from 34.73 in compound **6j** to 20.37 in compound **6c**. 3D interaction patterns of two active compounds **6j** and **6g** with IC_{50} values 7.19 and 8.11 µM respectively, and two inactive compounds **6a** and **6i** with IC_{50} values more than 100 µM, were depicted in Fig. 3.

Fig. 3a shows the docking interactions of compound **6j**. Docking score of this compound is 34.73 which is the highest score value among all compounds. Backbone structure of the compound made two pi-pi interactions via its aromatic rings: one pi-pi t-shaped interaction from imidazo [1,2-a]pyridine ring to Phe264 and one pi-pi stacked interaction from nitrobenzene group to His263. These two pi-pi interactions with these residues were among important ones. Other interaction types comprised pi-sigma and pi-alkyl interactions with Val283 and Ala286 respectively and pi-sigma interaction from imidazo [1,2-a]pyridine ring toVal283.

Docking interaction analysis of compound **6g**, Fig. 3b, shows the same pattern of pi-pi interactions with Phe264 and His263 from imidazo [1,2-a]pyridine ring and phenyl ring, respectively. Replacement of 3-nitro with 4-hydroxy group changed the orientation of compound **6g** in such a way that one hydrogen bond was made between carbonyl group and residue Val283. This additional hydrogen bond interaction did not cause any significant difference in activity against Tyrosinase in comparison to compound **6j**. Chemscore fitness value of this compound is a high value of 29.29. Compound **6g** made other interaction types such as a pi-cation from imidazo [1,2-a]pyridine ring to residue Arg268, a pi-alkyl interaction and a pi-sigma interaction between phenol and Ala286, and Val283 respectively that all further strengthened its binding towards Tyrosinase enzyme.

Another moderate to active compound is **6h** with IC_{50} value of 12.6 µM having ChemScore fitness value of 31.53. This compound made two important pi-pi interactions of the backbone with Phe264 and His263. In comparison to compound **6g** bearing 4-hydroxy moiety, compound **6h** has an additional hydroxyl in position 2 that donated a hydrogen bond to Asn260. This hydroxyl also made a metal-acceptor interaction with one Cu^{2+}. Another hydrogen bond interaction was formed between carbonyl group and Val283. A pi-alkyl interaction between Val283 and phenyl ring was also made, see Fig 3c.

In inactive compound **6i**, 3-hydroxy replaced 4-hydroxy in compound **6g**. This substitution got the backbone away from Phe264 in such a way that it was not able to make this critical pi-pi

interaction and just made pi-pi stacked interaction with His263. ChemScore fitness value of **6i** is 30.17 which might be resulted from other not that much important hydrophobic interactions it made like pi-alkyl with Ala286 and pi-sigma with Val248 inside the active site of Tyrosinase, see Fig. 3d.

In overall, inactive compounds, **6a, 6d** and **6i**, lacked one or two critical pi-pi interactions with His263 and Phe264 that resulted from a different orientation of these molecules in the enzyme active site. Presence of an electrondonating group such as hydroxyl in the para position of the phenyl ring makes compound able to form a metal-acceptor interaction with Cu^{+2} ion and also changes the orientation of the molecule to be able to form a hydrogen bond from carbonyl group to residue Val283.

4. **Conclusions**

In conclusion, we developed a series of imidazo [1,2-a]pyridine-2-carbohydrazide derivatives bearing various arylidene pendants as novel Tyrosinase inhibitors. Based on the obtained data, compounds **6j** and **6g** containing 3-nitro and 4-hydroxyl moieties were the most potent compounds against Tyrosinase with respective IC_{50} values of 7.19 μM and 8.11 μM. The results indicated that the inhibitory potential was dependent on the substituted moiety on the phenyl ring. Therefore, derivatives bearing an electrondonating such as hydroxyl and electron-withdrawing group such as nitro at the para and meta position of the phenyl ring exhibited considerable inhibitory activity, respectively. Molecular docking analysis results demonstrated that potent compounds **6g**, **6h** and **6j** had high docking score values and made two critical pi-pi interactions with Phe264 and His263. These results indicate that the compounds can serve as structural outlines and promising leads in order to design and develop novel effective Tyrosinase inhibitors.

Declaration of competing interest

The authors report no conflict of interest.

Credit Author Statement

Najmeh Edraki : principle investigator, supervision of the team in all research steps including data gathering, data analysis, and manuscript preparation/revision

Mehdi Khoshneviszadeh: Supervision of the design, synthetic steps and biological evaluation, manuscript preparation.

Tahereh Damghani: contribution in the data gathering, design and synthesis of compounds, data analysis, preparation of results, writing and revising of this manuscript.

Saba Hadaegh : contribution in the synthesis of compounds.

Mahsima khoshneviszadeh: Contribution in the biological and enzymatic assay..

Somayeh Pirhadi : Supervision and performance of *computational* docking and data analysis of this study.

Razieh Sabet : Supervision of synthetic parts.

Acknowledgment

The authors wish to thank the support of the Vice-Chancellor for Research, Shiraz University of Medical Sciences (Grant Number: 14006). This work was the PharmD thesis of Saba Hadaegh.

References

[1] T.F. Cestari, L.P. Dantas, J.C. Boza, Acquired hyperpigmentations, Anais brasileiros de dermatologia, 89 (2014) 11-25. DOI: https://doi.org/10.1590/abd1806-4841.20142353.

[2] S. Nouveau, D. Agrawal, M. Kohli, F. Bernerd, N. Misra, C.S. Nayak, Skin hyperpigmentation in Indian population: Insights and best practice, Indian journal of dermatology, 61 (2016) 487. DOI: 10.4103/0019-5154.190103.

[3] S. Dogra, R. Sarangal, Pigmentary disorders: An insight, Pigment International, 1 (2014) 5. DOI: 10.4103/2349-5847.135429.

[4] A. Lapedriza, K. Petratou, R.N. Kelsh, Neural crest cells and pigmentation, in: Neural Crest Cells, Elsevier, 2014, pp. 287-311. DOI: https://doi.org/10.1016/B978-0-12-401730-6.00015-6.

[5] A. Garcia-Jimenez, J.A. Teruel-Puche, P.A. Garcia-Ruiz, J. Berna, J.N. Rodríguez-López, J. Tudela, F. Garcia-Canovas, Action of 2, 2′, 4, 4′-tetrahydroxybenzophenone in the biosynthesis pathway of melanin, International journal of biological macromolecules, 98 (2017) 622-629. DOI: https://doi.org/10.1016/j.ijbiomac.2017.02.032.

[6] C.S. Nunes, K. Vogel, Tyrosinases—physiology, pathophysiology, and applications, in: Enzymes in Human and Animal Nutrition, Elsevier, 2018, pp. 403-412. DOI: https://doi.org/10.1016/B978-0-12-805419-2.00020-4.

[7] O. Nerya, R. Ben-Arie, T. Luzzatto, R. Musa, S. Khativ, J. Vaya, Prevention of Agaricus bisporus postharvest browning with tyrosinase inhibitors, Postharvest Biology and Technology, 39 (2006) 272-277. DOI: https://doi.org/10.1016/j.postharvbio.2005.11.001.

[8] T. Pillaiyar, M. Manickam, V. Namasivayam, Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors, Journal of enzyme inhibition and medicinal chemistry, 32 (2017) 403-425. DOI: https://doi.org/10.1080/14756366.2016.1256882.

[9] S.Y. Lee, N. Baek, T.-g. Nam, Natural, semisynthetic and synthetic tyrosinase inhibitors, Journal of Enzyme Inhibition and Medicinal Chemistry, 31 (2016) 1-13. DOI: https://doi.org/10.3109/14756366.2015.1004058.

[10] M. Loizzo, R. Tundis, F. Menichini, Natural and synthetic tyrosinase inhibitors as antibrowning agents: an update, Comprehensive Reviews in Food Science and Food Safety, 11 (2012) 378-398. DOI: https://doi.org/10.1111/j.1541-4337.2012.00191.

[11] T.-S. Chang, An updated review of tyrosinase inhibitors, International journal of molecular sciences, 10 (2009) 2440-2475. DOI: https://doi.org/10.3390/ijms10062440.

[12] F.A. Larik, A. Saeed, P.A. Channar, U. Muqadar, Q. Abbas, M. Hassan, S.-Y. Seo, M. Bolte, Design, synthesis, kinetic mechanism and molecular docking studies of novel 1-pentanoyl-3-arylthioureas as inhibitors of mushroom tyrosinase and free radical scavengers, European journal of medicinal chemistry, 141 (2017) 273-281. DOI: https://doi.org/10.1016/j.ejmech.2017.09.059.

[13] M.H.K. Nguyen, H.X. Nguyen, M.T.T. Nguyen, N.T. Nguyen, Phenolic constituents from the heartwood of Artocapus altilis and their tyrosinase inhibitory activity, Natural product communications, 7 (2012) 1934578X1200700214. DOI: https://doi.org/10.1177/1934578X1200700214.

[14] Z. Ashraf, M. Rafiq, S.-Y. Seo, M.M. Babar, Synthesis, kinetic mechanism and docking studies of vanillin derivatives as inhibitors of mushroom tyrosinase, Bioorganic & medicinal chemistry, 23 (2015) 5870-5880. DOI: https://doi.org/10.1016/j.bmc.2015.06.068.

[15] K. Sakuma, M. Ogawa, K. Sugibayashi, K.-i. Yamada, K. Yamamoto, Relationship between tyrosinase inhibitory action and oxidation-reduction potential of cosmetic whitening ingredients and phenol derivatives, Archives of pharmacal research, 22 (1999) 335-339. DOI: https://doi.org/10.1007/BF02979054.

[16] A. Hashimoto, M. Ichihashi, Y. Mishima, The mechanism of depigmentation by hydroquinone: a study on suppression and recovery processes of tyrosinase activity in the pigment cells in vivo and in vitro, Nihon Hifuka Gakkai zasshi. The Japanese journal of dermatology, 94 (1984) 797.

[17] Y.-R. Chen, R.Y.-Y. Chiou, T.-Y. Lin, C.-P. Huang, W.-C. Tang, S.-T. Chen, S.-B. Lin, Identification of an Alkylhydroquinone from Rhus succedanea as an Inhibitor of Tyrosinase and Melanogenesis, Journal of agricultural and food chemistry, 57 (2009) 2200-2205. DOI: https://doi.org/10.1021/jf802617a.

[18] A. Sasaki, Y. Yamano, S. Sugimoto, H. Otsuka, K. Matsunami, T. Shinzato, Phenolic compounds from the leaves of Breynia officinalis and their tyrosinase and melanogenesis inhibitory activities, Journal of natural medicines, 72 (2018) 381-389. DOI: https://doi.org/10.1007/s11418-017-1148-8.

[19] K. Tasaka, C. Kamei, S. Nakano, Y. Takeuchi, M. Yamato, Effects of certain resorcinol derivatives on the tyrosinase activity and the growth of melanoma cells, Methods and findings in experimental and clinical pharmacology, 20 (1998) 99-110. DOI: 10.1358/mf.1998.20.2.485637.

[20] L. Kolbe, T. Mann, W. Gerwat, J. Batzer, S. Ahlheit, C. Scherner, H. Wenck, F. Stäb, 4-n-butylresorcinol, a highly effective tyrosinase inhibitor for the topical treatment of hyperpigmentation, Journal of the European Academy of Dermatology and Venereology, 27 (2013) 19-23. DOI: https://doi.org/10.1111/jdv.12051.

[21] F. Awouters, J. Vermeire, F. Smeyers, P. Vermote, R. Van Beek, C.J. Niemegeers, Oral antiallergic activity in ascaris hypersensitive dogs: a study of known antihistamines and of the new compounds ramastine (R 57 959) and levocabastine (R 50 547), Drug Development Research, 8 (1986) 95-102. DOI: https://doi.org/10.1002/ddr.430080112.

[22] H. Huang, X. Ji, X. Tang, M. Zhang, X. Li, H. Jiang, Conversion of pyridine to imidazo [1, 2-a] pyridines by copper-catalyzed aerobic dehydrogenative cyclization with oxime esters, Organic letters, 15 (2013) 6254-6257. DOI: https://doi.org/10.1021/ol403105p.

[23] M. Bollini, J.J. Casal, D.E. Alvarez, L. Boiani, M. González, H. Cerecetto, A.M. Bruno, New potent imidazoisoquinolinone derivatives as anti-Trypanosoma cruzi agents: Biological evaluation and structure–activity relationships, Bioorganic & medicinal chemistry, 17 (2009) 1437-1444. DOI: https://doi.org/10.1016/j.bmc.2009.01.011.

[24] J.J. Kaminski, C. Puchalski, D.M. Solomon, R.K. Rizvi, D.J. Conn, A.J. Elliott, R.G. Lovey, H. Guzik, P. Chiu, Antiulcer agents. 4. Conformational considerations and the antiulcer activity of substituted imidazo [1, 2-a] pyridines and related analogs, Journal of medicinal chemistry, 32 (1989) 1686-1700. DOI: https://doi.org/10.1021/jm00128a005.

[25] Y. Rival, G. Grassy, A. Taudou, R. Ecalle, Antifungal activity in vitro of some imidazo [1, 2-a] pyrimidine derivatives, European journal of medicinal chemistry, 26 (1991) 13-18. DOI: https://doi.org/10.1016/0223-5234(91)90208-5.

[26] M. Abdel-Aziz, H.M. Abdel-Rahman, Synthesis and anti-mycobacterial evaluation of some pyrazine-2-carboxylic acid hydrazide derivatives, European journal of medicinal chemistry, 45 (2010) 3384-3388. DOI: https://doi.org/10.1016/j.ejmech.2010.04.025.

[27] A. Kajal, S. Bala, S. Kamboj, V. Saini, Synthesis, characterization, and computational studies on phthalic anhydride-based benzylidene-hydrazide derivatives as novel, potential anti-inflammatory agents, Medicinal Chemistry Research, 23 (2014) 2676-2689. DOI: https://doi.org/10.1007/s00044-013-0848-1.

[28] R. Narang, B. Narasimhan, S. Sharma, A review on biological activities and chemical synthesis of hydrazide derivatives, Current medicinal chemistry, 19 (2012) 569-612. DOI: https://doi.org/10.2174/092986712798918789.

[29] R. Sreenivasulu, K.T. Reddy, P. Sujitha, C.G. Kumar, R.R. Raju, Synthesis, antiproliferative and apoptosis induction potential activities of novel bis (indolyl) hydrazide-hydrazone derivatives, Bioorganic & medicinal chemistry, 27 (2019) 1043-1055. DOI: https://doi.org/10.1016/j.bmc.2019.02.002.

[30] Y.M. Ha, S.W. Chung, S. Song, H. Lee, H. Suh, H.Y. Chung, 4-(6-Hydroxy-2-naphthyl)-1, 3-bezendiol: a potent, new tyrosinase inhibitor, Biological and Pharmaceutical Bulletin, 30 (2007) 1711-1715. DOI: https://doi.org/10.1248/bpb.30.1711.

[31] http://www.chemaxon.com.

[32] N.M. O'Boyle, M. Banck, C.A. James, C. Morley, T. Vandermeersch, G.R. Hutchison, Open Babel: An open chemical toolbox, Journal of cheminformatics, 3 (2011) 33. DOI: https://doi.org/10.1186/1758-2946-3-33.

[33] G. Jones, P. Willett, R.C. Glen, A.R. Leach, R. Taylor, Development and validation of a genetic algorithm for flexible docking, Journal of molecular biology, 267 (1997) 727-748. DOI: https://doi.org/10.1006/jmbi.1996.0897.

[34] D.S. BIOVIA, Discovery studio visualizer, Release 2017, San Diego: Dassault Systèmes, 2016, to be found under http://accelrys. com/products/collaborative-science/biovia-discovery-studio/visualization-download. php (accessed Dec 12, 2017), (2017).

[35] S. Zolghadri, A. Bahrami, M.T. Hassan Khan, J. Munoz-Munoz, F. Garcia-Molina, F. Garcia-Canovas, A.A. Saboury, A comprehensive review on tyrosinase inhibitors, Journal of enzyme inhibition and medicinal chemistry, 34 (2019) 279-309. DOI: https://doi.org/10.1080/14756366.2018.1545767.

[36] J.P. Germanas, S. Wang, A. Miner, W. Hao, J.M. Ready, Discovery of small-molecule inhibitors of tyrosinase, Bioorganic & medicinal chemistry letters, 17 (2007) 6871-6875. DOI: https://doi.org/10.1016/j.bmcl.2007.10.014.

[37] İ.S. Doğan, A. Özel, Z. Birinci, B. Barut, H.E. Sellitepe, B. Kahveci, Synthesis of Some Novel 2-substitutedbenzyl-(4) 7-phenyl-1H-benzo [d] imidazoles in Mild Conditions as Potent Anti-tyrosinase and Antioxidant Agents, Archiv der Pharmazie, 349 (2016) 881-888. DOI: https://doi.org/10.1002/ardp.201600224.

[38] H.Y. Chung, M.H. Park, Y.M. Ha, Y.K. Han, J.Y. Park, Y.J. Park, J.A. Kim, J.Y. Lee, Y.M. Song, H.R. Moon, Compound having skin-whitening, anti-oxidizing and PPAR activities and medical use therefor, in, Google Patents, 2014.

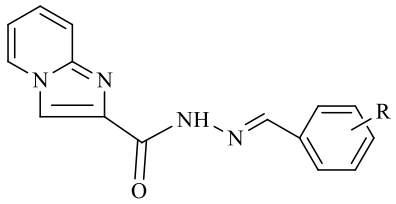
[39] W. Yi, R.-H. Cao, Z.-Y. Chen, L. Yu, L. Ma, H.-C. Song, Design, synthesis and biological evaluation of hydroxy-or methoxy-substituted phenylmethylenethiosemicarbazones as tyrosinase inhibitors, Chemical and Pharmaceutical Bulletin, 57 (2009) 1273-1277. DOI: https://doi.org/10.1248/cpb.57.1273.

[40] S. Son, H. Kim, H.Y. Yun, S. Ullah, S.J. Kim, Y.-J. Kim, M.-S. Kim, J.-W. Yoo, P. Chun, H.R. Moon, (E)-2-Cyano-3-(substituted phenyl) acrylamide analogs as potent inhibitors of tyrosinase: A linear β-phenyl-α, β-unsaturated carbonyl scaffold, Bioorganic & medicinal chemistry, 23 (2015) 7728-7734. DOI: https://doi.org/10.1016/j.bmc.2015.11.015.

[41] J. Hermanns, C. Pierard-Franchimont, G. Pierard, Skin colour assessment in safety testing of cosmetics. An overview, International journal of cosmetic science, 22 (2000) 67-71. DOI: 10.1046/j.1467-2494.2000.00021.x .

Table 1

Chemical structure, Tyrosinase inhibitory effects and molecular docking studies of synthesized compounds **6a-6j**.



compounds	R	Mw	ChemScore fitness value	Binding interactions with Tyrosinase active site	Yields*	IC_{50}(µm)±SE**
6a	2- methoxy	294	22.62	Hydrogen bonds: Val283	88%	> 100
6b	4- methoxy	294	23.93	Hydrogen bonds: Val283 Pi-pi interactions: His263,	83%	14.3±3.11
6c	3,4,5-trimethoxy	354	20.37	Hydrogen bonds: Arg268 Pi-pi interactions: Phe264	27%	11.10±1.55
6d	3-hydroxy-4-methoxy	310	28.47	Hydrogen bonds: Asn260, Val283	36%	> 100
6e	3-ethoxy-4-hydroxy	324	27.29	Pi-pi interactions: His263, Metal acceptor interaction: 1 Cu^{+2}	43%	81.9±9.75
6f	3-methoxy-4-acetoxy	352	28.49	Metal acceptor interaction: 2 Cu^{+2}	40%	41.70±2.40
6g	4-hydroxy	280	29.29	Hydrogen bonds: Ala283 Pi-pi interactions: His263, Phe264	63%	8.11±1.25
6h	2,4-dihydroxy	296	31.53	Hydrogen bonds: Asn260, Val283 Pi-pi interactions: His263, Phe264 Metal acceptor interaction: 1 Cu	21%	12.60±3.67
6i	3-hydroxy	280	30.17	Pi-pi interactions: His263	48%	> 100
6j	3-nitro	309	34.73	Pi-pi interactions: His263, Phe264	44%	7.19±2.56
Kojic Acid* **	-	-				9.64±0.50

*Reported yields are based on the yield of final step of synthesis.

** Values represent means ± SE of 3 independent experiments.*** Kojic Acid was used as a positive control.

Highlights

- A series of imidazo[1,2-a]pyridine 2-carbohudrazide derivatives bearing different arylidene pendantrs were designed and synthesized (10 compounds).
- Compounds bearing 3-nitro (6j) and 4-hydroxy (6g) moieties on the arylidene pendant exhibited the best Tyrosinase inhibitory activity with IC_{50} values of 7.19 and 8.11μM, respectively.
- Compounds 6j, 6h and 6g showed the potential of two critical pi-pi interactions with His263 and Phe264 in the active site of Tyrosinase.

The results indicated that 6j and 6g could be introduced as potent Tyrosinase inhibitors.

Design, Synthesis, In Vitro Evaluation and Molecular Docking Study of N'-Arylidene imidazo [1,2-a] pyridine -2-Carbohydrazide Derivatives as Novel Tyrosinase Inhibitors

Tahereh Damghani[1,2], Saba Hadaegh[1,2], Mahsima khoshneviszadeh[1], Somayeh Pirhadi[1], Razieh Sabet[2], Mehdi Khoshneviszadeh[1,2*], Najmeh Edraki[1*].

1. Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

2. School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

***Corresponding authors:**

Najmeh Edraki, PhrmD, PhD

Medicinal and Natural Products Chemistry Research Center, Shiraz university of Medical Sciences, Shiraz, Iran

Tel: +98 713 230 3872; Fax: +98 713 230 2225

E-mail: edrakin@sums.ac.ir, najmeh_edraki@yahoo.com

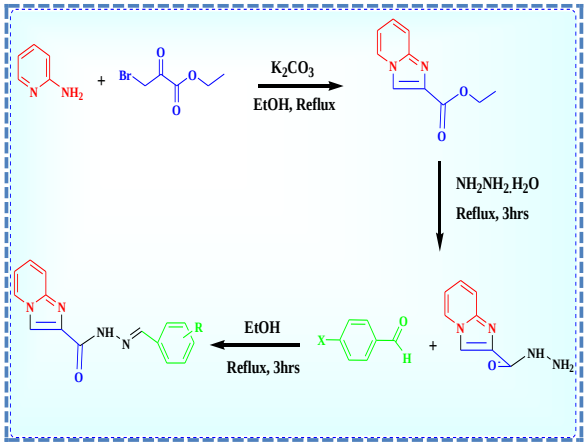
Mehdi Khoshneviszadeh, PhrmD, PhD

Medicinal and Natural Products Chemistry Research Center, Shiraz university of Medical Sciences, Shiraz, Iran

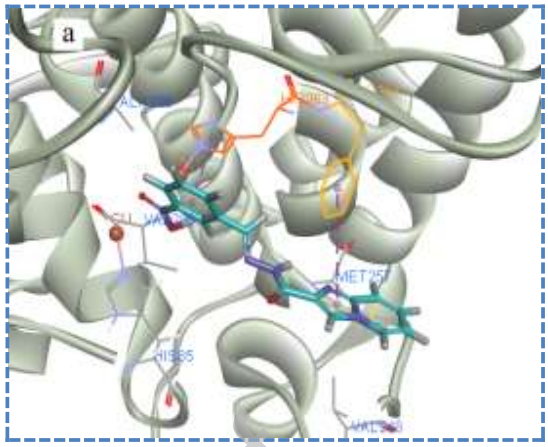
School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

E-mail: m.khoshneviszadeh@gmail.com

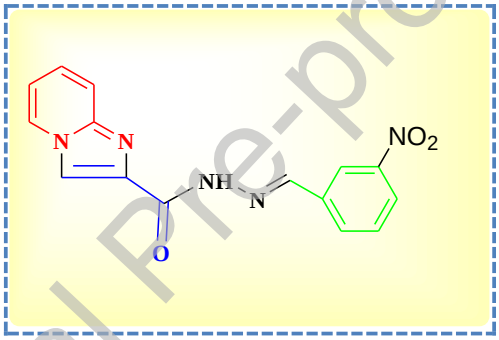
Graphical abstract



Synthesis route of Tyrosinase inhibitors



Molecular docking interaction inside Tyrosinase active site



Compound **6j** with IC_{50}= 7.19 µM

Abstract

A novel series of imidazo[1,2-a]pyridine 2-carbohudrazide derivatives bearing different arylidene pendantrs were designed, synthesized and evaluated for their inhibitory activity against mushroom Tyrosinase. It was found that compounds bearing 3-nitro (6j) and 4-hydroxy (6g) moieties on the arylidene pendant exhibited the best Tyrosinase inhibitory activity with IC_{50} values of 7.19 and 8.11μM, respectively. These results were comparable to that of kojic acid as the reference drug (IC_{50} = 9.64±0.5 μM). Additionally, molecular docking analysis was performed to study the interactions and binding modes of compounds 6j, 6h and 6g which are showing the potential of two critical pi-pi interactions with His263 and Phe264 in the active site of Tyrosinase. The results indicated that 6j and 6g could be introduced as potent Tyrosinase inhibitors that might serve as promising candidates in medicine, cosmetics or food industry.

Keywords: Tyrosinase inhibitor; imidazo [1,2-a]pyridine; docking; Kojic acid

5. Introduction

Melanin, the key pigment primarily responsible for the hair, eyes and skin pigmentation of human, is produced by melanocytes through melanogenesis. In regard to photoprotection, melanogenesis and skin pigmentation are the most significant factors in damage response to ultraviolet radiation from the sun and skin photocarcinogenesis.

The increased melanin synthesis and accumulation of these pigments occur in specific parts of the skin disorders including hyperpigmentation, lentigines, neurodegeneration associated with Parkinson's disease and skin cancer risk[1-3]

Though melanogenesis (the synthesis of melanin) is a very complex process represented by many enzymatic and chemical reactions, the enzymes such as Tyrosinase and other Tyrosinase-related proteins (TYRP1 and TYRP2) have a key role in melanin synthesis [4].

Tyrosinase a multifunctional copper-containing metalloenzyme with dinuclear copper ions, is responsible for the distinct reaction of melanin biosynthesis from tyrosine [5, 6]. Moreover Tyrosinase is the main factor for undesired browning of fruits and vegetables which leads to rapid degradation through the postharvest and handing process [7].

Considering the above mentioned points, controlling the activity of this enzyme by Tyrosinase inhibitors is an important effort for the treatment of hyperpigmentation disorders and fruit enzymatic browning. In addition, many Tyrosinase inhibitors are of great interest in the medical and cosmetic products as well as in food and environmental industries [8-10]. However, due to some limitations such as lack of safety, allergic reactions, low efficacy and poor bioavailability, development of effective and safe Tyrosinase inhibitors is still a field of great interest [11, 12].

Many natural and synthetic products such as simple phenolic and hydrazine-containing compounds can be classified as Tyrosinase inhibitors. for example vanillin [13] and its derivatives [14], hydroquinone [15, 16] and compounds of this type [17, 18] such as resorcinol (or resorcin) [19], 4-n-butylresorcinol [20**],** tropolon and kojic acid have been reported as possible phenolic inhibitors of the Tyrosinase in the scientific literature (Fig. 1).

Imidazo [1,2-a]pyridine have displayed a broad range of biological and pharmacological activities including anti-inflammatory [21], anticancer [22], anti-ulcer [23, 24] and antifungal [25]. Additionally, a number of hydrazides, acylated derivatives of hydrazine have been reported to

possess a number pharmaceutically effects such as antitumoral, anticonvulsant, antioxidant, anti-inflammatory and antimicrobial [26-29]. In this study, in order to the discovery of new Tyrosinase inhibitors, we report the synthesis and biological evaluation of imidazo [1,2-a]pyridine-2-carbohydrazide derivatives bearing various arylidene pendants. Additionally, molecular docking analysis of the synthetic compounds was carried out to find a perception of the ligand-receptor interactions of the compounds in the active site of Tyrosinase enzyme.

6. Materials and methods

2.1. Chemistry

All starting materials, reagents and solvents were purchased from the suppliers (Sigma-Aldrich, Fluka and Merck) and were used without more purification. Reaction progress was monitored by thin layer chromatography (TLC) on MERCK precoated silica gel 60-F254 (0.5 mm) aluminum plates and visualized under UV light (254 nm).

The melting points of title compounds were determined with Thermo Scientific Electrothermal digital apparatus (Thermo Fisher Scientific Inc.). The IR spectra obtained using Perkin-Elmer Spectrum RXI FTIR spectrophotometer (KBr disks). ^{1}H NMR (300 MHz) and ^{13}C NMR (75 Hz) spectra were measured using a Bruker 300 MHz NMR instrument by using DMSO-d6 as solvent and TMS as an internal standard. The chemical shifts (**δ**) are expressed in parts per million (ppm). The MS spectra were recorded using Agilent 7000 triple quadrupole mass spectrometer at an electron impact mode with an ionization voltage of 70 eV.

2.2. Synthesis

2.2.1. Procedure for the synthesis of ethyl imidazo [1,2-a]pyridine-2-carboxylate **(3)**

2-Aminopyridine (5 mmol) was added to 10 ml of ethanol and the mixture was stirred on ice bath. Then ethyl 3-bromo 2-oxopropanoat (7.5 mmol) was added and the mixture was refluxed for about 24 h. After cooling, the solid was filtered and washed with cold diethyl ether and recrystallized from ethanol.

Pale yellow solid; Yield: 90%, mp: 135-142 $^{\circ}$C. IR (KBr, cm^{-1}): 3086, 2986, 1729. ^{1}HNMR (300MHz, CD_3COCD_3): δ_H (ppm) 8.41 (d, J = 6 Hz, 1H, Ar-H), 8.30 (s, 1H, Ar-H), 7.43 (d, J = 9 Hz, Ar-H), 7.20(t, J = 6 Hz, 1H, Ar-H), 6.84 (t, J = 6 Hz, 1H, Ar-H), 4.22 (q,

2H,COO$\underline{CH_2}CH_3$), 1.23 (t, J = 9Hz, 3H, COOCH$_2\underline{CH_3}$) MS (EI) m/z (%): 190 (M+, 28), 145 (34), 118 (100), 91 (19), 78 (40).

2.2.2. Procedure for the synthesis of imidazo [1,2-a]pyridine-2-carbohydrazide **(4)**

A mixture of ethyl imidazo [1,2-a]pyridine-2-carboxylate (5 mmol) and hydrazine hydrate (4ml) was refluxed for 3 h. After cooling, the mixture of reaction was washed with cold ethanol and recrystallized from ethanol to give pure imidazo [1,2-a]pyridine-2-carbohydrazide.

White solid; Yield: 25%, mp: 192-198 ºC. IR (KBr, cm^{-1}): 3353, 3187, 3050, 1660. ^{1}HNMR (300MHz, $CDCl_3$): δ_H (ppm) 9.53 (s, 1H, CON$\underline{H}$), 8.58 (d , J = 6 Hz, Ar-H), 8.36 (s, 1H, Ar-H), 7.59 (d, J = 9 Hz, 1H, Ar-H), 7.33 (t, J = 9 Hz, 1H, Ar-H), 6.94 (t, J = 6 Hz, 1H, Ar-H), 4.48 (s, 2H, CONH$\underline{NH_2}$) MS (EI) m/z (%): 176 (M+, 100), 145 (100), 117 (45), 97 (34), 78 (39) .

2.2.3. Procedure for the synthesis of substituted derivative of imidazo [1,2-a]pyridine-2-carboxylic acid benzylidene-hydrazide **(6a-6j)**

Different derivatives of **(6a-6j)** were prepared via the reaction of appropriate imidazo [1,2-a]pyridine-2-carbohydrazide**(4)** (1.2 mmol) with benzaldehyde derivatives **(5a-5j)** (2 mmol) in chloroform/ethanol (9:1). This reaction mixture was stirred under reflux for 24 h. After completion of the reaction confirmed by TLC, the solid salts were separated by filtration and the filtrate was washed with n-hexane and further purified with appropriate solvent.

2.3.1 Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (2-methoxy-benzylidene)-hydrazide **(6a)**

White solid; Yield: 88%, mp: 110-114 ºC, IR (KBr, cm^{-1}): 3290, 3140, 3000, 2835, 2400, 1673, 1381. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.99 (s, 1H, CON$\underline{H}$), 8.92 (s, 1H, Ar-H), 8.62(d, 1H, J=7 Hz, Ar-H), 8.53 (s, 1H, N=C$\underline{H}$), 7.87 (d, 1H, J=7 Hz, Ar-H), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.39 (m, 2H, Ar-H), 7.09 (d, 1H, J=7 Hz, Ar-H), 7.02 (t, 2H, J=7 Hz, Ar-H), 3.86 (s, 3H, OC$\underline{H3}$). ^{13}CNMR (75MHZ):159.04, 158.29, 144.4, 144.08, 139.14, 131.87, 128.16, 127.01, 126.10, 123.19, 121.1, 177.78, 116.23, 113.79, 112.34, 56.22. MS (EI) *m/z* (%): 214 (M+, 9.9), 161 (68.82), 145 (66.6), 118 (100), 78 (42.21).

2.3.2 Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (4-methoxy-benzylidene)-hydrazide **(6b)**

White solid; Yield: 83%, mp: 174-178 ºC, IR (KBr, cm^{-1}): 3405, 3141, 2999, 2838, 2400, 1655, 1443. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm)) 11.83 (s, 1H, C=ONH), 8.63(d, 1H, J=6 Hz, Ar-H), 8.51 (s, 2H, Ar-H and N=CH),7.81 (d,1H, J=9 Hz, Ar-H),7.64 (d, 2H, J=8 Hz, Ar-H), 7.39 (t, 1H, J=8 Hz, Ar-H), 7.00-7.04 (m, 3H, Ar-H),3.80 (s, 3H, OCH_3) .^{13}CNMR (75MHZ):161.23, 158.75, 148.37, 144.38, 139.19, 129.14, 128.18, 127.49, 127.0, 117.72, 116.20, 114.79, 113.83, 55.74. MS (EI) *m/z* (%): 294.1 (M+, 17.88), 161 (69.43), 145 (63.15), 118 (100), 78 (36.82).

2.3.3. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3,4,5-trimethoxy-benzylidene)-hydrazide **(6c)**

White solid; Yield: 27%, mp: 221-225 ºC. IR (KBr, cm^{-1}): 3194, 3121, 2998, 2940, 2400, 1668, 1360, 1380, 1433. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.95 (s, 1H, C=ONH), 8.63 (d, 1H, J=7 Hz, Ar-H), 8.54 (s, 1H, Ar-H),8.51 (s, 1H, N=CH), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.39 (t, 1H, J=7 Hz Ar-H), 6.99-7.05 (m, 3H, Ar-H), 3.85 (s, 6H, $OCH_{3\ 3}$), 3.71(s, 3H, OCH_3). ^{13}CNMR (75MHZ): 158.96, 153.65, 148.34, 144.40, 139.61, 138.97, 130.50, 128.19, 127.15, 117.72, 116.35, 113.88, 104.66, 60.59, 56.45. MS (EI) *m/z* (%): 354.1 (M+, 21.5), 161 (53.75), 145 (59.13), 118 (100), 78 (29.02).

2.3.4. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-hydroxy-4-methoxy-benzylidene)-hydrazide **(6d)**

White solid; Yield: 36%, mp: 260-264 ºC IR (KBr, cm^{-1}): 3583, 3307, 3019, 2964, 2400, 1711. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.74(s, 1H, C=ONH), 9.32 (s, 1H, OH), 8.62 (d, 1H, J=7 Hz, Ar-H), 8.51 (s,1H, N=CH), 8.45(s, 1H, Ar-H), 7.62 (d, 1H, J=9 Hz, Ar-H), 7.38 (t, 1H, J=7Hz, Ar-H), 7.26(S, 1H, Ar-H), 6.88-7.04 (m, 3H, Ar-H), 3.81 (s, 3H, OCH_3). ^{13}CNMR (75MHZ):158.77, 150.17, 148.57, 147.33, 144.39, 139.18, 128.16, 127.83, 127.05, 120.64, 117.71, 116.14, 113.79, 112.83, 112.35, 56.03. MS (EI) *m/z* (%): 310 (M+, 31.57), 161 (63.12), 145 (86.26), 118 (100), 78 (38.92).

2.3.5. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-ethoxy-4-hydroxy-benzylidene)-hydrazide **(6e)**

White solid; Yield: 43%, mp: 139-143 ºC, IR (KBr, cm^{-1}): 3168, 2964, 2853, 2964, 2400, 1655. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.76 (s, 1H, C=ONH), 9.52(s, 1H, Ar-H), 8.62 (d, 1H, J=6 Hz, Ar-H), 8.53 (s, 1H, N=CH), 8.46 (s, 1H, Ar-H), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.39 (t, 1H, J=8 Hz, Ar-H), 7.29(s,1H, Ar-H), 6.84-7.04 (m, 2H, Ar-H), 6.5 (d, 1H, J=7 Hz, Ar-H), 4.06 (t, 2H, J=6 Hz, OCH_2), 1.34 (broad, 3H, CH_3). ^{13}CNMR (75MHZ): 158.70, 149.61, 149.04, 147.64, 144.32, 138.97, 128.19, 127.21, 126.32, 122.55, 117.62, 116.14, 115.96, 113.87, 110.72, 64.32, 15.19. MS (EI) *m/z* (%): 324 (M+, 19), 161 (50), 145 (70), 118 (100), 78 (35).

2.3.6. Synthesis of Acetic acid 4-[(imidazo [1,2-a]pyridine-2-carbonyl)-hydrazonomethyl]-2-methoxy-phenyl ester **(6f)**

White solid; Yield: 40%, mp: 208-212 ºC. IR (KBr, cm^{-1}): 3451, 3228, 3146, 3076, 2400, 1759. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.98 (s, 1H, C=ONH), 8.59-8.55 (m, 3H, Ar-H and N=CH), 7.64 (d, 1H, J=9 Hz, Ar-H), 7.45 (s, 1H, Ar-H), 7.39 (t, 1H, J=7 Hz, Ar-H), 7.26 (d, 1H, J=7 Hz, Ar-H), 7.18 (d, 1H, J=7 Hz, Ar-H), 7.03 (t, 1H, J=7Hz, Ar-H), 3.85 (s, 3H, OCH_3), 2.28 (s, 3H, $O=CH_3O$). ^{13}CNMR (75MHZ): 168.91, 159.07, 151.68, 147.81, 144.42, 141.21, 138.90, 133.87, 128.19, 127.17, 123.76, 120.98, 117.74, 116.39, 113.09, 110.18, 56.31, 20.87. MS (EI) m/z (%): 352 (M+, 8.8), 161 (86.65), 145 (71.11), 118 (100), 78 (38.88).

2.3.7. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (4-hydroxy-benzylidene)-hydrazide **(6g)**

Wihte solid; Yield: 63%, mp: 236-240 ºC. IR (KBr, cm^{-1}): 3243, 3242.7, 2400, 1627

^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.72 (s, 1H, C=ONH), 9.98 (s, 1H, OH), 8.62(d, 1H, J=6Hz, Ar-H), 8.48-8.51(m, 2H, Ar-H and N=CH), 7.63(d, 1H, J=9 Hz, Ar-H), 7.54 (d, 2H, J=9 Hz, Ar-H), 7.39 (t, 1H, J=7 Hz, Ar-H), 7.02 (t, 1H, J=7 Hz, Ar-H), 6.84 (d, 2H, J=7 Hz, , Ar-H). ^{13}CNMR (75MHZ): 159.80, 158.70, 148.80, 144.33, 138.99, 129.32, 128.19, 127.20, 125.91, 117.62, 116.28, 113.86. MS (EI) m/z (%): 280 (M+, 14.7), 161 (73.5), 145 (71.56), 118 (100), 78 (53.9).

2.3.8. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (2,4-dihydroxy-benzylidene)-hydrazide **(6h)**

White solid; Yield: 21%, mp: 300-305 ºC, IR (KBr, cm^{-1}): 3583, 3307, 3019, 2964, 2400, 1711. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 12.15 (s, 1H, C=ONH), 11.67 (s, 1H, OH), 10.02 (s, 1H, OH), 8.54-8.64 (m, 3H, Ar-H and N=CH), 7.65 (d, 1H, J=8 Hz, Ar-H), 7.40 (t, 1H, J=7 Hz, Ar-H), 7.22 (d, 1H, J=7 Hz, Ar-H), 7.03 (s, 1H, Ar-H), 6.35(m, 2H, OH). ^{13}CNMR (75MHZ): 161.10, 160.06, 158.54, 150.30, 144.42, 138.49, 132.13, 128.24, 127.30, 117.63, 116.31, 113.93, 111.01, 108.13, 103.17. MS (EI) *m/z* (%): 296 (M+, 14), 145(99), 118 (80), 78 (79).

2.3.9. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-hydroxy-benzylidene)-hydrazide **(6i)**

White solid; Yield: 48%, mp: 143-145 ºC. IR (KBr, cm^{-1}): 3544, 3148, 3019, 2400, 1644. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 11.90 (s, 1H, C=ONH), 9.67 (s, 1H, OH), 8.62 (d, 1H, J=6, Ar-H), 8.52-8.54 (m, 2H, Ar-H and N=CH), 7.63 (d, 1H, J=9 Hz, Ar-H), 7.38(t, 1H, J=7 Hz, Ar-H), 7.25 (t, 1H, J=7 Hz, Ar-H), 7.19 (broad, 1H, Ar-H), 7.08 (d, 1H, J=6 Hz, Ar-H), 7.02 (t, 1H, J=6 Hz, Ar-H), 6.84 (d, 1H, J=7Hz, Ar-H). ^{13}CNMR (75MHZ): 158.98, 158.11, 148.59, 144.39, 138.89, 136.22, 130.34, 128.19, 127.19, 119.21, 117.82, 117.69, 116.31, 113.88, 113.14. MS (EI) *m/z* (%): 280 (M+, 9.25), 161 (84.05), 145 (97.12), 118 (100), 78 (41.62).

2.3.10. Synthesis of Imidazo [1,2-a]pyridine-2-carboxylic acid (3-nitro-benzylidene)-hydrazide **(6j)**

Light yellow solid; Yield: 44%, mp: 258-262 ºC. IR (KBr, cm^{-1}): 3446, 3154, 3029, 2400, 2400, 1658. ^{1}HNMR (300MHz, DMSO-d6): δ_H (ppm) 12.20 (s, 1H, C=ONH), 8.70 (s, 1H, Ar-H), 8.57-8.62-(m, 2H, Ar-H and N=CH), 8.48 (s, 1H, Ar-H), 8.23 (d, 1H, J=7 Hz, Ar-H), 8.10 (d, 1H, J=6 Hz, Ar-H), 7.73 (t, 1H, J=6 Hz, Ar-H), 7.65 (d, 1H, J=8 Hz, Ar-H), 7.38 (s, 1H, Ar-H), 7.02 (s, 1H, Ar-H). ^{13}CNMR (75MHZ): 159.30, 148.63, 146.01, 144.45, 138.65, 136.77, 133.70, 130.91, 128.19, 127.21, 124.59, 121.25, 117.75, 116.59, 113.92. MS (EI) *m/z* (%): 309 (M+, 9.7), 161 (70.59), 145 (80.43), 118 (100), 78 (49.95).

2.7. Tyrosinase assay

Mushroom Tyrosinase (EC 1.14.18.1) (Sigma Chemical Co.) was tested according to a previous study with slight modifications using LDOPA as substrate [30]. Enzyme activity was checked by detecting dopachrome formation at 475 nm. The stock solutions of all test samples and kojic acid were prepared by dissolving in DMSO at 40mM and then diluted with phosphate buffer (pH=6.8) to the required concentrations. Initially, 10 µL of Tyrosinase (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. After the mixture was pre-incubated at 28 ºC for 20 min, 20 µL of L-DOPA solution (0.5 mM) was added to the phosphate buffer. DMSO in the absence of the test compounds was applied as the control, and kojic acid was used as a positive control. Each concentration was analyzed in three separate replicates. The inhibitory activity of the test compounds was expressed as the concentration that inhibited 50% of the enzyme activity (IC_{50}). The percent inhibition ratio was calculated according to the following equation:

Inhibition (%) = 100 - ($Abs_{control}$ - $Abs_{compound}$) / $Abs_{control}$

2.8. Molecular docking study

All compounds were sketched using MarvinSketch 18.20.0 [31] and energy of compounds was optimized using steepest descent algorithm by Open Babel 2.4.0 software [32]. GOLD 2018 docking program [33] was applied to dock all compounds using PDB code of 2Y9X. This structure is a Tyrosinase from Agaricus bisporus having tropolone as the native ligand in the binding site. Two Cu^{2+} metal ions exist in the binding site of Tyrosinase and were included for docking calculations. Protein structure was prepared using Discovery studio client [34] and all hydrogens were added. Binding site of the enzyme for docking was defined automatically by setting 8 Å around the coordinates of the native ligand tropolone. All available scoring functions in GOLD, namely CHEMPLP, ChemScore, ASP, and GoldScore were tried to re-dock tropolone inside the 2Y9X and then RMSD measure was calculated to validate and find the most appropriate scoring function for docking analyses.

3. Results and discussion

3.1. Design strategy

We designed the target compounds bearing imidazo [1,2-a]pyridine-carbohydrazid scaffold based on the structures of some potent Tyrosinase inhibitors reported in the literature [35, 36]. Some benzimidazole derivatives such as 2-(3-Methylbenzyl)-4(7)-phenyl-1*H* benzo[*d*]imidazole (**I**) illustrated Tyrosinase inhibitory activity with IC_{50} value of 37.86 μM [37]. The benzothiazole analogs like compound **(II)** have been reported as potential Tyrosinase inhibitors which demonstrated higher inhibition than kojic acid at 50 μM [38]. Additionally, the hydrazine-containing compounds such as compound **(III)** have been reported as effective Tyrosinase inhibitors (IC_{50}=1.93 μM) which exhibited better Tyrosinase inhibitor activity than kojic acid (Fig. 2) [39]. Based on these findings, herein we designed a series of imidazo [1,2-a]pyridine-2-carbohydrazide derivatives containing various arylidene pendants as tyroinase inhibitors via hybridization of imidazo pyridine core with hydrazine moiety as copper chelator. Furthermore, various phenolic moieties and its derivatives were introduced as a substituted benzylidene pendant to meet the essential requirements of Tyrosinase inhibitors and constructing structure–activity relationships (SARs).

3.2. Synthesis

The synthetic route to target compounds **6a–6j** is represented in Scheme 1. Reaction of 2-aminopyridine **(1)** and ethyl bromopyruvate **(2)** in refluxing ethanol resulted in ethyl imidazo [1,2-a]pyridine-2-carboxylate (**3**). In the next step, reaction of compound **(3)** with hydrazine hydrate under reflux condition afforded imidazo [1,2-a]pyridine-2-carbohydrazide **(4)**. Finally different substituted benzaldehyde derivatives **(5a-j)** were reacted with **2** in chloroform/ethanol (9:1). The resulting mixture was stirred under reflux and completion of the reaction was monitored by TLC to give compounds **6a–6j**.

3.3. Tyrosinase inhibitory activity

The inhibitory effect of all the synthesized derivatives were conducted on mushroom Tyrosinase enzyme by evaluation of their potential to inhibit enzymatic oxidation of L-Dopa. Kojic acid was used as the reference inhibitor according to the literature protocol [30, 40]. The chemical structure of the synthesized compounds and results are presented in Table 1.

The most potent compounds 6j and 6g exhibited considerable inhibitory potential with IC_{50} values of 7.19±2.56 μM and 8.11±1.25 μM, respectively against mushroom Tyrosinase compared to kojic acid with IC_{50} value of 9.64±0.50 μM. Compounds 6c, 6h, 6b, 6f and 6e showed moderate to weak inhibitory activities (IC_{50}s = 11.1-81.9 μM) while the rest of compounds (6a, 6d and 6i) were almost inactive.

Considering the obtained results from the SAR, there is a strong relation between the nature and position of the phenyl ring substitutions and Tyrosinase inhibition that can be shown as follows:

It seems that the presence of an electron donating group such as hydroxy and methoxy at the para position of the phenyl ring enhanced the inhibitory activity (compounds **6g** and **6b**). In addition, introduction of some groups such as nitro into the meta position of the phenyl moiety increased the anti-Tyrosinase potential of synthesized compound (compound **6j**). In contrast, compound **6i** bearing hydroxyl group on meta position of the phenyl ring was completely inactive. Furthermore, it was found that compound **6a** bearing 2-methoxy group on the phenyl ring was completely inactive while compound **6h** bearing 2,4 di hydroxyl residues exhibited the moderate Tyrosinase inhibitory activities. The comparison of substituents on different positions of phenyl ring indicated that compounds **6f**, **6e** and **6d** bearing different substitutes such as 3-methoxy-4-acetoxy, 3-ethoxy-4-hydroxy and 3-hydroxyl-4methoxy showed weak to inactive inhibitory activities; however, increasing the number of electrondonating group as in compound **6c** bearing 3,4,5 tri methoxy improved the activity. Consequently, the anti-Tyrosinase activity of **6j** and **6g** are higher than kojic acid and we hope that the imidazopyridine derivative would overcome some limitations of kojic acid such as formulation stability and skin penetration difficulties [41].

3.4. Molecular docking analysis

Molecular docking analysis was carried out to investigate interactions and binding poses of all synthesized compounds inside the binding site of Tyrosinase enzyme. RMSD value of redocking process of tropolone inside the active site of Tyrosinase with all available scoring functions in the GOLD was calculated in such a way that for ChemScore was 2.00 Å and for other scoring function algorithms was higher than 2.00 Å. Therefore, score values and binding modes of Tyrosinase inhibitors with the enzyme with PDB code 2Y9X was investigated by ChemScore fitness function. ChemScore fitness value of docking of all compounds along with their important interactions with amino acid residues inside the Tyrosinase active site were shown in

Table 1. ChemScore fitness values ranged from 34.73 in compound **6j** to 20.37 in compound **6c**. 3D interaction patterns of two active compounds **6j** and **6g** with IC_{50} values 7.19 and 8.11 µM respectively, and two inactive compounds **6a** and **6i** with IC_{50} values more than 100 µM, were depicted in Fig. 3.

Fig. 3a shows the docking interactions of compound **6j**. Docking score of this compound is 34.73 which is the highest score value among all compounds. Backbone structure of the compound made two pi-pi interactions via its aromatic rings: one pi-pi t-shaped interaction from imidazo [1,2-a]pyridine ring to Phe264 and one pi-pi stacked interaction from nitrobenzene group to His263. These two pi-pi interactions with these residues were among important ones. Other interaction types comprised pi-sigma and pi-alkyl interactions with Val283 and Ala286 respectively and pi-sigma interaction from imidazo [1,2-a]pyridine ring toVal283.

Docking interaction analysis of compound **6g**, Fig. 3b, shows the same pattern of pi-pi interactions with Phe264 and His263 from imidazo [1,2-a]pyridine ring and phenyl ring, respectively. Replacement of 3-nitro with 4-hydroxy group changed the orientation of compound **6g** in such a way that one hydrogen bond was made between carbonyl group and residue Val283. This additional hydrogen bond interaction did not cause any significant difference in activity against Tyrosinase in comparison to compound **6j**. Chemscore fitness value of this compound is a high value of 29.29. Compound **6g** made other interaction types such as a pi-cation from imidazo [1,2-a]pyridine ring to residue Arg268, a pi-alkyl interaction and a pi-sigma interaction between phenol and Ala286, and Val283 respectively that all further strengthened its binding towards Tyrosinase enzyme.

Another moderate to active compound is **6h** with IC_{50} value of 12.6 µM having ChemScore fitness value of 31.53. This compound made two important pi-pi interactions of the backbone with Phe264 and His263. In comparison to compound **6g** bearing 4-hydroxy moiety, compound **6h** has an additional hydroxyl in position 2 that donated a hydrogen bond to Asn260. This hydroxyl also made a metal-acceptor interaction with one Cu^{2+}. Another hydrogen bond interaction was formed between carbonyl group and Val283. A pi-alkyl interaction between Val283 and phenyl ring was also made, see Fig 3c.

In inactive compound **6i**, 3-hydroxy replaced 4-hydroxy in compound **6g**. This substitution got the backbone away from Phe264 in such a way that it was not able to make this critical pi-pi

interaction and just made pi-pi stacked interaction with His263. ChemScore fitness value of **6i** is 30.17 which might be resulted from other not that much important hydrophobic interactions it made like pi-alkyl with Ala286 and pi-sigma with Val248 inside the active site of Tyrosinase, see Fig. 3d.

In overall, inactive compounds, **6a, 6d** and **6i**, lacked one or two critical pi-pi interactions with His263 and Phe264 that resulted from a different orientation of these molecules in the enzyme active site. Presence of an electrondonating group such as hydroxyl in the para position of the phenyl ring makes compound able to form a metal-acceptor interaction with Cu^{+2} ion and also changes the orientation of the molecule to be able to form a hydrogen bond from carbonyl group to residue Val283.

4. **Conclusions**

In conclusion, we developed a series of imidazo [1,2-a]pyridine-2-carbohydrazide derivatives bearing various arylidene pendants as novel Tyrosinase inhibitors. Based on the obtained data, compounds **6j** and **6g** containing 3-nitro and 4-hydroxyl moieties were the most potent compounds against Tyrosinase with respective IC_{50} values of 7.19 μM and 8.11 μM. The results indicated that the inhibitory potential was dependent on the substituted moiety on the phenyl ring. Therefore, derivatives bearing an electrondonating such as hydroxyl and electron-withdrawing group such as nitro at the para and meta position of the phenyl ring exhibited considerable inhibitory activity, respectively. Molecular docking analysis results demonstrated that potent compounds **6g**, **6h** and **6j** had high docking score values and made two critical pi-pi interactions with Phe264 and His263. These results indicate that the compounds can serve as structural outlines and promising leads in order to design and develop novel effective Tyrosinase inhibitors.

Declaration of competing interest

The authors report no conflict of interest.

Credit Author Statement

Najmeh Edraki : principle investigator, supervision of the team in all research steps including data gathering, data analysis, and manuscript preparation/revision

Mehdi Khoshneviszadeh: Supervision of the design, synthetic steps and biological evaluation, manuscript preparation.

Tahereh Damghani: contribution in the data gathering, design and synthesis of compounds, data analysis, preparation of results, writing and revising of this manuscript.

Saba Hadaegh : contribution in the synthesis of compounds.

Mahsima khoshneviszadeh: Contribution in the biological and enzymatic assay..

Somayeh Pirhadi : Supervision and performance of *computational* docking and data analysis of this study.

Razieh Sabet : Supervision of synthetic parts.

Acknowledgment

The authors wish to thank the support of the Vice-Chancellor for Research, Shiraz University of Medical Sciences (Grant Number: 14006). This work was the PharmD thesis of Saba Hadaegh.

References

[1] T.F. Cestari, L.P. Dantas, J.C. Boza, Acquired hyperpigmentations, Anais brasileiros de dermatologia, 89 (2014) 11-25. DOI: https://doi.org/10.1590/abd1806-4841.20142353.

[2] S. Nouveau, D. Agrawal, M. Kohli, F. Bernerd, N. Misra, C.S. Nayak, Skin hyperpigmentation in Indian population: Insights and best practice, Indian journal of dermatology, 61 (2016) 487. DOI: 10.4103/0019-5154.190103.

[3] S. Dogra, R. Sarangal, Pigmentary disorders: An insight, Pigment International, 1 (2014) 5. DOI: 10.4103/2349-5847.135429.

[4] A. Lapedriza, K. Petratou, R.N. Kelsh, Neural crest cells and pigmentation, in: Neural Crest Cells, Elsevier, 2014, pp. 287-311. DOI: https://doi.org/10.1016/B978-0-12-401730-6.00015-6.

[5] A. Garcia-Jimenez, J.A. Teruel-Puche, P.A. Garcia-Ruiz, J. Berna, J.N. Rodríguez-López, J. Tudela, F. Garcia-Canovas, Action of 2, 2′, 4, 4′-tetrahydroxybenzophenone in the biosynthesis pathway of melanin, International journal of biological macromolecules, 98 (2017) 622-629. DOI: https://doi.org/10.1016/j.ijbiomac.2017.02.032.

[6] C.S. Nunes, K. Vogel, Tyrosinases—physiology, pathophysiology, and applications, in: Enzymes in Human and Animal Nutrition, Elsevier, 2018, pp. 403-412. DOI: https://doi.org/10.1016/B978-0-12-805419-2.00020-4.

[7] O. Nerya, R. Ben-Arie, T. Luzzatto, R. Musa, S. Khativ, J. Vaya, Prevention of Agaricus bisporus postharvest browning with tyrosinase inhibitors, Postharvest Biology and Technology, 39 (2006) 272-277. DOI: https://doi.org/10.1016/j.postharvbio.2005.11.001.

[8] T. Pillaiyar, M. Manickam, V. Namasivayam, Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors, Journal of enzyme inhibition and medicinal chemistry, 32 (2017) 403-425. DOI: https://doi.org/10.1080/14756366.2016.1256882.

[9] S.Y. Lee, N. Baek, T.-g. Nam, Natural, semisynthetic and synthetic tyrosinase inhibitors, Journal of Enzyme Inhibition and Medicinal Chemistry, 31 (2016) 1-13. DOI: https://doi.org/10.3109/14756366.2015.1004058.

[10] M. Loizzo, R. Tundis, F. Menichini, Natural and synthetic tyrosinase inhibitors as antibrowning agents: an update, Comprehensive Reviews in Food Science and Food Safety, 11 (2012) 378-398. DOI: https://doi.org/10.1111/j.1541-4337.2012.00191.

[11] T.-S. Chang, An updated review of tyrosinase inhibitors, International journal of molecular sciences, 10 (2009) 2440-2475. DOI: https://doi.org/10.3390/ijms10062440.

[12] F.A. Larik, A. Saeed, P.A. Channar, U. Muqadar, Q. Abbas, M. Hassan, S.-Y. Seo, M. Bolte, Design, synthesis, kinetic mechanism and molecular docking studies of novel 1-pentanoyl-3-arylthioureas as inhibitors of mushroom tyrosinase and free radical scavengers, European journal of medicinal chemistry, 141 (2017) 273-281. DOI: https://doi.org/10.1016/j.ejmech.2017.09.059.

[13] M.H.K. Nguyen, H.X. Nguyen, M.T.T. Nguyen, N.T. Nguyen, Phenolic constituents from the heartwood of Artocapus altilis and their tyrosinase inhibitory activity, Natural product communications, 7 (2012) 1934578X1200700214. DOI: https://doi.org/10.1177/1934578X1200700214.

[14] Z. Ashraf, M. Rafiq, S.-Y. Seo, M.M. Babar, Synthesis, kinetic mechanism and docking studies of vanillin derivatives as inhibitors of mushroom tyrosinase, Bioorganic & medicinal chemistry, 23 (2015) 5870-5880. DOI: https://doi.org/10.1016/j.bmc.2015.06.068.

[15] K. Sakuma, M. Ogawa, K. Sugibayashi, K.-i. Yamada, K. Yamamoto, Relationship between tyrosinase inhibitory action and oxidation-reduction potential of cosmetic whitening ingredients and phenol derivatives, Archives of pharmacal research, 22 (1999) 335-339. DOI: https://doi.org/10.1007/BF02979054.

[16] A. Hashimoto, M. Ichihashi, Y. Mishima, The mechanism of depigmentation by hydroquinone: a study on suppression and recovery processes of tyrosinase activity in the pigment cells in vivo and in vitro, Nihon Hifuka Gakkai zasshi. The Japanese journal of dermatology, 94 (1984) 797.

[17] Y.-R. Chen, R.Y.-Y. Chiou, T.-Y. Lin, C.-P. Huang, W.-C. Tang, S.-T. Chen, S.-B. Lin, Identification of an Alkylhydroquinone from Rhus succedanea as an Inhibitor of Tyrosinase and Melanogenesis, Journal of agricultural and food chemistry, 57 (2009) 2200-2205. DOI: https://doi.org/10.1021/jf802617a.

[18] A. Sasaki, Y. Yamano, S. Sugimoto, H. Otsuka, K. Matsunami, T. Shinzato, Phenolic compounds from the leaves of Breynia officinalis and their tyrosinase and melanogenesis inhibitory activities, Journal of natural medicines, 72 (2018) 381-389. DOI: https://doi.org/10.1007/s11418-017-1148-8.

[19] K. Tasaka, C. Kamei, S. Nakano, Y. Takeuchi, M. Yamato, Effects of certain resorcinol derivatives on the tyrosinase activity and the growth of melanoma cells, Methods and findings in experimental and clinical pharmacology, 20 (1998) 99-110. DOI: 10.1358/mf.1998.20.2.485637.

[20] L. Kolbe, T. Mann, W. Gerwat, J. Batzer, S. Ahlheit, C. Scherner, H. Wenck, F. Stäb, 4-n-butylresorcinol, a highly effective tyrosinase inhibitor for the topical treatment of hyperpigmentation, Journal of the European Academy of Dermatology and Venereology, 27 (2013) 19-23. DOI: https://doi.org/10.1111/jdv.12051.

[21] F. Awouters, J. Vermeire, F. Smeyers, P. Vermote, R. Van Beek, C.J. Niemegeers, Oral antiallergic activity in ascaris hypersensitive dogs: a study of known antihistamines and of the new compounds ramastine (R 57 959) and levocabastine (R 50 547), Drug Development Research, 8 (1986) 95-102. DOI: https://doi.org/10.1002/ddr.430080112.

[22] H. Huang, X. Ji, X. Tang, M. Zhang, X. Li, H. Jiang, Conversion of pyridine to imidazo [1, 2-a] pyridines by copper-catalyzed aerobic dehydrogenative cyclization with oxime esters, Organic letters, 15 (2013) 6254-6257. DOI: https://doi.org/10.1021/ol403105p.

[23] M. Bollini, J.J. Casal, D.E. Alvarez, L. Boiani, M. González, H. Cerecetto, A.M. Bruno, New potent imidazoisoquinolinone derivatives as anti-Trypanosoma cruzi agents: Biological evaluation and structure–activity relationships, Bioorganic & medicinal chemistry, 17 (2009) 1437-1444. DOI: https://doi.org/10.1016/j.bmc.2009.01.011.

[24] J.J. Kaminski, C. Puchalski, D.M. Solomon, R.K. Rizvi, D.J. Conn, A.J. Elliott, R.G. Lovey, H. Guzik, P. Chiu, Antiulcer agents. 4. Conformational considerations and the antiulcer activity of substituted imidazo [1, 2-a] pyridines and related analogs, Journal of medicinal chemistry, 32 (1989) 1686-1700. DOI: https://doi.org/10.1021/jm00128a005.

[25] Y. Rival, G. Grassy, A. Taudou, R. Ecalle, Antifungal activity in vitro of some imidazo [1, 2-a] pyrimidine derivatives, European journal of medicinal chemistry, 26 (1991) 13-18. DOI: https://doi.org/10.1016/0223-5234(91)90208-5.

[26] M. Abdel-Aziz, H.M. Abdel-Rahman, Synthesis and anti-mycobacterial evaluation of some pyrazine-2-carboxylic acid hydrazide derivatives, European journal of medicinal chemistry, 45 (2010) 3384-3388. DOI: https://doi.org/10.1016/j.ejmech.2010.04.025.

[27] A. Kajal, S. Bala, S. Kamboj, V. Saini, Synthesis, characterization, and computational studies on phthalic anhydride-based benzylidene-hydrazide derivatives as novel, potential anti-inflammatory agents, Medicinal Chemistry Research, 23 (2014) 2676-2689. DOI: https://doi.org/10.1007/s00044-013-0848-1.

[28] R. Narang, B. Narasimhan, S. Sharma, A review on biological activities and chemical synthesis of hydrazide derivatives, Current medicinal chemistry, 19 (2012) 569-612. DOI: https://doi.org/10.2174/092986712798918789.

[29] R. Sreenivasulu, K.T. Reddy, P. Sujitha, C.G. Kumar, R.R. Raju, Synthesis, antiproliferative and apoptosis induction potential activities of novel bis (indolyl) hydrazide-hydrazone derivatives, Bioorganic & medicinal chemistry, 27 (2019) 1043-1055. DOI: https://doi.org/10.1016/j.bmc.2019.02.002.

[30] Y.M. Ha, S.W. Chung, S. Song, H. Lee, H. Suh, H.Y. Chung, 4-(6-Hydroxy-2-naphthyl)-1, 3-bezendiol: a potent, new tyrosinase inhibitor, Biological and Pharmaceutical Bulletin, 30 (2007) 1711-1715. DOI: https://doi.org/10.1248/bpb.30.1711.

[31] http://www.chemaxon.com.

[32] N.M. O'Boyle, M. Banck, C.A. James, C. Morley, T. Vandermeersch, G.R. Hutchison, Open Babel: An open chemical toolbox, Journal of cheminformatics, 3 (2011) 33. DOI: https://doi.org/10.1186/1758-2946-3-33.

[33] G. Jones, P. Willett, R.C. Glen, A.R. Leach, R. Taylor, Development and validation of a genetic algorithm for flexible docking, Journal of molecular biology, 267 (1997) 727-748. DOI: https://doi.org/10.1006/jmbi.1996.0897.

[34] D.S. BIOVIA, Discovery studio visualizer, Release 2017, San Diego: Dassault Systèmes, 2016, to be found under http://accelrys. com/products/collaborative-science/biovia-discovery-studio/visualization-download. php (accessed Dec 12, 2017), (2017).

[35] S. Zolghadri, A. Bahrami, M.T. Hassan Khan, J. Munoz-Munoz, F. Garcia-Molina, F. Garcia-Canovas, A.A. Saboury, A comprehensive review on tyrosinase inhibitors, Journal of enzyme inhibition and medicinal chemistry, 34 (2019) 279-309. DOI: https://doi.org/10.1080/14756366.2018.1545767.

[36] J.P. Germanas, S. Wang, A. Miner, W. Hao, J.M. Ready, Discovery of small-molecule inhibitors of tyrosinase, Bioorganic & medicinal chemistry letters, 17 (2007) 6871-6875. DOI: https://doi.org/10.1016/j.bmcl.2007.10.014.

[37] İ.S. Doğan, A. Özel, Z. Birinci, B. Barut, H.E. Sellitepe, B. Kahveci, Synthesis of Some Novel 2-substitutedbenzyl-(4) 7-phenyl-1H-benzo [d] imidazoles in Mild Conditions as Potent Anti-tyrosinase and Antioxidant Agents, Archiv der Pharmazie, 349 (2016) 881-888. DOI: https://doi.org/10.1002/ardp.201600224.

[38] H.Y. Chung, M.H. Park, Y.M. Ha, Y.K. Han, J.Y. Park, Y.J. Park, J.A. Kim, J.Y. Lee, Y.M. Song, H.R. Moon, Compound having skin-whitening, anti-oxidizing and PPAR activities and medical use therefor, in, Google Patents, 2014.

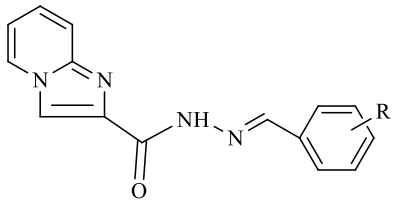
[39] W. Yi, R.-H. Cao, Z.-Y. Chen, L. Yu, L. Ma, H.-C. Song, Design, synthesis and biological evaluation of hydroxy-or methoxy-substituted phenylmethylenethiosemicarbazones as tyrosinase inhibitors, Chemical and Pharmaceutical Bulletin, 57 (2009) 1273-1277. DOI: https://doi.org/10.1248/cpb.57.1273.

[40] S. Son, H. Kim, H.Y. Yun, S. Ullah, S.J. Kim, Y.-J. Kim, M.-S. Kim, J.-W. Yoo, P. Chun, H.R. Moon, (E)-2-Cyano-3-(substituted phenyl) acrylamide analogs as potent inhibitors of tyrosinase: A linear β-phenyl-α, β-unsaturated carbonyl scaffold, Bioorganic & medicinal chemistry, 23 (2015) 7728-7734. DOI: https://doi.org/10.1016/j.bmc.2015.11.015.

[41] J. Hermanns, C. Pierard-Franchimont, G. Pierard, Skin colour assessment in safety testing of cosmetics. An overview, International journal of cosmetic science, 22 (2000) 67-71. DOI: 10.1046/j.1467-2494.2000.00021.x .

Table 1

Chemical structure, Tyrosinase inhibitory effects and molecular docking studies of synthesized compounds **6a-6j**.



compounds	R	Mw	ChemScore fitness value	Binding interactions with Tyrosinase active site	Yields*	IC_{50}(µm)±SE**
6a	2- methoxy	294	22.62	Hydrogen bonds: Val283	88%	> 100
6b	4- methoxy	294	23.93	Hydrogen bonds: Val283 Pi-pi interactions: His263,	83%	14.3±3.11
6c	3,4,5-trimethoxy	354	20.37	Hydrogen bonds: Arg268 Pi-pi interactions: Phe264	27%	11.10±1.55
6d	3-hydroxy-4-methoxy	310	28.47	Hydrogen bonds: Asn260, Val283	36%	> 100
6e	3-ethoxy-4-hydroxy	324	27.29	Pi-pi interactions: His263, Metal acceptor interaction: 1 Cu^{+2}	43%	81.9±9.75
6f	3-methoxy-4-acetoxy	352	28.49	Metal acceptor interaction: 2 Cu^{+2}	40%	41.70±2.40
6g	4-hydroxy	280	29.29	Hydrogen bonds: Ala283 Pi-pi interactions: His263, Phe264	63%	8.11±1.25
6h	2,4-dihydroxy	296	31.53	Hydrogen bonds: Asn260, Val283 Pi-pi interactions: His263, Phe264 Metal acceptor interaction: 1 Cu	21%	12.60±3.67
6i	3-hydroxy	280	30.17	Pi-pi interactions: His263	48%	> 100
6j	3-nitro	309	34.73	Pi-pi interactions: His263, Phe264	44%	7.19±2.56
Kojic Acid* **	-	-				9.64±0.50

*Reported yields are based on the yield of final step of synthesis.

** Values represent means ± SE of 3 independent experiments.*** Kojic Acid was used as a positive control.

Fig. 1

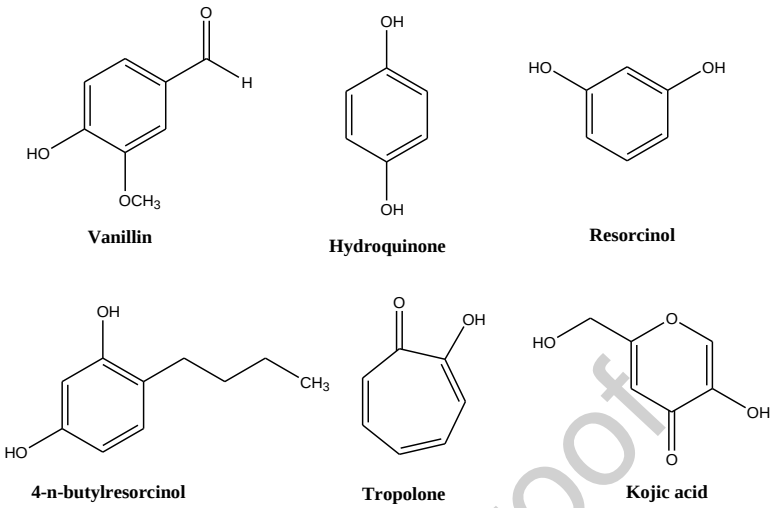


Fig. 1. Chemical structures of some well-known tyrosine inhibitors

Fig. 2

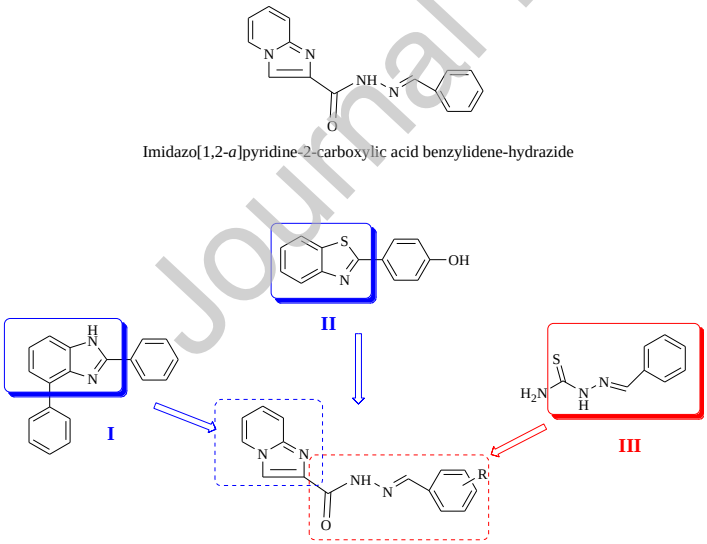


Fig. 2. Design of proposed Tyrosinase inhibitors of present work

Fig.3. 3D interaction pattern of compounds **6j (a), 6g (b), 6h (c)** and **6i (d)** inside the active site of Tyrosinase enzyme.

Fig.3a:

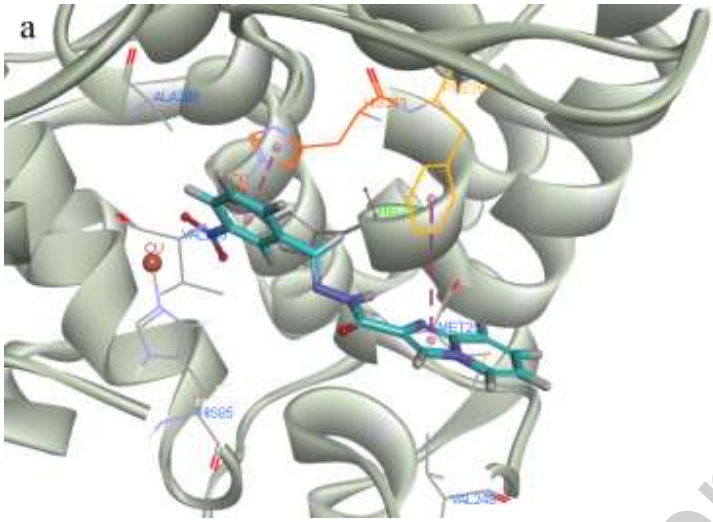


Fig.3b:

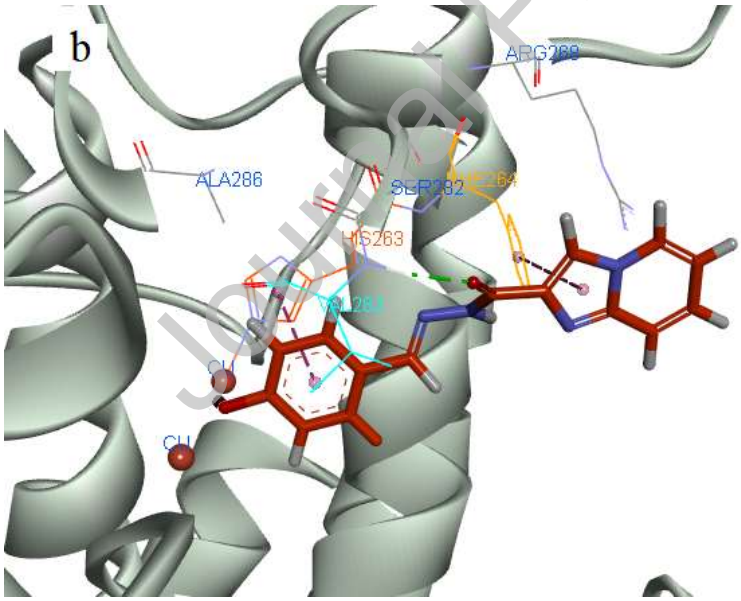


Fig. 3c:

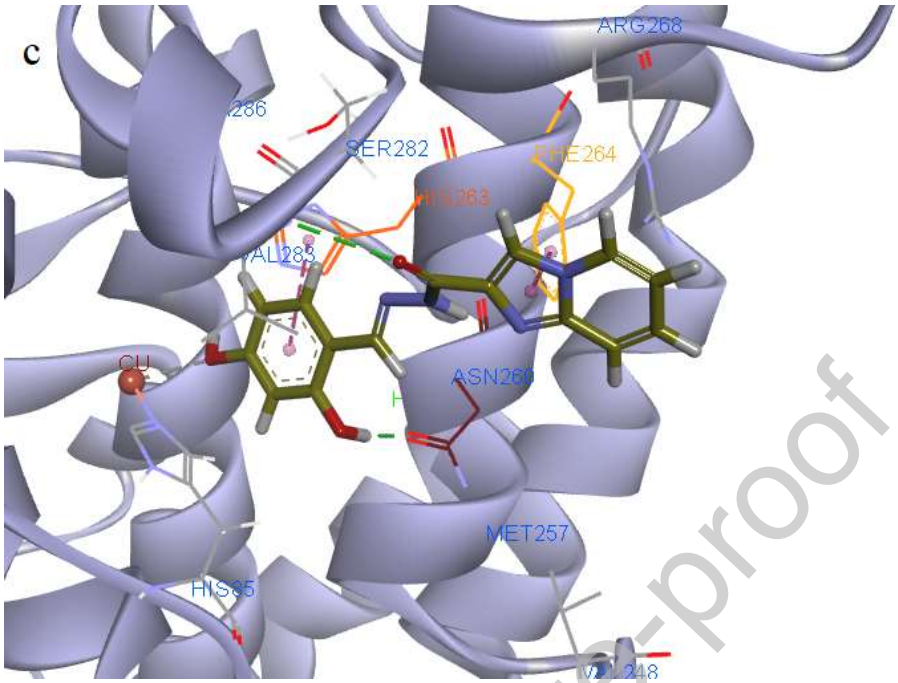
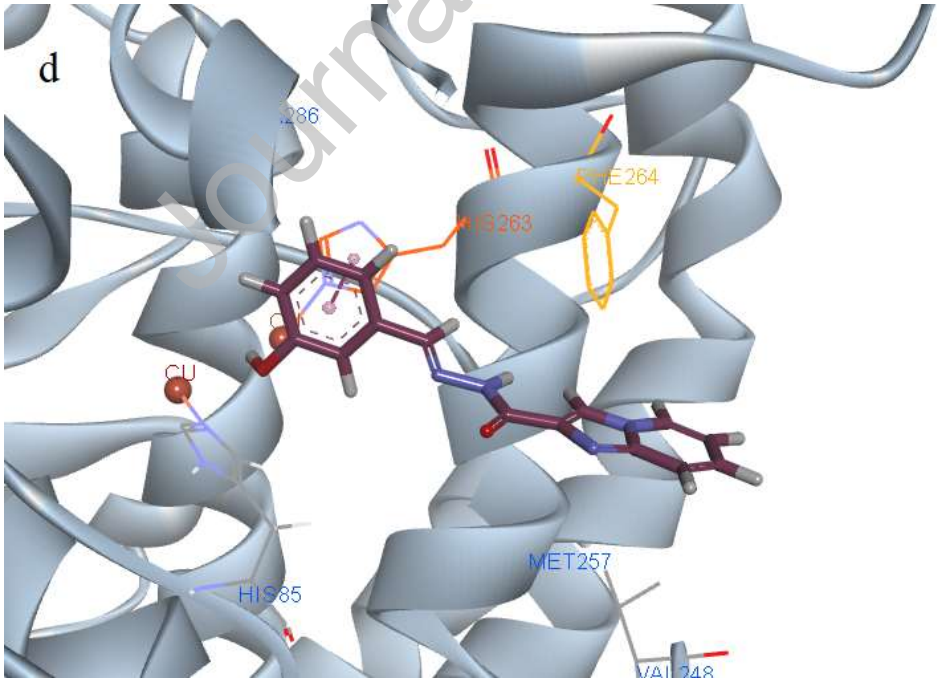
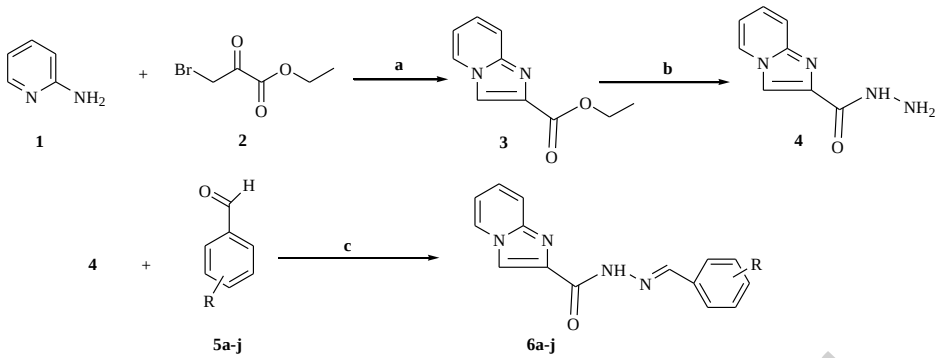


Fig. 3d:



Scheme 1:



6a; R= 2- methoxy
6b; R= 4- methoxy
6c; R= 3,4,5-trimethoxy
6d; R= 3-hydroxy-4-methoxy
6e; R= 3-ethoxy-4-hydroxy
6f; R= 3-methoxy-4-acetoxy
6g; R= 4-hydroxy
6h; R=2,4-dihydroxy
6i; R= 3-hydroxy
6j; R= 3-nitro

Scheme 1. Reagents and conditions: a). K_2CO_3, EtOH, Reflux. b). $NH_2NH_2.H_2O$, Reflux, 3hrs. c). EtOH, Reflux, 24hrs