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Structure–activity relationships of tyrosinase inhibitory combinatorial library of 2,5-disubstituted-1,3,4-oxadiazole analogues

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Abstract—Here the tyrosinase inhibition studies of library of 2,5-disubstituted-1,3,4-oxadiazoles have been reported and their structure–activity relationship (SAR) also have been discussed. The library of the oxadiazoles was synthesized under the microwave irradiation and was structures of these were characterized by different spectral techniques. From this study it could be concluded that for a better inhibition of tyrosinase, electronegative substitution is essential as most probably the active site of the enzyme contain some hydrophobic site and position is also very important for the inhibition purposes due to the conformational space. The electronegativity of the compounds is somewhat proportional to the inhibitory activity. The compound 3e (30-[5-(40-bromophenyl)-1,3,4-oxadiazol-2-yl]pyridine) exhibited most potent (IC_{50} = 2.18 μM) inhibition against the enzyme tyrosinase which is more potent than the standard potent inhibitor L-mimosine (IC_{50} = 3.68 μM). This molecule can be the best candidate as a lead compound for further development of drug for the treatments of several skin disorders.

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Key words

- Tyrosinase inhibitor; 2,5-Disubstituted-1,3,4-oxadiazole library; Melanin; Vitiligo; Hyperpigmentation; Depigmentation.

- Gel Electrophoresis, Enzyme, Catalysis, ELISA reader, ??????????

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Exercises

- Suggest few more suitable title for this article
- What should be the key words?
- How we can we improve the abstract?

Introduction

1. Introduction

Tyrosinase (E.C. 1.14.18.1), also known as polyphenol oxidase (PPO), is a multifunctional copper-containing enzyme, widely distributed in plants and animals. It catalyses the *o*-hydroxylation of monophenols and also the oxidation of *o*-diphenols to *o*-quinones. Tyrosinase is known to be a key enzyme for melanin biosynthesis in plants and animals. Therefore, tyrosinase inhibitors should be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation and also important in cosmetics for whitening and depigmentation after sunburn. In addition, tyrosinase is known to be involved in the molting process of insect and adhesion of marine organisms.[1] In insects, several functions of this enzyme have been reported in the generation of *o*-diphenols and quinones for pigmentation, wound healing, parasite encapsulation, and sclerotization and the enzyme is an alternative target site for the control of insect pests. In food industry, tyrosinase is responsible for the enzymatic browning reactions in damaged fruits during post-harvest handling and processing. Control of enzymatic browning during processing is important in fruit pulp manufacturing. In addition, tyrosinase inhibitors are becoming important constituents of cosmetic products that relate to hyperpigmentation. Therefore, there is a concerted effort to search for naturally occurring tyrosinase inhibitors from plant, because plants constitute a rich source of bioactive chemicals and many of them are largely free from harmful adverse effects.[2]

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Introduction

In recent years numbers of potent tyrosinase inhibitors have been reported from our and other groups. Very recently, we have reported two long chain esters, methyl 2β(2*S*)-hydroxyl-7(*E*)-tritriacontenoate and methyl 2β(2*S*)-*O*-β-D-galactopyranosyl-7(*E*)-tetratriacontenoate, showing strong to moderate inhibitory activities against tyrosinase.[3] In another paper we have reported that, (+)-androst-4-ene-3,17-dione and its five metabolic analogues having steroidal skeletons, namely androsta-1,4-diene-3,17-dione, 17β-hydroxyandrosta-1,4-dien-3-one, 11α-hydroxyandrost-4-ene-3,17-dione, 11α,17β-dihydroxyandrost-4-en-3-one and 15α-hydroxyandrosta-1,4-dien-17-one, exhibited moderate inhibitory activities against the enzyme.[4] Ahmad et al. in 2004 reported that, a new coumarinolignoid 8′-*epi*-cleomiscosin A together with the new glycoside 8-*O*-β-D-glucopyranosyl-6-hydroxy-2-methyl-4*H*-1-benzopyrane-4-one, exhibited strong inhibition against the enzyme tyrosinase, when compared to the standard tyrosinase inhibitors kojic acid and L-mimosine. The new coumarinolignoid exhibited two times more potency than that of the standard potent inhibitor L-mimosine.[5] Recently, Karbassi et al. reported the inhibition kinetics of two new synthetic bi-pyridine molecules, [1,4′]bipiperidinyl-1′-yl-naphthan-2-yl-methanone (**I**) and [1,4′]bipiperidinyl-1′-yl-4-methylphenyl-methane (**II**) of the catecholase activity of mushroom tyrosinase. The kinetics studies indicated that these are uncompetitive inhibitors and the values of the K_i are 5.87 and 1.31 μM for **I** and **II**, respectively, which showed high potency. Fluorescent studies confirmed the uncompetitive type of inhibition for these two inhibitors. They also suggested that, the inhibition mechanism presumably coming from the presence of a particular hydrophobic site which can accommodate these inhibitors. This site could be formed due to a probable conformational change that was induced by binding of substrate with the enzyme.[6]

Here in this paper, we have discussed the tyrosinase inhibitory activities of a library of 26 analogues of 2,5-disubstituted-1,3,4-oxadiazoles, which were synthesized using microwave-assisted combinatorial synthetic approach and finally their structure–activity relationships (SAR) also have been discussed.

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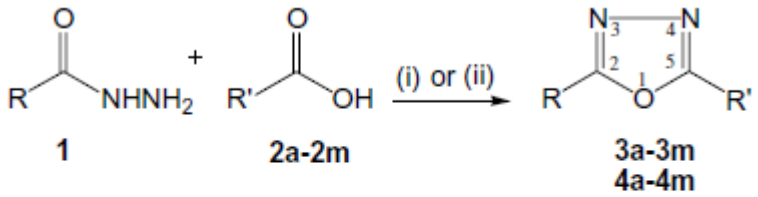
Results and Discussion

2. Results and discussion

2.1. General chemistry

The detailed chemistry and the synthetic parts of th
compounds have been reported recently and discusse
elsewhere.[7] Briefly, a number of commercially availab
hydrazides were treated with different carboxylic acids



3. R = 3-Pyridinyl, 4. R = 2-MeOC6H4

Scheme 1. Reagents: (i) $POCl_3$; (ii) $POCl_3$, Al_2O_3.

(**a**–**m**) in the presence of phosphorous oxychloride to afford 2,5-disubstituted-1,3,4-oxadiazoles **3** (**a**–**m**) and **4** (**a**–**m**) (Scheme 1). To establish the general validity of our newly developed method, several selected one-pot microwave-assisted syntheses were carried out. The reaction was found to proceed smoothly under microwave irradiation within 6–16 min whereas under reflux conditions in 4–10 h (shown in Tables 1 and 2). The products were isolated by simple cold aqueous work-up followed by either solvent extraction or precipitation and were finally purified by column chromatography wherever necessary to afford pure 2,5-disubstituted-1,3,4-oxadiazole. This method appeared to be the rapid and economical with wide range of applications.[7]

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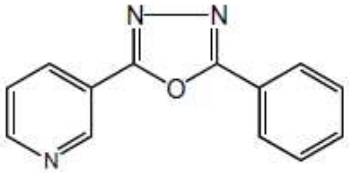
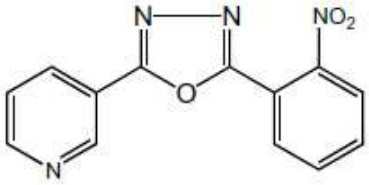
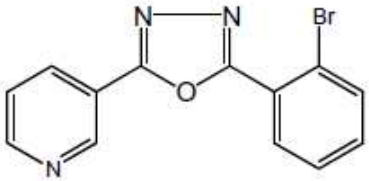
Results and Discussion

Table 1. Comparison between microwave-assisted and conventional method of synthesis of 2,5-disubstituted-1,3,4-oxadiazole **3** (**a**–**m**) in terms of time and yield

Sr.	R′	Structures	Microwave		Conventional	
			Time (min)	Yield (%)	Time (h)	Yield (%)
3a	C_6H_5		12	92	6	81
3b	*o*-$NO_2C_6H_4$		9	96	5	86
3c	*o*-BrC_6H_4		12	92	6	76

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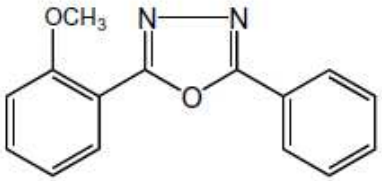
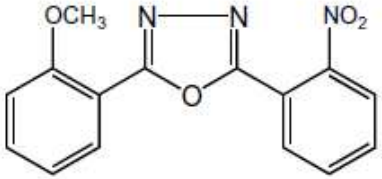
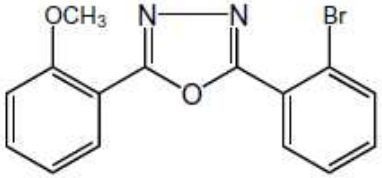
Results and Discussion

Table 2. Comparison between microwave-assisted and conventional method of synthesis of 2,5-disubstituted-1,3,4-oxadiazole **4** (**a**–**m**) in terms of time and yield

Sr.	R′	Structures	Microwave		Conventional	
			Time (min)	Yield (%)	Time (h)	Yield (%)
4a	C_6H_5		12	89	6	78
4b	*o*-$NO_2C_6H_4$		9	95	5	80
4c	*o*-BrC_6H_4		12	90	6	73

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Results and Discussion

2.2. Tyrosinase inhibition studies

In the present studies, two types of 26 derivatives of the oxadiazole basic skeleton have been studied to explain their inhibition patterns and structure–activity relationships (SAR) against the enzyme tyrosinase, which is a multifunctional copper-containing enzyme, widely distributed in plants and animals and catalyses the *o*-hydroxylation of monophenols and also the oxidation of *o*-diphenols to *o*-quinones.[1]

In one type of compounds, substitutions were changing at different positions of the phenyl ring at C-5 while keeping the pyridine ring constant at C-2. In another type of compounds, substitutions were changing at different positions of the phenyl ring while keeping the *o*-methoxy phenyl ring constant at C-2 position.

In a previous report it was found that 3-hydroxypyridine-4-ones is showing inhibition against tyrosinase.[8] This was established that alkyl substitution at position 2 in the aromatic ring minimizes the interaction with tyrosinase. Several phenolic compounds have been reported to have potent tyrosinase inhibitory activity.[9–11]

Compound **3a** exhibited potent tyrosinase inhibition and the IC_{50} value is 5.15 μM, where the IC_{50} value of reference tyrosinase inhibitor kojic acids (KA) is 16.67 μM. This compound was totally unsubstituted. When C-2″ position was substituted with $-NO_2$ group the resulting **3b** was showing highly potent (IC_{50} = 3.18 μM) inhibition against tyrosinase, when compared with highly potent reference tyrosinase inhibitor L-mimosine (LM) (IC_{50} = 3.68 μM). Due to the substitution of this $-NO_2$ group the resulting compound exhibited potent inhibition. But when the same phenyl ring was found to have bromine atom at C-2″ (**3c**, IC_{50} = 5.23 μM) and C-3″ (**3d**, IC_{50} = 6.04 μM)

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Results and Discussion

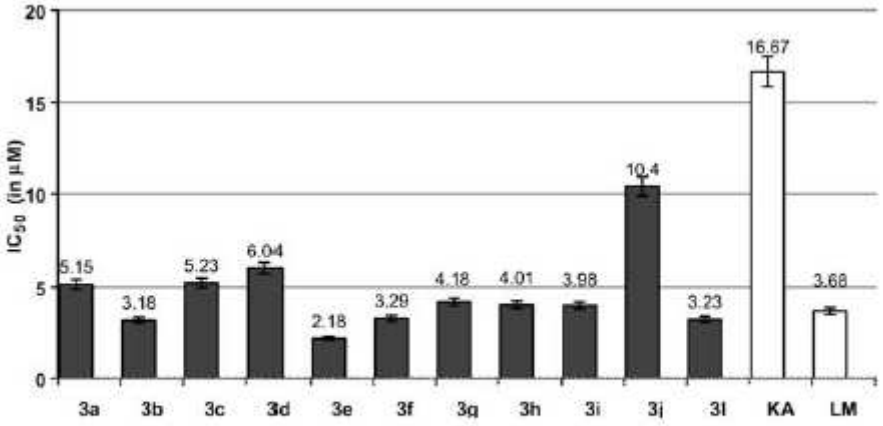

Figure 1. Graphical presentation of the comparative IC_{50} values of the series **3** (**a**–**m**) against the enzyme tyrosinase.

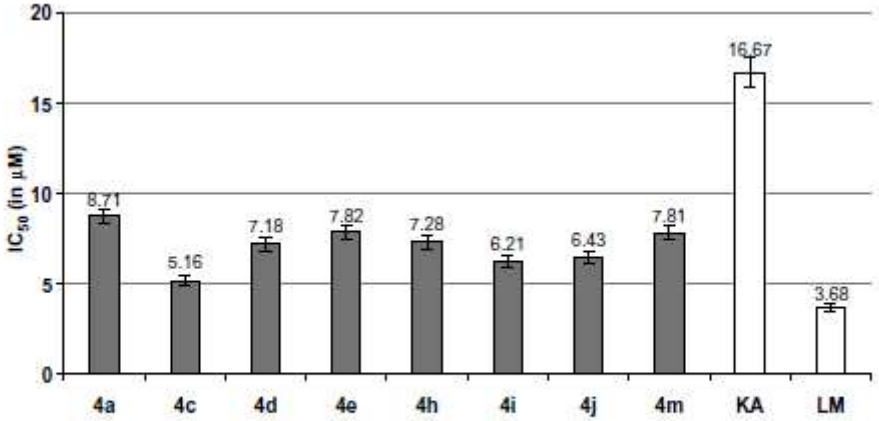

Figure 2. Graphical presentation of the comparative IC_{50} values of the series **4** (**a**–**m**) against the enzyme tyrosinase.

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Material and Methods

3. Materials and methods

3.1. General experimental

The ultraviolet spectra were measured in chloroform on a Lambda 5 UV/vis spectrophotometer (Perkin–Elmer). IR spectra (KBr discs or MeOH) were recorded on a Bruker FT-IR IFS48 spectrophotometer. EI mass spectra data were recorded with various MAT 711 (70 eV) spectrophotometers and data are tabulated as m/z ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using Bruker AC400 (500 and 400 MHz) spectrophotometer, respectively. Splitting patterns are as follows: s, singlet; d, doublet; dd, double doublets; t, triplet; m, multiplet. Chemical shifts are reported in δ (ppm) and coupling constants are given in hertz. The progress of all reactions was monitored by TLC, which was performed on 2.0×5.0 cm aluminum sheets precoated with silica gel $60F_{254}$ to a thickness of 0.25 mm (Merck). The chromatograms were visualized under ultraviolet light (254–366 nm) or iodine vapours.

3.2. Tyrosinase inhibition assay

Tyrosinase inhibition assays were performed in 96-well microplate format using SpectraMax® 340 (Molecular Devices, CA, USA) microplate reader according to the developed method earlier described by Hearing.[14]

First the compounds were screened for the *o*-diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. All the active inhibitors from the preliminary screening were subjected for IC_{50} studies. Briefly, all the compounds were dissolved in DMSO and finally the solvent mixture was 2.5%. Mushroom tyrosinase (30 units, 28 nM) was first preincubated with the compounds, in 50 nM Na–phosphate buffer (pH 6.8) for 10 min at 25 °C. Then the L-DOPA (0.5 mM) was added to the reaction mixture and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm (at 37 °C) of the formation of the DOPA chrome for 10 min.

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Spectral Data

3.3. Spectral data of the compounds

3.3.1. 3′-(5-Phenyl-1,3,4-oxadiazol-2-yl)pyridine (3a). Yield 92%; mp 112–114 °C; R_f = 0.34 (ethyl acetate–acetone, 9:1); UV (methanol): λ_{max} (log ε) 256 (2.32) nm^{-1}; IR (KBr) ν_{max}: 3073 (C–H), 1667 (C=N), 1557 (C=C), 1287 (C–O), 832, 659 (C–Br); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.21 (d, 1H, J = 1.1 Hz, H-2′), 9.13 (dd, 1H, $J_{6',5'}$ = 4.9 Hz, H-6′), 8.81 (br d, $J_{4',5'}$ = 8.3 Hz, 1H, H-4′), 8.59 (dd, 1H, $J_{5',6'}$ = 4.9, $J_{5',4'}$ = 8.3 Hz, H-5′), 7.63 (dd, 2H, $J_{2'',3''/6'',5''}$ = 7.8, $J_{2'',4''/6'',4''}$ = 2.3 Hz, H-2″/H-6″), 7.50 (t, 1H, J = 7.8 Hz, H-4″), 7.38 (t, 2H, J = 7.8, H-3″/5″); EI MS (m/z): 223 (M^+, 21), 145 (31), 106 (100), 77 (79), 78 (65), 68 (35), 51 (72). Anal. Calcd for $C_{13}H_9N_3O$: C, 9.95; H, 4.06; N, 18.82; O, 7.17. Found: C, 69.86; H, 3.97; N, 18.73; O, 7.08.

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Acknowledgement and References

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Thank you very much