Research Article

Tyrosinase Inhibitory Activity, 3D QSAR, and Molecular Docking Study of 2,5-Disubstituted-1,3,4-Oxadiazoles

Ramesh L. Sawant, Prashant D. Lanke, and Jyoti B. Wadekar

Department of Pharmaceutical Chemistry and PG Studies, College of Pharmacy Padmashri Dr. Vithalrao Vikhe Patil Foundation, PO MIDC, Vilad Ghat, Ahmednagar 414111, India

Correspondence should be addressed to Ramesh L. Sawant; sawantrl@yahoo.com

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In continuation with our research program, in search of potent enzyme tyrosinase inhibitor, a series of synthesized 2,5-disubstituted 1,3,4-oxadiazoles have been evaluated for enzyme tyrosinase inhibitory activity. Subsequently, 3D QSAR and docking studies were performed to find optimum structural requirements for potent enzyme tyrosinase inhibitor from this series. The synthesized 20 compounds of 2,5-disubstituted-1,3,4-oxadiazole series were screened for mushroom tyrosinase inhibitory activity at various concentrations by enzyme inhibition assay. The percentage enzyme inhibition was calculated by recording absorbance at 492 nm with microplate reader. 3D QSAR and docking studies were performed using VLife MDS 3.5 software. In the series 2,5-disubstituted-1,3,4-oxadiazoles enzyme tyrosinase inhibitory activity was found to be dose dependent with maximum activity for compounds **4c**, **4h**, **4m**, and **4r**. 3D QSAR and docking studies revealed that more electropositive and less bulky substituents if placed on 1,3,4-oxadiazole nucleus may result in better tyrosinase inhibitory activity in the series.

1. Introduction

Tyrosinase (E.C. 1.14.18.1), known as polyphenol oxidase (PPO), is a multifunctional, glycosylated, and copper-containing enzyme from the oxidase superfamily widely distributed in microorganisms, plants, and animals [1]. It is a nonessential amino acid made by the body that is a building block for several important neurotransmitters like epinephrine, norepinephrine, serotonin, and dopamine. It plays a key role in melanin biosynthesis catalyzing two divergent reactions: the hydroxylation of monophenols (cresolase or monophenolase activity) and the oxidation of *o*-diphenols (catecholase or diphenolase activity) into reactive *o*-quinones [2–4]. It is involved in human neuromelanin formation in the substantia nigra of the brain and dopamine neurotoxicity and contributes to Parkinson's disease-related neurodegeneration. In insects, tyrosinase is uniquely associated with different physiologically important biochemical processes, including sclerotization of the insect cuticle, defensive encapsulation and melanization of foreign organisms, and wound healing [5–10].

Tyrosinase inhibitors have become increasingly important in the field of medicine, agriculture, and cosmetics [11]. Presently, tyrosinase inhibitors have been a great concern due to its role in both mammalian melanogenesis and fruit or fungi enzymatic browning. Although melanin has photo-protective function in human skin, the accumulation of an abnormal amount of melanin in different specific parts of the skin resulting in more pigmented patches (melasma, freckles, ephelides, senile lentigines, etc.) might become an esthetic problem [12, 13]. In addition, enzymatic browning in fruit and fungi is undesirable, for example, fresh fruits, beverages, vegetables, and mushrooms which decrease the commercial value of the products [14]. These phenomena prompted us to design novel tyrosinase inhibitors with higher bioactivities that could be useful in skin whitening and antibrowning of foods.

In continuation with our earlier work, here we report enzyme tyrosinase inhibitory activity, three-dimensional quantitative structure activity relationship (3D QSAR) analysis, and molecular docking study with enzyme tyrosinase of

series of 2,5-disubstituted-1,3,4-oxadiazoles synthesized and reported by us [15].

2. Materials and Methods

2.1. Tyrosinase Inhibitory Activity. As an extension of our work, synthesized 20 analogues of 2,5-disubstituted-1,3,4-oxadiazole series (Table 1) were used to study their enzyme tyrosinase inhibitory activity. Tyrosinase inhibition activity was determined by the method described earlier [16]. The title compounds were dissolved in DMSO. The 96 well plate was prepared by applying 140 μ L of phosphate buffer (pH 6.8), 20 μ L of mushroom tyrosinase (48 units/mL), 20 μ L of sample, and 20 μ L of DL-DOPA (0.85 mM) to make the concentrations of 10, 20, 40, 80, and 100 μ g/mL. After incubation for 10 minutes, the enzyme activity was determined by measuring the absorbance at 492 nm using the microplate reader (Biotek Company, US). Kojic acid (1 mg/mL) was used as positive control. The percentage of tyrosinase inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100, \quad (1)$$

where, A and B are absorbance at 492 nm with and without test sample, respectively. The percentage tyrosinase inhibition at various concentrations of title compounds was calculated (Table 2), and logarithmic value of percentage inhibition at 100 μ g ($\text{Log } \%I_{100\mu\text{g}}$) was used as a dependant variable for the development of valid 3D-QSAR models.

2.2. Computational Details

2.2.1. Geometry Optimization. The 3D QSAR studies of 2,5-disubstituted-1,3,4-oxadiazole derivatives were carried out using VLife molecular design suite software version 3.5 (MDS 3.5) running on Pentium IV processor. Three-dimensional structures of title compounds were constructed and optimized their geometries to make the conformations with least potential energy using merck molecular force field (MMFF) and MMFF charge for the atom [17] followed by considering distance-dependent dielectric constant of 1.0 and convergence criteria (rms gradient) of 0.01 kcal/mol.

2.2.2. Alignment of Molecules. All molecules in the data set are aligned by template-based method where a template is built by considering common substructures in the series (Figure 1). Highly bioactive energetically stable conformation in the series is chosen as a reference molecule (Figure 2) on which other molecules are aligned (Figure 3).

2.2.3. Activity Prediction. The predictability of the QSAR model would be good if the values of biological activity predicted by the QSAR model do not appreciably differ from the observed results of biological activity for the given data set. Quality of selected models was further ascertained by r^2 , q^2 , and F test. The models were cross-validated by "leave one out" scheme and cross-validation correlation coefficient

TABLE 1: Structures of 2, 5-disubstituted-1,3,4-oxadiazoles.

Serial number	Compound	Structure
1.	4a
2.	4b
3.	4c
4.	4d
5.	4e
6.	4f
7.	4g
8.	4h
9.	4i
10.	4j
11.	4k
12.	4l

TABLE 1: Continued.

Serial number	Compound	Structure
13.	4m	
14.	4n	
15.	4o	
16.	4p	
17.	4q	
18.	4r	
19.	4s	
20.	4t	

q^2 was calculated. The model with high q^2 value is said to have high predictability.

2.2.4. 3D QSAR. Several 3D QSAR techniques such as comparative molecular field analysis (COMFA), comparative molecular similarity analysis (COMSIA), and k -nearest neighbor (k NN) [18] are being used in modern QSAR research. In the present study, molecular field analysis coupled with partial least squares (PLS) was applied to obtain a 3D QSAR model [19]. The calculated steric and electrostatic field descriptors were used as independent variables and $\log \%I_{100\mu g}$ values were used as dependent variables to derive the 3D QSAR models.

FIGURE 1: Template (ball and stick).

FIGURE 2: Reference molecule (compound 4j).

2.2.5. Docking. The crystal structure of mushroom tyrosinase (1wx2) was obtained from protein data bank and water molecules in the crystal structure were deleted. The optimized receptor was then saved as mol file and used for docking simulation.

2.2.6. Ligand Preparation. The 2D structures of the compounds were built and then converted into the 3D. The 3D structures were then energetically minimized up to the rms gradient of 0.01 using MMFF.

2.2.7. Identification of Cavities. By using cavity determination option of software, cavities of enzyme were determined. The cavities in the receptor were mapped to assign an appropriate active site. The basic feature used to map the cavities were the surface mapping of the receptor and identifying the geometric voids as well as scaling the void for its hydrophobic characteristics. Hence, all the cavities that are present in receptor are identified and ranked based on their size and hydrophobic surface area. Considering the dimensions and hydrophobic surface area, cavity-1 with

TABLE 2: Tyrosinase inhibition activity of title compounds.

Compound	Percentage inhibition of enzyme tyrosinase				
	10 μg	20 μg	40 μg	80 μg	100 μg
Kojicacid	7.86	15.22	28.95	42.52	54.01
4a	2.71	4.02	5.38	7.21	15.21
4b	2.61	3.62	7.51	10.12	8.91
4c	4.38	17.89	25.39	35.41	40.51
4d	4.91	15.67	23.77	32.18	36.13
4e	5.03	12.38	20.61	28.41	38.11
4f	2.71	5.54	7.81	10.02	12.02
4g	2.57	6.91	8.27	12.14	17.61
4h	6.57	18.37	28.16	34.59	39.71
4i	4.71	10.62	18.71	28.72	35.78
4j	3.68	15.12	21.91	27.83	33.81
4k	2.27	6.92	10.61	12.18	18.62
4l	3.11	12.62	20.68	25.51	32.56
4m	3.98	15.68	28.19	37.76	41.14
4n	2.51	10.16	12.72	16.22	22.16
4o	2.97	8.91	15.74	22.02	28.34
4p	4.18	15.71	17.81	22.61	31.57
4q	3.38	8.62	20.63	26.51	34.62
4r	4.61	15.76	28.63	36.44	47.92
4s	4.01	11.88	20.81	26.61	31.54
4t	4.62	10.81	15.42	20.98	33.02

TABLE 3: Selected QSAR model.

Model number	Equation	n	r^2	q^2	F test
1.	$\text{Log } \%I_{100\mu\text{g}} = -0.0005 - 147.0670 (\pm 10.4994) S_{793} + 0.0183 (\pm 0.0028) S_{757} + 0.0339 (\pm 0.0074) E_{550}$	16	0.8920	0.8187	33.0329

volume $219.531250 \text{ \AA}^3$ and surface area $340.284790 \text{ \AA}^2$ was found to be the best void as an active site.

2.2.8. Scoring Function. Distinction of good or bad docked conformation is based on scoring or fitness function. MDS uses fitness functions on only electrostatic and both steric and electrostatic interactions between receptor ligand as well as dock score scoring function. The dock score compute binding affinity of a given protein-ligand complex with known 3D structure.

3. Results and Discussion

3.1. Tyrosinase Inhibition Activity. The percentage inhibition of enzyme tyrosinase by compounds **4a–4t** is estimated at concentrations of 10, 20, 40, 80, and 100 μg and observed to be dose dependent. The compounds **4c**, **4h**, **4m**, and **4r**

FIGURE 3: Aligned molecule (stick model).

shows maximum where compounds **4b**, **4f**, **4g**, and **4k** shows minimum inhibition of enzyme tyrosinase from the series.

3.2. 3D QSAR. The model 1 describes the optimum structural feature for the tyrosinase inhibition activity as shown in Table 3. The training set of 16 molecules and test set of 4 molecules were used as described earlier. S_{793} , S_{757} , E_{550} are the steric and electrostatic field energy of interactions between probe (CH_3) and compounds at their

TABLE 4: Selected descriptors, actual and predicted activity of tyrosinase inhibitors.

Compound	Descriptors			Activity		
	S_793	S_757	E_550	Actual	Predicted	Residue
**4a	-0.00631	17.92055	2.079088	1.182129	1.325233	-0.1431
4b	-0.00557	10.99283	-0.7936	0.949878	0.992882	-0.043
4c	-0.00743	20.20807	2.151223	1.607562	1.533583	0.073979
4d	-0.00743	20.20807	2.151223	1.557868	1.533583	0.024285
4e	-0.00651	30	1.927336	1.581039	1.569836	0.011203
4f	-0.00574	19.12609	-1.80536	1.079904	1.131727	-0.05182
4g	-0.00629	18.09468	3.132391	1.245759	1.361143	-0.11538
**4h	-0.00574	10.46793	-0.80766	1.5989	1.007527	0.591373
**4i	-0.00744	19.83572	4.267113	1.55364	1.600056	-0.04642
4j	-0.00744	19.83572	4.267113	1.529045	1.600056	-0.07101
4k	-0.00654	30	-5.24257	1.26998	1.331729	-0.06175
4l	-0.0076	30	-5.36661	1.512684	1.483861	0.028823
4m	-0.00607	30	2.570345	1.614264	1.527344	0.08692
4n	-0.0069	6.383675	3.156899	1.34557	1.23779	0.10778
**4o	-0.00772	17.78855	1.660719	1.4524	1.516313	-0.06391
4p	-0.00772	17.78855	1.660719	1.499275	1.516313	-0.01704
4q	-0.00673	30	1.170805	1.539327	1.577306	-0.03798
4r	-0.00787	30	1.006801	1.680517	1.73882	-0.0583
4s	-0.00637	30	-2.43434	1.852554	1.805904	0.097768
4t	-0.00694	30	-2.22493	1.782629	1.694316	0.025586

** Test set compounds.

TABLE 5: Correlation matrix of $\log \%I_{100\mu\text{g}}$ with descriptor for model 1.

	$\log \%I_{100\mu\text{g}}$	E_550	S_757	S_793
$\log \%I_{100\mu\text{g}}$	1.000000			
E_550	0.256360	1.000000		
S_757	0.403622	-0.320018	1.000000	
S_793	-0.46893	-0.372145	-0.066491	1.000000

FIGURE 4: 3D QSAR model spatial grid points.

corresponding spatial grid points of 793, 757, and 550 as shown in Figure 4. Low residual value observed in case of training as well as test set (Table 4 and Figure 5) confirms reliability of the QSAR model 1. The selected descriptors have good correlation with biological activity and a low intercorrelation among them depicted in Table 5. As per model 1, steric descriptor S_793 with negative coefficients represent less bulky substituent is favourable; S_757 with positive coefficients represent more bulky substituent is favourable at 2, 5-disubstituted oxadiazole nucleus while electrostatic field descriptor E_550 with positive coefficient indicate electropositive groups is favourable for activity. Compound 4r in the dataset bearing less bulky and electropositive chloro(hydroxy)phenyl rings on oxadiazole nucleus is most active, whereas less electropositive substituted oxadiazole nucleus in compound 4b is least active which indicates that developed 3D QSAR model satisfies model requirements.

TABLE 6: Grid docking score of title compounds.

Sr. no.	Compound	Docking score (Kcal/mol)
1	4a	-4.8032
2	4b	-4.9936
3	4c	-5.2345
4	4d	-5.3241
5	4e	-5.1800
6	4f	-4.7232
7	4g	-4.8539
8	4h	-5.2711
9	4i	-5.3660
10	4j	-5.2453
11	4k	-5.3106
12	4l	-5.4441
13	4m	-5.0124
14	4n	-4.9321
15	4o	-5.4969
16	4p	-5.4867
17	4q	-5.3207
18	4r	-5.8169
19	4s	-5.1214
20	4t	-5.1318

FIGURE 5: Plot of predicted against observed tyrosinase inhibition activity (model 1).

3.3. Docking. Docking studies of the title compounds with enzyme tyrosinase yielded docking score ranging from -5.8169 to -4.7232 (Table 6) indicating stable enzyme-substrate interactions. The compound **4r** with best docking score (-5.8169) has shown highest inhibition of enzyme tyrosinase in the dataset. The compounds interact with enzyme tyrosinase by binding with SER142, SER146, GLY145, and GLY198 amino acid residues as shown in Figure 6. The tyrosinase inhibitory assay and molecular docking study reveals that 2,5-disubstituted-1,3,4-oxadiazoles inhibits enzyme tyrosinase may be because of its interaction with SER142, SER146, GLY145, and GLY198 amino acid residues.

FIGURE 6: Interaction of compound 4r with mushroom tyrosinase.

4. Conclusion

It can be concluded from the whole study that electropositivity is crucial for the inhibition of enzyme tyrosinase by 2,5-disubstituted-1,3,4-oxadiazoles. It would be worthwhile to synthesize a novel 1,3,4-oxadiazole analogues with less bulky and more electropositivity group as a better enzyme tyrosinase inhibitors.

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