

Tyrosinase inhibition: Conformational analysis based studies on molecular dynamics calculations of biperidine based inhibitors

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Abstract

Two series of variably *N*-substituted biperidines were synthesized by condensing various acid chlorides, alkyl halides and anhydrides with 1,4-biperidine. The new compounds were tested as tyrosinase inhibitors and a structure–activity relationship (SAR) study was carried out. Potent inhibition was observed in the case of the 4'-methylbenzyl substitution on this atom ($IC_{50} = 1.72 \mu M$) with this compound being a lead for future drug design. Additionally, calculations of the important QSAR molecular descriptors were done on the biperidine analogues after their 2 ps molecular dynamics (MD) simulations using molecular mechanics force field (MMFF) approaches. Using MD simulations potential and total energies were calculated for the energy minimized models of biperidine and the most active analogs **2**, **3**, **4**, **6**, **8** and **10**.

Keywords: *Biperidine, tyrosinase, melanin, hyperpigmentation, energy minimization, conformation, molecular dynamics, inhibition*

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 [1]. It catalyzes the *o*-hydroxylation of monophenols as well as the oxidation of *o*-diphenols to *o*-quinones. Tyrosinase is known to be a key enzyme for melanin biosynthesis in plants and animals. Tyrosinase inhibitors are clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation and also important in cosmetics for whitening and depigmentation after sunburn. Hyperpigmentation is associated with increased plasma melanocyte-stimulating hormone activity in peoples with insufficient production of glucocorticoids (Addison's disease). Recently, a number of inhibitors from natural products

have been used in cosmetics [1]. Tyrosinase plays a leading role in various biological and chemical processes [2,3]. So, there is a need to develop tyrosinase inhibitors to cure various skin diseases.

In continuation of our research work on bioactive molecules [4,5] we have synthesized a series of biperidine derivatives **2–12** and found them to be an active class of tyrosinase inhibitors which may provide lead compounds for future drug development.

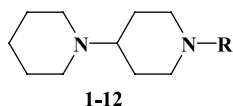
Materials and methods

General

Melting points were determined on a Büchi 434 melting point apparatus and are uncorrected. NMR

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Table I. Physical and tyrosinase inhibitory data for compounds 1–12.



Compounds	R	Physical Data	Yield (%)	IC ₅₀ (μM) ± S.E.M. ^a
1	H–	–	–	110.79 ± 0.05837
2		Yellow powder; M.P: 64–65°C; R _f : 0.15 (Acetic acid:Ethanol, 3:7)	67	29.94 ± 0.42889
3		Yellow powder; M.P: 64–65°C; R _f : 0.15 (Acetic acid:Ethanol, 3:7)	67	6.64 ± 0.35293
4		White solid; M.P: 265–266°C; R _f : 0.12 (Acetic acid:Ethanol, 3:7)	74	1.72 ± 0.04376
5		White powder; M.P: 253–255°C; R _f : 0.40 (Acetic acid:Ethanol, 3:7)	88	NA ^c
6		Yellow crystal; M.P: 85–86°C; R _f : 0.38 (Acetic acid:Ethanol, 3:7)	94	18.08 ± 0.34941
7		Reddish powder; M.P: 75–77°C; R _f : 0.42 (Acetic acid:Ethanol, 3:7)	97	NA ^c
8		White powder; M.P: 236–238°C; R _f : 0.44 (Acetic acid:Ethanol, 3:7)	80	8.76 ± 0.1186
9		Green crystal; M.P: 121–123° C; R _f : 0.34 (Acetic acid:Ethanol, 3:7)	90	NA ^c
10		Reddish powder; M.P: 91–92°C; R _f : 0.12 (Acetic acid:Ethanol, 3:7)	92	19.52 ± 0.00027
11		Liquid; R _f : 0.18 (Acetic acid:Ethanol, 3:7)	80	NA ^c
12		White crystal; M.P: 269–270°C; R _f : 0.14 (Acetic acid:Ethanol, 3:7)	91	NA ^c
Kojic acid ^b	–	–	–	16.67 ± 0.5190
L-Mimosine ^b	–	–	–	3.68 ± 0.02234

Notes: ^a standard error of the mean; ^b standard inhibitors; ^c inactive

spectroscopy was performed on a Bruker AVANCE 400 MHz. Elemental analysis was carried out on a Carlo Erba Strumentazione-Mod-1106 Italy. UV and IR spectra were recorded on Perkin-Elmer Lambda-5 UV/VIS and JASCO IR-A-302 spectrophotometers, respectively. A FINNIGAN MAT-311A Germany spectrometer was used to record mass spectra. Thin layer chromatography (TLC) was performed on pre-coated silica gel glass plates (Kieselgel 60, 254, E. Merck, Germany) and chromatograms were visualized by UV light at 254 and 365 nm or iodine vapors.

General procedure for the preparation of compounds 2–12

Various anhydrides, alkyl halides, aromatic and aliphatic acid halides (1.1 mmol) dissolved in dry THF (10 mL) was added drop wise to 1,4-bipiperidine (0.5 mmol) in dry THF (25 mL) and the resultant reaction mixture was stirred for 48 h under argon at ambient temperature. The reaction was monitored by TLC analysis and upon completion; the reaction mixture was evaporated under reduced pressure on a rotary evaporator. The resultant product, if solid, was washed with excess of dry THF and recrystallized with an appropriate solvent or in the case of liquids, distilled under reduced pressure. The compounds were characterized using different spectroscopic techniques including UV, IR, NMR and mass and elemental analysis.

Tyrosinase inhibition assay

Tyrosinase inhibition assays were performed in a 96-well microplate format using SpectraMax[®] 340 (Molecular Devices, CA, USA) microplate reader according to the method developed earlier [6].

Initially 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 to IC₅₀ studies. Briefly, the compounds were dissolved in DMSO to a

concentration of 2.5%. Thirty Units mushroom tyrosinase (28 nM) was first preincubated with the compounds, in 50 nM Na-phosphate buffer (pH 6.8) for 10 min at 25°C. Then 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 of the formation of the DOPACHrome for 10 min, at 37°C. The percent inhibition of the enzyme and IC₅₀ values of the active compounds were calculated using a program developed with Java and Macro Excel[®] 2000 (Microsoft Corp., USA) for this purpose. The following equation was used:

$$\text{Percent inhibition} = \frac{[\text{ABS}_{\text{Blank}} - \text{ABS}_{\text{sample}}]}{\text{ABS}_{\text{Blank}}} \times 100$$

Where ABS_{Blank} and ABS_{Sample} are the absorbances for the blank and samples, respectively. All the studies were done at least in triplicates and the results here represents the Mean ± S.E.M. (standard error of the mean). All the reagents, enzyme, substrate and reference compounds, were purchased from Sigma Chem Co., MO, USA.

Molecular modeling studies

Energy minimization. The 2D structures of the molecules were drawn with CS ChemDraw[®] Pro version 6.0 (CambridgeSoft Corporation), which were further changed into 3D forms and hydrogen atoms were added at the 3D structures with the help of HyperChem[®] Professional version 7.1 (HyperCube Inc., Gainesville, FL, USA).

Energy minimization experiments were carried out with HyperChem[®] using the Block-Diagonal Newton-Raphson algorithm at the RMS (root mean square) gradients of 0.1 Kcal/(Å mol) for different cycles *in vacuo* depending on the specific molecule. For rendering and refinement of the 3D structures of the molecules the Persistence of Vision (tm) Ray-Tracer (Pov-Ray[®]) MS windows XP[®] version was used [7,8].

Table II. Calculations of the molecular descriptors of the bipiperidine molecules.

Compounds	Grid surface area (Å ²)	Volume (Å ³)	Hydration energy (kcal/mol)	Log P	Polarizability (in Å ³)	Mass (in amu)
1	213.584	322.880	-0.692	1.106	15.214	149.131
2	236.807	370.302	-1.126	0.819	16.748	188.145
3	292.745	503.514	-0.526	1.192	29.785	296.244
4	277.111	461.456	-0.051	1.695	23.946	244.211
5	279.385	466.007	-0.619	0.966	24.584	260.211
6	242.374	377.607	-0.864	1.882	18.697	245.140
7	335.905	582.029	-0.384	2.634	32.144	332.277
8	281.234	467.202	-3.756	-0.244	25.400	314.254
9	320.741	544.520	-0.929	-1.050	26.633	332.231
10	309.903	515.303	-0.234	1.808	27.080	272.222
11	276.084	437.941	-1.697	1.689	19.951	228.166
12	267.547	439.741	-0.671	1.039	24.262	250.196

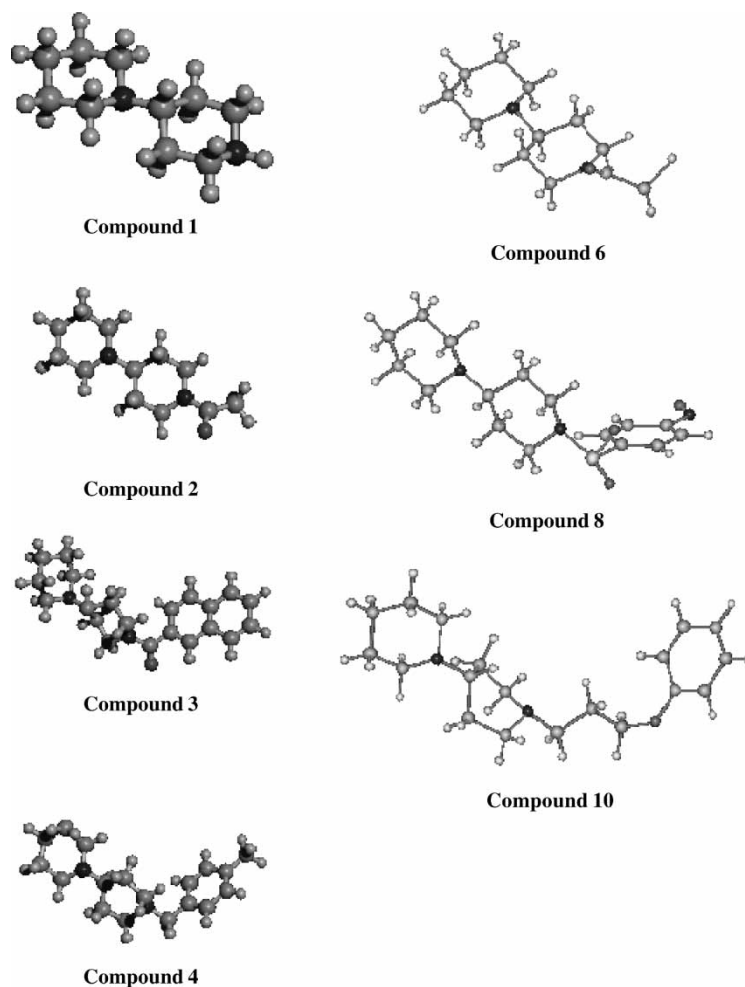


Figure 1. Energy minimized 3D models of the bipiperidine and its most active analogs.

Molecular dynamics (MD) simulations. The molecular dynamics (MM+, molecular mechanics force field, MMFF and Amber 94) simulation experiments were done using HyperChem[®] Professional version 7.1 (HyperCube Inc., Gainesville, FL, USA) on a Pentium[™] IV single processor computer using OS MS Windows XP[®] service pack 2 [4,5]. Electrostatic bond dipoles were taken for the option of MM+ simulations. In these experiments total run time was fixed for 1.0, heating and cooling times were 0.5 and step size was 0.001 pico second, respectively. The simulations were run *in vacuo* at temperatures of 300°K. Initial and final temperature was 0°K and the temperature step was 20°K. For the force field analysis, bond angle, torsion, non-bonded, electrostatic, and hydrogen bonded aspects were taken as the components for the MM+ simulations [7–9]. For each of the molecule the approximate CPU time for 2 ps MD simulation was about 4 h.

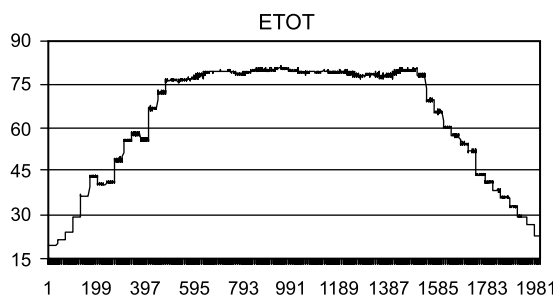
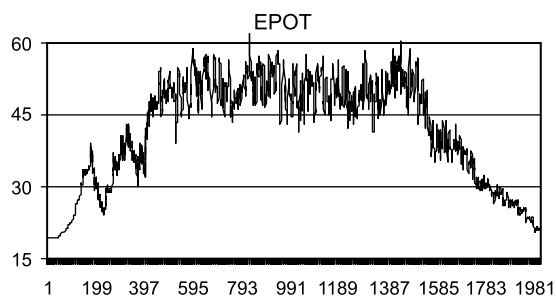
Calculation of molecular descriptors

Some molecular descriptors were calculated such as, net charge (not shown in Table II, as the values for all

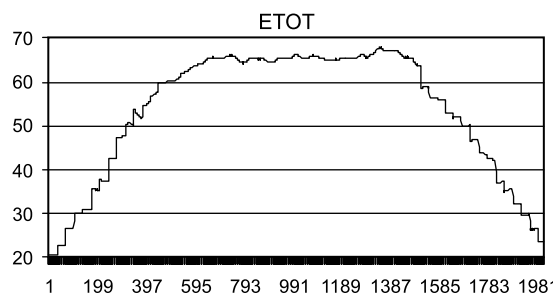
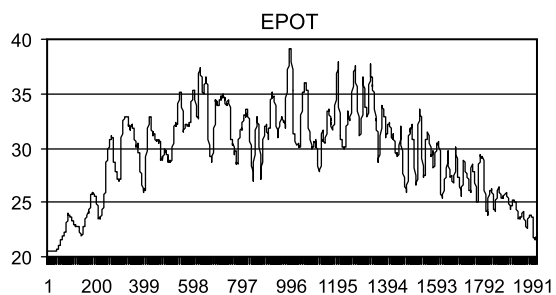
the compounds were calculated to be '0'), approximate and grid surface area, volume, hydration energy, log *P*, polarizability, refractivity and molecular mass using the QSAR module integrated with the program HyperChem[®] Professional version 7.1 (HyperCube Inc., Gainesville, FL, USA) on a Pentium[™] IV single processor computer using OS MS Windows XP[®] service pack 2 [4,5,7–9].

Results and discussion

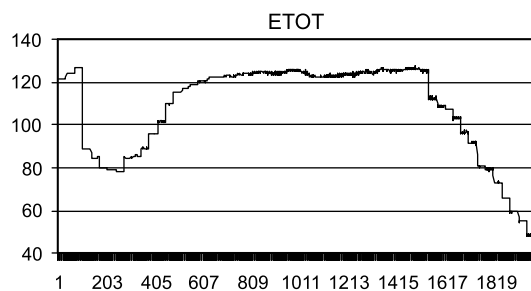
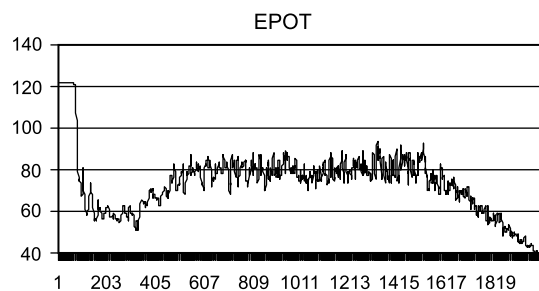
Tyrosinase inhibition studies were conducted on eleven *N*-substituted synthetic bipiperidine compounds 2–12 (Table I) were conducted and it was found that inhibitory activity changed with the substituent. The unsubstituted parent molecule 1 exhibited weak inhibition against the enzyme tyrosinase ($IC_{50} = 110.79 \mu\text{M}$), whereas, *N*-acetylation enhanced the inhibition ($IC_{50} = 29.94 \mu\text{M}$) as in compound 2, while the 2-naphthoyl derivative 3 also exhibited inhibitory properties ($IC_{50} = 6.64 \mu\text{M}$). 4'-Methylbenzoylation at the nitrogen as in compound 4 greatly increased activity ($IC_{50} = 1.72 \mu\text{M}$) and made this molecule the most active compound in the series.



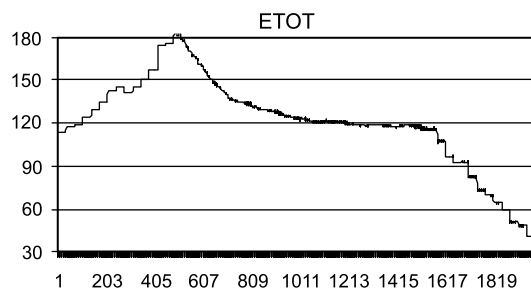
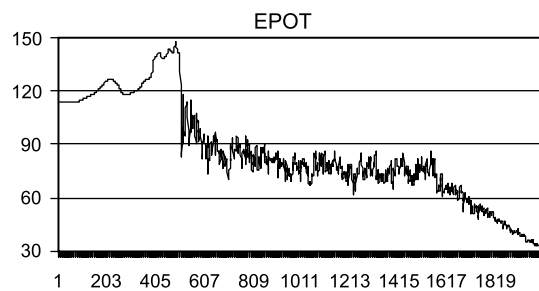
Compound 1



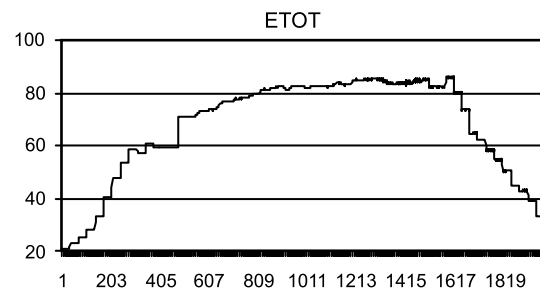
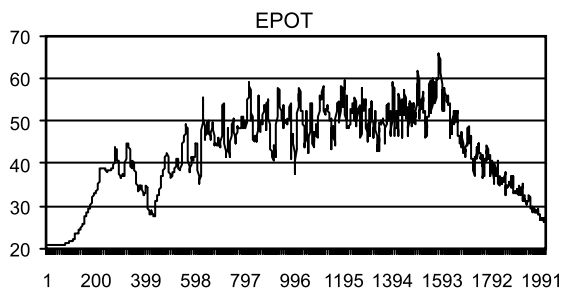
Compound 2



Compound 3



Compound 4



Compound 6

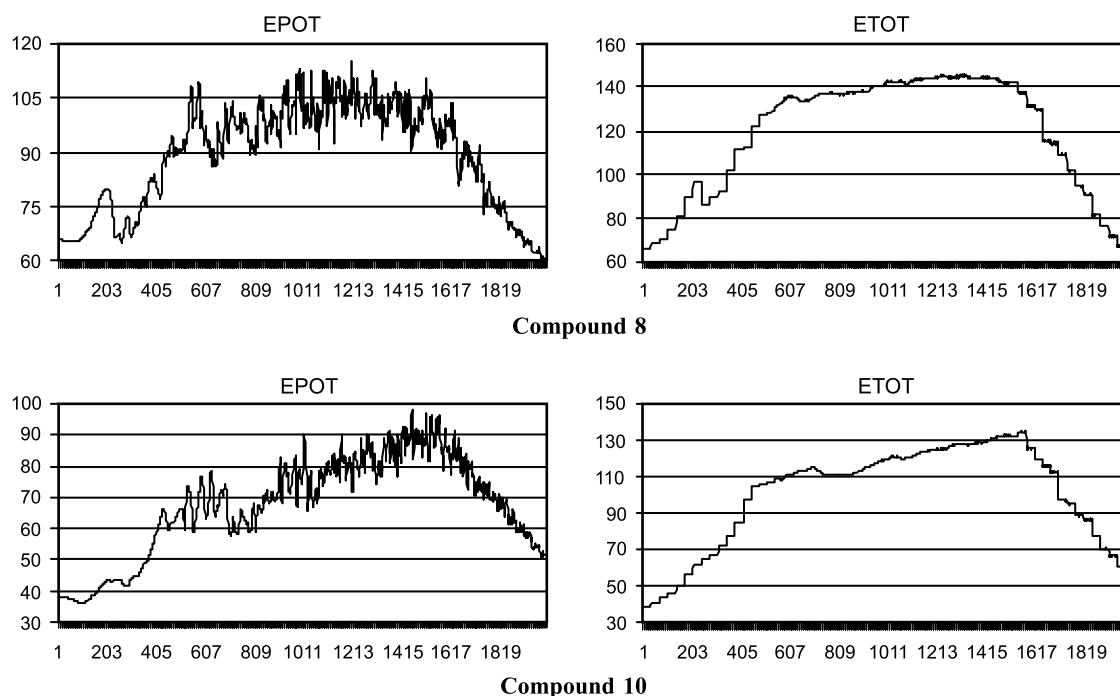


Figure 2. Energy [Potential (EPOT) and Total (ETOT)] calculations plots [time (x, in ps) vs energy (y, kcal/mol)] of Molecular Dynamics (MD) of compounds 1, 2, 3, 4, 6, 8 and 10, respectively.

The trifluoroacetylated derivative **6** exhibited moderate inhibition ($IC_{50} = 18.08 \mu M$), while, 4'-nitrobenzenesulfonylation and 3-phenoxypropylation at the *N*-atom (compounds **8** and **10**) led to moderate activity with $IC_{50} = 8.76 \mu M$, $IC_{50} = 19.52 \mu M$, respectively, whilst, benzoyl, myristoyl, 3',4',5'-trimethoxybenzoyl, methyl-3-propanoate and nicotinoyl substitution at the same position, as in compounds **5**, **7**, **9**, **11** and **12**, respectively, led to a remarkable decline in activity with completely inactivity. We have selected only eleven compounds to include in this communication for comparison purposes; other screened compounds were found to be inactive.

The tyrosinase inhibitory activities of these compounds were tested in reactions using L-DOPA as a substrate with reference to Kojic acid ($IC_{50} = 16.67 \mu M$) and L-mimosine ($IC_{50} = 3.68 \mu M$) (Table I). The Present study suggests that compound **4** might be a lead compound for further drug development for the management of several skin disorders.

Another purpose of this study was to identify the biologically active stable conformers of the biperidine analogues and to calculate the volume and several other very important molecular descriptors such as net charge, approximate and grid surface area, hydration energy, $\log P$, refractivity, polarizability and mass. Compound **4** exhibited excellent tyrosinase inhibitory activity ($IC_{50} = 1.72 \mu M$) when compared with the standard tyrosinase inhibitors kojic acid ($IC_{50} = 16.67 \mu M$) and L-mimosine ($IC_{50} = 3.68 \mu M$). In the energy minimized 3D model of the molecule, it showed 720 geometrical conformations at the RMS gradient of

0.1 kcal/(\AA^3 mol) and the final energy minimized structures are shown in Figure 1. Its approximate surface area was calculated as 277.111\AA^2 , volume as 461.46\AA^3 , and the hydration energy and $\log P$ were -0.051 kcal/mol and 1.695, respectively (Table II). Here, $\log P$ indicates the lipid solubility of the molecule [7]. From the molecular dynamic (MD) simulation experiments, kinetic, potential and total energy was calculated as presented in Figure 2. The highest kinetic energy calculated at the 514th step after 0.512 ps of simulation time was 96.54 kcal/mol. The highest potential energy calculated at the 494th step after 0.472 ps was 147.39 kcal/mol and the highest total energy calculated at the 510th step after 0.508 ps was 182.08 kcal/mol. The whole MD simulations were done with 2001 steps at 2.0 ps.

Acknowledgements

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