Supporting Information

Conjugated dienones from differently substituted cinnamaldehyde as highly potent monoamine oxidase-B inhibitors: Synthesis, biochemistry, and computational chemistry

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1. $^1$H-NMR, $^{13}$C-NMR and Mass Spectra of MK1 to MK15 (S3 – S32)

2. Computational Methodology (S33- S37)

3. REFERENCES (S38-S40)
MK1
1H_Scan CDCl3 [D:\Spectra] nmr 28
C13CFD CDC13 (D:\Spectra) nmr 28

BRUKER
AVANCE NEO
500 MHz NMR SPECTROMETER
SAIF, PANJAB UNIVERSITY,
CHANDIGARH

Current Data Parameters
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PROCNO  1
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DE  6.500 usec
TE  500.2 E
D1  2.000000000 sec
D11  0.000000000 sec
d2  1
SP1  125.7804233 MHz
MAX1  150
P0  3.33 usec
P1  10.00 usec
PM1  85.14059884 W
SP2  500.1220700 MHz
PM2  0.16490000 W
CFSBRG[2]  500.5058345 MHz
PD1  80.00 usec
PM3  20.79000031 W
PM4  0.32705000 W
PM3  0.16449000 W
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SF  125.7804285 MHz
MOD  0
SSS  0
LB  1.000 Hz
SB  1.40
MR5
Sage 65 TE:300MHz Bruker 480M Bruker 5Tm130

MR6
1H_8scan CDC13 (D:\Spectra) nmr 33

BRUKER
AVANCE NEO
500 MHz NMR
SPECTROMETER
SAIP, P.O.

Current Data Parameters
NAME August 2021
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PROCNO 1

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PROTOSE Z1949_B_0331
FULLPROG eg30
TD 65384
SOLVENT 1 H
NS 16
DS 9
SWH 14705.88 Hz
FIDRES 3.448786 Hz
AQ 2.2282240 sec
BD 100
DW 34000 ussec
DE 4.7 ussec
tE 300.1 K
DI 1.00000000 sec
TD1 500.173088 Hz
NHC1 16
tD 3.33 ussec
Pt 10.00 ussec
P121 20-3500000 W

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NDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

S14
MK7
C13CFD CDCl3 (D:\Spectra) nmr 34

BRUKER
AVANCE NEO
500 MHz NMR SPECTROMETER
SAIF, PANJAB UNIVERSITY, CHANDIGARH

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FIDRES 1.33333 Hz
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DG 100
DM 13.500 usec
DG 6.500 usec
TE 300.2 E
D1 2.00000000 sec
DG 0.03000000 sec
t0s 1
SPG1 125.780423 MHz
MAX1 13C
FG 3.33 usec
F1 10.00 usec
PLMR 85.14096084 W
SPG2 500.172900 MHz
MAX2 12.500000 W
CFW1 0.03000000
PLM2 20.35000000 W
PLM3 0.15449000 W
F2 - Processing parameters
S1 12756
SF 125.767544 MHz
NWM 0
SSB 0
LAB 1.40 Hz
S 1.40

MK7
13C-ACETIC Acid[13C] 2,2,2-DCH 2.0 Hz
C13CPD CDC13 (D:\Spectra) nmr 35
MK9
1H_Scan CDCl3 {D:\Spectra} nmr 36

MK9
1H_Scan CDCl3 {D:\Spectra} nmr 36

S20
MK12
1H_8scan CDCl3 {D:\Spectra} nmr 39

S26
MK15
1H_Scan CDCl3 {D:\Spectra} nmr 42

MK15
C13CPD CDCl3 {D:\Spectra} nmr 42

BRUKER
AVANCE NEO
500 MHz NMR SPECTROMETER
SAIF, PANJAB UNIVERSITY, CHANDIGARH

Current Date Parameters
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PROCNO 1

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NS 512
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FIDRES 1.350281 Hz
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RG 101
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DE 4.50 usec
DE2 0.000 usec
TE 0.000000 sec
D1 0.00000000 sec
D1L 0.00000000 sec
D0 125.780435 MHz
SP01 125.780435 MHz
AVC1 13C
PO 3.33 usec
F1 10.00 usec
PM1 3.1403848 W
SP02 500.172007 MHz
MDC2 12
CPDURG(2) walls85
PCHC 0.000000 W
PM2 20.97000031 W
PM1 0.32703000 W
PM1 0.08490000 W

F2 - Processing parameters
S1 0.5
S2 0.5
S3 125.780435 MHz
SMW NW SMS SB 1.0 Hz 1.40

S31
2. Computational Methodology

COMPUTATIONAL METHODOLOGY

Enzyme System preparation

The X-ray crystal structure of MAO-B was retrieved from RSCB Protein Data Bank\(^1\) with identification code 6RKB as shown in Figure S1\(^2\). The retrieved X-ray crystal structure was complexed with a styrylpiperidine analogue 1 and consisted of two chains (A and B) forming a homodimer. The complexed styrylpiperidine analogue 1 allowed for the identification of the inhibitor binding site of MAO-B. Chain B, water molecules, any non-standard residues were removed using UCSF Chimera\(^3\) to reduce computational cost. However, considering the cruciality of flavin adenine dinucleotide (FAD) cofactor in the function of MAO-B, it was retained in the prepared models. Hydrogen atoms were then added to the X-ray crystal structure saved for molecular docking. However, in preparing MAO-B for molecular dynamics (MD) simulations, hydrogen atoms were removed and the structure saved in pdb format.
**Figure S1:** X-ray crystal structure of MAO-B complex complexed with a styrylpiperidine analogue 1 and flavin adenine dinucleotide (PDB code: 6RKB)

**Ligand Preparation**

The two-dimensional (2D) structures of the synthesized compounds MK5, MK6, MK12, and MK14 were initially constructed using Marvin Sketch. The constructed 2D structures were then uploaded onto Avogadro software to generate the three-dimensional (3D) coordinates of each of the compounds. Afterward, the Avogadro software was also employed to perform energy minimization and optimization of each of the compounds. Final preparation of each of the compounds before molecular docking was carried out on UCSF Chimera, whereby hydrogen atoms and corresponding Gasteiger charges were added.

In preparing the compounds for molecular dynamics (MD) simulations, hydrogens atoms and corresponding AMBCC charges were added to each compound.

**Molecular docking**

AutoDock Vina was employed for the molecular docking of MK5, MK6, MK12, and MK14 into the inhibitor binding site of MAO-B. A grid box with coordinates $X=51.36$, $Y=154.89$, $Z=29.71$ for centre and $x$, $y$, and $z$ dimensions of 23.01, 22.86, and 26.04 for the size were used. Generated docking results...
were saved in pdbqt format. At the same time, the optimal geometric position with the best pose and energy score was selected for each compound and saved in a complex form with reference to MAO-B. The resultant complexes from the dockings were then prepared using UCSF Chimera and Molegro Molecular Viewer\(^7\) for Molecular Dynamics (MD) simulation. Intermolecular interactions were visualized using Discovery studio visualizer\(^8\).

**Molecular dynamics (MD) simulations**

MD simulation was performed using the GPU version of AMBER18\(^9\) with an integrated PMEMD module\(^10\), according to standard MD simulation protocols, which have been employed extensively in our previous reports\(^10\)–\(^14\). The compounds were parameterized using the ANTECHAMBER module\(^15\) of AMBER18, in which its atomic partial charges were generated. Parameterization with the ANTECHAMBER module was performed using the restrained electrostatic potential (RESP) and the General AMBER force field (GAFF) protocol\(^15\). The compounds were subsequently parameterized using the FF19SB AMBER force field\(^16\). Protonation of histidine residues was then performed using the pdb4amber files at a constant pH to prepare the enzyme structures for the LEAP process that followed. The LEAP module integrated with AMBER18 was then employed for neutralization of the entire system and for the addition of hydrogen atoms. Neutralization was carried out by adding an equivalent number of any of the counter ions, Na\(^+\), Cl\(^-\). Afterward, the topology and parameter files of the compounds, MAO-B, and complexes of MAO-B with each of compounds were then saved. The LEAP module also allowed for the addition of water molecules with a TIP3P orthorhombic box size of 8 \(\text{Å}\) to explicitly solvate each simulated system\(^17\),\(^18\). Prepared systems were then taken through a two-phase minimization process in which the initial minimization of 2000 steps was performed at 500kcal/mol restraint potential. The second minimization, referred to as a full minimization, involved 1000 steps at a steepest descent with no restraint. This was followed by the gradual thermalization of the systems with a temperature range of 0-300 K for 50 ps, after which each system was equilibrated for 500ps
while the temperature and pressure were kept constant at 300K and 1bar respectively using the Berendsen barostat. This was followed by MD production runs of 200 ns for each system, during which the SHAKE algorithm was used to constrict all atomic hydrogen bonds. The process of MD simulation involved a 1 fs time step initiation process while at 1 ps, coordinates of simulated files were saved. All trajectories generated over the simulation were subsequently analyzed using the PTRAJ and CPPTRAJ modules of AMBER18. Plots to interpret findings from the generated MD trajectories were also created using Microcal Origin, while UCSF Chimera was used to visualize and create relevant images. The trajectories were analyzed for root mean square deviation (RMSD), root mean square fluctuation (RMSF), surface accessible surface area (SASA), and binding free energy between the compounds and MAO-B. The binding free energy was estimated using the Molecular mechanics Poisson–Boltzmann surface area (MM/PBSA).

**Bind free energy calculations**

The MM/PBSA method was used to calculate binding free energy. The binding free energy \( \Delta G_{\text{bind}} \) was calculated from the following equations:

\[
\begin{align*}
\Delta G_{\text{bind}} & = G_{\text{complex}} - G_{\text{receptor}} - G_{\text{ligand}} \quad (1) \\
\Delta G_{\text{bind}} & = \Delta G_{\text{gas}} + \Delta G_{\text{sol}} - T \Delta S, \quad (2)
\end{align*}
\]

Where \( \Delta G_{\text{bind}} \) is considered to be the summation of the gas phase and solvation energy terms less the entropy \( (T \Delta S) \) term

\[
\Delta E_{\text{gas}} = \Delta E_{\text{int}} + \Delta E_{\text{vdw}} + \Delta E_{\text{elec}} \quad (3)
\]

\( \Delta E_{\text{gas}} \) is the sum of the AMBER force field internal energy terms \( \Delta E_{\text{int}} \) (bond, angle, and torsion), the covalent van der Waals \( \Delta E_{\text{vdw}} \) and the non-bonded electrostatic energy component \( \Delta E_{\text{elec}} \). The solvation energy is calculated from the following equation:

\[
G_{\text{sol}} = G_{\text{GB}} + G_{\text{non-polar}} \quad (4)
\]
The polar solvation contribution is represented as $G_{GB}$ and while $G_{\text{non-polar}}$ is the non-polar solvation contribution. $G_{\text{non-polar}}$ is calculated from the solvent assessable surface area (SASA), obtained by means of a 1.4 Å water probe radius. The surface tension constant ($c$) was set to 0.0072 kcal/mol and $b$ to 0 kcal/mol.  

3. REFERENCES

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