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Computational studies on the structural variations of MAO-A and MAO-B inhibitors - An *in silico* docking approach

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The neurological disorder is a concerning problem in the present social scenario. The malfunction of the monoamine oxidase (MAO) enzyme is the responsible factor behind this disorder because this enzyme regulates the metabolism of monoamine neurotransmitters. This work aimed to design and propose the best MAO inhibitors through extensive computational analysis so that the favourable drug-like molecules could be identified for future synthesis. The drugs selected in this study were three MAO-A inhibitors namely Moclobemide, Tolxatone and Brofaromine and two MAO-B inhibitors namely Selegiline and Rasagiline. By substituting hydrophilic and hydrophobic groups at the specified positions, structural variations were designed for each drug. The designed variations and their parent drugs were optimized (basis set is B3LYP/6-311G(d, p)) and the optimized structures were docked to the target using PyRx software. The binding energy of each variation was compared to that of parent drug. The drug-likeness, physicochemical properties (solubility, polarity, flexibility, gastrointestinal absorption, saturation *etc*.) and toxicity of the lower binding energy variations were analysed using the swissADME, Osiris property explorer and ProTox-II servers. The interacting residues of the enzymes were obtained from the LigPlot^{+} program. The safe and low binding energy variations with favourable drug properties are suggested for further drug research.

Keywords: Binding energy, Drug-likeness, Monoamine oxidase, Neurotransmitters, Optimization

Neurological disease is a disorder occurring in the nervous system. Millions and millions of people all over the world are the victims of neurological disorders like Epilepsy, Parkinson's disease, Alzheimer's disease *etc*. The studies held in the area of neurological disorders point out the fact that in India around 33 million suffer from neurological diseases and the chance of its occurrence is twice in rural areas[1]. Such disorders can affect the quality of life leading to lack of ability, economic loss, lack of social involvement *etc*.

Monoamines are the neuromodulators and the chemicals that allow the neurotransmission. The functions of monoamine neurotransmitters involve controlling sleep, moods, memory, learning, behaviour, motivation, dreams *etc*. Thus these neurotransmitters can be linked to depression-like mood disorders. Monoamine oxidase (MAO) is a collection of enzymes which hold the cofactor flavin adenine dinucleotide (abbreviated as FAD). Monoamine oxidase enzyme plays the role of speeding up (act as a catalyst) the oxidative deamination reaction of endogenous and exogenous monoamines into respective aldehydes[2]. The dysfunction of monoamine oxidase (high or low activity) is considered to be the factor responsible for neurological disorders. Because of the action of monoamine oxidase enzyme in regulating the metabolism of the neurotransmitter, it is the vital and generally studied targets of drugs that treat neurological disorders[3].

The targets selected in this work for the docking studies are MAO-A and MAO-B enzymes. For humans, there exist two sorts of monoamine oxidase *i.e* MAO-A that causes the oxidative deamination of monoamines like Serotonin, Adrenaline, Noradrenaline, Melatonin and MAO-B that deaminates the monoamines benzylamine, phenethylamine *etc*. Some monoamines like dopamine, tryamine *etc.* are disintegrated by both forms of MAO[4]. MAO-A inhibitors treat depression like challenging mood fluctuations very successfully[5]. MAO-B is treated as one of the targets for Central nervous system associated diseases[6].

In this work, three selected drugs namely Moclobemide, Toloxatone, and Brofaroamine are

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Suppl. Data available on respective page of NOPR

docked to MAO-A enzyme and two selected drugs namely Selegiline and Rasagiline are docked to MAO-B enzyme. The drug Moclobemide is considered as a successful MAO-A inhibitor[7]. The use of Moclobemide may be a beneficial choice to treat mood fluctuations related to Alzheimer's disease[8]. In an experiment conducted with ulcer diagnosed rats (due to indomethacin exposure) the anti-ulcer activity of Moclobemide is found out[9]. Due to the limited side effects, Moclobemide is regarded as a safe drug. Toloxatone is a reversible monoamine oxidase inhibitor with limited side effects compared to preceding drugs of the same category[10]. This drug falls in the third generation among all the drugs in the MAO inhibitor category. Toloxatone was launched as an anti-depressant in France in the late 1980s. Brofaroamine, an efficient anti-depressant drug is a reversible monoamine oxidase inhibitor which was synthesized during the early 1980s. The reversible hindering of the MAO enzyme by Brofaroamine safeguards the chance of serotonin syndrome to a little extent. The rate at which Brofaroamine is absorbed from the gut is slower when compared to Moclobemide. The drug Selegiline that irreversibly hinders the MAO-B, was introduced in the middle of the 1960s[11, 12]. Selegiline is generally employed to treat Parkinson's disease and depression a like mood swing disorders. Rasagiline that inhibits or hinders the MAO-B irreversibly, treats Parkinson's disease efficiently when taken alone or when taken with other associated drugs[13].

The main challenge in the field of drug synthesis is the economic loss due to the inadequate studies before the drug synthesis[14]. Extensive theoretical analysis has gained attention in the drug designing as the computer based studies reduces the economic loss and it helps to identify the unfavourable derivatives are they are omitted easily[15]. The present work aimed to shortlist a few best structural variations of the five selected MAO inhibitors. The best variations were initially identified through the Docking studies. Docking of the drug molecules to their respective macromolecule and examining the binding affinity value obtained for each docking process is a very vital step in the process of drug designing[16]. The structural variations were introduced to each drug, by the substitution of different groups (hydrophilic and hydrophobic) at the specified positions. The variations of each drug were then docked to the respective target. The binding free energy of each structural variation was compared to the binding free energy of their parent drug. All the structures possessing binding energy less than parent drugs were selected as they can be considered as promising structures. But only the binding energy evaluation is not adequate. So the structure of the variations were analysed to check if they are drug-like molecules. This was done by analysing whether their five Lipinski factors are in the required limit. The physicochemical properties of the variations were checked to pick up and omit the variation with poor drug properties. The toxicity of the variations was analysed and the toxic variations were omitted. The non-toxic, orally active, low binding energy variations with good drug-related properties are proposed in this work as the best structural variations of each selected drug.

Materials and Methods

Geometry optimization of drugs

The five selected MAO inhibitors and its structural variations were drawn using the visualization tool GaussView 5.0 (submits and reads the Gaussian input and output, respectively)[17]. The 9th model of the computational program Gaussian 09 was used to perform the abinitio studies[18]. For the optimization studies, the basis set used was B3LYP/6-311G (d, p)[19]. In this work, ChemDraw Ultra 12.0 software was employed to draw 2-dimensional images of selected drugs[20].

Active site prediction and Molecular Docking

The PDB structure of MAO-A (2BXR) and MAO-B (2VZ2) were taken from the RCSB data bank for proteins[21]. The Metapocket server and 3D Ligand site server are employed to find out the active amino acid residues and thereby the binding sites of both proteins[22]. The PyRx software (virtual screening software) was utilized to dock the selected drugs and its structural variations to the targets[23]. SDF forms of the optimized structures were taken for the docking process. The binding free energy of the best conformer was selected from the 9 docked conformers obtained from the docking software. Among the structural variations of each selected drug, the variations with the binding energy lower than parent drug were selected for further oral activity analysis, characteristics studies and toxicity analysis.

Oral activity

Drug-likeness of the variations was checked by analysing the five drug factors of Lipinski. As per Lipinski rule, the factors like Mass, Hydrogen donors, Log P, Refractivity and hydrogen donor atom

numbers should not be above 500 Dalton, 5, 5, 40-130, and 10, respectively[24]. Three or four of the five rules must fall in the required range for drug-likeness. It could be identified whether the variations fell were lipophilic or hydrophobic.

Physicochemical characteristics

By utilizing the server SwissADME (from Swiss Institute of Bioinformatics), various properties like flexibility, polarity, solubility, saturation, gastrointestinal absorption, blood-brain barrier *etc.* were analysed[25]. If for the variations, the TPSA value falls in between 20-130 $Å^2$, rotational bond falls below 9 and fraction csp^3 value are not lower than 0.25, they were categorized as the variations with optimal polarity, flexibility and saturation, respectively[26]. Abbot bio-availability score indicated the bioavailability of variations *i.e* the prospect of the variations to own atleast 10% rat's bioavailability or computable $caco_2$ permeability[27]. Gastrointestinal absorption was emanated from the model, white of a boiled egg[28]. The existence of a troublesome part in the molecule was checked by analysing the presence of PAINS alerts[29]. Esol model was used in this work to analyse the aqueous solubility of the structural variations and the results were obtained from SwissADME server[30].

Toxicity analysis

Oral rat toxicity (LD_{50}- how much of the materials in mg/kg is essential to kill half of the rats employed in the study) and the toxicity class number of the variations were analysed from the ProTox-II server[31]. The lethal dose ranges of $LD_{50} \leq 5$ (fatal), $5 < LD_{50} \leq 50$ (death), $50 < LD_{50} \leq 300$ (toxic), $300 < LD_{50} \leq 2000$ (harm), $2000 < LD_{50} \leq 5000$ (might be harmful) and $LD_{50} > 5000$ (safe) indicated the toxicity class from 1-6, respectively. The class from 1-6 showed the dose range at which the variation is toxic. It was analysed whether the parent drug and its variations were toxic in the same dose range. Using ProTox-II server, toxic conditions like cytotoxicity, carcinogenicity, immunotoxicity and mutagenicity of all the structural variations were analysed. Toxic conditions like reproductive effectiveness and irritation of the variations were studied using the Osiris property explorer.

Results and Discussion

The crystal structure of MAO-A (PDB id: 2BXR) with 3.00 Å resolution, taken from RCSB protein data bank, was used as the target for the drugs Moclobemide, Toloxatone, and Brofaromine. Only the chain A of this PDB, along with Flavin moiety was selected and used for the docking process. The validation of this crystal structure was carried out by employing the SAVES server. The Errat plot indicated that this PDB had a quality factor of 98.63 (Suppl. Fig. 1). Rampage throws light into the merit of protein structure[32]. The Ramachandran plot analysis (Suppl. Fig. 2) indicated that the residues found in most favorable, additionally permitted, generously permitted and not allowed regions are 88.2%, 10.1%, 0.9% and 0.8%, respectively.

Human MAO-B (PDB id: 2VZ2) with a resolution of 2.30 Å, obtained from the X-ray diffraction method (downloaded from protein bank), was employed as a docking target for the selected drugs Selegiline and Rasagiline. Only the chain A of this macromolecule, along with Flavin moiety was employed for the docking process. The validation of the PDB: 2VZ2, carried out employing the SAVES server gave an Errat plot with a quality factor of 95.71 (Suppl. Fig. 3). The Ramachandran plot obtained showed that the residues found in most favorable, additionally permitted, generously permitted and not allowed regions are 93%, 6.3%, 0.2%, and 0.5%, respectively (Suppl. Fig. 4). The VERIFY result indicated that 91.04% of amino acid residues exhibited averaged 3D-1D score ≥ 0.2 (80% indicates pass)[33, 34]. Thus both the PDB files were taken for docking due to their good quality.

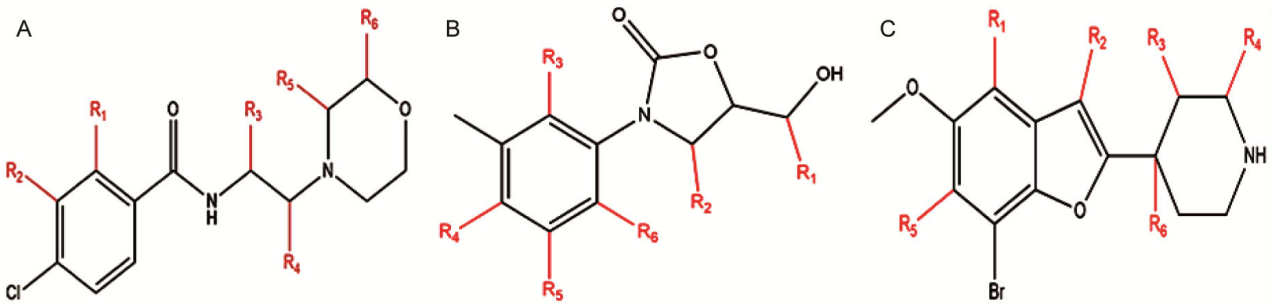

Fig. 1 — The two-dimensional representation of the substitutions on (A) Moclobemide; (B) Toloxatone; and (C) Brofaromine

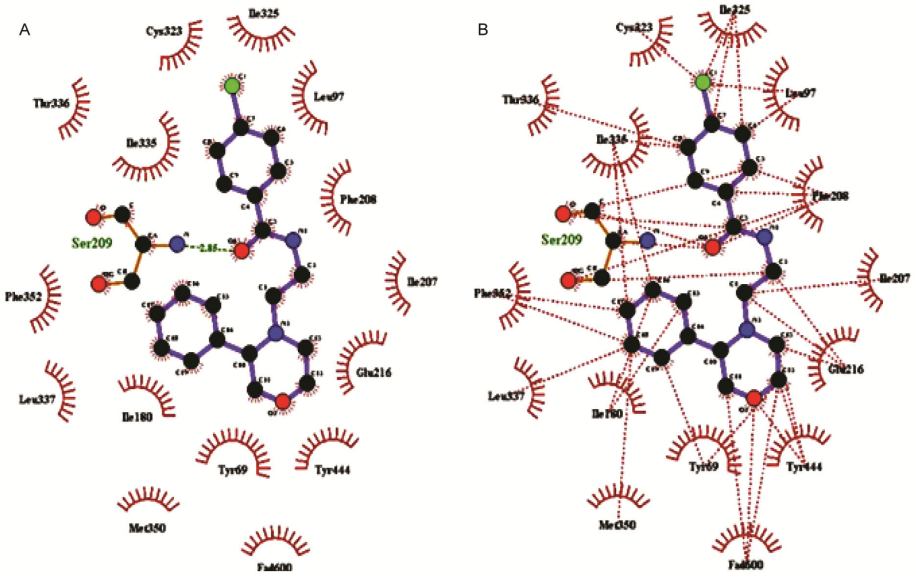

Fig. 2 — The interacting amino acid residues of MAO-A with structural variation 22, forming showing (A) hydrogen bond; and (B) hydrophobic interaction (LigPlot Digram)

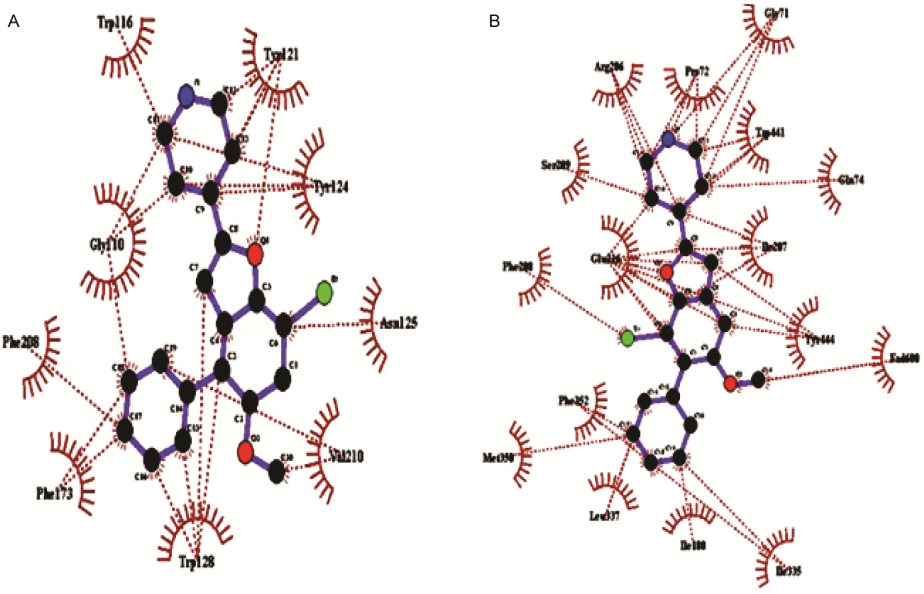

Fig. 3 — The interacting amino acid residues of MAO-A with structural variation 53, forming showing (A) hydrogen bond; and (B) hydrophobic interaction (LigPlot Digram)

Analysis of docking results

The images of Optimized structures of selected MAO-A inhibitor drugs Moclobemide, Toloxatone, and Brofaroamine are given in (Suppl. Fig. 5). The binding free energy obtained by docking Moclobemide, Toloxatone, and Brofaroamine with MAO-A were −7.7, −7.1 and −7.9 kcal/mol, respectively. The amino acid residues of MAO-A enzyme that has interacted with these three drugs are given in (Table 1). Moclobemide did not form a hydrogen bond with MAO-A enzyme.

The images of MAO-B inhibitor drugs Selegiline and Rasagiline, after optimization, are given in (Suppl. Fig. 6). The binding energy of Selegiline and Rasagiline after it docked with MAO-B were −6.1 and −6 kcal/mol, respectively. The interacting amino acid residues of MAO-B that has formed hydrophilic and hydrophobic bonding with Selegiline and Rasagiline are given in (Table 1). Both these drugs did not form hydrogen bond with MAO-B enzyme.

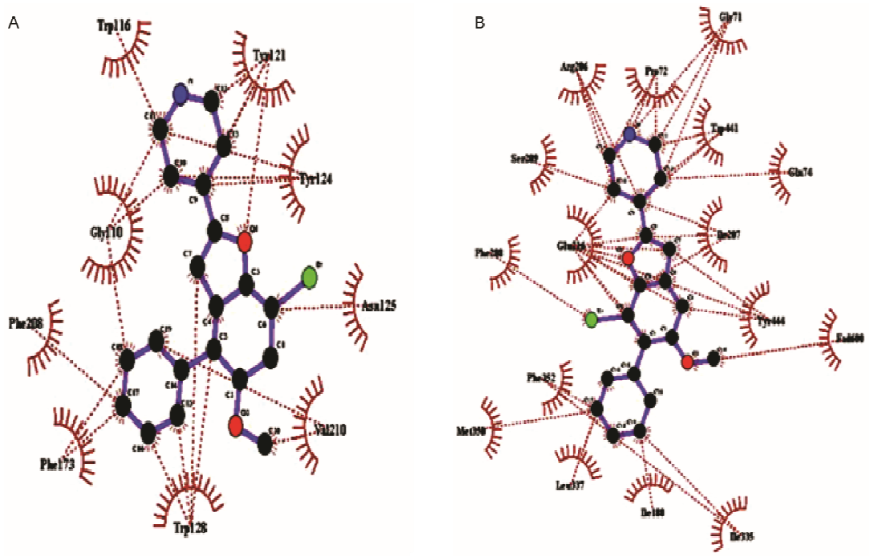

Fig. 4 — The hydrophobic interaction forming amino acid residues of MAO-A with (A) Structural variation 68; and (B) Structural variation 84 (LigPlot Digram)

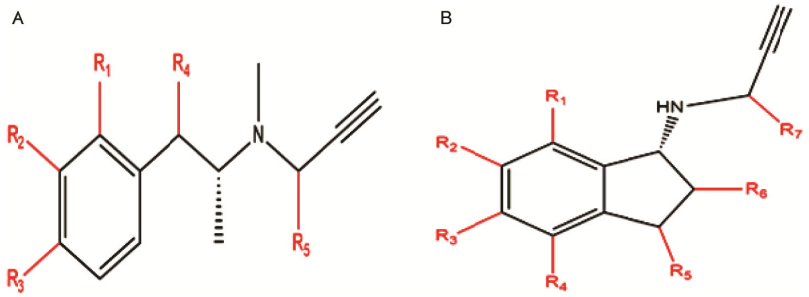

Fig. 5 — The two-dimensional representation of the substitutions on (A) Selegiline; and (B) Rasagiline

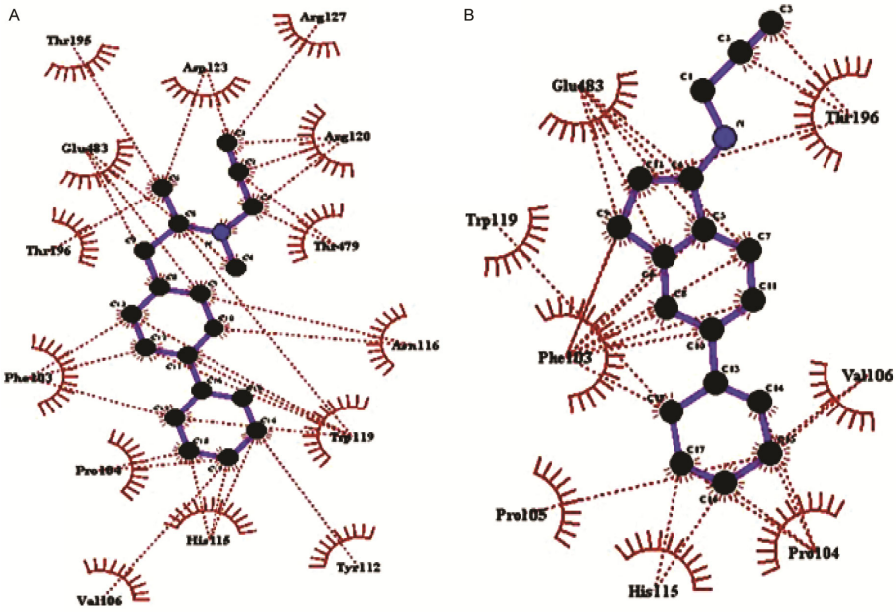

Fig. 6 — The hydrophobic interaction forming amino acid residues of MAO-B with (A) Structural variation 105; and (B) Structural variation 133 (LigPlot Digram)

Table 1— Interacting amino-acid residues

Drug	Hydrogen bond forming residues	Hydrophobic interaction forming residues
MAO-A with Moclobemide, Toloxatone, and Brofaroamine		
Moclobemide	-	Cys325, Thr336, Ile335, Ile325, Leu337, Phe208, Ser209, Glu216, Ile207, Phe352, FAD600, Tyr407, Tyr444
Brofaroamine	Ser209, Val210, Tyr407	Val210, Val93, Ser209, Phe208, Leu97, Leu337, Ile335, Thr336
Toloxatone	FAD600	FAD600, Phe352, Tyr69, Glu216, Leu337, Ser209, Phe208, Ile35, Thr336
MAO-B with Selegiline and Rasagiline		
Selegiline	-	Pro104, Val106, Glu483, Asn116, Arg120, Phe103, Trp119
Rasagiline	-	Lys302, Glu379, Tyr301, Glu303, Glu376, Phe305

Structural variations of Moclobemide

(Fig. 1A) is the two-dimensional image of the drug Moclobemide and in-order to obtain the structural variations, the substitution of various groups (hydrophilic and hydrophobic) were done at R_1, R_2, R_3, R_4, R_5, and R_6. The hydrophilic groups utilized for the substitution on Moclobemide were OH and NH_2. The hydrophobic groups utilized are CH_3, $C(CH_3)_3$, and C_6H_5. After docking with the MAO-A enzyme, the binding energy value possessed by each variation was compared to that of Moclobemide. From the docking results given in (Table 2), all the substitutions at R_1 and R_2 had given the lower energy structures and the binding energy from the hydrophobic group substitutions was much lower. The substitution of OH and C_6H_5 groups at R_3, two OH groups together at R_3 and R_4, two NH_2 groups together at R_3 and R_4 and the substitution of OH, NH_2, CH_3, and C_6H_5 at R_4, provided the structures with MAO-A binding energy much lower than that of Moclobemide. At R_5 and R_6, all the substitutions had provided lower binding energy structures. Here the two structures 22 and 25 obtained by the substitution of C_6H_5 group at R_5 and CH_3 group at R_6, respectively, had the lowest binding free energies compared to the other substitutions at these positions. All the variations except the compound 10 had MAO-A binding energy lower than Moclobemide (less than −7.7 kcal/mol) and are thus the promising structures. So the variation 10 is exempted from further analysis[35].

Table 3 gives information about amino acid residues (Obtained from LigPlot$^+$) of MAO-A enzyme that had formed hydrogen bonds and showed hydrophobic interaction with all the lower binding free energy variations. The compounds 2, 3, 6, 7, 16, 20, and 21 did not form hydrogen bond with the MAO-A enzyme. In the case of Moclobemide, hydrophobic interaction is the key interaction that had influenced the binding free energy values. Among all the substitutions from R_1 -R_6, the lowest binding energy structure was acquired by substituting C_6H_5 at R_5. *i.e* the variation 22 with a free binding energy of −9.8 kcal/mol The Ligplot diagram (both hydrophilic and hydrophobic interaction) of Variation 22 is given in (Fig. 2).

To know the oral activity of the structural variations, each variation was checked for its drug-likeness. All the variations satisfied all the five Lipinski conditions and thus they can be regarded as drug-like and orally active. The Log P value of the variation 12 was almost zero. So this molecule could be partitioned almost in the same ratio between the lipid and aqueous phase. The Log P values of the remaining Moclobemide variations were found in between 1-3. This shows that the variations are lipophilic (Suppl. Table 1).

On analysing the physicochemical characteristics (Suppl. Table 2) of the structural variations, all the variations were found to be polar, saturated, flexible and soluble with the TPSA, fraction csp^3, rotational bond number and ESOL log S values in the required optimal range, respectively. High gastrointestinal absorption and the absence of PAINS alert (nonspecific fragments) had made the variations very favourable. The compounds 4, 12, 13, 15, 19, and 24 were found to be impermeable to blood-brain barrier. All the variations possessed a bioavailability value of 0.55. This means the variations have drug-likeness. Thus the variations exhibited good drug properties.

From the informations about rat oral LD_{50} (lethal dose, 50%) and toxicity class number of the variations (Suppl. Table 3), it was found that the drug Moclobemide and all its variations belonged to the toxicity class 4 (LD_{50} in the range $300 < LD_{50} \leq 2000$, denoting the state of the harmful situation after swallowing). The variation 1 had an LD_{50} value of

Table 2 — Energy of Binding obtained for the substitutions on Moclobemide, Toloxatone, Brofaromine, Selegiline and Rasagiline

Variation No.	Substitution of hydrophilic and hydrophobic groups						Free energy of binding (kcal/mol)
	R_1	R_2	R_3	R_4	R_5	R_6	
Moclobemide	-H	-H	-H	-H	-H	-H	−7.7
1	-OH	-H	-H	-H	-H	-H	−7.9
2	$-CH_3$	-H	-H	-H	-H	-H	−8.1
3	$-C(CH_3)_3$	-H	-H	-H	-H	-H	−8.7
4	$-NH_2$	$-NH_2$	-H	-H	-H	-H	−8.2
5	-H	-OH	-H	-H	-H	-H	−7.8
6	-H	$-CH_3$	-H	-H	-H	-H	−7.8
7	-H	$-C(CH_3)_3$	-H	-H	-H	-H	−8.2
8	-H	$-C_6H_5$	-H	-H	-H	-H	−8.5
9	-H	-H	-OH	-H	-H	-H	−8.0
10	-H	-H	$-CH_3$	-H	-H	-H	−7.6
11	-H	-H	$-C_6H_5$	-H	-H	-H	−9.4
12	-H	-H	-OH	-OH	-H	-H	−8.1
13	-H	-H	$-NH_2$	$-NH_2$	-H	-H	−8.3
14	-H	-H	-H	-OH	-H	-H	−8.1
15	-H	-H	-H	$-NH_2$	-H	-H	−9.3
16	-H	-H	-H	$-CH_3$	-H	-H	−8.5
17	-H	-H	-H	$-C_6H_5$	-H	-H	−8.5
18	-H	-H	-H	-H	-OH	-H	−8.2
19	-H	-H	-H	-H	$-NH_2$	-H	−8.3
20	-H	-H	-H	-H	$- CH_3$	-H	−7.8
21	-H	-H	-H	-H	$-C(CH_3)_3$	-H	−8.1
22	-H	-H	-H	-H	$- C_6H22_5$	-H	−9.8
23	-H	-H	-H	-H	-H	-OH	−8.0
24	-H	-H	-H	-H	-H	$-NH_2$	−8.0
25	-H	-H	-H	-H	-H	$- CH_3$	−8.1
26	-H	-H	-H	-H	-H	$-C(CH_3)_3$	−7.8
	R_1	R_2	R_3	R_4	R_5	R_6	
Toloxatone	-H	-H	-H	-H	-H	-H	−7.1
27	-OH	-H	-H	-H	-H	-H	−7.6
28	$-NH_2$	-H	-H	-H	-H	-H	−7.5
29	-F	-H	-H	-H	-H	-H	−7.7
30	$-CH_3$	-H	-H	-H	-H	-H	−7.6
31	$-C(CH_3)_3$	-H	-H	-H	-H	-H	−8.3
32	$-C_6H_5$	-H	-H	-H	-H	-H	−9.4
33	-H	-OH	-H	-H	-H	-H	−7.4
34	-H	$-NH_2$	-H	-H	-H	-H	−7.2
35	-H	-F	-H	-H	-H	-H	−7.5
36	-H	$-CH_3$	-H	-H	-H	-H	−7.4
37	-H	$-C(CH_3)_3$	-H	-H	-H	-H	−8.0
38	-H	$-C_6H_5$	-H	-H	-H	-H	−7.2
39	-H	$-C_6H_{11}$	-H	-H	-H	-H	−8.0
40	-H	-H	-OH	-H	-H	-H	−7.0
41	-H	-H	$-NH_2$	-H	-H	-H	−7.0
42	-H	-H	-F	-H	-H	-H	−7.4
43	-H	-H	$-CH_3$	-H	-H	-H	−7.2
44	-H	-H	$-C(CH_3)_3$	-H	-H	-H	−8.4
45	-H	-H	-H	$-CH_3$	-H	-H	−7.7
46	-H	-H	-H	$-C(CH_3)_3$	-H	-H	−8.5
47	-H	-H	-H	$-C_6H_5$	-H	-H	−8.9
48	-H	-H	-H	-OH	-H	-H	−7.0

(*Contd.*)

Table 2 — Energy of Binding obtained for the substitutions on Moclobemide, Toloxatone, Brofaromine, Selegiline, and Rasagiline (*Contd.*)

Variation No.	Substitution of hydrophilic and hydrophobic groups						Free energy of binding (kcal/mol)
	R_1	R_2	R_3	R_4	R_5	R_6	
49	-H	-H	-H	$-NH_2$	-H	-H	−7.0
50	-H	-H	-H	-F	-H	-H	−7.2
51	-H	-H	-H	-H	$-CH_3$	-H	−7.7
52	-H	-H	-H	-H	$C(CH_3)_3$	-H	−7.8
53	-H	-H	-H	-H	$-C_6H_{11}$	-H	−9.1
54	-H	-H	-H	-H	-OH	-H	−7.3
55	-H	-H	-H	-H	$-NH_2$	-H	−6.6
56	-H	-H	-H	-H	-F	-H	−7.4
57	-H	-H	-H	-H	-H	$-CH_3$	−7.6
58	-H	-H	-H	-H	-H	CH_2CH_3	−6.6
59	-H	-H	-H	-H	-H	$-C_6H_5$	−7.4
60	-H	-H	-H	-H	-H	$-C_6H_{11}$	−7.4
61	-H	-H	-H	-H	-H	-OH	−7.4
62	-H	-H	-H	-H	-H	$-NH_2$	−6.6
63	-H	-H	-H	-H	-H	-F	−7.2
	R_1	R_2	R_3	R_4	R_5	R_6	
Brofaromine	-H	-H	-H	-H	-H	-H	−7.9
64	-OH	-H	-H	-H	-H	-H	−7.7
65	$-NH_2$	-H	-H	-H	-H	-H	−7.5
66	$-CH_3$	-H	-H	-H	-H	-H	−7.8
67	$-C(CH_3)_3$	-H	-H	-H	-H	-H	−8.0
68	$-C_6H_5$	-H	-H	-H	-H	-H	−8.5
69	-OH	-OH	-H	-H	-H	-H	−8.2
70	-H	-OH	-H	-H	-H	-H	−7.8
71	-H	$-NH_2$	-H	-H	-H	-H	−6.4
72	-H	$-CH_3$	-H	-H	-H	-H	−8.2
73	-H	$-C(CH_3)_3$	-H	-H	-H	-H	−7.5
74	-H	$-C_6H_5$	-H	-H	-H	-H	−8.9
75	-H	-H	$-CH_3$	-H	-H	-H	−7.8
76	-H	-H	$-C(CH_3)_3$	-H	-H	-H	−7.5
77	-H	-H	$-C_6H_5$	-H	-H	-H	−8.1
78	-H	-H	-OH	-H	-H	-H	−7.8
79	-H	-H	-H	$C(CH_3)_3$	-H	-H	−8.4
80	-H	-H	-H	$CH(CH_3)_2$	-H	-H	−8.0
81	-H	-H	-H	-OH	-H	-H	−7.7
82	-H	-H	-H	-H	$-NH_2$	-H	−8.0
83	-H	-H	-H	-H	$-CH_3$	-H	−8.3
84	-H	-H	-H	-H	$-C_6H_5$	-H	−8.5
85	-H	-H	-H	-H	$CH(CH_3)_2$	-H	−8.4
86	-H	-H	-H	-H	$-CH_2CH_3$	-H	−8.2
87	-H	-H	-H	-H	-F	-H	−8.2
88	-H	-H	-H	-H	-Br	-H	−7.7
89	-H	-H	-H	-H	-H	-OH	−8.0
90	-H	-H	-H	-H	-H	$-CH_3$	−8.4
	R_1	R_2	R_3		R_4	R_5	
Selegiline	-H	-H	-H		-H	-H	−6.1
91	$-CH_3$	-H	-H		-H	-H	−5.2
92	$-C(CH_3)_3$	-H	-H		-H	-H	−6
93	$-C_6H_5$	-H	-H		-H	-H	−7.5
94	$-C_6H_{11}$	-H	-H		-H	-H	−6.5

(*Contd.*)

Table 2 — Energy of Binding obtained for the substitutions on Moclobemide, Toloxatone, Brofaromine, Selegiline, and Rasagiline (*Contd.*)

Variation No.	Substitution of hydrophilic and hydrophobic groups							Free energy of binding (kcal/mol)
	R_1	R_2	R_3	R_4	R_5			
95	-OH	-H	-H	-H	-H			−4.9
96	$-CH_2C_6H_5$	-H	-H	-H	-H			−6.4
97	-H	$-CH_3$	-H	-H	-H			−6.0
98	-H	$-C(CH_3)_3$	-H	-H	-H			−5.1
99	-H	$-CH_2C_6H_5$	-H	-H	-H			−7.2
100	-H	$-C_6H_5$	-H	-H	-H			−6.2
101	-H	$-C_6H_{11}$	-H	-H	-H			−6.3
102	-H	-OH	-H	-H	-H			−5.7
103	-H	-H	$-CH_3$	-H	-H			−6.1
104	-H	-H	$-C(CH_3)_3$	-H	-H			−6.2
105	-H	-H	$-C_6H_5$	-H	-H			−7.8
106	-H	-H	$-C_6H_{11}$	-H	-H			−6.4
107	-H	-H	-OH	-H	-H			−4.9
108	-H	-H	$-CH_2C_6H_5$	-H	-H			−6.4
109	-H	-H	-H	$-CH_3$	-H			−5.3
110	-H	-H	-H	$-C_6H_5$	-H			−5.5
111	-H	-H	-H	-OH	-H			−5.4
112	-H	-H	-H	-H	-OH			−5.6
113	-H	-H	-H	-H	$-CH_3$			−5.8
114	-H	-H	-H	-H	$-C(CH_3)_3$			−5.8
115	-H	-H	-H	-H	$-C_6H_5$			−7.6
116	-H	-H	-H	-H	$-C_6H_{11}$			−6.6
117	-H	-H	-H	-H	$-CH_2C_6H_5$			−6.2
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	
Rasagiline	-H	-H	-H	-H	-H	-H	-H	−6.0
118	$-CH_3$	-H	-H	-H	-H	-H	-H	−5.8
119	$C(CH_3)_3$	-H	-H	-H	-H	-H	-H	−5.9
120	$-C_6H_5$	-H	-H	-H	-H	-H	-H	−6.6
121	$-C_6H_{11}$	-H	-H	-H	-H	-H	-H	−7.5
122	-OH	-H	-H	-H	-H	-H	-H	−5.9
123	$-NH_2$	-H	-H	-H	-H	-H	-H	−6.7
124	-H	$-CH_3$	-H	-H	-H	-H	-H	−6.1
125	-H	$C(CH_3)_3$	-H	-H	-H	-H	-H	−6.9
126	-H	$-C_6H_5$	-H	-H	-H	-H	-H	−7.5
127	-H	$-C_6H_{11}$	-H	-H	-H	-H	-H	−7.5
128	-H	-OH	-H	-H	-H	-H	-H	−5.7
129	-H	$-NH_2$	-H	-H	-H	-H	-H	−5.9
130	-H	-H	$-C(CH_3)_3$	-H	-H	-H	-H	−7.1
131	-H	-H	$-CH_2CH_3$	-H	-H	-H	-H	−6.2
132	-H	-H	$-C_6H_5$	-H	-H	-H	-H	−7.5
133	-H	-H	$-C_6H_{11}$	-H	-H	-H	-H	−8.0
134	-H	-H	-OH	-H	-H	-H	-H	−5.5
135	-H	-H	$-NH_2$	-H	-H	-H	-H	−5.9
136	-H	-H	-H	$-CH_3$	-H	-H	-H	−5.9
137	-H	-H	-H	$-C(CH_3)_3$	-H	-H	-H	-5.9
138	-H	-H	-H	$-CH_2CH_3$	-H	-H	-H	−5.9
139	-H	-H	-H	$-C_6H_5$	-H	-H	-H	−7.5
140	-H	-H	-H	$-C_6H_{11}$	-H	-H	-H	−7.3
141	-H	-H	-H	-OH	-H	-H	-H	−6.3
142	-H	-H	-H	$-NH_2$	-H	-H	-H	−7.6

(*Contd.*)

Table 2 — Energy of Binding obtained for the substitutions on Moclobemide, Toloxatone, Brofaromine, Selegiline, and Rasagiline

Variation No.	Substitution of hydrophilic and hydrophobic groups							Free energy of binding (kcal/mol)
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	
143	-H	-H	-H	-H	CH_2CH_3	-H	-H	−6.4
144	-H	-H	-H	-H	$-C_6H_5$	-H	-H	−7.0
145	-H	-H	-H	-H	$-C_6H_{11}$	-H	-H	−6.7
146	-H	-H	-H	-H	-OH	-H	-H	−5.6
147	-H	-H	-H	-H	$-NH_2$	-H	-H	−6.7
148	-H	-H	-H	-H	-H	-OH	-H	−6.4
149	-H	-H	-H	-H	-H	$-NH_2$	-H	−5.7
150	-H	-H	-H	-H	-H	-F	-H	−7.3
151	-H	-H	-H	-H	-H	$-C_6H_5$	-H	−7.3
152	-H	-H	-H	-H	-H	$C(CH_3)_3$	-H	−6.6
153	-H	-H	-H	-H	-H	CH_2CH_3	-H	−6.1
154	-H	-H	-H	-H	-H	-H	-OH	−6.6
155	-H	-H	-H	-H	-H	-H	$-NH_2$	−6.6
156	-H	-H	-H	-H	-H	-H	$C(CH_3)_3$	−6.4
157	-H	-H	-H	-H	-H	-H	CH_2CH_3	−6.1
158	-H	-H	-H	-H	-H	-H	$-C_6H_5$	−8.0

707 mg/kg and the remaining variations had an LD_{50} value of 1250 mg/kg, higher than the variation 1. Thus the variations are found safe at the safe dose range of Moclobemide.

The variations of Moclobemide were analysed for carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity using ProTox-II server. The irritation and the Reproductive effectiveness of the variations were checked using the Osiris property explorer (Suppl. Table 4). The results from the servers indicated that the variation 2 was an irritant and it showed reproductive effects. The variations 3, 7, and 25 were found to be immunotoxic with the probability score of activity 0.54, 0.54, and 0.56, respectively. All the variations except 3, 7, and 25 were found safe with the probability score of inactivity for Immunotoxicity, Mutagenicity, Carcinogenicity and Cytotoxicity in the range 0.77-0.99, 0.67-0.79, 0.56-0.66, and 0.57-0.75, respectively.

So from the analysis of the drug-related properties and the toxicities of the lower binding energy variations, the compounds 4, 12, 13, 15, 19, and 24 are not favourable because they are impermeable through blood-brain barrier. The structural variations 2, 3, 7, and 25 are toxic and so are not the promising structural variations of Moclobemide[36].

Structural variations of Toloxatone

(Fig. 1B) provides the 2D structure of Toloxatone. Structural variations were obtained by the substitution of hydrophilic groups and hydrophobic groups from R_1 to R_6. To obtain the variations that possess lower free energy than Toloxatone, the hydrophilic groups like OH, NH_2, F and hydrophobic groups such as CH_3, CH_2CH_3, C_6H_5, $C(CH_3)_3$, C_6H_{11} were substituted at given positions. The structures were optimized and docked to MAO-A enzyme (Table 2). At R_1 and R_2, the substitutions of all the hydrophilic and hydrophobic groups were favourable because the resultant structures had lower binding energy than Toloxatone. In both cases, hydrophobic substitution yielded the best low energy structures. At R_3, only the substitutions of hydrophobic groups gave the lower binding energy structures except in the case of substitution of the fluorine group. The same results were found for the substitutions at R_4. At R_5, the substitutions of all the groups except NH_2 and at R_6, the substitutions of all the groups except NH_2 and CH_2CH_3 had given the favourable structural variations with the MAO-A binding energy lower than that of Toloxatone. The compounds 40, 41, 48, 49, 55, 58, and 62 were omitted from the remaining studies as these compounds had higher MAO-A binding energy than Toloxatone. Rest all the compounds possessed the binding energy lower than −7.1 kcal/mol, and so they were analysed further[37].

Table 3 pointed out the residues of MAO-A enzyme, involved in forming hydrogen bond and hydrophobic interaction with lower binding energy variations. In the case of Toloxatone, the very important interaction that influenced the binding free energy is hydrophobic.

Table 3 — Amino acid residues of MAO-A that interacts with the variations of Moclobemide (1-26), Toloxatone (27-63), Brofaromine (67-90) forming hydrogen bond and hydrophobic interaction

Variation No	Hydrogen bond forming residues	Residues of MAO-A showing hydrophobic interaction
1	Ser209	FAD600, Tyr444, Ile335, Ile180, Phe352, Tyr407, Ile207, Phe208, Glu216, Ser209
2	-	Cys323, Leu97, Ser209, Ile325, Leu337, Thr336, Ile335, FAD600, Tyr407, Phe352, Tyr444, Phe208, Ile207, Glu216
3	-	Phe173, Asn125, Arg129, Val210, Gly110, Trp128, Phe177, Phe208, Thr205, Asp132, Thr204
4	Tyr444, Ile207, Arg206	FAD600, Tyr444, Asn181, Phe352, Tyr69, Ser209, Glu216, Trp441, Pro72, Thr73, Gly71
5	Ser209	Ser209, Phe208, Glu216, FAD600, Ile207, FAD600, Tyr444, Tyr407, Phe352, Ile335, Cys323, Ile325, Leu97
6	-	Cys323, Leu97, Ile325, Thr336, Phe208, Ile335Phe352, FAD600, Ile207, Tyr407, Tyr444, Glu216, Leu337
7	-	Tyr69, Ile207, Tyr444, Tyr407, Phe362, ile180, Ile335, Ile325Cys323, Thr336, Leu97, Leu337, Glu216, FAD600
8	Thr205	Asn125, Asp132, Arg129, Thr205, Thr204, Trp128, Phe177, Phe208, Gly110, Phe173
9	Thr407, Glu216, Ser209	Phe352, Leu337, Tyr407, Ile180, Asn181, Ile207, Glu216, Phe208, Ser209, Ile335, Cys323, Ile325, Thr336
11	Glu216	Tyr444, Tyr69, Phe352, FAD600, Tyr407, Ile207, Gln74, Gly71, Val70, Trp441, Glu216, Phe208, Ile335, Leu337, Leu97, Cys323
12	Tyr444, Glu216	Phe352, Tyr407, FAD600, Leu337, Phe208, Thr336, Cys323, Ile335, Leu97, Ile325, Ser209, Glu216, Ile207, Tyr444, Asn181
13	Glu216, Tyr444	Phe352, Tyr407, FAD600, Leu337, Phe208, Thr336, Cys323, Ile335, Leu97, Ile325, Ser209, Glu216, Ile207, Tyr444, Asn181
14	Ser209, Glu216	Thr336, Leu97, Ile325, Cys323, Leu337, Ile335, Ser209, Phe208, Tyr69, Ile207, Glu216, FAD600, Tyr407, Phe352, Tyr444
15	Tyr444, Ser209	Tyr444, Phe352, FAD600, Tyr407, Ile207, Leu337, Ser209, Leu97, Ile335 , Cys323, Ile325
16	-	FAD600, Phe352, Tyr444, Glu216, Tyr69, Thr336, Leu337, Ile335, Tyr407, Ser209, Phe208, Ile325, Leu97, Cys323
17	Ser209	Trp441, Tyr69, Glu216, Gly71, Tyr444, Gln74, Phe352, Cys323, Ile335, Leu337, Ser209, FAD600, Phe208, Ile325, Tyr407, Ile207, Tyr69
18	Tyr444, , Ile207, Asn181, Tyr407	Tyr444, Asn181, Ile207, Tyr407, Ile180, Phe208, Gln216, Ile325, Leu337, Ser209, Met324, Ile335, Cys323, Phe352, Thr336
19	Tyr444, , Ile207, Tyr407, Ser209	Asn181, Ile207, Glu216, Phe208, Ser209, Thr336.Ile335, Leu337, Cys323, Ile325, Thr336, Leu97, Val93
20	-	Tyr444, Ile335, Ile207, Ile180, Asn181, Tyr69, Phe208, Phe352, Glu216, Leu337, Thr336, Leu97, Val93
21	-	Cys323, Leu97, Thr336, Met324, Ile335, Ile325, Ser209, Phe208, Ile207, Leu337, Glu216, Phe352, Tyr69, Tyr444, FAD600
22	Ser209	Ile325, Cys323, Thr336, Leu97, Phe208, Ser209, Leu337, Ile207, Glu216, Met350, Phe352, Ile180, FAD600, Ile335, Tyr444, Tyr69
23	Asn181, Tyr407, Tyr444, Ser209	Thr336, Ile335, Cys323, Leu97, Ile325, Leu337, Phe208, Glu216, Ser209, Tyr407, Asn181, Ile180, Tyr444
24	Tyr444, Ser209	Ile180, Asn181, Tyr444, Phe352, Tyr407, Glu216, Ile335, Thr336, Phe208, Ser209, Leu337, Cys323, leu97, Ile325

(*Contd.*)

Table 3 — Amino acid residues of MAO-A that interacts with the variations of Moclobemide (1-26), Toloxatone (27-63), Brofaromine (67-90) forming hydrogen bond and hydrophobic interaction (*Contd.*)

Variation No	Hydrogen bond forming residues	Residues of MAO-A showing hydrophobic interaction
25	Ser209	FAD600, Phe352, Ile180, Tyr407, Tyr69, Glu216, Ile335, Leu337, Ser209, Thr336, Phe208, Cys323, Ile325, Leu97
26	Gln74, Ile207	Pro72, Gln74, Trp441, Gly71, Tyr444, FAD600, Ser209, Arg206, Gln215.Ile207, Glu216, Tyr69, Leu337
27	Asn181, Tyr444	Tyr444, Tyr407, Asn181, Tyr69, Phe352, Phe208, Leu337, Ile335, Thr336, Met350
28	Asn181, Tyr444	Tyr444, Tyr407, Ile180, Asn181, Phe352, Tyr69, Met350, Phe208, Thr336, Ile335, Leu337
29	Asn181, Tyr407, Tyr444	Asn181, Tyr407, Tyr444, Tyr69, Phe208, Ile335, Met350, Leu337, Thr336, Phe352
30	FAD600	FAD600, Phe352, Tyr407, Tyr69, Ser209, Leu337, Glu216, Ile335, Phe208, Thr336
31	Ser209	Glu216, Leu337, Ile325, Ile180, Ile335, Asn181, FAD600, Phe352, Tyr444, Tyr407, Phe208
32	Ser209, Glu216	Val93, Phe208, Cys323, Leu337, Ile325, Thr336, Phe352, Tyr407, FAD600, Tyr69, Tyr444, Glu216, Ser209
33	Asn181, Tyr407, Tyr444, Ile180	Asn181, Tyr444, Tyr407, Ile180, Tyr69, Leu337, Phe352, Phe208, Ile335, Thr336
34	Ser209	FAD600, Glu216, Tyr69, Phe208, Ile335, Val93, Leu337
35	Asn181, Tyr407, Tyr444	Asn181, Tyr407, Tyr444, Tyr69, Thr336, Ile180, Phe208, Phe352, Ile335, Leu337
36	FAD600	Asn181, Tyr407, Glu216, Tyr69, Phe208, Leu337, Phe352, Ile335, Ile180, Asn181
37	Asn181, Tyr407, Tyr444, Ile207	Asn181, Tyr407, Tyr444, Ile207, Tyr69, Glu216, FAD600, Ile180, Phe352, Ile335, Phe208, Leu337, Thr336
38	Phe208, Thr204	Gly110, Tyr124, Phe173, Val210, Phe177, Asn125, Trp128, Thr205, Phe208, Thr204
39	Tyr444, FAD600	Glu216, Ile207, Tyr69, Arg206, Phe208, Tyr407, Leu337, Ser209, Phe352, Tyr444
42	FAD600	Phe352, Tyr69, Tyr407, Glu216, Ser209, Phe208, Leu337, Ile335, Thr336.Phe352
43	Thr204, Thr205	Thr204, Thr205, Phe173, Gly110, Phe177, Trp128
44	Glu216	FAD600, Tyr444, Tyr69, Ser209, Phe208, Phe352, Met350, Leu337, Thr336, Ile335
45	Tyr407	FAD600, Tyr444, Ser209, Phe208, Thr356, Ile335, Leu337, Phe352, Ile180, Tyr407
46	Tyr407	Tyr407, FAD600, Tyr444, Ser209, Phe208, Ile335, Ile180, Phe352, Leu337, Thr336
47	Gln74, Glu216	Arg206, Val70, Gly71, Gln74, Glu216, Tyr69, FAD600, Tyr444, Ser209, Asn181, Ile180, Tyr407, Ile207
50	Thr205, Asn125	Arg129, Asn125, Thr205, Phe177, Trp128, Phe173, Thr204, Gly110, Val210, Phe208
51	Tyr444	Tyr407, FAD600, Leu337, Phe208, Ile335, Thr336, Met350, Tyr444
52	Thr205, Thr204	Thr204, Thr205, Trp128, Phe208, Phe173, Phe177, Gly110
53	Val210, Ser209	Gly214, Val93, Leu337, Met350, Ile335, Thr336, Tyr407, Phe352, Phe208, Tyr444, FAD600, Ser209, Glu216, Val210
54	Gln74, Glu216, Ile20, Asn81, Ile180	Gln74, Glu216, Ile207Asn81, Ile180, Val70, Gly71, Ser209, Tyr407, Tyr444, Phe352, Phe208, Tyr69

(*Contd.*)

Table 3 — Amino acid residues of MAO-A that interacts with the variations of Moclobemide (1-26), Toloxatone (27-63), Brofaromine (67-90) forming hydrogen bond and hydrophobic interaction

Variation No	Hydrogen bond forming residues	Residues of MAO-A showing hydrophobic interaction
56	FAD600	Tyr407, Tyr444, Phe352, Phe208, Ile335, Leu337
57	FAD600	FAD600, Tyr407, Ile207, Phe352, Ser209, Leu337, Glu216, Tyr69, Leu337, Thr336, Ile335, Phe208
59	Thr204, Thr205	Thr204, Thr205, Val210, Phe177, Phe208, Phe173, Trp128, Asn125
60	Thr204, Thr205	Thr204, Thr205, Val210, Phe177, Phe208, Phe177, Trp128, Asn125, Tyr121
61	Gln74, Ser209, Glu216, Tyr444, Asn181, Ile207	Val70, Gly71, Phe352, Tyr69, Ile180, Phe208
63	FAD600	FAD600, Phe352, Tyr407, Tyr69, Ser209, Glu216, Phe208, Leu337, Ile335, Thr336
67	-	Tyr124, Tyr121, Phe173, Gly110, Val210, Phe208, Thr204, Phe177, Trp128, Asn125
68	-	Trp116, Tyr121, Tyr124, Asn125, Val210, Trp128, Phe173, Phe208, Gly110
69	Ile207, Ser209, Tyr444	Glu216, Leu337, Ser209, Phe208, Phe352, Ile180, Ile335, Asn181, FAD600, Tyr444, Ile207, Ser209
72	Ser209	Leu337, ser209, Phe208, Glu216, FAD600, Phe352, Tyr69, Tyr407, Tyr444
74	Thr205	Gly110, Tyr124, Phe173, Trp128, Tyr121, Asn125, phe177, Thr205, Thr204, Asp132
77	Thr204	Thr204, Trp128, Phe173, Asn125, Phe208, Tyr124, Tyr121, Gly110, Val210, Arg109
79	Tyr407	Tyr407, Asn181, Tyr444, Phe352.Ile180, Phe208, Glu216, Ile35, Ser209, Leu337, Val93
80	-	Phe177, Phe173, Thr204, Phe208, Trp128, Gly110, Tyr124, Tyr121, Trp116
82	FAD600	Leu97, Cys323, Thr336, Phe208, Ile335, Leu337, Glu216, Tyr407, Tyr444, Tyr69, Phe352, FAD600
83	-	Tyr69, Tyr444, FAD600, Glu216, Phe208, Val210, Ser209, Leu337, Thr336
84	-	Ile335, Ile180, Leu337, Met350, Phe352, Phe208, FAD600, Tyr444, Glu216, Ile207, Ser209, Gln74, Trp441, Arg206, Pro72, Gly71
85	Tyr444	FAD600, Ile180, Tyr407, Ile207, Asn181, Glu216, Phe352, Phe208, Ile335, Leu337, Ser209, Leu97, Cys323, Ile325, Thr336
86	FAD600, Ser209, Val210	Val93, Leu97, Leu337, Phe208, Tyr69, Glu216, Ile335, Tyr444, Phe352, Ile180, Ser209, Val210
87	-	FAD600, Tyr444, Tyr69, Glu216, Ser209, Phe208, Val93, Leu337, Ile335, Ile180, Phe352
89	Thr336, Tyr407	Val93, Ser209, Val210, Phe208, Leu97, Ile335, Cys323, Leu337, Tyr407, Ile180, Phe352
90	Ser209	Ser209, Leu97, Val210, Phe208, Leu327, Cys323, Thr336, Ile335, Phe352, Ile180, Tyr407

In order to understand the drug-likeness of the favourable Toloxatone variations, five Lipinski factors of each variation were analysed using the SwissADME server. The result showed that the five Lipinski factors of all variations are within the required range. Thus all the structural variations that possessed the lower MAO-A binding free energy can be regarded as drug-like molecules. The Log P values of the variations 27 and 33 were found to be almost zero. The Log P values of the remaining variations were in between 2-5.5. This indicates that the variations possess optimal Lipophilicity (Suppl. Table 5).

The physicochemical properties of the promising variations of Toloxatone were studied using the SwissADME server. From the results, the variations were found to be polar and flexible with the TPSA values and rotational bond numbers of the variations in the optimal range, respectively. Except the variations 32, 38, 47, and 59, all the remaining molecules were found to be saturated with fraction csp3 values above 0.25. The variations possessed high gastrointestinal absorption. Among the favourable variations, the compounds 27, 28, 34, 54 and 61 were found impermeable to blood-brain

Table 4 — Amino acid residues of MAO-B that interacts with the variations of Selegiline (93-117) and Rasagiline (120-158), forming hydrogen and hydrophobic interaction

Variation No	Hydrogen bond forming residues	Residues of MAO-B involved in hydrophobic interaction
93	-	Arg127, Thr479, Thr195, Phe103, Thr196, Glu183, Asp123, Arg128, Asn116, Trp110, Hia115
94	-	Asn116, Pro105, Tyr112, His115, Pro104, Val106, Glu483, Phe103, Trp119
96	-	Ser160, Val106, Val92, Trp107, Tyr97, His90, Pro105, Pro98
99	-	Tyr112, Pro104, Phe103, Trp119, Thr196, Glu483, Thr479, Arg120, Asp123
100	-	Gly101, Arg100, Pro102, Glu483, Trp119, Phe103, Pro104
101	-	Tyr112, Phe103, Asn116, Glu483, Trp119, Arg120
104	-	Arg120, Thr479, Asp123, Thr196, Glu483, Trp119, Phe103, Thr195, Tyr112, His115, Pro104
105	-	Thr195, Asp123, Arg127, Glu483, Arg120, Thr196, Thr479, Phe103, Asn116, Trp119, Pro184, Val106, His115, Tyr112
106	-	Tyr112, Phe103, Glu483, Trp119, Arg120, Thr196, Asp123
108	Phe103	Arg100, Pro105, Phe103, Glu483, Trp119, Asn116, Thr479, Thr196
115	Glu483	Asp123, Thr196, Arg120, Trp119, Glu483, His115, Asn116, Phe103, Val106, Tyr112
116	-	His115, Asn116, Trp119, Phe103, Val106, Pro104, Tyr112, Glu483
117	-	Tyr97, Pro105, His90, Val106, Val92, Ser160, Lys93, Gln163
120	-	Phe103, Glu483, Tyr112, His115, Asn116, Trp119
121	-	Phe103, Glu483, Tyr112, His115, Asn116, Trp119, Pro104, His115
123	Tyr112	Tyr112, Phe103, Trp119, His115, Pro104, Pro105
124	-	Thr479, Glu483, Arg120, Thr196, Asn116, His115, Phe103, Trp119
125	Thr196	Thr478, Glu483, Thr196, Asn116, Phe103, Trp119
126	-	Pro104, val106, His115, Pro105, Phe103, Asn116, Trp119, Glu483
127	-	Arg120, Thr106, Thr479, Phe103, Trp119, Asn116, His115, Pro104
130	-	Thr478, Glu483, Thr196, Phe103, Trp119, Pro104
131	-	Thr106, Glu483, Pro102, Phe103, trp119, Pro104
132	Thr196	Thr478, Pro102, Phe103, Pro104, Trp119, Glu483, Asn116, Tyr112, His115
133	-	Glu483, Thr196, Trp119, Phe103, Val106, Pro105, His115, Pro104
139	-	Thr196, Trp119, Asp123, Thr479, Arg120, Arg484, Phe103, Asn116, Glu483
140	Lys348	Ala325, Leu345, Asp318, Asn170, Gly319, Glu320, Leu167, Thr166, Gln163, Lys162, Lys348
141	-	Tyr435, FAD1502, Gly434, Gln200, Phe343, Ile198, Leu171, Ile199, Cys172, Tyr326
142	Asp123	Trp119, Phe103, Thr196, Thr195, Glu483, Arg120, Asn116, Thr479
143	-	Thr196, Thr195, Phe103, His115, Trp119, Tyr112, Glu483, Pro104
144	Tyr112	Tyr112, His115, Pro104, Glu483, Phe103, asn116, Trp119
145	-	Asn116, Arg120, Trp119, Phe103, Thr479, Glu483, Thr195, Thr478, Pro102
147	Asp123, Thr195, Thr196	Thr196, Thr479, Arg484, Glu483, Arg120, asn116, Trp119, Phe103
148	Asn116	Tyr112, Phe103, Thr478, Thr479, Glu483, Thr196, Arg120, Trp119
150	-	Ile198, Leu171, Gln206, Pro102, Tyr326, Ile316, Ile199, Pro104, Phe168, Trp119, Leu164
151	Glu483	Tyr112, Glu483, Pro104, Val106, Phe103, Asn116, Trp119
152	-	Trp119, Asn116, Pro104, His115, Val106, Tyr112, Pro105, Phe103, Glu483
153	Glu483	Glu483, Phe103, Pro104, Trp119
154	FAD1502	FAD1502, Tyr435, Tyr398, Gly434, Cys172, Ile199, Ile198, Leu171, Tyr326, Gln206, Phe343, Tyr398
155	Glu483	Tyr112, Glu483, Phe103, Thr195, Arg120, Thr479, Trp119, Asn116, Thr196, Asp123, Thr479
156	-	Val106, Ser160, Val92, Gln163, Tyr97, Pro105, His90
157	-	Ser160, His90, Val106, Val92, Tyr97, Pro105, Trp107
158	Glu483	Arg120, Asp123, Asn116, Trp119, Thr196, Pro104, Phe103, Glu483

barrier (Suppl. Table 6). Overall the variations exhibits good drug properties.

The median rat lethal dose (LD_{50}) and the toxicity class number of all the favourable Toloxatone variations were studied using ProTox-II server (Suppl. Table 7). Toloxatone possessed an LD_{50} value of 1225 mg/kg. All the variations belonged to the same toxicity class of Toloxatone. Thus Toloxatone and all its variations fell in the toxicity class 4 (LD_{50} in the range $300 < LD_{50} \leq 2000$, that denotes harmful effects after swallowing in dose between 300-2000 mg). So the designed variations are safe in the dose at which Toloxatone is safe.

To analyse the toxicity of the variations, all variations were checked for carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity using ProTox-II server. The irritation and the reproductive effectiveness of the variations were checked using Osiris property explorer. The results obtained from the servers pointed out the variations 31 and 57 as reproductive effective and the variations 32, 43 and 44 as irritants (Suppl. Table 8). All the variations were found safe with a probability score of inactivity for immunotoxicity, mutagenicity, carcinogenicity and cytotoxicity in the range 0.95-0.99, 0.62-0.70, 0.52-0.64, and 0.53-0.65, respectively[38].

The compound 32 obtained by the substitution of C_6H_5 at R_1, had the lowest binding energy among all the substitutions. But this compound was found to be an irritant. The LigPlot diagram (both hydrophilic and hydrophobic interaction) of the next best variation 53 is given in (Fig. 3)

Thus from the detailed analysis carried out related to the drug properties and toxicities, the lower binding energy variations of Toloxatone like 27, 28, 34, 54 and 61 are impermeable through blood-brain-barrier. The variations 31, 33, 43, 44, and 57 were found to be toxic. So despite having lower MAO-A binding energy than Toloxatone, these toxic and non-permeable variations cannot be regarded as the favourable Toloxatone variations.

Structural variations of Brofaromine

(Fig. 1C) provides the two-dimensional figure of Brofaromine and the structural variations are obtained by the substitutions of different groups from R_1-R_6. The hydrophilic groups substituted on Brofaromine were OH, NH_2, Br and F. The hydrophobic groups substituted for the study were CH_3, CH_2CH_3, $CH(CH_3)_2$, $C(CH_3)_3$ and C_6H_5. The structural variations of Brofaromine are optimized and docked to MAO-A enzyme, to check the binding free energy of each structure (Table 2). From the docking results, substitutions of the hydrophilic groups were not found favourable in any of the positions R_1, R_2, R_3, and R_4. The substitutions of OH group together at R_1 and R_2 was favourable in providing the structure that binds to MAO-A more favourably than Brofaromine. OH group substitution at R_6 had decreased the MAO-A binding energy of Brofaromine. The substitution of NH_2 and F groups separately at R_5 had given two structures that bind more spontaneously than Brofaromine. The substitutions of hydrophobic groups $C(CH_3)_3$ and C_6H_5 at R_1, CH_3 and C_6H_5 at R_2, C_6H_5 at R_3 and $CH(CH_3)_2$, $C(CH_3)_3$ at R_4 (one group at a time) had decreased the MAO-A binding free energy of Brofaromine. At R_5, all the hydrophobic group substitutions had given positive results. At R_6, the substitution of hydrophobic group CH_3 had given favourable low binding energy structures. The compounds 67, 68, 69, 72, 74, 77, 79, 80, 82, 83, 84, 85, 86, 87, 89, and 90 possessed lower binding energy than Brofaroamine (more negative than −7.9 kcal/mol). So these structures are promising structures. Rest all the structures were omitted from the remaining analysis[39].

Table 3 indicated the residues of MAO-A enzyme that had formed hydrogen bonds and showed hydrophobic interaction (obtained from Ligplot$^+$) with all the promising variations of Brofaromine. The amino acid residues of MAO-A enzyme did not form hydrogen bond with the variations 67, 68, 80, 83, and 87. The table emphasised the hydrophobic interaction as the vital interaction that influenced the binding free energy of the variations of Brofaromine.

The oral activity of the structural variations that had lower binding free energy than Brofaromine was checked using the swissADME server. The log P values of the variations 67, 68, 74, 77, 79, 84, 85 were found slightly above 5. But these seven variations had other four Lipinski factors within the optimal required range. The remaining variations satisfied all the five rules of Lipinski. Thus all the variations are orally active and drug-like (Suppl. Table 9).

The pharmacological characteristics of all the low binding free energy variations were studied from SwissADME server. All the variations were found to have optimal polarity, flexibility and saturation as they possessed TPSA values below 75 $Å^2$, rotational

bond numbers below 9 and fraction csp3 above 0.25. All the variations were blood-brain barrier permeable and also they showed zero PAINS alert and high gastrointestinal absorption (Suppl. Table 10). Drug-likeness of the variations was confirmed by the bioavailability score of 0.55. Thus the variations exhibited good properties.

The rat oral LD_{50} (lethal dose, 50) and the toxicity class number of all the structural variations of Brofaromine were predicted using ProTox-II server (Suppl. Table 11). The results showed that Brofaromine possessed an LD_{50} value of 190 mg/kg and so it fell in toxicity class 3. All the variations except 77, 89, and 90 fell in the higher toxicity class number 4 (LD_{50} in the range $300 < LD_{50} \leq 2000$, that denoted harmful effects after swallowing in this dose range). Thus most of the Brofaromine variations were found safe in the dose at which Brofaromine was toxic. Only the compound 90 was predicted with 100% accuracy. The results of the remaining compounds had 67-68.07% accuracy in prediction.

To check whether the promising variations of Brofaromine exhibitted any toxic conditions, all variations were checked for carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity using ProTox-II server. The irritation and reproductive effects of the variations were analysed with Osiris property explorer. The compounds 69, 80, 82, and 84 were immunotoxic with a probability score of activity 0.92, 0.86, 0.65, and 0.91, respectively. The compound 82 was found to be carcinogenic and mutagenic with a probability score of activity 0.51 and 0.59, respectively. The compound 74 was found to be reproductively effective. None of the compounds were irritants (Suppl. Table 12). All the remaining variations were safe with a probability score of inactivity for immunotoxicity, mutagenicity, carcinogenicity and cytotoxicity in the range 0.61-0.91, 0.64-0.68, 0.53-0.61, and 0.55-0.61, respectively[40].

Among all the substitutions, the lowest binding free energy structure was the Variation 74 with a binding free energy of −8.9 kcal/mol. But it was found to be reproductively effective. The LigPlot diagram (hydrophobic interaction) of the next best Variations 68 and 84 is given in (Fig. 4)

Thus all the lower binding energy variations have very favourable drug related physico-chemical properties. But among the favourable variations, 69, 74, 80, 82, and 84 are toxic. So these five toxic variations are non-promising Brofaromine variations.

Structural variations of Selegiline

Figure 5A provides the two-dimensional figure of Selegiline and the structural variations are got by substituting hydrophilic and hydrophobic groups from R_1-R_5. The only hydrophilic group selected for the substitution on Selegiline is OH. The hydrophobic groups selected for the study were CH_3, $C(CH3)_3$, C_6H_5, C_6H_{11}, and $CH_2C_6H_5$. The structural variations obtained by the substitution of the selected groups from R_1 to R_5 were optimized and the optimized structures were docked to MAO-B enzyme. From the docking results given in (Table 2), the substitution of the OH (hydrophilic group) from R_1 to R_5 did not yield low binding energy structures. The substitution of heavy hydrophobic groups - C_6H_5, C_6H_{11}, and $CH_2C_6H_5$ (one at a time) were found favourable at R_1, R_2, R_3, and R_5 because the resultant structures had the MAO-B binding energy lower than that of Selegiline. The substitution of $C(CH_3)_3$ group had decreased the binding energy of Selegine only when this group was substituted at R_3 and at R_5. At R_4, none of the substitutions were found successful in yielding good low binding energy structures. The compounds 93, 94, 96, 99, 100, 101, 104, 105, 106, 108, 115, 116, and 117 had lower binding energy than Selegiline (more negative than −6.1 kcal/mol). So these variations are promising structures. The remaining structures were not taken for further analysis[41].

Table 4 listed the amino acid residues of MAO-B that had formed hydrogen bond and showed hydrophobic interaction with the low binding energy variations of Selegiline (Obtained from LigPlot$^+$). The table pointed out the hydrophobic interaction as the main interaction that had influenced the binding energy of all the structures. Only the variations 108 and 115 formed a hydrogen bond with the MAO-B enzyme. Among the structural variations of Selegiline, the lowest binding energy is shown by the compound 105, obtained by the substitution of C_6H_5 at R_3, (binding energy = −7.8 kcal/mol. The LigPlot diagram (hydrophobic interaction) of the variation 105 is given in (Fig. 6A).

In order to evaluate the drug-likeness of the favourable variations of Selegiline, the five Lipinski factors of the variations were analysed. The results pointed out all the variations as orally active molecules because all of them had five Lipinski factors within the optimal range. The Log P values of the variations fell in between 3-4.3. This indicates that the variations are Lipophilic. (Suppl. Table 13).

On analysing the physicochemical properties (obtained from SwissADME) possessed by all the low

binding energy variations, all the structural variations had very low TPSA values. The variations are saturated and flexible because the fraction csp^3 value and rotational bond numbers were above 0.25 and below 9, respectively. All the variations showed blood-brain barrier permeability and high gastrointestinal absorption. The variations showed no PAINS alerts (Suppl. Table 14). Thus the variations exhibited good drug properties[42].

The rat oral lethal dose of all variations along with their toxicity class number (obtained from Protox-II server) were studied for the lower binding energy variations of Selegiline (Suppl. Table 15). Selegiline fell in the toxicity class 4 (LD_{50} in the range $300 < LD_{50} \leq 2000$) with an LD_{50} of 385 mg/kg. The variations 94, 105, 108, 115, and 116 belonged to the toxicity class 3 (toxic effects if swallowed ($50 < LD50 \leq 300$). So these five variations were found toxic in the safe dose range of the drug Selegiline.

To verify the toxicity of the variations, all the variations were checked for carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity using ProTox-II server. The irritation and the reproductive effectiveness of the variations were checked using Osiris property explorer (Suppl. Table 16). The results showed that the variation 104 was both irritant and reproductively effective. The remaining variations were neither irritant nor reproductively effective. All the compounds were found to be free of carcinogenicity, immunotoxicity, mutagenicity and cytotoxicity with a probability score of inactivity in the range 0.63-0.67, 0.83-0.99, 0.73-0.78, and 0.70-0.85, respectively.

So from the analysis of the drug-related properties and the toxicities of the lower binding energy variations, only the variation 104 is toxic. So 104 is the only non-promising Selegiline variation.

Structural variations of Rasagiline

Figure 5B gives the 2D figure of the Rasagiline and the structural variations are got by substituting selected groups from R_1 to R_7. The hydrophilic and hydrophobic groups selected for the substitution at on Rasagiline were OH, NH_2, F, and CH_3, CH_2CH_3, $C(CH_3)_3$, C_6H_5, C_6H_{11}, respectively. The structures obtained by the substitution of these selected groups at the specified positions of Rasagiline were optimized and docked to the MAO-B enzyme. Only one group (either hydrophilic or hydrophobic) was substituted on Rasagiline for a structural variation. From the docking results (given in Table 2), OH group substitution on any of the four positions R_1, R_2, R_3, and R_5 had failed to decrease the MAO-B binding energy of Rasagiline. But the OH group substitution was found favourable at R_4, R_6, and R_7. NH_2 group substitution at all of the four positions R_1, R_4, R_5, R_6 had given the structures with binding energy more negative than that of Rasagiline. All the hydrophobic group substitutions were found favourable at R_2, R_3, R_5, R_6 and R_7 as the resultant structure had MAO-B binding energy lower than Rasagiline. Among all the hydrophobic groups, only the substitution of C_6H_5 and C_6H_{11} were favourable at R_1 and R_4, in providing lower binding energy structures. The structural variations 120, 121, 123, 124, 125, 126, 127, 130, 131, 132, 133, 139, 140, 141, 142, 143, 144, 145, 147, 148, 150, 151, 152, 153, 154, 155, 156, 157, and 158, that possessed lower binding energy than Rasagiline were taken for further analysis[43].

Table 4 listed the residues of MAO-B that had formed hydrophilic and hydrophobic interaction with the lower binding energy variations of Rasagiline. For Rasagiline, the main interaction that had influenced the binding free energy of all the variations is the hydrophobic one.

The drug-likeness of the favourable Rasagiline variations was checked using the SwissADME server. All the variations showed optimal values for the five Lipinski factors. Thus all the variations can be regarded as orally active molecules. All the compounds are lipophilic (Suppl. Table 17).

The physicochemical properties (obtained from SwissADME) of all the low binding energy variations of Rasagiline were analysed. The variations had low TPSA values. The variations were flexible with rotational bond numbers below 9. They showed high gastrointestinal absorption and zero PAINS alerts. The variations except 120, 126, 132, 139, 144, 151, and 158 were saturated molecules. All the variations showed blood-brain barrier permeability (Suppl. Table 18).

The rat oral LD_{50} (lethal dose, 50) and the toxicity class number of the variations of Rasagiline (obtained employing Protox-II server) were analysed. The Rasagiline fell in the toxicity class 3 and it had an LD_{50} of 250 mg/kg. Most of the designed variations fell in the higher toxicity class 4 ($300 < LD50 \leq 2000$ - harmful effects after swallowing in this dose range) or 5 ($2000 < LD50 \leq 5000$, that denotes 'could be harmful' situation after swallowing in this dose range). This shows that such variations are safe in the dose range at which Rasagiline is toxic (Suppl. Table 19).

To identify the toxicity of the structural variations of Rasagiline, all variations were checked for

carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, irritation and reproductive effectiveness (Suppl. Table 20). None of the variations were irritants and none of them showed reproductive effects. The compounds 120, 126, 132, 139, and 158 were mutagens with a probability score of activity 0.53, 0.63, 0.68, 0.53, and 0.56, respectively. All the remaining variations were found free of carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity with a probability score of inactivity in the range 0.63-0.70, 0.87-0.99, 0.52-0.78, and 0.58-0.85, respectively.

The compounds 133 (obtained by substituting C_6H_{11} at R_3) and 158 (obtained by substituting C_6H_5 at R_7) had the lowest binding free energies compared to all other substitutions. But the variation 158 was a mutagen. The LigPlot diagram (hydrophobic interaction) of the safe variation 133 is given in (Fig. 6B).

So from the detailed analysis of the drug properties, the lower binding energy Rasagiline variations have favourable physico-chemical properties. But the variations 120, 126, 132, 139, and 158 are mutagens. So despite their good drug properties, these five mutagens are the non-promising variations of Rasagiline[44].

Conclusion

In the area of drug designing, computational studies have gained greater attention because very in depth and fast drug screening is possible without much economic burden. A very extensive computation studies always benefits the synthesis with very promising drugs. In this present study, non-promising structural variations of the selected MAO-A and MAO-B drugs are omitted through the analysis of properties, drug-likeness and toxicities. Listing the favourable variations through such extensive screening will definitely increase the success rate of drug designing.

Among the designed structural variations of Moclobemide, the variation 10 was exempted from the analysis because of its higher MAO-A binding energy compared to Moclobemide. All the variations except 2, 3, 7, and 25 are safe because they are free from carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, irritation, and reproductive effectiveness. The variations 4, 12, 13, 15, 19, and 24 are non-favourable variations because they are impermeable to blood-brain-barrier. So on screening the variations based on drug properties, toxicities, and binding energies, the compounds 1, 5, 6, 8, 9, 11, 14, 16-18, 20, 21-23, and 26 are the promising structural variations of Moclobemide.

In the designed variations of Toloxatone, all the variations except 40, 41, 48, 49, 55, 58, and 62 had low binding energy than Toloxatone. So these seven variations were not analysed further. Among the lower binding energy variations, all the compounds except 31, 33, 43, 44, and 57 are non-toxic molecules. The variations 27, 28, 34, 54, and 61 are impermeable to blood-brain-barrier. So the variations 29, 30, 32, 35, 36, 37, 38, 39, 42, 45, 46, 47, 50, 51, 52, 53, 56, 57, 59, 60, and 63 with favourable drug properties, oral activity, safety and lower binding energy are promising structures for further drug studies.

Among the designed variations of Brofaromine, all the variations except 69, 74, 80, 82, and 84 are safe molecules. So the non-toxic structural variations of Brofaromine - 67, 68, 72, 77, 79, 83, 85, 86, 87, 89, and 90 which have favourable drug properties, oral activity and lower binding energy than Brofaromine are the promising structures for further drug studies.

In the matter of Selegiline, the non-promising variations 91, 92, 95, 97, 98, 102-104, 107, and 109-114 are omitted because of their higher MAO-B binding energy than Selegiline. Among the lower binding energy variations, all the compounds excluding 104 are non-toxic molecules. The structural variations of Selegiline 93, 94, 96, 99, 100, 101, 105, 106, 108, 115, 116, and 117 are promising structures for the future drug research because these are safe molecules with good physico-chemical properties and drug-likeness.

Among the designed variations of Rasagiline, all the low binding energy variations except the compounds 120, 126, 132, 139, and 158 are non-toxic molecules. They have favourable drug-properties and oral activity. Thus all the non-toxic variations 118-158, 118, 119, 121-125, 127-131, 133-138, and 140-157 are promising structural variations of Rasagiline.

This work involves in-depth analysis of the various factors associated with the drug molecule and we have come up with the promising variations of Moclobemide, Toloxatone, Brofaromine, Selegiline and Rasagiline. This work would definitely be beneficial for the future studies associated with the synthesis of MAO inhibitors.

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Conflict of interest

All authors declare no conflicts of interest.

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