A Computational Approach to Find Alternative Drugs for Managing Depression

By

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A thesis submitted to the Department of Pharmacy in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy(Hons)

Department of Pharmacy
Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

Student's Full Name & Signature:

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Approval

The thesis titled "*A Computational Approach to Find Alternative Drugs for Managing Depression*" submitted by Musharrat Shaheed Mubashira (16146005) of Spring2016 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on 1st March,2020.

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Ethics Statement

The study does not involve any kind of animal trial or human trial.

Abstract

Depression is the most prominent disorder in the field of neuropsychiatry affecting more than 300 million people worldwide, according to Global Burden of Disease report, 2020. Frequent occurrences of depressive episodes among the treated patients suggests that clinically used antidepressants have become resistant. As searching for a new drug can be time consuming and costly, an *in-silico* based study was conducted to repurpose approved drugs to be used in depression. Pathogenesis of depression shows that human monoamine oxidase A protein (MAOA) plays a key role in degrading notable neurotransmitters and so this protein was studied. Through molecular docking, binding affinity of around hundreds of drugs and some natural small molecules with the protein was evaluated. Furthermore, superimposition and protein-ligand interactions were visualized and assessed. It was found that Glimepiride, an anti-diabetic agent from the synthetic drugs and Curcumin from the natural small molecules have possible antidepressant properties.

Keywords: MAO-A; depression, protein, neurotransmitters, drugs, small molecules, molecular docking.

Dedication

Dedicated to my parents and to all individuals with depression.

Acknowledgement

To begin with, all praises and glory to Allah, He Who is Al-Hakeem, the One with the ultimate wisdom and He Who has guided me through my whole academic career.

I would like to show my heartfelt gratitude towards my respected supervisor, Professor Dr. Eva Rahman Kabir, chairperson of Department of Pharmacy, Brac University as she has helped me completing this thesis with her constant support, intelligence and assistance. Due to her consistent and excellent supervision, I finished my work in an effective and efficient manner. Her depth of knowledge and dedication towards work has inspired me in a great way. It was a great privilege conducting this thesis under her guidance.

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Last but not the least, I would like to thank my family for believing in me and my potentials and for staying beside me in reaching my goals.

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List of Acronyms

MAO-A	Mono Amino Oxidase A
WHO	World Health Organization
5-HTR	5- Hydroxy Triptamine Receptor
DPP-4	Dipeptyl peptidase- 4
5-HIAA	5- Hydroxyindolacetic acid
PDB	Protein Data Bank
BBB	Blood brain barrier
5-HT	5- Hydroxy Triptamine

Chapter 1

Introduction

1.1 Depression

Depression is categorized as the single largest factor of global disability, affecting over 300 million people, according to WHO (Stringaris, 2017). It is one of the most prevalent mental disorder that usually remains undertreated and underdiagnosed, which pictures the urge to expand the scope of contemporary drug screening methods for depression (Eichstaedt *et al.*, 2018). The prevalence of this disorder turns out to be quite similar if we compare high-income countries (5·5%) with low-income and middle-income countries (5·9%), suggesting that depression occurs neither for having a modern complex life-style nor for poverty (Malhi and Mann, 2018).

Depression initially starts with having feelings of anxiety. These problems can be chronic or repetitive and often lead to substantial disablement, hampering an individual's ability to do day to day regular tasks and responsibility. In the worst scenario, this mental illness leads the way to suicide (Dine and Dine, 2010). WHO stated that, approximately 800 000 people die due to suicide every year (*Depression,* 2019).

Depression can be a lifelong recurrent illness. Major depressive disorder (MDD) having patients refers to those that are not any more symptomatic and have retrieved their usual function after having an episode or occurrence of major depression (Malhi and Mann, 2018). Around 20–30% of patients of MDD face a chronic course of disease, developing a poor quality of life with increased cost and care utilization (Bennabi *et al.*, 2019). This disease has been linked to comprehensive cognitive dysfunction which correlates in the areas of information processing speed, attention, executive functioning and memory (Gudayol-ferré and Duarte-rosas, 2019). MDD has heterogeneous symptoms which eventually makes it

complex to treat. These symptoms reduce individual's ability to conduct daily life function and rapidly increase the likelihood of suicide (Chesney, Goodwin and Fazel, 2014). There is a strong challenge for physicians to treat patients with MDD effectively as there remains a complex network of symptoms. Although all antidepressant drugs have quite similar efficacy rates, patient response regarding these drugs varies (Culpepper, Muskin and Stahl, 2015). Pharmacologic approaches still remain the keystone of treatment, but response rates varies and remission of the symptoms is seen in minority of sufferers (from 30 to 45%) (Bennabi *et al.*, 2019).

Symptoms mostly include:

- Depressive mood
- Anhedonia (not feeling good or pleasurable in normally pleasurable tasks)
- Feelings of guilt or worthlessness
- Suicidal anticipation
- Fatigue or feeling of tiredness
- Hypersomnia (excessive sleep) or insomnia (lack of sleep)
- Weight and appetite (gain or loss)
- Reduced focus or concentration, or indecisiveness

Psychomotor retardation (slowing down of thoughts and physical movements) or agitation (Malhi and Mann, 2018).

1.2 Rationale for finding new potential agents for major depressive disorder:

The key treatment strategy for alleviating the symptoms of depression is to enhance different monoaminergic neurotransmitters, for instance dopamine, norepinephrine and serotonin, but in actual fact, this effect is frequently seen to work 4 weeks after the initiation of treatment.

About the first generation of antidepressants, they were believed to work by enhancing central serotonergic and noradrenergic activity, but they were reported to be equally ineffective. Afterwards, second-generation antidepressants that were acknowledged to focus a single particular neurochemical system (dopamine, norepinephrine, or serotonin) also have low success rate in treating patients with depression.

Furthermore, various antidepressants in a same particular class (e.g. different selective serotonin-reuptake inhibitors, SSRIs) may not give similar result in the same patient; in particular, a patient may show good response to Fluoxetine but may not response to Escitalopram.

In short, most of the antidepressants do not work rapidly — a feature that remains critical in treating individuals with suicidal thoughts (Coccaro, 2019).

Therefore, it can be clearly seen that, there is a tremendous necessity for developing more effective, rapid acting anti-depressant agents that are suitable for all types of patients with depression.

1.3 Rationale for Drug Repurposing:

As traditional de novo drug discovery takes several years (10-17 years) to develop and reach the market, new expeditious approaches to find new drug candidates is much needed. There are some undesirable drawbacks of the traditional approach of drug discovery as:

- It is a time-consuming process.
- Huge investment in development.
- Number of new drugs developed by this method has significantly decreased over the past decades.
- Drug testing procedures and clinical trials which are lengthy.
- High failure rates (Shim and Liu, 2014)

For this reason, to find a potent candidate in an effective and efficient manner, repurposing of existing drugs has been the most popular choice lately. Drug repurposing (also known as therapeutic switching, 'repositioning') indicates recognizing novel therapeutic indications of drugs, those have formerly acquired regulatory acceptance or been frequently tested in different phases of clinical trials for other disease or disorder indications. There is a huge advantage in scrutinizing approved drugs, or medications that have been previously evaluated in different clinical trials. For instance, different pharmacokinetics properties, toxicology results, medicinal chemistry and dosing profiles are already documented from previous clinical trials and use. This accelerates the process in finding new indications and eventually minimizes the risk of failure because of poor pharmacokinetic properties or serious adverse effects or side effects for which novel drug entities are usually discarded (approximately 90%). As a consequence, repurposed drugs can effectively reach the clinic in less time and with lower clinical development costs relative to novel drug entities (Lago and Bahn, 2019).

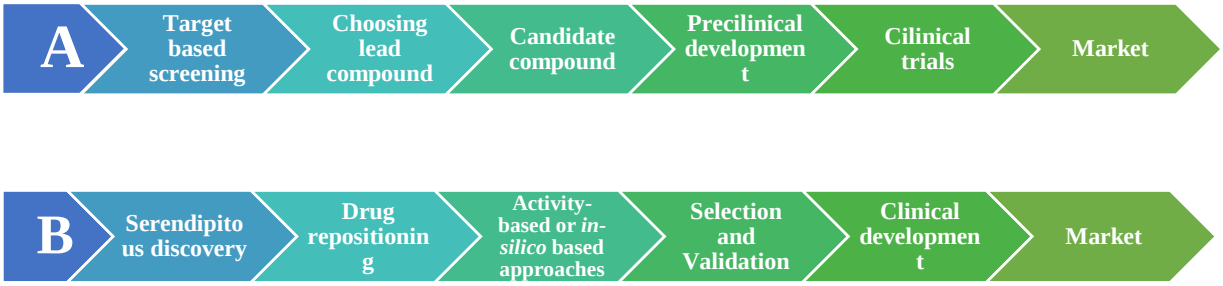


Figure 1: Flowchart representing (A) Traditional drug discovery and development and (B) drug repurposing (Kim, 2015)

1.4 Targeted Protein: Human Mono Amine Oxidase A (MAO-A)

Since the discovery of monoamine oxidase (MAO) during 1960's, scientists have been trying to decipher the underlying mechanisms of MAO in mental disorders, notably depression. In addition to that, inhibitors of MAO have been considered as important agents to treat different psychiatric conditions (Duncan, Johnson and Ou, 2012). Out of the several theories that explains the neurobiological causes of depressive symptoms, one well-documented theory is the serotonin theory (also known as monoamine theory or monoamine hypothesis of depression). The theory hypothesized that decreased 5-HT (5-hydroxytryptamine) levels in brain eventually cause different depressive symptoms and MAOA is the lead protein in degrading 5-HT to 5- hydroxyindoleacetic acid (5-HIAA). MAO-A is often used as a biological marker in human brain imaging studies of psychiatric diseases, including depression.

Monoamine oxidase (MAO) plays catalytic role by oxidizing monoamines. For a long time, this action has been viewed as an interesting drug target site for different neuropsychiatric disorder (Lee *et al.*, 2017). MAO has two isoforms, they are: MAO-A and MAO-B. These are synthesized by prominent genes, and these two have different substrate specificity. For instance, MAO-A particularly deaminates serotonin. On the other hand, MAO-B deaminates

benzylamine and phenylethylamine (Youdim, Edmondson and Tipton, 2006). Moreover, for depression and anxiety, MAO-A is selectively targeted, whereas MAO-B is particularly targeted in Alzheimer's and Parkinson's diseases. Hence, MAO-A inhibitors have been extensively studied in the field of depression (Lee *et al.*, 2017) . However, MAOA inhibitors are scarcely used now-a-days for depression since they have potential side effects (Higuchi, Soga and Parhar, 2017).

1.5 Mechanism of Action of MAO-A

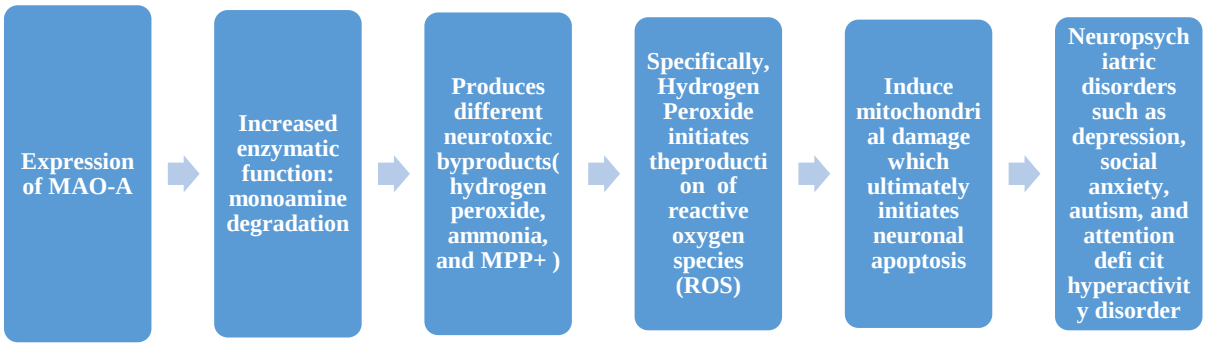


Figure 2: Mechanism of Action of MAO-A in Major Depressive Disorder (Duncan, Johnson and Ou, 2012)

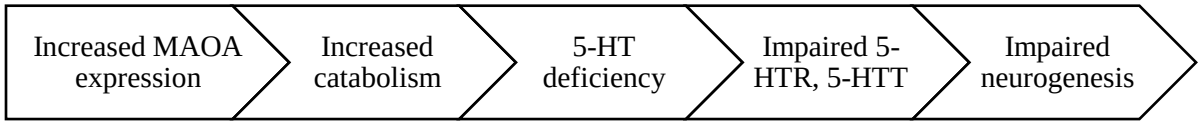


Figure 3: Significance of MAO-A in Major Depressive Disorder. Here, MAOA = MonoAmine Oxidase A, 5-HT= 5-Hydroxytryptamine, 5-HTR= 5-Hydroxytryptamine Receptor (Naoi, Maruyama and Shamoto-Nagai, 2018).

1.6 Selection of Drugs

Different drugs have been found to have alternative therapeutic indications. For instance, repurposing CNS drugs for treating cancer(Abdelaleem *et al.*, 2019). Therefore, to find possible candidates for depression, different classes of drugs were select to learn whether they have anti-depressant properties or not.

1.6.1 Anti-Diabetic Drugs

Anti-diabetic drugs are used either alone (as monotherapy) or in combination. They are generally safe and well tolerated by patients. The mode of action of these drugs are not identical to each other as they vary in pathways and doses. However, as a whole, anti-diabetic drugs have one certain objective which is to lower blood sugar/glucose levels. Therefore, almost all of the mechanisms of action of these drugs are either directly or indirectly connected with glucose metabolism (Meneses *et al.*, 2015).

There are different classes of anti-diabetic drugs. These include (1) drugs that potentiate insulin production in the pancreas (sulfonylureas, meglinitides), (2) drugs that increase insulin sensitivity of the target organs (thiazolidinediones, biguanides), and (3) drugs that lower the rate of glucose absorption in gastrointestinal tract (DPP4 inhibitors) (Hossain and Pervin, 2018).

Among the anti-diabetic agents, Glimepiride is one of the sulfonylureas that is used for the treatment of type-2 diabetes. Sulfonylureas has been used broadly for about 50 years. They are divided into 2 groups or generations. Among them, first generation sulphonylureas, for instance Chlorpropamide and Tolbutamide, are no longer in use due to their adverse hypoglycemic effect. On the other hand, second generation drugs (such as Gliclazide, Glimepiride, Glipizide) are cost-effective, efficacious and have a high tolerance rate by patients. (Sola *et al.*, 2015). The safety and efficacy of sulphonylureas (second generation) in type 2 diabetes are evaluated thoroughly by different noncomparative and comparative studies. Glimepiride works as an insulin secretagogues, which means it stimulates the secretion of insulin by pancreas. It binds to the receptors, situated on the ATP (adenosine triphosphate) dependent potassium channels on pancreatic cell surface. Later, the channels are closed causing membrane depolarization which eventually results in efflux of potassium,

influx of calcium and release of insulin (Davis, 2004).In comparison to other sulphonylureas, glimepiride has less side effects including adverse cardiovascular reactions (Nakamura *et al.*, 2014).

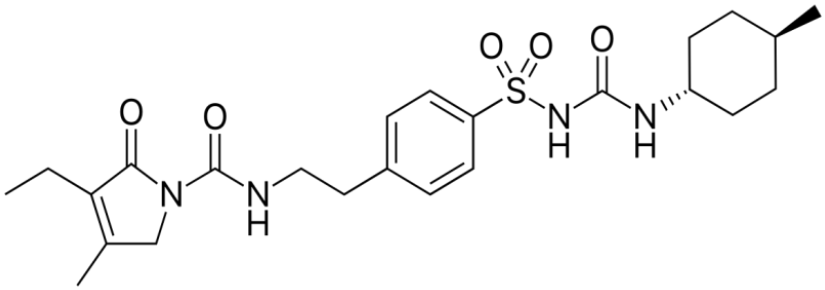


Figure 4: 2D structure of Glimepiride

Another important class of anti-diabetic agents is the DPP-4 inhibitors. Denagliptin and Omarigliptin are selective DPP-4 (dipeptidyl peptidase-4) inhibitor with potent hypoglycemic activity. DPP-4 inhibitors have showed good cardiovascular safety. They inhibit the secretion of glucagon (a peptide hormone which increase glucose level) by elevating the levels of GLP-1 (glucagon like peptide-1) without an inherent hypoglycaemia risk. Currently, sulphonylureas have been increasingly replaced by DPP-4 inhibitors. They are now used as a second line therapy if diabetes treatment with metformin fails (Gallwitz, 2019).

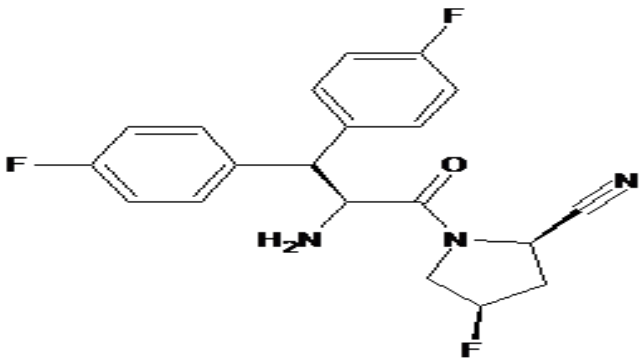


Figure 5: 2D structure of Denagliptin

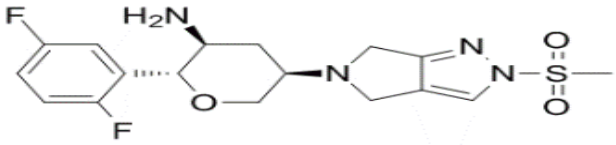


Figure 6: 2D structure of Omarigliptin

1.6.2 Anti-hypertensive drugs

Adult hypertension is usually treated with different antihypertensive medication therapy as well as with change in lifestyle measures (S.Aronow, 2018). To get an effective outcome, multiple antihypertensive drug therapy with least side effects is given. A total of five major pharmacological categories of antihypertensive drugs are used: diuretics, beta blockers, calcium channel blockers, angiotensin II receptor antagonists, angiotensin converting enzyme inhibitors (Laurent, 2017).

1.6.3 Anti-Histamines

To treat allergic conditions, antihistamines are extensively used. Due to histamine release, itching and inflammation occurs. Antihistamines are useful in treating these patient symptoms. They usually bind to the histamine receptors on cell surface. Later, they inactivate the stabilization of receptors and relief from the symptoms (Randall and Hawkins, 2018).

First generation antihistamines like chlorphenyramine, terfenadine show more cholinergic and sedative activity than second generation antihistamines (bilastine, rupatadine, desloratadine). Second generation antihistamines have less side effects than the first ones (Kuna *et al.*, 2016).

1.6.4 Natural Small Molecules

Natural small molecule compounds are usually secondary metabolites extracted from plants. They have diversified structures, types, actions and stable resources. Among them, those with

clinical efficacy and effective pharmacological actions have been extensively used clinically for treating different human diseases (Du, 2018).

1.7 Aim of the study

The aim of the study is to propose new potent candidates from approved synthetic drugs and natural molecules to be used in major depressive disorder.

Chapter 2

Methodology

To find the potential candidates, molecular docking was performed and further evaluation was done using different software and tools of computational biology. Three different classes of drugs (anti-diabetic, anti-hypertensive and anti-histamines) and different natural small molecules were screened rigorously to get the desired result.

For molecular docking, selected protein (macromolecule) and drugs (ligands) were prepared separately in Autodock Vina. Regarding the selection of protein, some factors were taken into considerations:

- The protein must have a full crystal structure.
- There should be no mutations in the structure.
- The protein must be from the organism: *Homo sapiens*.
- Low value for resolution was chosen. As structures with low resolution has been found useful in predicting annotation and function (Zhang *et al.*, 2018).
- Recent published protein was chosen (latest publication).

Based on these factors and as MAO-A was targeted for depression, PDB ID: 2z5x was focused as the desired protein structure.

All the drugs and natural small molecules were taken as 3D structure from PubChem. No further modification was done.

Table 1: MAOA Protein information obtained from RCSB PDB

RCSB PDBID	Protein Name	Mutation	Chain	Publication year	Resolution
2z5x	Crystal Structure of Human Monoamine Oxidase A with Harmine	0	A	2008	2.2 Å

2.1 Software and Tools used

Different software and tools were used or validation, screening and visualizing the protein-ligand model. These software and tools enhanced the quality and accuracy of this screening of candidates. In addition, they made the *in-silico* investigation expeditious.

PyMOL ((1.7.4)

PyMOL is basically a molecular graphics tool, that has been extensively used for 3D visualization of small molecules, nucleic acids, proteins, surfaces, electron densities and trajectories. It is a cross-platform tool for advanced visualization and functional analysis. PyMOL plays a noteworthy role in virtual screening of protein or ligands, molecular simulations and ligand-protein modelling. The computational field for discovering new drug candidates for different targets has been using this tool successfully (Yuan, Chan and Hu, 2017).

Open Babel GUI (2.4.1)

Open Babel is a chemical toolbox that can speak different languages of chemical data. Open Babel can interconvert more than 110 formats. To represent a broad molecular and chemical

data, a library is needed which applies a large-scale algorithms of cheminformatics – Open Babel is one such tool which has this type of library (Boyle *et al.*, 2011).

Discovery Studio (16.1.0.15350)

Discovery studio can design, stimulate and analyze small molecule, macromolecule, analyze receptor-ligand interactions, pharmacophore modeling *(Portfolio, 2000).*

Autodock Vina (1.5.6)

Compared to other molecular docking tools, Autodock Vina has high speed magnitude (around two orders). It calculates the grid maps automatically and gives a result that is transparent and understandable to the user (Trott and Olson, 2010).

2.2 Working Process

To begin with, the structure of the protein was downloaded from RCSB PDB (protein data bank) as pdb format. The protein was curated in PyMOL and then further prepared as pdbqt file in Autodock Vina.

In case of the drugs and natural small molecules, all the structures were downloaded from Pubchem. Using Open Babel GUI, the sdf files of the drugs were converted to pdb files and were saved.

After the protein and the drugs were prepared and saved, molecular docking was done in Autodock Vina. Drugs that showed binding affinities which were higher than the standard drug (Mirtazapine) were selected for further evaluation.

Furthermore, selected drugs were superimposed with the standard drug in PyMOL. All superimposed drugs were then evaluated through Discovery Studio where non-bonded interactions between protein and ligands were observed. Finally, admetSAR properties of the

selected drugs were checked regarding their absorption, distribution, metabolism, excretion and toxicity.

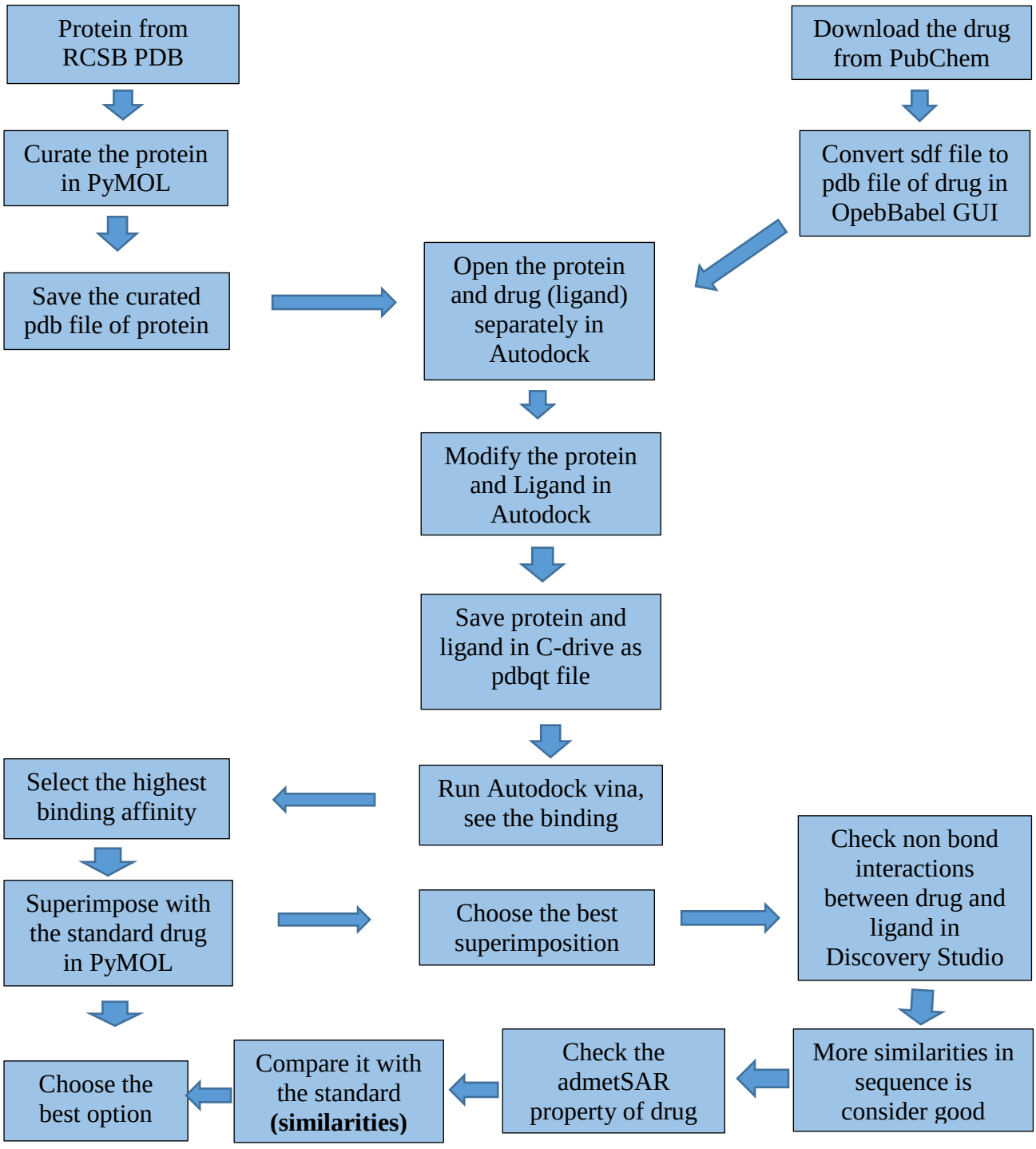


Figure 7: Steps involved in molecular docking and in-silico screening.

Chapter 3

Validation and Result

The protein of interest was taken from RCSB PDB, a protein data bank. RCSB PDB provides validated protein. Therefore, the structure of the protein was not further validated. The results obtained from this study are discussed further.

3.1 Protein structure

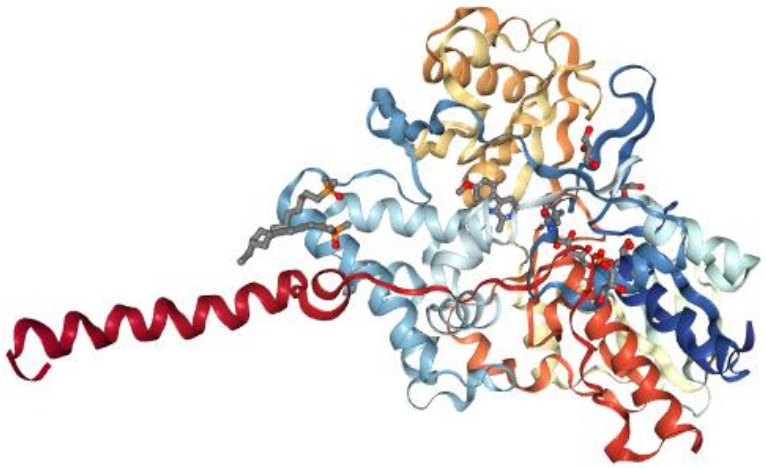


Figure 8: 3-dimensional structure of MAO-A protein obtained from RCSB PDB, PDBID: 2z5x (Crystal Structure of Human Monoamine Oxidase A with Harmine)

The 3-D model of the structure was visualized in PyMOL.

3.2 *In-silico* screening of synthetic drugs and natural molecules

All the structures of ligands were downloaded from PubChem. They were saved as SDF files. Using Open Babel GUI, the structures were converted to PDB files. Further process was done with these PDB files. In case of molecular docking, rigid docking was done as it gave higher binding affinity value than flexible docking. Binding affinities were noted down for anti-diabetic drugs, anti-hypertensive drugs, antihistamines and different natural small molecules.

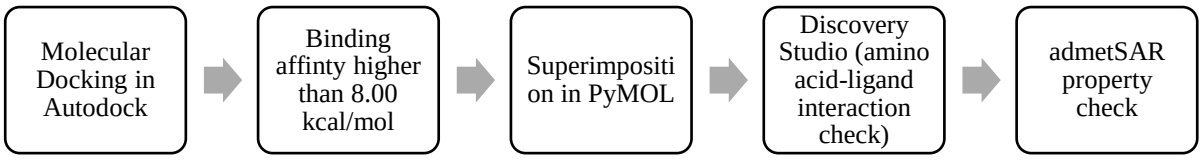


Figure 9: Steps involved in screening

3.2.1 Selection of standard drug

Mirtazapine is a noradrenergic and specific serotonergic antidepressant (NaSSA) that is approved in many counties for use in the treatment of major depression. Monotherapy with mirtazapine 15-45 mg/day leads to rapid and sustained improvements in depressive symptoms in patients with major depression, including the elderly. It is as effective as other antidepressants and may have a more rapid onset of action than selective serotonin reuptake inhibitors (SSRIs). Mirtazapine is generally well tolerated in patients with depression (Croom, Perry and Plosker, 2009).

Mirtazapine was chosen as the standard drug. Its binding affinity was 8.00 kcal/mol. For this reason, repurposed drugs having a binding affinity above 8.00kcal/mol were chosen for further screening process.

3.2.2 Rigid docking results of synthetic drugs and natural small molecules

Around 100 drugs of three different classes (anti-diabetic, anti-hypertensive, antihistamines) and some natural small molecules were docked using rigid docking. As flexible docking result showed poor binding affinities, rigid docking results were taken. The binding affinities are listed below in table 3, 4, 5 and 6.

Here, reference drug and standard drug are used interchangeably.

Table 2: Rigid docking results of anti-diabetic drugs with MAO-A (2z5x)

SL no	Anti- Diabetic Drugs with MAOA (PDBID:2z5x)	Binding affinity (kcal/mol)
1	ABT(341)- analog 17	-11.5
2	ABT (341)- analog 22	-9.4
3	Denagliptin	-9.1
4	Glimepiride	-9.7
5	Gliquidone	-9.3
6	Glisoxepide	-9.2
7	Omarigliptin	-8.4

For anti-diabetic drugs, only 7 drugs showed higher binding affinities than the reference drug Mirtazapine. Furthermore, only 3 among them superimposed with the standard drug: Glimepiride, Denagliptin and Omarigliptin. Others were then discarded from the screening process.

Table 3: Rigid docking results of anti-hypertensive drugs with MAO-A (2z5x)

SL no	Anti-hypertensive Drug with MAOA (PDBID:2z5x)	Binding affinity (kcal/mol)
1	Bendroflumethazide	-10.00
2	Cyclothiazide	-8.4
3	Efonidipine	-10.5
4	Ketanserin	-9.4
5	Rescinnamine	-10.5
6	Losartan	-9.1

6 anti-hypertensive drugs were found to have higher binding affinities than the standard drug, Mirtazapine. Again among them, only Efonidipine and Cyclothiazide superimposed with the reference drug. So, these two were selected for further evaluation.

Table 4: Rigid docking results of antihistamines with MAO-A (2z5x)

SL no.	**Antihistamines with MAOA (PDBID:2z5x)**	**Binding affinity (kcal/mol)**
1	Ebastine	-10.9
2	Fexofenadine	-10.3
3	Levocabastine	-9.1
4	Bilastine	-9.2

In case of antihistamines, 4 drugs in total showed higher binding affinities. However, the superimposition result was not so satisfied. Only Fexofenadine superimposed with the reference drug. Others were immediately eliminated for further evaluation.

Table 5: Rigid docking results of natural small molecules with MAO-A (2z5x)

SL no	**Natural small molecule with MAOA (PDBID:2z5x)**	**Binding affinity (kcal/mol)**
1	Catechin	-10.1
2	Curcumin	-9.5
3	Epicatechin gallete	-9.8
4	Epicatechin	-11.0
5	Epigallocatechin	-10.1
6	Theaflavin	-11.3

Natural small molecules showed pretty good results. 6 of them showed higher binding affinities in rigid docking. Out of them, Curcumin, Epicatechin, Epigallocatechin and Theaflavin superimposed well with the reference drug. They were selected for further evaluation.

3.2.3 Superimposition of ligands with standard drug

The drugs that were found to have higher binding affinity than the standard drug Mirtazapine, were selected. These drugs were further evaluated in PyMOL to check whether they superimpose with the standard one or not. The drugs that superimposed appropriately with the standard drug were taken for further evaluation, others were discarded.

3.2.3.1 Superimposition of Anti-diabetic drugs with Mirtazapine

Only 3 drugs were found to have superimposed with the standard drug. These are Glimepiride, Denagliptin and Omarigliptin.

Table 6: Anti-diabetic superimposition with Mirtazapine in PyMOL

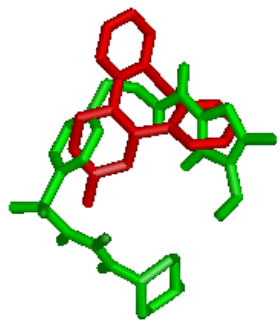
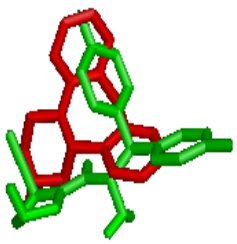
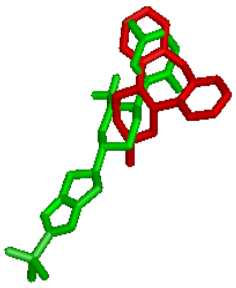
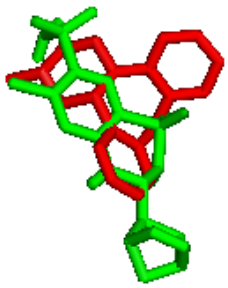
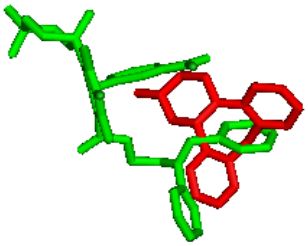
		

Figure 10: Superimposition of Glimepiride (green) and Mirtazapine (red)	*Figure 11: Superimposition of Denagliptin (green) and Mirtazapine (red)*	*Figure 12: Superimposition of Omarigliptin (green) and Mirtazapine (red)*

3.2.3.2 Superimposition of Anti-hypertensive drugs with Mirtazapine

Out of all, only two drugs were seen to superimpose. These are Efonidipine and Cyclothiazide.

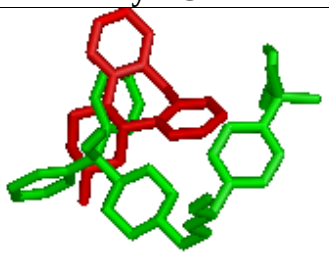
Table 7: Anti-hypertensive superimposition with Mirtazapine in PyMOL

 *Figure 13: Superimposition of Cyclothiazide and Mirtazapine* *Red= Mirtazapine, green= Cyclothiazide*	 *Figure 14: Superimposition of Efonidipine and Mirtazapine* *Red= Mirtazapine, green= Efonidipine*

3.2.3.3 Superimposition of Anti-histamines with Mirtazapine

Among all the anti-histamine drugs, only Fexofenadine superimposed with the standard drug.

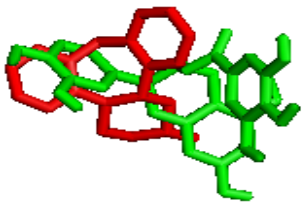
Table 8: Anti-Histamines superimposition with Mirtazapine in PyMOL

Drug name	Superimposition with Mirtazapine in PyMOL
Fexofenadine	 *Figure 15: Superimposition of Fexofenadine and Mirtazapine* *Red= Mirtazapine, green= Fexofenadine*

3.2.3.4 Superimposition of Natural small molecules with Mirtazapine

Superimposition in PyMOL showed that four natural small molecules superimpose with Mirtazapine. These are curcumin, theaflavin, epicatechin gallate and epigallocatechin. These superimpositions are showed in a table.

Table 9: Natural small molecules superimposition with Mirtazapine in PyMOL

<table>
<tr>
<td> 
Figure 16: Superimposition of Epicatechin gallate and Mirtazapine

Red= Mirtazapine, green= Epicatechin Gallete</td>
<td> 
Figure 17: Superimposition of Curcumin and Mirtazapine

Red= Mirtazapine, green= Curcumin</td>
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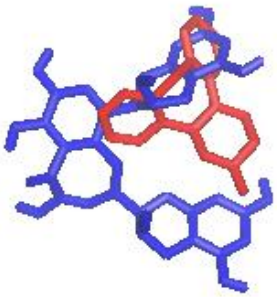
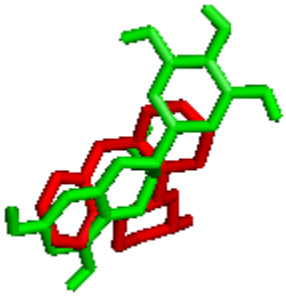
<td> 
Figure 18: Superimposition of Theaflavin and Mirtazapine

Red= Mirtazapine, blue= Theaflavin</td>
<td> 
Figure 19: Superimposition of Epigallocatechin and Mirtazapine

Red= Mirtazapine, blue= Epigallocatechin</td>
</tr>
</table>

3.2.4 Non-bond interaction visualization through Discovery Studio

Drugs that superimposed well with the standard drug were further evaluated by Discovery Studio.

Table 10: List of drugs and natural molecules superimposed

Anti-Diabetic Drugs	**Anti-Hypertensive Drugs**	**Anti-Histamines**	**Natural Small Molecule**
Glimepiride	Efonidipine	Fexofenadine	Theaflavin
Denagliptin	Cyclothiazide		Curcumin
Omarigliptin			Epigallocatechin
			Epictechin Gallete

Discovery Studio is useful in visualizing the bonds between the amino acids of the proteins and the ligands, their category and type of bond, their length and distance. To measure the similarities and dissimilarities between the standard drug and the repurposed drugs, non-bond interactions were visualized in Discovery Studio ("Discovery Studio Predictive Science Application, Dassault Systèmes BIOVIA", 2020).

Hydrophobic interactions and hydrogen bonding are two weak intermolecular interactions that plays a key role in stabilizing ligands (energetically-favored) within a protein structure (Varma *et al.*, 2010). Again the hydrophobic bonds are very crucial for any bond as they can increase binding affinity greatly by around 3.2 times when single methyl group is added. Therefore, hydrogen and hydrophobic bonds will be considering as a good parameter for strong interaction.

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3.2.4.1 Non-bond interactions between MAO-A (2z5x) with Mirtazapine

For Mirtazapine (standard drug), amino acid GLU185 can be seen to bond with the OE1 ligand from the drug. In addition to that, aa (amino acid) ASP328 is bonded with Carbon and Oxygen atoms and aa GLU329 is bonded with Nitrogen atom of the ligand. Other amino acids can be seen form bonds with UL (Unknown Ligand). All the distance range from 2-5. There are four hydrophobic bonds and one hydrogen bond suggesting strong interaction. The types of bonds were carbon-hydrogen bond, amide-pi stacked bond, alkyl and pi-alkyl bond.

Table 11: Protein-ligand interaction between MAOA (2z5x) with Mirtazapine

Protein-ligand interaction	Distance	Type	Catagory of Bond
:UNL1:H7 - A:GLU185:OE1	2.87919	Carbon Hydrogen Bond	Hydrogen Bond
A:ASP328:C,O;GLU329:N - :UNL1	4.76908	Amide-Pi Stacked	Hydrophobic
A:ARG172 - :UNL1	5.0404	Alkyl	Hydrophobic
:UNL1 - A:LEU176	4.66861	Pi-Alkyl	Hydrophobic
:UNL1 - A:ARG172	4.24596	Pi-Alkyl	Hydrophobic

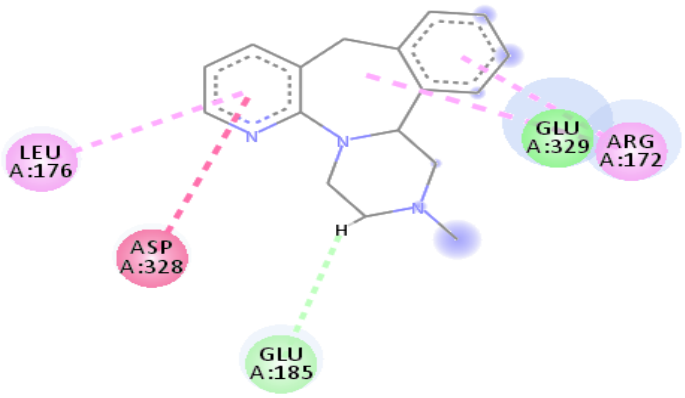

Figure 20: Non Bond Interaction of Mirtazapine with MAO-A (2z5x) 2D structure

3.2.4.2 Non-bond interactions between MAO-A (2z5x) with

Anti-Diabetic drugs

Table 12: Protein-ligand interaction between MAO-A (2z5x) and 3 anti-diabetic drugs (Glimepiride, Denagliptin, Omarigliptin)

Ligand	Protein-ligand interaction	Distance	Type	Catagory of Bond
Glimepiride	A:**ASP328**:N - :UNL1:O	3.06824	Conventional Hydrogen Bond	Hydrogen Bond
	:UNL1:H24 - A:**ARG172**:O	2.65355	Carbon Hydrogen Bond	Hydrogen Bond
	A:**GLU185**:OE1 - :UNL1	3.67781	Pi-Anion	Electrostatic
Denagliptin	A:**GLU329**:CD - :UNL1:F	3.39571	Halogen (Fluorine)	Halogen
	A:**GLU329**:N - :UNL1	4.15437	Pi-Donor Hydrogen Bond	Hydrogen Bond
	:UNL1 - A:**ARG172**	5.14088	Pi-Alkyl	Hydrophobic
	:UNL1 - A:**LEU176**	5.24335	Pi-Alkyl	Hydrophobic
Omarigliptin	:UNL1:H - A:**GLU185**:OE1	2.18303	Conventional Hydrogen Bond	Hydrogen Bond
	:UNL1:H6 - A:**GLU185**:OE1	2.93863	Carbon Hydrogen Bond	Hydrogen Bond
	A:**ARG172**:O - :UNL1:F	2.77466	Halogen (Fluorine)	Halogen
	:UNL1 - A:**LEU176**	4.8608	Pi-Alkyl	Hydrophobic

For Glimepiride, there are three amino acids that are similar to the standard drug These are ASP328, ARG172 and GLU 185. Only these threes are shown in the table. Among them, amino acid GLU185 is bonded with the OE1 ligand from the drug. There are, in total, four hydrogen bonds and three hydrophobic bonds were found, suggesting strong interaction. Thus, Glimepiride can be selected.

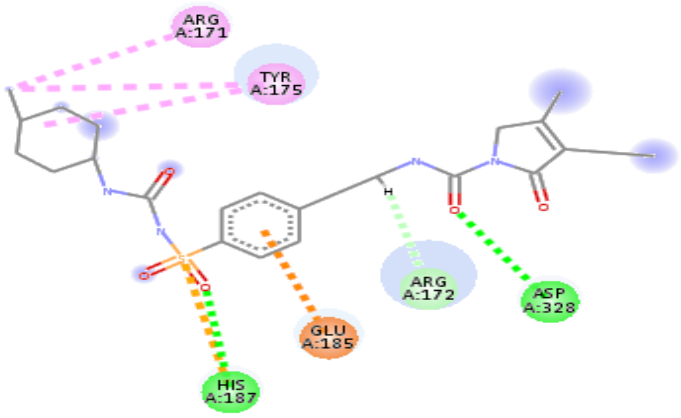

Figure 21: Non Bond Interaction of Glimepiride with MAO-A (2z5x) 2D structure

There are three amino acid similarities of Denagliptin with the standard drug. These are GLU 329, ARG 172 and LEU 176. There are two hydrophobic and one hydrogen bond. Furthermore, there is one halogen bond. The hydrophobic bonds are pi-alkyl type. The distance range is 3.39 to 5.24.

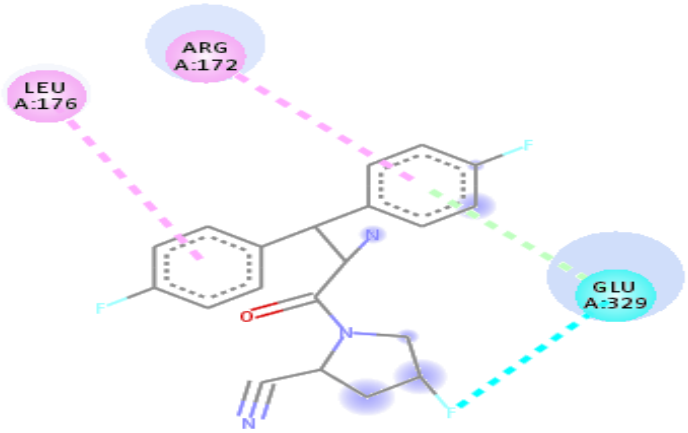

Figure 22: Non Bond Interaction of Denagliptin with MAO-A (2z5x) 2D structure

There are four hydrogen bonds and one hydrophobic bond in Omarigliptin. Amino acid GLU185 is bonded with the ligand OE1 of the drug. Other two amino acids ARG172 and LEU176 is bonded with UL (unknown ligands). The distances are within 2-5. There are four similar amino acids in compare to the standard one, Mirtazapine.

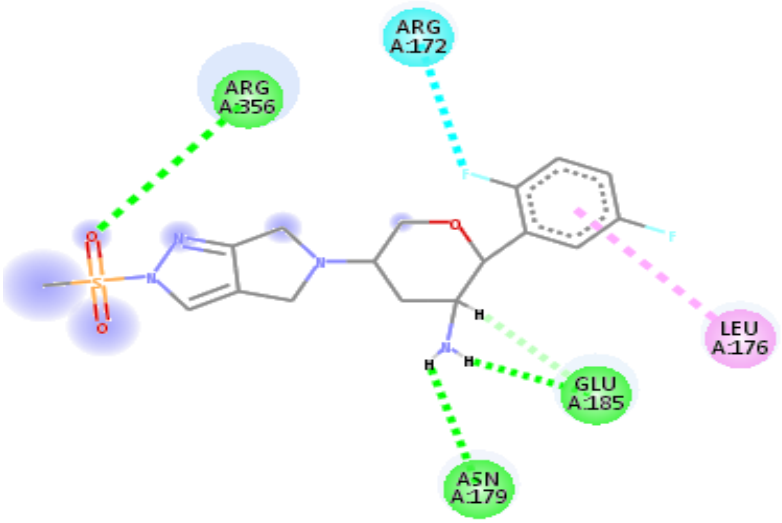

Figure 23: Non Bond Interaction of Omarigliptin with MAO-A (2z5x) 2D structure

3.2.4.3 Non-bond interactions between MAO-A (2z5x) with

Anti-hypertensive drugs

Table 13: Protein-ligand interaction between MAO-A (2z5x) and 2 anti-hypertensive drugs (Efonidipine and Cyclothiazide)

Ligand	Protein-ligand interaction	Distance	Category	Type
Efonidipine	A:**ARG172**:NH2 - :UNL1	4.59906	Electrostatic	Pi-Cation
	:UNL1 - A:**ARG172**	5.22547	Hydrophobic	Pi-Alkyl
	:UNL1 - A:**LEU176**	5.19564	Hydrophobic	Pi-Alkyl
Cyclothiazide	A:**ASP328**:N - :UNL1:O	2.94346	Hydrogen Bond	Conventional Hydrogen Bond
	:UNL1:H - A:**GLU185**:OE1	2.45174	Hydrogen Bond	Conventional Hydrogen Bond
	A:**GLU329**:OE1 - :UNL1	4.26998	Electrostatic	Pi-Anion
	A:**ARG172** - :UNL1	4.81234	Hydrophobic	Alkyl

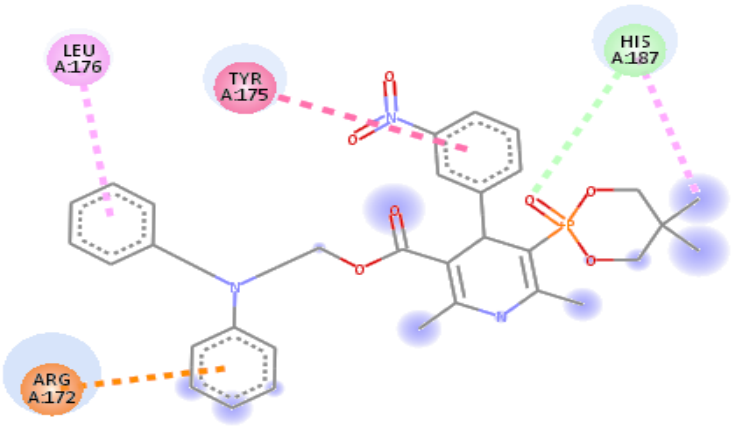

Figure 24: Non Bond Interaction of Efonidipine with MAO-A (2z5x) 2D structure

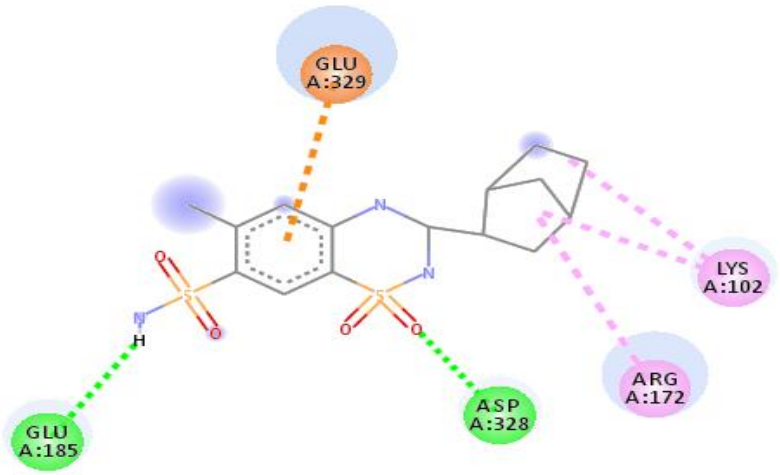

Figure 25: Non Bond Interaction of Cyclothiazide with MAO-A (2z5x) 2D structure

3.2.4.4 Non-bond interaction between MAO-A (2z5x) with Natural small molecules

Four natural molecules: Curcumin, Epicatechin gallete, Epigallocatechin and Theaflavin were evaluated in Discovery studio as they showed higher binding affinities and better superimposition.

Table 14: Protein-ligand interaction of MAOA (2z5x) with Natural Small Molecules

Ligand	Protein-ligand interaction	Distance	Type	Category of Bond
	A:**ARG172**:NH1 - :UNL1:O	3.24374	Conventional Hydrogen Bond	Hydrogen Bond
	:UNL1:H18 - A:**GLU185**:OE1	2.49554	Carbon Hydrogen Bond	Hydrogen Bond
	:UNL1:H19 - A:**GLU329**:OE1	2.58311	Carbon Hydrogen Bond	Hydrogen Bond

Curcumin	A:**ASP328**:OD1 - :UNL1	4.68238	Pi-Anion	Electrostatic
	A:**GLU329**:OE1 - :UNL1	3.90714	Pi-Anion	Electrostatic
	:UNL1 - A:**LEU176**	5.35452	Pi-Alkyl	Hydrophobic
Epicatechin Gallete	A:**ARG172**:NE - :UNL1:O	3.24532	Conventional Hydrogen Bond	Hydrogen Bond
	:UNL1:H - A:**GLU329**:OE1	2.05906	Conventional Hydrogen Bond	Hydrogen Bond
	A:**ARG172** - :UNL1	4.81727	Alkyl	Hydrophobic
	:UNL1 - A:**LEU176**	5.12713	Pi-Alkyl	Hydrophobic
Theaflavin	A:**ARG172**:NH2 - :UNL1:O	3.1442	Conventional Hydrogen Bond	Hydrogen Bond
	A:**GLU329**:N - :UNL1:O	3.05785	Conventional Hydrogen Bond	Hydrogen Bond
	:UNL1 - A:**ARG172**	5.15425	Pi-Alkyl	Hydrophobic
	:UNL1 - A:**LEU176**	4.68897	Pi-Alkyl	Hydrophobic
Epigallo catechin	A:**ASP328**:N - :UNL1:O	3.16791	Conventional Hydrogen Bond	Hydrogen Bond
	A:**GLU329**:OE1 - :UNL1	4.09196	Pi-Anion	Electrostatic
	A:GLU327:C,O;**ASP328:**N - :UNL1	4.85697	Amide-Pi Stacked	Hydrophobic
	:UNL1 - A:**LEU176**	5.13047	Pi-Alkyl	Hydrophobic
	:UNL1 - A:**ARG172**	4.21644	Pi-Alkyl	Hydrophobic

There are six amino acids that showed similarities with the standard drug, Mirtazapine. These are: ARG172, GLU185, GLU1329, ASP328, GLU329, LEU176. Among them, GLU185 and

GLU329 is bonded with OE1 ligand and ASP328 is bonded with OD1 ligand from the Natural Small Molecule, Curcumin. There are four hydrogen bonds and one hydrophobic bonds suggesting strong interaction.

Curcumin, also known as diferuloylmethane, is the primary curcuminoid in turmeric (*Curcuma longa*), which is a rhizomatous plant. Curcumin has reportedly shown anti-inflammatory and anti-oxidant properties. As a result, it has been used widely in inflammatory diseases such as arthritis, rheumatoid arthritis, in many chronic disease like diabetes, cancer or inflammatory bowel disease (Fusar-Poli *et al.*, 2019).

In addition to that, recent studies have showed that, Curcumin, if given as an adjunctive therapy, gives potent antidepressant effects with major depressive disorder (MDD). The study was conducted in MDD patients and the symptoms started to improve 12 to 16 weeks after treatment beginning. Curcumin was well-tolerated and safe, having no prominent side effects. (Kanchanatawan *et al.*, 2018)

Thus, it can be said that, Curcumin is a potent candidate to fight depression.

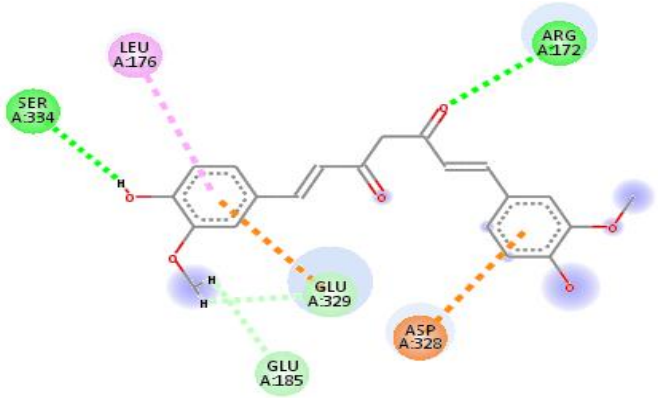

Figure 26: Non Bond Interaction of Curcumin with MAO-A (2z5x) 2D structure

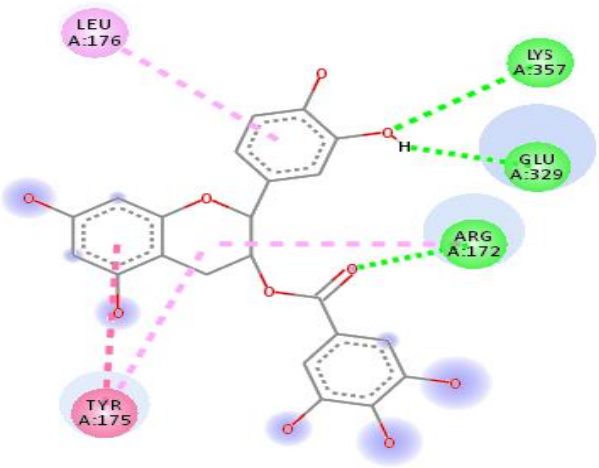

Figure 27: Non Bond Interaction of Epicatechin Gallete with MAO-A (2z5x) 2D structure

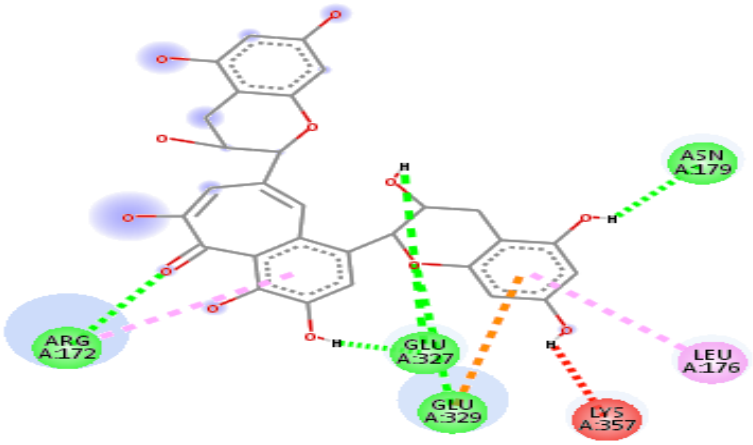

Figure 28: Non Bond Interaction of Theaflavin with MAO-A (2z5x) 2D structure

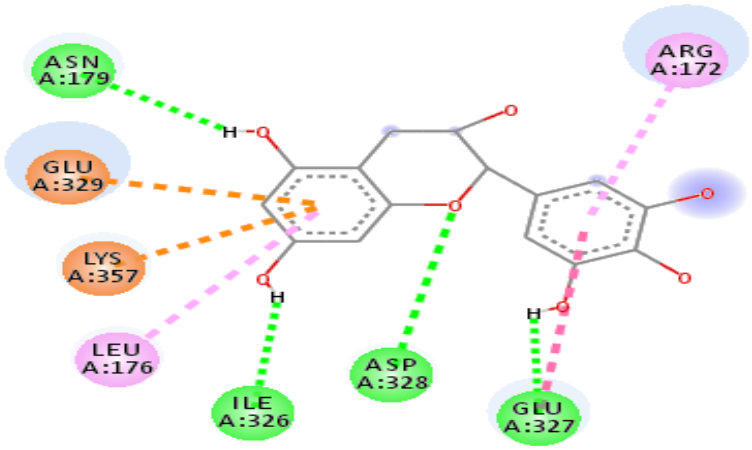

Figure 29: Non Bond Interaction of Epigallocatechin with MAO-A (2z5x) 2D structure

3.2.5 Evaluation of Pharmacokinetic Properties in admetSAR

Lastly, we checked the admetSAR to see the pharmacokinetic properties of the drugs. admetSAR was developed as a comprehensive source and free tool for the prediction of chemical ADMET properties (Yang *et al.*, 2019).

For better success rate, a drug theoretically should cross the blood brain barrier (BBB) to treat depression (O'Brien *et al.*, 2012). Therefore, the drugs that crosses the BBB are selected as a potent candidate for treating depression, others are discarded.

Different model results were analyzed. Among them are BBB (blood brain barrier), Caco-2 permeability, human intestinal absorption, AMES toxicity and carcinogens. The standard drugs (Mirtazapine) along with the repurposed one's pharmacokinetic properties are given here.

Table 15: ADMET Properties of Mirtazapine

MODEL	RESULT	PROBABILITY
Blood-Brain Barrier	BBB+	0.9855
Human Intestinal Absorption	HIA+	0.9873
Caco-2 Permeability	Caco2+	0.7283
AMES Toxicity	Non AMES toxic	0.8079
Carcinogens	Non-carcinogens	0.9742
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.7532

Mirtazapine is a blood-brain barrier (BBB+) positive drug. The probability ratio is 0.9855. Mirtazapine is absorbed in human intestine (0.9873%). For human Ether-a-go-go related gene inhibition, it is a weak inhibitor. It is proved to be a non-toxic and a non-carcinogen.

Table 16: ADMET Properties of Glimepiride

MODEL	RESULT	PROBABILTY
Blood-Brain Barrier	BBB+	0.7322
Human Intestinal Absorption	HIA+	0.9860
Caco-2 Permeability	Caco2-	0.6809
AMES Toxicity	Non AMES toxic	0.6392
Carcinogens	Non-carcinogens	0.7301
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.7714

Glimepiride is also a BBB+ drug. It crosses the blood-brain barrier. The probability percentage is 0.7322. Like Mirtazapine, it is also absorbed through human intestine. Caco-2 permeability is negative for Glimepiride. It works as a weak inhibitor in human ether-a-go-go-related gene inhibition. It is a non-carcinogen and a non AMES toxic drug.

Table 17: ADMET Properties of Omarigliptin

MODEL	RESULT	PROBABILITY
Blood-Brain Barrier	BBB+	0.7813
Human Intestinal Absorption	HIA+	1.0000
Caco-2 Permeability	Caco2-	0.6015
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.8472
	Inhibitor	0.5588
AMES Toxicity	Non AMES toxic	0.5749
Carcinogens	Non-carcinogens	0.6805

Omarigliptin crosses the blood-brain barrier at a probability percentage of 0.7813. Through human intestinal, it is absorbed. It shows no carcinogenic effect or any toxic effects.

Table 18: ADMET Properties of Denagliptin

MODEL	RESULT	PROBABILITY
Blood-Brain Barrier	BBB+	0.9880
Human Intestinal Absorption	HIA+	0.9946
Caco-2 Permeability	Caco2+	0.5095

Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9819
AMES Toxicity	Non AMES toxic	0.7196
Carcinogens	Non-carcinogens	0.8549

Denagliptin is blood brain barrier positive. The probability ratio is higher than Glimepiride and Omarigliptin. It is also a weak inhibitor in inhibiting human ether-a-go-go related genes. It has no AMES toxicity and does not act as a carcinogen.

Table 19: ADMET Properties of Curcumin

MODEL	**RESULT**	**PROBABILITY**
Blood-Brain Barrier	BBB+	0.6162
Human Intestinal Absorption	HIA+	0.9539
Caco-2 Permeability	Caco2+	0.7093
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9421
Carcinogens	Non-carcinogens	0.9040
AMES Toxicity	Non AMES toxic	0.9132

Cucumin crosses the blood-brain barrier. It is permeable through caco-2 cell line. It is highly absorbed via human intestine. It shows negative result as a carcinogen and as AMES toxic substance. On the other hand, Denagliptin is a dipeptidyl peptidase IV (DPP-IV) inhibitor. In 2006, it had entered the phase III clinilal trials as anti-diabetic agent at GlaxoSmithKline. However, the development of the drug was put on hold as it showed long term toxicity in its

preliminary data (DENAGLIPTIN, New Drug Approvals, GSK, 2014). For this reason, Denagliptin was discarded. Both Glimepiride and Omarigliptin are safe in use with low hypoglycemic control and well tolerated by patients. As we can see Glimepiride has higher binding affinity than Omarigliptin (Glimepiride: -9.7 kcal/mol, Omarigliptin: -8.4 kcal/mol), Glimepiride was finally chosen as the best possible repurposed synthetic candidate other than Curcumin.

To sum up, it may be said that, after vigorous screening of three different classes of drugs and list of natural molecules by different soft wares and tools, one anti-diabetic drug namely Glimepiride and one natural small molecule, Curcumin was found to be potent candidates as an anti-depressant agent.

Chapter 4

Discussion and Conclusion

4.1 Discussion

The neurobiology underlying depression has not been fully identified, but is thought to result from molecular and cellular abnormalities that interact with genetic and environmental factors. This complexity and heterogeneity have made it difficult to define, diagnose, and treat this widespread illness. Currently available antidepressants, although widely prescribed for depression and other mood and anxiety related illnesses, have significant limitations, including a long time lag for a therapeutic response (weeks to months) and low response rates (only a third respond to the first drug prescribed, and up to two thirds after multiple trials, often taking months to years). This is particularly problematic for an illness associated with high rates of suicide (Duman and Voleti, 2012).

To find new candidates as antidepressants, drug repurposing was done. Out of various pathways of depression, neurotransmitter degradation by MAO (Mono Amine Oxidase) was focused and so MAO-A protein was selected. The protein structure (Crystal Structure of Human Monoamine Oxidase A with Harmine, PDBID: 2Z5X) was taken from RCSB PDB (Protein Data Bank). The structure was validated using different web servers and tools like ProSA web, ERRAT, Verify 3D, RAMPAGE Ramachandran Plot. Using PyMol, the pure structure of the protein was obtained by removing all ligands and oxygen atoms (bonded with the protein). For taking a standard drug, Mirtazapine was taken. The protein and the ligands (repurposed drugs) were prepared as pdbqt file in AutoDock Vina. Molecular docking (Rigid Docking) was done in Autodock Vina with three different classes of drugs namely Anti-diabetics, Anti-Hypertensive, Anti- Histamines and a number of natural molecules. The drugs that gave higher binding affinity than the standard one was taken for further evaluation.

Superimposition in PyMOL, visualization of the 3D model of the ligand in Discovery Studio (ligand-amino acid interaction, category and type of bonds, their distances), and lastly ADMET properties were checked to select the best candidates. Out of all drugs, Glimepiride, Danagliptin, Omarigliptin passed all the requirements. Their binding affinities were -9.7, -9.1 and -8.4 (kcal/mol) respectively. These drugs were chosen as they showed higher binding affinities than the standard one (Mirtazapine: -8.00 kcal/mol). Among the natural molecules, curcumin was selected (binding affinity: -9.5). These molecules were selected for further evaluation as they superimposed quite well. The 3D model of the complex was then visualized through Discovery Studio where amino acid-ligand interaction, their bond type and category, their distance were evaluated. Some of the amino acids that were common between the standard and the combination were GLU185, ASP328, GLU329, ARG172 and LEU176. The category of the bonds was mostly: hydrophobic and hydrogen bonds. The distance range was kept 2 to 5. Lastly, ADMET (Absorption, Distribution, Metabolism, Excretion, Toxicity) properties of each candidate were predicted by admetSAR. The factors that were investigated were blood brain barrier, human intestinal absorption, caco-2 permeability, AMES toxicity, carcinogens etc. Denagliptin was discarded as it proved to cause long-term toxicity. In addition, Glimepiride was chosen the best possible synthetic drug over Omarigliptin. Among the natural molecules, Curcumin was the chosen as the best option. Thus, this *in silico* study paved the path to repurpose existing approved drugs and natural molecule for the treatment of major depressive disorder.

4.2 Conclusion

Through *in-silico* molecular docking screening, this study showed that Glimepiride, an existing approved anti-diabetic synthetic drug and Curcumin, a natural small molecule have anti-depressant properties. They both showed higher binding affinities than the standard drug Mirtazapine. Furthermore, their interaction with the protein and their pharmacokinetic

properties were better than the standard drug. Hence, it can be said that, this study put forward a possibility of new drug development for depression.

4.3 Future work

This study was an *in-silico* study, hence, further in-vitro and in-vivo study should be conducted to confirm the effectivity of the proposed drugs. Additionally, molecular dynamic simulation should be done to see the physical movements of the protein and ligand while binding, their duration of binding and their specific binding sites.

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