KADIR HAS UNIVERSITY
SCHOOL OF GRADUATE STUDIES
PROGRAM OF COMPUTATIONAL BIOLOGY AND BIOINFORMATICS

IN SILICO SCREENING OF POTENT HISTON DEMETHYLASE 1 (LSD1) ENZYME INHIBITOR

Maryam Muhsin Al-Rikabi

Master Thesis

Istanbul, January 2020

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Master Thesis

Submitted to the Graduate School of Science and Engineering of
Kadir Has University in partial fulfillment of the requirements for the degree of
Master of Science in the Program of Computational Biology and Bioinformatics

Istanbul, January 2020

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IN SILICO SCREENING OF POTENT HISTON DEMETHYLASE1 (LSD1) ENZYME INHIBITOR

ABSTRACT

Histone lysine specific demethylase (LSD1) is one of the main enzymes which regulates histone demethylation which in return regulates different epigenetic processes such DNA replication and transcription also gene silencing. Moreover, recent studies have made a direct yet unclear link between LSD1 and the development of several diseases such viral infections, neurodegenerative diseases and most commonly cancer. An overexpression of the enzyme has been observed in different types of cancer including; acute myeloid leukemia (AML), breast cancer, lung cancer and prostate cancer. This observation led to the development of two LSD1 inhibitors; Tranylcypromine and 2-[4-methoxy-phenyl] Cyclopropylamine yet both have demonstrated low selectivity against the enzyme therefore this study along with many others solo focus on finding more potent LSD1 inhibitors through applying newly developed computer aided drug design (in silico) approaches. In this study Zinc15 database was screened in order to obtain pre-synthesized potential lead compounds. 40 thousand compounds were obtained, prepared and docked in two phases, firstly with PyRx autodock vina software and afterwards the compounds that have passed the first evaluation were further docked in autodock4 software and a total of 24 compounds have shown potential with a binding energy of -8.00 kcal/mol and less. Later on, Discovery Studio Visualizer software was used to generate 2D and 3D diagram pictures of the enzyme – ligand complex to further display and investigate the ligand interactions in the enzymes binding pocket.

Keywords: LSD1, In silico, CADD, Cancer, Autodock, PyRx, Zinc15 database.

IN SILICO TARAMAYLA GÜÇLÜ POTANSIYEL HISTONE DEMETHYLASE 1 (LSD1) İNHIBITÖRLERININ BULUNMASI

ÖZET

Histon lisinspesifik demetilaz (LSD1) histon demetilasyon düzenleyen ana enzimlerden biridir, farklı epigenetik süreçleri düzenler örneğin DNA çoğaltma ve transkript ve gen susturma. Ayrıca, son çalışmalarda birçok hastalığın gelişmesinde LSD1 doğrudan ama belirsiz bir bağlantı yapmış, viral enfeksiyonlar, nörodejeneratif hastalıklar ve en yaygında kanser.

LSD1 enziminin seviyesi yüksek ekspresyonu gözlemlenen kanser türleri (AML), meme kanseri, akciğer kanseri ve prostat kanseri. bu gözlemler sonuconda iki LSD1 inhibitörleri gelişmiştir yol açtı; Tranylcypromine and 2-[4-methoxy-phenyl] Cyclopropylamine fakat ikiside enzime karşı düşük seçicilik gösterdiler. bu sebeple bu çalışma birçok diğer çalışmalar ile birlikte solo odaklanan daha seçici ve potansiyeli yüksek özgün LSD1 inhibitörleri tasarlamak için yeni geliştirilen bilgisayar destekli ilaç tasarım (in silico) yöntemi kullanılır. Çalışmada Zinc 15 veri bankasında bulunan 40.000 bileşik kullanılmıştır hazırlanan ve iki aşamada yerleştirildi, PyRx (autodockVina) sonra ilk değerlendirmeden geçtiğinde bileşikler daha fazla yerleştirildi. Autodock 4 programı kullanılarak sonucunda bağlanma enerjileri -8.00 kcal\mol ‘dan daha iyi 24 bileşik elde edilebilmiştir. Sonradan, enzimi bağlayıcı cebinde ligand etkileşimleri daha fazla görüntü ve araştırma için Discovery Studio Visualizer programı kullanılarak enzim – ligand kompleksi 2D ve 3D diyagramı resimleri oluşturuldu.

Anahtar Sözcükler: LSD1, In silico, CADD, Kanser, Autodock, PyRx, Zinc15 veritabanı.

ACKNOWLEDGMENT

In the beginning I would like to offer my deepest gratitude to my supervisor **Prof. Dr. Kemal Yelekci** for his advice, guidance, understanding and continuous support. And I want to extend this gratitude to all faculty members whom have taught me during this journey and to all my colleagues and friends who have supported and aided me with their help and advice.

To my parents and siblings, all I am today is credited to your endless love and support.

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LIST OF SYMBOLS/ ABBREVIATIONS

CADD …… Computer aided drug design

LSD1 …….. Lysine Specific Demethylase 1

FAD ……... Flavin adinine dinucleotide

TCP …… Tranylcypromine

AML …….. Acute Myeloid Leukemia

H3K4 ……..……. Histone H3 lysine K4

CoREST ……..… Transcriptional co-repressor protein

NCBI …….. National Center for Biotechnology Information

MAO-A …….. Monoamine oxidase A

MAO-B …….. Monoamine oxidase B

ER …… Estrogen receptor

FAD …… Food and Drug Administration

PDB …… Protein Data Bank

IC50 …… Inhibitory concentration

Ki ……. Inhibitor constant.

GPF …… Grid Parameters File

DPF …… Dock Parameter File

GLG ……... Grid log file

DLG …… Dock log file

1.OBJECTIVE

In recent years a link between aberrant over expression of LSD1 enzyme and cancerous cells development have been discovered (Xiaoli Fu et al., 2017). And ever since this discovery a search for a way to inhibit the enzyme and thus aiding in cancer inhibition was the drive of many studies and researches which diverse between intensively studying LSD1 and its biological characteristics and designing and developing selective inhibitors that can bind and reversibly or irreversibly inhibit its activity.

the objective of this study is to implement in silico approaches in the race to inhibit LSD1 through screening large databases of thousands of compounds, in this study Zinc15 database was screened for potential leads, in short periods of time in order of discovering new potential lead compounds that can fit in the substrate binding pocket of LSD1 and inhibiting its activity which in the future might lead to the development of novel cancer treatment with lesser side effect and better pharmacokinetic and pharmacodynamics properties.

2. INTRODUCTION

2.1 Drug Discovery

The core of pharmaceutical industry is the desire to improve the therapeutic value and safety of existing treatments and discovering new drugs. This aided in the appearance of drug research during the 20th century. And basing on the fact that drug discovery is "a patient-oriented science" which aims to improve the quality of living of the patient either by designing new drugs or improving the potency or side effect of already existing drugs along with the new advances in science and the development of new technologies all together pushed into realizing the importance of enzyme targeting to either inhibit its action or modify the enzyme feedback mechanism which have a direct effect on disease inhibition. (Ratti and Trist, 2001) (Meidrum and Roughton, 1933).

Table (2.1): Important discoveries in the field of medicine, right from 19th century to 21st century (Martis and Somani, 2012)

Year of Discovery	Drug Name	Category
1806	Morphine	Hypnotic agent
1899	Aspirin	Analgesic and Anti-pyretic agent
1922	Insulin	Anti-Diabetic agent
1928	Penicillin	Antibiotic
1960	Chlordiazepoxide	Tranquillizer
1971	L-dopa	Anti-Parkinson agent
1987	Artemisinin	Anti-malarial agent
1998	Sildenafil	Erectile Dysfunctioning treatment
1999	Celecoxib, Rofecoxib	Selective COX-2 inhibitors
1999	Zanamavir, Oseltamivir	Anti-influenza agents
2001	Imatinib	Leukemia treatment

However, developing of treatments is a long, complex and expensive process that can take up to 12 or 14 years and will also requires a strong financial aid. The reason behind that is the fact that the drug discovery process for the development

of new drugs combines several long steps from biological and chemical research to toxicity and side effect studies and then several steps of pre-clinical and clinical trials which might take up to 8 years until the drug might be finally approved by the food and drug administration (FDA) and the follow up process of manufacturing, distribution and following up the feedbacks on its activity and possible side effects (Congreve et al., 2005).

In later years, computer-aided drug design approaches (CADD) have been incorporated into the process of drug discovery by facilitating the earlier stages of lead identification and optimization, through several techniques such as pharmacophore mapping, virtual screening and molecular docking. CADD approaches were also incorporated in the pre-clinical trials through ADMET in silico prediction for instance, which is set to predict the drug's absorption, distribution, metabolism, excretion, and toxicity properties in the organism. Such methods have proved to be more efficient and reduced the overall time and money generally required in pharmaceutical industry (Martis and Somani,, 2012) (Augen, 2002**).**

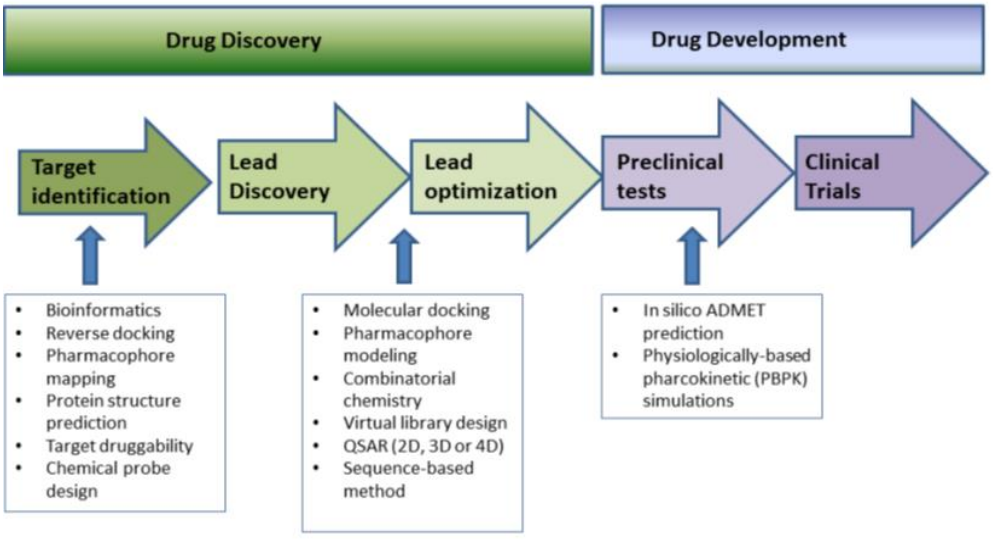

Figure (2.1): Computational drug discovery approaches applied in various stages of the drug discovery and development pipeline (Chunxia, 2016).

2.1.1 Virtual Screening

Virtual screening is a rational approach used for identifying new compound hits from large database libraries of compounds. It is used in order to computationally evaluate these libraries and come out with a selection of small number of candidates which have high percentage of being active.

Virtual screening can either be ligand based or structure base. For the ligand base it depends on the information from structural and biological activity of known compounds, a concept based on the idea that molecules with similar structure most likely have the similar biological activity. While structure-based virtual screening depends on the three-dimensional (3D) structure of the target. X-ray crystallography or NMR are used in the presence of 3D structure of the target and in its absence methods such as Homology modeling is applied to gain required information for screening libraries also Molecular docking is the most common method used along with structural-based Virtual Screening (Gimeno et al. 2019).

On the other hand, different types of libraries can be used in virtual screening. Some of these libraries contain hypothetical(virtual) compounds. others although containing actual compounds, not all of them are public or commercial, some of them are private (in house libraries). The Zinc library is one of the public libraries with a huge demand, also aside from the zinc library there are other libraries as well such as ChemBL, PubChem, and BindingDB libraries (Lopez-Vallejo et al., 2011).

2.1.2 Molecular Docking

Molecular Docking approach is used for the prediction of the most favorable binding conformation and interactions of the ligand in the protein binding pocket to achieve the minimum free binding energy in terms of binding affinity through the use of different scoring functions that are applied via many Docking tools such as CHEM Score, AutoDock4, and GOLD (Dar and Mir, 2017) .

Docking is performed in different patterns, flexible protein-ligand docking, flexible protein-protein, flexible ligand-rigid protein or hydrophobic docking (Shore, 2012). These patterns based on two different theories that explains the ligand-receptor mechanism. the Key and lock theory by (Fischer, 1894) and the fit theory by Koshland (1963). Computationally, the flexibility of the protein is more favorable, nevertheless while docking it can become very challenging (Alonso, Bliznyuk and Gready, 2006).

2.2 Cancer

Cancer is an aberrant growth of normal body cells which may lead to death. Normal body cell would start dividing uncontrollably and, in some cases, new unneeded cells start to form. Cancer is classified as a genetic disease, it can breakout in any organ or tissue in the body due to DNA damage which leads to mutations in normal genes and turn them to cancerous genes also the loss or damage of tumor suppressor genes which control cell growth and along with oncogenesis expression can be two other reasons for cancer arising and spreading to other tissues and organs in the body (Imran et al., 2017).

Several internal or external factors can lead to genomic mutations such as gene duplication, insertion, deletion, or chromosome translocation and due to these mutations, an over expression of certain proteins occurs which have a direct link to cancer formation. These factors include viral infection, chemical or radiation exposure, injury, certain types of diseases, alongside smoking and dietary factors that have shown a direct link to cancer development and treatment (Croce, 2008).

According to the National Cancer Institute (NCI) (https://www.cancer.gov/), there were 14.1 million new cancer cases and 8.2 million cancer-related deaths in 2012 around the world with the number of new cases expected to rise in 2030 to 23.6 million. Based on NCI statics cancer is one of the main causes of death worldly.

The most common types of cancer are lung cancer, breast cancer, leukemia, and prostate cancer. Methods of treatment differ according to the type and degree of spreading to other tissues. Some cancer types such as brain cancer are more difficult to treat by conventional cancer treatment methods with 5-year survival rate (people might live five years after the tumor is found) of 34% for men and 36% for women due to the complexity of the brain anatomy. Others, although can be eliminated by conventional methods such as surgery and chemotherapy, these methods carry with them pain to the patient and harm to body tissues (https://www.asco.org/).

On the other hand, financially, cancer research is considered one of the most important aspects of science. According to (Eckhouse et al., 2008) $14 billion is spent annually in both public and private sectors for cancer research. Although it can be a financial burden, yet cancer research has proven its effectivity through helping to understand cancer pathogenesis, identifying new treatments and therapeutic drugs, and realizing country specific epidemiology. Moreover, when the calculating the overall direct costs of dealing with cancer in all stages and the costs of premature death and also the informal care expenditures, an analysis from the European Union of the costs related to cancer shows that €18.8 billion or 15% of cancer treatment costs go to lung cancer and followed by breast cancer with approximate of 12% expenditures (Aggarwal et al., 2016).

2.2.1 Cancer Treatment

The type of cancer treatment depends on the type of cancer, some cancers are treated with one type treatment others require a combination of treatments, mainly surgical intervention, chemotherapy, and/or radiation. Other treatments include hormone therapy, immunotherapy and targeted therapy (Arruebo et al., 2011).

Cancer treatment can cause number of side effects on the tissue or organ because while it kills the aberrant cells, it also kills or slow the growth of healthy cells that grow and divide rapidly. This damage to the healthy cells can be obvious in

symptoms such as mouth sores, nausea and vomiting, diarrhea, fatigue, hair loss, and many other.

On the desire to minimize these side effects and perform higher accuracy in treating cancer, targeted therapy was discovered.

On the other hand, in 1940 a direct link was established between nutrition and cancer pathogenesis and it was found that nutritional habits reduced occurrence of cancer in mice (Tannenbaum, 1940). Diet is accounted for 30% of cancers approximately which hypothetically makes it the second cause of cancer that might be avoided after tobacco in industrial countries. While 60% of cancer incidence in developing countries are linked to diets low in vegetables and fruits. Different studies have investigated the relationship between cancer development and treatment and following a diet low on cholesterol, and high in plant-based options with specific defensive micronutrients (Patel et al., 2018).

2.2.2 Targeted Therapy

The advances in drug discovery had led to the significant improvements in cancer treatment by discovering and designing new therapeutic agents as targeted drugs. Targeted therapy targets the changes that happens in the cell, specifically the proteins that is responsible for the tumors fast grow and spread and inhibit its effect. The drawback of this treatment is the resistance that can be formed by the cancer cells, which requires the use of chemotherapy or radiation in combination with the drugs administrated as targeted therapy.

The incorporation of modern technologies such as computer aided drug design (CADD), researchers were able to have a better and clearer view on the cells and tissues and thus designing more accurate and specific drugs to avoid the use of chemotherapy or radiation and to also minimize or eliminate any side effects that might arise. CADD enabled researchers to screen large libraries in short periods of times to come up with the compounds of the best predicted activities and then optimizing lead compounds to increase to achieve higher binding affinity and

better ADMET properties. on the other hand, CADD eased in designing novel compounds (Mandal et al., 2009)

A wide range of drugs with different targets inside the cell with the aim of inhibiting cancer have been developed and approved. In present time, more than 15 types of cancer have FDA approved drugs in table (2.2) a variety of these drugs are displayed. However, new challenges keep arising for the cancer treatments, for instance not all cancer cells have the right target for the targeted therapy, also the tumor cells might develop another way to overcome the treatment.

Table (2.2): Variety of FDA approved targeted therapy drugs, their targets and potential side effects.

Drug	Target	Side effect
tamoxifen (Nolvadex)	Breast cancer 1.inhibits the binding of estradiol to estrogen receptors. 2. up-regulates the production TGFb. 3. down-regulates (IGF-1)	1.increased tumor or bone pain 2.hot flashes 3.nausea 4.depression … etc.
imatinib mesylate (Gleevec®)	Leukemia binds to an intracellular pocket within TK, inhibiting ATP binding and prevents phosphorylation and activation of growth receptors.	1.Low blood counts 2.Nausea and vomiting. 3.Edema 4.Muscle cramps and bone pain. ... etc.
crizotinib (Xalkori®)	Lung cancer 1.binds to and inhibits ALK kinase and ALK fusion proteins. 2.inhibits c-Met kinase and disrupts the c-Met signaling pathway.	1.Edema 2.Constipation. 3.Fatigue, Dizziness. 4.Increased liver enzymes … etc.
everolimus (Afinitor®)	Brain cancer binds to the immunophilin FK Binding Protein-12 (FKBP-12) generating a complex that binds to and inhibits the activation of (mTOR)	1.Infection. 2.Fatigue. 3.Swelling or edema. 4.Nausea, vomiting … etc.

2.3 Lysine Specific Histone Demethylase 1 (LSD1)

LSD1, belongs to the LSD family of histone demethylase enzymes which regulates the process of histone lysine methylation along with methyltransferases. In fact LSD1 was the first identified histone demethylase and its discovery have led to the understanding of the importance of histone tail methylation in the cell through intensively studying both the biochemical and biological properties of LSD1 (Maiques-Diaz and Somervaille, 2016).

LSD1, also known as KDM1A, is linked with more than 60 protein regulatory genes and thus it is believed to facilitates large number of cell signaling pathways and it also has a crucial role in regulating key cellular processes by demethylating mono- and di-methylated H3Lys4 and H3Lys9, as both of them are involved in DNA epigenetic regulation (co-activators or co-repressors) (Majello et al., 2019).

Moreover, LSD1 is a flavine adenine dinucleotide (FAD) depended enzyme which resides in the amine oxide (AO) domain of LSD1 and poses the catalytic activity of the enzyme by acting as the enzyme's cofactor. LSD1 structurally resembles other members of FAD depended enzymes such as monoamine oxidase (MAOs) and poly amine oxidase (PAOs) and mostly it resembles its homolog LSD2, also known as KDM1B, the resemblance is in their amino acid sequence and 3D structure which consists of the AO domain and also the SWIRM (Swi3p, Rsc8p, and Moira) domain that is special for all chromatin – related enzymes. However, what drive the specificity of LSD1 is its coiled – coil elongated Tower domain which projects from the AO domain and it is not find in the other monoamine oxidase enzymes and it is of most importance as it's where the CoREST (REST corepressor 1) protein binds and in turn thought to facilitate the binding of LSD1 to the nucleosome by acting as a linker between the nucleosomal DNA and LSD1 with the aid of the Tower domain on LSD1 and two SANT domains on the CoREST protein (Niwa and Umehara, 2017).

Figure (2.2) displays the 3D structure of LSD1 enzyme and its domains which are explained above and highlights the position of the FAD cofactor and the substrate binding site.

The FAD cofactor plays a crucial role in LSD1 activity as it is responsible for the oxidative demethylation of Lys4 after the methylated histone lysine are deproteinized by Lys661 and the hydride are transferred by a water bridge to FAD. Thus, any mutations at Lys66 or interruption of the intrapeptide hydrogen bonds present between Arg2 at the substrate binding pocket and histone3 can crash the network and inhibit LSD1 activity (Xiaoli Fu et al., 2017).

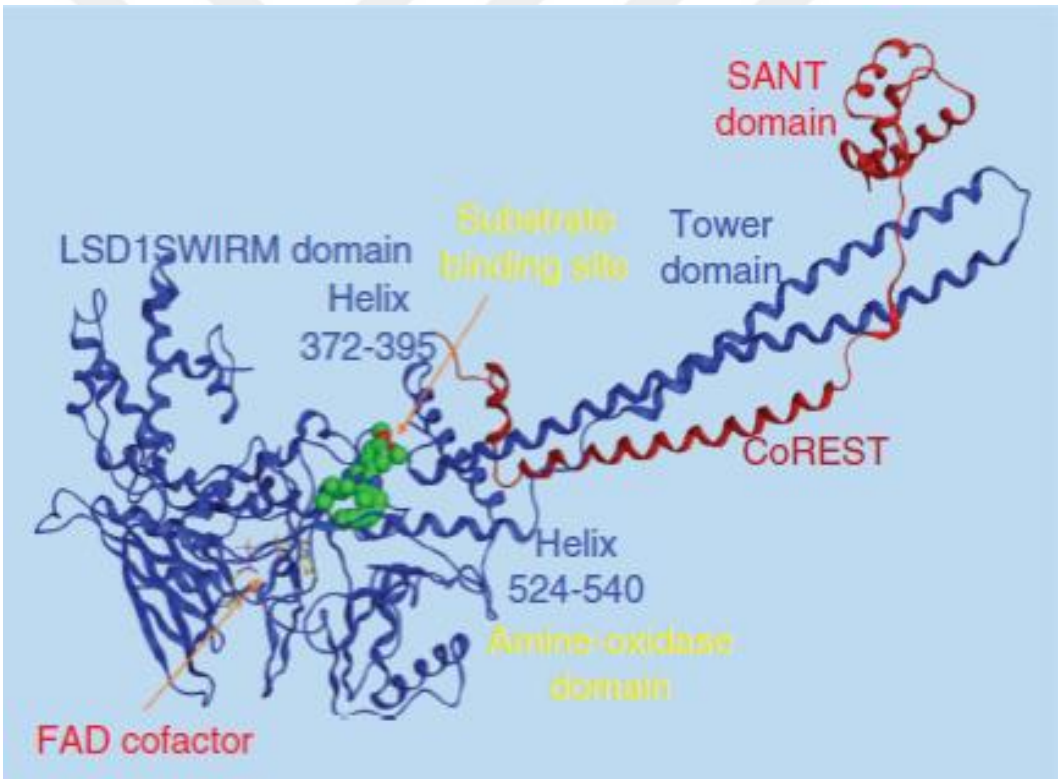

Figure (2.2): The 3D structure of LSD1. FAD domain (blue) interacted with SWIRM (red) Tower domain is extended from the spherical core shaping helix turn helix motif, to provide the ability for interaction with other proteins (Xiaoli Fu et al., 2017).

In recent years, a link between LSD1 and several diseases has been found which emphasized the importance of closely examining it and identifying all its features. One of the most important diseases linked to LSD1 is cancer. LSD1 is found to have a primal role in maintaining cancer stem cells features. Furthermore, it is observed that an overexpression of the enzyme can be found in numerous cancer cells and that it is responsible for its differentiation, multiplication, migration to other tissues, invasion of healthy cells and low prognosis. And on the contrary, when the enzyme is experiencing dysfunctionality the body cells gets disrupted which leads to diseases such as cardiovascular diseases, inflammations and infections, neurodegenerative disease and, as mentioned earlier, cancer (Xiaoli Fu et al., 2017).

Table (2.3): Molecular information of LSD1 as acquired from NCBI. http://www.ncbi.nlm.gove/gene/23028

Official name	KDM1A
Official symbol	Histone Lysine-specific Demethylase
Alternate names	AOF2, CPRF, KDM1, and BHC110
Primary source	HGNC:HGNC:29079
Organism	*Homo sapiens*
Gene type	Protein coding

2.3.1 LSD1 And Cancer

Over the years it has been noticed a change in histone methylation in cancer cells (Song et al., 2016). And as mentioned earlier, methyltransferase and demethylase modify cell's DNA chromatin, hence cell epigenetic. Cell epigenetic refers to the phenotypic changes of inherited characteristics of the genome without any changes in the actual DNA. Epigenetic have a direct role in cell regulation and hence importance in cell development, differentiation of immune cells and cytokinetic expression (Mayer et al., 2002) and according to (Maiuri and

O'Hagan, 2016), histone modifications, non-coding RNAs, and DNA methylation are the prime leaders of cell regulations. LSD1 being one of the demethylating enzymes by demethylating histone lysine residue which promotes transcriptional repression and moreover it was discovered that LSD1 methylate/demethylate particular lysine residues on non-histone proteins such as p53, DNMT1, STAT3, RB1, MEFD2, MTA1, HIF-1α, and AGO2 as a result of its demethylation mechanism and thus having a role in regulating these non-histone proteins, and as a result an overexpression of LSD1 have been observed in many cancer types such as acute myeloid leukemia (AML), lung cancer, breast cancer, prostate cancer, ovarian cancer, and pancreatic cancer (Chen et al., 2015) (Majello et al., 2019).

It has also been observed through expression profiling that inhibition of LSD1 in cancer cells would mainly targets the replication mechanism and cell cycle by disturbing EGFR (epidermal growth factor receptor) signaling pathways this will lead to the inhibition of call proliferation, differentiation, migration and the invasion mechanism of the cancer cells and ultimately in some cases the death of the cancer cells (Song et al., 2016) (Fang et al., 2019).

2.3.1.1 LSD1 And Acute Myeloid Leukemia (AML)

AML is heterogeneous hematopoietic malignant disorder. The occurrence of AML is not completely understood yet it can be specified by incomplete differentiation of myeloid progenitors (blast cells) accumulating in the blood and in the bone marrow (Wouters and Delwel, 2016). AML blasts morphologically range in their size from being little larger than lymphocytes to having the same size of monocytes or larger than it. Also, AML blasts can express the same common differentiation (CD) markers that exists on healthy undifferentiated myeloid cells including CD13, CD33, and CD34. Moreover, AML can co-express antigens specifically found in T or B cells including terminal deoxynucleotide transferase (TdT). AML is considered a disease with various genetic prospects, yet it can be classified to seven subtypes according to the World Health

Organization (WHO): (1) Repetitive genetic abnormalities AML; (2) Myelodysplasia-related changes AML; (3) Therapy related; (4) Not otherwise specified (NOS); (5) Myeloid sarcoma; (6) Down syndrome caused myeloid proliferations; (7) Blastic plasmacytoid dendritic cell neoplasm (Saultz and Garzon, 2016).

Incomplete differentiation of blast cells has a direct link to dysfunctionality of the epigenetic mechanism. KIT tyrosine kinase receptor is transmembrane a145 kDa protein and it has a very important role in regular hematopoiesis. KIT mutations are less than 5% of AML cases yet it possesses higher relapse risk and lower overall survival (OS) rates (Paschka et al., 2006). And as its been mentioned by Goardon et al. (2001), LSD1 has a direct and primary role in AM Leukemia developments as it has been noticed that a high level of LSD1 expression in the less differentiated c-kit + AML subtype and a lesser level of LSD1 expression in the differentiated c-kit- AML subtype.

2.3.1.2 LSD1 And Breast Cancer

Breast cancer is the most common malignancy in the world and the most common among women with occurrence percentage of 22.9% and an incidence rate of 1.6 million case per year, moreover depending on the degree of cancerous cells spreading from the tumor of origin location, the mortality rate can be as high as 90% the farther from the origin of tumor the new cancer colonies have formed (Yoosuf et al., 2020) (De Cicco et al., 2019). Breast cancer is a hormonal based cancer, an over expressed estrogen receptor alpha (ER+) is highly linked to its occurrence in 80% of the cases (Gao et al., 2019). An active ER signals the transcription of certain genes which have importance in tumor development and growth. Currently ER+ breast cancers aims to inhibit ER signaling pathways by either targeting estradiol synthesis or by competitively binding to estrogen receptor itself and thus inhibiting its activity (Patel et al., 2019). According to Lim et al. (2010) when LSD1 is inhibited in the cell, a down regulation of several genes that have a direct link to tumorigenesis development such as CCNF,

CDCA7, and MK167 which are responsible for cell cycle transition, cell division cycle and cellular proliferation respectively and thus the inhibition of LSD1 will consequentially lead to inhibition of breast cancer.

2.3.1.3 LSD1 And Lung Cancer

Lung cancer is a malignant solid cancer caused by abnormal and uncontrollable growth of lung tissues cells. Lung cancer appeared more visible after cigarette smoking emerged. In the 1950s the first solid evidence on the link between smoking and lung cancer development was established and published by Doll and Hill (1956). Benzo [α] pyrene is one of 73 known carcinogens that are found in cigarette smoke along with NNK, 1,3-butadiene and polonium-210 which is a radioactive isotope of polonium. these elements can cause a DNA damage and consequently leading to epigenetic changes affecting cell proliferation, apoptosis and DNA repair process. And as more deterioration accumulates the risk of lung cancer increases. When carcinogens causes mutation of oncogenes or tumor suppressor genes it induces cancer formation such as mutations in the K-ras oncogene which is accountable for up to 30% of lung cancer cases, and EML4-ALK tyrosine kinase fusion gene mutations also abnormalities of DNA methylation, histone tail modification and RNA regulations which lead to deactivation of tumor suppressor genes (Mustafa et al., 2016).

A high level of LSD1 expression have also been observed in lung cancer. In fact, the level of LSD1 expression is two times higher in cancerous cells than in healthy lung cells. An inhibition of LSD1 will lead to higher H3K9 acetylation level and E-cadherin expression which in turn suppresses development of lung cancer (Nair et al., 2010).

2.4 Inhibition of LSD1

Ever since the discovery of LSD1 and realizing its direct and important role in epigenetic, cell differentiation and proliferation specifically its role in several diseases such as cancer, LSD1 has gained more and more attention from scientist. In silico methods have also been implemented which of course fast forwarded the process. Assays such as ligand/substrate-based assays, PPI based assays and byproducts-based assays have been used along with virtual screening which allowed the examination of large numbers of LSD1 potential ligands for the inhibition of the biological activity of LSD1 enzyme in very short periods of time (Guanjun et al., 2018).

One of LSD1 main features is its resemblance to MAO-A and MAO-B enzymes which drew attention to the ability of using MAO-A and MAO-B inhibitors to inhibit LSD1 activity as well. Moreover, the ability to design dual action inhibitors that can act on all three enzymes in the same time, considering all of them belong to the methyltransferase and demethylase groups of enzymes as mentioned above, is also of great pharmacological importance such as ORY-2001 which is under investigation as a dual action inhibitor for LSD1/MAO-B enzymes for the treatment of Alzheimer disease, relapsing-remitting multiple sclerosis (RRMS), and secondary progressive multiple sclerosis (SPMS) (Fang et al., 2019). However, LSD1 has a feature of its own that separates it from MAO-A and MAO-B enzymes structurally. While the FAD binding site of all three enzymes (LSD1, MAO-1, and MAO-B) is very similar, the substrate binding site of LSD1 is a larger and a hydrophilic region in contrast with the other two MAOs. Therefore, the base to selectively inhibit LSD1 enzyme is also available (Xiaoli Fu et al., 2017).

Nowadays, several synthetic drug candidates for LSD1 inhibition are being investigated in different stages of clinical and pre-clinical trials. Table (2.4) lists number of these drugs.

Table (2.4): Synthetic drugs for LSD1 inhibition in clinical and pre-clinical trials (Hosseine and Saverio, 2017)

Compound	Cancer Type	Stage	Trials No.
GSK2879552	AML and lung Cancer	Phase I/IIa	NCT02034123, NCT02177812, NCT01943851, NCT01587703
ORY-1001	Leukemia	Phase I/IIa	EudraCT Number-2013–002447–29
4SC-202	AML, ALL, lymphoytic leukemia	Phase I	NCT01344707
Tranylcypromine trentinoin (in combination with ATRA)	AML	Phase I/IIa	NCT02717884, NCT02261779, NCT02273102
IMG-7289 With and without ATRA	AML, ALL, lymphoytic leukemia	Phase I	NCT02842827
INCB59872	AML, lung cancer	Phase I/IIa	--
SP2577	AML, breast cancer	Phase I	NCT02712905
SP2509	Ewing and AML	Pre-clinical	--
SP2577	AML	Pre-clinical	--

2.4.1 Tranylcypromine as LSD1 Inhibitor

Until now, only tranylcypromine (TCP) (Schmidt and McCafferty, 2007) (Huang, 2007) and cyclopropylamine (Yujiang, 2004) (Metzger, 2005) have been describes as inhibitors of LSD1.

Tranylcypromine was first identified and used as a treatment for depression as an inhibitor for MAOs enzymes agents with no regards to its anti- cancer activity (Akdoğan, Erman and Yelekçi, 2011). However, in 2017 tranylcypromine inhibition activity of LSD1 against AML cells of MLL-AF9 in mouse modules and in the in vitro cell line was discovered (Hosseine and Minucci, 2017). Now a days TCP is used as a notable module or scaffold for designing irreversible

inhibitors for LSD1 activity. It is suggested that alterations to the phynel ring and the amine group of TCP leads to significant improvements of its potency against LSD1 (Fang et al., 2019).

TCP mechanism of inhibition depends on the interference with FAD covalently and a TCP-FAD adduct is generated when cyclopropyl ring of TCP binds with C-4a of FAD via Carbon-Carbon bond and a single electron transferred and either atropaladehyde or cinnamaldehyde is formed as a result. Fig. (2.3) and Fig. (2.4) demonstrates the inhibition mechanism of LSD1 by TCP respectively.

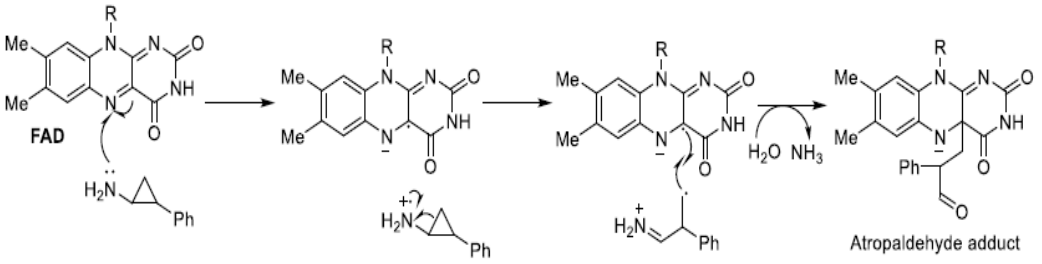

Figure (2.3): TCP mechanisms in LSD1 inhibition atropaladehyde pathway (Jernej, 2015).

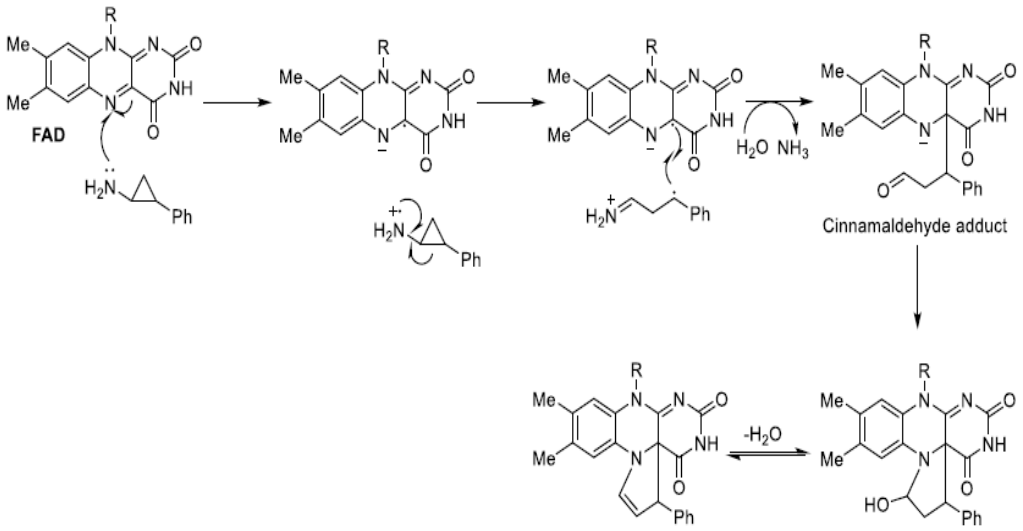

Figure (2.4): TCP mechanisms in LSD1 inhibition cinnamaldehyde pathway (Jernej, 2015).

3. MATERIALS AND METHODS

3.1 Introduction

The computer aided drug design approach was used in this study in order to generate novel LSD1 inhibitors. The in-silico applications including virtual screening and Autodocking were applied after the preparation and validation of the protein LSD1 enzyme crystal structure. The steps followed in conducting this study are stated below:

1- Protein validation

2- Database preparation

3- Docking: a- PyRx
b- AutoDock

4- Generating 2D and 3D diagrams

3.2 Protein Validation

The first and main step is the validation of the protein, in this study LSD1 enzyme 3D crystal structure in code name 5LGT with 3.0 A° resolution was acquired from the protein data bank (PDB) (Berman *et al.*, 2002) (http://www.rcsb.org). 5LGT crystal structure was then cleaned by the use of BIOVIA discovery studio (Accelerys 4.5, Inc.) software to prepare it for docking (http://www.accelerys.com). First water molecules as well as the non-interaction ions were removed to avoid any unrequired interferences with the docking process then missing hydrogen atoms were added to the structure. Lastly, clean geometry tool kit was used to optimize the force field.

Usually during the protein preparation, any interacting ligands are also removed but in the case of LSD1; FAD ligand is kept for its role as a cofactor for the

enzyme mechanism of action that we are aiming to inhibit. FAD importance is stated above in the literature review chapter.

Secondly, known inhibitors for the validating of the enzyme were obtained and prepared. Known inhibitors for LSD1 with good experimental values of IC50 and Ki were chosen from literature as references for LSD1 inhibition when they bind to the substrate binding site and then comparing the results obtained with the values acquired from literature. These known inhibitors 3D structures were obtained and further processed with the use of BIOVIA discovery studio software by adding hydrogen atoms and optimizing force field energy with clean geometry tool. Table 3.1 presents the known inhibitors used for the validation of 5LGT along with their IC50 and Ki values as mentioned in literature.

Table (3.1): Known inhibitors for 5LGT validation from literature

Name	**IC50**	**Reference**
RN-2	IC50= 70 nM	(Neelamegam et al., 2012)
RN-1	IC50= 10-70 nM	(Neelamegam et al., 2012)
IC	IC50= 10 μM	(Sharma et al., 2010)
Compound 1	Ki =29 nm	(Niwa et al., 2018)
GSK-2879552	IC50= 38 nM	(Mohammad et al. 2015)
CBB1007	IC50= 5.27μM	(Wang etal., 2011)
D compound	Ki=2.4 ± 0.63 Mm	(Khan, Suzuki and Miyata, 2013)
G compound	Ki =0.009 μM	(Khan, Suzuki and Miyata, 2013)

GSK2699537	IC50= 16 nM	(Smitheman et al., 2015; Mohammed et al.,2015)
6w3	IC50 = 160 nm	(Vianello et al., 2017)
F compound	Ki =0.005 μM	(Khan, Suzuki and Miyata, 2013)
Namoline	IC50=51 μM.	(Willmann et al., 2012)

Autodock version 4.2 was used for 5LGT validation assessment using semi empirical force field. Auto-dock automated docking tools (ADT) was used to generate docking files based on Ligand-Receptor Binding. The docking files include (1) PDBQT files for both the ligand and receptor. (2) Grid parameter file in which a map that contains the potential energy for each atom of the ligand and receptor. (3) Docking parameter file which contains the names of both receptor and ligand in their pdbqt format, plus docking and search parameters.

The GPF parameter for defining the 3D space volume around the protein binding site for each ligand was 55 A° 55 A° 55 A° and the XYZ coordination used were -66.531, -37.003, -35.506 respectively. Lamarckian Genetic algorithm was chosen as a last step with number of energy evaluation set to 25 million and GA runs to 20.

On the command line two commands (autogrid4 -p complex.gpf -l complex.glg and autodock4 -p complex.dpf -l complex.dlg) were applied to generate. glg and .dlg files that describes minimum and maximum energy in each grid map and contains the output results of docking respectively.

3.3 Database Preparation

Zinc15 database is a noncommercial database developed with access to over 120 million compounds ready for virtual screening. All compounds are available freely in their ready to dock, 3D structures (Sterling and Irwin, 2015). Zinc15 platform, Tranches, offers a feature of choosing compounds according with Lipinski's rule of five. Meaning all compounds (1) must be under the molecule weight of 500 Dalton (2) have Hydrogen-bond donors less than 5 (3) Hydrogen-bond acceptor less than 10 (4) their lipophilicity log P less than 5 (5) contain rotatable bonds less than 4 (Goodwin, Bunch and McGinnity, 2017).

Biovia Discovery studio, build 3D database toolkit was used for further filtration and obtaining best results according to BIOVIA DS fit value.

3.4 Docking

3.4.1 PyRx

The filtered results obtained from BIOVIA DS were docked initially with PyRx tool for screening by autodock vina (https://pyrx.sourceforge.io) for further filtration. PyRx was used first because it's an easily used docking wizard which will aid in speeding the process by autodocking only compounds that pass the first docking phase with binding energy -8.0 kcal\mol or lower. The reason is that PyRx is a user-friendly interface with ability to dock 50 compounds in each run (Zolfaghari, 2017). PDB format of each compound that had a fit value over 3.5 in BIOVIA DS filtration where uploaded as ligands along with 5LGT as macromolecule to PyRx autodock vina tool. Results were obtained and compounds that passed the binding energy criteria were further docked by Autodock 4.2.

3.4.2 AutoDock

The second docking phase was the use of AutoDock 4.2 version tool. Compounds were manually docked with 5LGT in order to obtain the binding energy scores and Ki values. Grid box dimensions for the GPF file were set to 55 A° 55 A° 55 A° and the XYZ coordination to -66.531, -37.003, -35.506 respectively. for the DPF files Lamarckian genetic algorithm was applied and 20 GA runs performed for each compound with 25 million evaluations.

Afterwards Autodock script was run in terminal to randomly detect torsions angels and obtain results as .dlg files which contain information about all 20 runs performed by Autodock and from which the binding energy and estimated inhibition constant Ki of the best run were obtained.

3.5 Generating 2D and 3D diagrams

Biovia Discovery studio visualizer's receptor-ligand interaction tool was used for the generating of both 2D and 3D diagrams of the 24 autodocked results for furthered examination of their interactions with 5LGT receptor. Coordinations were obtained from the .dlg files and added to the pdb file of 5LGT and processed with discovery studio to obtain first the 2D diagrams and then 3D diagrams for the ligands with interesting interactions.

4. RESULTS AND DISCUSSION

4.1 Protein Validation

Validating the protein acquired from protein data base in its crystal structure is the first and most important step in any CADD based work as it lays the base for the other steps as mentioned above. 5LGT was prepared and docked with known inhibitors accessed from literature to validate its crystal structure. The main criterion for the protein validation is to pass -8.00 kcal/mol binding energy threshold when autodocked against known inhibitors obtained from literature. As a result to the docking procedure, all acquired known inhibitors docked with 5LGT enzyme 'subject of the test', passed the threshold with binding energy range between -8.21 kcal/mol and -11.27 kcal/mol as presented in table (4.1), hence giving a green light to continue working with 5LGT as a crystal structure of LSD1 enzyme in this study.

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Table (4.1): Known inhibitors docking results against 5LGT crystal structure of LSD1

Known Inhibitor	IC50 / Ki values from literature (nm)	Binding Energy (kcal/mol)	Ki values (nm)
Compound 1	Ki =29	-10.62	16.44
GSK2699537	IC50= 16	-8.85	325.18
GSK-2879552	IC50= 38	-10.51	19.82
RN-1	IC50= 10-70	-9.36	137.84
RN-2	IC50= 70	-10.37	24.84
IC	IC50= 10 μm	-9.66	150.26
CBB1007	IC50= 5.27μM	-9.40	68.30

D compound	Ki=2.4 ± 0.63 μM	-9.14	439.09
G compound	Ki =0.009 μM	-10.70	586.22
6w3	IC50 = 160 nm	-10.30	13.02
F compound	Ki =0.005 μM	-8.82	2.20
Namoline	IC50=51 μM.	-8.71	480.08

4.2 Database Screening and Docking

Zinc.15 data base with 600 thousand compounds was screened, and 40 thousand compounds were obtained and filtered with PyRx autodock vina docking tool as it has been mentioned in the previous chapter and 100 compounds were able to achieve the threshold goal of -8.00 kcal/mol binding affinity or less in PyRx. These 100 compounds were then prepared for further docking with Autodock software and as a result of the docking process 24 compounds have shown potential LSD1 inhibition activity with binding energies of a range between -8.21 kcal/mol for ZINC000005687158 compound being the least significant binding result and -11.27 kcal/mol for ZINC000012246801 compound as the most significant binding result. In table (4.2) all 24 compounds are demonstrated with their binding energy results from docking with Autodock software along with their Ki values also obtained from Autodock, their molecule weight (MWt) and last their lipophilicity (LogP). Both MWt and LogP information were obtained from the zinc.15 database website.

Table (4.2): 24 docked compounds with significant binding energy

	Ligand	Binding Energy kcal/mol	Ki nM	MWt Dalton	Log P
1	ZINC000025765273	-9.74	72.80	342.398	4.957
2	ZINC000252430948	-9.23	172.85	325.452	4.963
3	ZINC000224772079	-8.61	484.39	325.452	4.629
4	ZINC000001470099	-10.54	18.90	345.527	4.737
5	ZINC000224737150	-9.06	228.03	345.442	4.831
6	ZINC000224722229	-8.77	370.16	347.499	4.853
7	ZINC000033648004	-9.10	212.76	334.419	4.571
8	ZINC000224978372	-8.42	675.61	338.451	4.636
9	ZINC000032752547	-8.98	261.19	334.419	4.636
10	ZINC000033603612	-8.68	434.01	348.398	4.802
11	ZINC000224369627	-8.67	438.04	341.451	4.501
12	ZINC000224761650	-9.19	184.90	339.479	4.715
13	ZINC000033601697	-9.21	177.07	348.402	4.774
14	ZINC000033737726	-9.25	165.09	348.446	4.88
15	ZINC000033721741	-8.76	382.25	348.446	4.88
16	ZINC000224737801	-9.44	119.72	337.463	4.708
17	ZINC000031923896	-9.89	56.26	329.443	4.52
18	ZINC000003449908	-8.39	706.30	339.372	4.603
19	ZINC000005687158	-8.21	966.06	208.265	-0.122
20	ZINC000035900121	-8.55	536.29	223.32	0.045
21	ZINC000042680039	-8.67	443.55	225.336	0.293
22	ZINC000000123376	-9.99	47.50	349.43	4.54
23	ZINC000000123384	-9.28	158.89	349.43	4.54
24	ZINC000012246801	-11.27	5.45	344.458	4.834

4.3 Generating 2D and 3D Diagrams

For further examination of the interactions between the ligands and the binding pocket amino acids of 5LGT, 2D structures were generated for all 24 compounds. And for further investigation, 3D structures of the compounds which have showed the best favorable interactions in 2D structures were generated also for a clearer understanding of its interactions in the binding pocket of 5LGT. Figure (4.1) and figure (4.2) demonstrates the interactions between ZINC000012246801 ligand compound and 5LGT in 2D structure and 3D structure images which highlights the presence of several important interactions such as van der waals , a salt bridge, an attractive charge and a pi-anion interactions between ASP555, ASP556 and GLU559 and both the piperidine ring and the benzene ring. Also, a conventional hydrogen bond is formed between ASP556 and the hydrogen atom from the benzene ring. Other interactions are also formed including pi-pi T-shaped, pi-alkyl, amide-pi stacked between PRO808, ALA809 and the benzene ring of the ligand and between PHE506 and the pyridine ring. These interactions together give an idea of the high binding energy of the ligand mentioned with 5LGT which is -11.27 kcal/mol.

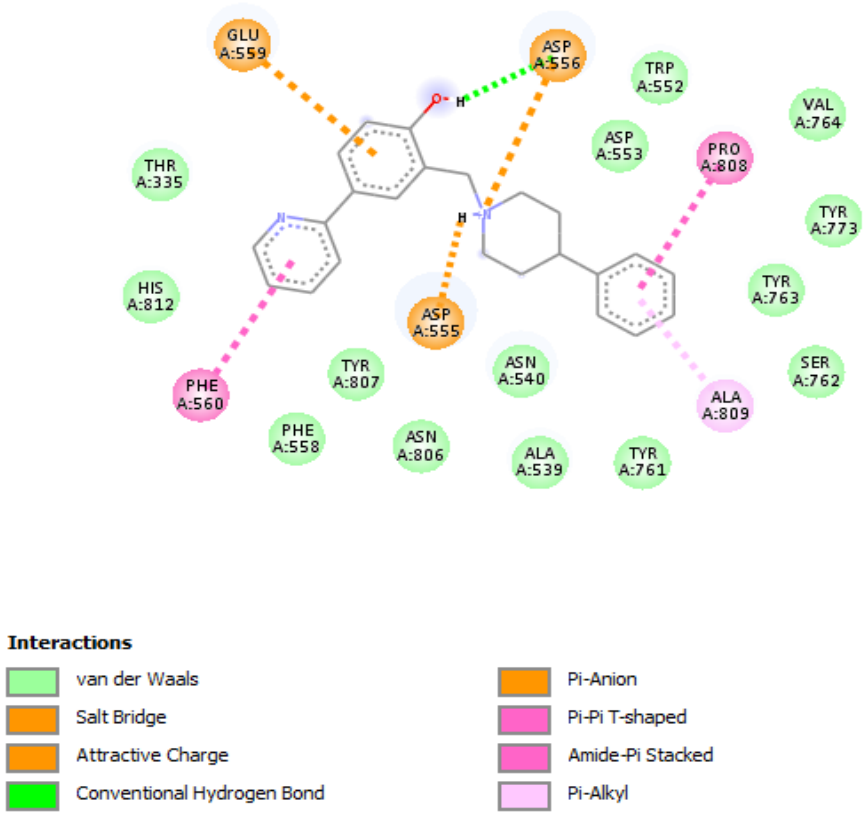

Figure (4.1) 2D diagram of the chemical interactions between ZINC000012246801 with 5LGT receptor

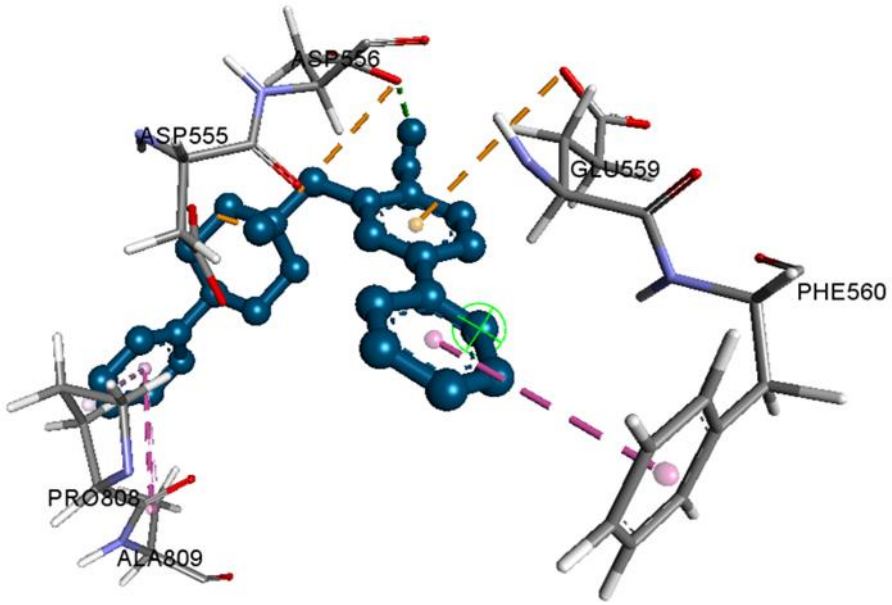

Figure (4.2) 3D diagram of the chemical interactions between ZINC000012246801 with 5LGT receptor

Three other compounds; ZINC000031923896, ZINC000042680039, and ZINC000035900121 with binding energies of -9.89 kcal/mol, -8.67 kcal/mol, and -8.55 kcal/mol respectively appeared to form salt bridge interactions with 5LGT binding pocket in their 2D structures as shown in their images in figures (4.3), (4.4), and (4.5) respectively. However, none of them had pi interactions except for ZINC000031923896 compound with the presence of pi-anion, pi-pi T-shaped, and pi-alky, also out of the three compounds mentioned it has the higher binding energy of -9.89 kcal/mol which leads to presuming that the formation of a salt bridge interaction along with pi-anion interactions between ASP555 and both the nitrogen on the 1,2,3,4-tetrahydroisoquinoline ring and the benzene ring along with a conventional hydrogen bond between the same residue and the ligand leads to better binding of the compounds to 5LGT binding pocket.

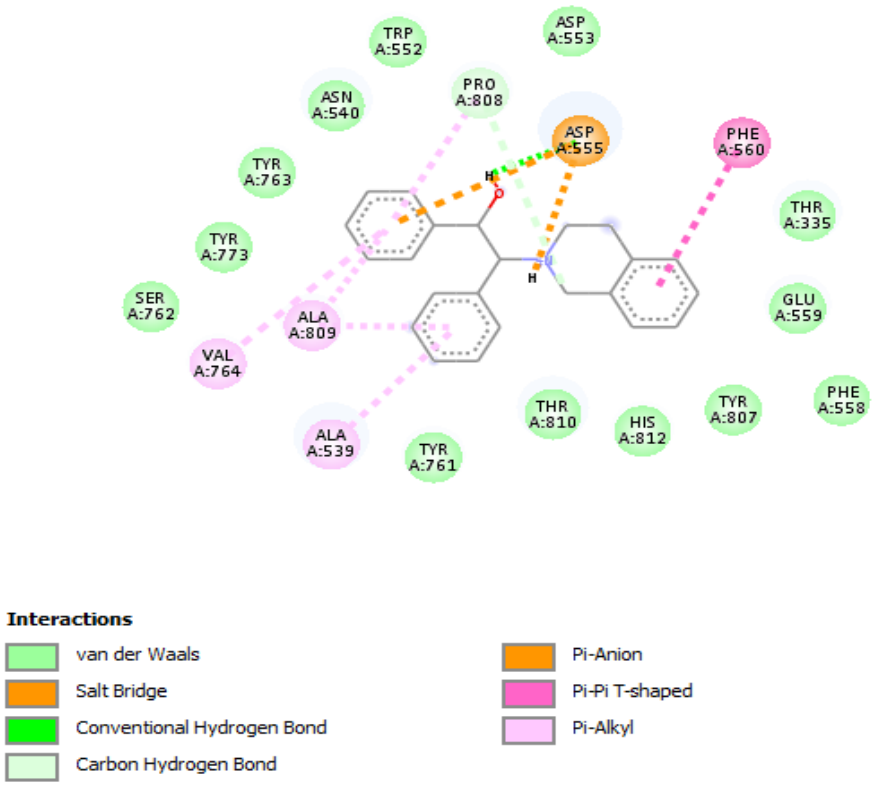

Figure (4.3) 2D diagram of the chemical interactions between ZINC000031923896 with 5LGT receptor

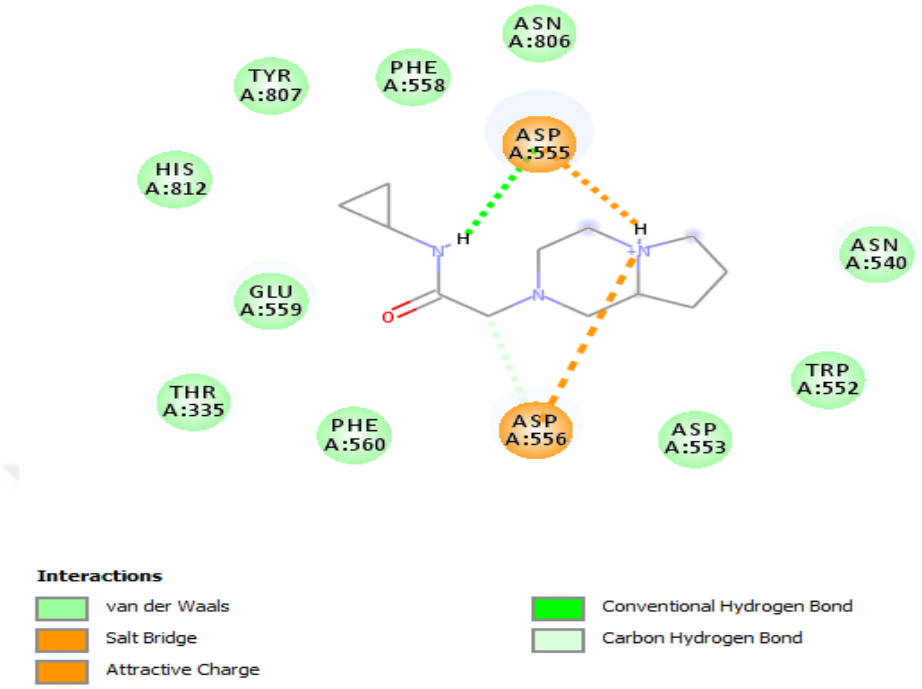

Figure (4.4) 2D diagram of the chemical interactions between ZINC000042680039 with 5LGT receptor

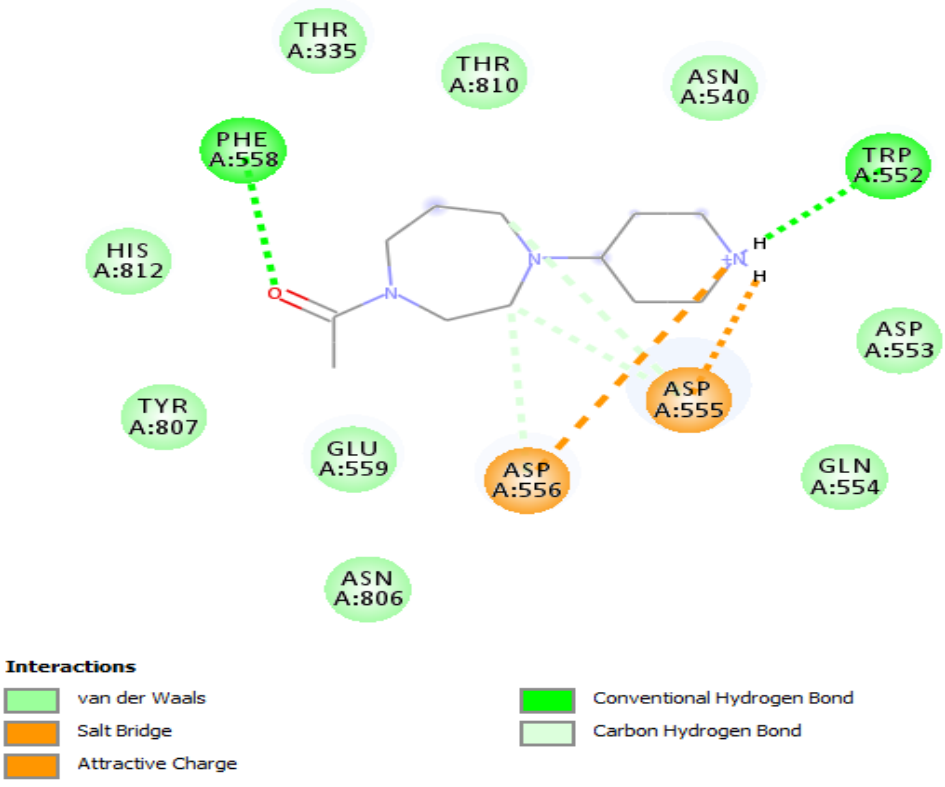

Figure (4.5) 2D diagram of the chemical interactions between ZINC00004268001 with 5LGT receptor

9 other compounds with code names ZINC000001470099, ZINC000224737150, ZINC000033648004, ZINC000224761650, ZINC000033601697, ZINC000033737726, ZINC000224737801, ZINC000031923896, ZINC000000123376, and ZINC000000123384 demonstrated in figures from (4.6) to (4.15) having a binding energy between -9.06 kcal/mol and -10.54 kcal/mol have presented potential by forming interactions such as pi-anion, pi-pi T-shaped, and pi-alkyl with 5LGT binding pocket residues as observed in their 2D diagrams along with the presence of an abundance of van der waals interactions and conventional hydrogen bonds.

One compound in particular, with code name ZINC000001470099, among the other nine compounds have presented interesting ability with a binding energy of -10.54 kcal/mol. When 2D and 3D diagrams, figures (4.6) and (4.7) respectively, were generated for it and abundance of van der waals interactions were observed along with pi- anion and attractive charge interactions between ASP555 and both the nitrogen on piperidine ring and the benzyne ring, and a conventional hydrogen bond between TRP552 and an oxygen atom on the ZINC000001470099 ligand, also alkyl and pi-alkyl interactions between PRO808, ALA809, HIS812 and PHE560 and both the piperidine ring and the cyclohexane ring of the ligand.

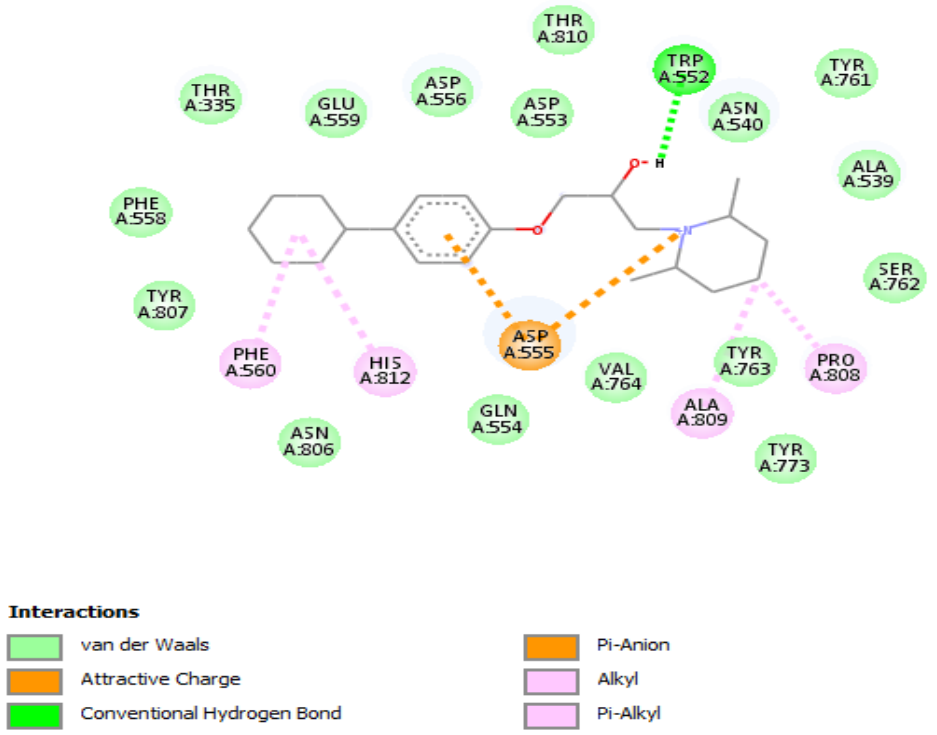

Figure (4.6) 2D diagram of the chemical interactions between ZINC000001470099 with 5LGT receptor

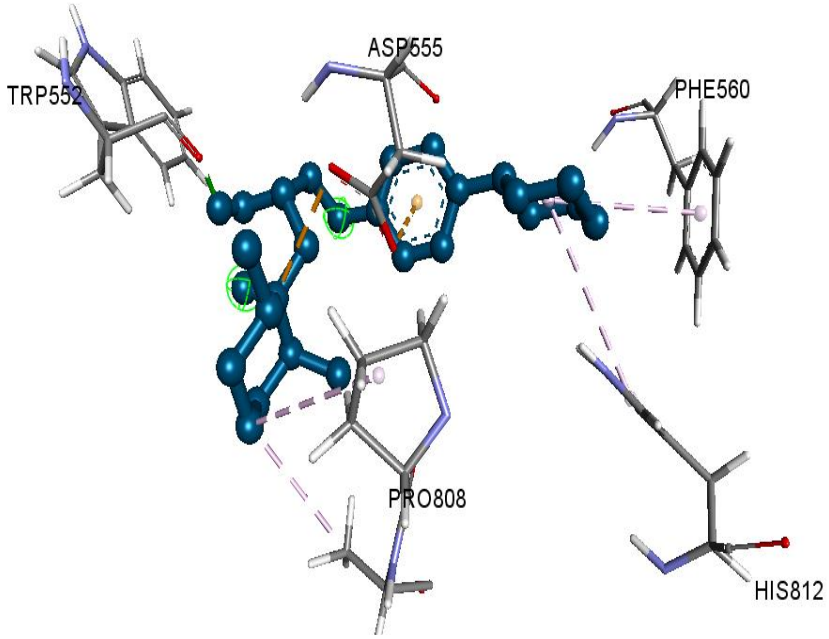

Figure (4.7) 3D diagram of the chemical interactions between ZINC000001470099 with 5LGT receptor

Figure (4.8) of the ZINC000033648004 compound 2D interactions in 5LGT substrate binding site also represents potential by forming 3 conventional hydrogen bonds between ASP555, PRO808 and ASN540 from the binding site and the ligand. Also 2 pi-anion interactions are formed between ASP555 and the ring of the ligand. Moreover, pi-donor hydrogen bond, pi-sigma. Pi-pi T shaped, pi-alkyl and van der waals interactions are also formed which presumably strength the binding of the ligand to 5LGT.

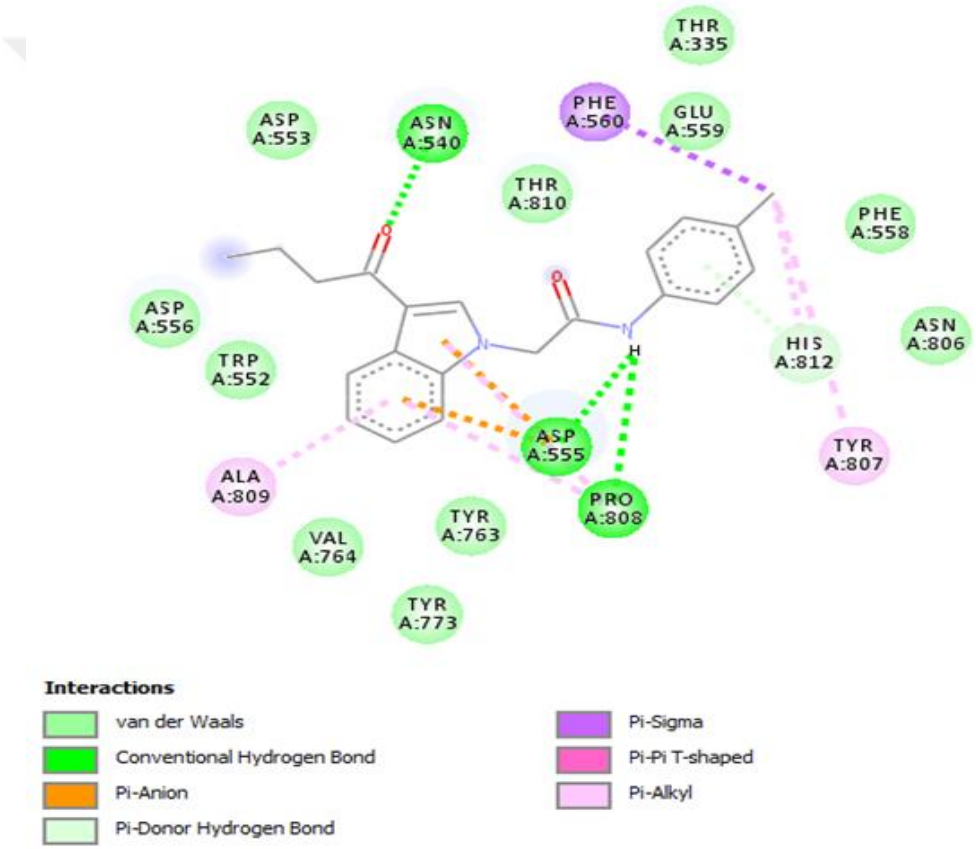

Figure (4.8) 2D diagram of the chemical interactions between ZINC000033648004 with 5LGT receptor

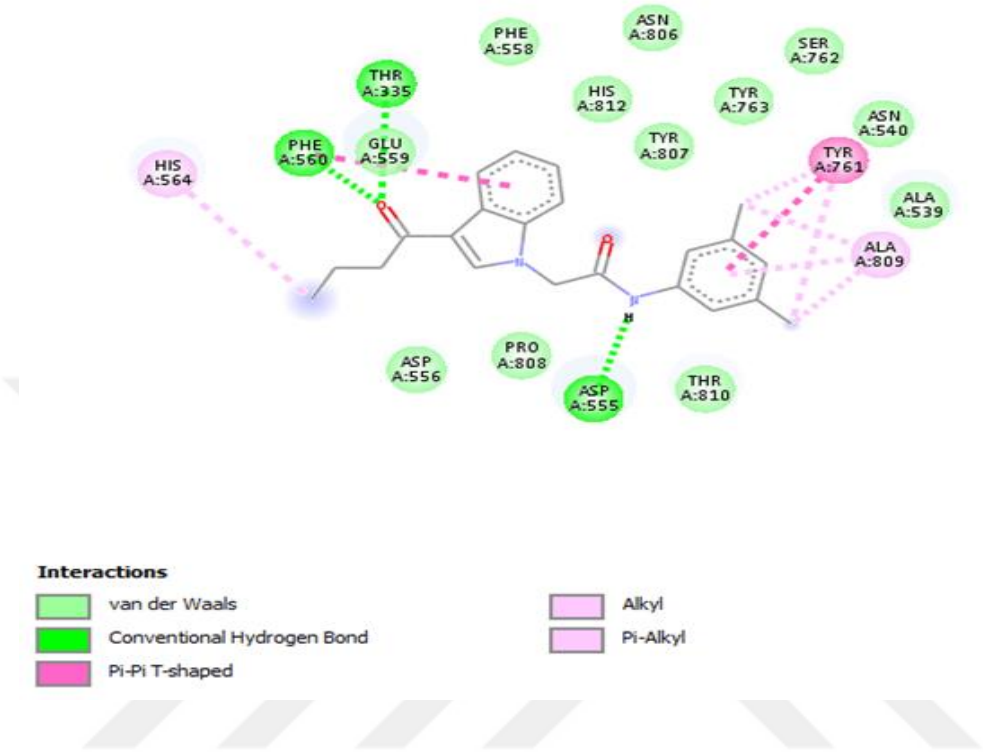

Figure (4.9) 2D diagram of the chemical interactions between ZINC000033737726 with 5LGT receptor

ZINC000033737726 compound in figure (4.9) had 3 conventional hydrogen bonds between the ligand and ASP555, THR335 and PHE560 on the binding site of 5LGT.

While compound ZINC000000123376 in figure (4.10). also presented good interactions between ASP55 and the ligand in form of attractive charges and pi-anion interactions with abundance of van der waals interactions and 2 conventional hydrogen bonds

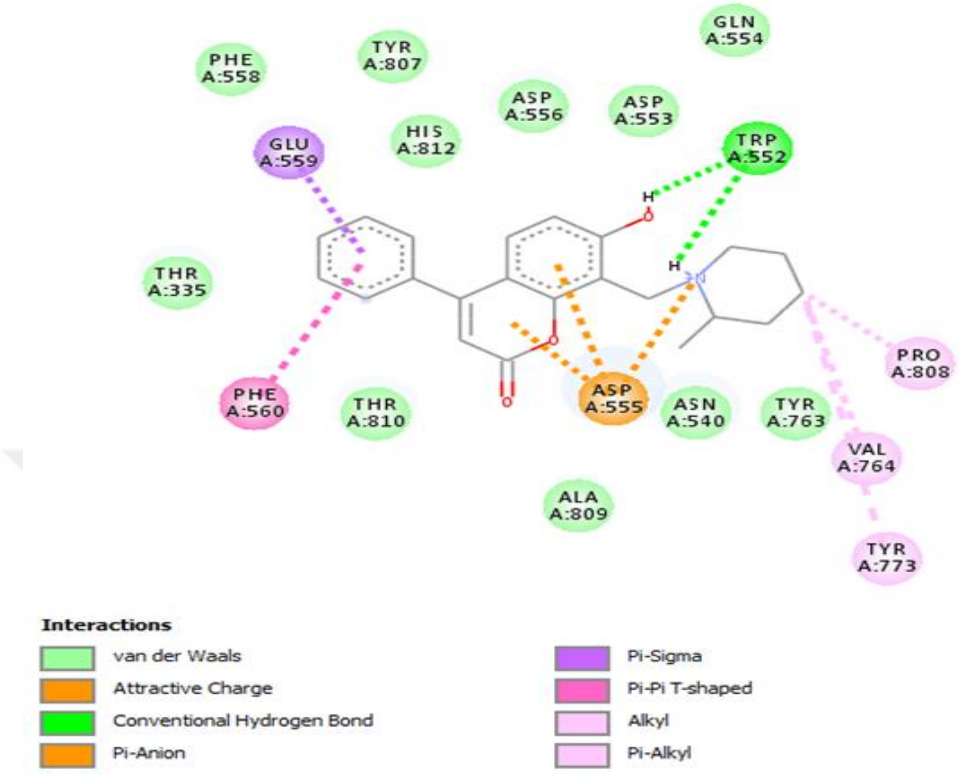

Figure (4.10) 2D diagram of the chemical interactions between ZINC000000123376 with 5LGT receptor

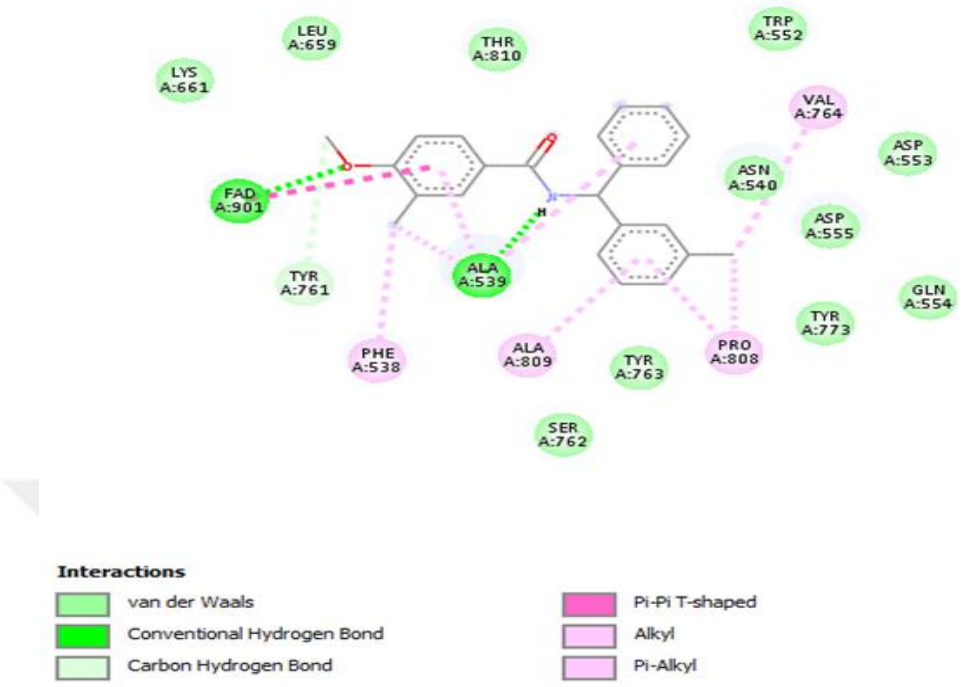

Figure (4.11) 2D diagram of the chemical interactions between ZINC000224737150 with 5LGT receptor

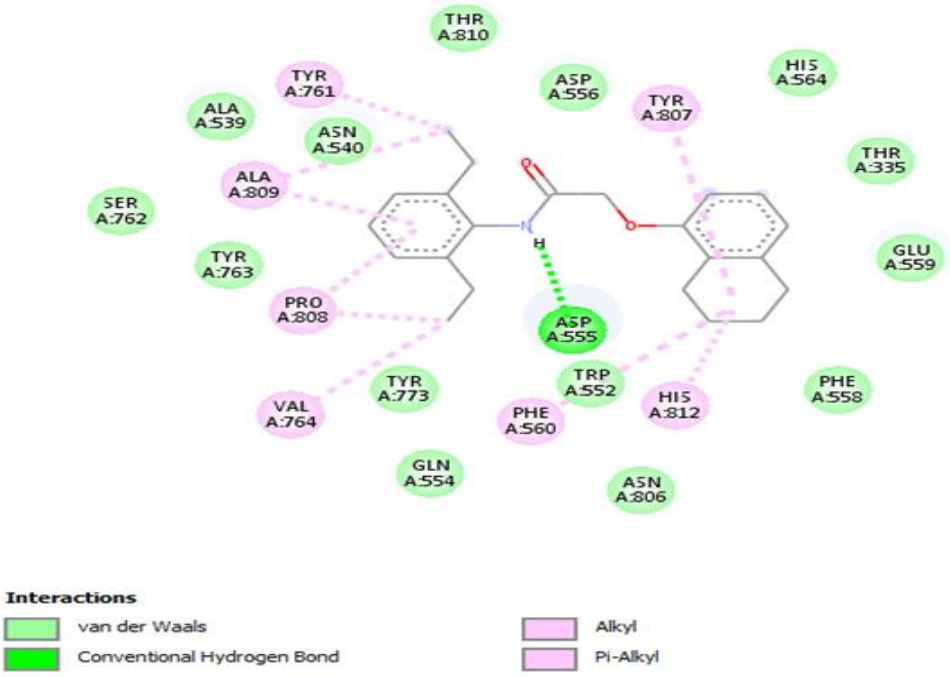

Figure (4.12) 2D diagram of the chemical interactions between ZINC000033601697 with 5LGT receptor

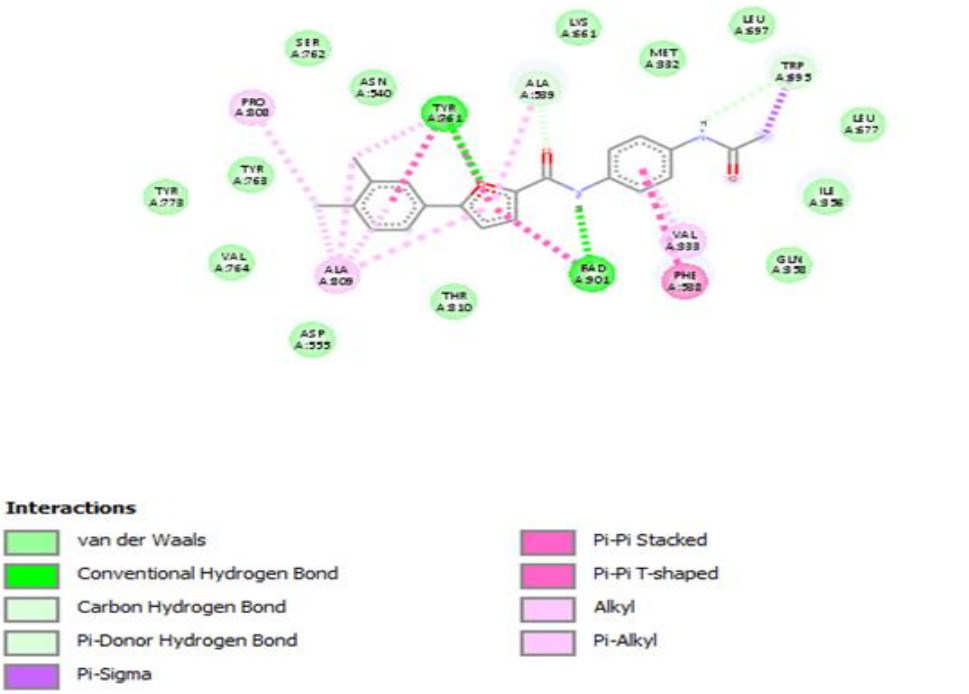

Figure (4.13) 2D diagram of the chemical interactions between ZINC000224737801 with 5LGT receptor

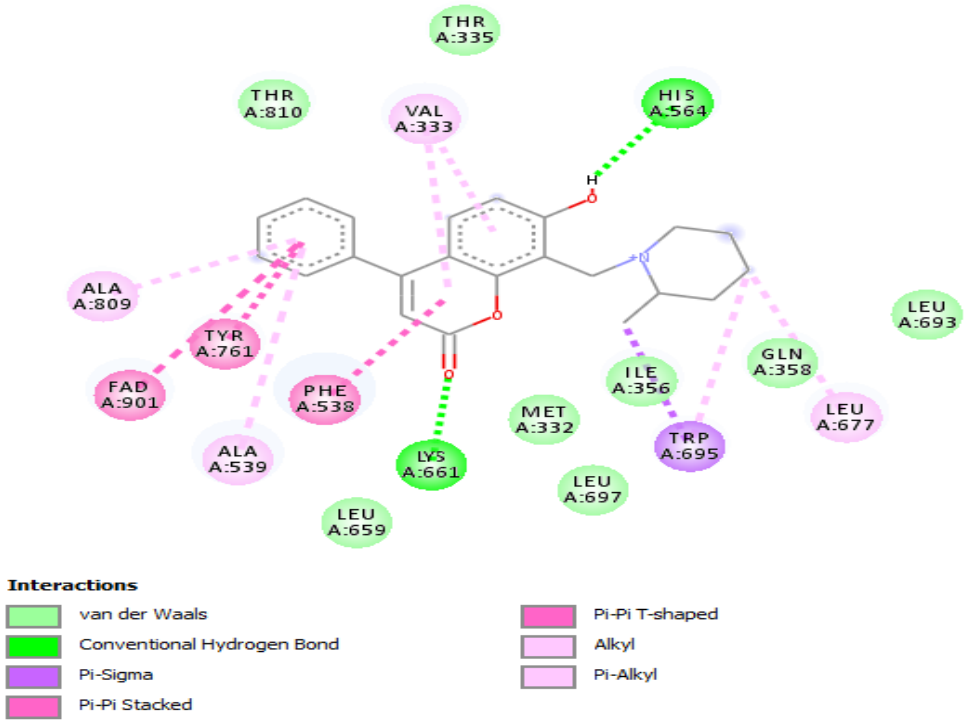

Figure (4.14) 2D diagram of the chemical interactions between ZINC000224761650 with 5LGT receptor

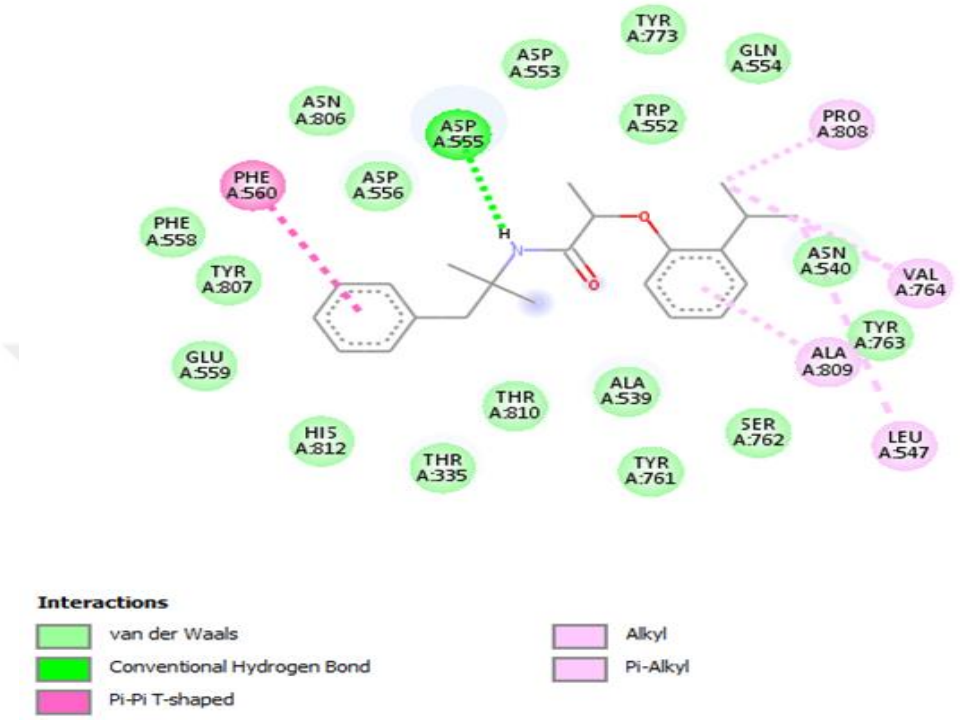

Figure (4.15) 2D diagram of the chemical interactions between ZINC000000123384 with 5LGT receptor

Of all the 24 compounds obtained, only one compound ZINC000252430948, presented in figure (4.16), with binding energy of -9.23 kcal/mol has showed unfavorable acceptor- acceptor interaction along with two conventional hydrogen bonds one of them between ALA539 and a hydrogen atom of the compound and the other one between the FAD cofactor and oxygen atom of the compound, also the presence of van der waals attractions in abundance with alkyl and pi-alkyl interactions between ALA809 and PRO808 and the bicyclo[10.1.0]trideca-4,8-diene ring.

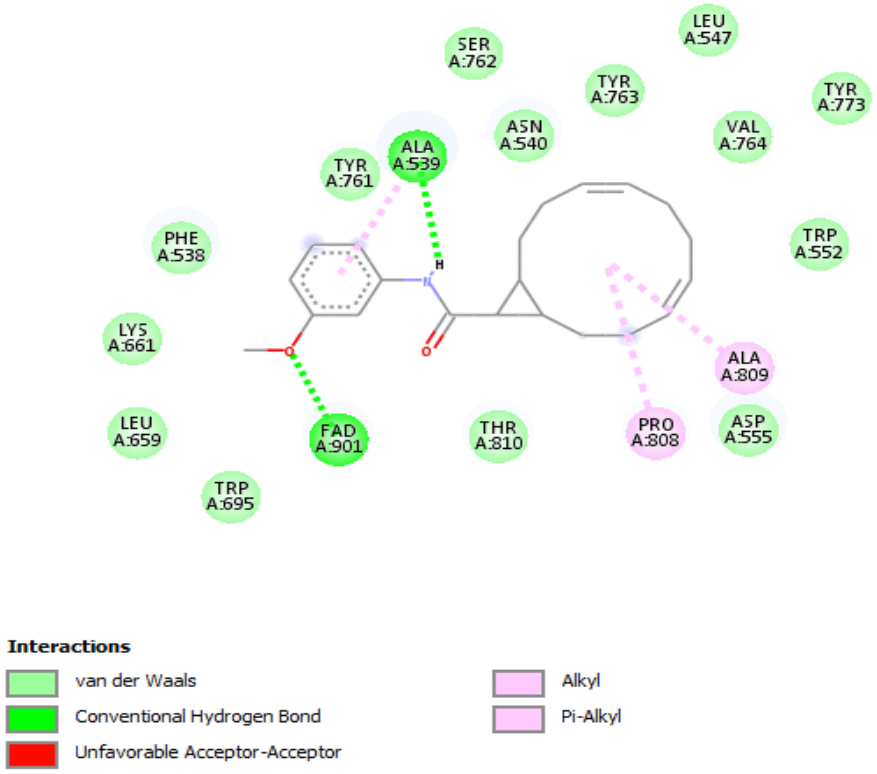

Figure (4.16) 2D diagram of the chemical interactions between ZINC000252430948 with 5LGT receptor

5. CONCLUSION AND RECOMMENDATIONS

As mentioned in an earlier chapter LSD1 enzyme possess a great epigenetic role in the cell and thus in the development of different diseases, most importantly several types of cancer. For this reason new studies are paying a grave attention to find or design a ligand that can bind to it and inhibit its activity.
This study as well focused on the ability to find a chemical structure that has all the required characteristics to presumably inhibit LSD1 enzyme through applying in silico approaches . As mentioned in the results section 24 compounds have been found to have the ability to fit in the binding pocket of the enzyme with different interactions, most commonly van der waals, conformational hydrogen bonding and Pi alkyl interactions also few compounds made salt bridge interactions mainly between ARG555 from the binding site of LSD1 and the candidate drug like ligands. These compounds had a molecular weight ranging between 200 and 400 Daltons, and although the binding pocket of LSD1 is known to be big enough for higher molecular weight ligand, hence higher binding energy, this being one of the characteristics that distinguish LSD1 from other demethylase enzymes. However, some of the most recognized inhibitors for LSD1 are of small molecular weight which are tranylcypromine and cyclopropylamine which is why a great focus was given to smaller molecule weight compounds in this study.

Conclusively, regardless of these 24 compounds promising binding energy results, it’s recommended to further study their interactions and their absorption, distribution, metabolism, excretion, and toxicity should be conducted as well as in vitro and in vivo conformations. considering that one of them might be a candied for LSD1 inhibition.

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