

KADIR HAS UNIVERSITY
SCHOOL OF GRADUATE STUDIES

**NOVEL INHIBITOR DESIGN FOR HISTONE
DEMETHYLASE 1 (LSD1) ENZYME USING MOLECULAR
MODELING**

MARWAH ALSHAFEAY

MASTER THESIS

ISTANBUL, May, 2019



**NOVEL INHIBITOR DESIGN FOR HISTONE
DEMETHYLASE 1 (LSD1) ENZYME USING MOLECULAR
MODELING**

MARWAH ALSHAFEAY

MASTER THESIS

Submitted to the School Of Graduate Studies
Of Kadir Has University in Partial Fulfillment of the Requirements for the Degree of
Master in the Program of Computational Biology and Bioinformatics

ISTANBUL, MAY, 2019

DECLARATION OF RESEARCH ETHICS /
METHODS OF DISSEMINATION

I, Marwah Alshafeay hereby, declare that;

- This Master's thesis is my original work and that due references have been appropriately provided on all supporting literature and resources.
- This Master's Thesis contains no material that has been submitted or accepted for a degree or diploma in any other educational institution.
- I have followed *Kadir Has University Ethics Principle Prepared in Accordance With the Council of Higher Education's Ethical Conduct Principles*.

In addition, I understand that any false claim in respect of this work will result in disciplinary action in accordance with University regulations.

Furthermore, both printed and electronic copies of my work will be kept in Kadir Has Information Centre under the following condition as indicated below.

- The full content of my thesis/project will not be accessible for years two. If no extension is required by the end of this period, the full content of my thesis will be automatically accessible from everywhere by all means.



.....
MARWAH ALSHAFEAY

21.5.2019

KADIR HAS UNIVERSITY
SCHOOL OF GRADUATE STUDIES

ACCEPTANCE AND APPROVAL

This work entitled NOVEL INHIBITOR DESIGN FOR HISTONE DEMETHYLASE 1 (LSD1) ENZYME USING MOLECULAR MODELINGS prepared by Marwah ALshafeay has been judged to be successful at the defense exam on 21.5.2019 and accepted by our jury as master's thesis.

APPROVED BY:

Prof. Dr. Kemal YELEKÇİ (Advisor)
Kadir Has University

Asst. Prof. Şebnem EŞSİZ GÖKHAN
Kadir Has University

Dr.Öğr. Üyesi Vildan ENİSOĞLU ATALAY
Uskudar University

I certify that the above signatures belong to the faculty members named above.

K. Yelekcı
Şebnem Eşsiz Gökhan
N. Enisoğlu
Sinem Akgül Açıkmeşe

Dean of School of Graduate Studies

DATE OF APPROVAL: 21.5.2019

TABLE OF CONTENT

ABSTRACT	i
ÖZET	i
ACKNOWLEDGMENT	iii
LIST OF TABLES	iv
LIST OF FIGURES	v
LIST OF ABBREVIATIONS	vii
1. INTRODUCTION	1
1.1 Overview	1
1.2 Problem Statement and Its Significance.....	2
1.3 Objectives of the Study	3
1.4 A Brief Description of Research Methodology.....	3
1.5 Scope of the Research.....	4
1.6 Thesis Organization.....	4
2. LITERATURE REVIEW	6
2.1 Introduction	6
2.2 Cancer.....	6
2.2.1. Epigenetic	7
2.2.1 Cancer Treatment	10
2.3 Histone Lysine-specific Demethylase 1 (LSD1).....	13
2.3.1 The Role of LSD1 in Cancer.....	16
2.3.2 The Association of LSD1 in Acute Myeloid Leukemia (AML)	17
2.3.3 The Association of LSD1 in Lymphoid Leukemia	18
2.3.4 The Association of LSD1 in Solid Cancers.....	19
2.4. LSD1 Inhibitors	19
2.4.1 Tranycypromine LSD1 inhibitor	21
2.5 Drug Design Techniques	22
2.5.1 Molecular Docking.....	23

2.5.2 Virtual Screening.....	24
2.5.3 Pharmacophore modeling.....	25
2.5.4 ADMET prediction in rational drug design.....	25
3. METHODS AND MATERIAL.....	27
3.1 Introduction	27
3.2 Protein Validation.....	28
3.3. Virtual screening.....	30
3.4 Structure Based Pharmacophore Modeling	31
3.5 Auto Dock	31
3.6 ADMET Prediction Test.....	32
4. RESULTS.....	33
4.1. Introduction	33
4.2 Protein Validation.....	33
4.3 Virtual Screening.....	34
4.4 Structure Based Pharmacophore Modeling	37
4.5 ADMET Prediction	41
5. CONCLUSION AND RECOMMENDATIONS.....	52
REFERENCES.....	54

NOVEL INHIBITOR DESIGN FOR HISTONE DEMETHYLASE 1 (LSD1) ENZYME USING MOLECULAR MODELING

ABSTRACT

Cancer is the most lethal disease among all known diseases and comprises the largest segment of death causes. Although many factors have direct involvement in cancer triggering, still the complete understanding behind the main cause of cancer is unrevealed. Recent studies have shown that the epigenetic process plays an ultimate role in cancer initiation. DNA methylation and histone modification are considered the most common systems that might cause epigenetic changes. Histone lysine specific demethylase (LSD1) has proved to have a significant impact and involvement in a wide range in the epigenetic process including gene silencing, DNA transcription, DNA replication, and DNA repairing. In addition, it has been noticed that LSD1 enzyme level is increased in many cancer types such as AML, breast cancer, prostate cancer, and many other cancers. However, extensive attention has been drawn toward developing safe and effective LSD1 inhibitors, meanwhile, only two drugs are described as LSD1 inhibitors 2-[4-methoxy-phenyl] cyclopropylamine and Tranylcypromine which they are not very selective. Therefore, the main objective of the research is to design and develop specific and selective inhibitors for LSD1 using molecular modeling *in silico*. Potential lead compounds were obtained using virtual screening and structure based pharmacophore. Zinc15 database was used in this research; more than 60 thousand compounds were screened. As results of different analysis using PyRx (autodock), several hundred compounds shown better binding energy values than -9kcal/mol. However, 20 compounds out of total obtained compounds shown significant binding energy, and desirable chemical interaction at the active site of the enzyme. Pharmacokinetics properties of the 20 selected compounds were investigated by applying ADMET prediction assay. All 20 compounds passed the ADMET prediction criteria and they can serve as drug candidates for advance optimization across the design of safe, effective, and selective LSD1 inhibitors.

Keywords: Epigenetic, LSD1, Tranylcypromine, molecular modeling, Zinc15 database.

YENİ INHİBİTÖR TASARIMI HISTONE DEMETHYLASE 1 (LSD1) MOLEKÜLER MODELLEME KULLANIMI ENZİM

ÖZET

Kanser bilinen hastalıkların en öldürücü ve ölüm nedenlerinin büyük bir kısmını kapsar. Kanseri tetikleyen birçok faktörün bilinmesine rağmen ana neden tam olarak henüz anlaşılmiş değildir. Son yıllarda yapılan çalışmalardan epigenetik süreçlerin kanser başlangıcında önemli rol oynadıkları bulunmuştur.

DNA'nın metilasyonu ve histon modifikasyonları en yaygın sistemler olarak epigenetik değişimlere sebep olurlar. Histon lisinspesifik demetilaz (LSD1) gen susturmada, DNA transkripsiyonunda, DNA replikasyonunda ve DNA hasar tamiri gibi geniş bir yelpazede epigenetik proseslerde görev almaktadır. Bunların yayında LSD1 enziminin seviyesi AML, meme kanseri, prostat kanser ve birçok diğer kanser tiplerinde anlamlı bir şekilde yükseldiği görülmüştür. LSD1 enzimine etkili ve güvenli inhibitör tasarlanması için yoğun bir ilgi çekmemesine rağmen sadece çok seçici olmayan iki ilaç; 2-[4-methoxyphenyl]cyclopropylamine ve tranlycypromine onay almıştır. Çalışmamızın amacı *in silico* yöntemle daha seçici ve potansiyeli yüksek özgün LSD1 inhibitörleri tasarlamak ve geliştirmektir.

Sanal tarama ve yapıya dayalı farmakofor modelleme yaklaşımları kullanılarak potansiyel lider bileşikler elde edilmiştir. Çalışmada Zinc 15 veri bankasında bulunan 60,000 bileşik kullanılmıştır. PyRx (autodockVina) kullanılarak değişik analizler sonucunda bağlanma enerjileri -9 kcal/mol 'dan daha iyi birkaç yüz bileşik elde edilebilmiştir. Bu yaklaşımlardan LSD1 enzimini inhibe eden 20 bileşik bağlanma enerjileri ve 2D resimlerden enzimin aktif kısmına konumlanma gibi istenilen özellikleri karşılayabilecek niteliklere sahip olduğu tespit edilmiştir. Seçilen 20 bileşiklerin farmakokinetik özellikler ADMET testleri uygulanarak kontrol edilmiştir.

Anahtar Sözcükler: Epigenetik, LSD1, Tranlycypromine, moleküler modelleme, Zinc15 veritabanı.

ACKNOWLEDGMENT

Foremost, I would present my gratitude and thanks to my supervisor, **Prof. Dr. Kemal YELEKÇİ**, for his guidance and encouragement. He was a spiritual father not only a supervisor. I had the honor to be his student. At the same time, I hugely indebted my husband, **Mr. GHASSAN ALI**, who demonstrate a senior deal of supporting, patience and understanding, and definitely, my sincere appreciation to my parents, **Mr. ABD ALI ALSHAFFEAY** and **Mrs. AHLAM JASSIM**, for the absolute support and enormous love they have offered me throughout my life. Ultimately many thanks to all **my teachers** and **colleagues** for their help during my studies.

LIST OF TABLES

Table	Title	Page
2.1	Some approved chemo- therapeutic with their mechanisms	12
2.2	molecular details of LSD1 from	14
2.3	some histone lysine demethylases involved in different cancers	16
2.4	LSD1 inhibitors under clinical and pre-clinical investigation	20
3.1	Known LSD1 inhibitors that used for validation	29
4.1	Binding energies of redocked known inhibitors	34
4.2	The ten selected compounds that possess significant binding energies were obtained via PyRx	35
4.3	lists the 10 chosen compounds that obtained from structure based pharmacophore modeling according to ADMET prediction	39



LIST OF FIGURES

Figure	Title	Page
1.1	Schematic diagram of brief illustration on research methodology	4
2.1	Modification on histone and DNA	9
2.2	different modifications of histone by writers, readers and erasers proteins ..	10
2.3	chemical structure of Taxol (Paclitaxel)	11
2.4	chemical structure of Platinol-AQ (Cisplatin)	11
2.5	chemical structure of Taxotere (Docetaxel)	12
2.6	The 3D structure of LSD	14
2.7	TCP mechanisms in LSD1 inhibition atropaladehyde pathway	21
2.8	mechanisms in LSD1 inhibition cinnamaldehyde pathway	21
3.1	Flow diagram of the study	27
4.1	Chemical structures of ten compounds that obtained via PyRx	36
4.2	Hypothesis number 4	37
4.3	Hypothesis number 10	38
4.4	The chemical structures of the 10 chosen compounds that obtained from structure based pharmacophore modeling	40
4.5	The ten compounds that obtained from virtual screening which they passed ADMET criteria	42
4.6	The ten compounds out of 200 compounds that obtained from structure base pharmacophore modeling which they passed ADMET criteria.....	43
4.7	The 2D of the chemical interactions between compound zinc_19925080 with 3ZMS receptor	44
4.8	The 3D of the chemical interactions between compound zinc_19925080 with 3ZMS receptor	45
4.9	The 2D of the chemical interactions between compound zinc_19925082with 3ZMS receptor	46

4.10	The 3D of the chemical interactions between compound zinc_19925082with 3ZMS receptor.....	47
4.11	The 2D of the chemical interactions between compound ZINC000170602679 and 5LGT receptor.....	48
4.12	The 3D of the chemical interactions between compound ZINC000170602679 and 5LGT receptor	49
4.13	The 2D of the chemical interactions between compound ZINC000225395750 and 5LGT receptor	50
4.14	The 3D of the chemical interactions between compound ZINC000225395750 and 5LGT receptor	51

LIST OF ABBREVIATIONS

LSD1:	Lysine Specific Demethylase 1
FAD:	Flavin adinine dinucleotide
TCP:	Tranylcypramine
ADMET:	Absorption, Distribution, Metabolism, Elimination And Toxicity
AML:	Acute Myeloid Leukemia
TFS:	TFs: proteins transcription factor protein
H3K4:	histone H3 lysine K4
MT	microtubule inhibitors
PTMs:	The process of histone post-translation modifications
JMJc:	Jumonji C Demethylase
CoREST:	transcriptional co-repressor protein
HDAC:	histone deacetylase
NCBI:	National Center for Biotechnology Information
T-ALL:	T-cell lymphoblastic leukemia
ER:	Estrogen Receptor
MAO-A:	Monoamine oxidase A
MAO-B:	Monoamine oxidase B
NMR:	Nuclear Magnetic Resonance
ER	Estrogen receptor
IUPAC:	International Union of Pure Applied Chemistry
FAD:	Food and Drug Administration
PDB:	Protein Data Bank
IC ₅₀ :	inhibitory concentration
K _i :	Inhibitor constant.
GPF:	Grid Parameters File
DPF:	Dock Parameter File
GLG:	Grid log file
DLG:	Dock log file

1. INTRODUCTION

1.1 Overview

Cancer is known as abnormal and uncontrollable growth of cells leads to serious complications to the human organs or death. Cancer occurrence is directed by many factors such as environment circumstances, lifestyle and genetic changes. Epigenetic is defined as the study of heritable changes in gene activity and expression. Epigenetic is the process in which cell differentiated and specialized into different cells as well as into different tissues. In contrast, epigenetic is believed to have a considerable effect in term of rising cancer risk. However, DNA methylation and histone modification are considered the most common systems that might cause epigenetic changes(Egger *et al.*, 2004). In addition to the involvement of the well-known factors such as lifestyle, age, and diseases status in the epigenetics initiation, still there are many significant influences factors that aid to trigger the process. Histone lysine specific demethylase (LSD1) has proved to have a significant impact and involvement in a wide range in the epigenetic process including gene silencing, DNA transcription, DNA replication and DNA repairing (AKDOĞAN *et al.*, 2011). However, the chemical structure of LSD1 is flavin adenine dinucleotide dependent that catalyzes oxidative removal one or two methyl group from H3K4 resulting in freeing two compounds namely; formaldehyde and hydrogen peroxides(Shi *et al.*, 2004) and (Metzger *et al.*, 2005). Although, LSD1 is essential for stem cells balancing and normal differentiation, but it has shown to be overexpressed in various types of cancers and it is believed to be a malignancy trigger. Furthermore, decreasing the level of LSD1 might lessen the ability of cancer cells to grow and migrant (Lv *et al.*, 2012).

Since the last decade, considerable attention has been drowning toward designing and finding such LSD1 inhibitors that would be effective and safe. Yet, the first generation of LSD1 as same as many drugs had pros and cons, the prime side effect of the first generation is represented in being fairly toxic and unfavorable to study (Schenk *et al.*, 2012)and(Harris

et al., 2012). Up to date, only two approved drugs namely; tranlylcypromine, and 2-[4-methoxy-phenyl]cyclopropylamine(Schmidt and McCafferty, 2007)and(Shi et at., 2004)are labeled as LSD1 inhibitors. However, both drugs the mechanism of them against cancer is not specific and the experimental data regarding those drugs is poor (AKDO~GAN et al., 2011), therefore the development of a new generation of LSD1 inhibitors with procession a direct outcome against different types of cancer has become necessary.

In the present study, thousands of potent compounds were investigated in silico by applying different molecular and computational recognitions techniques. Thus, this study is one among only a few studies that carried out in term of finding computationally effective and novel LDS1 inhibitors by providing an explanation on binding site and interaction residues. In this work, more than 60,000 compounds of zinc 15 database were screened using PyRx virtual screening tool software. However, 950 eligible compounds were chosen depending on their selectivity, for further investigation by auto-dock and ADMET tools. Nevertheless, only10 out of 950 compounds showed a significant inhibition activity against LSD1. In addition, in term of pharmacophore modeling, structure-based was applied in order to obtain the most common chemical interaction of the receptor-ligand complex. In the same context, 10 qualified compounds were chosen among the hundreds after sequencing screening of the output compounds depending on their binding energy and ADMET Prediction as well.

1.2 Problem Statement and Its Significance

Although, huge efforts have been spent experimentally to design and to find a specific LSD1 inhibitor with a demonstration of anti-cancer activity, only two drugs have been stated as LSD1 inhibitors. However, both approved drugs are not specific for cancer inhibition. In the same contrast, the experimental assays such as enzyme coupled FRET and LC-MS assays are considered costly and required special instruments(Zhenget *al.*, 2017). Conversely, the demand on using computational techniques has been increased and more attention has been paid in the area of drug design and pharmaceuticals due to cost

effectiveness as well as, less time consuming. However, in the present study zinc15 database was screened to discover a new class of lsd1 inhibitors by using molecular modeling techniques.

1.3 Objectives of the Study

- i. To reveal a new class of inhibitors of Lysine Specific Demethylase 1
- ii. To create novel inhibitors possess high potency with less side effect and ideal pharmacokinetic and pharmacodynamics properties for cancer curing.
- iii. To identify potential inhibitors by screening thousands of compounds of zinc 15 library
- iv. To search for candidate inhibitors via structure base pharmacophore modeling
- v. To investigate the possibility of generating promising LDS1 inhibitors those match the ADMET Prediction standers.

1.4 A Brief Description of Research Methodology

In this section, a brief clarification on the steps that applied in the current study is sketched in Figure 1.1 in order to provide a general sight on the major steps. However, comprehensive illustrations with all necessary details are mentioned in chapter three in this thesis.

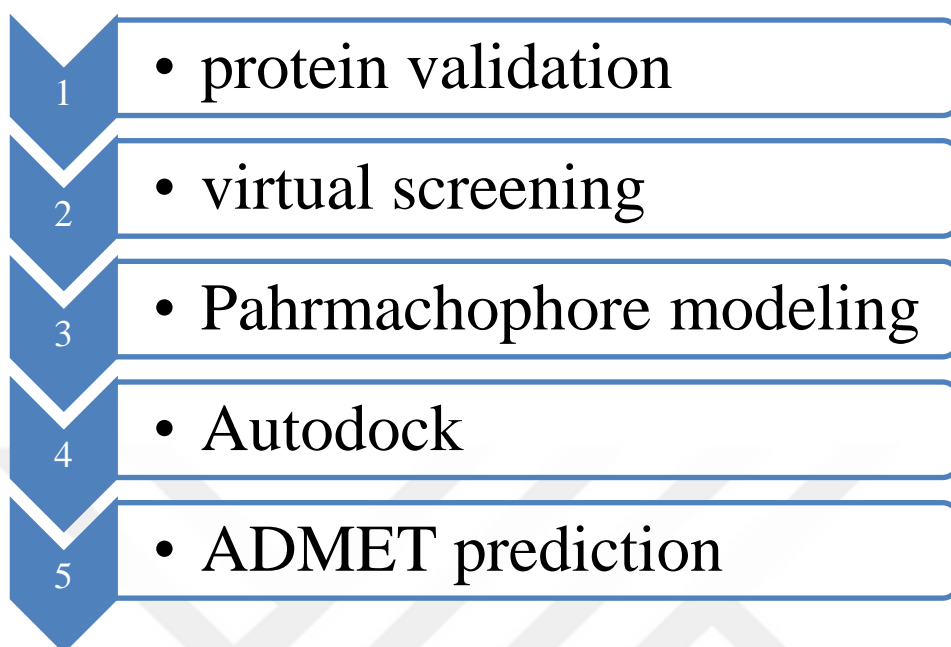


Figure 1.1: Schematic diagram of brief illustration on research methodology

1.5 Scope of the Research

The current study aims to design novel LSD1 inhibitors; hence this work composes several steps which restrict to the assigned objectives. Different reliable computational techniques were involved to achieve desirable results starting from obtaining a crystal structure of LSD1 followed by, protein validation and the others needed techniques accordingly. However, 64,000 compounds were screened in term of significance outcome, whereas only 10 compounds shown encouragement to proceed for further analyses.

1.6 Thesis Organization

This thesis composes of four chapters, starts with the introduction which in turn provides an overview on the current research, problem statement and its significance that highlights the major issue of the rule of LSD1inhibitors in cancer treatment, followed by the objectives that might help to develop safe and promising inhibitors. Chapter two is the literature review that deals with the previous works which might help to overcome the obstacles that

could be faced, as well as it covers all the research aspects and phases. Chapter three is the methodology which explains the sequence of different approaches and tools that applied in the current study. Finally, chapter four comprises the results that obtained and the discussion of entire achieved results.



2. LITERATURE REVIEW

2.1 Introduction

This chapter contains six main sections that cover all research aspects that followed in the current study and exhibits the many previous studies related in order follow the guidelines and avoid as well as overcome all obstacles that occurred in these studies. This chapter initiates with a brief definition of cancer, and how LSD1 acts as a trigger in many cancer types especially AML, in addition to the description of the most known LSD1 inhibitors. Then view on the LSD1 inhibitors that designed experimentally and in silico with details. Finally, the last section of this chapter highlights in silico drug design and the approaches of computational drug design.

2.2 Cancer

Cancer is a well-known term used to describe an aggressive and uncontrolled process of cell growth lead to a fatal end. Although, a numerous factors such as genetic, lifestyles, environment and genetics have been approved their direct involvement in the cancer process, the full understanding of cancer development is unrevealed. According to the World Health organization in 2018, 22 % of total cancer death ascribed to the smoking tobacco, while 10 % due to lifestyles includes a poor diet, alcohol consumption and deficiency of physical activity. Genetic changes on the other hand, are not less potential factors than other factors, which lead to cancer. These changes include different mutations that occur in the nucleotides of DNA genomic sequences.

However, there are more than 100 types of cancer and they vary in term of progression and cell origin. Some of these cancers have viable process form tumour while other types have hidden process such as Leukaemia. Moreover, methods of different types of cancer treatment differ from type to another. Breast cancers for example, are treated by

chemotherapy or tumour eradication. In contrast, Leukaemia has different patterns of treatment depending on its types.

Although, some cancer types are considered curable by conventional methods, but still these methods carry a number of drawbacks and side effects. The most recent studies have focused on innovated methods such as in silico drug design to bring effective and safe drugs.

2.2.1. Epigenetic

The term epigenetic refers to the heritable phenotype changes in the genome that does not involve any changes that occur in the DNA sequences. These changes have ultimate effects on gene expression as well as function, and it is strongly suggested it is linked with the environment (Mayer *et al.*, 2002). Epigenetic is an essential process in embryonic development, immune cell differentiation, and cytokine expression. In early embryo development, the epigenetic process begins. DNA methylation marks are almost totally erased with few regions exception, which is important for gene expression stability, this process it occurs precisely at the zygote level (Oswald *et al.*, 2000). In addition, epigenetic plays an important role in gene expression in the embryonic development stage. Two different sorts of epigenetic protein complex namely polycomb and trithorax protein have direct involvement in gene expression during embryonic development. PRC1 and PRC2 are protein belongs to polycomb protein group, and these proteins regulate the expression of Hemeobox genes (HOX), which in turn these encodes the anatomical differentiation and pattern (Margueron and Reinberg, 2011) and (Kennison, 2002)

Significance role of epigenetic expands to immune cell differentiation. During T cell facing antigenic presenting cell, a number of transcription factors are re-localized, deactivated or activated, initiate changes in gene expressions, which determine the T cell into different types, include Th1, Th2, Th17 and Treg. The whole process of T cell differentiation is controlled by the epigenetic process (Wilson, Rowell and Sekimata, 2009). In addition, the

epigenetic process is believed to have a strong interfering with the suppression of certain types of cytokines. Moreover, epigenetic possesses ability over gene expressing that responsible for macrophage cells differentiation and polarization (Mosser and Edwards, 2008).

According to (Maiuri and O'Hagan, 2016), three molecular namely; histone modifications non-coding RNAs, and DNA methylation have prime influence in gene regulation and expression. Nucleosomes are two structures that form DNA, which they form out of DNA wrapped around histone proteins. Histone protein, on the other hand, has long tail-like structure that can be modified and many changes can be occurred such as transcription and chromatin compaction through these modifications in histone. These modifications have a panel of forms including methylation, acetylation, phosphorylation and my other forms (Cosgrove, Boeke and Wolberger, 2004). Figure 2.1 illustrates the histone modifications and DNA.

Methylation on histone tail can have either repressive or activating influence depending on the methylated residue. Histone trimethylation H3 at lysine 27 is mediated by an enzyme called methyltransferase can have a repression effect on the transcriptional process of many polycomb proteins (Cao *et al.*, 2002), figure 1 illustrates the histone modifications and DNA.

In addition, these changes affect directly or indirectly the transcriptional process via interfering with protein complex binding that can change chromatin compacting as well as accessibility, or via changing the recruitment mechanisms of TFs proteins (Tessarz and Kouzarides, 2014). In the same context, LSD1 (lysine specific histone demethylase 1) aims at removing Methylation from histone lysine residue that promotes transcriptional repression process through reducing H3K4me1 and H3K4me2 marks (Forneris *et al.*, 2008). Nevertheless, another modification on the surface of histone can occur which can influence transcription on DNA-based process (Lawrence, Daujat and Schneider, 2016).

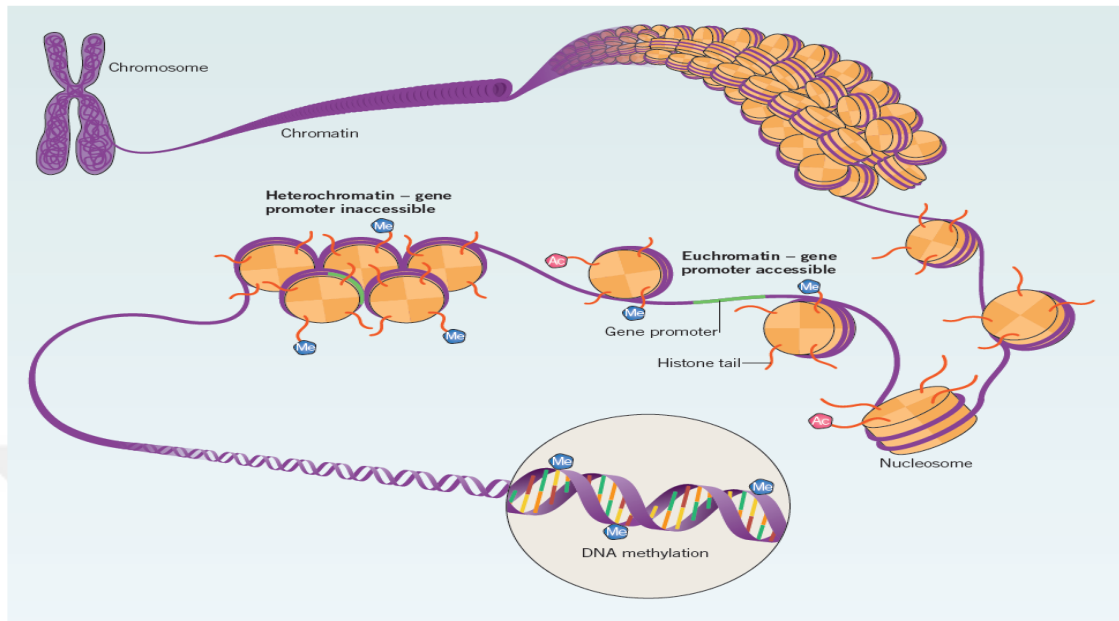


Figure 2.1: modification of histone and DNA (Müller-knapp and Brown, 2014)

However, epigenetic changes are ascribed to many factors such as lifestyle and environmental circumstances like exposure to toxins. Therefore, it is highly suggested that epigenetic mechanisms involved in many pathologies include cancers. Yet, the epigenetic mechanisms can be categorized depending on the different proteins that conducting these modifications. These proteins are called writer, reader and eraser according to their mode of action. Writers aim at catalysing the addition of chemical group(s) that existed on histone and they know as marks. Whereas, readers are assigned to specific marks or act as coordinator of read-erase and/or read-write mechanisms. On the other hand, erasers have the ability over removing epigenetic marks to reverse the gene expression changes that occurred by marks, hence the changes by marks are not always permanent (Müller-knapp and Brown, 2014). Figure 2.2 explains the different modifications of histone by writers, readers, and erasers proteins.




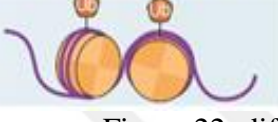
	READ	WRITE	ERASE
	Bromodomains; Tandem PHD domains	Histone acetyltransferases	Histone deacetylases
	Chromodomains; Tudor domains; MBT domains; ZF-CW proteins; Ankyrin repeats; WD40; PWWP	Histone methyltransferases	Lysine demethylases (KDMs); JMJD6 (arginine demethylase)
	14.3.3 proteins; BRCT proteins; Chromoshadow domains	Kinases (RSK2; AMPK; ATM/ATR; Aurora B kinase; JAK2; PKC)	Protein Tyr phosphatases; Protein Ser/Thr phosphatases
	Unknown	Ubiquitin E2 conjugases; Ubiquitin E3 ligases	Deubiquitinating enzymes

Figure 22: different modifications of histone by writers, readers and erasers proteins(Müller-knapp and Brown, 2014)

2.2.1 Cancer Treatment

Despite different approaches that used in cancer treatment and how far they are effective, still early cancer diagnosis is considered the most important factor in the treatment. Early cancer diagnosis could enhance the chance of recovery. However, there are many known cancer treatment approaches such as surgery, radiation, and chemotherapy. These approaches depend mainly on the different types of cancer, breast cancer for example, is mostly treated by surgery, and meanwhile, thyroid cancer is treated by radiation. Furthermore, chemotherapy is considered effective in many types of Leukaemia and lung cancer.

Wide ranges of chemotherapy drugs are currently available for curing and treating different types of cancer. Although, chemotherapy drugs are varied in term of their different mechanisms patterns but still they share the same goal, which is cell multiplication. Vincristine, Colchicine, and Vinblastine are microtubule (MT) inhibitors, these drugs bind or interfering with tubulin, which aim at, inhibit MT polymerization to block mitosis. Taxol (Paclitaxel) is a natural product belongs to terpenes group, it is extracted from the bark of *Taxus brevifolia* and it is one of the most well-known chemotherapy drug that targets cell

division, in other words, it blocks cell division by stabilizing MT(Cooper, 2000). Figure 2.3 demonstrates the chemical structure of Paclitaxel.

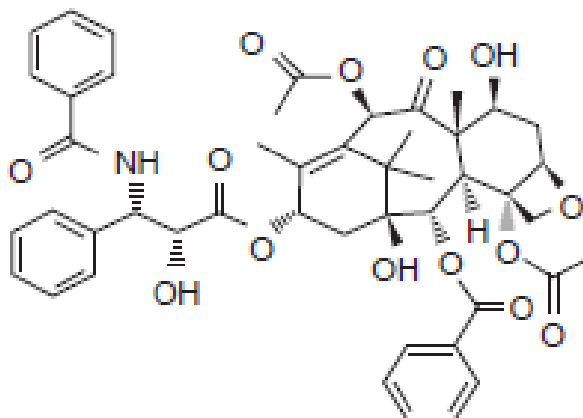


Figure 2.3: chemical structure of Taxol (Paclitaxel)

Platinol-AQ (figure 2.4) and Temodar are anticancer drugs belong to Alkylating, they intend to have direct binding to the cell genome and inhibit DNA replication. Another mechanisms of chemotherapeutic drugs is anti-metabolites. 6-mercaptopurine and 5-fluorouracil are anti-metabolites drugs, which they cause systematic cell damage during S-phase in cell division. Furthermore, Anthracyclines therapeutics are subcategorized as anti-tumor antibiotics, they affecting DNA by intermediating with certain enzymes that essential for DNA replication. Nevertheless, Taxotere (figure 2.5)and, Olaparib have different mechanisms, which they are mitotic inhibitor and PARP inhibitor respectively(Stilgenbauer *et al.*, 2016).

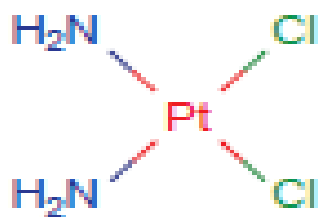


Figure 2.4: chemical structure of Platinol-AQ (Cisplatin)

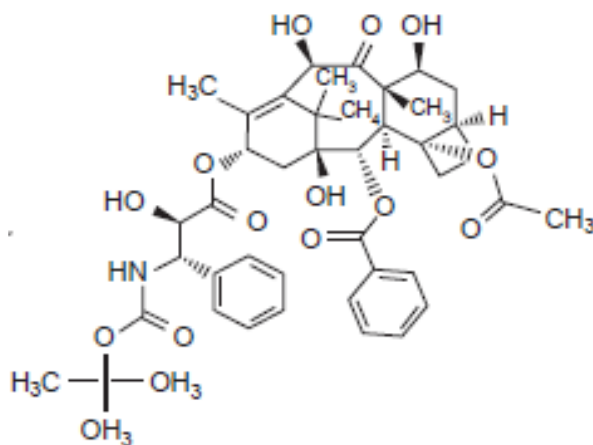


Figure 2.5: chemical structure of Taxotere (Docetaxel)

Yet, dozens of approved chemotherapy are commercially available used for different cancer treatment purposes with different mechanisms of action. Table 2.1 illustrates some of the chemotherapeutic with their mechanisms.

Table 2.1: Some approved chemo- therapeutic with their mechanisms

Scientific name	Commercial name	Mechanisms	Reference
Tarceva	Erlotinib	EGFR tyrosine kinase inhibitor (TKI) inhibitors	(Han <i>et al.</i> , 2012)
Trexall	Methotrexate	antimetabolites, antipsoriatics, antirheumatics	(Fulda, Friesen and Debatin, 1998)
Avastin	Bevacizumab	VEGF/VEGFR inhibitors	(Sandler <i>et al.</i> , 2006)
Iressa	Gefitinib	EGFR inhibitor	(Anderson <i>et al.</i> , 2001)
Alimta	Pemetrexed	antimetabolites	(Hanna <i>et al.</i> , 2004)

2.3 Histone Lysine-specific Demethylase 1 (LSD1)

Chromatin is formed of DNA compacted by histone and a vast number of proteins. Certain histone modifications organize chromatin structure as well as chromatin dynamic and DNA function, eventually lead to control of certain gene expression (Meghan et al., 2016). These modifications in histone particularly occur on histone tail and could be achieved by adding molecules to the specific amino acid on N-terminus. Hence, the sequence of histone tail would be modified which in turn have an enormous influence on the transcriptional process, DNA repair and many other necessary processes(Kouzarides, 2007). The process of histone post-translation modifications (PTMs)can be explained as some enzymes catalyze and remove methyl group in either arginine or lysine that existed on the histone tail (Pedersen and Helin, 2010).

Two families of histone demethylase enzymes have been well studied and identified namely, Histone Lysine specific Demethylase (LSD) and Jumonji C Demethylase(JMJc), and it is believed they recognize H3K4me as a substrate. LSD1 is *Homo sapiens* (human) was discovered and described for the first time in 2004, and it is comprised out of three domains namely, N-terminal (SWAIRM), C-terminal Flavin adenine dinucleotide (FAD) and tower domain. The unique shape of LSD can be simplified as the FAD structure is highly associated with WAIRM via hydrophobic interaction forming a spherical core, meanwhile, tower structure is extended from the spherical core shaping helix turn helix motif(figure 2.6). However, It has been recorded and documented that, LSD1 involved and contributed in many essential biological processes include chromosomes separation (Lv *et al.*, 2010),control cell proliferation(Cho *et al.*, 2011), adipogenesis(Musri *et al.*, 2010),embryonic development (Foster *et al.*, 2010)and other functions. Despite all necessary biological functions of LSD1, it can be acted as an oncogene factor; it could promote cancers, cell migration and cell invasion (10). In addition, LSD1 is highly associated with transcriptional co-repressor protein (CoREST) and histone deacetylase (HDAC) (Kozub *et al.*, 2017).

National Center for Biotechnology Information (NCBI) has provided molecular details of LSD1 as mentioned in table 2.2.

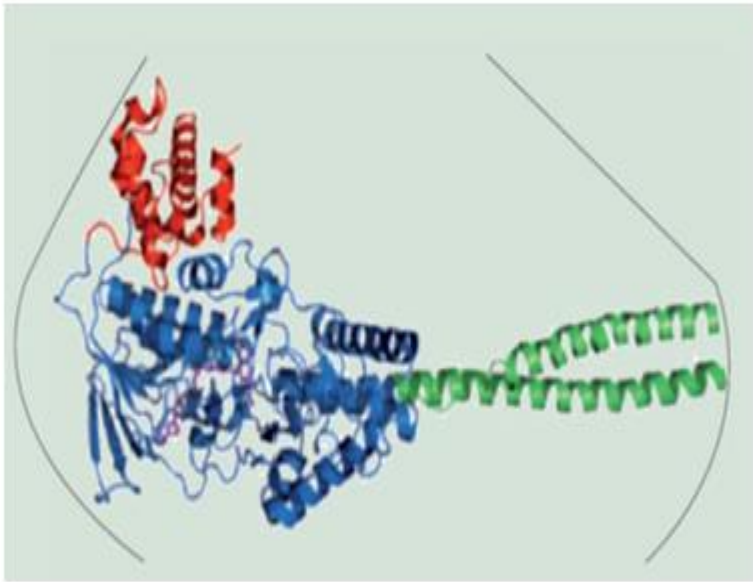


Figure 2.6: The 3D structure of LSD. It composed of FAD domain (blue) which is strongly interacted with SWIRM (red) shaping the spherical core. Tower domain is extended from the spherical core shaping helix turn helix motif, to provide the ability for interaction with other proteins.

Table 2.2: molecular details of LSD1 from NCBI. <http://www.ncbi.nlm.gov/gene/23028>

Official symbol of LSD1	KDM1A
Official name	Histone Lysine-specific Demethylase
Primary source	HGNC:HGNC:29079
Ensembl	ENSG00000004487 MIM:609132
Gene type	Protein coding
Organism	<i>Homo sapiens</i>
Alternate names	AOF2, CPRF, KDM1, and BHC110

LSD1 as mentioned earlier in this section has many biological activities. These activities are extended to hormones regulation, hematopoietic differentiation, and other activities. Importantly, LSD1 plays a significant role in hormone receptor mediated gene-expression, throughout demethylation of histone and DNA chromatin modeling. LSD1 accurately leads Estrogen Receptor mediated transcription over both gene promoter and enhancer site. Hence, Estrogen Receptor participates in chromatin binding in order to interfere with RNA polymerase to initiate transcription.(Perillo *et al.*, 2008).Nevertheless, LSD1 contributes to formation of hydrogen peroxide, which induces three enzymes namely local oxidative oxygen DNA damage, 8-Oxoguanine DNA glycosylase, and topoisomerase II β . These enzymes are important to promote gene expression (Hu *et al.*, 2008)Moreover, LSD1 considerable controls hematopoiesis by binding to the regulation region of hematopoietic factors and regulates their activity. Hemangioblast development is a necessary event to start hematopoietic differentiation. This process takes place when LSD1 down regulating of Etv2 gene expression at the early stage of hematopoiesis(Ferrari-Amorotti *et al.*, 2013).One of the most convincing suggestions made by Kerenyi et al in 2013 states that LSD1 is an irreplaceable factor for hematopoiesis differentiation and deletion could cause severe pancytopenia.

It is has been strongly suggested that overexpression of LSD1 has high connection with triggering many cancers and it could be the prime cause of many cancer types. Finally, LSD1 is simply can be defined as an enzyme that has the ability to remove the methyl

group from histone H3K4me1, H3K4me2, H3K9me1, and H3K4me2. However, in the following section in this chapter, the role LSD1 in cancer-promoting and its association with different cancer types will be discussed in details.

2.3.1 The Role of LSD1 in Cancer

Many studies demonstrated that LSD1 is strongly associated and being overexpressed in many types of cancers. However, every single cancer cells undergo a metabolic shifting from mitochondrial metabolism into glycolytic metabolism, this considered a sort of adaptation to new microenvironment to sustain the cell productivity(Heiden, Cantley and Thompson, 2009). In this pathway LSD1 is required in many types of cancer cells, by repressing of mitochondrial respiration associated gene such as EHHADH and PPARGC1A, via binding to their promoters and subsequent H3K4 demethylation, since LSD1 depletion leads to glucose reduction uptake which in turn lead to mitochondrial respiration as well as oxidative phosphorylation (Sakamoto *et al.*, 2015).

It is important to notice that, many histone lysine demethylases are involved with different cancer types. Table 2.3 provides a list of some histone lysine demethylases involved in different cancers.

Table 2.3: List some histone lysine demethylases involved in different cancers

Histone demethylase	Substrate	Cancer	Reference
LSD1	H3K4 H3K9	Prostate, breast, bladder, lung, ovarian, Colorectal, AML, pancreatic, hepatocellular, , gallbladder \cancers	(Heidenblad <i>et al.</i> , 2008)
LSD2	H3K4	Urethral carcinoma	(Vogt <i>et al.</i> , 1999)
JARID1C	H3K4	Prostate and renal cancers	(Stein <i>et al.</i> , 2014)
JARID1D	H3K4	Prostate cancer	(Perinchery <i>et al.</i> , 2000)
FBXL10	H3K36	Bladder, leukemia pancreatic cancers	(Kottakis <i>et al.</i> , 2011)
JMJD2A	H3K9 H3K36	Bladder and breast cancer	(Pryor <i>et al.</i> , 2011)
JMJD2B	H3K9 H3K36	Malignant peripheral nerve sheath tumor	(Pryor <i>et al.</i> , 2011)
PHFE	H3K9	Breast, head and neck squamous cell carcinoma	(Barradas <i>et al.</i> , 2009)
UTX	H3K27	Multiple myeloma, chronic myelomonocyticleukemia, breast cancer, esophageal squamous cell carcinoma, renal clear cell carcinoma	(Van Haaften <i>et al.</i> , 2009)

2.3.2 The Association of LSD1 in Acute Myeloid Leukemia (AML)

AML is can be defined as heterogeneous hematopoietic malignant cell and it is can be characterized by the accumulation of incomplete differentiation of blast cells in both blood and bone marrow. Although the occurrence of AML is not fully understood, there are pieces of evidence refer to the main mechanism of this type of leukemia. The vast studies in the genetic area have been providing the molecular keys of AML. However, AML is highly associated with deregulation of epigenetic mechanism, and thus could be happened by

somatic mutation in the certain gene and mutated transcriptional regulation(Wouters and Delwel, 2016).

However, as mentioned previously LSD1 level significantly increased in many cancers. In leukemia, LSD1 is overexpressed in the less differentiated subtype of AML like M1, in comparison with well differentiated AML subtypes, according to (Goardon et al., 2001). In addition, the expression of LSD1 is much higher in c-kit⁺AML (less differentiated subtype) than c-kit⁻(differentiated AML).Gene set enrichment analysis has strongly suggested that there is a panel of subsets genes activate the process of oncogenic associated with MLL-AF9 subtype leukemia and these genes however, are regulated by LSD1 (main). In the same context, the LSD1 is required to maintain and develop types of leukemia caused by fusion protein AMLL-AF9 in mice module(Goardon *et al.*, 2011).Furthermore, the role of LSD1 is more explicit in Acute Promyelocytic Leukemia (M3FAB), which is more leukemia morphological differentiated subtype. According to (main), although the cells that do not rely on LSD1 for survival, still LSD1 is considered has the prime role in AML controlling.

2.3.3 The Association of LSD1 in Lymphoid Leukemia

T-cell acute lymphoma represents about 20 percentages and 15 percentages in both adult and pediatric respectively of all cancer types. Regular mutations of Notch1 are commonly occurring in T-cell lymphoblastic leukemia (T-ALL), which in turn leads to Notch pathway activation. LSD1 possesses a dual activity as repressor or activator of Notch-mediated T-ALL. LSD1 acts as co-repressor when linked with CSL-repressor via removing H3K4me2 substrate at Notch target, and this process takes place in case of Notch absence. Meanwhile, LSD1 demonstrate the co-activator activity of Notch1 upon Notch activation by ensuring efficient H3K9me2 demethylation(Yatim *et al.*, 2012). Yet, many studies found that TAL1 is highly expressed in T-ALL. However, TAL1 requires LSD1-CO-REST complex to initiates the process of repression its target gene in T-cell lymphoblastic leukemia (T-ALL) (Kerenyi et al., 2013) and(Li *et al.*, 2012)

2.3.4 The Association of LSD1 in Solid Cancers

LSD1 has been showing great involvement with solid cancers and its overexpression is linked with poor prognosis. LSD1 expression takes place in both normal and cancer cells, with different expression levels. For instance, in lung cancer, LSD1 expression is away higher by doubles than in normal lung cells, and it is highly believed it is associated with poor prognosis, which maintains malignant cells proliferation, migration, and invasion. Whereas, knocking down LSD1 leads to increases H3K9 acetylation level and E-cadherin expression resulting in suppression of lung cancer cells proliferation.(Nair *et al.*, 2010).In addition, LSD1 has become an indicator of many solid cancers. It has been found that high the level of LSD1 increased in pancreatic cancer, and depletion in its levels could repress cancer cells proliferation and activity(Qin *et al.*, 2014)

Many recent studies proposed that LSD1 is a useful biomarker tool for aggressive negative Estrogen Receptor (ER) breast cancer. LSD1 manipulates many proliferative genes such as p21, CCNA2, and ERBB2. LSD1 inhibition and knocking down leads to down-regulating certain genes such as MK167, CCNF and CDCA7 and many other genes which possesses a significant role in cell proliferation and tumorigenesis, thus, breast cancer would be inhibited (Lim *et al.*, 2010)

2.4. LSD1 Inhibitors

Since the last decade, tremendous effort has been spent toward screening LSD1 inhibitor in the view of the fact that LSD1 has a significant role in term of epigenetic process. The demand for developing novel and effective inhibitors has been increasing due to the critical function of LSD1. However, many assays for LSD1 inhibitors screening are available such as target-based assay, substrate-based assay, byproduct-based assay and PPI-based assay. Target-Based Assay, virtual screening is reliable for a large number of LSD1 ligands screening in silico, which is preferable for being cost-effective and high throughput.

MAO-A and MAO-B are two enzymes encoded by MAO-A gene and MAO-B gene respectively and their function is to catalyze the oxidative deamination of amines. These have a similar sequence as LSD1. Therefore, the latest studies aim at investigating MAO-A and MAO-B inhibitors against LSD1 as well as to evaluate their activity as potential LSD1 inhibitors. Although, only two drugs so far namely tranylcypromine (Schmidt and McCafferty, 2007),(Huang *et al.*, 2007) and cyclopropylamine(Shi *et al.*, 2004); (Metzger *et al.*, 2005)are described as LSD1 inhibitors, many synthesized LSD1 inhibitors are currently investigated and evaluated in clinical and pre-clinical trials. Table 2.4 lists LSD1 inhibitors undergo to both clinical and pre-clinical investigation.

Table 2.4: LSD1 inhibitors under clinical and pre-clinical investigation (Hosseine and Saverio, 2017)

Compound	Status	Cancer	Trials
GSK2879552	Phase I/IIa	AML and lung cancer	NCT02034123, NCT02177812, NCT01943851, NCT01587703
ORY-1001	Phase I/IIa	Leukemia	EudraCT Number- 2013-002447-29
Tranylcypromine trentinoin (in combination with ATRA)	Phase I/IIa	AML	NCT02717884, NCT02261779, NCT02273102
4SC-202	Phase I	AML, ALL, lymphocytic leukemia	NCT01344707
IMG-7289 With and without ATRA	Phase I	AML, ALL, lymphocytic leukemia	NCT02842827
SP2577	Phase I	AML	NCT02712905
INCB59872	Phase I/IIa	AML, lung cancer	--
SP2577	Pre-clinical	AML, breast cancer	---
SP2509	Pre-clinical	Ewing and AML	---

2.4.1 Tranlycypromine LSD1 inhibitor

Tranlycypromine (TCP) is known as an anti MAOs agent with no confirmation regarding its anti-cancer activity (Akdoğan, Erman and Yelekçi, 2011). Previously, the main purpose of TCP was to treat depression issues and then has become an effective LSD1 inhibitor. The mechanism of TCP is interfering with FAD domain covalently resulting in adduction with the flavin ring (main). To illustrate, TCB-FAD adduct is formed by binding cyclopropyl ring (TCP) with C-4a, (FAD) via C-C bond by transferring single electron which can be resulted in either atropaldehyde or cinnamaldehyde, figures 2.7 and 2.8 respectively.

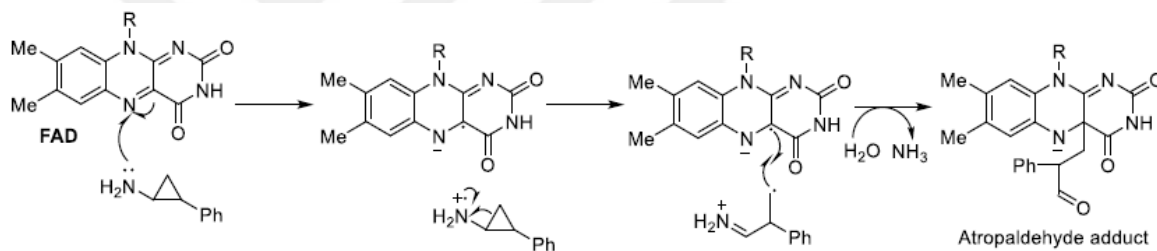


Figure 2.7: TCP mechanisms in LSD1 inhibition atropaldehyde pathway (Jernej, 2015).

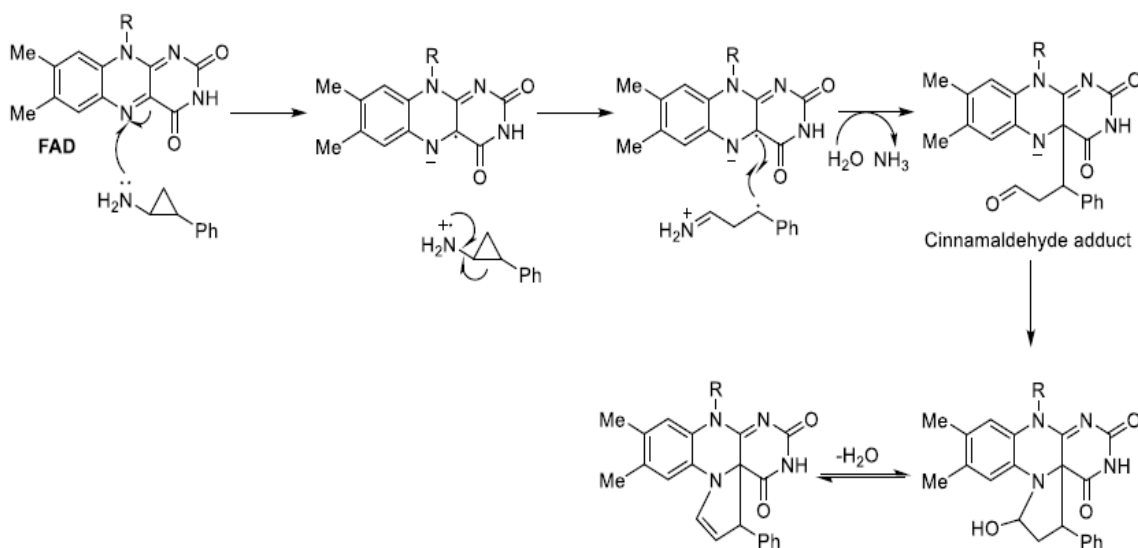


Figure 2.8: TCP mechanisms in LSD1 inhibition cinnamaldehyde pathway (Jernej, 2015).

The inhibition activity of TCP was found against AML cell of MLL-AF9 in mouse models and in the vitro cell line. (Hosseine and Minucci, 2017) mentioned that, MLL-AF9 cell line was shown a delay pattern of starting secondary AMLs in the vitro when incubated with 5 μ M of tranlycypromine for long 5 days. However, according to (Hosseini and Minucci, 2017), TCP is not the first choice as an LSD1 inhibitor, and they ascribed that to the selectivity and potency, therefore new TCP derivatives have emerged. Two analogs of tranlycypromine; Trans-N-((2,3-dehydrobenzo[b][1,4] dioxon-6-yl) methyl) -2-phenylcyclopropan-1-amine and trans-N-((2-methoxypyridin-3-yl) methyl)-2-phenylcyclopropan-1-amine, demonstrate more efficiency in term of selectivity as well as, potency and they are photocopied of LSD1 KD and TCP treatment (Somerville and Cleary, 2006) and (Harris et al., 2012). Another reason led to the emphasis on find or synthesis more effective inhibitor that, the FAD-depended of LSD1 is quite larger with MAOs even they share the same sequence.

2.5 Drug Design Techniques

Drug design is a term refers to the process of discovering or developing a new medication depending on the biological facts, and it is referred as rational drug design (Madsen, 2002). Human genome sequencing technologies have been developed and advanced to serve the medication purposes and therefore a considerable number of new therapeutic has been discovered. In addition, a high outcome of crystallography and computational approaches has vastly contributed to drug discovery. These approaches beside nuclear magnetic resonance (NMR) lead to better understanding the atomic structures of proteins and protein-ligand complexes have always been contributing to drug design (Langer and Hoffmann, 2005). The cost-effectiveness and convenience of using the bio-computational methods are properties made these techniques more desirable in screening and investigate new medications (Mandal, Moudgil and Mandal, 2009). Different structure based drug design methods are accurate, precise, and possess the necessary features to design ligands with target receptors or enzymes. These features include specific interactions, of high affinity

ligand with the interaction of protein along with cellular function and process, which in turn definitely lead to achieving desired pharmacological impacts (Urwyler, 2011).

However, many molecular approaches are available such as docking, virtual screening pharmacophore and ADMET as well as they can be applied individually or as a combination depending on the purpose and the intention of the research. Finally, it is important to mention that, only promising compounds that determined by these computational approaches would be proceeded to be synthesized, thus these approaches are valuable in term of traducing cost, time and efforts.

2.5.1 Molecular Docking

Molecular docking is considered a precious tool in drug design since the development of the first algorithm in the early 1980s (Lopez-Vallejo *et al.*, 2011).The correct binding mechanisms of the ligand are always is a big challenge in silico drug design. Docking modules aim at the prediction of ligands in the binding pocket of the target, which provides a precise view and rational concept of the interaction occurs between the enzymes and their inhibitors(Zhang *et al.*, 2015);(Yao *et al.*, 2014). Two main steps in the docking process, first the prediction of ligands confirmation, ligand position and ligand orientations, this step is called pose, while the second step is the assessment of the binding affinity. However, the binding energy can be predicted through docking via applying different scoring function.

Key and lock theory was found by Fischer a German scientist (Fischer, 1894),this theory illustrates the concept of ligand-receptor mechanism which it states that the ligand fits into the binding site of the receptor. (Kollman, 1994), state that both ligand and receptor are considered and treated as rigid compounds based on key-lock theory Later on, a more convincing theory had emerged “fit theory” by Koshland(1963). This theory claims that the ligands and receptors are considered flexible compounds rather than being rigid compounds, based on the assumption of protein pass through many conformational changes allow the ligand fit into the protein. This theory is more preferable in term of computational

drug design as both ligands and proteins must be treated as flexible compounds during docking, taking into the consideration the dynamic behavior of proteins mechanisms in the biological events (Abdulla). Nevertheless, during the docking process, the receptor flexibility particularly in the backbone is challenging since the protein tends to be dynamic not static (Alonso, Bliznyuk and Gready, 2006).

Docking tools employ a range of methods search to discover ligand conformational space and these methods can be divided into systematic, stochastic torsional conformational and molecular dynamic simulation. In the systematic methods, matching algorithm for example which aim at fitting a certain ligand into the predicated binding site after determining all degrees of freedom (Brint and Willett, 1987). Furthermore, Incremental construction methods drag the ligand into the active site in such an incremental pattern, and this method has been used in dock4.0 (Ewing *et al.*, 2001). Stochastic torsional conformational aims at evolving new low energy conformers by searching rotatable bonds such as genetic algorithm and genetic Monte Carlo (Hart and Read, 1992). Finally, for unrevealing the energy landscape of certain molecule, molecular dynamic simulation methods are used. Yet, the panel of docking including Autodock, Dock, FlexX, Glide, GOLD, Surflex, ICM Cdocker, and many other tools are used in the research related to the rational drug design depending on the purpose of use and the methodologies of different researchers.

2.5.2 Virtual Screening

The process of developing, discovering and modifying medications is complex and need enormous efforts. Computational chemistry and the viability of high throughput screening have improved and facilitated the process as well as, shortened time consuming. Virtual screening is either structure based approach or ligand based approach. Structure based approach relies on the 3D structure of the different biological molecules to screen through large chemical compounds library to obtain the different compounds with similar bioactivity. 3D structure in turn can be categorized into X-ray, NMR and homology modeling.

On the other hand, ligand based approach is relays on the provided information related to ligands as a template to identify the different structures (Walters, Stahl and Murcko, 2002).Ligand based approach can be categorized as well into pharmacophore which provides a set of structurally diverse ligands that could be bound with assigned receptors through comprehensive information that existed in ligands, and into another approach that depends totally on the 2D structure. In the second category of ligand-based approach, 2D structure similarity analysis methods are used for screening (Willett, Barnard and Downs, 1998) . However, different approaches of virtual screening are used for different purposes. In addition, the choice of using each approach is eventually related to the nature, goals and circumstances of the research.

2.5.3 Pharmacophore modeling

The word of pharmacophore is driven from (phoros) the necessary features that responsible for the biological activity of the certain drug (pharmacon), and it was first defined by Ehrlich in 1909 as a molecular framework. Later on the International Union of Pure Applied Chemistry (IUPAC), has defined the pharmacophore as “ensemble of steric and electronic features that are necessary to ensure the optimal supra-molecule interactions with a specific biological target for triggering or blocking the biological response”. However, these features are represented in H-bond (recipient or donor), aromatic ring, Ionized groups (PI,NI), metal groups (M) and other futures that important for matching different chemical structures sharing same properties to recognize promising and novel ligand. The process of developing such a pharmacophore model is formed out five main steps and these steps are ligands selection, conformational analysis, molecular superimposition, abstraction, and validation. The use of pharmacophore modeling application has sharply increased in drug design since it facilitates the process of drug discovery and drug screen.

2.5.4 ADMET prediction in rational drug design

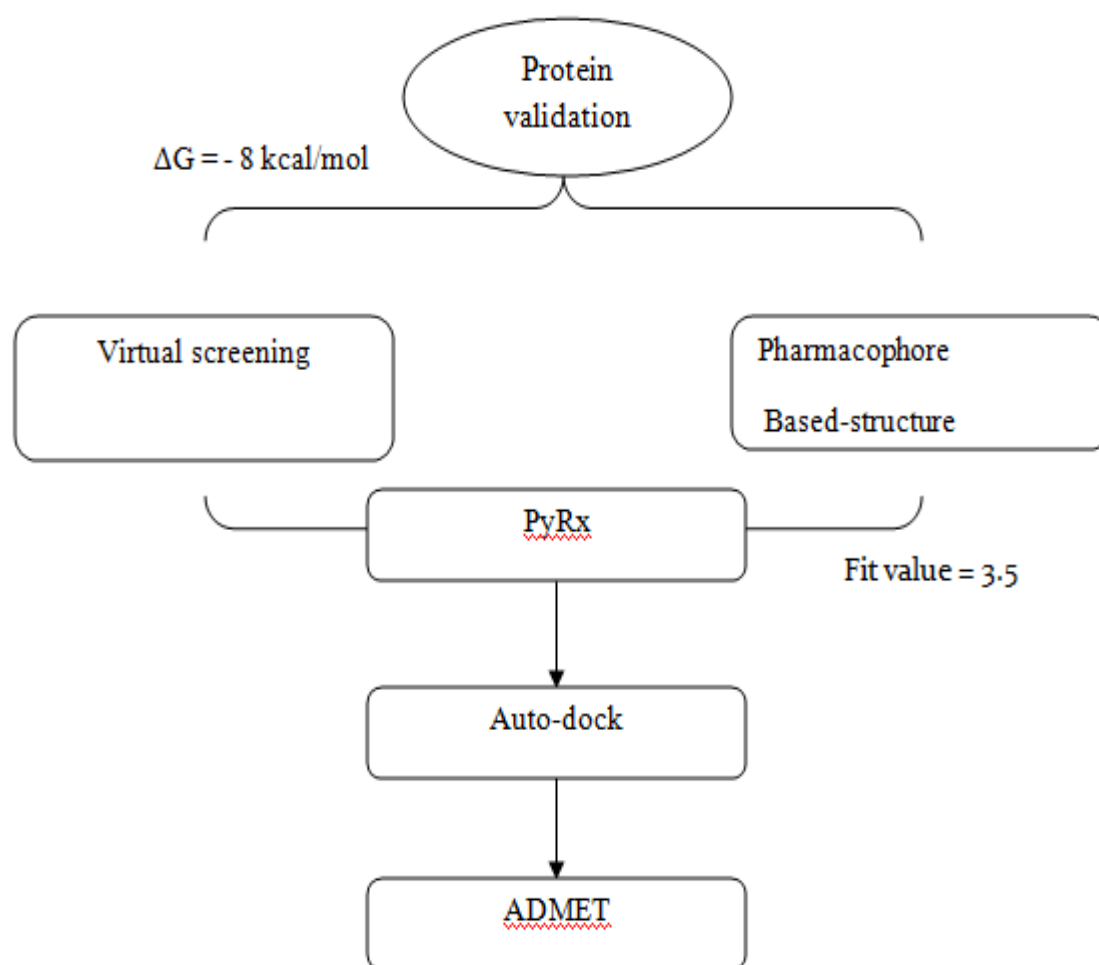
Such features are irreplaceable in drug development, drug modification and drug discovery, which they are absorption, Distribution, Metabolism, Elimination and Toxicity. These properties determine whether the drug candidate is worth for proceeding or not and the term of ADMET is used in both *in silico* and *in vitro* drug screening. ADMET profile utilizes a statistic analysis for prediction, experimental data and molecular description to initiate a complex process (Biswas et al., 2006;(Li *et al.*, 2007).

Due to the strict instructions and regulations that made by Food and Drug Administration (FDA), and European Agency for the Evaluation of Medicinal Products (EMA) regarding drugs toxicity, the priority of drug investigating has been given to *in silico*, since these organizations highly recommend that do not involve the animal in the drug assessment(Benz, 2007)However, high cost and longtime consumption of drug ADMET predication *in vitro*is another reason of giving the priority to drug screening *in silico*.

3. METHODS AND MATERIAL

3.1 Introduction

In this chapter, all methods used in the study of generating LSD1 inhibitors using molecular modeling are explained accordingly. A series of *in silico* applications include protein validation, followed by virtual screening, pharmacophore modeling, and then AutoDock, and finally ADMET prediction, which were applied sequentially in order to reach desired results. The major steps were followed from previous studies with slight modification.



3.1 Flow diagram of the study

3.2 Protein Validation

Protein validation or protein preparation is an essential step for inhibitors investigation. Protein validation aims to clear up the target by removing water molecular, ligands and non-interactions ions, due to these factors might interfere with the dock process. However, in the present study, two crystal structures of LSD1 (codes 3ZMS and 5LGT their resolutions are 2.96 Å and 3.0 Å respectively) were extracted from Protein Data Bank (PDB) (Berman *et al.*, 2002)(<http://www.rcsb.org>). Both structures were cleaned up by removing water molecules and non-interaction ions, while FAD was kept since it behaves as enzyme co-factor. Eventually, the optimization of force-field protein's geometry took place and then submitted into the Clean Geometry tool-kit of BIOVIA discovery studio (Accelrys 4.5, Inc.) software (<http://www.accelerys.com>).

The known inhibitors with significant experimental values of IC₅₀ or Ki were subjected to molecular docking. Hydrogen atoms were added into these inhibitors before being processed, and then clean geometry protocol was applied for more optimization. Known LSD1 inhibitors that used in the study are listed in table 3.1.

Table 3.1: Known LSD1 inhibitors that used for validation

Name of inhibitor	IC ₅₀	Reference
RNA1-2HCL	IC ₅₀ = 70 nM	(Neelamegam et al., 2012) and(Vatikus et al., 2015)and(Cui et al., 2015)
RNA1-HCL	IC ₅₀ = 10-70 nM	(Neelamegam et al., 2012; Vatikus et al., 2015; Cui et al., 2015)
GSK-HCL	IC ₅₀ = 16 nM	(Smitheman et al., 2015; Mohammed et al., 2015)
GSG-2HCL	IC ₅₀ = 16 nM	(Neelamegam et al., 2012)
IC	IC ₅₀ = 10 μ M	(Sharma et al., 2010)
CBB1007	IC ₅₀ = 5.27 μ M	(Wang et al., 2011)
GSK2879552	IC ₅₀ = 38 nM	(Kimberly et al., 2015)
Namoline	51 μ M.	(Willmann <i>et al.</i> , 2012)
D compound	K _i =2.4 \pm 0.63 Mm	(Khan, Suzuki and Miyata, 2013)
E	K _i =0.61 \pm 0.13 Mm	(Khan, Suzuki and Miyata, 2013)
F	K _i =0.005 μ M	(Khan, Suzuki and Miyata, 2013)
G	K _i =0.009 μ M	(Khan, Suzuki and Miyata, 2013)
8WC	K _i =29 nm	(Niwa <i>et al.</i> , 2018)
5W3	IC ₅₀ = 160 nm	(Vianello <i>et al.</i> , 2017)

In the present research Autodock version 4.2 (18) was applied using a semi-empirical force field based. First, PDBQT files for both protein targets (3ZMS and 5LGT) were created that contain hydrogen atoms with partial charges. In other words, 3ZMS.PDB and 5LGT.PDB files were transferred into 3ZMS.PDBQT and 5LGT.PDBQT respectively. In the same context, all 14 ligands were treated in the same way. AutoDock 4.2 version uses molecular model mechanisms for enthalpic calculations and contributions such as hydrogen bonds and vdW as well as, it uses an empirical model for entropic changes upon binding (Tatar et al., 2011). The Protein-ligand grid parameters file (GPF) was generated. The 3D space of

volume around the binding site of protein was defined. Then AutoGrid 4.2 was used in order to generate different maps and grid files. The final output of the protein-ligand PDBQT complex in AutoDock is protein-ligand docking file (DPF). Finally, all parameters files were prepared for docking, 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. However, glg file describes minimum and maximum energy in each grid map, while dlg file contains the output and results of docking. Molecular docking estimates the free binding energy hence, all processed inhibitors demonstrated free energy of -8.0 kcal/mol or less .

3.3. Virtual screening

Zinc 15 database was used for database setting and to obtain the candidate lead compounds. Zinc database was chosen for screening due to it is a free database, obtainable includes 230 million compounds, easy to access, and fast to search for analogs and all compounds are available as 3D format as well as, in other formats (Irwin and Shoichet, 2005). All zinc compounds are classified according to their molecular weight, logP factor and other parameters. However, in this step the screening was performed through the database in order to detect the best binding affinity between the compound and target protein, all molecules were obtained as SDF files. All chosen compounds from zinc library were undergone to filtration method. Lipinski “rule of five” filtration method was applied which is considered the most widespread substantial procedure used for filtration purposes. Lipinski “rule of five” is a set of rules that select the compounds that have sensible absorption properties rather than the compounds that do not have. These five rules can be listed as (1) molecular weights of less than 500 Da (2) hydrogen-bond donors fewer than 5 (3) hydrogen-bond acceptors fewer than 10 (4) lipophilicity log P less than 5 (5) rotatable bonds less than 4 (Goodwin, Bunch and McGinnity, 2017) .

After filtration took place by biovia discovery studio, more than 64,000 lead compounds were subjected to PyRx tool for screening by autodockvina. Initially, the selected

compounds and target protein 3ZMS were converted into PDBQT format to be ready for docking. The grid box size dimension was 50 Å × 50 Å × 50 Å and the coordinate (XYZ) was (-5.186, 53.959, and 82.131). After the run had been finished for all compounds, only the compounds with the binding energy of -9.0 kcal/mol or less were subjected for manual docking one by one by Autodock4.2

3.4 Structure Based Pharmacophore Modeling

Structure based approach was applied to achieve the most ideal compounds that could serve as potential inhibitors for LSD1 enzyme, crystal structure code 5LGT was used which combined with native ligand code 6W3. For protein preparation, BIOVIA Discovery Studio 4.5 (Accelrys, Inc.) was applied to remove the water molecules for pharmacophore generating process. Then protein setting in receptor-ligand pharmacophore protocol took place, thus the different 10 hypothesis appeared in the binding site of the protein and its native ligand, which represent the most common chemical interaction features between receptor and ligand.

However, screening 3D database protocol applied using BIOVIA Discovery Studio 4.5 (Accelrys, Inc.) for each hypothesis. Zinc15 was used in this screening. More than six million compounds had been screened against target protein code 5LGT. The compounds that had fit value more than 3.5 were selected and considered for virtual screening using PyRx tool in order to dock all selected compounds with the target protein. In addition, only the compounds that had binding energy of -8 kcal/mol were considered and re-docked to achieve desirable requirements using AutoDock 4.2.

3.5 Auto Dock

All led compounds identified via virtual screening and pharmacophore were subjected for manual docking using AutoDock 4.2 for higher certainty. Each set of compounds was docked into the binding pocket of their respective protein. The grid box dimensions were

50A° 50A° 50A° and 55A° 55A° 55A° for both 3ZMS and 5LGT respectively. The Lamarckian genetic algorithm was used for conformational search, and for each compound, 20 independent runs are performed. The programs randomly detected torsion angles of rotatable bonds. However, ADMET prediction was applied after dock was done for all compounds.

3.6 ADMET Prediction Test

As mentioned previously, ADMET is a short name for the following terms, Absorption, Distribution, Metabolism, Elimination and Toxicity, which they are the standards that the drug design depends on. All drug-likeness candidates must pass the ADMET required properties (Kumar, 2013) and (Wadood *et al.*, 2014)

In the present study, BIOVIA Discovery Studio protocol was assigned. ADMET PAS 2D “polar surface area” against ADEMT ALOGP98 is the algorithm of the partition coefficient between the n-octanol and water. The pharmacokinetic properties of potential leads of all drug-likeness candidates that obtained via virtual screening and structure base pharmacophore were identified by ADMET prediction.

4. RESULTS

4.1. Introduction

The goal of the current study is to investigate and to design novel inhibitors for Lysine SpecificDemethylase 1 enzyme (LSD1). Wherefore, all efforts were concentrated to achieve desirable results through steady and according to sequences of methods and work. It is well known there are only two described drugs as LSD1 inhibitors namely tranlycypromine and cyclopropylamin, which they are not ultimately specific. Hence, the demand of generating new potential LSD1 inhibitors with good pharmacokinetic properties, non-toxic and possess less side effects has been increased, which is considered a big challenge in drug design. However, in the present work *in silico* approach was used to overcome the problems by screening a library of perceptible-chemical compounds to select candidates depending on the inhibition ability and energy values. In addition, for more confirmation structure based pharmacophore modeling was performed to check the chemical interactions groups between the ligands and the receptors.

4.2 Protein Validation

Known inhibitors that under clinical trial and pre-trial which possess experimental values were docked with target protein to confirm the crystal structure validity, taking into consideration of the binding energy of -8.0 kcal/mol or less. The results have shown the binding energy of known inhibitors were significant which varied between -10.76 kcal/ mol and -8.05 kcal/mol. Table 4.1 shows the results of the binding energy of docking known inhibitors. The binding energy value of know inhibitors that obtained by docking showed desirable results to move for further investigations.

Table 4.1: Binding energy of redocked known inhibitors

Inhibitor	experimental IC ₅₀ or Ki values	Binding energy	Ki values
RN1_2HCL	IC ₅₀ = 70 nM	-9.14 kcal/mol	199.41 nM
RN1_HCL	IC ₅₀ = 10-70 nM	-9.06 kcal/mol	228.08 nM
GSK_HCL	IC ₅₀ = 16 nM	-8.2Kcal/mol	1.47 uM= 1470 nm
GSK_2HCL	IC ₅₀ = 16 nM	-8.16Kcal/mol	1.89 uM = 1890 nm
IC	IC ₅₀ = 10 μM	-9.30 kcal/mol	152.26 nM
CBB1007	IC ₅₀ = 5.27μM	-9.74 kcal/mol	73.09 nM
GSK2879552	IC ₅₀ = 38 nM	-9.01 kcal/mol	248.99 nM
Namoline	IC ₅₀ =51 μM.	-8.62 kcal/mol	479.83 nM
D compound	Ki=2.4 ± 0.63 Mm	-8.4Kcal/mol	698.08 nM
E compound	Ki=0.61 ± 0.13 Mm	-8.09 kcal/mo	1.32 uM
F compound	Ki =0.005 μM	-8.05Kcal/mol	2.20 uM
G compound	Ki =0.009 μM	-8.50 kcal/mol	586.22 Nm
8WC	Ki =29 nm	-8.81 kcal/mol	428.68 nM
6w3	IC ₅₀ = 160 nm	-10.76 kcal/mol	13.02 nM

4.3 Virtual Screening

In silico screening and molecular modeling was utilized to develop an idealistic drugs that possess high activity and an outstanding pharmacokineticsof LSD1. To create novel class of inhibitors for the target enzyme LSD1, more than 60 thousand of chemical compounds were screened from zinc 15 database using PyRx tool. As we mentioned previously, only the compounds with binding energy of -9.0 kcal/mol or less would be selected for further evaluation. Thus 950 compounds have shown required binding energy value for the present research. These compounds were re-docked with the 3ZMS target protein using AutoDock 4.2 and then submitted for ADMET Prediction. Ten compounds out of all screened compounds shown desirable and significant binding energy within required pharmacokinetics properties. The compounds with their binding energy, Ki values, molecular weights, and log P(s) are listed in table 4.2, and their chemical structures are listed in figure 4.1. However, compound ZINC000000197090 demonstrated the most significant binding energy value of -10.60 kcal/mol while, compound zinc_20283934 showed acceptable binding energy of -8.22 kcal/mol. Even there is a slight fluctuation in the

binding energy among the ten chosen compounds, still all of them possess desirable binding energy and they are considered encouraged to proceed for further investigation.

Table 4.2: The ten selected compounds that possess significant binding energy were obtained via virtual screening approach.

Compound	Binding energy Kcal/mol	Ki nM	M.W Dalton	Log P
zinc_19925080	-8.66	447.34	428.493	-0.375
zinc_19925082	-9.27	161.64	428.493	-0.375
zinc_20283934	-8.22	940.47	373.457	-0.044
zinc_20326400	-8.90	301.01	440.304	-0.075
zinc_22924087	-10.23	31.49	426.521	-0.214
ZINC000023213990	-9.62	89.35	372.429	-0.064
zinc_24263007	-8.60	494.88	363.418	-0.15
ZINC000067742250	-10.24	31.19	378.488	-0.804
ZINC000021703310	-9.01	249.42	441.488	-0.063
ZINC000000197090	-10.60	16.98	335.412	-1.046

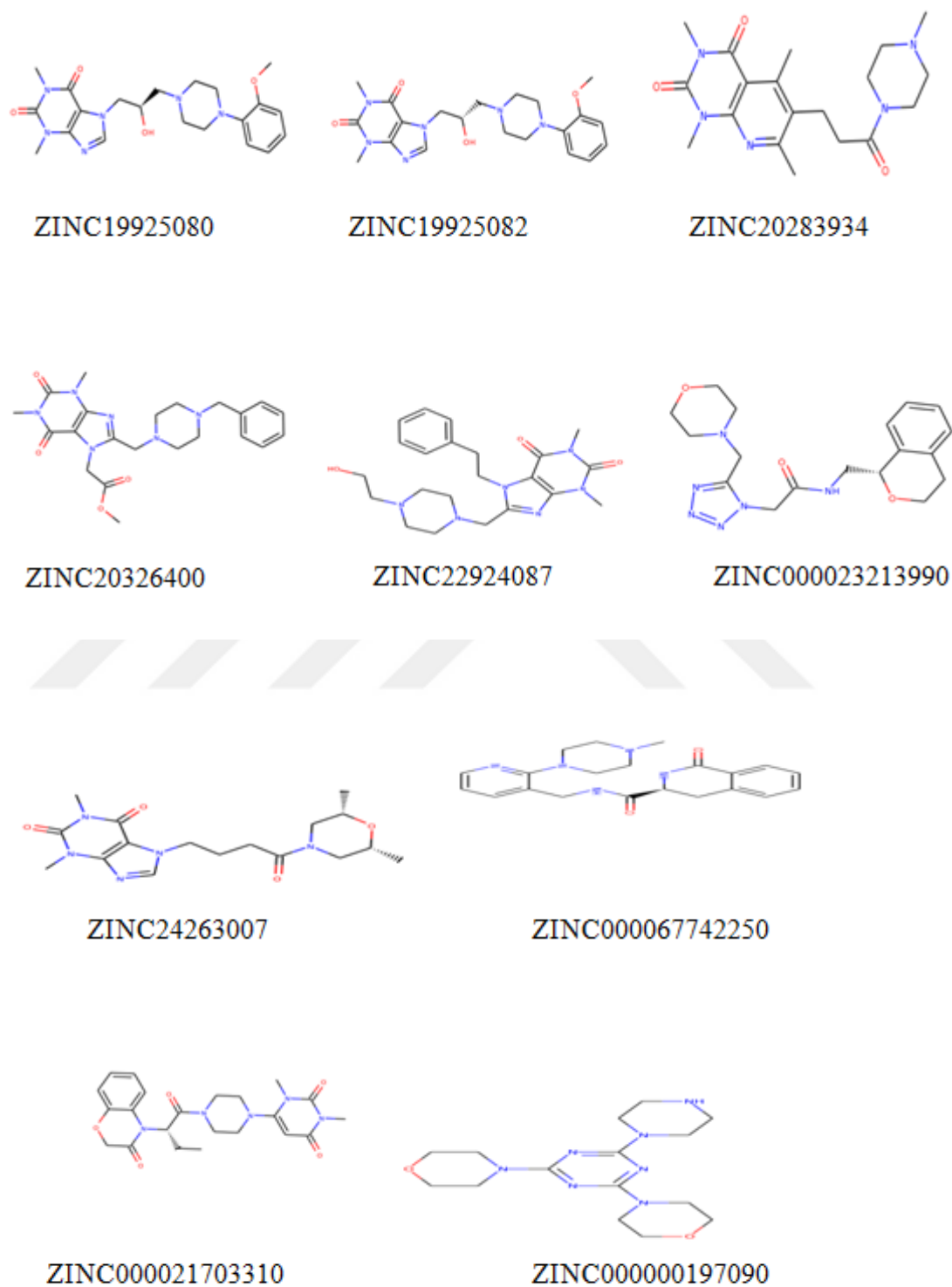


Figure 4.1 : The chemical structures of ten compounds that obtained via virtual screening approach.

4.4 Structure Based Pharmacophore Modeling

In term of identification, structure bases pharmacophore modeling was applied. Structure based pharmacophore was aimed at the identification of common chemical interactions between the receptor and ligand, thus 5LGT crystal structure protein was used for identification. Ten hypotheses had been obtained from receptor ligand pharmacophore generation and then all these hypotheses were screened by 3D database protocol using zinc 15 database via BIOVIA Discovery Studio. However, hypothesis number 4 and number 10 demonstrated the best chemical features as explained in figures 4.2 and 4.3 respectively.

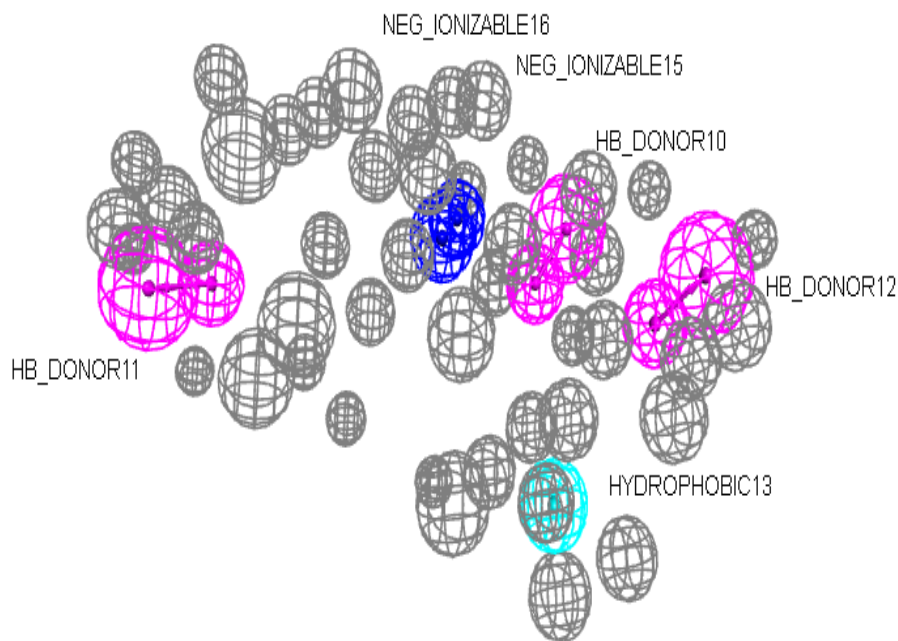


Figure 4.2: Hypothesis number 4.

The figure shows the pharmacophore model of receptor-ligand of chemical interaction features. These features can be defined as hydrogen bond donor (red color), hydrophobic effect (turquoise color) and NEG- ionizable (blue color).

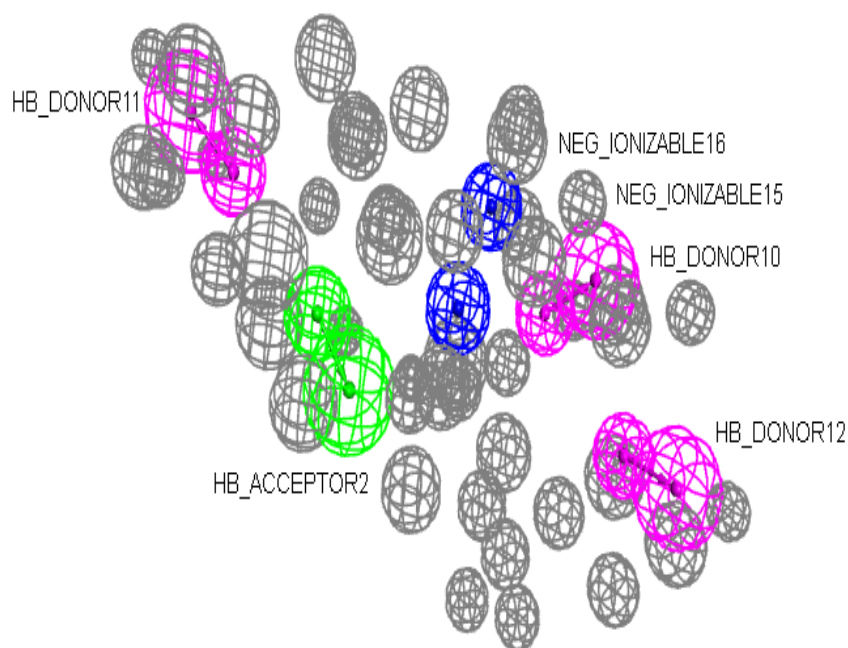


Figure 4.3: Hypothesis number 10.

The figure shows the pharmacophore model of receptor-ligand of chemical interaction features. These features can be defined as hydrogen bond donor (red color), hydrogen bond acceptor (green color and NEG- ionizable (blue color).

The output of the 3D database was several thousand compounds that have had a fit value of 3.5 or more with a selectivity score of 18.418. These compounds were subjected to virtual screening using PyRx tool. However, more than 200 compounds shown binding energy of -9.0 kcal/mol or less, then these compounds were re-docked using AutoDock 4.2 for more confirmation, and the results showed all compounds had desirable binding energy values. Finally, the best 10 compounds as shown in table 4.3 below were chosen according to ADMET prediction and their chemical structure are shown in figure 4.4.

Table 4.3 lists the 10 chosen compounds that obtained from structure based pharmacophore modeling according to ADMET prediction

No.	compounds	Binding energy kcal/mol	Ki nm	M.W Dalton	Pi
1	ZINC000010233861	-10.9	9.62	425.46	0.946
2	ZINC000021156768	-11.59	3.18	427.505	0.84
3	ZINC000021807965	-10.85	11.23	439.52	0.239
4	ZINC000064991903	-10.33	26.58	429.91	0.75
5	ZINC000170602678	-9.81	64.49	425.55	0.625
6	ZINC000170602679	-9.19	183.58	425.55	0.625
7	ZINC000225393486	-11.38	4.59	432.56	0.438
8	ZINC000225395750	-11.09	7.37	427.50	0.774
9	ZINC000225396116	-9.41	127.61	429.51	0.878
10	ZINC12817117	-10.43	22.67	393.44	0.636

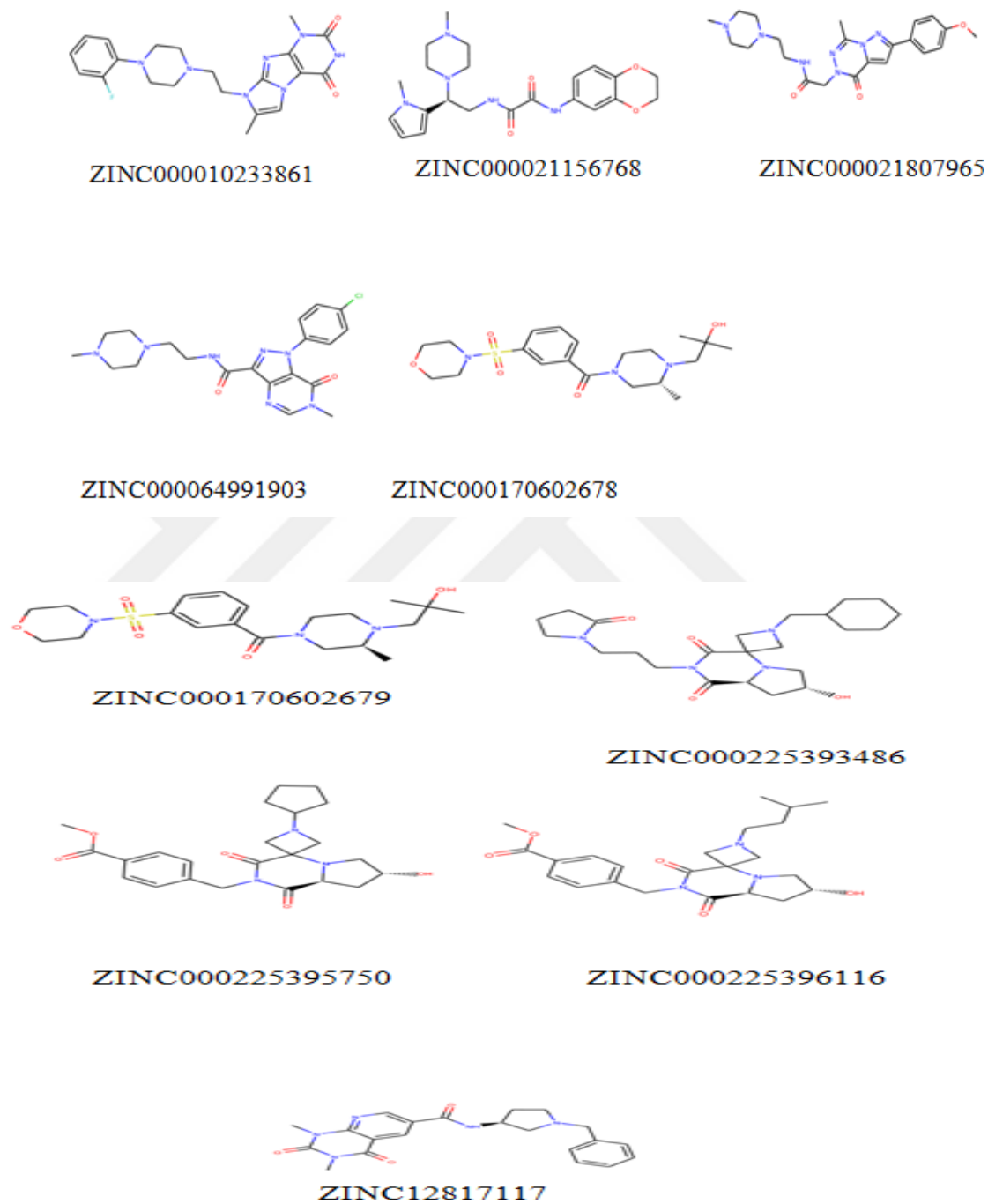


Figure 4.4: The chemical structures of the 10 chosen compounds that obtained from structure based pharmacophore modeling

4.5 ADMET Prediction

ADMET is an irreplaceable approach to check the absorption, distribution, metabolism, elimination, and toxicity of any drug's pharmacokinetics. Furthermore, all drug candidates shall pass the ADMET required standards. In the present study, however, BIOVIA Discovery Studio protocol was assigned. ADMET PAS 2D "polar surface area" against ADEMT ALOGP98 is the algorithm of the partition coefficient between the n-octanol and water. The final 950 compounds that obtained through virtual screening approach only 10 compounds were passed ADMET. Figure 4.3 shows the 10 compounds that matched the criteria of ADMET.

Furthermore, more than 200 compounds obtained from structure base pharmacophore modeling were undergone to ADMET prediction. Ten compounds out of 200 compounds demonstrated significant results that compatible with ADMET prediction (figure 4.4). Those 20 compounds that obtained from virtual screening as well as from structure base pharmacophore modeling are considered the promising candidates that could be modified as a potential drug.

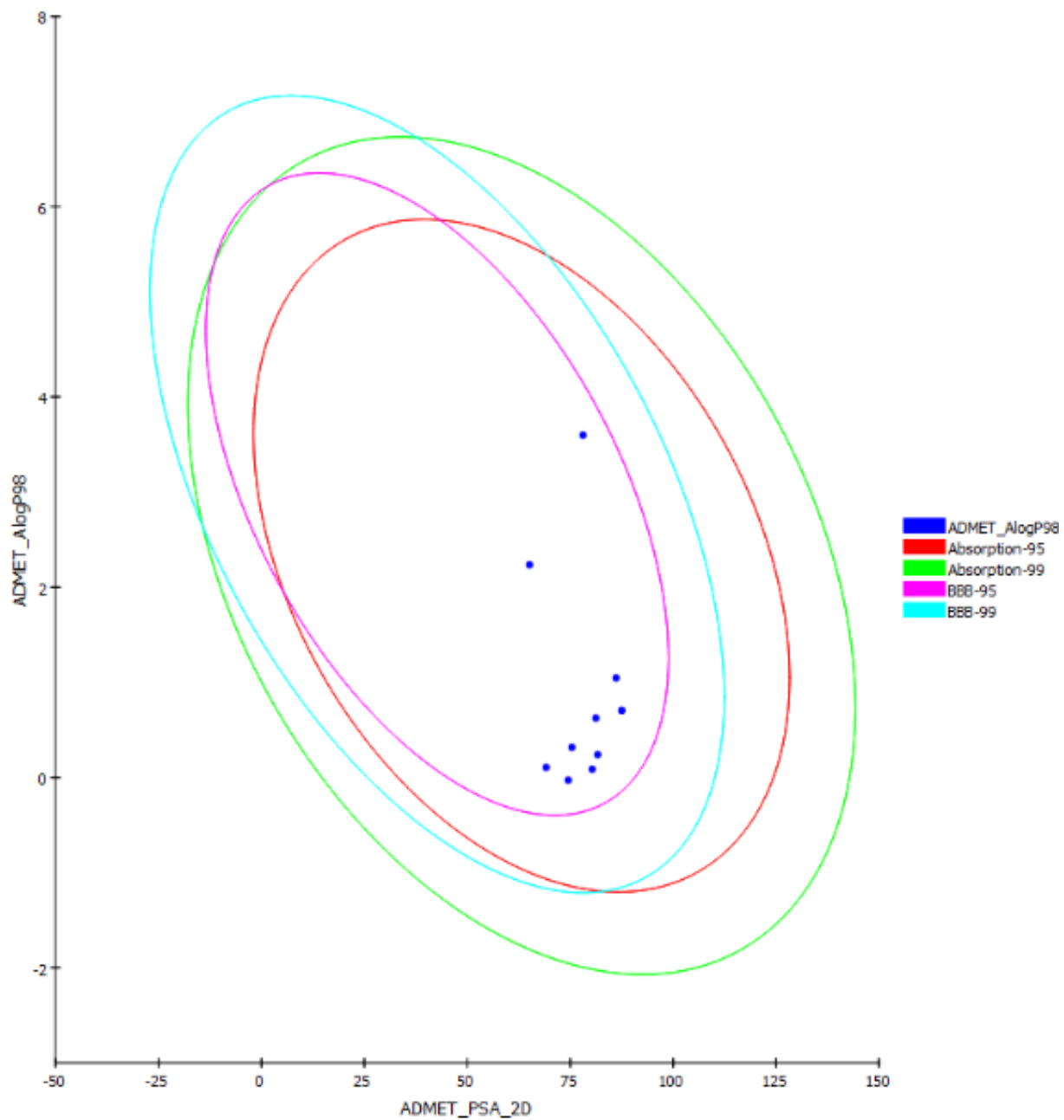


Figure 4.5: The ten compounds that obtained from virtual screening which they passed ADMET criteria

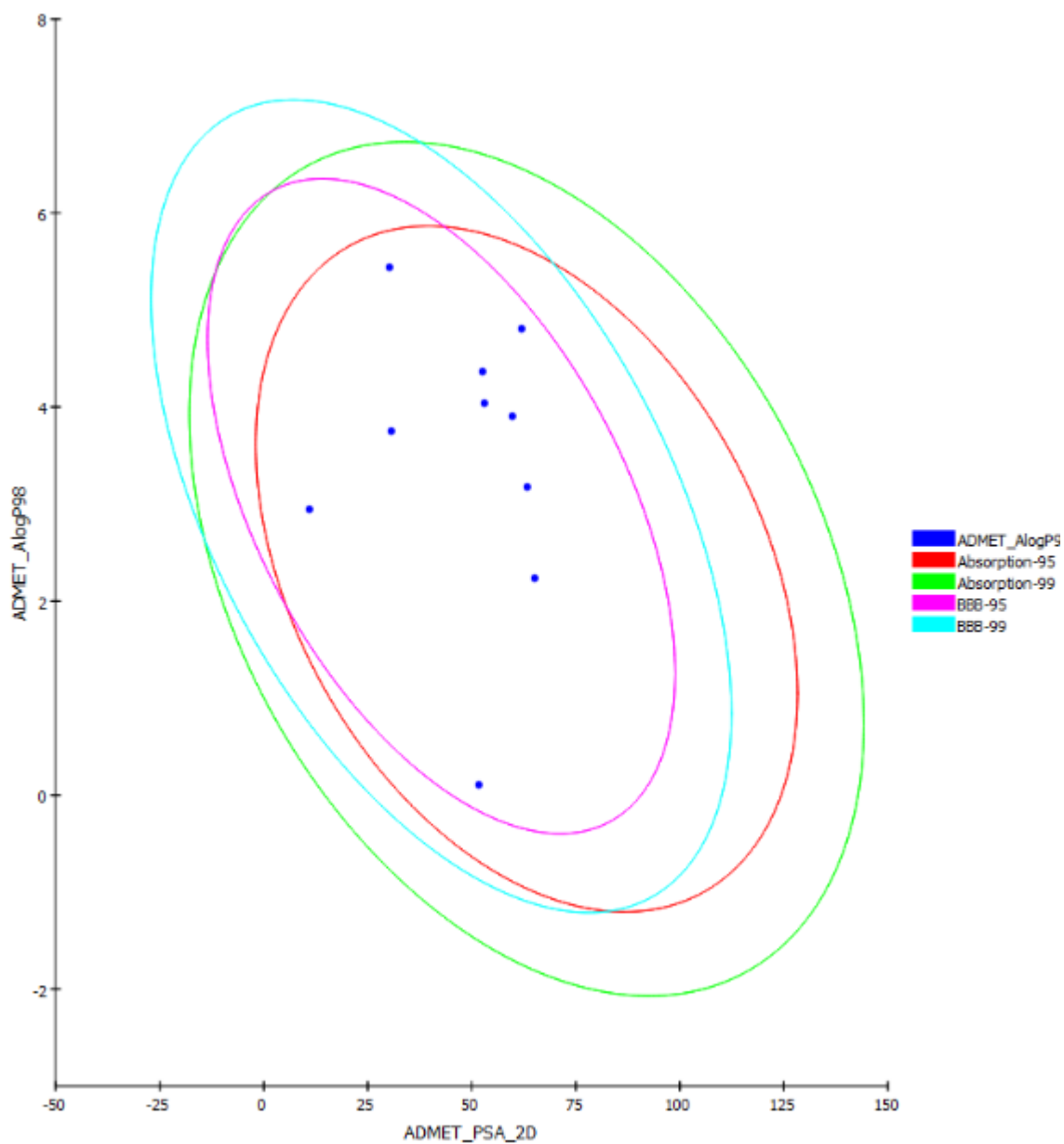


Figure 4.6: The ten compounds out of 200 compounds that obtained from structure base pharmacophore modeling which they passed ADMET criteria

However, zinc_19925080 compound shown desirable chemical interaction with 3ZMS receptor, which demonstrated different types of interactions include conventional hydrogen

bond, van der waals, carbon hydrogen bond, Pi-Anion, Pi-Sigma, Pi-Lone Pair, Pi-Pi T-shape, Alkyl and Pi-Alkyl are illustrated as 2 D and 3D diagrams in the figures 4.5 and 4.6 respectively.

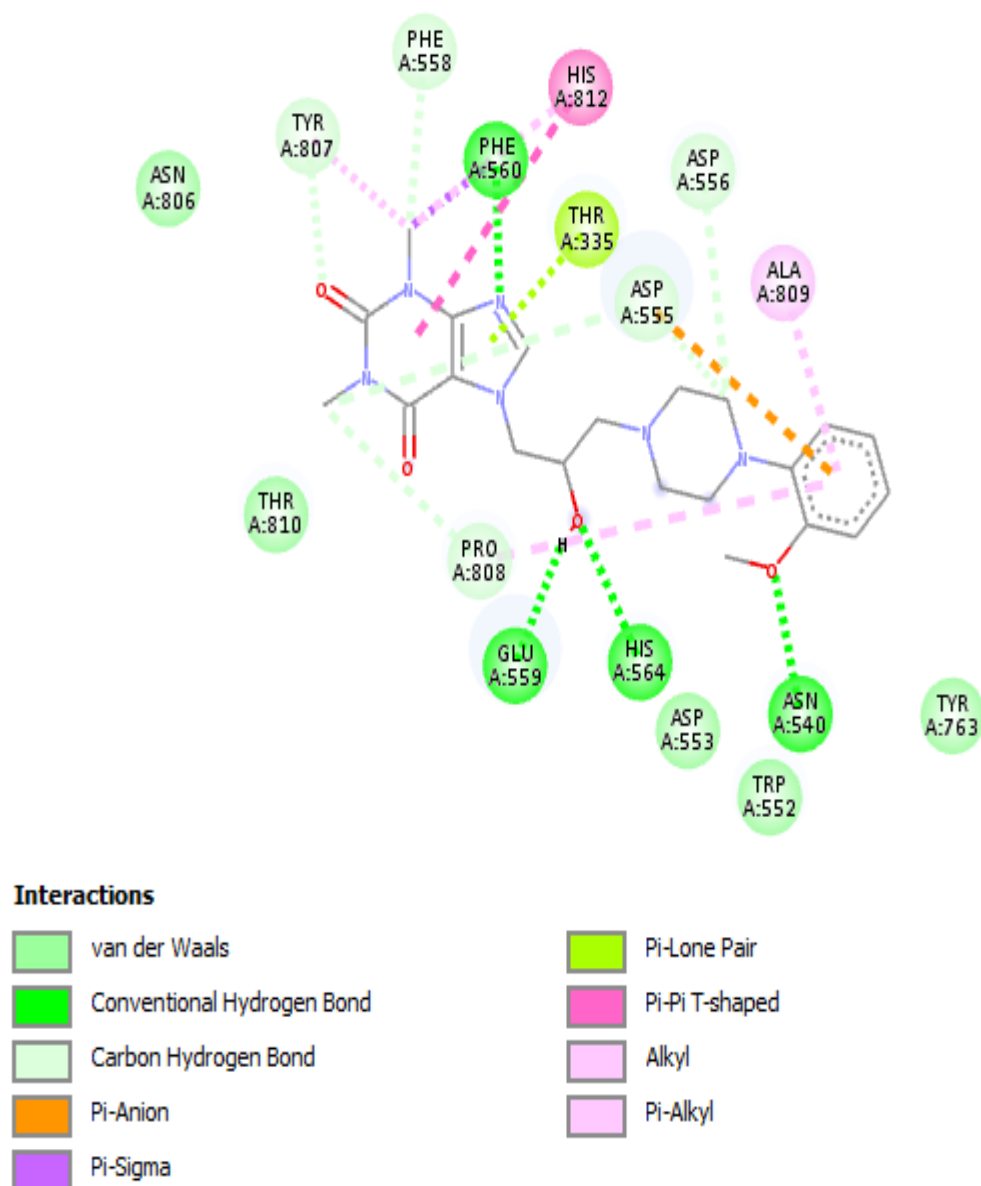


Figure 4.7: The 2D diagram of the chemical interactions between compound zinc_19925080 with 3ZMS receptor

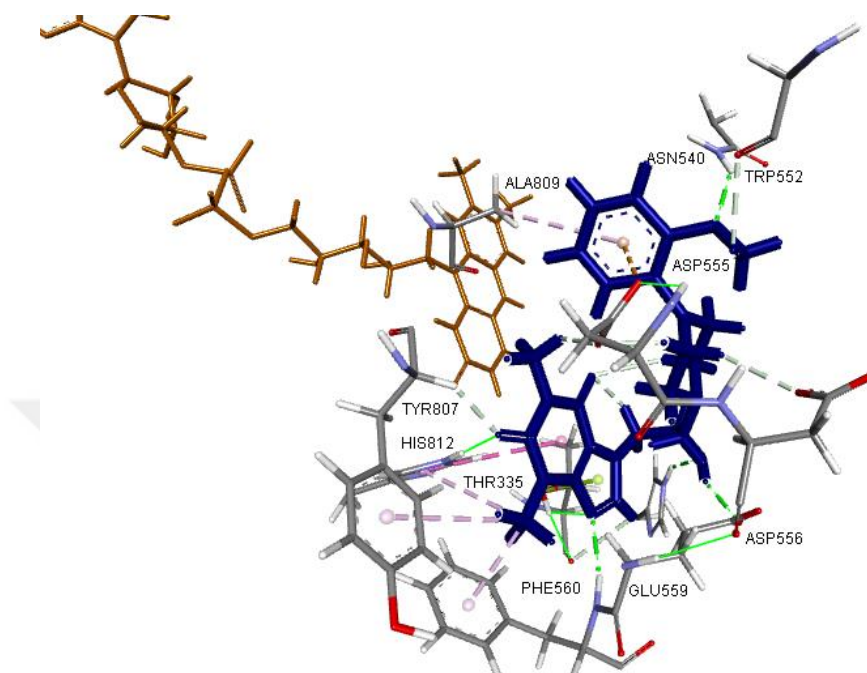


Figure 4.8: The 3D diagram of the chemical interactions between compound zinc_19925080 with 3ZMS receptor

In addition, zinc_19925082 shown another desirable chemical interactions with 3ZMS receptor via 2D and 3D visualization as explained in the figures 4.7 and 4.8 respectively.

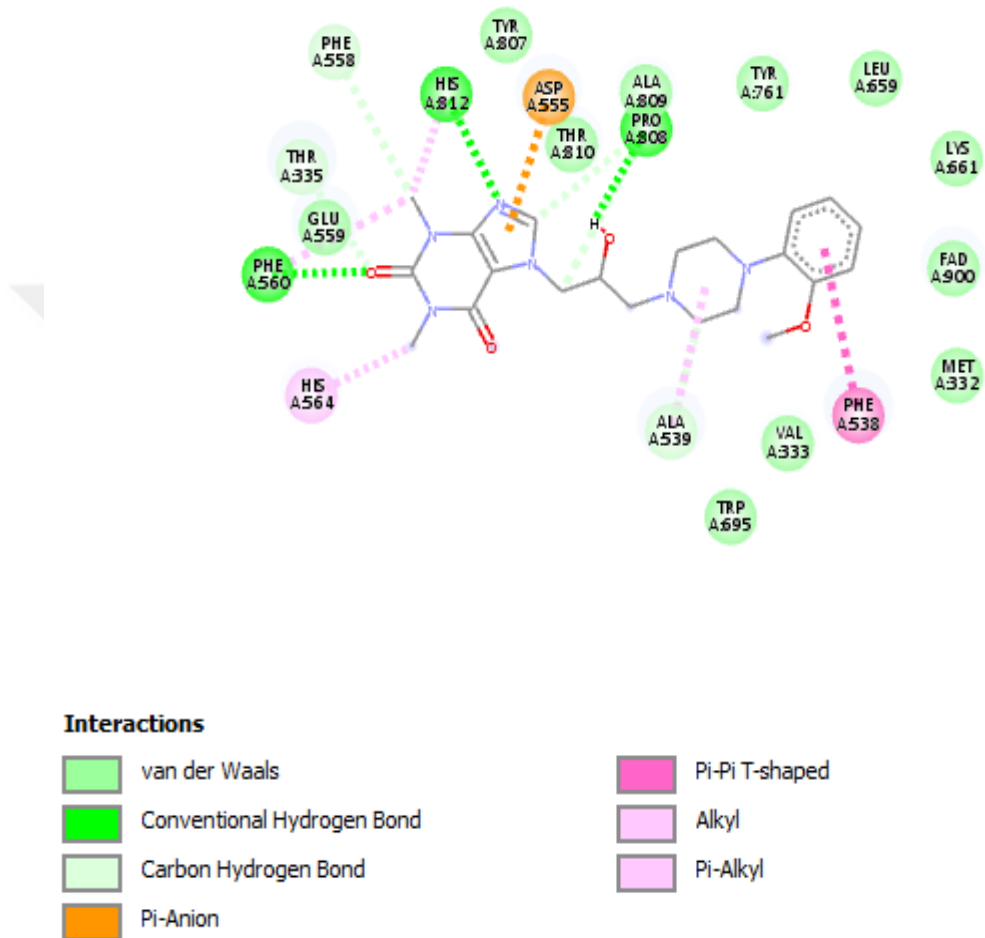


Figure 4.9: The 2D diagram of the chemical interactions between compound zinc_19925082 with 3ZMS receptor

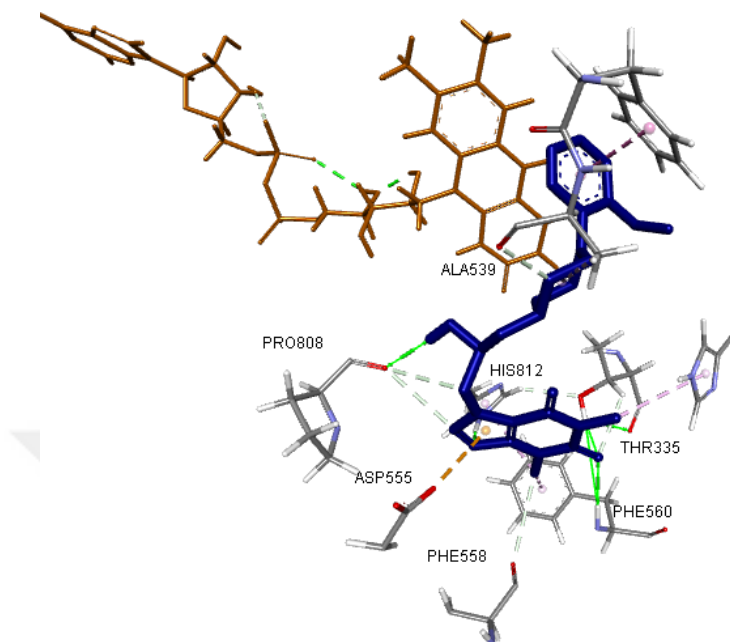


Figure 4.10: The 3D diagram of the chemical interactions between compound zinc_19925082 with 3ZMS receptor

On the other hand, the 2D and 3D structures of compound ZINC000170602679 demonstrated significant chemical interactions with 5LGT include four conventional hydrogen bond, van der waals, carbon hydrogen bond, Pi-Anion, Pi-Sigma, Pi-Lone Pair, Pi-Pi T-shape, Alkyl and Pi-Alkyl as shown in figures 4.8 and 4.9 respectively.

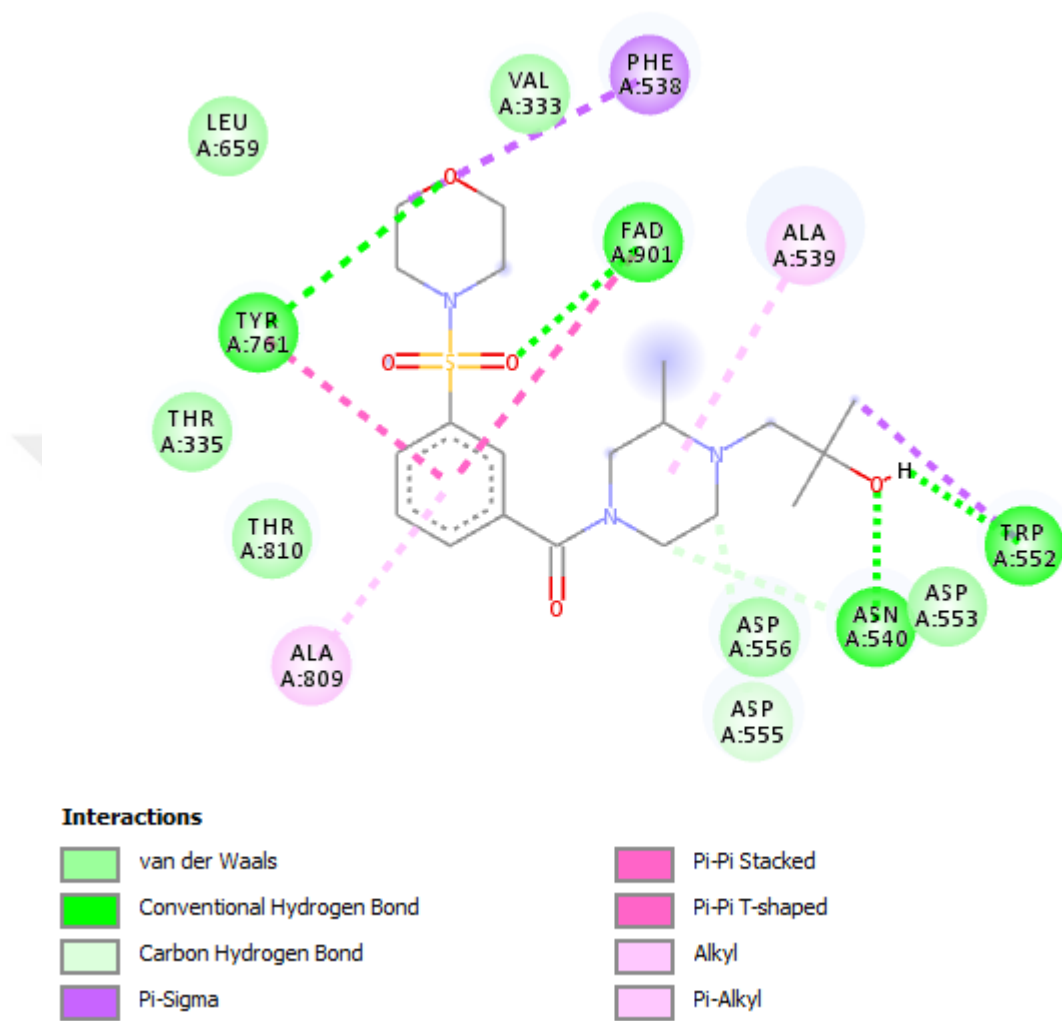


Figure 4.11: The 2D diagram of the chemical interactions between compound ZINC000170602679 and 5LGT receptor

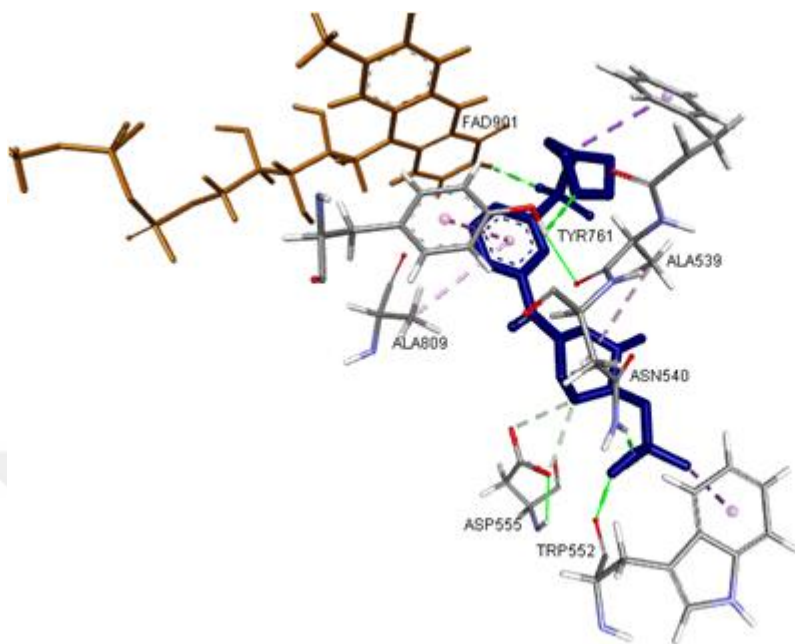


Figure 4.12: The 3D diagram of the chemical interactions between compound ZINC000170602679 and 5LGT receptor

Furthermore, compound ZINC000225395750 is as same as compound ZINC000170602679 in term of chemical interaction with 5LGT with the difference of possessing 3 conventional hydrogen bonds instead of 4 in compound ZINC000170602679. Figures 4.10 and 4.11 show the 2D and 3D diagrams of the chemical interactions of compound ZINC000225395750 with 5LGT.

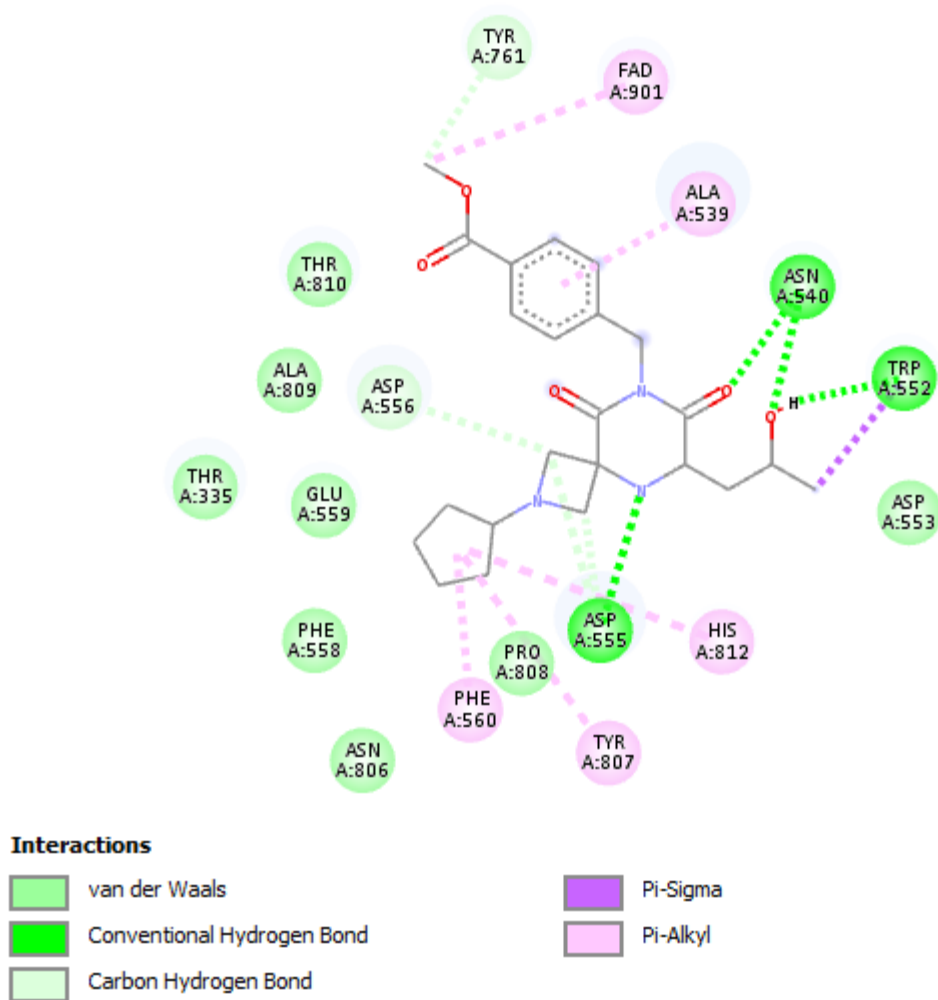


Figure 4.13: The 2D diagram of the chemical interactions between compound ZINC000225395750 and 5LGT receptor

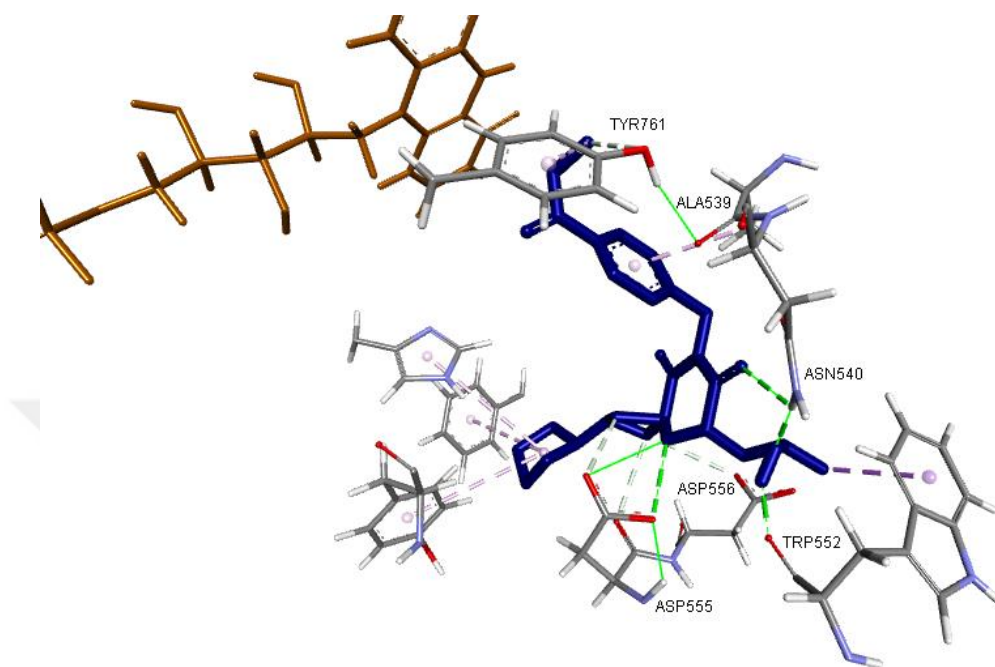


Figure 4.14: The 3D diagram of the chemical interactions between compound ZINC000225395750 and 5LGT receptor

5. CONCLUSION AND RECOMMENDATIONS

Epigenetic changes have considerable involvement in many pathogeneses including cancer initiation. DNA methylation and histone modification have been found to be the most common systems that might cause epigenetic changes, whereas Lysine Specific Demethylase 1 (LSD1) is one of the most common systems that might cause epigenetic. LSD1 has been involving in the diversity of pathological processes such as tumor-genesis and immune system weakening. Up-regulation of LSD1 has a direct contribution on cancer maintenance and its level is increased in Acute Myeloma Leukemia (AML), breast cancer, prostate cancer, and many other cancers. Therefore, since the last decade, a constant effort has been spent to create and develop LSD1 inhibitors that could be safe and effective. However, up to date only two drugs namely tranilcypromine and cyclopropylamine have are described as LSD1 inhibitors, where these drugs are not ultimately specific and they do not have direct effect against cancer.

Therefore, the current study aims to develop and create novel, potent and effective LSD1 inhibitors. Hence, virtual screening, pharmacophore modeling, molecular docking and ADMET prediction were included as a combination of different *in silico* approaches to attain the goal of the present study.

A series of known LSD1 inhibitors were docked in order to match their binding energy that obtained experimentally for protein validation. Zinc 15 database was an essential phase in both virtual screening (PyRx) and structure base pharmacophore. Hence, Zinc 15 database compounds that undergo to Lipinski's "Rule of Five" were screened to design new a class of inhibitors that possess potency in term of LSD1 inhibition. After various analysis took place via PyRx (autodockVina) to obtain potential lead compounds, several hundred initial compounds that shown low energy values of -9 kcal/mol and less were subjected to higher evaluations. These compounds and then re-docked with the respective protein using AutoDock 4.2 which was the fundamental step of visualizing the process that leads to obtaining punctual estimating of energy in active site between target and ligand.

Pharmacokinetics properties in drug design and drug discovery are irreplaceable and it is considered challenging. All successful drugs candidates are determined by good ADMET profile, which is the estimation of drug's adsorption, distribution, metabolism, elimination and toxicity. Therefore, ADMET prediction *in silico* was applied on the 950 compounds that obtained via screening in order to determine their novelty. A total of 20 compounds out of 950, which they were obtained from both virtual screening (10 compounds) and structure base pharmacophore (10 compounds) displayed significant chemical interactions with receptor through 2D structure and had stability due to their low free binding energy, yet all 20 compounds passed the ADMET prediction test.

Depending on the current analysis in this research, these 20 compounds, which they are listed in the figures 4.1 and 4.4 in chapter four, are considered the promising candidate as LSD1 inhibitors. Therefore, further conformational studies *in vitro* and *in vivo* for the 20 chosen compounds are highly recommended to investigate their potency in terms of mechanisms and pathway.

REFERENCES

- Akdoğan, E. D., Erman, B. and Yelekçi, K. (2011) 'In silico design of novel and highly selective lysine-specific Histone demethylase inhibitors', *Turkish Journal of Chemistry*. doi: 10.3906/kim-1102-985.
- Alonso, H., Bliznyuk, A. A. and Gready, J. E. (2006) 'Combining docking and molecular dynamic simulations in drug design', *Medicinal Research Reviews*. doi: 10.1002/med.20067.
- Anderson, N. G. *et al.* (2001) 'ZD1839 (Iressa), a novel epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, potently inhibits the growth of EGFR-positive cancer cell lines with or without erbB2 overexpression', *International Journal of Cancer*. doi: 10.1002/ijc.1557.
- Barradas, M. *et al.* (2009) 'Histone demethylase JMJD3 contributes to epigenetic control of INK4a/ARF by oncogenic RAS', *Genes and Development*. doi: 10.1101/gad.511109.
- Benz, R. D. (2007) 'Toxicological and clinical computational analysis and the US FDA/CDER', *Expert Opinion on Drug Metabolism & Toxicology*. Taylor & Francis, 3(1), pp. 109–124. doi: 10.1517/17425255.3.1.109.
- Berman, H. M. *et al.* (2002) 'The protein data bank', *Acta Crystallographica Section D: Biological Crystallography*. doi: 10.1107/S0907444902003451.
- Brint, A. T. and Willett, P. (1987) 'Algorithms for the Identification of Three-Dimensional Maximal Common Substructures', *Journal of Chemical Information and Computer Sciences*. doi: 10.1021/ci00056a002.
- Cao, R. *et al.* (2002) 'Role of histone H3 lysine 27 methylation in polycomb-group silencing', *Science*. doi: 10.1126/science.1076997.
- Cho, H. S. *et al.* (2011) 'Demethylation of RB regulator MYPT1 by histone demethylase LSD1 promotes cell cycle progression in cancer cells', *Cancer Research*. doi: 10.1158/0008-5472.CAN-10-2446.
- Cooper, G. M. (2000) *The Cell: A Molecular Approach. 2nd edition*, Sinauer Associates.

doi: 10.1016/B978-0-12-387738-3.00003-2.

- Cosgrove, M. S., Boeke, J. D. and Wolberger, C. (2004) 'Regulated nucleosome mobility and the histone code', *Nature Structural and Molecular Biology*. doi: 10.1038/nsmb851.
- Egger, G. *et al.* (2004) 'Epigenetics in human disease and prospects for epigenetic therapy', *Nature*. doi: 10.1038/nature02625.
- Ewing, T. J. A. *et al.* (2001) 'DOCK 4.0: Search strategies for automated molecular docking of flexible molecule databases', *Journal of Computer-Aided Molecular Design*. doi: 10.1023/A:1011115820450.
- Ferrari-Amorotti, G. *et al.* (2013) 'Inhibiting interactions of lysine demethylase LSD1 with snail/slug blocks cancer cell invasion', *Cancer Research*. doi: 10.1158/0008-5472.CAN-12-1739.
- Fischer, E. (1894) 'Einfluss der Configuration auf die Wirkung der Enzyme. II', *Berichte der deutschen chemischen Gesellschaft*. doi: 10.1002/cber.189402703169.
- Fornieris, F. *et al.* (2008) 'LSD1: oxidative chemistry for multifaceted functions in chromatin regulation', *Trends in Biochemical Sciences*. doi: 10.1016/j.tibs.2008.01.003.
- Foster, C. T. *et al.* (2010) 'Lysine-Specific Demethylase 1 Regulates the Embryonic Transcriptome and CoREST Stability', *Molecular and Cellular Biology*. doi: 10.1128/mcb.00521-10.
- Fulda, S., Friesen, C. and Debatin, K.-M. (1998) 'Molecular determinants of apoptosis induced by cytotoxic drugs', *Klinische Pädiatrie*, 210(04), pp. 148–152. doi: 10.1055/s-2008-1043870.
- Goardon, N. *et al.* (2011) 'Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia', *Cancer Cell*. doi: 10.1016/j.ccr.2010.12.012.
- Goodwin, R. J. A., Bunch, J. and McGinnity, D. F. (2017) 'Mass Spectrometry Imaging in Oncology Drug Discovery', in *Advances in Cancer Research*. doi: 10.1016/bs.acr.2016.11.005.
- Van Haaften, G. *et al.* (2009) 'Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer', *Nature Genetics*. doi: 10.1038/ng.349.

- Han, S. Y. *et al.* (2012) 'Marsdenia tenacissima extract restored gefitinib sensitivity in resistant non-small cell lung cancer cells', *Lung Cancer*. doi: 10.1016/j.lungcan.2011.06.001.
- Hanna, N. *et al.* (2004) 'Randomized phase III trial of pemetrexed versus docetaxel in patients with non-small-cell lung cancer previously treated with chemotherapy', *Journal of Clinical Oncology*. doi: 10.1200/JCO.2004.08.163.
- Harris, W. J. *et al.* (2012) 'The Histone Demethylase KDM1A Sustains the Oncogenic Potential of MLL-AF9 Leukemia Stem Cells', *Cancer Cell*. doi: 10.1016/j.ccr.2012.03.014.
- Hart, T. N. and Read, R. J. (1992) 'A multiple- start Monte Carlo docking method', *Proteins: Structure, Function, and Bioinformatics*. doi: 10.1002/prot.340130304.
- Heiden, M. G. Vander, Cantley, L. C. and Thompson, C. B. (2009) 'Understanding the warburg effect: The metabolic requirements of cell proliferation', *Science*. doi: 10.1126/science.1160809.
- Heidenblad, M. *et al.* (2008) 'Tiling resolution array CGH and high density expression profiling of urothelial carcinomas delineate genomic amplicons and candidate target genes specific for advanced tumors', *BMC Medical Genomics*. doi: 10.1186/1755-8794-1-3.
- Hosseini, A. and Minucci, S. (2017) 'A comprehensive review of lysine-specific demethylase 1 and its roles in cancer', *Epigenomics*. doi: 10.2217/epi-2017-0022.
- Hu, Q. *et al.* (2008) 'Enhancing nuclear receptor-induced transcription requires nuclear motor and LSD1-dependent gene networking in interchromatin granules', *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.0810634105.
- Huang, Y. *et al.* (2007) 'Inhibition of lysine-specific demethylase 1 by polyamine analogues results in reexpression of aberrantly silenced genes', *Proceedings of the National Academy of Sciences*. doi: 10.1073/pnas.0700720104.
- Irwin, J. J. and Shoichet, B. K. (2005) 'ZINC - A free database of commercially available compounds for virtual screening', *Journal of Chemical Information and Modeling*. doi: 10.1021/ci049714+.
- Kennison, J. A. (2002) 'The Polycomb and Trithorax Group Proteins of Drosophila: Trans-

- Regulators of Homeotic Gene Function', *Annual Review of Genetics*. doi: 10.1146/annurev.genet.29.1.289.
- Khan, M. N. A., Suzuki, T. and Miyata, N. (2013) 'An Overview of Phenylcyclopropylamine Derivatives: Biochemical and Biological Significance and Recent Developments', *Medicinal Research Reviews*. doi: 10.1002/med.21269.
- Kollman, P. A. (1994) 'Theory of macromolecule-ligand interactions', *Current Opinion in Structural Biology*. doi: 10.1016/S0959-440X(94)90315-8.
- Kottakis, F. *et al.* (2011) 'FGF-2 Regulates Cell Proliferation, Migration, and Angiogenesis through an NDY1/KDM2B-miR-101-EZH2 Pathway', *Molecular Cell*. doi: 10.1016/j.molcel.2011.06.020.
- Kouzarides, T. (2007) 'Chromatin modifications and their function.', *Cell*. doi: 10.1016/j.cell.2007.02.005.
- Kozub, M. M. *et al.* (2017) 'LSD1, a double-edged sword, confers dynamic chromatin regulation but commonly promotes aberrant cell growth', *F1000Research*. doi: 10.12688/f1000research.12169.1.
- Kumar, C. (2013) 'An Insight to Drug Designing by In Silico approach in Biomedical Research', *Jphmr.Com*, 1(2), pp. 63–65. Available at: <https://pdfs.semanticscholar.org/ae77/3b5bbbb89402412824e3a0569e005e49013a.pdf> (Accessed: 30 April 2019).
- Langer, T. and Hoffmann, R. (2005) 'Virtual Screening An Effective Tool for Lead Structure Discovery', *Current Pharmaceutical Design*. doi: 10.2174/1381612013397861.
- Lawrence, M., Daujat, S. and Schneider, R. (2016) 'Lateral Thinking: How Histone Modifications Regulate Gene Expression', *Trends in Genetics*. doi: 10.1016/j.tig.2015.10.007.
- Li, Q. *et al.* (2007) 'A large descriptor set and a probabilistic kernel-based classifier significantly improve druglikeness classification', *Journal of Chemical Information and Modeling*. doi: 10.1021/ci700107y.
- Li, Y. *et al.* (2012) 'Dynamic interaction between TAL1 oncoprotein and LSD1 regulates TAL1 function in hematopoiesis and leukemogenesis', *Oncogene*. doi:

10.1038/onc.2012.8.

- Lim, S. *et al.* (2010) 'Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology', *Carcinogenesis*. doi: 10.1093/carcin/bgp324.
- Lopez-Vallejo, F. *et al.* (2011) 'Integrating Virtual Screening and Combinatorial Chemistry for Accelerated Drug Discovery', *Combinatorial Chemistry & High Throughput Screening*. doi: 10.2174/138620711795767866.
- Lv, S. *et al.* (2010) 'LSD1 is required for chromosome segregation during mitosis', *European Journal of Cell Biology*. doi: 10.1016/j.ejcb.2010.01.004.
- Lv, T. *et al.* (2012) 'Over-expression of LSD1 promotes proliferation, migration and invasion in non-small cell lung cancer', *PLoS ONE*. doi: 10.1371/journal.pone.0035065.
- Maiuri, A. R. and O'Hagan, H. M. (2016) 'Interplay Between Inflammation and Epigenetic Changes in Cancer', in *Progress in Molecular Biology and Translational Science*. doi: 10.1016/bs.pmbts.2016.09.002.
- Mandal, S., Moudgil, M. and Mandal, S. K. (2009) 'Rational drug design', *European Journal of Pharmacology*. doi: 10.1016/j.ejphar.2009.06.065.
- Margueron, R. and Reinberg, D. (2011) 'The Polycomb complex PRC2 and its mark in life', *Nature*. doi: 10.1038/nature09784.
- Mayer, W. *et al.* (2002) 'Demethylation of the zygotic paternal genome', *Nature*. doi: 10.1038/35000656.
- Metzger, E. *et al.* (2005) 'LSD1 demethylates repressive histone marks to promote androgen-receptor- dependent transcription', *Nature*. doi: 10.1038/nature04020.
- Mosser, D. M. and Edwards, J. P. (2008) 'Exploring the full spectrum of macrophage activation.', *Nature reviews. Immunology*. doi: 10.1038/nri2448.
- Müller-knapp, S. and Brown, P. J. (2014) 'Open Access Drug Discovery Epigenetic Probes', *Tocris scientific review*.
- Musri, M. M. *et al.* (2010) 'Histone demethylase LSD1 regulates adipogenesis', *Journal of Biological Chemistry*. doi: 10.1074/jbc.M110.151209.
- Nair, S. S. *et al.* (2010) 'PELP1 is a reader of histone H3 methylation that facilitates

- oestrogen receptor- α target gene activation by regulating lysine demethylase 1 specificity', *EMBO Reports*. doi: 10.1038/embor.2010.62.
- Niwa, H. *et al.* (2018) 'Crystal Structure of LSD1 in Complex with 4-[5-(Piperidin-4-ylmethoxy)-2-(p-tolyl)pyridin-3-yl]benzotrile.', *Molecules (Basel, Switzerland)*, 23(7), p. 1538. doi: 10.3390/molecules23071538.
- Oswald, J. *et al.* (2000) 'Active demethylation of the paternal genome in the mouse zygote', *Current Biology*. doi: 10.1016/S0960-9822(00)00448-6.
- Pedersen, M. T. and Helin, K. (2010) 'Histone demethylases in development and disease', *Trends in Cell Biology*. doi: 10.1016/j.tcb.2010.08.011.
- Perillo, B. *et al.* (2008) 'DNA oxidation as triggered by H3K9me2 demethylation drives estrogen-induced gene expression', *Science*. doi: 10.1126/science.1147674.
- Perinchery, G. *et al.* (2000) 'Deletion of Y-chromosome specific genes in human prostate cancer', *Journal of Urology*. doi: 10.1016/S0022-5347(05)67774-9.
- Pryor, J. G. *et al.* (2011) 'Microarray comparative genomic hybridization detection of copy number changes in desmoplastic melanoma and malignant peripheral nerve sheath tumor', *American Journal of Dermatopathology*. doi: 10.1097/DAD.0b013e31820dfcbf.
- Qin, Y. *et al.* (2014) 'LSD1 sustains pancreatic cancer growth via maintaining HIF1 α -dependent glycolytic process', *Cancer Letters*. doi: 10.1016/j.canlet.2014.02.013.
- Sakamoto, A. *et al.* (2015) 'Lysine demethylase LSD1 coordinates glycolytic and mitochondrial metabolism in hepatocellular carcinoma cells', *Cancer Research*. doi: 10.1158/0008-5472.CAN-14-1560.
- Sandler, A. *et al.* (2006) 'Paclitaxel–Carboplatin Alone or with Bevacizumab for Non–Small-Cell Lung Cancer', *New England Journal of Medicine*. doi: 10.1056/nejmoa061884.
- Schenk, T. *et al.* (2012) 'Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-trans-retinoic acid differentiation pathway in acute myeloid leukemia', *Nature Medicine*. doi: 10.1038/nm.2661.
- Schmidt, D. M. Z. and McCafferty, D. G. (2007) 'trans-2-phenylcyclopropylamine is a mechanism-based inactivator of the histone demethylase LSD1', *Biochemistry*. doi:

10.1021/bi0618621.

- Shi, Yujiang *et al.* (2004) 'Histone demethylation mediated by the nuclear amine oxidase homolog LSD1', *Cell*. doi: 10.1016/j.cell.2004.12.012.
- Somervaille, T. C. P. and Cleary, M. L. (2006) 'Identification and characterization of leukemia stem cells in murine MLL-AF9 acute myeloid leukemia', *Cancer Cell*. doi: 10.1016/j.ccr.2006.08.020.
- Stein, J. *et al.* (2014) 'KDM5C is overexpressed in prostate cancer and is a prognostic marker for prostate-specific antigen-relapse following radical prostatectomy', *American Journal of Pathology*. doi: 10.1016/j.ajpath.2014.05.022.
- Stilgenbauer, S. *et al.* (2016) 'Venetoclax in relapsed or refractory chronic lymphocytic leukaemia with 17p deletion: a multicentre, open-label, phase 2 study', *The Lancet Oncology*. doi: 10.1016/S1470-2045(16)30019-5.
- Tessarz, P. and Kouzarides, T. (2014) 'Histone core modifications regulating nucleosome structure and dynamics', *Nature Reviews Molecular Cell Biology*. doi: 10.1038/nrm3890.
- Urwyler, S. (2011) 'Allosteric Modulation of Family C G-Protein-Coupled Receptors: from Molecular Insights to Therapeutic Perspectives', *Pharmacological Reviews*. doi: 10.1124/pr.109.002501.
- Vianello, P. *et al.* (2017) 'Thieno[3,2- *b*]pyrrole-5-carboxamides as New Reversible Inhibitors of Histone Lysine Demethylase KDM1A/LSD1. Part 2: Structure-Based Drug Design and Structure–Activity Relationship', *Journal of Medicinal Chemistry*, 60(5), pp. 1693–1715. doi: 10.1021/acs.jmedchem.6b01019.
- Vogt, T. *et al.* (1999) 'Deficiency of a novel retinoblastoma binding protein 2-homolog is a consistent feature of sporadic human melanoma skin cancer', *Lab Invest*.
- Wadood, A. *et al.* (2014) 'In-silico drug design: An approach which revolutionarised the drug discovery process', *OA Drug Design and Delivery*. doi: 10.13172/2054-4057-1-1-1119.
- Walters, W. P., Stahl, M. T. and Murcko, M. A. (2002) 'Virtual screening—an overview', *Drug Discovery Today*. doi: 10.1016/s1359-6446(97)01163-x.
- Willett, P., Barnard, J. M. and Downs, G. M. (1998) 'Chemical similarity searching',

- Journal of Chemical Information and Computer Sciences*. doi: 10.1021/ci9800211.
- Willmann, D. *et al.* (2012) 'Impairment of prostate cancer cell growth by a selective and reversible lysine-specific demethylase 1 inhibitor', *International Journal of Cancer*. doi: 10.1002/ijc.27555.
- Wilson, C. B., Rowell, E. and Sekimata, M. (2009) 'Epigenetic control of T-helper-cell differentiation', *Nature Reviews Immunology*. doi: 10.1038/nri2487.
- Wouters, B. J. and Delwel, R. (2016) 'Epigenetics and approaches to targeted epigenetic therapy in acute myeloid leukemia', *Blood*. doi: 10.1182/blood-2015-07-604512.
- Yao, Y. *et al.* (2014) 'Design, synthesis, and biological evaluation of 1, 3-disubstituted-pyrazole derivatives as new class I and IIb histone deacetylase inhibitors', *European Journal of Medicinal Chemistry*. doi: 10.1016/j.ejmech.2014.09.024.
- Yatim, A. *et al.* (2012) 'NOTCH1 Nuclear Interactome Reveals Key Regulators of Its Transcriptional Activity and Oncogenic Function', *Molecular Cell*. doi: 10.1016/j.molcel.2012.08.022.
- Zhang, S. *et al.* (2015) 'Synthesis, Biological Evaluation, and Computer-Aided Drug Designing of New Derivatives of Hyperactive Suberoylanilide Hydroxamic Acid Histone Deacetylase Inhibitors', *Chemical Biology and Drug Design*. doi: 10.1111/cbdd.12554.
- Zheng, Y.-C. *et al.* (2017) 'An Overview on Screening Methods for Lysine Specific Demethylase 1 (LSD1) Inhibitors', *Current Medicinal Chemistry*. doi: 10.2174/0929867324666170509114321.