1.1. METHODS OF COMPUTER-AIDED DRUG DESIGN AND DEVELOPMENT

Since the second half of the twentieth century, researchers have concentrated on the study of the relationship between the molecular structures of chemical compounds and their biological activities in order to reach the new drug active compounds. These new techniques have gained an increasingly important role in the development of new chemical compounds as drugs, the attainment of more ideal compounds, and the identification of mechanisms of activity.

There are two basic motion points for computer aided drug design.

1- **Target =** Receptors, enzymes or nucleic acids
2- **Effector =** There may be natural endogenous substances or drugs which occupy the active site of the target and affect the target positively or negatively.

Computer aided drug design and development studies are examined in two groups.

1- Methods based on Effector (Ligand) Structure
   - Quantitative Structure-Activity Relationships (QSAR) Analyses
   - Pharmacophore Analysis
2- Methods based on Target Structure
   - Molecular Docking

In the design based on ligand structure, it is aimed to interpret the structure of the receptor by making use of the structure of the molecules which affect. In the design based on target structure, it is aimed to design of molecules which can affect by the action of known receptor structure.

1.1.1. Methods based on Effector Structure

1.1.1.1. Quantitative Structure-Activity Relationships (QSAR)

Quantitative Structure-Activity Relationships (QSAR) are the processes of describing the relationship between the molecular properties and biological activities of chemical compounds with the mathematical methods.

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1.1.1.2. Pharmacophore Analysis

In cases the 3D structure of the receptor is unknown, that is frequently encountered in drug design, the structures of the ligands are well characterized by molecular modeling studies. Thus, it can be precisely revealed the conformations that play a role in the biological activity. Then, the structure of the receptor can be extracted or 'mapped' from such ligands. Hereby, it is possible to design new drug candidates by using existing structure-activity relationships.

The pharmacophore is defined as the basic functional groups required for the biological activities of ligands. In other words, the pharmacophore is the spatial regulation of the structural elements required for a given biological activity.

Pharmacophore model shows the molecular properties, including the three-dimensional structure of the molecule, such as hydrophobic groups, charged or ionizable groups, hydrogen bond donors and acceptors. To form the pharmacophore model, their common properties are determined by superimposing the compounds on one another.

These properties include chemical functions that resembled with the characteristical properties and required for receptor binding. The compounds that are compatible with the generated pharmacophore model can be used as 3D databases and as a result, it is provided suggestion of new active candidate molecules.

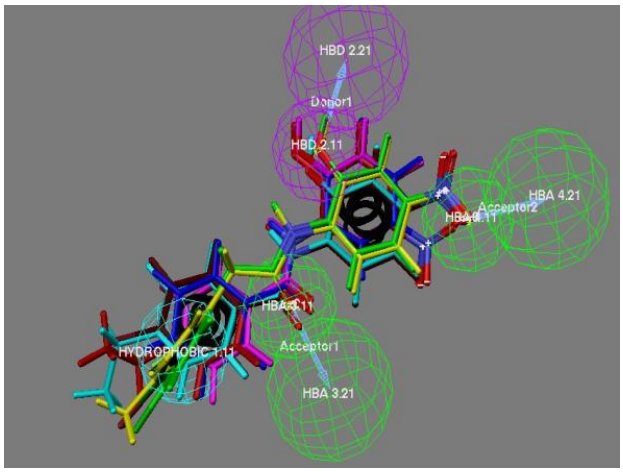


Figure 4.5. A pharmacophore model consisting of two hydrogen bond acceptors, one hydrogen bond donor and one hydrophobic feature and the compounds mapping with this model.

With the pharmacophore analysis, it is aimed to interpret the structure of the receptor by means of the structure of the effective molecules. While this method is used;

- The structure of the receptor is unknown
- The mechanism of activity is known or unknown
- Ligand and its biological activity are known.

The properties of pharmacophore groups

Hydrogen bond acceptor feature: Atoms carrying free electron pairs
Ex: O, N, S...
Hydrogen bond donor feature: Atoms carrying at least one H atom
Ex: -OH, -NH-, R_3NH^+, -SH...
Hydrophobic feature:

- Nonpolar groups increase hydrophobic feature.

Ex: -R, -X, -Ar

- Polar groups decrease hydrophobic feature.

Ex: -COOH, -OH, $-NH_2$
Ring aromatic feature: Rings in an aromatic structure
Ex: -Ar
benzene, thiophene, prydine, naphtalene...
Electrostatic feature: The atoms in the form of anion or cation
Ex: $-NH_3^+$ (positive ionizable group)
$-COO^-$ (negative ionizable group)

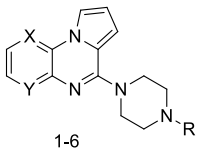
Pharmacophore Analysis on Serotonin 5-HT$_3$ Receptor Partial Agonists

Serotonin 5-HT$_3$ receptors have been investigated as potential therapeutic targets due to the effects of the formation of numerous physiological events. If this receptor is activated by agonists, the effects are observed such as;

- in the central nervous system; nausea and vomiting in the brain stem, anxiety and paralysis
- in the peripheral nervous system; stimulation of nerve cells (autonomic and pain nerves) and vomiting.

Serotonin 5-HT$_3$ receptor partial agonists are used in various gastrointestinal system disorders, particularly irritable bowel syndrome (spastic colon).

Table 4.4. Serotonin 5-HT$_3$ receptor agonists used as study set



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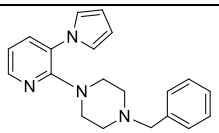

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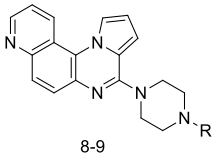

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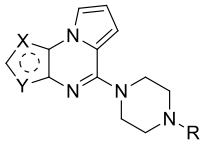

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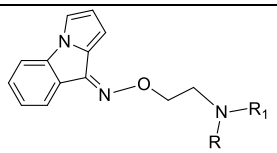

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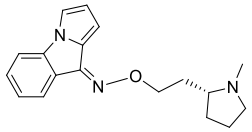

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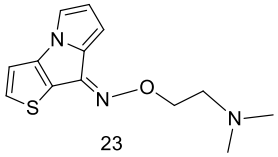

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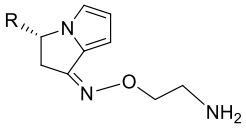

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Compounds	X	Y	R	R_1	Activity (-log IC_{50})
1	N	C	H	-	8.83
2	N	C	allyl	-	11.40
3	N	C	benzyl	-	12.09
4	N	C	4F-benzyl	-	8.59
5	C	C	Benzyl	-	9.45
6	C	C	4F-benzyl	-	8.62
7	-	-	-	-	7.34
8	-	-	allyl	-	7.71
9	-	-	benzyl	-	7.51
10	S	C	H	-	7.92
11	S	C	CH_3	-	6.09
12	S	C	allyl	-	8.58
13	S	C	benzyl	-	8.85
14	S	C	4F-benzyl	-	7.67
15	C	S	CH_3		6.62
16	C	S	allyl	-	9.04
17	C	S	benzyl	-	8.34
18	C	S	4F-benzyl	-	8.93
19	-	-	H	H	6.66
20	-	-	CH_3	CH_3	8.24
21	-	-	benzyl	H	6.54
22	-	-	-	-	7.85
23	-	-	-	-	8.46
24	-	-	thienyl	-	6.05
25	-	-	phenyl	-	6.00

1. Create a conformational databank

- In the study, 25 compounds with known biological activity and 250 inactive compounds which structurally resemble these 25 compounds from databank containing 19000 different compounds are used.
- For the 275 compounds in the study set generated, a conformational analysis study is initiated and different conformation of these compounds is obtained.
- Biological activity values of formed conformers are input.

2. Generate the pharmacophore

A pharmacophore model with two aromatic rings, a hydrogen bond acceptor, a hydrogen bond donor and cationic site features is shown on the compound with the highest biological activity.

3. Pharmacophore analysis

The 275 molecules in the study set and their conformers are mapped with the resulting pharmacophore model.

4. Evaluation of results

Quantitative data obtained in the result of mapping the compounds with the pharmacophore model in the study set are examined. The compatibility of the molecules with the pharmacophore model is explained by the RMSD value.

RMSD (Root-mean-square deviation): It is a value that measures the difference between the actual observed value and the predicted value by generating a model. The closer this value is to zero, the higher the compatibility with the molecular pharmacophore model.

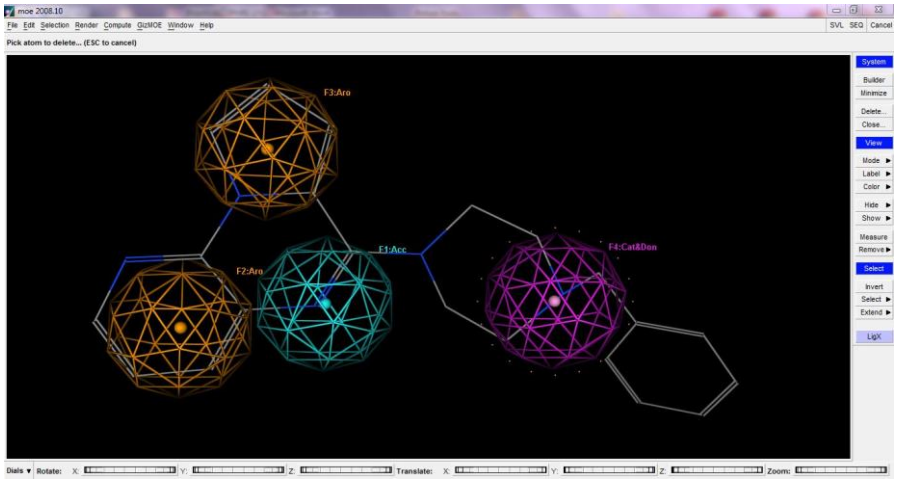

Figure 4.6. The pharmacophore model generated as a result of the study

Orange: ring aromatic (RA), light blue: Hydrogen bond acceptor (HBA), purple: Hydrogen bond donor (HBD) and cationic center

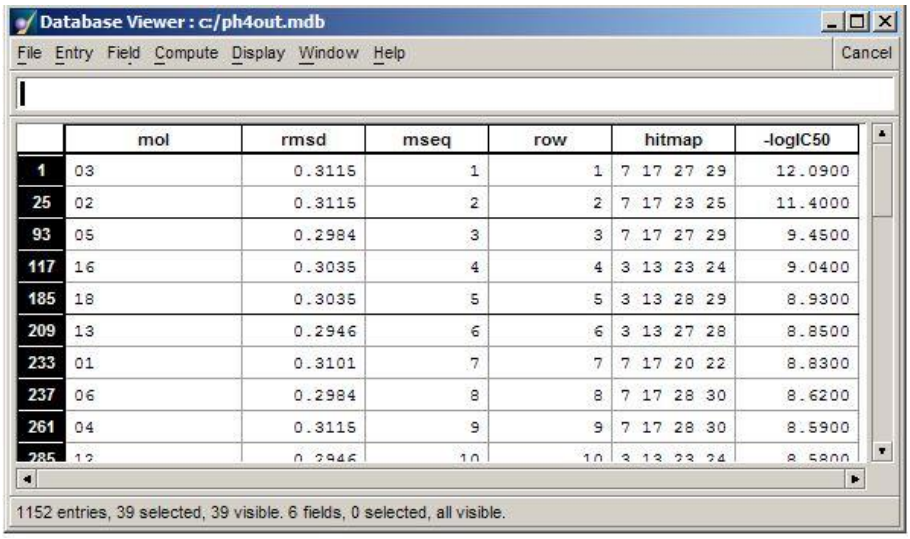

Figure 4.7. Results obtained as a result of pharmacophore analysis

Questions

1) What is the pharmacophore model? How to generate?
2) What are the most important features that emerged in the pharmacophore model that you generated on partial agonists of serotonin 5-HT_3 receptors?
3) Design a new molecule that can be a serotonin 5-HT_3 receptor partial agonist, based on the pharmacophore model you generated on serotonin 5-HT_3 receptor partial agonists.

1.1.2. Methods of Target Structure

1.1.2.1. Molecular Docking

The prominence in the drug design of protein-ligand docking methods is disputable. In drug design, docking studies between small molecules and receptors become increasingly important.

The structure that is considered as a receptor in docking studies has protein structure. X-ray crystallography and nuclear magnetic resonance (NMR) techniques are mostly used to describe the structure of the receptor. the structure of this protein can be determined by sending X-rays onto a protein that can be obtained as a crystal. In the more complex NMR method, the conformation of the protein dissolved in the water environment can be detected. However, the NMR method requires highly complex solutions. Thus, the preferred method is X-ray crystallography.

Molecular docking techniques are frequently used to investigate how drugs or drug candidates with enzymes, nucleic acids, and receptor proteins are compatible with each other in computer aided rational drug design. In the docking studies, binding energies to the receptor that is known 3D structure can be determined and the position of the ligand in the binding site of the receptor can be visualized. This can be useful for understanding the type of binding and designing smaller and more compatible ligands that target proteins. In other words, docking is as a key-lock relationship between the proper conformation of the ligand and the receptor. Ligands are usually flexible and have many different conformations in the solvent. The most commonly used method of docking is the docking process between the rigid active site of the protein and a series conformation of the ligand. Figure 4.8 and Figure 4.9 show protein-drug interactions.

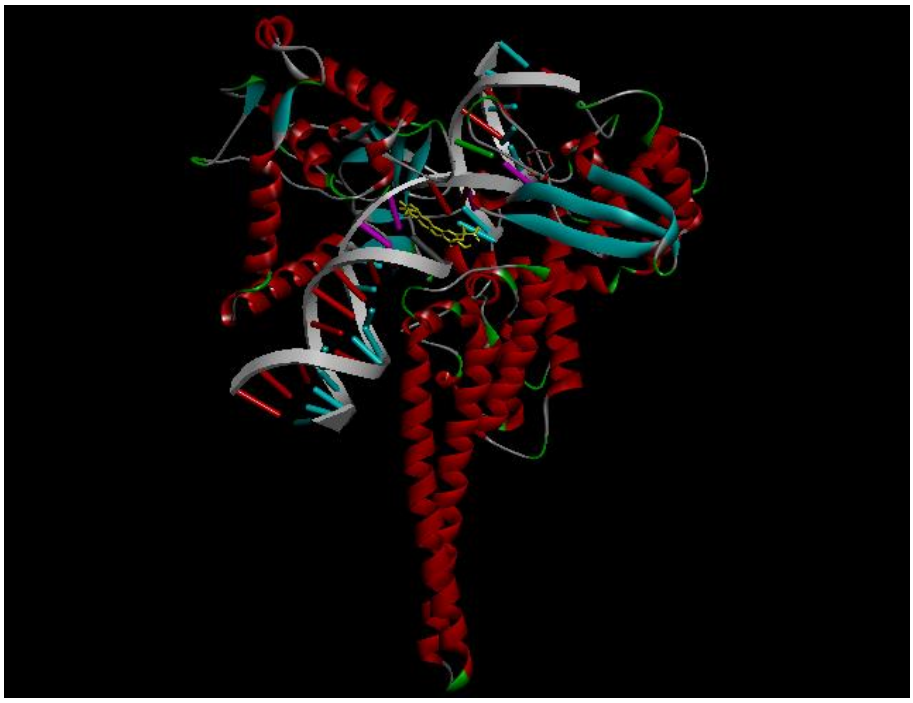

Figure 4.8. Interaction of Topotecan (yellow) with Topoisomerase I enzyme ve DNA (Pdb: 1K4T)

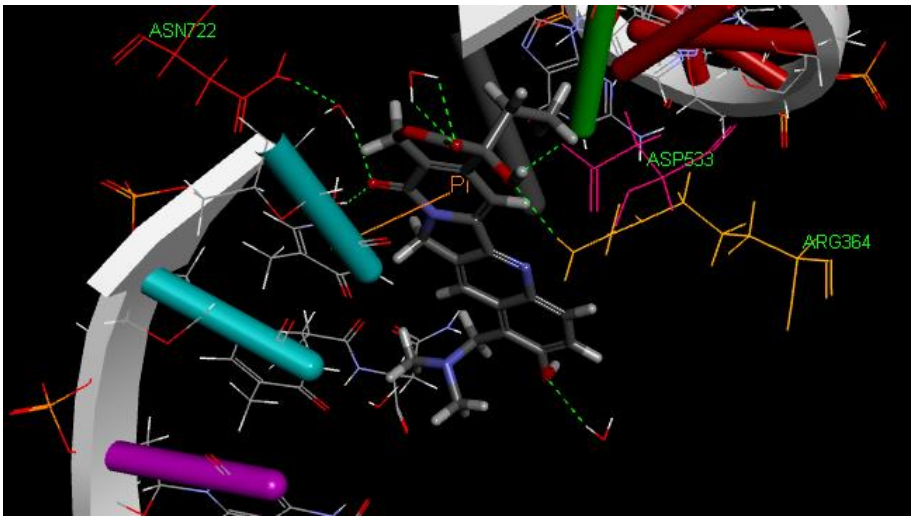

Figure 4.9. Docking pose of Topotecan (Hydrogen bonds and pi interactions)