T. R.
GAZİANTEP UNIVERSITY
GRADUATE SCHOOL OF HEALTH SCIENCES

DESIGN OF EFFECTIVE CARBONIC ANHYDRASE INHIBITORS BY USING IN SILICO PHARMACOPHORE ANALYSIS

Nur GÜREL

MASTER THESIS

DEPARTMENT OF BIOINFORTMATICS AND DEVELOPMENT BIOLOGY

SUPERVISOR
Assoc. Prof. Dr. Tuğba TAŞKIN TOK

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Approval of the Graduate School of Health Sciences

Prof. Dr. Mehmet TARAKÇIOĞLU

Director of Graduate School of Health Science

I certify that this thesis satifies all the reqirements as a thesis for the degree of Master of Science of Philosophy.

Assoc. Prof. Dr. Tuğba TAŞKIN TOK

Head of Bioinformatics and Computational Biology Department

This is to certify that we have reasd this thesis and that in our consensus/majority opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science of Philosophy.

Assoc. Prof. Dr. Tuğba TAŞKIN TOK

Supervisor

This thesis has been read and accepted, in scope and quality, as a thesis for the degree of Master of Philosophy.

Examining Committee Members	**Signature**
Assoc. Prof. Dr. Tuğba TAŞKIN TOK	.....
Assoc. Prof. Dr. Zafer ÇETİN	.....
Assoc. Prof. Dr. Belgin ÖZER	.....

ii

DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Nur GÜREL

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TABLE OF CONTENTS

LIST OF FIGURE

LIST OF TABLES

LIST OF ABBREVIATIONS

ADME/T	Absorption, Distribution, Metabolism, Excretion and Toxicity (ADME/T)
CADD	Computer Aided Drug Design
SBDD	Structure Based Drug Design
LBDD	Ligand Based Drug Design
CA	Carbonic Anhydrase inhibitör
hCA-I	Human Carbonic anhydrase I
hCA-II	Human Carbonic anhydrase II
DMPK	Drug Metabolism and Pharmacokinetics
QSAR	Quality Structure Activity Relationship

ABSTRACT

DESIGN OF EFFECTIVE CARBONIC ANHYDRASE INHIBITORS BY USING IN SILICO PHARMACOPHORE ANALYSIS

Nur GÜREL

Master of Science Thesis, Department of Bioinformatics and Computational Biology
Supervisor: Assoc. Prof. Dr. Tuğba TAŞKIN TOK

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Pharmacophore modeling is a successful yet very diverse subfield of computer-aided drug design. The concept of the pharmacophore has been widely applied to the rational design of novel drugs. In this paper, we review the computational implementation of this concept and its common usage in the drug discovery process. Carbonic anhydrases (CAs) are zinc containing metalloenzymes including sixteen different isoforms. These enzymes differ in their subcellular localization, catalytic activity and susceptibility to different classes of inhibitors. The previous studies have also indicated that the CA I and II levels were also higher in several cancer types, such as higher cytosolic erythrocte levels in stomach, prostate, lung and ovary tumors; also in hematological diseases such as leukemia. In addition, CA II has come to the forefront by being expressed in the endothelium of neovessels in some cancer tissues, including melanoma, esophageal, renal cancers. Therefore, it is aimed to investigate and design reliable and potential carbonic anhydrase inhibitors against to multi target; CA I and II by using biological data from our team for treatment of cancer.

Keywords: Pharmacophore, Carbonic Anhydrase, HipHop, urea; thiourea

ÖZET

İN SİLİKO ORTAMDA ERKİN KARBONİK ANHİDRAZ İNHİBİTÖRLERİNİN FARMAKOFOR TEKNİĞİ İLE TASARLANMASI

Yüksek Lisans Tezi, Biyoinformatik ve Bilişimsel Biyoloji Anabilim Dalı

Tez Danışman: Doc. Dr. Tuğba TAŞKIN TOK

Temmuz 2019, 35 Sayfa

Farmakofor modelleme, bilgisayar destekli ilaç tasarımında başarılı ancak çok çeşitli bir alt alandır. Farmakofor kavramı, yeni ilaçların rasyonel tasarımına yaygın olarak uygulanmıştır. Bu yazıda, bu kavramın hesaplamalı uygulanmasını ve ilaç keşif sürecinde ortak kullanımını gözden geçirdik. Karbonik anhidrazlar (CA), on altı farklı izoformu içeren, çinko içeren metaloenzimlerdir. Bu enzimler subselüler lokalizasyonları, katalitik aktiviteleri ve farklı inhibitör sınıflarına duyarlılıkları bakımından farklılık gösterir. Önceki çalışmalar, hCA-I ve hCA -II seviyelerinin, mide, prostat, akciğer ve yumurtalık tümörlerinde daha yüksek sitozolik eritrosit seviyeleri gibi bazı kanser türlerinde daha yüksek olduğunu; ayrıca lösemi gibi hematolojik hastalıklarda. Ek olarak, hCA-II, bazı kanser dokularında, melanom, özofagus, böbrek kanserleri de dahil olmak üzere bazı kanser dokularındaki endotelyumda ifade edilerek ön plana çıkmıştır. Bu nedenle, çoklu hedefe karşı güvenilir ve potansiyel karbonik anhidraz inhibitörlerinin araştırılması ve tasarlanması; hCA-I ve hCA-II, kanser tedavisi için ekibimizdeki biyolojik verileri kullanarak amacımız olmuştur.

Anahtar Sözcükler: Farmakofor, Karbonik anhidraz, HipHop, üre;tiyoüre

1. INTRODUCTION

Pharmacophore modeling is a successful yet very diverse subfield of computer-aided drug design. The pharmacophore definition has been widely performed to the rational drug design. In this thesis, we use the computational techniques to investigate potent carbonic anhydrases (CAs) inhibitors in the drug discovery process. Carbonic anhydrases are zinc containing metalloenzymes. These enzymes have different their subcellular localization, catalytic activity and susceptibility. The literatures indicated that the CA I and II levels were also higher in several cancer types, such as stomach, prostate, lung and ovary tumors. Additionally, CA II causes in some cancer tissues, like melanoma, renal cancers. Because of these, it is aimed to elucidate and design rational and lead carbonic anhydrase inhibitors against to each target; CA I and II based on the biological data from our team for treatment of cancer.

Computational methods including pharmacophore mapping, quantitative structure-activity relationship (QSAR) modeling, molecular docking and virtual screening (VS) have proven their usefulness in pharmaceutical investiation for the determination of new chemical entities [1]. The pharmacophore modeling involves ligand-based and structure-based was major areas in drug design.

These applications are useful because they rationalize a lot number of experimental data and resent for saving both time and cost in the drug process. Moreover, in silico applications are extremely economical than other application experimental and activity investigations.

2. GENERAL INFORMATION

Biological systems are the most complicate sort of systems we know and molecular recognition between ligands and their target macromolecules lie at the centre of many of the steps talking part in biological environmental. The quick increase in the number of targets with known three-dimensional structure has turn on the probability to find the proper binding modes of their natural ligands in their bindings, one of the keys towards intelligences the proteins function treatment. Protein doing is modulated by binding of a small molecule to active regions on the protein and the discomfort of such regulation is mostly a reason for a disease [2]. Hence, many medicines try by inhibiting the action of proteins with improved activity. Also, the suitable action of malfunctioning or non-functioning proteins causing a illness can sometimes be restored by the binding a certain chemical.

The development of a novel medicine is a highly costly, hard process; this part is named “lead detection” and outputs a ligand to be further optimized for an increased interest and particular. With increased interest and particular, the nominee medicine should also represent optimal pharmacokinetic properties, including its absorption, distribution and metabolism in the alive system, along with its extraction and fastener of toxicity. It is after all these canons are met that the drug enters diverse process of clinical testing before being confirm and marketed.

While finding a strong and high-interest lead ligand in a rapid and secure way is already defiance quite difficult to handle with as is, the major rise in the number of medicinal targets without known small molecule ligands made present in the flow “post-genome era” has made the exploration for a lead build even a bigger defiance [3]. In the part, most medicines were found either by definition of the real component from classical remedies, variance of native ligands or by serendipitous exploration. However, novel discovery touches are based on sensibility the molecular and physiological control mechanisms of the illness. In the big search for a lead compound, there are two important touches to facilitate these tangles: high-throughput (experimental) scanning [4] -in vitro testing- and computer aid level desing [5] – in silico testing- of big compound bookcases.

The operate by which a novel drug from identified to marketed is applied as the improving bond or “pipeline” and occur of a number of several state (Figure 2.1). It can be splitted into two big states: drug find and drug progress. The first state, drug exploration, can be further classified into three diverse steps: target discovery, lead estimation, and lead optimization. In

this state, a range of trials and works are planned to implemented initial certificate of a biologic target, as well as research for a only molecule to check the action.

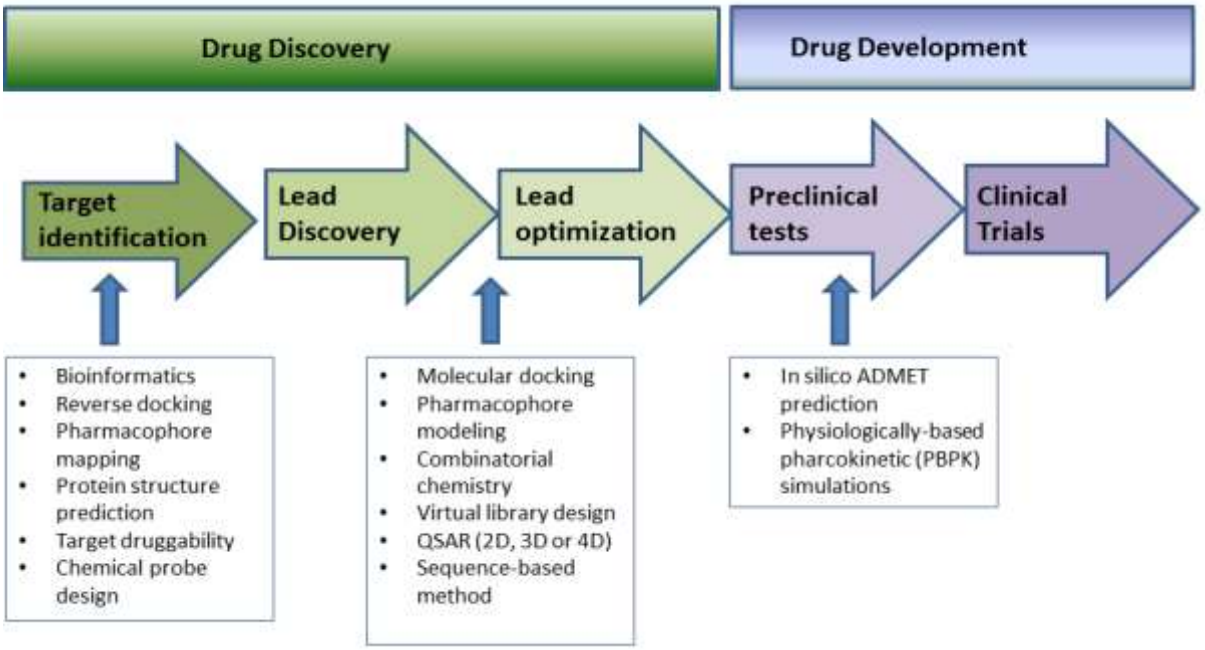


Figure 2. 1. Multiple computational drug discovery approaches have been applied in various stages of the drug discovery and development pipeline, including target identification, lead discovery and optimization, and preclinical tests.

CADD tools have been used in nearly every state, many exchanges the strategy and pipeline for drug find (Figure 2.1). Even though the classical practise of CADD is in lead find and optimization, today the practise spread in the sense of target certificate and approval, and forwards preclinical works, mostly through ADME/T prediction. In the medicine find and improving treat, CADD is usually performed for three important purposes: (1) screen great molecule bookcase into smaller sets of molecules, for experimentally test only the compounds with highest estimated activities; (2) catch up the optimization of lead molecules, in order to rise the linking care or optimize medicine metabolism and pharmacokinetic (DMPK) properties including ADME/T; (3) purpose new compounds, either by "adding/modifying" functional groups in early molecule or by connective together parts into new molecules.

An sample of enforce CADD in different states of the dmedicine find procedure is pictorial in Figure 2.2. We initiate from applying homology modelling to create a target, pursed by molecular dynamics (MD) simulations to optimize the target. Then the protein model is prepared for docking of compound bookcase to define possible binders [6].

Once the possible binders are identified, combinatorial chemistry can be used to generate a series of derivatives. However, if there is no target structure available, a QSAR pharmacophore can be generated based on ligand structure and activity information, where key pharmacophore features can be achieved for searching the same classes of binders to the target. Further, the DMPK properties of the binders, such as ADME/T can also be predicted by CADD tools and used to compare with bio-assay data. If a compound can pass all the steps above, then it becomes a drug candidate for the following clinical trials.

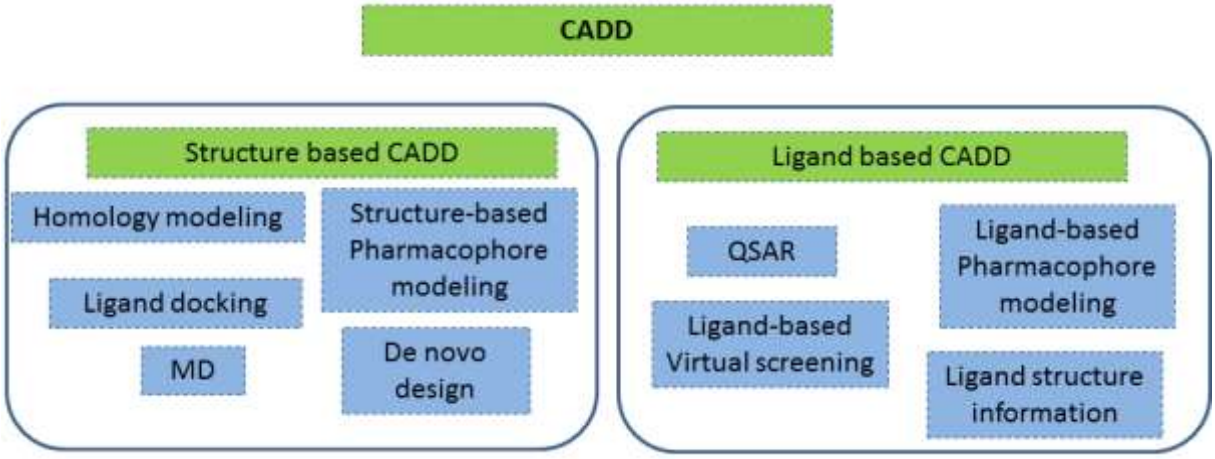


Figure 2. 2. CADD is classified into two groups based on the availability of target structure information.

2.1 Computer Aided Rational Design

Virtual screening is a computational method to scan large numbers of small molecules to see whether they bind to a target protein and function in the desires manner, using the available information about the target, binding mechanism or the known or hypothetical binding mode. It is a complementary approach to experimental discovery methods that aims to enhance and accelerate the lead discovery process. Drug discovery research for both hit identification and lead optimization has shifted towards computational methodologies, which are able to handle millions of molecules in a much shorter time compared to experimental techniques/approaches. The increase in the number of known protein structures and the enormous chemical space of conceivable small molecules has drawn particular attention to virtual screening techniques[7].

Even though virtual screening is a newly emerging approach, the advances in computer technology and methodology promoted its success, and there are already several drugs that were developed and optimized fully or partially with rational design techniques (Table 2.1)

Table 2. 1. List of drugs

Drug	**Target**	**Disease or Infection**
Dorzolamide	Carbonic Anhydrase	Glaucoma
Imatinib (Gleevec)	Tyrosine Kinase	Some types of cancer
Cimetidine	Histamine H2 Reseptor	Peptidic Ulcers
Zolpidem	GABAA Receptor	Insomnia, Brain Disorders
Zanamivir	Neuraminidase	Prophylaxis of influenza
Raltegravir	HIV Integrase	HIV Infection
Enfuvirtide	HIV Transmembrane Protein	HIV Infection

Drug discovery is such a difficult problem that every relevant technique has to utilized to its best advantage. All computational techniques may provide different strategies, useful insights, new suggestions for molecular structures to synthesize and cost-effective virtual analysis prior to synthesis. The strategy to be pursued in rational design strictly depends on the availability of the three-dimensional structure of the biological target. Therefore, computer-aided drug discovery CADD techniques can be grouped in classes: ligand-based and structure-based (often also called target or receptor-based) methods [8].

2.2 Ligand-Based Methods

Despite the increase in the number of proteins with known 3-D structure, there are still a fair number of drug targets without the structure information. However, if there is at least one known active ligand validated thought a cell culture assay for a target, ligand- based computational techniques that do not require the target structure and binding site geometry can be employed. The main idea followed in ligand-based drug design is that ligand structural similarity or similarity of steric and electrostatic features implies similar activity, which allows deriving the required properties for active molecules from the analysis of already known active ligand(s). While not requiring a target structure can be advantageous for ligand-based methods., with respect to structure-based methods, it can at the same time be a drawback not to be able to integrate ligand-target complementarity information in the drug design process[9]. On one hand ligand-based methods have low computational complexity; however, on the other hand they do not allow a chemically diverse set of results due to restriction by the known ligand(s). The ligand-based techniques range from rather simple similarity searches- usually applied if there is only one known active ligand to more sophisticated methods like pharmacophore modelling.

2.2.1 Ligand-Based Pharmacophore Modeling

In computer-aided drug design, one approach to distinguish potentially active from inactive compounds in a database of small molecules is to use the knowledge of the physical and chemical properties of the target binding site or set of known actives. Pharmacophores are ensembles of these physical and chemical features that are necessary for optimal interactions between a specific biological target and a ligand to enhance or inhibit the target function. The most common pharmacophore features include being aromatic, hydrophobic, hydrogen bond donor, hydrogen bond acceptor, an anion or a cation [10]. Thus, based on which pharmacophoric features are used, pharmacophore modeling approaches can be categorized in two, whether the structure properties of the target protein and/or the binding site are known, namely structure-based pharmacophore models, or a set of known active ligands which bind to the same region in the target protein are known, namely ligand-based pharmacophore modeling.

For ligand-based pharmacophore modeling, the information of the target protein or the binding site is to not be needed; the model can be created from a set of known actives. At the same time, it is crucial that all the known ligands should bind to the same region of the target protein. Ligand-based pharmacophore model creation is basically finding the common chemical and physical features of the known ligands to be used as a “quary” to search for molecules fitting the model in a small molecule database.

If there are no data available about binding conformation of the known ligands to the target protein, finding the active conformations may be quite challenging. In this instance, all the conformers of the ligands should be created and aligned to find the best alignment. The alignment can be done first by superimposing the most rigid compounds and then adjusting the remaining compounds accordingly with computational tools or manually [11].

To prevent any bias, the known molecules should preferably be derivatives of the different structures. Once the alignment is completed, the pharmacophore model can be generated via determining the features that are present in all the molecules. The model can then be used to search a database of molecules, resulting in a qualitative ranking based on how well the molecules fit the model.

2.3. Structure-Based Method

Structure-based methods (also called target or reseptor-based methods) use structural information about the target, e.g., crystal structure derived by NMR, or homology models.

The main assumptions of structure-based design is that good inhibitors must possess significant structural and chemical complementarity to their target receptor. In cases where the information about the target protein structure and/or the binding site is available, structure-based virtual screening techniques can be applied without any information about known active ligands.

2.3.1 Structure-Based Pharmacophore Modeling

The alternative to generating pharmacophore models from known ligands is to generate them from the target binding site. This method is preferred if there are not any or enough known ligand and also prevents any errors that might arise from the restrictions of a training set of known ligands. In structure-based pharmacophore modeling, it is crucial to examine the binding site intensively and deduce the interactions that play major roles in ligand binding and action mechanism of the target. Since the pharmacophore features are derived from the residues of the active site and the compounds whose pharmacophore features match the properties of the active target binding site are considered to be more active than the other compounds, the matching ligands should have the corresponding pharmacophore features. For instance, if a hydrogen bond donor feature is defined on a residue of the target binding site, then a corresponding hydrogen bond acceptor feature should be included in the pharmacophore model that is used for small molecule database search .

2.4. Carbonic Anhydrase

According to the last studies it has been proofed that the solid tumors extracellular pH is more acidic than the regular tissue. However, the intracellular pH is like normal cells or even a louse more basic. To regulate the Ph gradient between the intracellular and extracellular compartments the tumor cells secrete ion transort proteins, like, H+- ATPase, Cl-/HCO3- exhanger etc.[12]

Many tumours also express The CAs, the Zn(II) dependent enzymes catalyzing the hydration of carbon dioxide to from bicarbonate and a proton.

$$CO_2 + H_2O \longrightarrow H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Carbonic anhydrases are among the most studied members of a large family of metalloenzymes.

Carbonic anhydrases (CAs) are ubiquitous zinc-containing metalloenzymes reşated in the catalysis of a simple however necessary physiological reaction: the reversible hydration of carbon dioxide to bicarbonate ion and proton. These enzymes play a fundamental role in great importance physiological processes involved to respiration and acid-base regulation,

electrolyte secretion in a variety of tissues/organs, and biosynthetic reactions (e.g. gluconeogenesis, lipogenesis and ureagenesis). A few studies have demonstrated that aberrant levels or activities of CAs have been often consolidated with different human diseases. As a results, CA isozymes have been identified as potential drug targets for the design of inhibitors or activators with clinical administrations.

Inhibition of CAs has emerged as a promising approach for the treatment of a variety of disorders such as glaucoma, epilepsy, obesity, and cancer. Recent studies have also pointed out the importance of CAs for the design of anti-infective agents with a novel mechanism of action.

Among thiadiazole derivatives, acetazolamide (AAZ) (N-(5-sulfamoyl-1,3,4-thiadiazole-2-yl) acetamide), a potent carbonic anhydrase inhibitor, is used in the treatment of glaucoma, acute mountain sickness, epileptic seizures.15,24

On the other hand, furan-based hydrazone derivatives were also reported to exhibit notable inhibitory effects on hCA-I.

Prompted by the afore-mentioned findings and in the continuation of our ongoing research in the field of design, synthesis and biological evaluation of thiadiazole derivatives as hCA inhibitors,herein we reported the synthesis and inhibitory effects of a new series of N'-(5-arylfuran-2-yl)methylene-2-[(5-(phenylamino)-1,3,4-thiadiazol-2-yl)thio] acetohydrazide derivatives on hCA I and hCA II. Ultimately, all compounds were docked to the active sites of hCA-I and hCA-II with AAZ in order to speculate the possible binding modes of these molecules in the active sites of these enzymes.

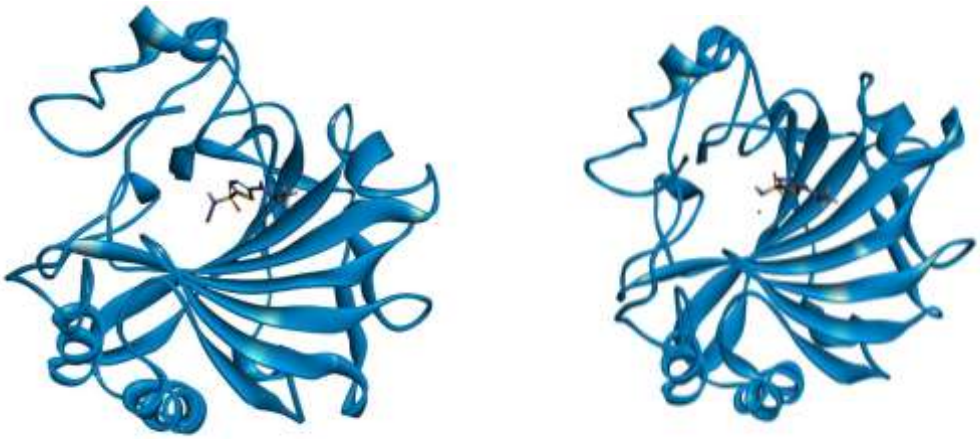


Figure 2. 3. The 3-D structure of hCA-I and hCA-II.

It must be noted that using this protocol can generate pharmacophores that are common to a set of active ligands and optionally it can add excluded volumes to the pharmacophore model by entering a series of inactive compounds. In fact, the purpose of a pharmacophore model is to provide a set of steric and electrostatic features that are vital for an optimal interaction with specific biological target.

3. MATERIALS AND METODS

In this thesis, we selected twenty five compounds having urea and thiourea framework. These compounds were synthesized and their biological activities were examined by our team. Beside their biological activity data, pharmacophore analyses were applied to define the optimal pharmacophore map to the human carbonic anhydrase I and human carbonic anhydrase II active sites. The 3D pharmacophore model was validated using a data set of 25 CAs (hCA-I and hC -II). The chemical structures of training dataset and dataset compounds along with their biological activity are presented in Table 3.1.

Firstly, binding sites of the targets were examined by using pharmacophore protocol in Discovery Studio 3.5. Then the selected ligands were analysed by using common feature pharmacophore subprotocol in DS 3.5. After then, fragment based design was performed to occur potential inhibitor(s) against to CAs. The all computational techniques were done with help of Discovery Studio 3.5.

In HipHop, conformational resilience of compounds is addressed by performing conformational analysis above pharmacophoric hypothesis generation and considering in turn each single conformer of all the compounds.

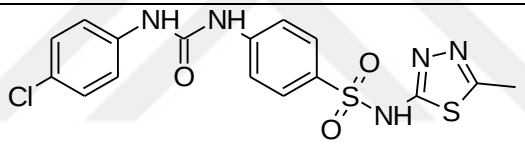
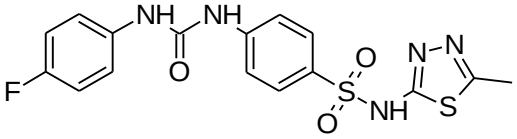
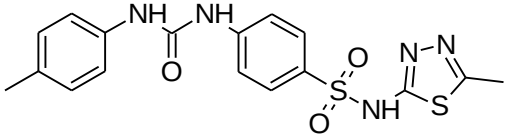
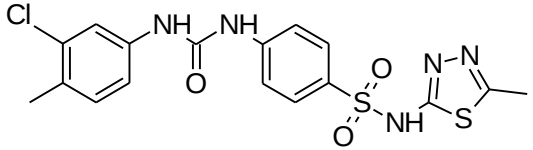
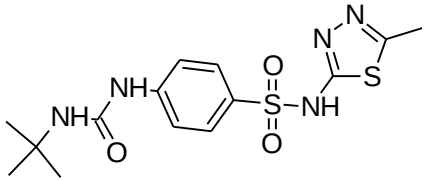
For each of the dataset compounds, a database was generated using the 'best' option and default structure generation parameters were elective. The HipHop pharmacophore map was based on the alignment of common features pharmacophore present in highly effective compounds.

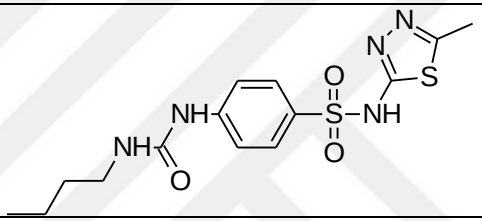
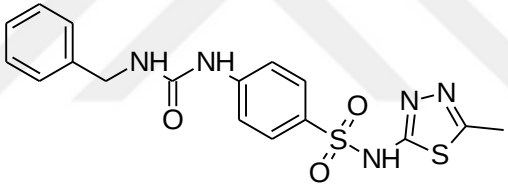
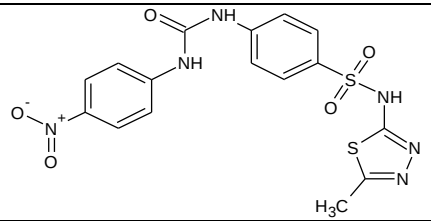
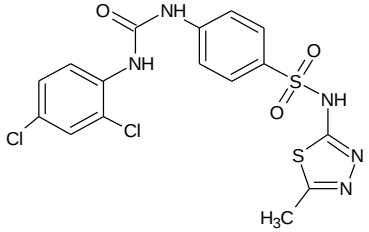
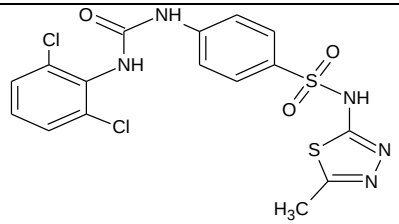
The hypotheses are ranked on the basis of the number of datas fitting the pharmacophore and the frequency of its occurrence. The pharmacophore of the mapping between a compound and a hypothesis is indicated by the pharmacophoric fit value.

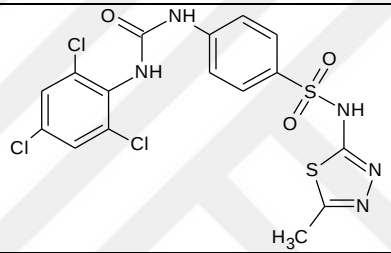
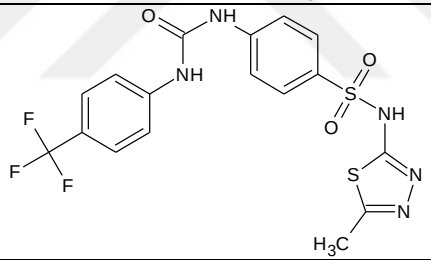
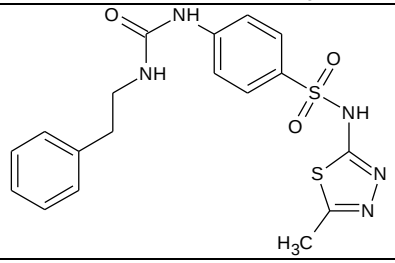
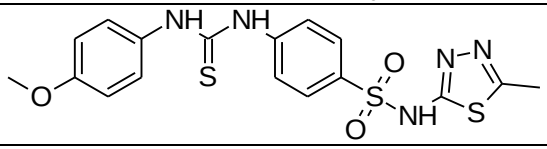
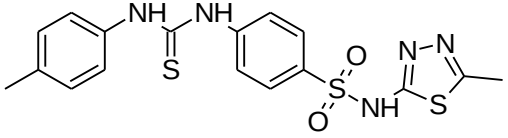
Present methodology of 3D HipHop pharmacophore model development was sufficient. While, in the ligand-based 3D pharmacophore model we could not achieve the bioactive conformation of the studied compounds in the absence of the experimental 3D structure of human carbonic anhydrases structure. So the structure generation protocol acknowledged by us in the existing ligand-based technique as explained above was proved.

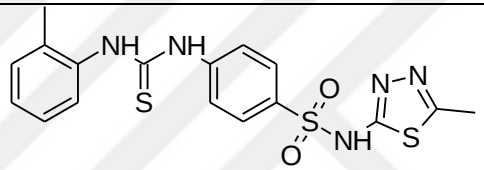
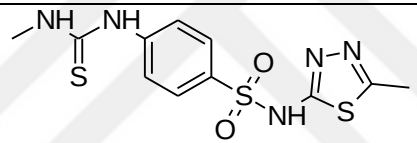
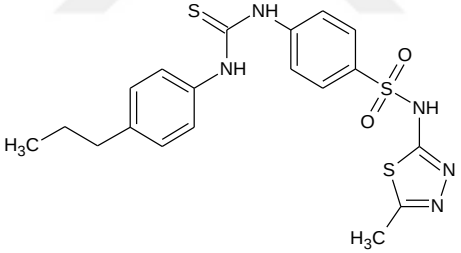
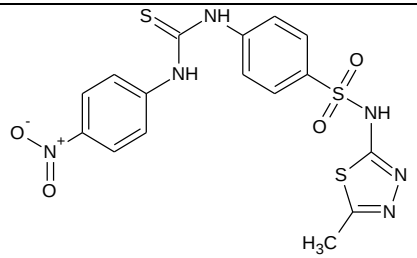
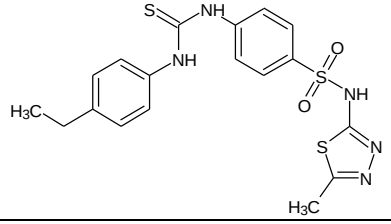
The obtained results were shown and evaluated in Results and Discussion part of the thesis.

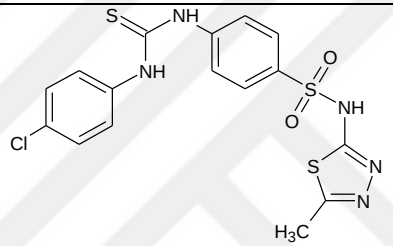
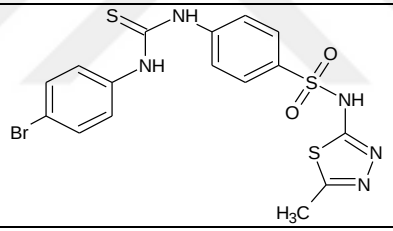
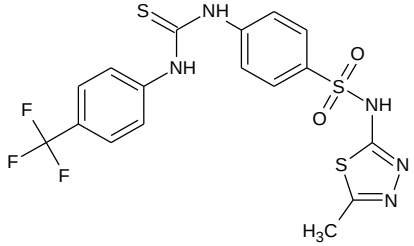
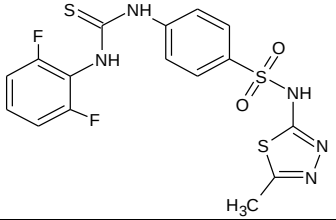
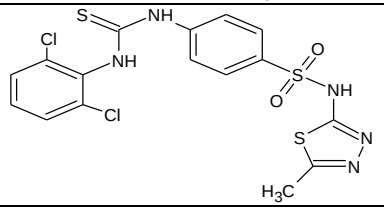
Table 3. 1. The chemical structure of different CAs along with its IC50

Yayında verilen kod	Açık Formülü	Kapalı Formülü	hCA-I Inhibition[a] *IC_{50} (nM)*	hCA-II Inhibition[b] *IC_{50} (nM)*
1		$C_{16}H_{14}ClN_5O_3S_2$	40.9	21.2
2		$C_{16}H_{14}FN_5O_3S_2$	6.16	3.4
3		$C_{17}H_{17}N_5O_3S_2$	7.02	4.32
4		$C_{17}H_{16}ClN_5O_3S_2$	2.19	-
5		$C_{14}H_{19}N_5O_3S_2$	10.25	1.65

6		$C_{14}H_{19}N_5O_3S_2$	6.08	1.696
7		$C_{17}H_{17}N_5O_3S_2$	2.88	0.45
8		$C_{16}H_{14}N_6O_5S_2$	2.37	6.71
9		$C_{16}H_{13}Cl_2N_5O_3S_2$	3.48	11.17
10		$C_{16}H_{13}Cl_2N_5O_3S_2$	4.99	9.34

11		$C_{16}H_{12}Cl_3N_5O_3S_2$	15.65	13.36
12		$C_{17}H_{14}F_3N_5O_3S_2$	6.44	17.95
13				
14		$C_{17}H_{17}N_5O_3S_3$	0,172	0,147
15		$C_{17}H_{17}N_5O_2S_3$	0,144	0,109

16		$C_{17}H_{17}N_5O_2S_3$	-	-
17		$C_{11}H_{13}N_5O_2S_3$	0,259	0,175
18		$C_{19}H_{21}N_5O_2S_3$	0.142	0.124
19		$C_{16}H_{14}N_6O_4S_3$	3.14	5.6
20		$C_{18}H_{19}N_5O_2S_3$	2.53	7.61

21		$C_{16}H_{14}ClN_5O_2S_3$	3.74	4.01
22		$C_{16}H_{14}BrN_5O_2S_3$	2.42	1.52
23		$C_{17}H_{14}F_3N_5O_2S_3$	0.095	0.057
24		$C_{16}H_{13}F_2N_5O_2S_3$	8.78	2.33
25		$C_{16}H_{13}Cl_2N_5O_2S_3$	6.68	8.37

4. RESULTS AND DISCUSSION

In order to determine the pharmacophore properties in the active regions of the target structures, the result of the pharmacophore model protocol was obtained as Figure 4.1. Following this, 25 new synthesized compounds have been assigned a common pharmacophore map. The results obtained for each target are given in Figures 4.2 and 4.3. To prove the accuracy of the pharmacophore models found, the reliability of the reference compound was proven by the best matching.

The correct representation of the 3D-chemical features and the appropriate sampling of the conformational space for the 3D pharmacophore mapping were performed. These include, hydrogen bond donors/acceptors, hydrophobic, hydrophobic aliphatic/aromatic, and charged centers, with the default definitions of the chemical features being customizable.

A maximum of five types of chemical features can be specified for 3D HipHop pharmacophore map generation. The number of appearances of a particular chemical feature was customizable for a minimum of zero and maximum of three respectively. In the HipHop pharmacophore map, the chemical features that have directionality (hydrogen bond donor and hydrogen bond acceptor) are described using two points. On the other hand, nondirectional features such as charged centers, ring aromatic features tures and aliphatic hydrophobic regions are represented by single points respectively. A maximum of five types of chemical features can be specified for 3D HipHop pharmacophore map generation. The number of appearances of a particular chemical feature was customizable for a minimum of zero and maximum of three respectively. In the HipHop pharmacophore map, the chemical features that have directionality (hydrogen bond donor and hydrogen bond acceptor) are described using two points. On the other hand, nondirectional features such as charged centers, ring aromatic features tures and aliphatic hydrophobic regions are represented by single points respectively.

Structures 1–25 were investigate for their in vitro inhibitory effects on human CA-I and human CA-II and IC50 values were calculated for all datas by Marmamara University departmen of Pharmacology studies team. (Table 3.1). The reference compound was selected AAZ.

All training dataset compounds (CAs) were taken as the reference compound (AAZ) by allotting each of them ‘‘Principal” value of 2 and ‘‘MaxOmitFeat” value of 0. This was to ensure that all chemical features present in them will be captured while generating 3D pharmacophoric hypotheses, and is presented in Table 4.1.

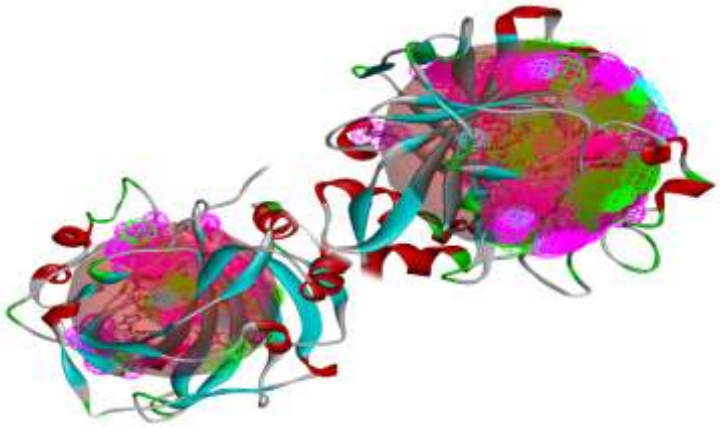


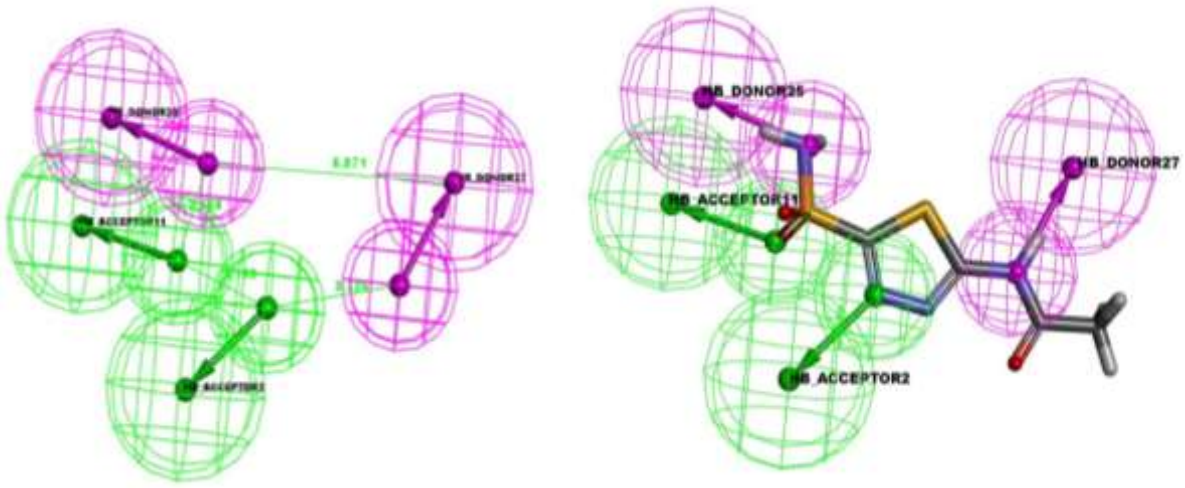
Figure 4. 1. Pharmacophore features of active sites for hCA-I andhCA-II.

Table 4. 1. The 3D pharmacophore modeling results in hCA-I

Name	Fitvalue	Principal	MaxOmitFeat	HB_Acceptor	HB_Acceptor2	HB_Donor25	HB_Donor27	Pharmprint
AAZ	3.9870	2	0	1	1	1	1	'1111'
6	2.4537	2	0	1	1	1	1	'1111'
12	2.4533	2	0	1	1	1	1	'1111'
9	2.4530	1	1	1	1	1	1	'1111'
2	2.4529	2	0	1	1	1	1	'1111'
13	2.4527	0	2	1	1	1	1	'1111'
5	2.4526	0	2	1	1	1	1	'1111'
1	2.4525	0	2	1	1	1	1	'1111'
24	2.4525	0	2	1	1	1	1	'1111'
16	2.4523	0	2	1	1	1	1	'1111'
18	2.4523	0	2	1	1	1	1	'1111'
4	2.4523	1	1	1	1	1	1	'1111'
10	2.4523	1	1	1	1	1	1	'1111'
11	2.4522	0	2	1	1	1	1	'1111'
7	2.4521	1	1	1	1	1	1	'1111'
20	2.4520	1	1	1	1	1	1	'1111'
17	2.4520	0	2	1	1	1	1	'1111'
8	2.4516	1	1	1	1	1	1	'1111'
19	2.4515	1	1	1	1	1	1	'1111'
3	2.4514	0	2	1	1	1	1	'1111'
14	2.4504	0	2	1	1	1	1	'1111'
15	2.4499	0	2	1	1	1	1	'1111'
21	2.4334	1	1	1	1	1	1	'1111'
22	2.4135	1	1	1	1	1	1	'1111'
23	2.3860	0	2	1	1	1	1	'1111'
25	2.3641	2	0	1	1	1	1	'1111'

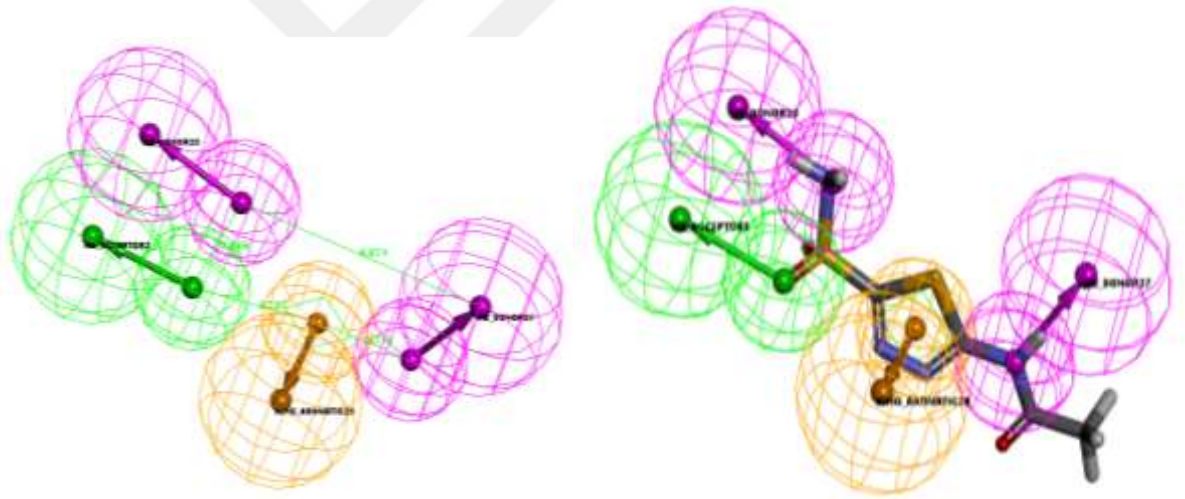
Table 4. 2. The 3D pharmacophore modeling results in hCA-II.

Name	Fitvalue	Principal	MaxOmitFeat	HB_Acceptor3	HB_Donor25	HB_Donor27	Ring_Aromatic28	Pharmprint
AAZ	3.9870	2	0	1	1	1	1	'1111'
8	3.7880	2	0	1	1	1	1	'1111'
2	3.7853	0	2	1	1	1	1	'1111'
4	3.7631	0	2	1	1	1	1	'1111'
1	3.7618	0	2	1	1	1	1	'1111'
5	3.7602	0	2	1	1	1	1	'1111'
17	3.7569	0	2	1	1	1	1	'1111'
20	3.7552	2	0	1	1	1	1	'1111'
14	3.7531	0	2	1	1	1	1	'1111'
18	3.7503	0	2	1	1	1	1	'1111'
9	3.7488	0	2	1	1	1	1	'1111'
6	3.7481	0	2	1	1	1	1	'1111'
3	3.7469	1	1	1	1	1	1	'1111'
13	3.7455	0	2	1	1	1	1	'1111'
11	3.7423	0	2	1	1	1	1	'1111'
10	3.7339	1	1	1	1	1	1	'1111'
7	3.7301	0	2	1	1	1	1	'1111'
12	3.7284	0	2	1	1	1	1	'1111'
16	3.7169	0	2	1	1	1	1	'1111'
15	3.7072	0	2	1	1	1	1	'1111'
24	3.7067	0	2	1	1	1	1	'1111'
19	3.6974	2	0	1	1	1	1	'1111'
21	3.6914	1	1	1	1	1	1	'1111'
22	3.6711	0	2	1	1	1	1	'1111'
23	3.5451	0	2	1	1	1	1	'1111'
25	3.4370	2	0	1	1	1	1	'1111'



(a) (b)

Figure 4. 2. The best pharmacophore model for hCA-I.



(a) (b)

Figure 4. 3.The best pharmacophore model for hCA-II.

In this study, we generated 10 hypotheses from 25 ligands to obtain the best common feature for carbonic anhydrases inhibitors. The best pharmacophore model for hCA-I is composed of the feature which are 2 hyrogen bond acceptor, 2 hyrogen donor. The other human CAs the obtained pharmacophore model including 1 hyrogen bond acceptor, 2 hyrogen donor and 1 ring aromatic, [Figure 4.2(a) and Figure 4.3(a)]. In the meantime, these data were validated by using ligand pharmacophore mapping subprotocol of DS. Additionally, referance ligans, AAZ

(5-acetamido-1,3,4-thiadiazole-2-sulfonamide) is used to evaluate the calculated results, as given in Figure 4.2(b) and Figure 4.3(b)

5. CONCLUSIONS

The obtained pharmacophore models for each human CAs is going to be illuminated and help for future done experimental and clinical studies. To design of potent human CAs inhibitors will be done and applied by using in this pharmacophore analysis.

3D Pharmacophore model development of CAs was developed using a HipHop module in Discovery software [15]. In silico techniques offer attractive advantages over experimental methods, especially for membrane/channel receptors. These are difficult to purify/ crystallize for solving the 3D structure of T-type channel receptor using either X-ray or NMR techniques.

So, instead of structure-based modeling study, ligand-based pharmacophore modeling approach was adopted in the present study.

3D model should be robust and significant enough to distinguish between selective and non-selective CAs.In the present study, various ligand-based 3D pharmacophoric strategies were adopted by us to develop HipHop pharmacophore models using different CAs. Multi-target inhibitors for CA I and II enzymes are going to be experimentally designed and studied to treatment of cancer.

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RESUME

Nur Gürel was born in 1991 in Gaziantep. She completed his primary, secondary and high school education in Gaziantep. She graduated from Mersin University, Faculty of Engineering, Department of Chemical Engineering. In 2016, she started her master's degree in Gaziantep University, Institute of Health Sciences, Department of Bioinformatics and Scientific Biology.