Journal of Pharmaceutical Research International

32(29): 84-91, 2020; Article no.JPRI.61677
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Discovery of Carbonic Anhydrase Inhibitors through Molecular Docking as Novel Anticancer Agents

Ahmed Alharbi[1*], Waleed Mansi Alshammari[2] and Turki A. K. Alreshidi[3]

[1]Department of Clinical Laboratory Scinces, Colloge of Applied Medical Sciences, University of Hail, Hail, Kingdom of Saudi Arabia.
[2]King Khaled Hospital, Ministry of Health, Hail, Kingdom of Saudi Arabia.
[3]Hail Regional Laboratory, Ministry of Health, Hail, Kingdom of Saudi Arabia.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i2930886
Editor(s):
(1) Dr. Mohamed Fathy, Assiut University, Egypt.
Reviewers:
(1) K. Hemalatha, Mother Theresa Post Graduate & Research Institute of Health Sciences, India.
(2) Ana Mayra Pereira da Silva, Federal University of Mato Grosso, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61677

Original Research Article

Received 02 August 2020
Accepted 07 October 2020
Published 20 November 2020

ABSTRACT

The cancer is the world's most silent and life-threatening diseases, which may arise in most common people without any indication at any age and result in uncontrolled growth and metastasis. In the current manuscript, we targeted the discovery of novel carbonic anhydrase IX inhibitors. The discovery is based on computational techniques based on direct and indirect drug design. We screened nearly 500000 compounds from the zinc database to identify the top 1000 compounds with indirect drug design techniques while the top 200 were docked for the interactions and scoring functions. The top 12 out of these were reported in the manuscript, which showed higher binding scores than the standard compounds with selectivity based on interaction. These leads may be the future drugs for anticancer agents through carbonic anhydrase inhibitors.

Keywords: Cancer; carbonic anhydrase inhibitors; virtual screening.

**Corresponding author: E-mail: am.alharbi@uoh.edu.sa;*

1. BACKGROUND

Cancer is one of the world's most life-threatening illnesses, which contains uncontrolled growth and metastasis. The essential factors that cause multiple cancers are dysfunctional oncogenes, microRNA genes, and tumor suppressor genes [1]. Cancer is the world's second-largest cause of death, causing an estimated 9.6 million deaths in 2018, or one in six [2]. The most common types of cancer in men are lung, prostate, colorectal, stomach and liver cancer, while breast, colorectal, lung, cervical and thyroid cancers are most common in women [https://www.who.int/health-topics/cancer].

Uncontrolled proliferation is recognized in most cancer cells as the key enzymes and proteins that regulate cell division and development are deregulated [3]. Many chemotherapy drugs affect different cancer cells, but toxicity, poor selectivity, and poor tolerance indicate the need for new cancer agents with improved cytotoxicity and lower adverse reactions. Human carbonic anhydrases (hCAs) belong to the family of carbonic anhydrases, and these hCAs have 16 isoforms in turn. CA (I, II, III, VII and XIII) are cytosolic, CA (IV, IX, XII, XIV and XV) are transmembrane bound, CA Va and Vb are mitochondrial, and CA VI are salivary and colostrum-secreted. CA VIII, X and XI are catalytically inactive isoforms called CA-related (CARPs) proteins [4,5]. hCA IX and XII are over-expressed in cancer cells since they are tumor-associated, transmembrane-bound enzymes, often hypoxic tumors, low in normal cells. Besides, overexpression leads to the development of cancer cells, proliferation, angiogenesis and metastases [6,7]. An anticancer agent can selectively inhibit tumor-associated hCAs IX and XII over cytosolic CAs such as hCA I and II to exhibit potent cytotoxicity with no adverse effects. The development of new anticancer agents targeting tumor-associated hCA isozymes (hCA IX and XII) will also be an effective and successful technique for cancer therapy [8]. Coumarin (2H-chromen-2-one) is benzene and 2-pyrone fused heterocyclic ring structure. It belongs to the plant secondary metabolite family Neo flavonoids. The coumarins are capable of generating non-covalent interactions. Hydrogen bonding, hydrophobic, electrostatic, metal coordination, van der Waals power, etc. with different active protein and enzyme sites show a wide range of pharmacological activities such as antimicrobial, anti-inflammatory, anti-tuberculosis, anti-hyperlipid, antiviral, antidepressant, anti-oxidant, anti-cancer, etc. [9,10]. There is a heterocycle of

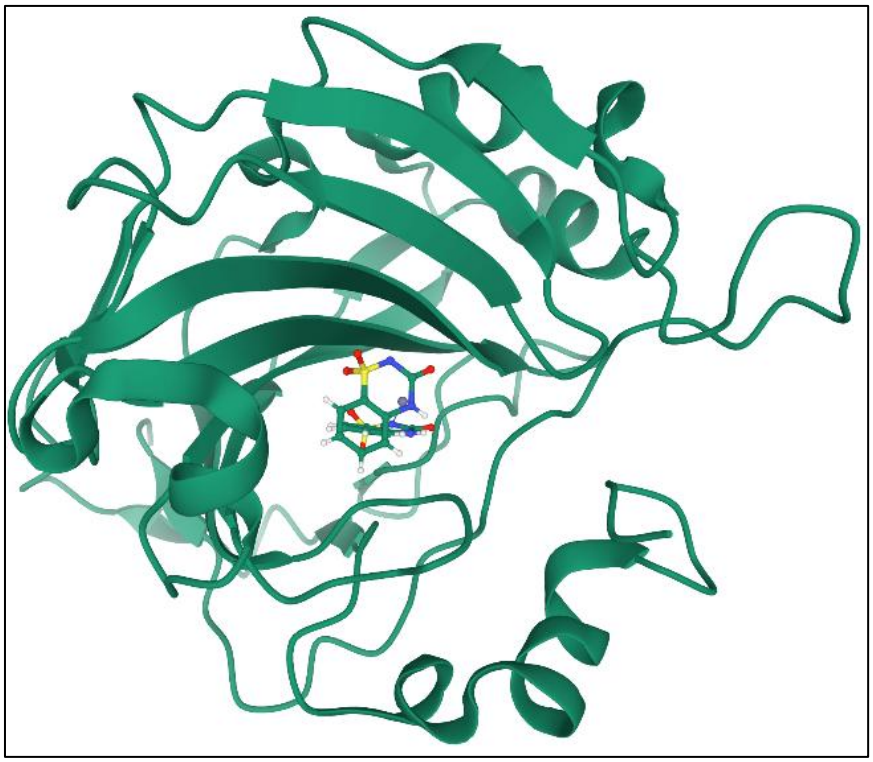


Fig. 1. 3D structure of Carbonic anhydrase 9 (CAIX) in complex with SB4197

coumarin in a variety of well-established prescription drugs with different therapeutic activities [11]. Crystal structure of CAIX in complex with SB4197 is available in RCSB protein databankwith PDB ID 6U4T [12]. Maresca, A & Supuran CT. et al., stated that coumarins (1) were a new class of non-zinc-mediated carbonic anhydrase inhibitors and proposed that the coumarin hydrolysis product, a derivative of cis-2-hydroxy-cinnamic acid, but not the coumarin moiety bound in the active site of the enzyme. Umbelliferone (7-hydox coumarin, 2) and its derivatives were also identified by the same research group as selective inhibitors of IX and/or XII over CA I and CA II [13]. Aikaterini P &Supuran CT. et al., have reported a novel 6- and 7-substituted coumarins (3) as selective tumor-associated CA IX and XII inhibitors [14].

In the current scenario where the potent drugs with protein specificity are to be discovered for the target. In this manuscript, we targeted CAIX to find possible leads for the target through our state-of-the-art techniques in drug design and discovery through direct and indirect drug design approaches. The hierarchical protocol for virtual screening was used as per the reported articles [15-17].

2. MATERIALS AND METHODS

2.1 Molecular Docking

In Molegro Virtual Docker (MVD) program, molecular docking was performed using the MolDock module [14] MolDock 's scoring method for molecular docking is based on piecewise linear potentials (PLPs) [15] using protein with PDB ID 6U4T. A reclassification process has been applied to the top-rated poses to improve the accuracy of docking. For this analysis, the search algorithm 'MolDock SE' was used and population size of 50 and a maximum number of iterations of 1500 were set as parameters. Other parameters were kept as default with the number of runs as 10. Since MVD is based on an evolutionary algorithm, repeated docking runs do not lead to exactly the same poses and interactions. To resolve this inherent arbitrariness, ten successive runs were carried out and the best three poses were used to imagine further interactions as previously stated by us.

2.2 Database Preparation

The database of compounds was prepared using the Chemdraw Ultra 12 and minimizing the molecules in sdf format using the online minimization tools for computational studies. The database of ~500000 compounds from the NCI database was prepared and minimized for the docking experiments using the state of the art techniques of indirect drug design approach not reported in this manuscript. The top 200 compounds were used in direct drug design using Molegro Virtual Docker. The prepared database was added with standard ligands for further verification of the docking protocol.

2.3 Docking Validation

The validation of the docking was carried out using the co-complexed ligand re-docking to compare the docking poses as well as docking scores. The SB4197 was redocked in the binding site of CAIX to observe the pose as well as hydrophobic, electrostatic and steric interactions.

3. RESULTS AND DISCUSSION

3.1 Molecular Docking

The molecular docking was conducted using the MVD 6.0 software and its standard docking protocol was followed [16-18]. The binding site was defined 15A around the co-crystal ligand. The amino acids viz. His94, His96, Leu198, Val207, Val147, Thr200, Thr199 were concentrated as the center of interactions for the database ligands. The internal standard ligands viz. SB4197 and SLC0111 were analyzed for their interactions as well as their reported pi-pi stackings.

3.2 Database Screening

The database screening of the prepared molecules in .sdf format in the template generated pharmacophore query efavirenz from 6U4T gives rise to several unknown compounds that have not yet documented for anti-HIV activity were predicted active. The structures of some of them are shown in Table 1. The preliminary filtration by Lipinski's rule of five resulted in the selection of the top 200 hits from Zinc databases. The *SB4197 and SLC0111* (Fig. 4) were docked as reference ligand in the binding site of 6U4T using Molegro docking protocols. The Moldock score and rerank scores were employed for analysis of the various scores.

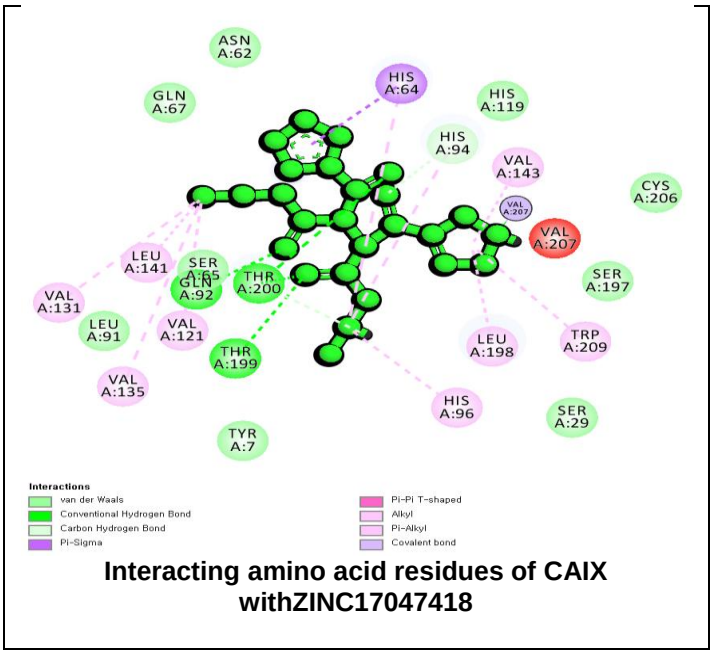
The docking reveals the fact that the Inhibitor binding was stabilized by the formation of

hydrogen bonds between the inhibitor and the Thr199 side chain hydroxyl in addition to inhibitor sulfonamide and T199 backbone amide. It was also observed that the binding was supported by VDW interactions with active site residues such as Val121, Val131, Val135, Leu141, Val143, Leu198, Thr200, Pro202.

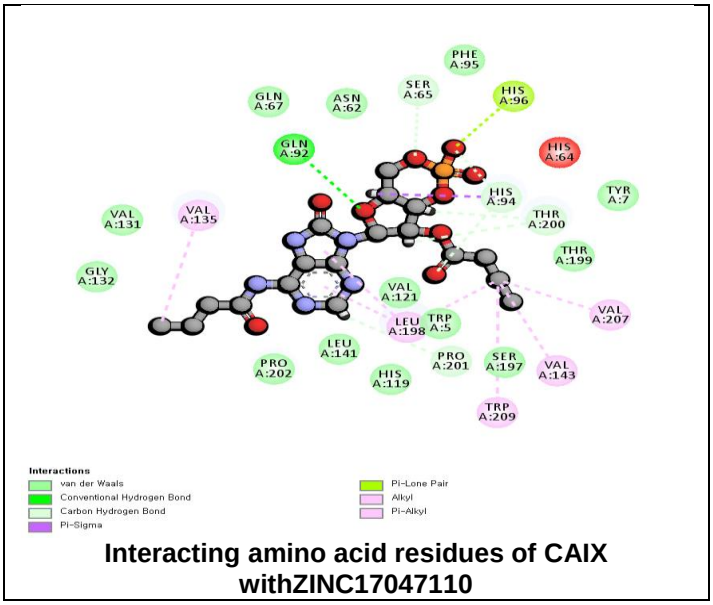
The CAIX has a wider hydrophobic pocket, which is packed with substrates and several inhibitors due to residue Val131. The lower steric hindrance to this residue when the active site is opened allows it possible to bind bulkier aromatic compounds, such as those which have been found in these studies and recorded in the MS. Strong compatibility with the hydrophobicity of substitutions has been found. The presence of halogen further increases the amount or frequency of VDW and hydrophobic interactions with the active site debris around the site.

The standard ligands bound with the protein showed all the above-mentioned interactions. The validated model was then used to screen the database of new chemical entities for the targeted proteins. The top identified compounds from the screening were analyzed for the interactions at the binding site. The major interactions of the screened ligands were found with *His94, His96, Val147, Leu198, Thr199, Thr200, Val207* (Figs. 2 and 3). Similarly, the leads screened from the dataset were docked in the same binding site using both GOLD and Molegro docking protocols. Table 1 enlists the ligands retrieved after the docking along with their GOLD, Moldock scores, binding affinity, and mapping scores with the pharmacophore model, respectively. The top 12 leads with higher Gold score and Moldock scores than the reference was identified. All the selected ligands show important binding interactions with the Thr199 and Thr200. The docking analysis clearly shows the important interaction of identified leads withinthe hydrophobic pocket and bound the enzyme in a mode comparable to standard ligands.

The core of both structures involves energetically stable conformation in the binding site and the hydrophobic and electrostatic Val135, Val143, Leu198, Val-207, Trp209 interactions due to carbonyl oxygen in the hydrophobic side chain of the identified lead. The better scores in terms of docking for these ligands were due to additional interactions of leads which tend to stabilize binding addition to important core interactions. Thus, these potential leads comprising important pharmacophore features required for selective reverse transcriptase inhibition. The comparable docking figures showed the interactions of reference compound and top screened lead with important interactions and the respective binding scores in terms of Moldock score are presented in Table 1. The physicochemical properties and ADME analysis performed by SwissADMEwebtools is depicted in Table 3.

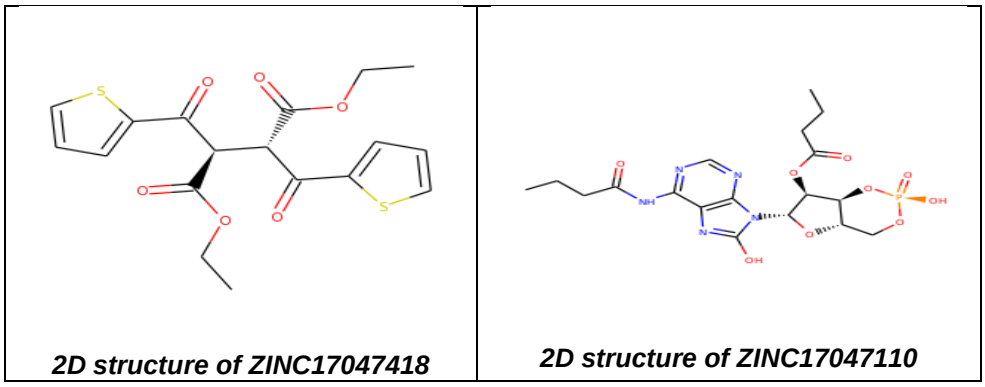


Interacting amino acid residues of CAIX withZINC17047418



Interacting amino acid residues of CAIX withZINC17047110

Fig. 2. Interacting amino acid residues of CAIX with top-ranked compounds



2D structure of ZINC17047418 ***2D structure of ZINC17047110***

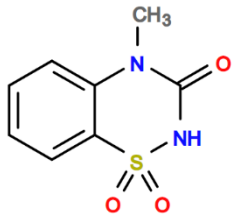
Fig. 3. 2D structure of top tanked compounds

Table 1. Identified leads from the virtual screening protocol

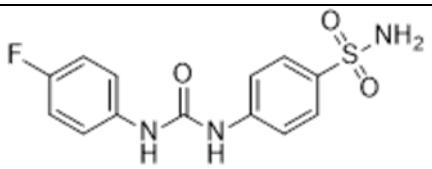
Ligand	MolDock score	Rerank score
ZINC01617871	-131.675	-105.023
ZINC01617872	-117.866	-95.6381
ZINC01762554	-142.621	-103.56
ZINC01762878	-133.309	-102.66
ZINC17046863	-129.66	-74.5905
ZINC17046870	-130.32	-102.909
ZINC17046908	-101.678	-89.5864
ZINC17047110	**-136.654**	**-107.691**
ZINC17047112	-93.3434	-83.4629
ZINC17047116	-108.115	-95.5304
ZINC17047118	-110.743	-93.7983
ZINC17047418	**-135.444**	**-106.036**
SB4197	**-82.6339**	**-64.2225**
SLC0111	**-116.309**	**-93.6411**

Table 2. Physicochemical properties and ADME of top screened compounds

Molecule	MW	GI absorption	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	log Kp (cm/s)	Lipinski violations	Leadlikeness violations	Synthetic accessibility
ZINC01617871	282.25	High	No	No	No	No	No	No	-7.62	0	1	2.42
ZINC01617872	224.21	High	No	No	No	No	No	No	-6.67	0	1	1.83
ZINC01762554	298.26	Low	No	No	No	No	No	No	-9.48	1	0	4.25
ZINC01762878	314.32	Low	No	No	No	No	No	No	-9.2	0	0	4.21
ZINC17046863	430.48	Low	No	No	No	No	No	No	-8.13	0	1	5.02
ZINC17046870	339.35	Low	No	No	No	No	No	No	-8.84	1	0	4.6
ZINC17046908	344.32	High	No	No	Yes	No	No	No	-8.61	0	0	4.23
ZINC17047110	485.39	Low	No	Yes	No	No	No	Yes	-10.13	1	2	5.13
ZINC17047112	415.3	Low	No	No	No	No	No	No	-10.7	1	1	4.68
ZINC17047116	415.3	Low	No	No	No	No	No	No	-10.7	1	1	4.68
ZINC17047118	415.3	Low	No	No	No	No	No	No	-10.7	1	1	4.68
ZINC17047418	394.46	Low	No	No	Yes	Yes	Yes	No	-5.97	0	3	3.85



SB4197 (4-methyl-1lambda~6~,2,4-benzothiadiazine-1,1,3(2H,4H)-trione)



SLC0111 (4-Hydroxy-3-(3-(phenylureido)benzenesulfonamides)

Fig. 4. Structure of SB4197and SLC0111

4. CONCLUSION

The carbonic anhydrase IX played an important role in cancer treatment. In this study, we identified about 200 novel leads for the target and out of which the top 12 molecules were prioritized based on their indirect drug design studies validated through docking experiments. These leads were verified for their affinity at the targeted protein and validated for their interactions at the binding site. The identified leads have higher binding scores in terms of moldock and rerank scores and as compared to literature, these leads may be the future molecules for the treatment of cancer through carbonic anhydrase inhibitors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Croce CM. Oncogenes and cancer. N. Engl. J. Med. 2008;358:502–511.
2. New Global Cancer Data: GLOBOCAN; 2018. Available:https://www.uicc.org/new-globalcancer-data-globocan-2018
3. Mareel M, Leroy A. Clinical, cellular and molecular aspects of cancer invasion. Physiol. Rev. 2003;83:337–376.
4. Supuran CT. Carbonic anhydrases: Novel therapeutic applications for inhibitors and activators. Nat. Rev. Drug Discovery. 2008;7:168–181.
5. Alterio V, Di Fiore A, D'Ambrosio K, Supuran CT, De Simone G. Multiple binding modes of inhibitors to carbonic anhydrases: How to design specific drugs targeting 15 different isoforms? Chem. Rev. 2012;112:4421–4468.
6. Supuran CT, Alterio V, Di Fiore A, Ambrosio KD, Carta F, Monti SM, De Simone G. Inhibition of carbonic anhydrase IX targets primary tumors, metastases and cancer stem cells: Three for the price of one. Med. Res. Rev. 2018;38:1799–1836.
7. Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, Sibtain A, Wilson GD, Turley H, Talks KL, Maxwell PH, Pugh CW, Ratcliffe PJ, Harris AL. Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer Res. 2000;60:7075–7083.
8. Ivanov S, Liao SY, Ivanova A, Danilkovitch-Miagkova A, Tarasova N, Weirich G, Merrill MJ, Proescholdt MA, Oldfield EH, Lee J, Zavada J, Waheed A, Sly W, Lerman MI, Stanbridge EJ. Expression of hypoxia-inducible cell-surface transmembrane carbonic anhydrases in human cancer. Am. J. Pathol. 2001;158:905–919.
9. Peng XM, Damu GLV, Zhou CH. Current developments of coumarin compounds in medicinal chemistry. Curr. Pharm. Des. 2013;19:3884–3930.
10. Thakur A, Singla R, Jaitak V. Coumarins as anticancer agents: A review on synthetic strategies, mechanism of action and SAR studies. Eur. J. Med. Chem. 2015;101:476–495.
11. Emami S, Dadashpour S. Current developments of coumarin-based anti-cancer agents in medicinal chemistry. Eur. J. Med. Chem. 2015;102:611–630.

12. Bua Silvia, et al. A sweet combination: Developing saccharin and acesulfame K structures for selectively targeting the tumor-associated carbonic anhydrases IX and XII. Journal of Medicinal Chemistry. 2020;63(1):321-333.
DOI: 10.1021/acs.jmedchem.9b01669
13. Maresca A, Supuran CT. Coumarins incorporating hydroxy- and chloro-moieties selectively inhibit the transmembrane, tumor-associated carbonic anhydraseisoforms IX and XII over the cytosolic ones I and II. Bioorg. Med. Chem. Lett. 2010;20:4511–4514.
14. Thomsen R, Christensen MH. MolDock: A new technique for high-accuracy molecular docking. J Med Chem. 2006;49:3315-3321.
15. Gehlhaar DK, Bouzida D, Rejto PA. Fully automated and rapid flexible docking of inhibitors covalently bound to serine proteases. International Conference on Evolutionary Programming, City; Springer. 1998;449-461.
16. Ahmad Khurshid, et al. Targeting Caspase 8: Using structural and ligand-based approaches to identify potential leads for the treatment of multi-neurodegenerative diseases. Molecules (Basel, Switzerland). 2019;24(9);1827.
DOI: 10.3390/molecules24091827
17. Saeed Mohd, et al. Predicted binding of certain antifilarial compounds with glutathione-S-transferase of human Filariids. Bioinformation. 2013;9(5):233-7.
DOI: 10.6026/97320630009233
18. Balaramnavar VM, et al. Eur J Med Chem. 2014;87:578.
PMID: 25299681.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61677