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In silico Identification of Novel Potential BACE-1 Inhibitors for Alzheimer's Disease Treatment: Molecular Docking, Pharmacophore Modeling and Activity and Synthetic Accessibility Predictions

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Authors' contributions

This investigation was performed in collaboration with all authors. Authors AAP, KRS, AESS and LISH designed the study, wrote the protocol, involved in writing the first draft, participated in data collection. Authors AAP, FSB and LISH managed the literature search, analyses of the study and manuscript preparation. Authors CHTPS, LISH and CBRS performed data interpretation and were actively involved in reading the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Alzheimer's Disease (AD) is a progressive neurodegenerative disease accompanied by loss of memory and cognition. With its causes still unknown, one of the main hypotheses related to its pathogenesis is the amyloidal, where the abnormal metabolism of amyloid precursor protein (APP), in this case cleaved by β-secretase enzyme (BACE-1), generates sAPPβ, subsequent action of β-secretase generates β-amyloid. This gives the β-secretase importance as a therapeutic target of

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AD, since their inhibition can control the onset and progression of the disease. This work intends to propose three new compounds with inhibitory activity for BACE-1 that may be potential drug candidates for AD treatment.
Place and Duration of Study: Laboratory of Modeling and Computational Chemistry (LMCC) at Federal University of Amapá (UNIFAP), Macapá, Brazil, between January 2014 and February 2015.
Methodology: First, we selected a group of inhibitors deposited in the BindingDB database as well as a crystallographic protein solved in the Protein Data Bank (PDB). Then we performed a prediction of ligand binding sites of BACE-1. To propose the binding mode of the inhibitor with the enzyme, molecular docking and molecular interactions analyses were performed. New proposals with potential inhibitory activity of BACE-1 in addition to a pharmacophore perception calculation as well as biological activity and synthetic accessibility predictions were made.
Results: A group of 40 inhibitors was selected from the database BindingDB, which were submitted to molecular docking simulation (for verification of the possible binding modes with the biological target), when analyzing the results of molecular docking, the hydrogen bond proved dominant over the others (approximately 74%). For pharmacophore perception calculation, the following characteristics were observed: a hydrophobic group, three aromatic groups, three donors and seven hydrogen bond acceptors. The target protein had its regions of the binding site predicted, and the most likely ligand binding site agree with the one already reported in the literature as the catalytic region of BACE-1. This allowed us to model three proposals that, in turn, had their predicted biological activities for BACE-1 as well as their synthetic accessibility.
Conclusion: Results showed that the proposals are promising BACE-1 inhibitors, with suitable drug-like properties, for future AD tretament.

Keywords: Alzheimer's disease; BACE-1 inhibitors; in silico drug design; molecular docking; pharmacophore derivation; prediction of activity; synthetic accessibility.

1. INTRODUCTION

As a rule, there is no replacement of dead neurons in the adult central nervous system (CNS), or neurons terminals capable to regenerate when their axons are interrupted. Thus, a pathologic process that causes neuronal degeneration has, in general, irreversible consequences [1].

In the context, one finds Alzheimer's Disease (AD), which presents itself as the major cause of cognitive decline in adults, particularly in the elderly. AD can be seen as a progressive neurodegenerative disease which is accompanied by loss of memory and cognition [2,3]. The AD causes remain unknown but characteristic histopathological changes are observable, such as: (a) The formation of senile plaques, also known as neuritic plaques, deriving from abnormal metabolism of amyloid precursor protein (APP); and (b) neurofibrillary tangles, formed from the collapse of neuronal cytoskeleton due to hyperphosphorylation of tau protein [2,4].

A possible therapeutic target for AD is the β-secretase (BACE-1), which represents the first protease cleaved in the APP degradation process, leading to production of β-amyloid (βA). BACE-1 is a 501 amino acid protein that contains an N-terminal of 21 amino acids followed by a proprotein domain comprising amino acids 22-45. The luminal domain of the protein is along the residues 46-460 and followed by a transmembrane domain of 17 residues and 24 amino acids residues forming a short cytosolic tail [5].

Thus, the BACE-1 enzyme is important in drug discovery as a first therapeutic target for AD. This occurs because, reduces the β-amyloid production, which become a promising target to control and progression of AD [6] and your catalytic site comprises the amino acids Asp32, Thr72, Gln73, Phe108, Asp228, Thr231 and Arg235 [7].

In this work, we studied 40 most active BACE-1 inhibitors, available in the BingindDB database as well as used a selected crystal structure of BACE-1, analyzed physicochemical properties as well as interactions in order to propose a drug candidate for future AD treatment.

2. METHODOLOGY

2.1 Selection of Inhibitor Group and Crystallographic Enzyme

For this work, knowledge of compounds already reported in literature that have inhibitory activity

of β-secretase is crucial. For this, we used the BindingDB database [8]. BindingDB is a collection of ligands with 1,058,945 substances, whose structures can be downloaded, in addition to information about inhibition constant (KI), dissociation constant (KD), measure of efficacy (IC_{50}), half maximal effective concentration (EC_{50}), thermodynamics, amongst others [8].

A search on BindingDB for BACE-1 inhibitors reveals 4522 hits compounds, and we selected the 40 most active inhibitors (compounds were sorted in crescent order of Ki, ranging from 0,017 to 3.0). For further methodology, these inhibitors structures were geometry optimized and energy full minimized using the semiempirical AM1 method, thus implemented in the HyperChem v.80.6 software [9].

The selected crystallographic structure of BACE-1 was experimentally resolved and deposited in the Protein Data Bank (PDB) under the code 4IVT at 1.6 Å resolution [7].

2.2 Prediction of Binding Sites

Prediction of possible BACE-1 ligand binding sites was carried out using the Q-SiteFinder webserver [10] which has a method based on purely energetic criterion: calculating van der Waals interaction energy of a methyl group with the submitted protein. At Discovery Studio Visualizer 4.0 [11] protein structure (PDB: 4IVT) was prepared for the identification of possible catalytic sites, this preparation consisted of removing the inhibitor from the crystallographic complex structure, generating a file containing only the enzyme, which in turn was submitted to the server.

2.3 Molecular Docking Procedures

Molecular docking aims to predict binding modes of protein-ligand complexes, defining the preferred orientation of a molecule with respect to the other [12,13]. In this methodological procedure we used AutoDock Vina software [14], ranking the possible poses of the ligand inside the enzyme catalytic site according to an affinity score function (defined as affinity energy in kcal/mol). With the help of Discovery Studio Visualizer 4.0 [11], inhibitors selected from the BindingDB database [8] were downloaded and the BACE-1 structure in complex with the inhibitor N-{N-[4-(acetylamino)-3,5-dichlorobenzyl]carbamimidoyl}-2-(H-indol-1-yl) acetamide (VTI) was splitted, generating two files that contain separately the enzyme and the inhibitor.

Regarding the enzyme structure, non-polar hydrogen atoms were added as well as Gasteiger atomic charges. Another important step carried out was to define the Grid Box that defines the region of the molecule in which the software will be free to engage the ligand. The dimensions of the grid box, with size x=24 Å, y=24 Å and z=24 Å enough to cover all the active site region reported in literature by Zou et al. [7], as the ligand binding region, were: x=23.014, y=23.954 and z=1.946.

2.4 Interactions Analyses

The models generated with highest affinity energy values by AutoDock Vina [14] were selected and a study about average distances of the observed interactions using Discovery Studio Visualizer 4.0 software [11] was performed. The maximum distance parameters used were: (a) 3.4 Å for conventional hydrogen interactions; (b) 3.8 Å for carbonic hydrogen interactions - in this case the hydrogen is attached to a carbon and interacts directly with fluorine, nitrogen or oxygen; (c) 5.6 Å for electrostatics; e (d) 6.0 Å for hydrophobic [15,16]. The software also has the ability to recognize, using its own parameters, other types of intermolecular interactions.

2.5 Pharmacophore Perception Calculation

32 highly active compounds (with the lowest Ki values) were selected, for pharmacophore perception calculation, using the web server PharmaGist [17], which detects the pharmacophoric groups by multiple and flexible alignment of the ligands used. The pharmacophoric features that can be observed in the results are: hydrophobic groups, aromatic groups, hydrogen bond donors, hydrogen bond acceptors, negative ionizable and positive ionizable groups, sorting the alignments by a score function.

It is also possible to choose one of the ligands as a pivot (rigid) for alignment of the other ones. In this study we used the compound CID_46888954 as pivot frame due to presenting the best affinity for the target protein (-10.7 kcal/mol) in the molecular docking experiments.

2.6 New Proposals

Since the steric and electronic features of the pharmacophore were calculated for the selected BACE-1 inhibitors, they were aligned by PharmaGist [17]. Investigating all the possible interactions after the docking procedures, three compounds were designed based on the structure CID_46888954, using the ChemSketch software [18], which are proposals of novel β-secretase inhibitors candidates with drug-like properties.

2.7 Biological Activity Predictions

Biological activity predictions were carried out for the proposals using the web software PASS [19] (http://www.akosgmbh.de/pass/index.html), which predicts with high accuracy (70-80%) up to 2000 biological activities for chemicals. Predicting possible given compound activity is necessary for indicating, with certain property, if the proposals drawn up follow the path proposed in the methodology, in order to achieve the research objectives.

2.8 Synthetic Accessibility

These three proposals had their synthetic accessibility investigated using the Sylvia software [20], which estimates how accessible is the synthesis of a specific compound, sorting the results as easy, medium or hard synthetic accessibility.

3. RESULTS AND DISCUSSION

As a first step, a selected BACE-1 crystal structure was downloaded from PDB as well as potent inhibitors reported in the BindingDB database [8]. The 4IVT crystal structure [7] was selected (BACE-1 in complex with the inhibitor VTI) (Fig.1), while the group of inhibitors was selected regarding those of lowest Ki values (Fig. 2).

The BACE-1 structure (inhibitor removed) was submitted to the Q-SiteFinder webserver [10] in which potential protein binding sites were identified using a probe group methyl, indicating 10 possible regions which could be bind ligands. In Fig. 3 the results obtained using the Q-SiteFinder can be visualized, where the region of the enzyme most likely to be the catalytic site is shown.

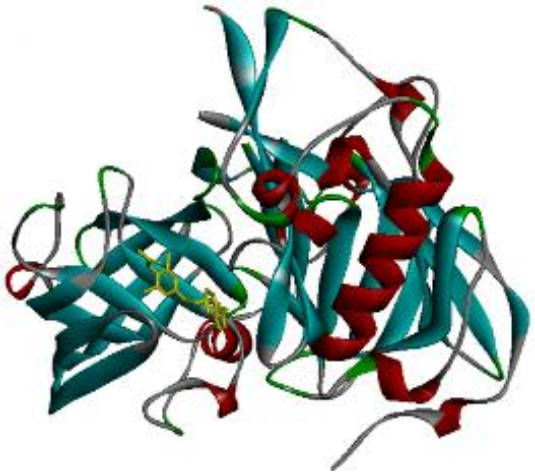

Fig. 1. BACE-1 structure (in cartoon) in complex with the inhibitor VTI (highlighted in yellow)

Since the structural information about of the both biological target and ligands are provided but not all the complexes, molecular docking procedures were carried out in order to propose potential binding modes for these inhibitors.

In the first docking simulation performed here we have used as a ligand the crystallographic inhibitor VTI, in order to verify, by calculating the Root Mean Square Deviation (RMSD), if the AutoDock [14] could reproduce the crystallographic pose. The RMSD for the best obtained pose was equal to 0.7108 Å (Fig. 4), thus validating the docking calculations, whereas values lower than 2 Å are successfully accepted [21].

For analysis for the potential of interactions between the inhibitor and BACE-1 amino acid residues, we used the parameters described in section 2.3.

In the Table of the supplementary data it is possible to identify the different interactions considered in the docking procedure. However, the hydrogen bonds account for approximately 74.0% of all the observed interactions, two-thirds of these being conventional interactions and the rest are due to carbonic HBond. The electrostatic bonds account for 14%, while the hydrophobic comprise 11.0% of total binding. The other interactions add up to 1.0%.

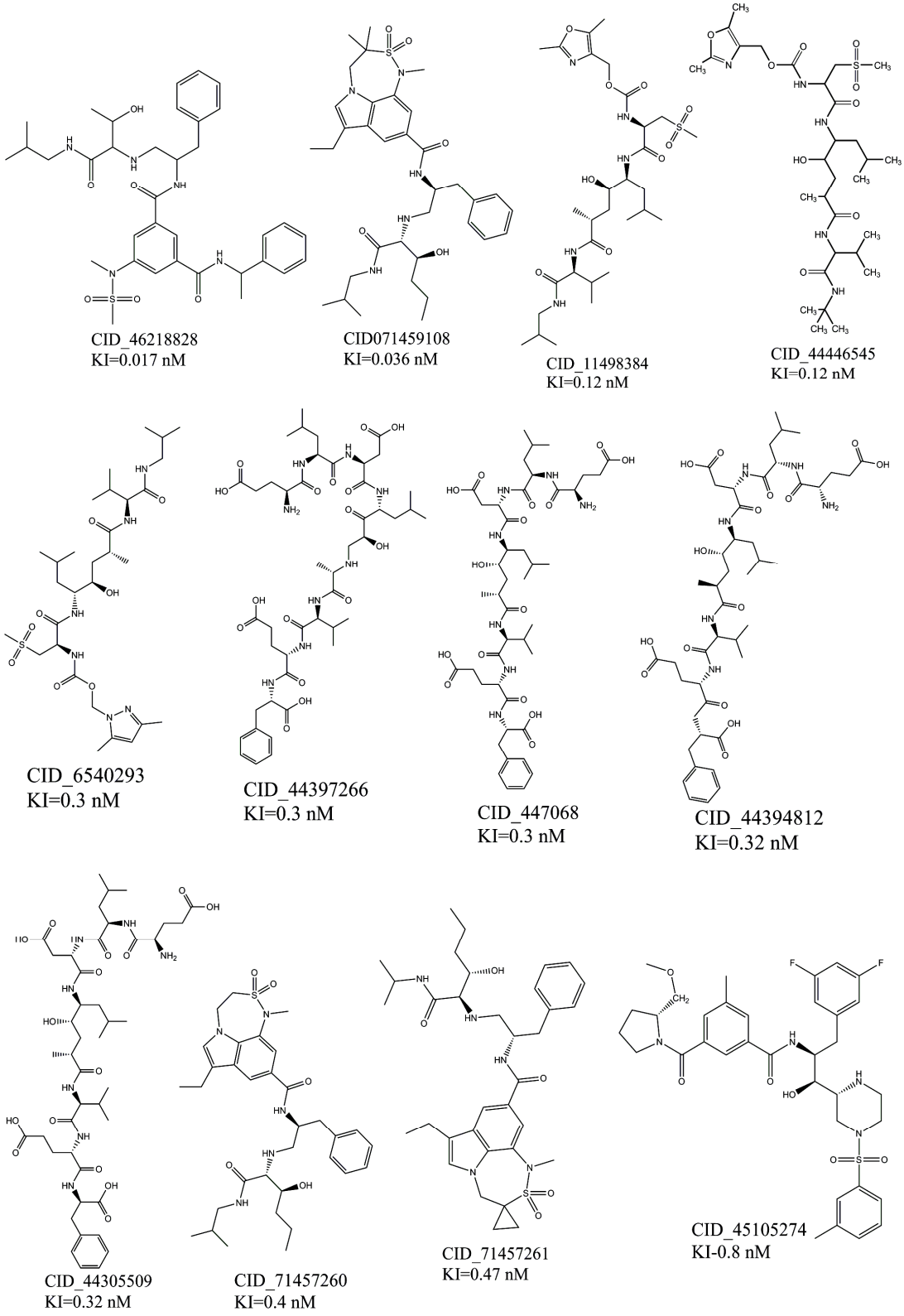

Fig. 2 Continuation

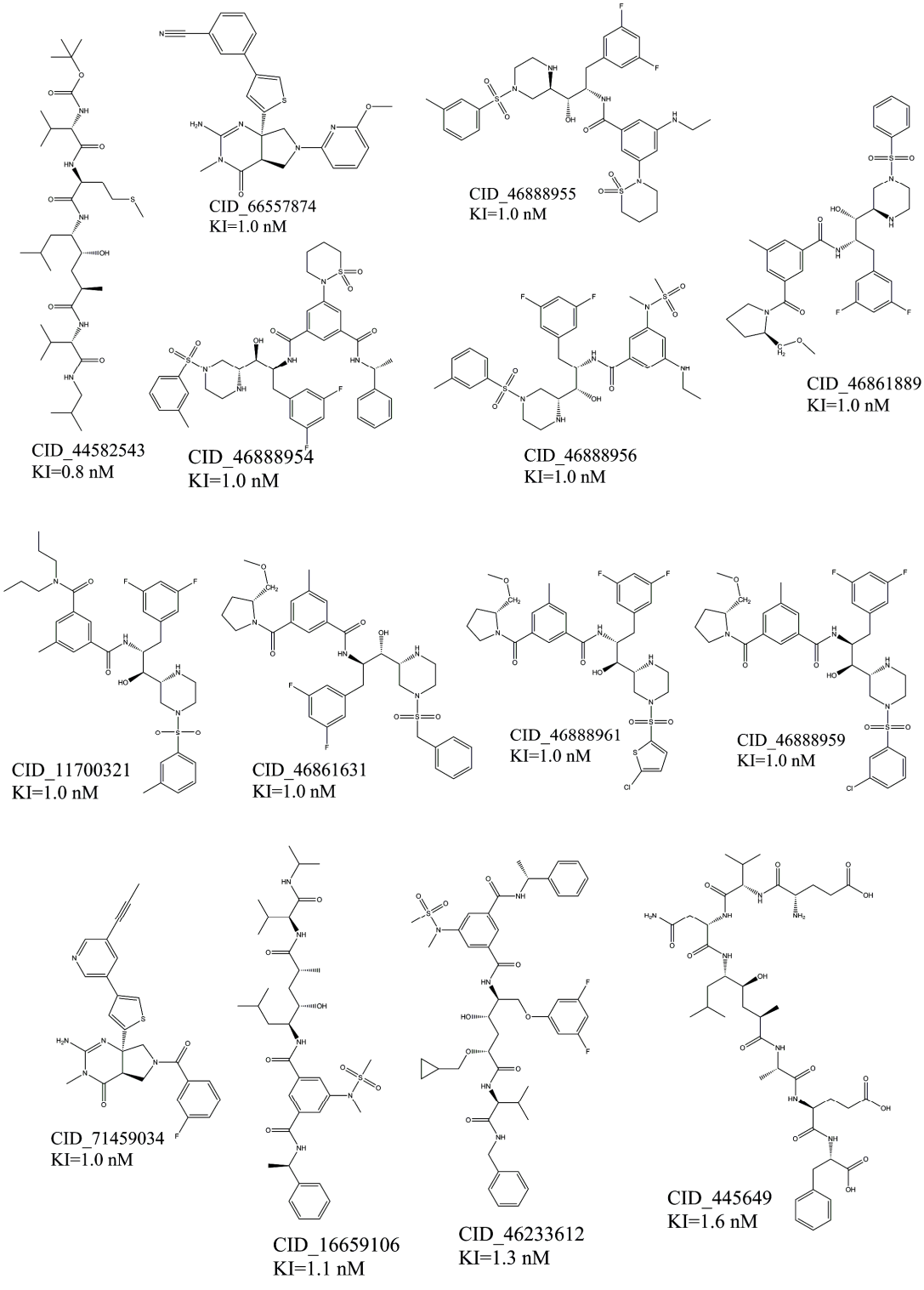

Fig. 2 Continuation

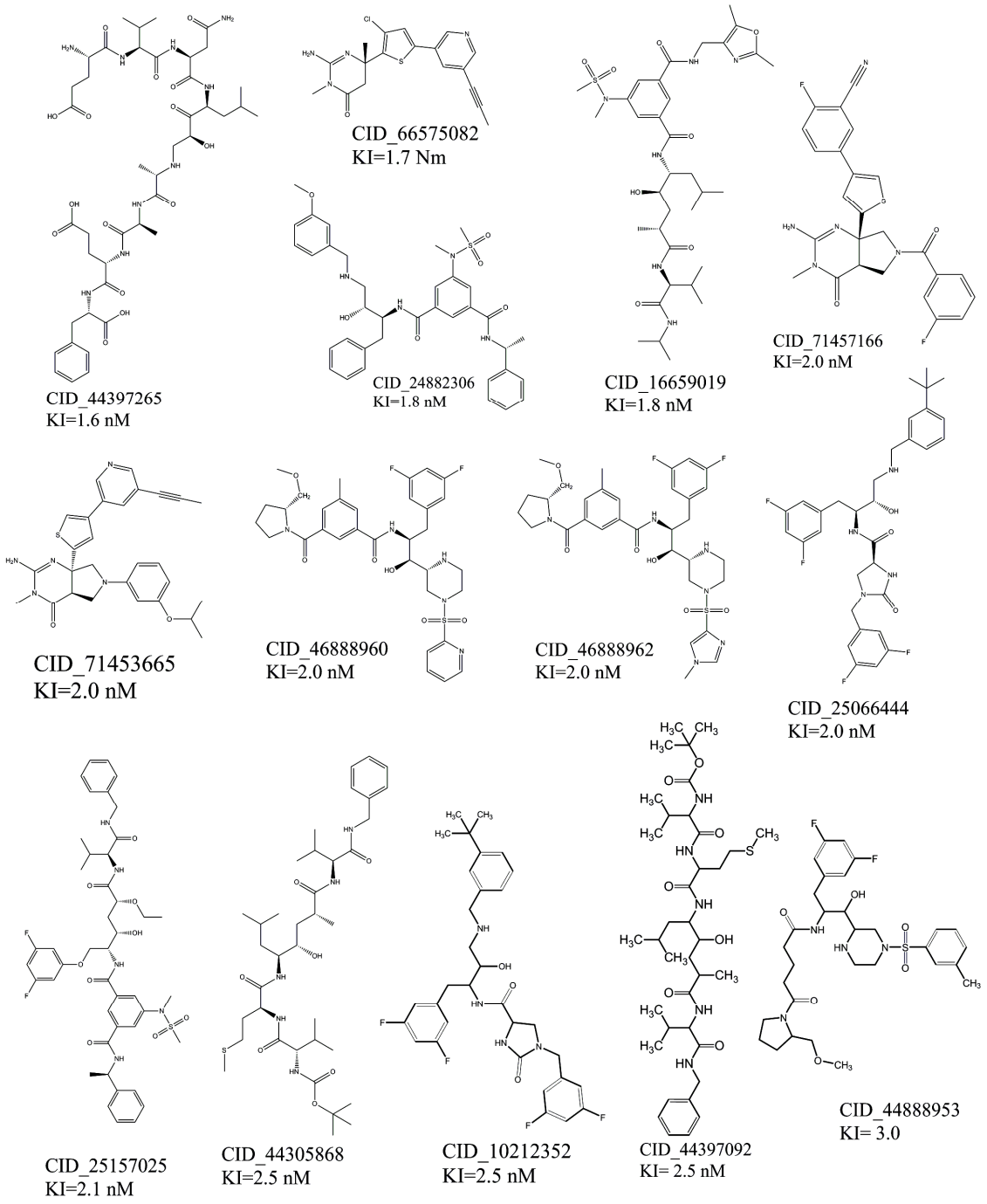

Fig. 2. BACE-1 inhibitors selected in the BindingDB database

From this perspective, one can see that hydrogen bonds play a very important role in the formation of the receptor-ligand complex, since they are present in all the couplings provided by AutoDock Vina [14]. Another interesting point in the results is that all hydrophobic interactions occurred between an inhibitor and Phe108. Table in the supplementary data more clearly elucidates the interactions, when they occur, between each amino acid of the BACE-1 catalytic site (Asp32, Thr72, Gln73, Phe108, Asp228, Thr231 and Arg235) [7] and the ligands,

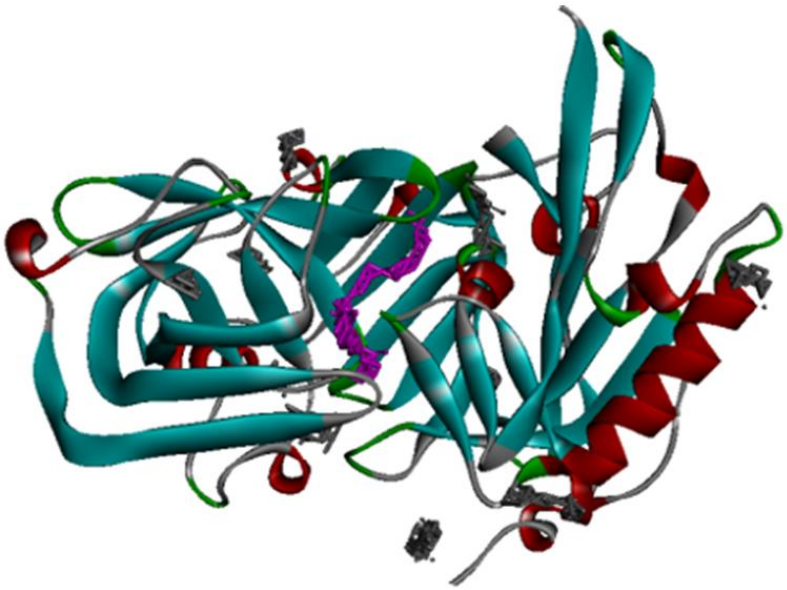

Fig. 3. Output of the web server Q-SiteFinder superimposed to the BACE-1 structure (in cartoon representation): region most likely to contain the catalytic site of the protein is depicted in magenta; other regions less likely are shown in gray

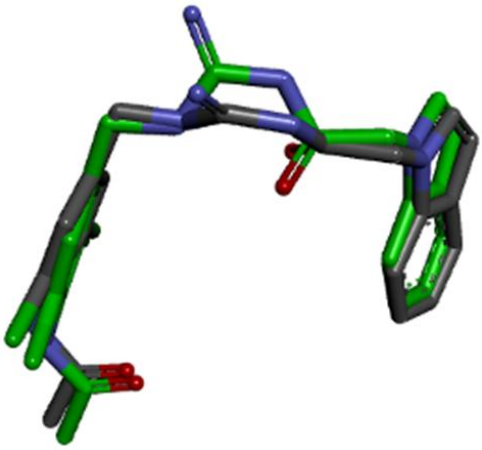

Fig. 4. VTI inhibitor: superposition of the top-ranked pose generated with AutoDock Vina (carbon atoms in green stick) with the crystallographic pose (carbon atoms in gray stick), with RMSD of 0.7108 Å

the distance between them, the atoms involved and the affinity energy measured while performing the docking procedures. It was observed that compounds containing low values of Ki (compounds of highest activity) showed not necessarily the best result docking, since there is no an ideal Score function for any docking procedure.

Based on the results generated with the docking simulation, it was observed that the compound CID_46888954 (Ki= 1.0 nM) was the best one that interact with BACE-1, showing binding affinity of -10,7 kcal/mol. Regards to BACE-1 active site residues, such inhibitor interacts with the amino acids Gln73, Asp228, Thr231 and Arg235, through hydrogen bonds (both conventional and with carbon) and electrostatic interaction (Fig. 5). According to the docking simulation, results indicated that Asp32 does not interact with the selected inhibitors.

One of the main points regarding the prediction of molecular interactions, crucial in computational drug design process, is the pharmacophore perception calculation [22]. The pharmacophore is the set of steric and eletronic features that is necessary to occur the interactions between a ligand and a specific biological target structure and to promote or to block its biological response [23].

Several pharmacophoric hypotheses were generated using the web server PharmaGist [17], where the 32 most active hits were selected from the web database BindingDB [8], so that potential pharmacophore groups (features) were detected in a common structural moiety/pattern. The features that can be observed in the results generated using such server are: hydrophobic

groups, aromatic groups, hydrogen bond donors, hydrogen bond acceptors, negative and positive ionizable groups, with the models and respective alignments are sorted by a score function.

PharmaGist [17] allows us to select a ligand that it will serve as a pivot molecule (or reference) for aligning the other ones. We have selected the molecule CID_46888954 as the pivot molecule due to its highest theoretical affinity with the target (-10,7 kcal/mol), thus indicated in the molecular docking experiments. The best model generated has a score of 61,306 and tack 5 inhibitors (CID_46888954, CID_46888959, CID_46888961, CID_45105274 e CID_46861889), having the following characteristics: a hydrophobic group, three aromatic, three hydrogen bond donors and seven acceptors (Fig. 6).

These results guided the design of novel proposals obtained by molecular changes applied into the structure of the compound CID_46888954. Three proposals were made based on this one, for which two properties violated the Lipinski rule, which states that drugs that have oral bioavailability, in general, must have: molecular weight ≤ 500 daltons, log *P* ≤ 5, number of hydrogen bond donor groups ≤ 5 and the number of hydrogen bond acceptor groups ≤ 10 [24], namely, molecular weight and number of hydrogen bond acceptor groups (Table 1).

The docking analyses allowed us the observation of the moieties of the ligand interacting with the target enzyme, while the pharmacophore hypotheses allowed us to know which regions of the molecule could be maintained for optimization purposes (Fig. 7).

The first proposal aimed to reduce the molecular weight of the compound, since the observance of this property is extremely important in drug design since the drug needs to cross the blood brain barrier to reach its site of action. In this proposition, we tried to remove idle regions that had no interaction with the catalytic BACE-1 amino acids (radicals attached to C9 and C17) (Fig. 8).

In Proposal 2, the goal was also molecular weight decrease and removal of acceptor hydrogen bond groups, the two parameters of Lipinski's Rule [24] violated by the prototype compound. Thus, the observed changes in this proposal are: withdrawing radical attached to N37 and replacement of N37 by a carbon (Fig. 8).

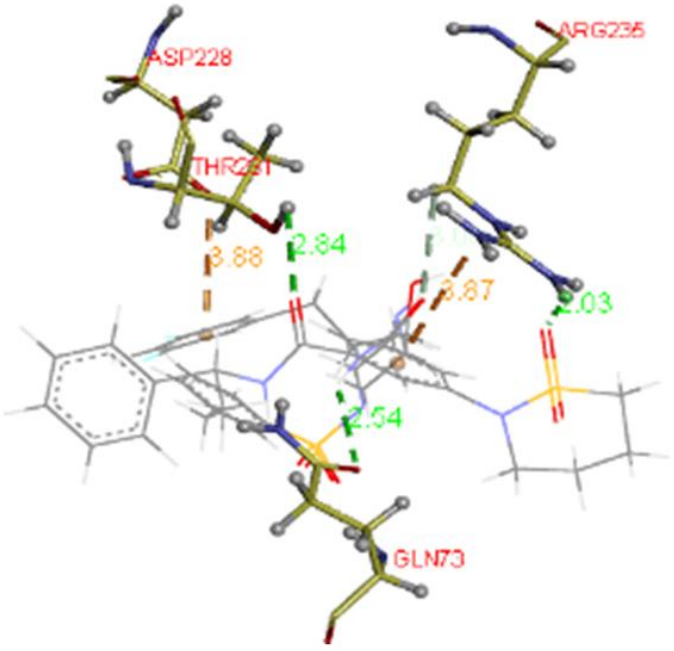

Fig. 5. Molecular docking result reveals compound CID_46888954 doing strongest interaction with BACE-1

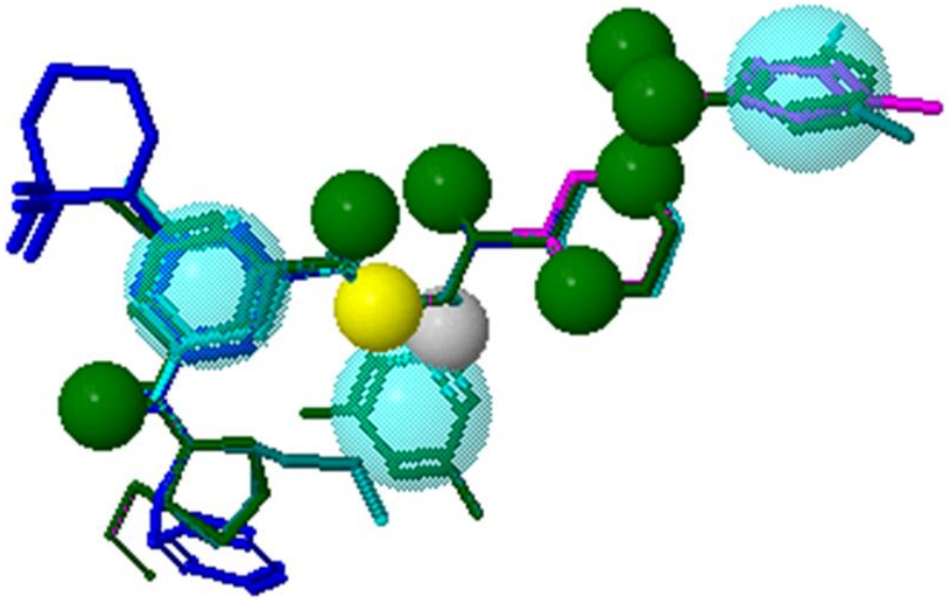

Fig. 6. Pharmacophore model obtained by the alignment of 5 of the 32 inhibitors of BACE-1 (previously selected from the BindingDB web server), generated using the web server PharmaGist

Table 1. BACE-1 inhibitors selected from the web database BindingDB and physicochemical parameters calculated according to the RULE of Lipinski

No.	Compound	Molecular weight	H Bond donors	H Bond acceptors	LOG P	Violations
1	CID_46888954	809.9414	4	12	4.2	2
2	CID_71457166	491.5124	1	7	2.3	0
3	CID_66557874	458.5355	1	7	2.3	0
4	CID_46888955	705.8353	4	12	3.4	2
5	CID_46888960	671.7545	3	11	2.6	2
6	CID_46861889	670.7664	3	10	3.3	1
7	CID_46888956	679.7981	4	12	2.9	2
8	CID_46888962	674.7584	3	11	1.7	2
9	CID_46888959	705.2114	3	10	3.9	1
10	CID_71453665	499.6275	1	6	3.5	0
11	CID_46861631	684.793	3	10	3.2	1
12	CID_46888961	711.2392	3	11	4.3	2
13	CID_45105274	684.793	3	10	3.7	1
14	CID_11700321	670.8095	3	9	5.0	1
15	CID_25066444	600.6469	4	8	4.7	1
16	CID_24882306	658.8069	4	8	4.0	1
17	CID_71459034	487.5486	1	6	2.2	0
18	CID_46218828	665.8426	5	8	3.6	1
19	CID_71459108	653.875	4	7	4.5	1
20	CID_46233612	892.0188	5	12	5.4	3
21	CID_16659019	706.893	5	10	3.2	1
22	CID_25157025	865.9815	5	12	5.0	2
23	CID_6540293	658.8502	5	9	2.9	1
24	CID_46888953	636.7502	3	10	1.9	1
25	CID_71457260	625.8218	4	7	3.9	1
26	CID_71457261	637.8325	4	7	3.7	1

No.	Compound	Molecular weight	H Bond donors	H Bond acceptors	LOG P	Violations
27	CID_16659106	701.9162	5	8	4.3	1
28	CID_445649	892.9921	12	15	-2.9	3
29	CID_44305868	721.9904	6	8	5.5	3
30	CID_44394812	935.0685	11	16	-0.7	3
31	CID_11498384	659.8349	5	10	2.8	1
32	CID_447068	936.0566	12	16	-1.0	3
33	CID_44397092	721.9904	6	8	5.5	3
34	CID_44397265	936.0168	13	17	-6.4	3
35	CID_44397266	979.0813	13	18	-4.4	3
36	CID_44446545	659.8349	5	10	2.5	1
37	CID_10212352	721.9904	6	8	5.5	3
38	CID_44582543	687.9742	6	8	5.4	3
39	CID_66575082	372.8718	1	4	2.1	0
40	CID_44305509	936.0566	12	16	-1.0	3

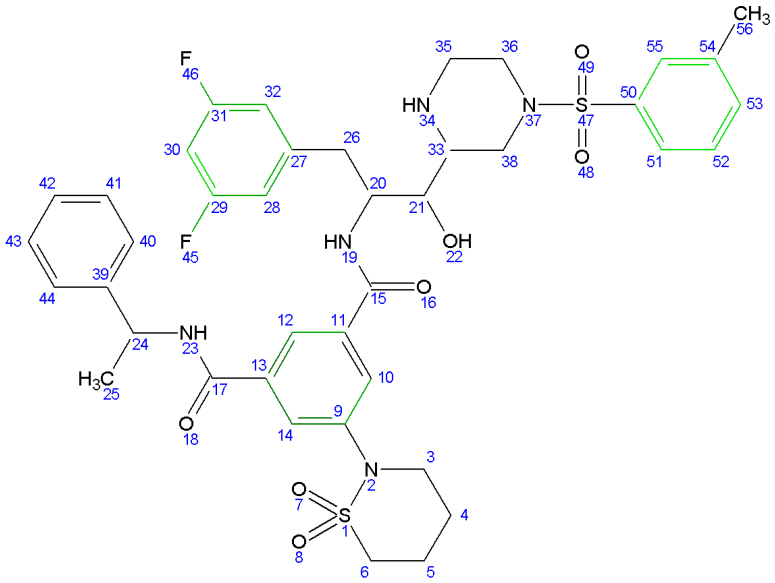

Fig. 7. Structure of compound CID_46888954

In order to have a compound that respects the properties of Lipinski's rule violated by compound CID_46888954 [24], the same modifications brought the Proposal 2 were then maintained (except replacement of N37 by a carbon atom), with summing of three other modifications, as follows: removal of the methyl group linked to C24, the radical attached to C9 and replacement of N23 by a carbon. This allow us to design a proposal with molecular weight lower than 500 Da (491,57, according to the calculation of Discovery Studio Visualizer 4.0 software [11], and fewer hydrogen bond acceptors groups (Fig. 8).

The pharmacophore of each molecule generally was maintained, but another point to be noted in the proposals 2 and 3, is that the aromatic ring attached to the C24 showed no interaction on the docking analyses. However, it was maintained due to be close to Phe108, increasing the probability that a hydrophobic interaction occurs in such region.

After defining the structure of the proposals, they were submitted to the PASS software [19] for biological activity prediction, as shown in Table 2. PASS lists the results informing of the biological activity that the compound may take, potential activity (Pa) and its potential of inactivity (Pi). The values that Pa and Pi may take vary from 0 to 1, and the best results expected for the Pa proposal are those approaching 1, while Pi of compound is presented in a more satisfactory way when it tends to 0 [19].

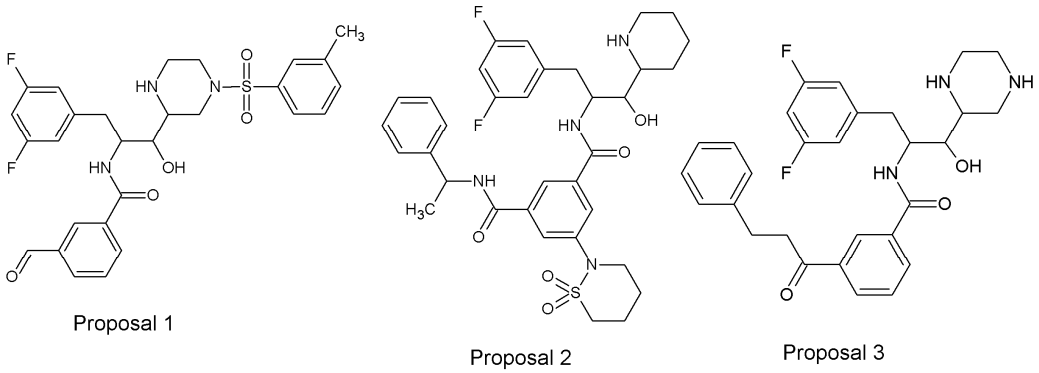

Fig. 8. Proposals of molecular changes to the inhibitor CID_46888954

Table 2. Prediction of activity and inactivity for the inhibitor with the highest binding affinity for BACE-1, and derivative proposals

Compound	Pa	Pi
CID_46888954	0.995	0.000
Proposal 1	0.675	0.002
Proposal 2	0.972	0.000
Proposal 3	0.300	0.002

Predictions of biological activity for these compounds reveal that compound CID_46888954 has the highest potential activity in comparison to the other ones. In the meantime, it is important to remember that Proposal 2 has a Pa value relatively satisfactory, whereas Pi tends to 0 with high relevance. This proposal also counts with the fact to be itself modeled in order to respect physicochemical, steric and electronic properties important to the purpose for which these molecules were designed.

After checking the biological activity of the three proposals, results were obtained using the Sylvia software [20] for the synthetic accessibility. Proposals 1 and 2 had their accessibility predicted as difficult (the software generated red background for both, with values equal to 6.32 and 7.10, respectively), whereas the Proposal 3 showed yellow background, being considered middle synthetic accessibility (5,71).

4. CONCLUSION

Based on the results here obtained, design of novel potential BACE-1 inhibitors showed to be promising for future AD treatment, and the computational tools here used relevant efficacy to this aim. Undoubtedly, we can infer also, that the knowledge of the pharmacophore model and analysis of the results of molecular docking enable a more accurate understanding of how a potential novel inhibitor drug candidate (with its structure based on these ligands) will interact with the biological target, before its assay. In this study, docking procedures demonstrated that hydrogen bonds account for approximately 74.0% of all observed interactions, 14% of electrostatic bond, 11% hydrophobic bond and 1% other interactions between inhibitors and catalytic site comprises the amino acids Thr72, Gln73, Phe108, Asp228, Thr231 and Arg235 of BACE-1 enzyme. The next steps of study will be docking with proposals, synthesis of the compounds and *in vitro* test activity assays.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rang HP, Dale MM, Ritter JM. Pharmacology. 6th ed. Rio de Janeiro: Guanabara Koogan; 2007. Portuguese.
2. Forlenza OV. Pharmacological treatment of Alzheimer disease. Rev Psic Clín. 2005;32(3):137-148. Portuguese
3. Shen L, Liu G, Tang Y. Molecular docking and 3D-QSAR studies of 2-substituted 1-indanone derivatives as acetylcholinesterase inhibitors. Acta Pharm Sin. 2007;28(12):2053-2063.

4. Selkoe JD, Lansbury JJRP. Biochemistry of Alzheimer's and Prion Disease. In: Siegel GJ, Agranoff BW, Albers RW, et al. editors. Basic Neurochemistry: Molecular, cellular and medical aspects. 6th ed. Philadelphia: Lippincott-Raven; 1999.
5. Citron M. Strategies for disease modification in Alzheimer's disease. Nat Rev Neurosci. 2004;5:677-685.
6. Ghosh AK, Bilcer G, Hong L, Koelsch G, Tang J. Memapsin 2 (beta-secretase) inhibitor drug, between fantasy and reality. Curr Alzheimer Res. 2007;4:418-422.
7. Zou Y, Li L, Chen W, Chen T, Ma L, Wang X, Xiong B, Xu Y, Shen J. Virtual screening and structure-based discovery of indole acylguanidines as potent β-secretase (BACE1) inhibitors. Molecules. 2013;18(5): 5706-5722.
8. Liu T, Lin Y, Wen X, Jorissen RN, Gilson MK. BindingDB: a web-accessible database of experimentally determined protein-ligand binding affinities. Nucleic Acids Res. 2007;35(1):198-201.
9. Chemplus: Modular extensions for HyperChem Release 6.02, Molecular Modeling for Windows, HyperClub, Inc., Gainesville; 2000.
10. Laurie ATR, Jackson RM. Q-SiteFinder: an energy-based method for the prediction of protein-ligand biding sites. Bioinformatics. 2005;21(9):1908-1916.
11. Dietrich SW. Burger's medicinal chemistry and drug discovery: principles and practice. 5th ed. New York: John Wiley; 1995.
12. Lengauer T, Rarey M. Computational methods for biomolecular docking. Cur Op in Struc Bio. 1996;6(3):402-406.
13. Morgon NH, Coutinho K. Theoretical chemistry and molecular modeling methods. 1st ed. São Paulo: Livraria da Fisica; 2007. Portuguese.
14. Trott Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comp Chem. 2010; 31:455-461.
15. McGaughey GB, Gagnes M, Rappe AK. π-Stacking interactions: Alive and well in proteins. J Biol Chem. 1998;273(25): 15458-15463.
16. Bissantz C, Kuhn B, Stahl M. A Medicinal Chemist's guide to molecular interactions. J Med Chem. 2010;53:5061-5084.
17. Schneidman-Duhovny D, Dror O, Inbar Y, Nussinov R, Wolfson HJ. PharmaGist: a webserver for ligand-based pharmaco-phore detection. Nucl. Acids. Res. 2008;36:W223-W228.
18. ACD/Chemsketch Freeware, Advanced Chemistry Development, Inc, Toronto, ON, Canadá; 2010.
19. Filimonov DA, Poroikov VV. Bioactive compound design: Possibilities for industrial use. 1st ed. Oxford: BIOS Scientific Publishers; 1996.
20. Boda K, Seidel T, Gasteiger J. Structure and reaction based evaluation of synthetic accessibility. J Comput Aided Mol Des. 2007;21(6):311-325.
21. Cole JC, Murray CW, Nissink JWM, Taylor RD, Taylor R. Comparing protein–ligand docking programs is difficult. Proteins: Struc, Func Bioinf. 2005;60:325–332.
22. Dror O, Shulman-Peleg A, Nussinov R, Wolfson HJ. Predicting molecular interactions in silico: I. A guide to pharmacophore identification and its applications to drug design. Curr Med Chem. 2004;11(1):71-90.
23. Wermuth CG, Ganellin CR, Lindberg P, Mitscher LA. Glossary of terms used in medicinal chemistry. Pure & Appl Chem. 1998;70(5):1129-1143.
24. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computacional approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Del Rev. 1997;23:3-25.

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