Screening for New Agonists Against Alzheimer's Disease

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Abstract: To find new drug candidates for treating Alzheimer's disease, we used the similarity search technique and GTS-21 as a template to search the Traditional Chinese Medicines Database. The high-score molecules thus obtained were compared with the template through the flexible alignment. Those molecules which had good alignment with GTS-21 were selected for conducting the docking studies aimed at the α7 nicotinic acetylcholine receptor. The CHARMM22 force field was taken to compute the partial charge and the TABU search was adopted to operate the docking process. The docking results thus obtained were used to compare with that of GTS-21. Those molecules which had better docking results than that of GTS-21 were singled out for further consideration. Finally, it was found through an in-depth structural analysis that Mol 7235 might be a promising candidate for further modification by experiments to make it become an effective drug for treating Alzheimer's disease.

Key Words: Alzheimer's disease, α7 receptor, GTS-21, pharmacophore alignment, docking, traditional chinese medicines database

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of the senile dementia. AD is characterized by a neurodegenerative, dementia-inducing disorder that results in a progressive and ultimately fatal loss of mental capacity. The development of AD is accompanied by the gradual spread of sticky plaques and clumps of tangled fibers that disrupt the delicate organization of nerve cells in the brain, undermining the normal communication among brain cells. AD is a kind of protein structure disease, which is initiated by the incorrect fold of protein structure [1-3]. According to some recent reports, GTS-21 (3-[(2,4-dimethory)-benzylidene]-anabaseine) could be used to treat the Alzheimer's disease in some degree [4-6]. GTS-21 is one of the synthetic benzylidene derivatives of anabaseine. After being taken orally, it can selectively stimulate α7 nAChRs (nicotinic acetylcholine receptors), entering into the patient's brain so as to greatly improve the cognition function and memory [7]. By studying the hydroxyl metabolites of GTS-21, some scientists found that these hydroxyl metabolites were also able to improve the cognition function [8]. However, molecular modeling studies suggest that the volumes of GTS-21 and its metabolites might be somewhat larger than optimal for fitting into the active pocket of the α7 receptor [9]. To improve the efficiency, we should search for a new compound with a smaller volume while maintaining the key active groups so that it can enter into the brains of patients more quickly and stimulate the α7 nAChR more effectively. Computational approaches, such as homology modeling, structural alignment, pharmacophore search, and molecular docking, can provide very useful insights for biomedicine studies and drug discovery [10-12]. The present study was initiated in an attempt to dig out the desired compounds from the information buried in the Traditional Chinese Medicines Database. The similarity search, flexible alignment and molecular docking techniques were used in this study.

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METHODS AND THEORIES

1. The First Screening - Similarity Search and the Flexible Alignment

The first screening was conducted to exclude those molecules which did not match the template molecule so as to reduce the number of compounds to be considered for the next step. The database used here was the Traditional Chinese Medicines Database (TCMD 2003.1) [13], which was developed by the Institute of Process Engineering Chinese Academy of Science. 9127 different compounds from various sources of traditional Chinese medicines were included in the database and their 3D structures are constructed by the geometry optimization using CHARMM22 force field [14]. Taiho Pharmaceutical Company's new drug GTS-21 currently in phase III clinical trials was adopted as the template molecule [4,9], and similarity search was made in the TCMD. Before performing the search, the fingerprints of all the compounds concerned were computed. The particular features thus obtained would depend on the topology of the chemical graph or even the 3D conformation of the compound. Different fingerprint scheme, which was represented by a set of features derived from the structure of a given molecule, would emphasize a different molecular attribute. The 2D structure and 3D structure of GTS-21 are given in Fig. (**1-A**) and Fig. (**1-B**), respectively.

The above search process led our focus on 57 compounds, of which, however, some molecules were too large in volume in comparison with the template molecule and were hence excluded for further studies. The remaining molecules were then subjected to the Lipinski's "rule of 5"

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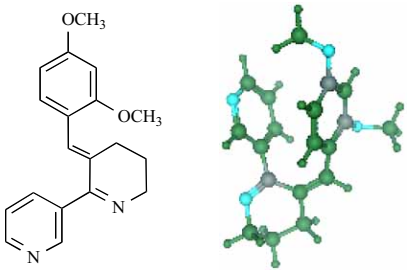

Fig. (1). The 2D structure of (A) GTS-21, and (B) 3D structure of GTS-21 rendered with its pharmacophore, where turquoise, green and gray represent pharmacophores of hydrogen bond acceptors, hydrophobic areas/aromatic centers and others, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

[15]. The number of qualified compounds was reduced to 21, which are shown in Fig. (**3**).

2. Flexible Alignment

Before flexible alignment was performed, the molecular fingerprints should be computed. The fingerprints [16] represent a set of features derived from the structure of a molecule. The particular features calculated from the structure depend on the topology of the chemical graph or even a 3D conformation. Different fingerprint schemes emphasize different molecular attributes according to the design philosophy of the fingerprint system. Comparisons between molecules are then reduced to comparing sets of features and measuring the degree to which the sets overlap.

CHARMM22 force field was used to compute the partial charges of all the molecules concerned. During the flexible alignment, the energies of any two molecules to be aligned with each other were subject to minimization. The single bonds revolving and Welden Transfer were permitted [17]. The alignment was performed by the SAMM software developed in our Lab, in which the alignment results were evaluated by the probability density in terms of the Gaussian function. The isotropic (spherically symmetric) Gaussian, or Normal, probability density has the functional form as given below

$$f(x) = s^3 (2\pi)^{-3/2} \exp\left(-\frac{1}{2} |x - x_0|^2 s^2 \right) \qquad (1)$$

where $s = (a/r)$, with a representing the parameter for the density broadness, r the van der Waals redius, and x_0 the center (and mean) of the density. Generally speaking, the smaller the value of s, the better the alignment result of the two molecules.

3. The Second Screening - Docking

Molecule docking is placing a small molecule ligand at the active site of a macromolecular target, and searching for the optimal conformation and orientation, which makes the ligand and the receptor match best. In addition, the affinity of ligand and receptor includes some kinds of fields, such as electronic field, hydrophobic field, three-dimensional field and hydrogen bond interaction field and so on. In this study, α7 dimmer was used as our receptor [10,18], to which the 21 compounds were docked receptively.

In this paper, the flexible docking method was used. During the docking procedure, the macromolecular target remains rigid while the small molecule is flexible so that the small molecule can move freely in the active pocket of the target. The docking program generates a set of conformations by making the atom coordinates of the small drug molecule change randomly. At last, a good conformation fitting for the active pocket can be found. In the docking study, the CHARMM22 force field parameters were used to compute the interactions, and conduct the simulations.

RESULTS

The α7 receptor belongs to nicotine acetylcholine receptor, which is a channel-linked receptor in reaction. Nicotine takes effects on patients' tissue *via* interactions with a family of ligand-gated ion channels [19,20]. The α7 nAchR includes 502 amino residues. Shown in Fig. (**2A**) is the α7 dimer complexed with GTS21. A close view of the interaction between the active site of the α7 dimer and GTS21 is given in Fig. (**2B**).

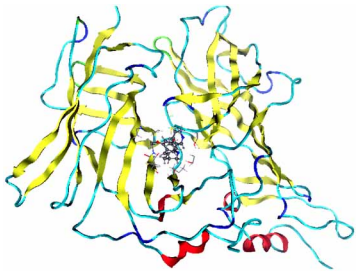

Fig. (2A). Plot of α7 dimer complexed with GTS-21, where backbone is shown in ribbon and GTS-21 in ball-and-stick.

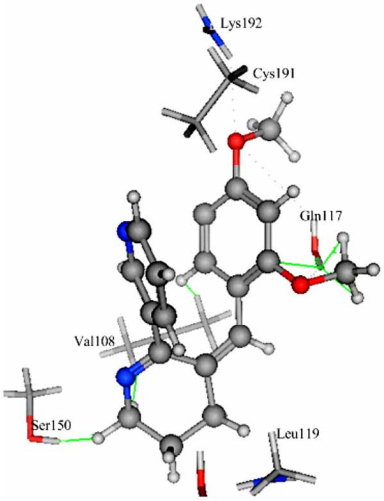

Fig. (2B). A close view of the interaction between the active site of the á7 dimer and GTS-21, where the residues of α7 are expressed by sticks, and the GTS-21 molecule by ball-and-stick. The hydrogen bonds are expressed by grey dashes, and van der Waal's interactions between GTS21 and α7 dimer by green dashes. The atoms C, N and O are colored in grey, green and red, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

Shown in Table **1** are the results obtained by making the flexible alignment of the 21 molecules retrieved from the first screening with GTS-21, respectively. For each of the 21 molecules, a conformation with closest alignment with GTS-21 is obtained. The molecules are ranked according to their scores of the best conformation.

Subsequently, we docked the 21 compounds to the α7 receptor, and the best docking energies are selected and listed in Table **2**.

From Table **2**, we can see that there are twelve compounds whose binding energies with the α7 receptor are lower than that of GTS-21. The structures of the twelve compounds are given in Fig. (**3**).

DISCUSSION

The total energy of each complex includes the following types of energy: electrostatic interaction energy, vdw (van der Waal) energy, and docking binding energy. The docking results show that there are twelve molecules with smaller binding energies with the α7 receptor than that of GTS-21. The order is as follows: Mol 3719 < Mol 7475 < Mol 7235 < Mol 6950 < Mol 5760 < Mol 2779 < Mol 6358 < Mol 7232 < Mol 1195 < Mol 259 < Mol 2780 < Mol 6591 < GTS-21. Generally speaking, if a molecule with a lower binding energy than GTS21 to the α7 receptor will have better activity than GTS21. We did analysis of the details of how the twelve molecules bind with α7 receptor. However, an in-depth investigation by analyzing the molecular surfaces, hydrogen bond interactions, and van der Waals interactions indicates that the reason for some molecules having smaller binding energies is because their volumes are so small that they even cannot fully occupy the active pocket of receptor. This kind of binding, although with lower energies, will not play an important role in inhibiting the receptor. For example, Mol 3719 and Mol 7232 belong to this category. In contrast, it was found that Mol 259 and Mol 7235 had pretty good docking results. There is a hydrogen bond formed between Mol 259 and α7 receptor, and there are two hydrogen

Table 1. **The Alignment Results of GTS-21 and Candidate Molecules (Energy Unit: Kcal/mol)**

NO.	Molecular Code	Molecular Formula	U (Kcal/mol)	Score
1	3719	$C_{13}H_{14}N_2O$	29.4227	205.8987
2	7475	$C_{12}H_{17}NO_2$	28.5646	206.8678
3	6358	$C_{18}H_{21}NO_3$	27.0565	208.5393
4	262	$C_{19}H_{18}N_2O_3$	31.7268	210.1534
5	259	$C_{19}H_{18}N_2O_4$	28.4479	210.3360
6	7232	$C_{20}H_{24}N_2O_2$	28.0710	212.2219
7	1533	$C_{18}H_{23}NO_2$	35.7885	213.2113
8	1050	$C_{17}H_{16}NO_2$	31.5572	215.7870
9	6950	$C_{17}H_{21}NO_3$	38.4268	216.0683
10	668	$C_{17}H_{17}NO_2$	28.5902	216.9570
11	6384	$C_{18}H_{19}NO_2$	36.5002	219.2484
12	7913	$C_{18}H_{20}NO_2$	35.1515	219.6831
13	7235	$C_{20}H_{22}N_2O_2$	38.7083	220.1114
14	6409	$C_{19}H_{21}NO_2$	35.6145	220.6131
15	5760	$C_{19}H_{23}NO_2$	32.3637	221.5634
16	1195	$C_{17}H_{21}NO_4$	34.4875	221.9186
17	4835	$C_{18}H_{19}NO_4$	30.7730	222.0914
18	6591	$C_{21}H_{22}NO_4$	33.3342	226.9437
19	2780	$C_{20}H_{24}N_2O_2$	32.8059	230.0133
20	2049	$C_{22}H_{24}NO_4$	36.8731	230.7464
21	2779	$C_{20}H_{24}N_2O_2$	32.8370	236.8218

Note that the molecular code in Table 1 is the serial number of every molecule in TCMD. U is the potential energy of the aligned GTS-21 and the corresponding compound. The score is calculated as - kT log F + U, where U is the average potential energy of the molecules in the alignment and *F* is the probability density overlap function. Lower score values imply better alignments.

Table 2. List of the Interaction Energies of the 21 Molecules with α7 Receptor (Kcal/mol); for Facilitating Comparison, the Corresponding Interaction Energy for GTS-21 is also Included

NO.	Mol. code	U-total	U-ligand	U-ele	U-vdw	U-binding	rank
0	GTS-21	217.6556	97.8056	1.9603	117.8056	119.7659	*
1	3719	24.2040	43.0843	0.4059	-19.2862	-18.8803	1
2	7475	45.4261	43.1015	0.5679	1.7567	2.3246	2
3	6358	124.3798	79.6247	-0.4291	45.1842	44.7551	7
4	262	564.4979	95.0280	-1.2546	470.7245	469.4699	21
5	259	130.5845	79.4029	-0.0570	51.2386	51.1816	10
6	7232	141.2957	92.9069	3.9776	44.4112	48.3889	8
7	1533	232.3058	79.4731	2.0672	150.7655	152.8327	15
8	1050	230.7747	82.6223	-0.5540	148.7064	148.1524	14
9	6950	107.6726	88.9367	-1.4516	20.1876	18.7359	4
10	668	277.8524	76.8132	-0.1787	201.2179	201.0392	16
11	6384	305.2453	103.3830	0.6304	201.2319	201.8623	17
12	7913	227.4877	98.1400	-2.1494	131.4971	129.3477	13
13	7235	122.6469	114.5269	-1.5313	9.6513	8.1200	3
14	6409	545.0681	177.7781	0.8476	366.4424	367.2900	19
15	5760	113.1379	83.7659	-0.4092	29.7812	29.3720	5
16	1195	142.4597	92.8446	-0.9771	48.6380	49.6151	9
17	4835	323.0897	74.4852	2.0184	246.5861	248.6045	18
18	6591	191.9690	110.1863	2.2719	79.5108	81.7827	12
19	2780	177.9996	119.0684	1.8888	57.0425	58.9312	11
20	2049	487.0133	109.7154	0.6817	376.6162	376.6162	20
21	2779	149.4081	108.3359	0.7206	40.3516	41.0722	6

Note that in Table 2 U-ligand represents the ligand self-energy; U-vdw the Van der Walls contribution; U-ele the electrostatic energy; U-binding (binding energy) = U-ele + U-vdw; U-total (total energy) = U-binding + U-ligand.

bonds formed between Mol 7235 and α7 receptor. So we come to the conclusion that Mol 7235 is a much more potent inhibitor for the α7 receptor than any other molecules in TCMD. Accordingly, Mol 7235 might be a quite promising candidate for drug development against Alzheimer's disease. Some important features about Mol 7235 are illustrated in Figs. (**4-6**).

As is well known, the distance between hydrogen donor and receptor is within 3.5Å (see, e.g., [21,22]). Many lines of evidences indicate that hydrogen bonding interaction between ligand and receptor plays a key role in the binding mechanism (see, e.g., [23-28]). It can be seen from Fig. (**4**) that the hydrophobic and hydrophilic surface of Mol 7235 and the residues of the α7 receptor match very well to each other (green is the hydrophobic area, and blue the hydrophilic area), and the volume of Mol 7235 fully fits the active pocket of the α7 receptor. As shown in Fig. (**5**), there is a hydrogen bond (2.30 Å) formed between the oxygen of Mol 7235 and the nitrogen atom of Gln-57 of the α7 receptor. There is another possible hydrogen bond (2.81 Å) between the oxygen of Mol 7235 and the nitrogen atom of Gln-117 of the receptor. Furthermore, among the residues of the α7 receptor, there are some additional hydrogen bonds, such as the one between the sulphur atom of Cys-191 and the oxygen atom of Gyr-190 and the one between the sulphur atom of Cys-190 and the oxygen atom of Gyr-190. These hydrogen bonds would make the complex of Mol 7235 and its receptor more stable. Moreover, as shown in Fig. (**5**), the distance between hydrogen atom of Cys-191 of the α7 receptor and the carbon atom A of Mol 7235 is 2.08Å, and the distance between the hydrogen atom of Trp-149 and the carbon atom B of Mol 7235 is 2.13 Å. Hence the van der Waals force would play an important role in this range, making the complex more stable. Also, a very good structural alignment of Mol 7235 with GTS-21 can be seen in Fig. (**6**), further supporting the deduction that Mol 7235 is a hopeful drug candidate for Alzheimer's disease.

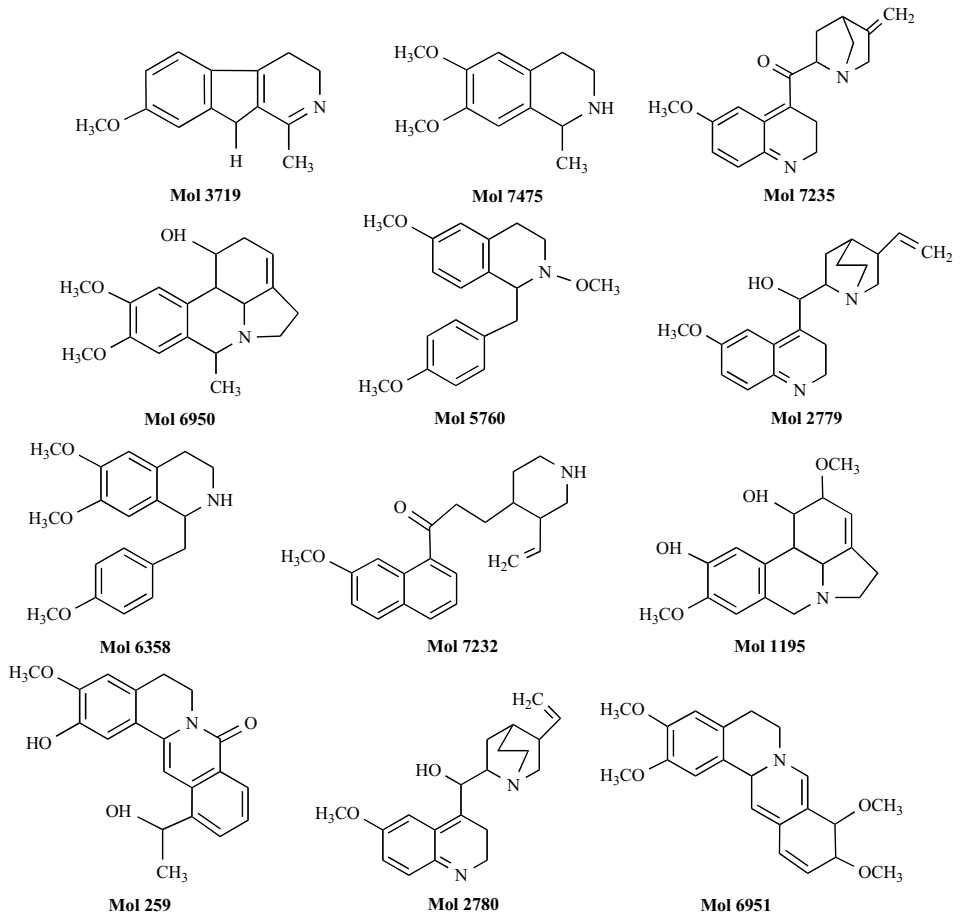

Fig. (3). The structures of the12 compounds whose binding energy is lower than that of GTS-21.

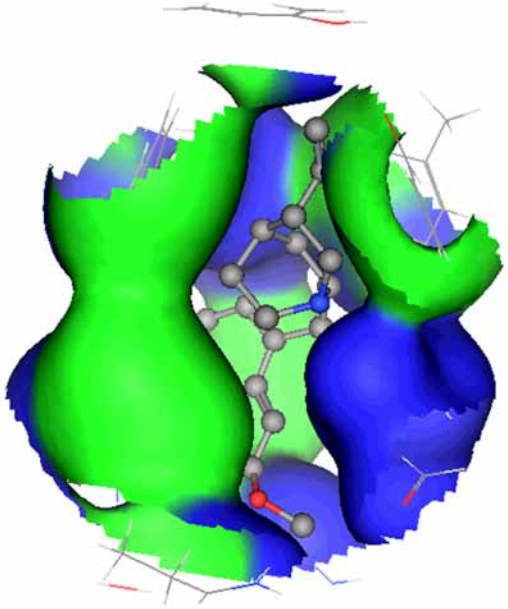

Fig. (4). The molecule interfaces of Mol 7235 and the α7 receptor. The hydrophobic surface is colored green, and the hydrophilic surface colored blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

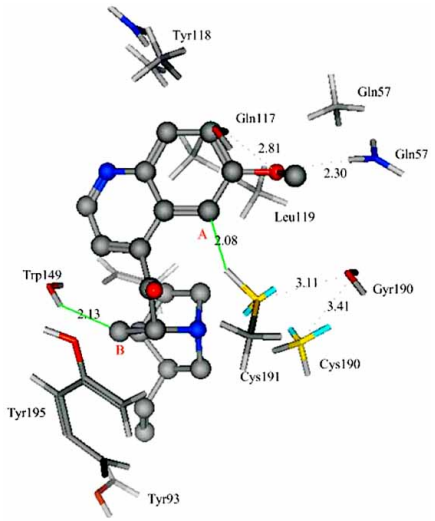

Fig. (5). Atomic details about the binding interaction between Mol 7235 and the α7 dimer, where the grey broken line is hydrogen bond, the green line shows the van der Waals interaction distance (in the unit of Å). The atoms C, N, O and S are colored in grey, green, red, and yellow, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

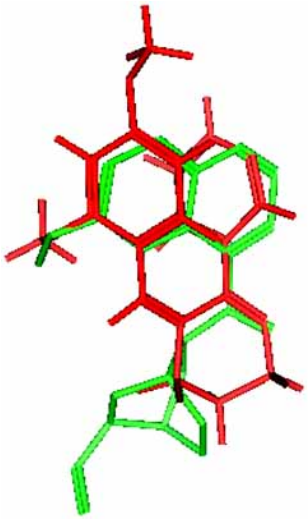

Fig. (6). The alignment of GTS-21 (red) and Mol 7235 (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

CONCLUSION

Mol 7235 might be a good candidate for further modification by experiments to make it become an effective drug against Alzheimer's disease. One of the key steps in modifying Mol 7235 is to deal with its bridge cycle, which seems a little too bulky for the active pocket of the α7 receptor.

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