Discovery of natural products for dual pharmacology CETP inhibitors and niacin receptor agonists

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***Abstract*—Dyslipidemia is a leading causative factor in cardiovascular diseases, and the traditional modulating lipid drugs mainly focus on reducing Low Density Lipoprotein Cholesterol (LDL-C). However, the increase of High Density Lipoprotein Cholesterol (HDL-C) also has gradually become an important focus on modulating lipid drugs. It is universally acknowledged that the drugs for significantly increasing HDL-C act on either the niacin receptor or cholesteryl ester transfer protein (CETP). Therefore, by comprehensively considering advantages and shortness of these two drug targets, compounds which act on the dual targets were studied in this paper. To be specific, a HipHop pharmacophore model for CETP inhibitors was built firstly, and then the pharmacophore model was validated internally and externally. The best pharmacophore model for CETP inhibitors included one hydrogen bond acceptor, four hydrophobic groups and two ring aromatics. In addition, the common basic structure of niacin receptor agonists was analyzed, and the novel basic structure was designed by bioisosterism principle. Afterward, the database of niacin receptor agonists, including 214 compounds, was established by fragment searching from traditional Chinese medicine database (TCMD, version 2009) and Lipinski' rules. Finally, five natural products with dual targets activity were gained by using CETP inhibitors pharmacophore model to screen the molecular database of niacin receptor agonists, which provided the study of dual-targets drug design with a reliable utility.**

Keywords—pharmacophore; virtual screening; dyslipidemia; fragment searching; niacin; CETP; TCM; bioisosterism

I. INTRODUCTION

Dyslipidemia is a crucial factor for cardiovascular diseases, such as coronary heart diseases, atherosclerosis and so on. The traditional drugs for modulating lipid include HMG-CoA reductase inhibitors, peroxisome proliferator receptor-alpha (PPAR-α) agonists, Niemann-Pick C1 like 1 protein (NPC1L1) inhibitors and so on. Most of drugs are able to reduce Low Density Lipoprotein Cholesterol (LDL-C) and triglyceride. However, studies have demonstrated that there is also a negative correlation between the level of High Density Lipoprotein Cholesterol (HDL-C) and the occurrence of coronary heart disease and stroke [1, 2]. So a significant way of modulating lipid has been gradually generated, which is to increase HDL-C.

It is accepted that the drug targets for observably increasing HDL-C include niacin receptor and Cholesterol ester transfer protein (CETP) at present [3]. Wherein, niacin receptor agonists, frequently used in clinic, are able to effectively increase HDL-C. Meanwhile, they can inhibit the hydrolysis of lipids, improve the HDL level of body and lower the levels of serum total cholesterol, LDL and triacylglycerol. However, high doses of niacin receptor agonists can cause flushing and elevate levels of uric acid in the blood [4]. CETP is a kind of hydrophobic glycoprotein, mainly involved in a series of metabolic process for secretion, transport and elimination of lipoprotein. Due to the pharmacological activities for significantly increasing HDL-C level, CETP inhibitors are novel lipid-lowering drugs under clinical research stage. However, the clinical trials of torcetrapib, an experimental CETP inhibitors, showed that it may cause the risk of cardiovascular side effect, such as hypertension, hyperaldosteronism and so on [5]. To the best knowledge, the beneficial feature for CETP inhibitors is typically aromatic ring structure with nitrogen and oxygen. Also, small molecular weight and specific basic structure are features of niacin receptor agonists. Although both two targets have a significant effect on improving HDL-C, they have side effects at the same time. Accordingly, it is feasible for natural products to screen dual-target active compounds, due to the structural diversity, better biological activity, minor side effects and multi-target therapeutic effect [6, 7].

Pharmacophore is a mathematical set including pharmacodynamic feature of the compounds acting on the same target and the form of spatial arrangement. Wherein, Common Feature Pharmacophore Generation modules (HipHop) algorithm is usually used for establishing qualitative ligand-based pharmacophore model and able to generate common chemical feature based on three-dimensional spatial arrangement of active compounds in training set. Fragment searching is a kind of virtual screening methods, based on the principle that compounds with similar molecular fragments may have similar pharmacological effects. The query structure is selected based on the common or important structural fragments and functional fragments of drug molecules acting on the same targets, so it is easy for discovery of the similar pharmacological active compounds [8].

In this paper, the HipHop pharmacophore model of CETP inhibitors is established by Discovery Studio software (version 4.0), and the optimal pharmacophore model is determined by internal and external validation. Meanwhile, the molecular fragments of niacin receptor agonists are designed based on the structure of niacin and the bioisosterism principle. Then the molecular database is established based on fragment-based searching from traditional Chinese medicine database (TCMD, version 2009). Finally, the database of niacin receptor agonists

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is screened by using optimal pharmacophore model and the leading compounds with dual-target activity are gained.

II. MATERIALS AND METHODS

A. Pharmacophore modeling of CETP inhibitors

1) Dataset of training and testing

The dataset for CETP inhibitors pharmacophore construction was derived from The Binding Database (http://www.bindingdb.org/bind/index.jsp). 98 compounds were selected, of which activities were tested by human pharmacological model. Wherein, 10 highly active compounds were selected as training set. The other active compounds were combined with non-active compounds on the ratio of 1:3, in order to build testing set for validation. Non-active compounds were derived from MDL Drug Data Report database (MDDR, Version 2007.2). All compounds were optimized to the lowest energy conformations by Best method. The relative energy threshold was set to 20kcal/mol and the maximum number of conformations was set to 255. The name and structure of active compounds in training set were shown in Fig.1.

2) Pharmacophore generation

The pharmacophore models for CETP inhibitors were established by HipHop. The Principal values of 10 highly active compounds were set to 2. The MaxOmitFeat value of CHEMBL303954, CHEMBL479527 and CHEMBL1081288 were set to 1 and others were set to 0. Misses, FeatureMisses and CompleteMisses were selected to 1, 1 and 0 respectively. Maximum Excluded Volumes value was selected to 5. All other parameters were automatically set to the default value.

3) Model evaluation

The HipHop pharmacophore models were ordered according to Rank score, which was used as internal validation of models. Rank score was calculated by the ratio of active compounds and random compounds, which were both aligned with pharmacophore. The more active compounds and less random compounds, the higher Rank score was. Higher Rank score meant better alignment between pharmacophore and active compounds and more reliable pharmacophore model.

Ligand Profiler module was used for external validation and virtual screening. The hypothesis models were evaluated by using testing set. Several indices were counted, such as hits of active compounds (Ha), hits of total compound (Ht), total number of active compounds (A) and total number of compounds (D). Hit rate of Active compounds (A% value) represented the ability to distinguish active compounds from total compounds and identify effective index (E value) expressed the ability to distinguish the active compounds and non-active compounds. The more E value was, the stronger the ability to distinguish the active and non-active compounds was. Comprehensively considering the evaluation results of E and A value on pharmacophore, Comprehensive Appraisal Index (CAI) was used to evaluate the screening ability of pharmacophore [9, 10].

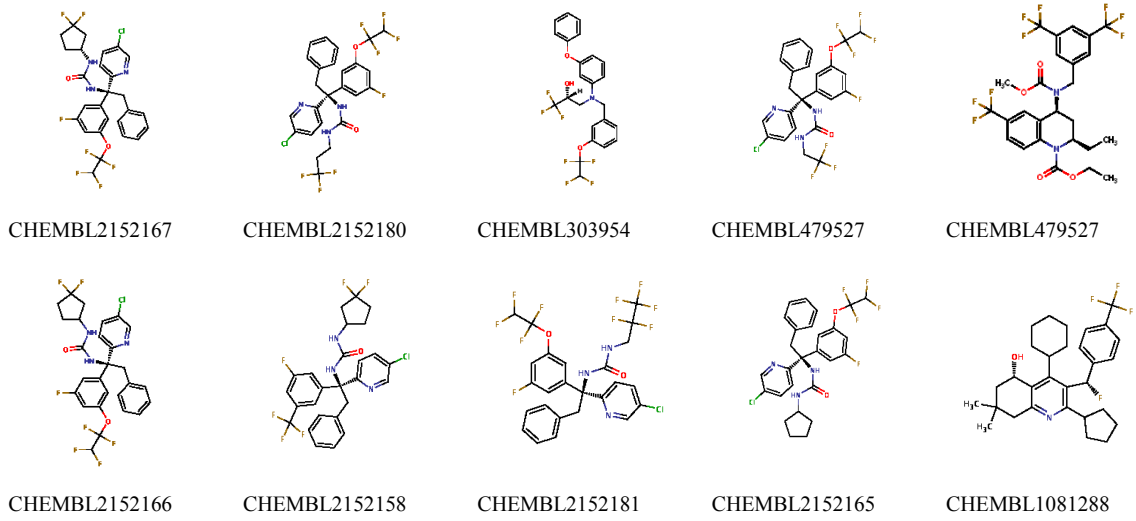

Fig1. The structure of train set compounds

B. Generation of niacin receptor agonists set and virtual screening

The common basic structure types of niacin receptor agonists included picolinate and pyrazine carboxylic acid, acifran, pyrazole-3-carboxy and isoxazole. 6 sorts of functional molecular fragments were selected and showed in Fig.2.

Bioisosterism, which is a commonly used method for drug design, includes classical bioisosteres and non-classical bioisosteres [11]. Based on hydride displacement laws, the classical bioisosteres contain 5 kinds of types, namely univalent, (e.g. X,-OH,-CH3), bivalent (e.g. -CH2-,-O-,-NH2), trivalent (e.g. =N-, =CH-), tetravalent (e.g. =C=) and ring equivalence. The non-classical bioisosteres contain 3 kinds of types, namely ring and non-ring structure, exchangeable groups (e.g. -SONH2, -COOH), reverse groups (-COOR,-

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OCOR). Furthermore, studies were shown that carboxyl of picolinate and pyrazine carboxylic acid was essential group for activity. If carboxyl is replaced by amide or its location is changed, the activity of compounds will disappear. Therefore, functional molecular fragments of niacin receptor agonists were designed according to rules and constraints of bioisosterism.

The compounds in TCMD were searched by small molecular fragments which were gained by functional fragments and bioisosterism principle. Then the hits were screened by Lipinski's rule and niacin receptor agonists database were established. Finally, the compounds of niacin receptor agonists were minimized by conformation analysis and were screened by the best pharmacophore of CETP inhibitors. In this paper, virtual screening was performed using Search 3D Database module, wherein the search mode was set to Best method and other parameters were set to default.

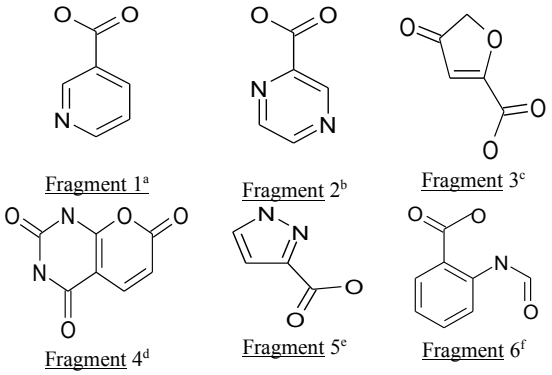

Fig.2 The functional fragments acting on niacin receptor

a: niacin; b: acipimox; c: acifran; d: pyridopyrimidine ketone; e: pyrazole-3-carboxy; f: anthranilate

III. Results and Discussion

A. Result of pharmacophore modeling

1) Pharmacophore model

Ten highest scoring pharmacophore models of CETP inhibitors were obtained, and the rank score value was from 158.90 to 145.28. The main features included hydrogen bond acceptor, hydrogen bond acceptor lipid, hydrophobic group, hydrophobic aromatic group and aromatic ring. The result was shown in Table 1.

2) Model Validation

A% value and CAI were calculated by testing set, and the result was shown in Table 1. Among the CETP hypothesis, the Rank score for CETP-1 and CETP-2 was higher and the occasionality of alignment between training set and pharmacophore was less. Besides, higher N value and CAI in CETP-1 was shown. So CETP-1 model was determined as the best pharmacophore model by comprehensively considering each parameter. This model included one hydrogen bond acceptor group, four hydrophobic groups and two aromatic ring group. In addition, N value was 4.07, A% value was 0.44 and CAI was 1.80. The best pharmacophore model was showed in Fig.3.

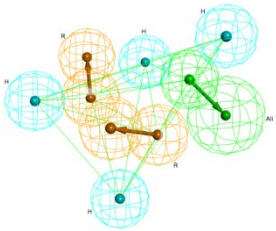

Fig 3. HipHop pharmacophore model of CETP inhibitors

TABLE 1. THE EVALUATION RESULTS FOR EACH PHARMACOPHORE HYPOTHESIS

Hypo	Feature	Rank score	Ha	Ht	A%	N	CAI
CETP-1	RR HHHH Ali	158.90	39	44	0.44	4.07	1.80
CETP-2	RRHHHHAli	150.85	36	43	0.41	3.84	1.57
CETP-3	RRHHHHA	148.85	36	45	0.41	3.67	1.50
CETP-4	HHHHHAli	148.85	38	43	0.43	4.06	1.75
CETP-5	HHHHHAliA	146.85	38	47	0.43	3.71	1.60
CETP-6	HHHHHAliA	146.16	39	43	0.44	4.16	1.85
CETP-7	HHHHHAA	146.11	38	53	0.43	3.29	1.42
CETP-8	RHaroHHHHAli	146.11	33	52	0.38	2.91	1.09
CETP-9	RHaroHHHHAli	145.45	38	56	0.43	3.12	1.35
CETP-10	RHaroHHHHAli	145.28	33	51	0.38	2.97	1.11

B. Virtual Screening

Six functional molecular fragments were modified using bioisosterism principle to obtain 22 novel molecular fragments. And molecular fragments as query structure were used to search TCMD, and 354 natural products were gained. Then 214 compounds were gained by Lipinski's rules and database of niacin receptor agonists was established after structure optimization. Five higher active compounds were obtained by CETP-1 three-dimensional pharmacophore searching. Partial of the hit was showed in Table 2.

TABLE 2. PARTIAL DUAL-TARGET COMPOUNDS

compound	source	fitvalue
Anisotine	Adhatoda vasica	1.41
Adhatodine	Adhatoda vasica	1.57

Anisotine and adhatodine are alkaloids derived from Adhatoda vasica Nees. Chemical structures of these two compounds, which were mapped with the best pharmacophore, were showed in Figure 4. Wherein, molecular fragment of

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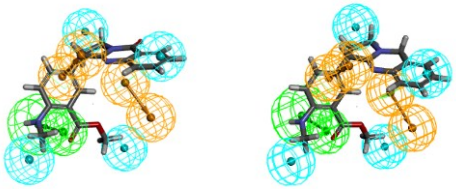
anthranilate was exposed in two molecules. Muhammad [12] used diabetic rats to research anti-diabetic activity of the roots and leaves of Adhatoda vasica Nees. The experimental results showed that ethanol extract of the roots of Adhatoda vasica Nees had a significant ability to increase HDL-C and decrease LDL-C.



Anisotine Adhatodine

Fig 4. Matching graph between hits and CETP-1 model

IV. Conclusions

In this study, bioisosterism principle, fragment screening and Lipinski's rules was used to construct database of niacin receptor agonists by preliminarily screen TCMD. Meanwhile, highly active CETP inhibitors were used to generate HipHop pharmacophore. The best pharmacophore model was used to screen niacin receptor agonist database for dual pharmacology natural products. These dual-target molecules of CETP and niacin receptor provide the basis and guidance for designing modulating-lipid drugs to increase HDL-C, reduce LDL-C and treat dyslipidemia.

Acknowledgment

Authors would like to acknowledge the financial support by the National Natural Science Foundation of China (No. 81173522).

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