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Pharmacophore Model Generation of thrombin Inhibitors

Xing Wang[1], Yanling Zhang[1], Yuhong Xiang[2], Zhenzhen Ren[1], Yanjiang Qiao[1]*

[1]School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing 100102, China; [2]Department of Chemistry, Capital Normal University, Beijing 100048, China.

Email: yjqiao@263.net

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ABSTRACT

Thrombin, an important factor of clotting system, take part in a variety of physiological actions, such as blood clotting, anticoagulation, thrombosis and fibrinolysis. Inhibiting thrombin is a pivotal and effective step for the prophylaxis of venous and arterial thrombosis, as well as prevent myocardial infarction for high-risk patients. In this study, a three dimensional pharmacophore model was generated for the molecules which are responsible for vasodilation activities targeting thrombin. Ten compounds with known thrombin-inhibiting activity values were selected as training set to generate the hypothesis using GALAHAD program in SYBYL 7.0 software. The best hypothesis comprises five pharmacophore features: four hydrophobes groups and one positively charged group. It has been further validated towards a test set including known activity compounds obtained from Binding Database, the values of effectively active hit A% and comprehensive evaluation index CAI are respectively 63.33% and 2.34, the pharmacophore was proven to be successful in discriminating active and inactive inhibitors. Furthermore, the pharmacophore model was used as a 3D query to screen TCMD (Version 2009) database and 6 hit compounds of higher predicted activity were the reported cardiovascular activities, which may be useful for further study.

Keywords: Cardiovascular Disease; Thrombin; Pharmacophore; Virtual Screening

1. Introduction

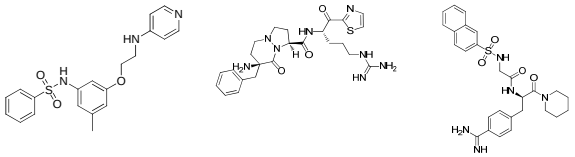
Thrombin, a "trypsin-like" serine protease protein, is an important factor of clotting system. It take part in a variety of physiological actions, such as blood clotting, anticoagulation, thrombosis and fibrinolysis [1-3]. Moreover, previous studies have demonstrated that thrombin is one of the key factors for cancer developing, such as PAR1, an important type of thrombin, mediates the enhancement effects of thrombin in angiogenesis, invasion and metastasis, growth of cancer [4-6]. These facts have raised great interests to find an effective, safe, and orally available thrombin inhibitors, such as hirudin [7], dabigatran [8], rivaroxaban and apixaban [9], which could be useful anticoagulant drugs for the prophylaxis of venous and arterial thrombosis, as well as prevent myocardial infarction for high-risk patients [10].

In this paper, we have developed a pharmacophore model whose purpose is to identify the critical pharmacophoric features necessary for potent thrombin inhibitors. Further, hypothesis was evaluated by a test set with known activity compounds, the effectively active hit A% and comprehensive evaluation index CAI were used to assess the model. Finally, the pharmacophore model was used as a 3D query to screen TCMD (Version 2009) database, 52 compounds were hit and the 6 hit compounds of higher predicted activity were the reported cardiovascular activities, which may be useful for further study.

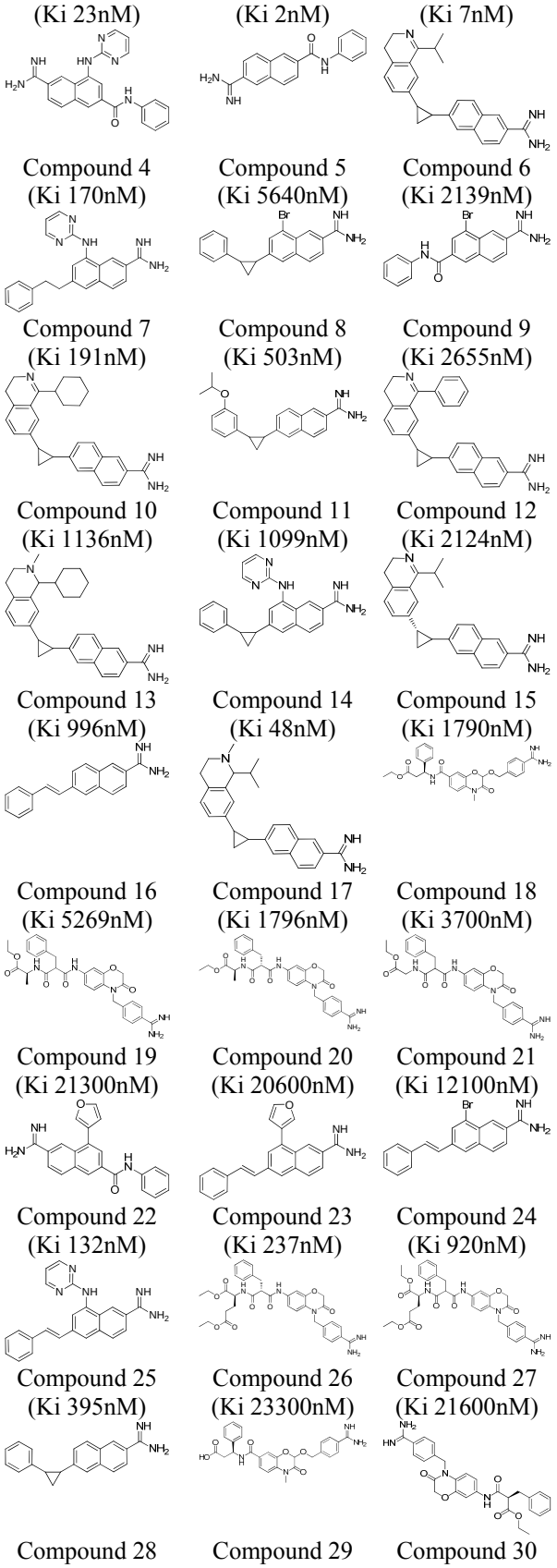
2. Materials and Methods

2.1. Compounds and biological data

Compounds 1～30, which can inhibit thrombin, were taken from the literatures [11-13] and served as the database in the pharmacophore modeling. The structures and inhibitory activities are listed in Figure 1. The chemical structures were drawn in ISIS-Draw software and saved in SYBYL mol2 format, then all the 2D structures were converted to 3D structures in SYBYL 7.0 software.



Compound 1 Compound 2 Compound 3



(Ki 1890nM) (Ki 14900nM) (Ki 16300nM)

Figure 1. Chemical structure of thrombin inhibitors.

2.2. Modeling tool

All the pharmacophore modeling calculations were carried out by using the GALAHAD module implemented in SYBYL 7.0 (Tripos Inc.) software, the UNITY module was used to perform a flex search for the potential inhibitors. All the tasks were running on Xeon Intel(R) X5460 CPU 3.16 GHz, RAM Memory 12 GB under the Windows XP system.

2.3. Pharmacophore studies

GALAHAD (Genetic Algorithm with Linear Assignment of Hypermolecular Alignment of Datasets) uses Tripos' proprietary technology [14-15] to generate pharmacophore hypotheses and alignments from sets of ligand molecules that bind at a common target site. Before establishing the model, all the structures of ligands were checked, hydrogen atoms were added and a minimization procedure was implemented using the MMFF94 force-field. GALAHAD was run for 130 generations with a population size of 100. The rest of the parameters were set as default values. Only models with 8 ligands with contribution to the consensus feature were considered. The generated models were evaluated by a test database containing 111 compounds, including 30 active compounds and 81 non-active compounds.

2.4. Virtual Screening

The pharmacophore models built within the Catalyst software can be used as queries for 3D database screening. Virtual screening of such databases can serve two main purposes: first, validating the quality of the generated pharmacophore models by selective detection of compounds with known thrombin inhibitory activity, and second, finding novel, potential leads suitable for further development. In this study, the best pharmacophore model was used to search TCMD (Version 2009) to quest for the potential inhibitors of thrombin from traditional Chinese medicine.

3. Results and Discussion

3.1. Generation of pharmacophore hypotheses

GALAHAD models were derived by using ten active ligands as a training set [compounds 4-6, 8, 9, 15, 17, 18, 25, 28], 20 pharmacophore models were derived by using the training set after GALAHAD run. All the 20 models were evaluated successively by the test database constructed previously. Table 3 shows the predictable results of the test database for models with all the eleven ligands with contribution to the consensus feature.

In order to intuitively understand the meaning of indicators used to evaluate the performance of the models, the schematic diagram was listed (Figure 2) and the parameter values for each pharmacophore model generated were listed in Table 1. D is for the total number of compounds in test database and A represents the number of active compounds. Ht is the total number of hit compounds from test database and Ha represents the number of active hit compounds from test database, A% represents the ability to identify active compounds from test database, Y% represents the proportion of active compounds in the hit compounds. N, the index of effective identification, is used to evaluate the ability of the models to identify active compounds from the non-active compounds. CAI, a comprehensive evaluation index, is used to identify the best pharmacophore model. Higher value of CAI is considered to be the better model. In this study, MODEL_016, with the highest value of CAI was considered to be the best model.

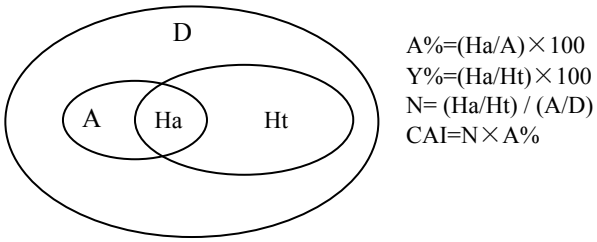


Figure 2. The schematic diagram of indicators used to evaluate the pharmacophore model

Table 1. The parameter values for each pharmacophore model

	Ht	Ha	A%	Y%	N	CAI
MODEL_01	12	12	40.00	100.00	3.70	1.48
MODEL_02	17	17	56.67	100.00	3.70	2.10
MODEL_03	14	14	46.67	100.00	3.70	1.73
MODEL_04	19	19	63.33	100.00	3.70	2.34
MODEL_05	9	9	30.00	100.00	3.70	1.11
MODEL_06	29	21	70.00	72.41	2.68	1.88
MODEL_07	17	17	56.67	100.00	3.70	2.10
MODEL_08	16	16	53.33	100.00	3.70	1.97
MODEL_09	6	6	20.00	100.00	3.70	0.74
MODEL_10	19	19	63.33	100.00	3.70	2.34
MODEL_11	17	17	56.67	100.00	3.70	2.10
MODEL_12	19	19	63.33	100.00	3.70	2.34
MODEL_13	19	19	63.33	100.00	3.70	2.34
MODEL_14	19	19	63.33	100.00	3.70	2.34
MODEL_15	14	14	46.67	100.00	3.70	1.73
MODEL_16	**19**	**19**	**63.33**	**100.00**	**3.70**	**2.34**
MODEL_17	11	11	36.67	100.00	3.70	1.36
MODEL_18	17	17	56.67	100.00	3.70	2.10
MODEL_19	14	14	46.67	100.00	3.70	1.73
MODEL_20	13	13	43.33	100.00	3.70	1.60

MODEL_016 is displayed in Figure 3, cyan and red spheres indicate hydrophobes group and positively charged charged group, respectively. MODEL_016 includes five pharmacophore features: four hydrophobes groups and one positively charged group.

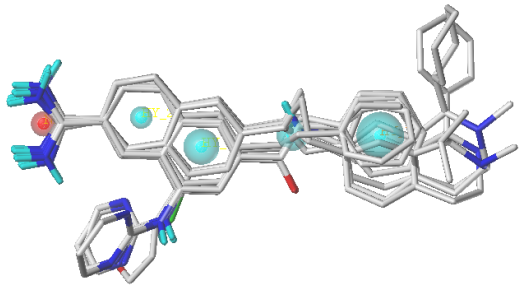


Figure 3. Pharmacophore MODEL_016 and molecular alignment of the compounds used to elaborate the model.

3.2. Virtual Screening

Pharmacophore MODEL_016 was used to screen TCMD (Version 2009). TCMD screening yielded a hit list of 52 compounds and the 6 hit compounds of higher predicted activity (Bufotoxin, Cyclovirobuxine, Cyclovirobuxine D, Leurosine, Methoxyadiantifoline and Thaliadanine) were the reported cardiovascular activities, such as vasodilator, anti-arrhythmic, cardiac, coronary vasodilator and anti-hypertension, et al. Cyclovirobuxine mapped to MODEL_016 is showed in Fig 4.

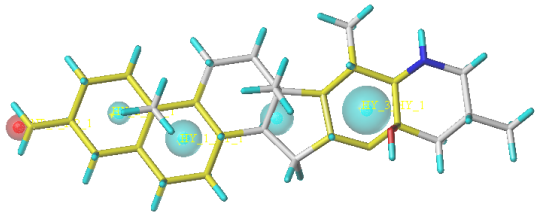


Figure 4. MODEL_016 mapped with Cyclovirobuxine

4. Conclusions and Future Work

In this study, we have built the pharmacophore model of thrombin inhibitors using GALAHAD module implemented in SYBYL 7.0 software. The optimal pharmacophore model contains 5 pharmacophore features: four

hydrophobes group and one positively charged charged group. The external validation shows that the values of effectively active hit A% and comprehensive evaluation index CAI are respectively 63.33% and 2.34, which can prove that the pharmacophore model is reliable and available. The virtual screen results of TCMD (Version 2009) database shows that the pharmacophore model has the ability of determining vasoactive compound related to thrombin, and the validation experiments needs to be further studied.

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