Pharmacophores Generation of multiple Anti-inflammatory Targets and Identification of Active Compounds in Chinese herbs

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***Abstract*—Chinese herbs always have activity on multiple targets. For the identification of potential anti-inflammatory compounds from Chinese herbs, six targets, which are mostly associated with inflammatory, were selected as following: Cox-2 (cyclooxygenase 2), PDE4B (phosphodiesterase 4B), p38α MAPK (p38α mitogen-activated protein kinase), JNK3 (c-Jun N-terminal kinases 3), ICE (interlenkin-1β converting enzyme) and iNOS (inducible type of nitric oxide synthase). Structure-based pharmacophore models of the inhibitors of each target were generated by LigandScout based on complexes from the PDB (Protein Data Bank). Based on the screening results of MDDR (MDL Drug Data Report database) and a new metric CAI (comprehensive appraisal index), the best models for each target were defined and used to identify the potential anti-inflammatory compounds from Chinese herbs. Six compounds and sixteen herbs were obtained that can act on multiple targets. The traditional function of the most hit herbs was 'heat-clearing and detoxifying', which has been experimentally demonstrated to have anti-inflammatory activity.**

Keywords- Structure-based pharmacophore; Multiple targets; anti-inflammatory; Chinese Herbs; Cyclooxygenase 2; Phosphodiesterase 4B; Interleukin-1β converting enzyme; p38α mitogen-activated protein kinase; c-Jun N-terminal Kinases 3; inducible type of Nitric Oxide Synthase

I. INTRODUCTION

Inflammation is one of the most common and complex pathological processes in human diseases. By drugs acting on one target, the anti-inflammatory effect maybe not very clear and always accompanied with side effects. Chinese herbs can act on multiple targets by multiple ingredients, which make wide anti-inflammatory effect and fewer side effects. Structure-based pharmacophore (SBP) model is constructed by interpreting the interaction between the target and ligand. The generation process of the SBP models is quickly and the models possess a high specificity. Based on the anti-inflammatory mechanism, the following targets were selected: Cyclooxygenase 2 (Cox-2), Phosphodiesterase 4B (PDE4B), p38α mitogen-activated protein kinase (p38α MAPK), c-Jun N-terminal kinases 3 (JNK3), Interlenkin-1β converting enzyme (ICE) and inducible type of nitric oxide synthase (iNOS). The SBP models of six targets were generated and the active compounds in Chinese herbs were then identified.

COX-2 is an important catalytic enzyme in the arachidonic acid metabolism pathway and mediates the inflammatory reaction [1]. IL-1β, which is associated with a variety of inflammatory diseases, was converted by ICE [2]. JNK3 is principally present in the brain and cardiac muscle [3], and activated in various inflammatory diseases. PDE4B inhibitors have anti-inflammatory activity, and can avoid the side effects caused by the inhibition of PDE4D by nonselective inhibitors [4]. P38α MAPK plays a key role in the inflammatory cytokine biosynthesis pathway, and can active or increase the inflammatory response [5]. The induction of iNOS can increase the synthesis of large amount of NO, which can cause inflammation, asthma, and other diseases. According to the inhibition mechanism, the iNOS inhibitor can be divided into L-arginine competitive inhibitor and specificity cofactor antagonists. In present study, L-arginine competitive inhibitors were used for the SBP model generation. The inhibitors of COX-2, ICE, JNK3, PDE4B, P38α MAPK and iNOS are therefore expected to retain anti-inflammatory efficacy.

II. DATA AND METHOD

A. Pharmacophore model generation

The structures of the complexes of the targets and ligands were collected from the Protein Data Bank (PDB) database for the pharmacophore models generation. In present study, 17 human PDE4B complexes, 10 mouse COX-2 complexes, 29 human p38α MAPK complexes, 8 human ICE complexes, 38 mouse iNOS complexes and 25 human JNK3 complexes were selected, and all structures were analyzed by x-ray diffraction method with a resolution of less than 3Å (listed in table I).

LigandScout software was used to construct the SBP models by identifying the pharmacophore features and excluded volume between the ligands and receptors. Database searching ability was used to evaluate the SBP models. In present study, a metric CAI value (Comprehensive Appraisal Index) was used to define the best models, which was

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described as CAI=E×A%=(Ha/A)× (Ha/Ht)/(A/D), where the enrichment factor (E) and percent ratio of the actives in the hit list (A%) were both the most common used metrics for the evaluation of pharmacophore models [6], and D is the number of compounds in the database, A is the number of active compounds in the database, Ht is the number of compounds in the hit list, and Ha is the number of active compounds in the hit list.

B. In silico screening

In present study, MDDR (MDL Drug Data Report: Version 2007.2) was used as the testing database to evaluate the pharmacophore models, and the higher CAI value is considered to be the better model. The MDDR contains 177,981 molecules, in which 293 compounds with clearly labeled PDE4 inhibition activity, and 972 COX-2 inhibitors, 238 p38α MAPK inhibitors, 411 ICE inhibitor, 523 mouse L-arginine competitive iNOS inhibitors, 61 JNK3 inhibitors. Then the best SBP models will be used to screen the Traditional Chinese Medicine Database (TCMD 2009), which contains 23033 compounds from the Chinese herbs.

For all the compounds in both databases, a maximum of 250 3D low energy conformers were generated in Catalyst software. The pharmacophore models were then adjusted and converted into Catalyst software, and the BEST Flexible Searching method was performed for virtual screening.

III. Results and Discussion

The CAI values of each pharmacophore models were shown in Table I. The best models with the highest CAI value of each target were also selected. The best model of COX-2 inhibitors was generated from the 3LN0 complexes, while 1W84 for P38α MAPK, 1RWM for ICE, 1DF1 for iNOS, 3FI2 for JNK3, 1XMU for PDE4B, with the CAI vales were 1.72, 8.07, 3.49, 4.68, 5.44, 9.55 respectively (Shown in Figure 1). The best models were then performed for the virtual screening.

1091 compounds were hit by the six SBP models. Only six compounds have activity on multiple targets (listed in Table II). The hit list was further analyzed to find the source Chinese herbs that contained the potential active compounds. As listed in Table III, sixteen herbs were obtained the compounds that can act on multiple targets. The traditional function of the herbs was then discussed. The hit Chinese herbs mostly have the efficacy of 'heat-clearing and detoxifying', which has been experimentally demonstrated to have anti-inflammatory activity.

In summary, six targets were selected in present study, and the SBP models of the COX-2 inhibitors, p38α MAPK inhibitor, ICE inhibitor, JNK3 inhibitor, iNOS inhibitor and PDE4B inhibitor were generated and used for the virtual screening for potential anti-inflammatory compounds from Chinese herbs. Six compounds and sixteen herbs were obtained that can act on multiple targets. Molecular docking and ADME/T prediction can be furtherly used to increase the accuracy of the identification. In vitro or in vivo experiments should also be carried out to estimate the anti-inflammatory activity of hit compounds.

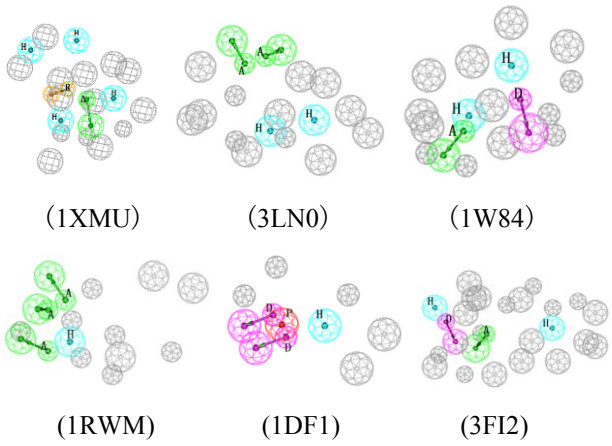

Fig. 1. Pharmacophore models of anti-inflammatory targets. (best model of PDE4B inhibitor (1XMU), COX-2 inhibitor (3LN0), P38α MAPK inhibitor (1W84), ICE inhibitor (1RWM), iNOS inhibitor (1DF1) and JNK3 inhibitor (3FI2); A means Hydrongen Bond Acceptor, D means Hydrogen Bond Donor, H means Hydrophobic group, P means Postive Ionizable group)

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TABLE I FEATURES OF THE BEST PHAMACOPHORE MODELS

PDB ID	*Features*	*Ha*	*Ht*	*CAI*	*PDB ID*	*Features*	*Ha*	*Ht*	*CAI*	*PDB ID*	*Features*	*Ha*	*Ht*	*CAI*
COX2														
1PXX	3H1A	677	88553	0.98	3NT1	2H2A	595	40556	1.64	3PGH	3H1A	620	49528	1.46
3LN0	2H2A	700	53532	1.72	3NTB	2H2A	492	32259	1.41	4COX	3H1A	499	45425	1.03
3LN1	2H2A	512	39328	1.26	3NTG	3H1A	634	57286	1.32	6COX	3H1A	584	41678	1.54
3MQE	2H2A	680	66516	1.31										
p38α MAPK														
1A9U	3H1R	158	46269	1.70	1OVE	1R1A2H	116	52021	0.81	1WBW	1D1A2H	108	6879	5.33
1BL6	3H1A	155	27254	2.77	1OZ1	2H2A	168	37290	2.38	1YQJ	3H1A	154	29937	2.49
1BL7	2H2A	152	23351	3.11	1W7H	1A2H1D	107	13232	2.72	1ZYJ	2H2A	124	26097	1.85
1BMK	3H1A	172	53168	1.75	1W82	3H1A	138	32446	1.84	1ZZ2	1D3H	63	8027	1.55
1DI9	3H1A	195	71567	1.67	1W83	2H2A	103	15579	2.14	1ZZL	3H1A	124	22440	2.15
1KV1	3H1A	180	65911	1.54	1W84	1D1A2H	138	7413	8.07	2BAJ	3H1A	146	34704	1.93
1KV2	2H2A	110	9626	3.95	1WBN	2H2A	119	15558	2.86	2BAK	3H1D	116	29457	1.44
1M7Q	1A2H1R	138	60626	0.99	1WBS	1A1D2H	104	15028	2.26	2BAL	1A1D2H	106	18045	1.96
1OUK	3H1A	134	28474	1.98	1WBT	2H2A	116	20270	2.09	2BAQ	1D1A2H	110	14084	2.70
1OUY	3H1A	138	44478	1.35	1WBV	1D3H	128	26307	1.96					
ICE														
1RWK	1D4A	171	17535	1.76	1RWO	3A1H1D	160	8614	3.13	1RWX	2A1R2H	159	10000	2.66
1RWP	3H1A	106	6717	1.76	1RWM	3A1H	139	5841	3.49	1BMQ	1H4A	113	6505	2.07
1RWN	1H4A	243	41146	1.51	1RWW	1D1H3A	229	53216	1.04					
iNOS														
1N2N	2A2P	83	2863	1.57	2NOS	2D2P	0	5	0	3E6L	1A1D2H	140	33947	0.38
1DD7	3H	310	136178	0.46	2ORO	4H	168	74063	0.25	1R35	3A3D2P1N1H	156	3477	4.55
1DF1	1H1P2D	55	421	4.68	2ORP	4H	166	73345	0.24	3E6O	2D2H	83	3415	1.31
1DWW	1A2D1P	61	1274	1.90	2ORQ	1D2H	385	90850	1.06	3E6T	1P3H	230	27689	1.24
1M8D	1A1D1H	348	94220	0.84	2ORR	3H	313	137723	0.46	3E7I	2H2D	147	5041	2.79
1M8E	1D1P1H	200	16415	1.59	2ORS	3H	311	139154	0.45	3E7T	1P3H	236	28133	1.29
1M9T	1D1P1H	199	23671	1.09	2ORT	3H	311	138119	0.46	3EAI	1A1D1H	304	72070	0.83
1QW6	2P1H1A	117	3892	2.29	2Y37	1P1A2H	151	26933	0.55	3EBD	HDDH	142	3852	3.41
1QWC	2D2H	183	10136	2.15	3E65	2H2D	137	4262	2.87	3EBF	1D2P1H	8	440	0.09
3E6N	2D2H	89	4322	1.19	3E67	2D1H	174	11273	1.75	3NOD	2A2P	71	1596	2.06
1VAG	1R1P3H	83	4750	0.94	3E68	2D1H	178	11804	1.75	3NQS	1A1D1H	275	87672	0.56
2BHJ	1A2H	333	154434	0.47										
JNK3														
1PMN	3H1A	34	21715	2.55	2P33	1A2H1D	29	12909	3.12	3FV8	3H1A	46	65200	1.55

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1PMQ	3H1A	42	27055	3.12	2R9S	3H1A	34	16803	3.29	3G90	1D3H	25	6763	4.42
1PMU	2A2H	42	34866	2.42	2WAJ	3H1A	40	35586	2.15	3G9L	3H1A	38	27724	2.49
1PMV	2H1D1A	39	18783	3.87	2ZDT	3H1A	39	46570	1.56	3KVX	4H	26	54826	0.59
2B1P	3H1D	27	14229	2.45	2ZDU	2A2H	38	33725	2.05	3OY1	1D3H	37	17019	3.85
2EXC	3H1A	38	28054	2.46	3DA6	1D1A2H	22	29863	0.78	3PTG	3H	48	113141	0.97
2O0U	2H1D1A	27	13285	2.62	3FI2	1A2H1D	19	3174	5.44	3TTI	3H1A	38	24587	2.81
2O2U	2H1A1D	21	7074	2.98	3FI3	3H1A	44	45570	2.03	3TTJ	2H2A	34	25883	2.14
2OK1	1D2H1A	35	22027	2.66										
PDE4B														
3G45	3H	284	129063	1.30	2QYL	3H1N	26	9669	0.15	3LY2	2A3H	165	14104	4.00
1RO6	2A2H	206	27448	3.21	3HMV	1A1D2H1R	25	1953	0.66	1XMU	1A4H1R	102	2258	9.55
1XLX	3A1H	197	29060	2.77	1XM4	2A2H1R	131	8009	4.44	1Y2H	1A5H	87	3668	4.28
1XLZ	2A1H1N	17	5203	0.12	1XM6	2A3H	127	4729	7.07	1Y2J	1A4H1P	8	550	0.24
1XOS	2A2H	223	45356	2.27	3D3P	1A3H1R	150	5804	8.04	3GWT	1A1D3H1R	9	597	0.28
1XOT	1A3H	251	67709	1.93	3FRG	1A1D1H1R	98	18625	1.07					

TABLE II FEATURES OF THE BEST PHAMACOPHORE MODELS

	COX2	ICE	JNK3	P38	PDE4B
Artemisetin			√	√	√
Danshenxinkun D	√		√		
3',4'－Dihydroxywogonin			√		√
Isoarcapillin			√	√	√
Polygalaxanthone VI	√	√	√		
Triptinin A	√				√

TABLE III FEATURES OF THE BEST PHAMACOPHORE MODELS

Herb (pinyin)	Species	COX2	ICE	JNK3	PDE4B	P38
Bei Mu	*Fritillaria cirrhosa* D. Don, *Fritillaria unibracteata* Hsiao et K.C. Hsia, *Fritillaria przewalskii* Maxim., *Fritillaria delavayi* Franch.	√	√			
Chai Hu	*Bupleurum chinense* DC., *Bupleurum scorzonerifolium* Willd.	√	√			
Chen Pi	*Citrus reticulate* Blanco			√	√	√
Chuan Xiong	*Ligusticum chuanxiong* Hort.	√		√		
Chuan Xin Lian	*Andrographis paniculata* (Burm. F.) Nees	√	√	√		
Dan Shen	*Salvia miltiorrhiza* Bge.	√		√	√	
Fang Feng	*Saposhnikovia divaricate* (Turcz.) Schischk.		√	√		
Gan Cao	*Glycyrrhiza uralensis* Fisch., *G.* inflate Bat., *G. glabra* L.	√	√	√		√
Huang Hua Hao	*Artemisia annua* L.	√		√	√	√
Huang Qin	*Scutellaria baicalensis* Georgi			√	√	√
Huo Xiang	*Agastache rugosa* (Fisch. Et Mey.) O. Ktze.			√	√	√

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Yan Hu Suo	*Corydalis yanhusuo* W. T. Wang	√			√	
Yin Chen Hao	*Artemisia capillaris* Thunb., *Artemisia scoparia* Waldst. Et Kit.			√	√	√
Yin Yang Huo	*Epimedium brevicornum* Maxim., *Epimedium sagittatum* (Sieb. et Zucc.) Maxim., *Epimedium pubescens* Maxim., *Epimedium wushanense* T.S.Ying, *Epimedium koreanum* Nakai		√		√	√
Zhi Zi	*Gardenia jasminoides* Ellis		√	√	√	√
Lei Gong Teng	*Tripterygium wilfordii* Hook f.	√	√		√	

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