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Network pharmacology-based prediction of the active ingredients and potential targets
of Mahuang Fuzi Xixin decoction for application to allergic rhinitis

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Abstract

Ethnopharmacological relevance: Certain herbal formulae from Traditional Chinese Medicine (TCM) are effective for treating and preventing diseases in clinical practice. Mahuang fuzi Xixin Decoction (MFXD) is a TCM that is used to treat allergic rhinitis (AR); however, the active ingredients and potential targets of its action against AR remain unclear. Therefore, further investigation is required.

Methods: A network pharmacology approach comprising drug-likeness evaluation, oral bioavailability prediction, multiple drug target prediction, and network analysis has been used in this study.

Results: The comprehensive systematic approach was successfully to identify 41 bioactive ingredients in MFXD, while 37 potential targets hit by these ingredients related to AR. Moreover, wherein four predicted ingredients possess anti-inflammatory effects were found by this technique.

Conclusions: Our works successfully predict the active ingredients and potential targets of MFXD for application to allergic rhinitis and helps to illustrate mechanism of action on a systematic level. This study not only provides new insights into the chemical basis and pharmacology of MFXD but also demonstrates a feasible method for discovering potential drugs from herbal medicine.

Keywords: Traditional Chinese Medicine, Mahuang Fuzi Xixin Decoction, Allergic rhinitis, Network pharmacology

Chemical compounds studied in this article:

Pseudoephedrine (PubChem CID: 7028);

Herbacetin (PubChem CID: 5280544);

Salsolinol (PubChem CID: 91588).

Dibutyl phthalate (PubChem CID: 3026).

1. Introduction

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa, induced by an immunoglobulin E (IgE)-mediated reaction in allergen-sensitized subjects. The most frequent symptoms include sneezing, rhinorrhea, nasal congestion, and nasal pruritus. AR can diminish quality of life in some patients and it generates considerable costs to society (Canonica et al., 2007; Schatz, 2007; Schoenwetter et al., 2004). The current treatments for AR, which are limited to nasal corticosteroid, antihistamine, and anti-allergen and anti-leukotriene immunotherapy, could be improved, and the search for more effective drugs is ongoing (Leung and Hon, 2008). Traditional Chinese Medicine (TCM) is an attractive area in which researchers could develop multi-target combination drugs for prevention and treatment of AR (Yang et al., 2012; Yen et al., 2015). As a matter of fact, TCM contain numerous active ingredients and target several diverse genes or proteins, it is too complex to be detected solely by conventional experimental method (Wang et al., 2013). There is an urgent need to develop new and appropriate approaches to address this problem.

Network pharmacology, combined with pharmacology and pharmacodynamics, is a novel research field which is implicated in the application of omics and systems biology-based technologies (Liang et al., 2014). As a major tool in network pharmacology, the network analysis based on widely existing databases allows us to form an initial understanding of the action mechanisms within the context of systems-level interactions (Liu and Sun, 2012; Tao et al., 2013). Since TCM herbal formula has been considered as a multi-component and multi-target therapeutics which potentially meets the demands of treating a number of complex diseases in an integrated manner, the methodologies of network pharmacology are suitable for pursuing a priori knowledge about the combination rules embedded in formula (Li et al., 2010). Thus the application of network

pharmacology to TCM provides new possibilities to understand the interactions among active interactions and relevant targets which in turn highlight the mechanism of action (Tao et al., 2013; Zhao et al., 2015). It is also a promising method for discovering potential drugs from herbal medicine (Hasan et al., 2012; Li et al., 2014)

Mahuang Fuzi Xixin Decoction (MFXD) is an effective treatment for allergic reactions and inflammation related to hypersensitivity (Type I and IV), as demonstrated in previous experiments (Hashimoto et al., 1994; Hikino et al., 1980a). MFXD is an extract of an herbal prescription consisting of three kinds of herbal medicines, *Herba Ephedrae* (Mahuang in Chinese, the dried herbaceous stems of *Ephedra sinica* Stapf., *Ephedra intermedia* Schrenk & C.A. Mey. or *Ephedra equisetina* Bunge), *Radix Aconiti Lateralis* (Fuzi in Chinese, the lateral root of *Aconitum carmichaelii* Debeaux) and *Asarum heterotropoides* (Xixin in Chinese, the root and rhizome of *Asarum heterotropoides* f. *mandshuricum* (Maxim) Kitag.) in a ratio of 4:1:3, which was first described in *Shokanron*, one of the Chinese medicinal classics. MFXD has been widely used in Asia over many years for treating the symptoms of cold, nasal congestion, and especially AR. Both Mahuang and Xixin are known to have anti-allergic activity and Fuzi exerts an anti-inflammatory effect (Hashimoto et al., 1994; Hikino et al., 1980a; Hikino et al., 1980b). MFXD significantly inhibited carrageenin-induced hind foot edema and cotton pellet-induced granulation tissue growth in rats, it may exert anti-inflammatory effects through preventing mediator release from mast cells and macrophages (Ikeda et al., 1998). MFXD also inhibited the IL-4 and IFN- γ production, it may exert immunomodulation effects (Ikeda et al., 2000). However, three fundamental questions regarding MFXD remain unanswered, including the following: 1) what are the active ingredients of the formula, 2) what are the specific targets of the active ingredients, 3) what are new discoveries of

MFXD using network pharmacology technique.

In the present work, a network pharmacology approach, combining drug-likeness evaluation, oral bioavailability prediction, multiple drug target prediction as well as other network pharmacology techniques, was employed to attempt to answer these questions. Specifically, we identified the active ingredients of MFXD and their targets when it is used to treat AR. Moreover, wherein four predicted ingredients possess anti-inflammatory effects were firstly found by this technique.

2. Materials and methods

2.1 Database building

We compiled the identities of the chemical ingredients in the three medicines that MFXD is composed of by using (1) TCM Database@Taiwan (<http://tcm.cmu.edu.tw/>) and (2) Traditional Chinese Medicines for Systems Pharmacology Database and Analysis Platform (TCMSP, available online: <http://sm.nwsuaf.edu.cn/lsp/tcmsp.php>) (Ru et al., 2014).

2.2 Oral bioavailability prediction

For an administered drug, Oral bioavailability (OB) describes the ratio of the amount absorbed into systemic circulation when dosed orally compared to the amount in blood upon intravenous dosing. OB is a good indicator of the efficiency of oral administration for drug delivery into systemic circulation (Liu et al., 2013), and an essential pharmacokinetic parameter for screening candidate compounds that have the potential to be further developed into drugs (Li et al., 2012b). A reliable in silico model OBioavail 1.1 (Xu et al., 2012) which integrates the metabolism (P450 3A4) and transport (P-glycoprotein) information was used to predict the OB value of herbal ingredients. The optimal model was based on a dataset of 805 structurally diverse drug and drug-like molecules that have been critically evaluated for their OB in humans. We selected $OB \geq 30\%$ as a screening threshold

(the OB properties of all compounds in MFXD are presented in the TcmSPTM). The OB threshold used here was chosen mainly because (1) it enables maximum extraction of information from MFXD with the least number of components, and (2) the model obtained using this threshold can be reasonably explained by the reported pharmacological data.

2.3 Drug-likeness prediction

Drug-likeness (DL) is a qualitative property of chemicals that describes how the pharmacokinetic and pharmaceutical properties of compounds, such as solubility and potency, correspond in the majority of known drugs (Walters and Murcko, 2002). DL analysis is used to evaluate whether a compound is drug-like or not. In order to identify drug-like compounds, we applied a database-dependent model using the Tanimoto coefficient (Yamanishi et al., 2010) to calculate the DL (see Eq. (1)) of each compound in MFXD.

$$T(x, y) = \frac{x \cdot y}{\|x\|^2 + \|y\|^2 - x \cdot y} \quad (1)$$

In this equation, x represents the molecular parameters of herbal ingredients, and y represents the average molecular properties of 6511 molecules in DrugBank database (<http://www.drugbank.ca/>). The DrugBank database is a unique bioinformatics and cheminformatics resource that currently contains 6511 compounds, which are either Food and Drug Administration (FDA) approved drugs or chemicals undergoing clinical trials that will potentially become licensed drugs. We removed any molecule with a DL index < 0.1 . We assessed both of these variables because only those compounds with OB and DL above our thresholds are likely to exert desirable pharmaceutical activities. The molecules that satisfied both DL and OB thresholds were regarded as candidate compounds.

2.4 Target prediction

Predicting interactions between drugs and target proteins can help decipher the underlying

biological mechanisms. Compound-target mappings were obtained from two sources. Experimentally validated drug-target pairs were retrieved from the HIT database (<http://lifecenter.sgst.cn/hit/>). For compounds without validated targets, a reliable in silico SysDT model (Yu et al., 2012) (which efficiently integrates a large amount of chemical, genomic, and pharmacological data, and applies two powerful methods: Random Forest (RF) and Support Vector Machine (SVM)) was used to predict potential targets. The dataset used to build models comprised 6511 drugs and 3987 targets with known compound–protein interactions in the DrugBank database. Here, the compound-target interactions with SVM score ≥ 0.8 and RF score ≥ 0.7 were extracted for further research. Finally, all targets obtained from the above two steps were sent to Therapeutic Target Database (TTD; <http://bidd.nus.edu.sg/group/cjttd>) and PharmGKB (available online: <http://www.pharmgkb.org>) databases to eliminate the noises and overlaps.

2.5 Network construction and analysis

Drug-target and target-disease networks can be used to identify active ingredients and potential targets, understand action mechanisms, and explore new clinical applications (Gu et al., 2011). We constructed our networks as follows: (1) the ‘candidate compound–candidate target (cC–cT) network’ was constructed by linking candidate compounds with all of their candidate targets; (2) the ‘potential compound–potential target (pC–pT) network’ was established by connecting potential compounds with their potential targets, which were related to inflammation or regulation of the immune system; and (3) in the ‘target–disease (T–D) network’, the potential targets were connected with related diseases obtained from the PharmGKB and TTD. Cytoscape (<http://www.cytoscape.org/>) was used to generate all networks, and the topological properties of the networks were analyzed using Network Analyzer.

2.6 Anti-inflammatory effects of four predicted ingredients

Four predicted ingredients (M137, M188, M234 and M289) were investigated by using the production of prostaglandin E2 (PGE₂) in IgE-activated rat basophilic leukemia (RBL-2H3) cells. The compounds for the experiment were purchased from the National Institute for Food and Drug Control (Beijing, China; purity $\geq$ 99.0%) and Forever-Biotech(Shanghai, China, purity $\geq$ 99.0%), the RBL-2H3 cells were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China) . The RBL-2H3 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (both from Gibco, Carlsbad, USA) and antibiotics (100U/mL penicillin and 100 μ g/mL streptomycin) at 37°C and in 5% CO₂ conditions. The cells were first incubated in a 24-well plate (2 $\times$ 10⁵ cells/well) at 37°C overnight. Subsequently, the cells were washed with PBS and then further incubated with dinitrophenol (DNP) -IgE (with a final concentration of 10 μ g/mL) for 24 h. The cells were then incubated with M137, M188, M234, and M289 for 1 h, before 10 μ L of antigen (DNP-bovine serum albumin (BSA) with a final concentration of 100ng/mL) was added to each well and they were incubated at 37°C for another 4 h. Finally, the supernatant was collected, and the inhibitory activity of the compounds was determined by analyzing PGE₂ levels using an enzyme-linked immunosorbent assay kit (Bangyi Technologies Inc., Shanghai, China).

3. Results and discussion

3.1 Oral bioavailability and drug-likeness

We identified 367 chemicals, including 234 in Mahuang, 65 in Fuzi, and 68 in Xixin. Detailed information about these ingredients is provided in Table S1. In this study, we identified 54 candidate compounds (Table S1) from the 367 chemicals (14.71%) that possessed both sufficient DL ($\geq$ 0.10)

and OB ($\geq 30.0\%$). Some compounds of them have already been experimentally validated with various biological activities. For examples, β -sitosterol (M298, DL=0.75, OB=45.04%) exerts anti-inflammatory effect through suppressing oedema formation by 52% with dose of 0.5 mg/ear in mice model (Navarro et al., 2001), and the anti-inflammatory activity of β -sitosterol is involved in arachidonic acid cascade (Moreno, 2003). In addition, luteolin (M231, DL=0.25, OB=36.16%) can block the MAPK and NF- κ B signaling pathways to inhibit the inflammatory mediators release, and then exhibits an anti-inflammatory effect (Kang et al., 2010; Qiusheng et al., 2004). Kaempferol (M162, DL=0.24, OB=41.88%) also exhibits anti-inflammatory efficacy by inhibition of iNOS protein and mRNA expression, as well as of NO production and NF- κ B activation (Hamalainen et al., 2007).

It is worth noting that protocatechuic acid (M3, DL=0.1, OB=19.3%), apigenin (M205, DL=0.21, OB=23.06%) had a relatively low OB value, but are known to produce effective anti-allergic activity or promote activation of the immune system (Hsieh et al., 2005; Li and Zhang, 2013). Ephedrine (M233, DL=0.03, OB=43.35%) was the major pharmacological compound identified, followed by pseudoephedrine (M234, DL=0.03, OB=52.25%), and methylephedrine (M235, DL=0.04, OB=63.64%) (Andraws et al., 2005; Pellati and Benvenuti, 2008). Notably, the OB of ephedrine in this study was similar to the value previously reported (about 45.2%) (Takagi et al., 2006). Indeed, the present data show that almost all ephedra alkaloids are orally bioavailable (with OB $\geq 30\%$) but less drug-like (DL ≤ 0.04). Similarly, despite the concentration-dependent anti-allergic effects of methyleugenol (M327, OB=73.36), it had a DL of just 0.04% (Hashimoto et al., 1994). Because the potential pharmacological effects of compounds were considered in this study, these additional 6 compounds were also regarded as candidate compounds. In summary, 60

ingredients of MFXD were screened and regarded as candidate compounds.

3.2 Target identification

Indisputably, herbal medicines exert their therapeutic effects through the synergistic effects of multiple compounds and targets. Thus, besides exploring active ingredients, the therapeutic targets exploration is also a necessity. Thanks to the application of systematic model based on the RF and SVM methods, presently the binding of candidate components of MFXD to those targets that are related with inflammation and/or the enhancement of immune system have been studied. 43 compounds (Table 1) in 60 candidate compounds yield 280 candidate targets (Table S2). The obtained targets were further subjected to the Uniprot, Drugbank and TTD databases to check if they are relevant with inflammation or the regulation of immune system. Finally, 37 potential targets (Table 2) are reserved and 2 candidate compounds (M59, M223) without any relevant targets are removed, indicating that most of the candidate compounds we screened previously have effective therapeutic actions.

Table 1
43 compounds from MFXD, and corresponding predicted oral bioavailability(OB) and drug-likeness(DL)

No.	Compound	OB (%)	DL	Medicine
M3*	Protocatechuic acid	25.37	0.04	Mahuang
M4*	Bis(2-methoxyethyl) phthalate	55.66	0.15	Mahuang
M53*	Leucopelargonidin	57.97	0.24	Mahuang
M55*	Clovene	46.49	0.11	Mahuang
M59	Patchoulane	52.71	0.11	Mahuang
M63*	Cedreneoxide	82.69	0.14	Mahuang
M83*	Linoleic Acid	41.90	0.14	Mahuang
M137*	Herbacetin	36.07	0.27	Mahuang
M154*	α -gurjunene	52.57	0.10	Mahuang
M160*	Resivit	30.84	0.27	Mahuang
M161*	Rutin	47.00	0.28	Mahuang
M162*	Kaempferol	65.70	0.24	Mahuang/Xixin
M163*	Linolenic acid	45.01	0.15	Mahuang
M167*	(E,7S,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	33.82	0.13	Mahuang

M168*	Delphinidin	40.63	0.28	Mahuang
M175*	Diisobutyl phthalate	49.63	0.13	Mahuang
M188*	Dibutyl phthalate	64.54	0.13	Mahuang
M189*	(1R,4R)-4-isopropyl-1,6-dimethyltetralin	17.47	0.08	Mahuang
M190*	Palmitic acid	19.30	0.10	Mahuang
M205*	Apigenin	23.06	0.21	Mahuang
M215*	Farnesylacetone	37.84	0.10	Mahuang
M223	(-)-Isoledene	49.01	0.10	Mahuang
M227*	Quercetin	46.43	0.28	Mahuang
M231*	Luteolin	36.16	0.25	Mahuang
M232*	Epicatechin	48.96	0.24	Mahuang
M233*	Ephedrine	43.35	0.03	Mahuang
M234*	Pseudoephedrine	52.25	0.03	Mahuang
M235*	Methylephedrine	63.64	0.04	Mahuang
M236*	Alpha-Linoleic acid	41.90	0.14	Fuzi
M247*	Delphin_qt	57.76	0.28	Fuzi
M251*	Deltoin	46.69	0.37	Fuzi
M254*	Deoxyandrographolide	56.30	0.31	Fuzi
M257*	Karanjin	69.56	0.34	Fuzi
M289*	Salsolinol	36.83	0.16	Fuzi
M298*	β -sitosterol	45.04	0.75	Fuzi
M307*	4,9-Dimethoxy-1-vinyl-beta-carboline	65.30	0.19	Xixin
M308*	Caribine	37.06	0.83	Xixin
M317*	Pentadecylic acid	20.18	0.08	Xixin
M318*	Cryptopin	78.74	0.72	Xixin
M319*	Sesamin	56.55	0.83	Xixin
M327*	Methyleugenol	73.36	0.04	Xixin
M336*	[(1S)-3-[(E)-but-2-enyl]-2-methyl-4-oxo-1-cyclopent-2-enyl]	62.52	0.31	Xixin
M345*	(3S)-7-hydroxy-3-(2,3,4-trimethoxyphenyl)-2,3-dihydrochromen-4-one	48.23	0.33	Xixin

* Represent potential compounds

Table 2
The information of 37 potential targets

No.	Protein Name	Gene Name
T31	Arachidonate 5-lipoxygenase	ALOX5
T37	Beta-1 adrenergic receptor	ADRB1
T38	Beta-2 adrenergic receptor	ADRB2
T57	Cell division protein kinase 2	CDK2
T61	CGMP-inhibited 3',5'-cyclic phosphodiesterase A	PDE3A
T84	Dipeptidyl peptidase 4	DPP4
T94	Epidermal growth factor receptor	EGFR
T96	Estrogen receptor	ESR1
T110	Glucocorticoid receptor	NR3C1

T116	Glycogen synthase kinase-3 beta	GSK3B
T119	Heat shock protein 90	HSP90
T125	High affinity immunoglobulin epsilon receptor subunit beta	MS4A2
T131	Inhibitor of nuclear factor kappa-B kinase subunit alpha	CHUK
T138	Intercellular adhesion molecule 1	ICAM1
T141	Interleukin-1 alpha	IL-1A
T142	Interleukin-1 beta	IL-1B
T145	Interleukin-2	IL-2
T146	Interleukin-4	IL-4
T147	Interleukin-6	IL-6
T148	Interleukin-8	IL-8
T152	Leukotriene A-4 hydrolase	LTA4H
T164	Muscarinic acetylcholine receptor M1	CHRM1
T180	NF-kappa-B inhibitor alpha	NFKBIA
T182	Endothelial Nitric oxide synthase	NOS3
T183	Inducible Nitric oxide synthase	NOS2
T194	Peroxisome proliferator activated receptor gamma	PPARG
T195	Peroxisome proliferator-activated receptor alpha	PPARA
T196	Peroxisome proliferator-activated receptor delta	PPARD
T200	Poly [ADP-ribose] polymerase 1	PARP1
T207	Prostaglandin E synthase	PTGES
T209	Prostaglandin G/H synthase 1	COX-1
T210	Prostaglandin G/H synthase 2	COX-2
T234	Signal transducer and activator of transcription 1-alpha/beta	STAT1
T251	Transcription factor AP-1	JUN
T254	Transcription factor p65	RELA
T260	Trypsin-1	PRSS1
T262	Tumor necrosis factor	TNF

3.3 Network Construction and Analysis

3.3.1 cC-cT network for MFXD

Based on target prediction, the cC-cT network (Fig.1) comprised 322 nodes (43 candidate compounds and 280 candidate targets) and 944 edges. The targets in the outer circle show much less interactions with candidate compounds than those in the inner circles. This indicates that many candidate targets are hit by only one candidate compounds, but some can be modulated by multiple ligands rather than single compound. This finding indicated that many candidate targets were linked to only one candidate compound, but that some targets could be modulated by multiple ligands.

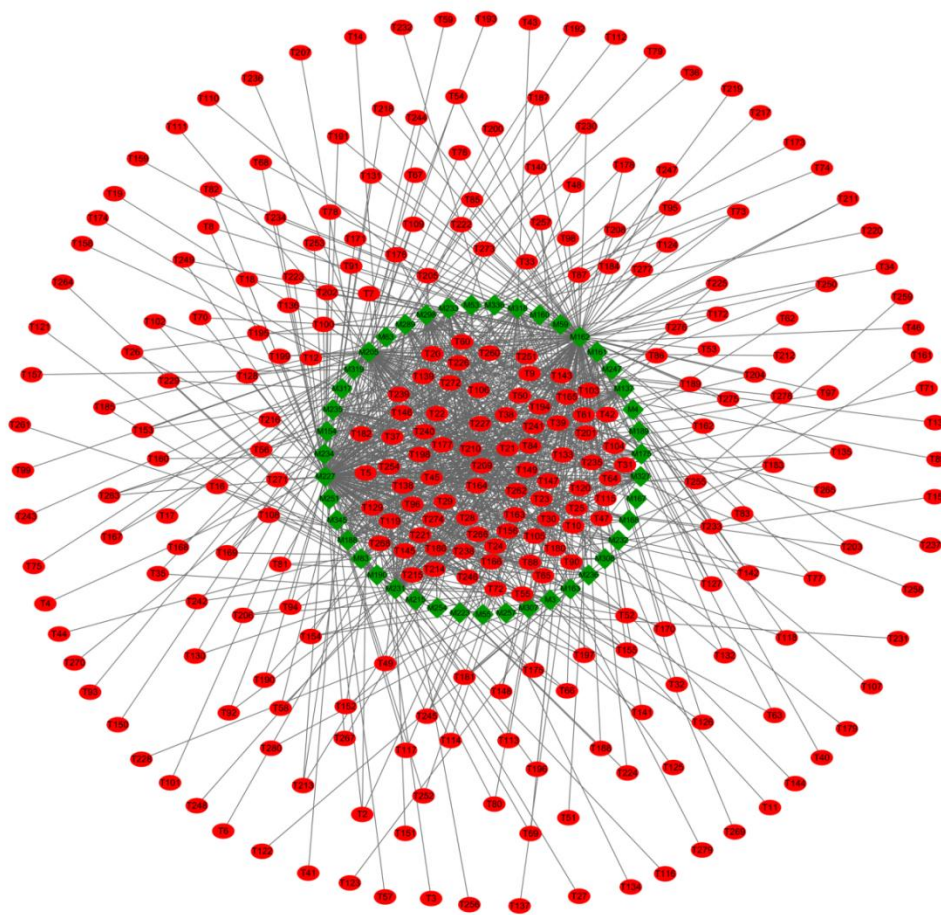


Fig.1 Network of 43 candidate compounds (M) predicted to have 280 candidate protein targets (T). The green diamond represents the compounds, while red circles delineate the protein targets.

3.3.2 pC-pT network: detecting the key players of MFXD in AR treatment

We further investigated the relationships between the active ingredients and their targets by deleting compounds without targets. The remaining 43 candidate compounds and their 37 potential targets were used to generate a bipartite graph in a pC-pT network (Fig.2). This network represents a global view of the potential compounds (green diamonds) and targets (red circles) in MFXD, and it comprised 78 nodes (41 potential compounds and 37 potential targets) and 233 edges. The centralization (0.389) and heterogeneity (1.097) of the pC-pT network indicated that certain compounds or targets were more central and important in the network than others. For example,

prostaglandin G/H synthase 2 (COX2, T209) had the largest number of drug interactions (36), while glucocorticoid receptor (NR3C1, T110) had only one.

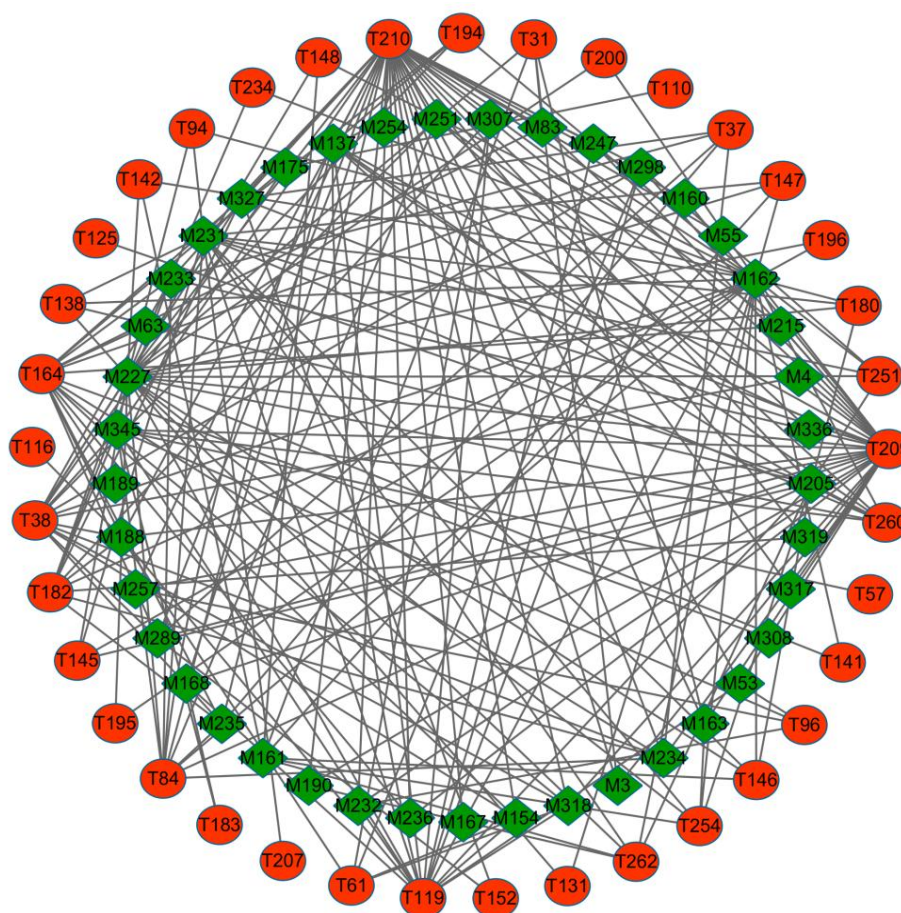


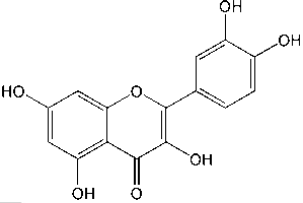
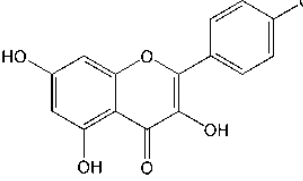
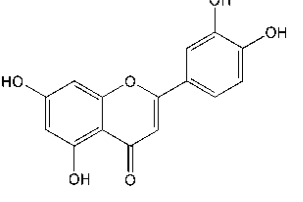
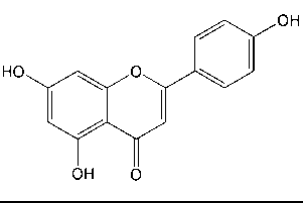
Fig.2 Network of 41 potential compounds (M, green diamond) predicted to have and 37 potential targets (T, red circles).

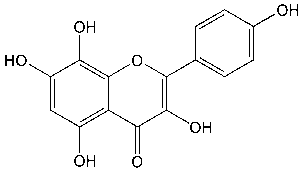
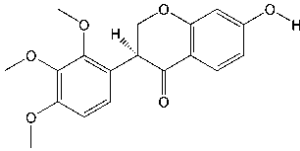
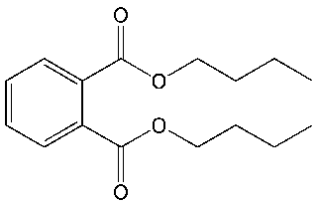
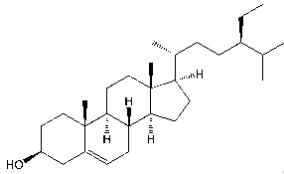
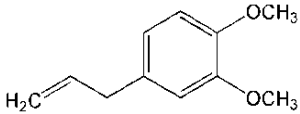
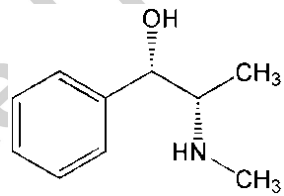
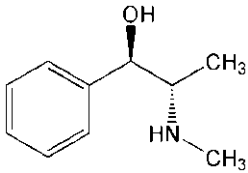
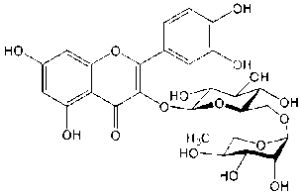
Degree and betweenness centrality are two key topological parameters that characterize the most influential nodes in a network, and we used these to determine the importance of active ingredients and action targets (the nodes in our network), and the extent of their influence on the spread of information through the network. The majority of compounds were linked with more than one target. Among the 43 active ingredients in our network, several compounds may be essential (Table 3). For example, quercetin (M227) exhibits the largest number of targets connections (26), followed by kaempferol (M162) with 25 potential targets and β -sitosterol (M298), methyleugenol (M327),

ephedrine (M234) and pseudoephedrine (M233) with 7 potential targets. These high-degree nodes (hubs) are likely to play a more important role in treating an allergy or enhancing the immune system. For example, the ephedrine alkaloids, i.e., ephedrine (M233) and pseudoephedrine (M234), are predicted to act on α - and β -adrenergic receptors, which is supported by evidence that these compounds are the primary active ingredients that target adrenergic receptors (Blechman et al., 2004). Interestingly, of all the 41 potential compounds, 13 compounds possess degree larger than 6.0 under an average value of 5.846 in which 10 have been experimentally validated with anti-inflammation and /or immunomodulation bioactivities. We suggest that our high hit rate ($>70\%$) indicates the reliability and rationality of our network analysis, which has identified the key compounds in MFXD.

Table 3

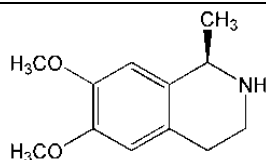
Structure and bioactivity of compounds with high degree in pC-pT network

NO	Name	structure	Degree ^a	Bioactivity(PMID)
M227	Quercetin		26	Anti-inflammation (18549926) Immunomodulation (17100629)
M162	Kaempferol		25	Anti-inflammation (17666881) Immunomodulation (21621872)
M231	Luteolin		16	Anti-inflammation (25692285) Immunomodulation (17100629)
M205	Apigenin		13	Anti-inflammation (25800110) Immunomodulation (17100629)

M137	Herbacetin		11	
M345	(3S)-7-hydroxy-3-(2,3,4-trimethoxyphenyl)chroman-4-one		10	
M188	Dibutyl phthalate		8	Immunomodulation (23480346)
M298	β -sitosterol		7	Anti-inflammation (11379762)
M327	Methyleugenol		7	Anti-inflammation (20815773)
M234	Pseudoephedrine		7	Immunomodulation (21631396)
M233	Ephedrine		7	Anti-inflammation (23604046)
M161	Rutin		7	Anti-inflammation (25753322) Immunomodulation (19010542)

M289

Salsolinol



6

^a The parameter of the nodes (molecules) were calculated by the Network Analyzer. PMID represented PubMed unique Identifier.

We found that 37 potential targets were involved in the various pathogenic processes of AR, which include the inflammatory response and immune suppression. Among these targets, we identified the hub nodes for further analysis. The 12 targets with the highest betweenness values (from high to low) were COX-2 (T210), prostaglandin G/H synthase 1 (COX-1, T209), heat shock protein 90 (HSP90, T119), muscarinic acetylcholine receptor M1 (CHRM3, T164), beta-2 adrenergic receptor (ADRB2, T38), dipeptidyl peptidase 4 (DDP4, T84), endothelial nitric oxide synthase (NOS3, T182), trypsin-1 (PRSS1, T260), tumor necrosis factor (TNF, T262), transcription factor p65 (RELA, T254), arachidonate 5-lipoxygenase (ALOX5, T31) and interleukin-4 (IL-4, T146), and The same ten targets had the highest degree values, but the order was slightly different. We suggest that compounds or targets such as these, with high degree and betweenness values, are the key the ingredients of MFXD when applied as a treatment for diseases.

3.3.3 Illustrating the mechanism of action of MFXD in AR treatment based on compound-target interactions

By analyzing potential targets in Table 2, two interesting phenomena were observed. On one hand, MFXD may have similar anti-inflammatory pharmacological mechanisms to western medicines by target FDA-approved anti-inflammatory therapeutic targets such as COX-2, COX-1, ALOX5, intercellular adhesion molecule 1(ICAM1), peroxisome proliferator activated receptor gamma (PPARG) and peroxisome proliferator-activated receptor delta (PPARD). COX-2 had the highest number of compound-target interactions, and is likely to play a key role in the treatment of

AR. Indeed, COX-2 is associated with inflammation and its expression increases in the airway epithelium and submucosal inflammatory infiltrate to induce sputum in allergic patients (Chacon et al., 2005; Taha et al., 2000). The formation of bioactive leukotrienes (LTs) is catalyzed by ALOX5, an important enzyme in the arachidonic acid cascade, with the aid of the ALOX5-activating protein, FLAP. LTs are inflammatory mediators that play a pathophysiological role in diseases such as asthma and AR (Funk, 2001; Werz and Steinhilber, 2006). On the other hand, two categories of inflammation closely-related proteins are found among these targets, i.e., pro-inflammatory cytokines (such as IL-1A, IL-1B, IL-2, IL-6 and TNF) and anti-inflammatory cytokines (like IL-4), which indicates that MFXD may also regulate these two categories of targets to exert synergistic or additive anti-inflammatory effect.

In our network, we also found that a number of targets such as DDP4, glycogen synthase kinase-3 beta (GSK3B), HSP 90, inducible Nitric oxide synthase (NOS2), PPARG and PRSS1 were involved in enhancing the immune reaction, which is maybe another critical mechanism of MFXD on AR. DDP4 is also known as lymphocyte cell surface protein CD26 and plays an important role in T-cell immunity, DPP4 inhibitors can suppress pathogenic effects of Th1 and Th17 cells and up-regulate Th2 cells and regulatory T cells. (Zhao et al., 2014). GSK3B and PPARG are closely related to nuclear factor-kappa B (NF- κ B), which plays an important role in the expression of numerous cytokines and adhesion molecules that improve the immune response (Chen et al., 2002; Ghosh et al., 1998; Hoeflich et al., 2000). HSP 90 and PRSS1 are somewhat connected to macrophages that may help produce potent cytokines like TNF- α and IL-6, which in turn recruit many kinds of immune cells that promote immunity (Kim et al., 2012; Koshikawa et al., 1998; Li et al., 2012a). Interestingly, there are a total of 41 compounds interacted with these targets and most of them belong to lignans

that may promote the activity of macrophages and the production level of IL-2 by T lymphocytes, thus enhancing the immunologic function.

3.4 Target-disease network

Most complex diseases are triggered by an unbalanced modulating network, in which multiple genes or their products stay in a state of dysfunction. Additionally, proteins that are targeted by a drug tend to associate with various diseases. Therefore, we constructed a T-D network to explore additional potential therapeutic effects of MFXD. We compiled the relevant diseases by mapping the potential targets into the TTD. In the T-D network (Fig.3), 28 targets were related to 138 diseases that were classified into 27 groups according to Medical Subject Headings (MeSH, <http://www.nlm.nih.gov/mesh/>). For example, AR, asthma, and obstructive airway disease were classified as respiratory tract diseases; breast cancer, colorectal cancer, and melanoma were related to neoplasm. Among the 138 diseases, we found most were classified as neoplasms (26.8%), respiratory tract diseases (10.1%), cardiovascular diseases (9.42%), and pathological conditions, signs, and symptoms (6.5%). This suggests that MFXD could potentially be used for clinical treatment of the symptoms of these diseases. Therefore, our findings provide new information on possible clinical uses for MFXD and highlight the potential for drug discovery from TCM.

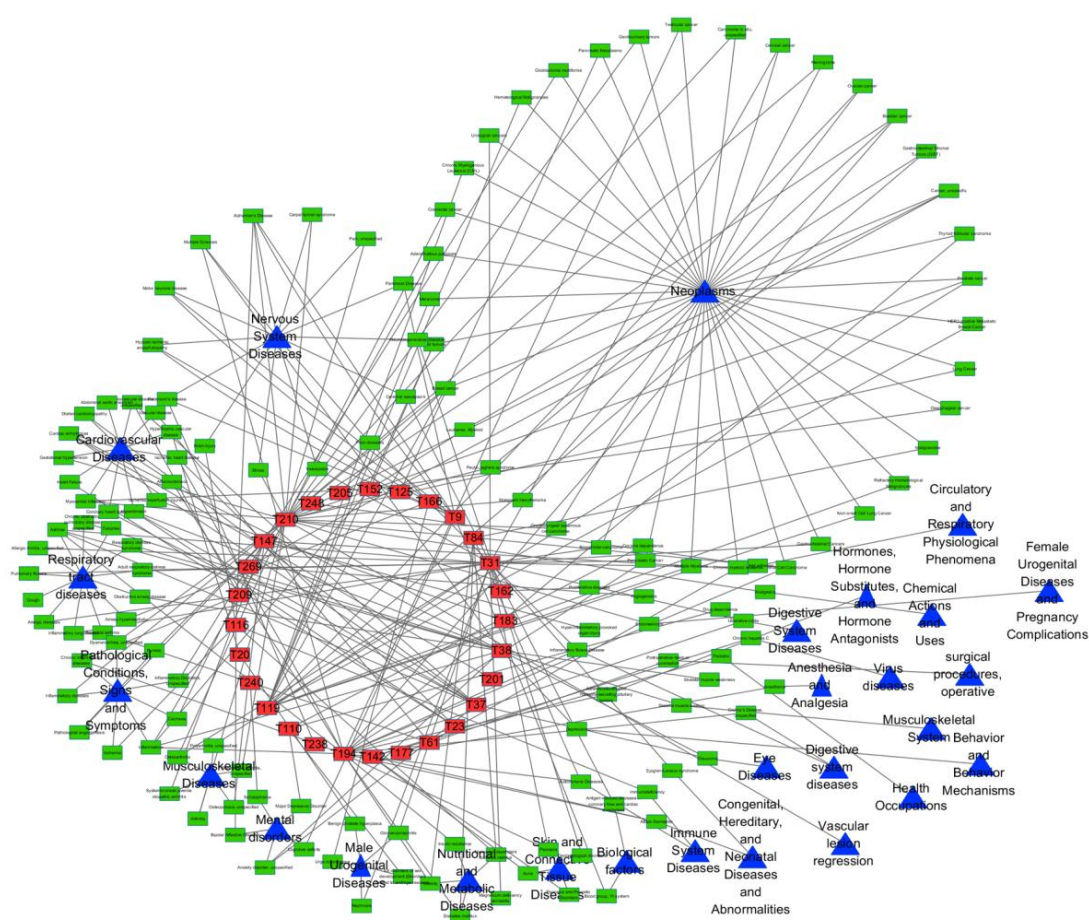


Fig.3 Target-disease network. Red circle and green rectangle represent potential targets and diseases, respectively, 138 disease terms were organized into 27categories (blue triangles) according to MeSH.

3.5 Anti-inflammatory effect of four predicted ingredients

As shown in Fig.4, PGE_2 was significantly inhibited by pretreatment with M137 and M289 in a dose-dependent manner. And high doses of M188 (5, 10 μM) and M234 (2, 5 μM) also significantly inhibit PGE_2 production. Most of anti-inflammation effects of these predicted active ingredients in Table 3 were confirmed by published literature; these four predicted ingredients were not previously reported. Mast cells are one of the major effector cells in allergic inflammatory reactions. Activated mast cells can produce histamine, as well as a wide range of other pro-inflammatory mediators such

as TNF- α , IL-1 β , PGE₂, and LTB₄. RBL-2H3 cells have been widely used to study IgE-Fc ϵ RI interactions, which are related to the intracellular signaling pathway in degranulation (Funaba et al., 2003; Ikawati et al., 2001; Ortega et al., 1988). PGE₂ production was used as a measuring index because in the pathway of PGE₂ production, it is widely known that COX-2 was rate-limiting enzymes, and drugs targeting the proteins could directly inhibit PGE₂ production. Therefore, we may conclude that the four compounds possess anti-inflammation effects in IgE-activated RBL-2H3 cells. The suppressive effect of these compounds on PGE₂ levels may also affect anti-allergic activity because PGE₂ contributes to asthma development and inflammation associated with type 2 cytokines such as IL-4 and IL-5 (van der Pouw Kraan et al., 1995). We also find methyleugenol (M327) markedly inhibit PGE₂ production and decreases the activation of COX-2 as predicted in pC-pT network (Fig.2)(Tang et al., 2015).

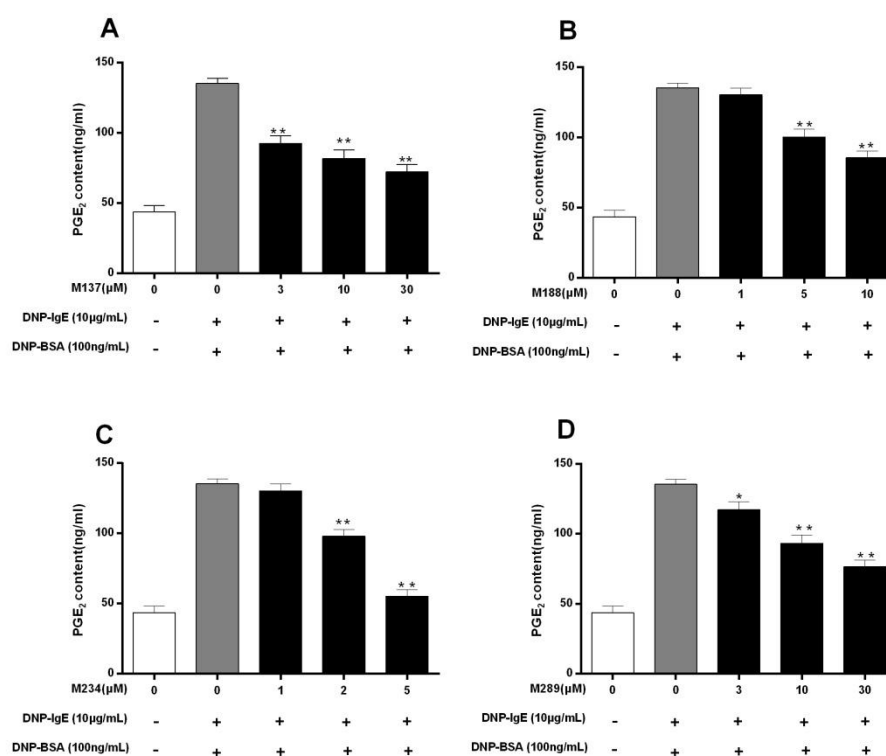


Fig.4 Effect of M137, M188, M234 and M289 on PGE₂ production in IgE-activated RBL-2H3 cells. RBL-2H3 cells were seeded on a 24-well plate in DMEM with 10% FBS at 37°C overnight, and then the cells were washed,

and further incubated with DNP-IgE for 24 h. The cells were incubated with M137, M188, M234 and M289 for 1 h, and then stimulated by DNP-BSA for 4 h. The analyzing PGE₂ levels using an ELISA kit. Data represent the mean $\pm$ SD of three independent experiments and differences between mean values were assessed by one-way ANOVA. * $p < 0.05$, ** $p < 0.01$ indicate significant differences compared with the DNP-BSA-treated group.

4. Conclusion

TCM is a multi-component synergistic system of medicine that can play a therapeutic role in the treatment of various diseases. Therefore, the research approach applied to TCM should be different from that of western medicine. In this study, we applied a network pharmacology method that integrated DL evaluation, OB prediction, multiple drug target prediction, and network analysis to predict the active ingredients and potential targets of MFXD in relation to its action against AR. In addition, some of predicted active ingredients and target relationships were confirmed by a consulting published literature. And four predicted ingredients possess anti-inflammatory effects were found by this technique. Using the example of MFXD, this study shows that network pharmacology is a powerful approach for identifying bioactive ingredients and mechanisms of action in TCM herbal formulae. Our results not only provide new insights for a deeper understanding of the chemical basis and pharmacology of MFXD (and perhaps TCM in general) but also demonstrate an efficient method for potential discovery of drugs originating from herbal medicine. However, because our method cannot discriminate whether an ingredient directly binds with a target or affects the target indirectly, we acknowledge that additional research is required to test the effects of MFXD ingredients in relation to different diseases.

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graphical abstract

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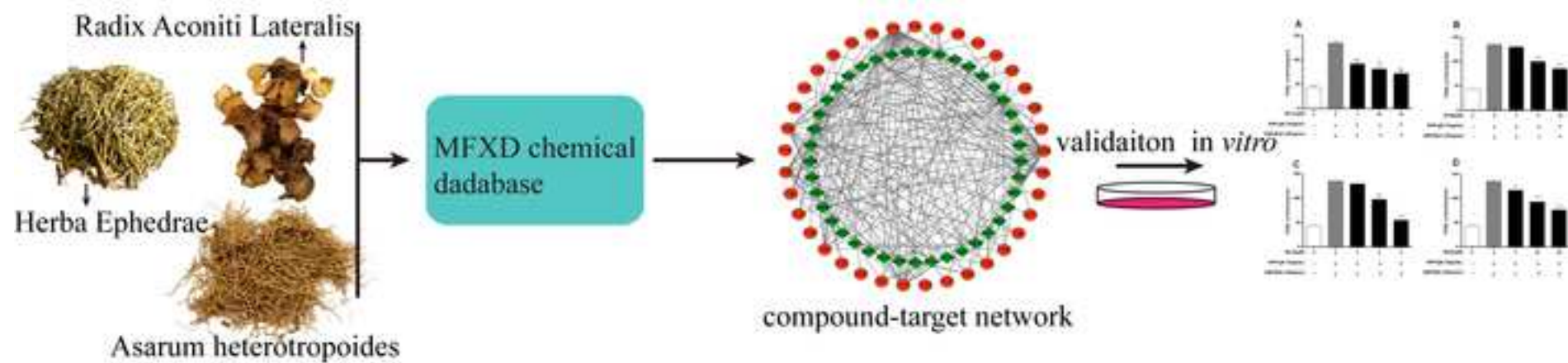


Table S1 366 Compounds from MFXD with corresponding DL and OB values

No.	Compound	OB (%)	DL
M1	Apple oil	22.26	0.03
M2	4-hydroxybenzenecarboxylic acid	30.15	0.03
M3*	protocatechuic acid	31.37	0.24
M4*	Bis(2-methoxyethyl) phthalate	55.66	0.15
M5	2-Hexenol	64.54	0.01
M6	(2S,4S)-4-methyl-2-(2-methylprop-1-enyl)oxane	37.82	0.03
M7	2,3,4-Trimethyl-5-phenyloxazolidine	57.1	0.06
M8	(1R,2S,3S,5S)-2,6,6-Trimethylnorpinan-3-Ol	53.95	0.06
M9	2-Methylpent-2-en-1-ol	99.8	0.01
M10	(E)-oct-3-en-1-yne	44.97	0.01
M11	1,2,3-trimethylcyclohexane	52.94	0.02
M12	(2S)-heptane-1,2-diol	29.91	0.01
M13	1,3,4-trimethyl-3-cyclohexene-1-carboxaldehyde	56.58	0.03
M14	2,5-Dimethylpyrazole	50.33	0.01
M15	1-Phenyltetrazole-5-thiol	13.13	0.04
M16	2-octyldodecan-1-ol	15.08	0.14
M17	p-Nitrosotoluene	55.71	0.02
M18	(2R)-1-ethoxypropan-2-ol	38.46	0.01
M19	Ethyltrimethylethylene	44.64	0.01
M20	3,3,6,9,9,10-hexamethyl-1-decene	5.87	0.06
M21	2-methyl-butanol-1-ol	84.22	0.01
M22	Tiglaldehyde	75.53	0
M23*	3,7,1,5-tetramethyl-2-Hexadecen-1-ol	32.89	0.13
M24	Pentenoic acid	61.28	0.01
M25	2-ethylidene-1,1-dimethyl-cyclopentane	48.6	0.02
M26	3,7-dimethyl-cyclopenta cyclooctene	46.51	0.06
M27	3,8-menthadiene	40.26	0.02
M28	3-methylhexa-2,4-diene	48.23	0.01
M29	2-Methyl-1-naphthol	60.3	0.05
M30	3-methyl-6-(1-methylethyl-idene)-cyclohexene	30.03	0.02
M31	3-METHYLPHENANTHRENE	17.11	0.11
M32	p-Toluyl chloride	54.03	0.02
M33	4-Oxo-1,4-dihydroquinoline-2-carboxylic acid	44.72	0.08
M34	Methyl 5-methylhexanoate	20.2	0.02
M35	Ethylheptylacetylene	20.66	0.02
M36	(S)-1-Boc-3-methylpiperazine	1.06	0.05
M37	Methylbenzylamine	59.64	0.02
M38	1,3-p-Menthadien-7-al	40.7	0.03
M39	[(2S)-6-methylhept-5-en-2-yl] acetate	32.07	0.03
M40	Allyloxybenzene	49.3	0.02
M41	(Z)-2-Phenyl-2-butene	39.71	0.02
M42	Methylene	7.57	0

Table S2 (to be continued)

No.	Compound	OB (%)	DL
M43	m-Ethylacetophenone	20.8	0.03
M44	Sulphuric acid Diethyl ester	72.36	0.02
M45	Ephedroxane	2.03	0.06
M46	Ethyl 2-decenoate	58.16	0.04
M47	Methyl 2-decenoate	19.29	0.03
M48	carvenone	51.37	0.03
M49	isobutyric acid	75.37	0.01
M50	()-N-Methylephedrine	63.64	0.04
M51*	O-benzoyl-L-(+)-pseudoephedrine	65.17	0.13
M52	Trans-ionone	40.6	0.05
M53*	leucopelargonidin	57.97	0.24
M54	1,5,8-trimethyl-1,2-dihydro-naphthalene	53.22	0.06
M55*	Clovene	46.49	0.11
M56	Trichloroicosylsilane	14.53	0.23
M57	1,5-dimethyl-4-(2-hexenyl)benzene	6.69	0.05
M58	[(1S,4S)-4-isopropyl-1-cyclohex-2-enyl]methanol	47.34	0.03
M59*	patchoulane	52.71	0.11
M60	1-propenyl-2-vinyl-4-methylcycloheptane	35.45	0.04
M61	Promyr	21.01	0.11
M62	Dehydroxylinalool oxide A	36.85	0.03
M63*	Cedreneoxide	82.69	0.14
M64	p-xylene	48.74	0.01
M65	[(3R)-3,7-dimethylocta-1,6-dien-3-yl] acetate	27.73	0.04
M66	(-)-Myrtenol	49.98	0.06
M67	vanillic acid	35.47	0.04
M68	6-Hydroxykynurenate	49.28	0.09
M69	Nonanal	40.28	0.02
M70	Cymol	27.2	0.02
M71	(L)-alpha-Terpineol	48.8	0.03
M72	Arachic acid	16.66	0.19
M73	(1R,5R,7S)-4,7-dimethyl-7-(4-methylpent-3-enyl)bicyclo[3.1.	16.23	0.09
M74	(4S)-4-isopropylcyclohexene-1-carbaldehyde	40.36	0.03
M75	1,8-cineole	39.73	0.05
M76	geraniol	23.93	0.02
M77	(-)-alpha-Pinene	46.25	0.05
M78	(S)-p-Mentha-1,8-dien-7-al	39	0.03
M79	(-)-nopinene	44.84	0.05
M80	NERYLACETATE	25.94	0.04
M81	octanol	21.06	0.01
M82	Camphor	67.17	0.05
M83*	Linoleic Acid	41.9	0.14
M84	benzyl alcohol	58.68	0.01

Table S2 (to be continued)

No.	Compound	OB (%)	DL
M85	Methyl laurate	21.75	0.05
M86	myristic acid	21.18	0.07
M87	Oktadekan	9.81	0.09
M88	PENTADECYLIC ACID	20.18	0.08
M89	Hypnon	48.19	0.02
M90	Linalool	49.37	0.04
M91	UPL	8.52	0.11
M92	NON	26.74	0.03
M93	Furol	34.35	0.01
M94	Tetracosane	8.28	0.24
M95	Dihydro-beta-ionone	26.25	0.05
M96	Methyl stearate	16.8	0.16
M97	Izoforon	44.98	0.03
M98	FMT	33.26	0
M99	ZINC02169908	23.3	0.1
M100	(Z)-caryophyllene	30.29	0.09
M101	L-Bornyl acetate	65.52	0.08
M102	(R)-linalool	39.8	0.02
M103	p-Ocimene	15.06	0.02
M104	Moslene	33.02	0.02
M105	Tereton	40.17	0
M106	toluene	42.58	0.01
M107	thymol	41.47	0.03
M108	hexanoic acid	73.08	0.01
M109	(6R)-6-isopropyl-3-methyl-1-cyclohex-2-enone	53.88	0.03
M110	tricin	27.86	0.34
M111	DEP	52.19	0.07
M112	Allocymene	14.89	0.02
M113	p-Cymen-8-ol	32.26	0.03
M114	1-terpineol	49.83	0.03
M115	WLN: T5OJ BVO1	49.41	0.02
M116	7-oxabicyclo-2.2.1-heptane,1-methyl-4-[1-methylethyl]-	60.92	0.04
M117	BOX	31.55	0.02
M118	tetramethylpyrazine	20.01	0.03
M119	1(3H)-Isobenzofuranone, 3-butyl-3a,4,5,6-tetrahydro-, cis(-)-	65.03	0.07
M120	cinnamic acid	19.68	0.03
M121	L-Limonen	30.09	0.02
M122	3-Hexenol	62.74	0.01
M123	beta-Ionone	20.63	0.05
M124	alpha-humulene	22.98	0.06
M125	(Z,Z)-farnesol	41.14	0.06
M126	Sulcatone	26.36	0.01

Table S2 (to be continued)

No.	Compound	OB (%)	DL
M127	Peruviol	29.61	0.06
M128	farnesol	28.44	0.06
M129	Isobutyl benzoate	47.76	0.04
M130	GBL	76.91	0.01
M131	o-Thymol	43.28	0.03
M132	beta-Citronellol	38.89	0.02
M133	(1S,5S)-1-isopropyl-4-methylenebicyclo[3.1.0]hexane	46.21	0.04
M134	Nonacosanol	10.57	0.43
M135	Picein	9.48	0.19
M136	Piceol	36.8	0.03
M137*	Herbacetin	36.07	0.27
M138	PCR	51.99	0.01
M139	6-Methylheptan-2-one	39.99	0.01
M140	(1R,2R,4R)-Dihydrocarveol	51.17	0.03
M141	caprylic acid	16.4	0.02
M142	Amylol	76.16	0
M143	lauric acid	23.59	0.04
M144	nonanoic acid	40.51	0.02
M145	beta-Eudesmol	26.09	0.01
M146	Isobutyral	25.51	0
M147	PEY	25.7	0.1
M148	naphthalene	27.55	0.03
M149	Heptanol	17.22	0.01
M150	(E)-oct-2-en-4-one	64.62	0.01
M151	Pyruvophenone	35.93	0.03
M152	m-Methylacetophenone	40.63	0.02
M153	calacorene	16.2	0.08
M154*	α-gurjunene	52.57	0.1
M155	(2R)-2-ethylhexan-1-ol	26.12	0.01
M156	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	11.71	0.02
M157	Cuminol	42.57	0.03
M158	2-Caren-10-al	44.74	0.05
M159	2-PENTADECANONE	7.41	0.06
M160*	Resivit	30.84	0.27
M161*	rutin	47.0	0.28
M162*	kaempferol	41.88	0.24
M163*	linolenic acid	45.01	0.15
M164	acetic acid	47.87	0
M165	heptanoic acid	13.38	0.01
M166	Methyl octylate	18.71	0.02
M167*	(E,7S,11R)-3,7,11,15-tetramethylhexadec-2-en-1-ol	33.82	0.13
M168*	delphinidin	40.63	0.28

Table S2 (to be continued)

No.	Compound	OB (%)	DL
M169	2-Ethyl-p-xylene	20.6	0.02
M170	2,6,10-trimethyl-dodecane	37.8	0.03
M171	.alpha.-Ionene	29.59	0.06
M172	Nonacosane	8.12	0.39
M173	DBF	37.28	0.08
M174	76897_FLUKA	48.67	0.01
M175*	DIBP	49.63	0.13
M176	Octacosanol	10.7	0.41
M177	2,3-dimethyl-1-pentene	32.08	0.01
M178	(-)-Terpinen-4-ol	81.41	0.03
M179	delta-amorphene	17.95	0.08
M180	Clorius	45.99	0.02
M181	Azulol	15.15	0.07
M182	Eciphin	43.35	0.03
M183*	alloaromadene	53.46	0.1
M184	Psi-ephedrin	52.25	0.03
M185	hexanal	55.71	0.01
M186	1-hexanol	22.04	0.01
M187	PENTYLFURAN	54.59	0.02
M188*	DBP	64.54	0.13
M189	(1R,4R)-4-isopropyl-1,6-dimethyltetralin	17.47	0.08
M190*	palmitic acid	19.3	0.1
M191	styrene	29.55	0.01
M192	(S)-Matsutake alcohol	40.11	0.01
M193	trans-2-nonenal	19.18	0.02
M194	Safranal	39.56	0.04
M195	beta-Cyclocitral	40.36	0.03
M196	trans-2,4-decadienal	51.03	0.02
M197	Geranylacetone	18.66	0.04
M198	Methyl carvacrol	31.76	0.04
M199	HMF	45.07	0.02
M200	2-Methylpentan-3-one	73.99	0.01
M201	MIK	17.74	0.01
M202	p-coumaric acid	43.29	0.04
M203	EEE	45.02	0
M204	OXA	63.21	0
M205*	apigenin	23.06	0.21
M206	ZINC02042359	16.19	0.04
M207	Methyl heptoate	19.45	0.02
M208	(E)-Pent-2-en-1-ol	71.87	0
M209	stearic acid	17.83	0.14
M210	Damascenone	36.43	0.05

Table S2 (to be continued)

No.	Compound	OB (%)	DL
M211	Heptadekan	8.64	0.07
M212	Henicosane	8.41	0.15
M213	eth	55.22	0
M214	2,6-Dimethylnaphthalene	19.21	0.05
M215*	Farnesylacetone	37.84	0.1
M216	methyl palmitate	18.09	0.12
M217	Tricosane	8.33	0.21
M218	tetradecane	15.94	0.04
M219	Tybraine	44.08	0.04
M220	Usaf cs-6	66.05	0.03
M221	(-)-N-Methylpseudoephedrine	37.12	0.04
M222	alpha-Farnesene	21.7	0.05
M223*	58870_FLUKA	49.01	0.1
M224	beta-Bisabolene	29.59	0.06
M225	Ethylpalmitate	18.99	0.14
M226	cuminal	38.29	0.03
M227*	quercetin	11.4	0.28
M228	(2R)-2-methylbutan-1-ol	85.35	0
M229	n-Triacontanol	10.46	0.45
M230	cinnamaldehyde	31.99	0.02
M231*	luteolin	36.16	0.25
M232*	Epicatechin	48.96	0.24
M233*	Ephedrine	43.35	0.03
M234*	Pseudoephedrine	52.25	0.03
M235*	Methylephedrine	63.64	0.04
M236*	EIC	41.9	0.14
M237	myristic acid	21.18	0.07
M238	uracil	42.53	0.02
M239	mesaconitine	8.7	0.25
M240	benzoylmesaconine	8.55	0.27
M241*	11,14-eicosadienoic acid	39.99	0.2
M242	13-Deoxo-13 alpha-acetyloxy-1-deoxynortaxine I	25.68	0.79
M243	14-Deoxy-11,12-didehydroandrographolide	13.6	0.32
M244	Delcorine	7.52	0.36
M245	Delphamine	13.74	0.66
M246	Delphin	2.61	0.67
M247*	Delphin_qt	57.76	0.28
M248	Delsemine B	18.32	0.18
M249	Delsoline	17.23	0.63
M250	Deltamine	9.62	0.37
M251*	Deltoin	46.69	0.37
M252*	DemethyldeLavaine A	34.52	0.18

Table S2 (to be continued)

No.	Compound	OB (%)	DL
M253*	DemethyldeLavaine B	34.52	0.18
M254*	Deoxyandrographolide	56.3	0.31
M255	14-acetyltalatisamine	13.64	0.63
M256*	karakoline	51.73	0.73
M257*	Karanjin	69.56	0.34
M258	TMPEA	47.54	0.06
M259	Neojiangyouaconitine	9.83	0.26
M260*	Neokadsuranic acid B	43.1	0.85
M261	Senbusine A	22.26	0.71
M262	Senbusine B	14.54	0.71
M263	Senbusine C	19.29	0.66
M264	Tamarixinol	12.18	0.59
M265*	2,7-Dideacetyl-2,7-dibenzoyl-taxayunnanine F	39.43	0.38
M266	beiwutine	14.88	0.24
M267	benzoylaconine	12.83	0.25
M268	benzoylhypaconine	8.7	0.29
M269*	benzoynapelline	34.06	0.53
M270	chasmaconitine	8.65	0.26
M271	chasmanine	15.77	0.67
M272	coryneine	3.55	0.05
M273	denudatine	16.55	0.67
M274*	6-Demethyl-desoline	51.87	0.66
M275*	deoxyaconitine	30.96	0.24
M276	fuzitine	25.79	0.54
M277	hetisine	29.12	0.59
M278*	(R)-Norcoclaurine	82.54	0.21
M279	hokbusine A	21.74	0.26
M280*	ignavine	84.08	0.25
M281*	isotalatizidine	50.82	0.73
M282*	jesaconitine	33.41	0.19
M283	Aconitine	7.87	0.23
M284	Zoba EG	66.14	0.02
M285	neoline	13.32	0.69
M286	Zoba 3GA	65.8	0.02
M287	UNAL	67.62	0.02
M288	Pyrrolezanthine	11.99	0.12
M289*	Salsolinol	36.83	0.16
M290	songorine	24.46	0.72
M291	talatizamine	16.94	0.72
M292*	(3R,8S,9R,10R,13R,14S,17R)-3hydroxy-4,4,9,13,14-pentm	41.52	0.22
M293*	Carnosifloside I_{qt}	38.16	0.8
M294	STOCK1N-53073	8	0.58

Table S2 (to be continued)

No.	Compound	OB (%)	DL
M295	Delavaconitine	9.91	0.37
M296	Delbruline	13.6	0.36
M297	Delbrusine	7.51	0.33
M298*	beta-sitosterol	36.91	0.75
M299*	hypaconitine	31.39	0.26
M300	Arachic acid	16.66	0.19
M301	Cymol	27.2	0.02
M302	Carvacryl acetate	29.98	0.05
M303	Dodecatetraenamide, N-(2-methylpropyl)-	48.46	0.09
M304	Sarisan	45.6	0.07
M305	epicamphor	50.47	0.05
M306	Kakoul	63.1	0.07
M307*	4,9-dimethoxy-1-vinyl-5b-carboline	65.3	0.19
M308*	Caribine	37.06	0.83
M309	Estriol	27.32	0.36
M310	ACon1_002321	5.16	0.68
M311	Myristelaidic acid	38.48	0.07
M312	DHNQ	41.43	0.08
M313	Sarmentolside	11.03	0.61
M314	1,8-cineole	39.73	0.05
M315	(-)-alpha-Pinene	46.25	0.05
M316	CAM	67.17	0.05
M317	Pentadecylic acid	20.18	0.08
M318*	Cryptopin	78.74	0.72
M319*	sesamin	56.55	0.83
M320	sesamol	66.34	0.03
M321	Croweacin	53.28	0.07
M322	(+/-)-Isoborneol	86.98	0.05
M323	D-Camphene	34.98	0.04
M324	Myrcene	24.96	0.02
M325	Safrol	45.34	0.05
M326	Moslene	33.02	0.02
M327*	Methyleugenol	73.36	0.04
M328	(1S,4R,5R)-1-isopropyl-4-methyl-4-bicyclo[3.1.0]hexanol	27.22	0.05
M329	TML	59.98	0
M330	Hemo-sol	39.84	0.02
M331	()-alpha-Terpineol	46.3	0.03
M332	delta-Terpineol	55.11	0.03
M333	Terragon	36.59	0.03
M334	()-Borneol	81.8	0.05
M335	Campherenol	18.25	0.09
M336*	[(1S)-3-[(E)-but-2-enyl]-2-methyl-4-oxo-1-cyclopent-2-enyl]	62.52	0.31

Table S2 (to be continued)

No.	Compound	OB (%)	DL
M337	Cynanuriculoside A	14.92	0.03
M338	(-)-beta-Phellandrene	40.44	0.02
M339	Myristicin	17.99	0.07
M340	Tereben	29.62	0.02
M341	(1S,5S)-1-isopropyl-4-methylenebicyclo[3.1.0]hexane	46.21	0.04
M342	3,4,5-Trimethoxytoluene	23.73	0.04
M343	Elemicin	21.94	0.06
M344	nicotiflorin	3.64	0.73
M345*	(3S)-7-hydroxy-3-(2,3,4-trimethoxyphenyl)chroman-4-one	48.23	0.33
M346	Myricadiol	13.58	0.77
M347	Safynol	34.21	0.06
M348	2-[(1R,3S,4S)-3-isopropenyl-4-methyl-4-vinylcyclohexyl]propan-2-ol	19.03	0.07
M349	naphthalene	27.55	0.03
M350	Azaron	38.39	0.06
M351	Cynanuriculoside A _{qt}	53.07	0.09
M352*	kaempferol	41.88	0.24
M353	3,5-Dimethoxytoluene	20.87	0.03
M354	Eucarvone	53.14	0.03
M355	Eugenone	18.7	0.1
M356	Astragalin	14.03	0.74
M357	O-Methylthymol	47.65	0.04
M358	β-terpineol	47.89	0.03
M359	MYS	13.98	0.05
M360	(E)-9-Isopropyl-6-methyl-5,9-decadiene-2-one	60.25	0.05
M361	2-[(1R)-4-methyl-1-cyclohex-3-enyl]propan-2-yl acetate	33.05	0.05
M362	()-beta-Pinene	44.77	0.05
M363	ACETIC ACID,BORNYL ESTER	67.15	0.08
M364	Germacrene B	18.69	0.06
M365	beta-Bisabolene	29.59	0.06
M366*	ZINC05223929	31.57	0.83
M367	5-isopropyl-2-methylbicyclo[3.1.0]hex-2-ene	47.19	0.04

* The candidate compounds

Table S2 The information of all the 280 candidate targets

No.	Candidate Target
T1	2,4-dienoyl-CoA reductase, mitochondrial
T2	26S proteasome non-ATPase regulatory subunit 3
T3	3,2-trans-enoyl-CoA isomerase, mitochondrial
T4	3-hydroxy-3-methylglutaryl-coenzyme A reductase
T5	5-hydroxytryptamine 2A receptor
T6	5-hydroxytryptamine receptor 3A
T7	72 kDa type IV collagenase
T8	78 kDa glucose-regulated protein
T9	Acetylcholinesterase
T10	Acetyl-CoA carboxylase 1
T11	Actin, cytoplasmic 1
T12	Activator of 90 kDa heat shock protein ATPase homolog 1
T13	Adenomatous polyposis coli protein
T14	Adenylate cyclase type 2
T15	Alcohol dehydrogenase 1B
T16	Alcohol dehydrogenase 1C
T17	Aldo-keto reductase family 1 member C3
T18	Aldose reductase
T19	Alpha- and gamma-adaptin-binding protein p34
T20	Alpha-1A adrenergic receptor
T21	Alpha-1B adrenergic receptor
T22	Alpha-1D adrenergic receptor
T23	Alpha-2A adrenergic receptor
T24	Alpha-2B adrenergic receptor
T25	Alpha-2C adrenergic receptor
T26	Amine oxidase [flavin-containing] A
T27	Amyloid beta A4 protein
T28	Androgen receptor
T29	Apoptosis regulator BAX
T30	Apoptosis regulator Bcl-2
T31	Arachidonate 5-lipoxygenase
T32	Aryl hydrocarbon receptor
T33	ATP-binding cassette sub-family G member 2
T34	ATP-citrate synthase
T35	Baculoviral IAP repeat-containing protein 4
T36	Bcl2 antagonist of cell death
T37	Beta-1 adrenergic receptor
T38	Beta-2 adrenergic receptor
T39	Beta-lactamase
T40	C5a anaphylatoxin chemotactic receptor
T41	Calcium-activated potassium channel subunit alpha 1
T42	Calmodulin

Table S3 (to be continued)

No.	Candidate Target
T43	cAMP and cAMP-inhibited cGMP 3',5'-cyclic phosphodiesterase 10A
T44	cAMP-dependent protein kinase inhibitor alpha
T45	Carbonic anhydrase II
T46	CASP8 and FADD-like apoptosis regulator
T47	Caspase-3
T48	Caspase-7
T49	Caspase-8
T50	Caspase-9
T51	Catalase
T52	Cathepsin D
T53	Caveolin-1
T54	C-C motif chemokine 2
T55	CD40 ligand
T56	Cell division control protein 2 homolog
T57	Cell division protein kinase 2
T58	Cell division protein kinase 4
T59	Cell division protein kinase 6
T60	Cellular tumor antigen p53
T61	cGMP-inhibited 3',5'-cyclic phosphodiesterase A
T62	Choline-phosphate cytidylyltransferase A
T63	Claudin-4
T64	Coagulation factor VII
T65	Coagulation factor Xa
T66	Collagen alpha-1(I) chain
T67	C-reactive protein
T68	C-X-C motif chemokine 10
T69	C-X-C motif chemokine 11
T70	C-X-C motif chemokine 2
T71	Cyclin-A2
T72	Cyclin-dependent kinase inhibitor 1
T73	Cyclin-dependent kinase inhibitor 2A, isoforms 1/2/3
T74	Cytochrome c
T75	Cytochrome P450 19A1
T76	Cytochrome P450 1A1
T77	Cytochrome P450 1A2
T78	Cytochrome P450 1B1
T79	Cytochrome P450 2B6
T80	Cytochrome P450 3A4
T81	Cytochrome P450-cam
T82	DDB1- and CUL4-associated factor 5
T83	Delta-type opioid receptor
T84	Dipeptidyl peptidase IV

Table S3 (to be continued)

No.	Candidate Target
T85	DNA gyrase subunit B
T86	DNA topoisomerase 1
T87	DNA topoisomerase 2-alpha
T88	DNA topoisomerase II
T89	Dolichyl-phosphate beta-glucosyltransferase
T90	Dopamine D1 receptor
T91	Dual oxidase 2
T92	E3 ubiquitin-protein ligase Mdm2
T93	Endothelin-converting enzyme 1
T94	Epidermal growth factor receptor
T95	E-selectin
T96	Estrogen receptor
T97	Estrogen sulfotransferase
T98	ETS domain-containing protein Elk-1
T99	Eukaryotic translation elongation factor 1 epsilon-1
T100	Eukaryotic translation initiation factor 6
T101	Fatty acid synthase
T102	Ferrichrome-iron receptor
T103	G1/S-specific cyclin-D1
T104	G2/mitotic-specific cyclin-B1
T105	Gamma-aminobutyric acid receptor subunit alpha-1
T106	Gamma-aminobutyric-acid receptor alpha-2 subunit
T107	Gamma-aminobutyric-acid receptor alpha-5 subunit
T108	Gamma-aminobutyric-acid receptor subunit alpha-6
T109	Gap junction alpha-1 protein
T110	Glucocorticoid receptor
T111	Glucose-6-phosphatase
T112	Glucose-6-phosphate 1-dehydrogenase
T113	Glutathione S-transferase Mu 1
T114	Glutathione S-transferase Mu 2
T115	Glutathione S-transferase P
T116	Glycogen synthase kinase-3 beta
T117	Heat shock factor protein 1
T118	Heat shock protein beta-1
T119	Heat shock protein HSP 90
T120	Heme oxygenase 1
T121	Hepatocyte growth factor receptor
T122	Hepatocyte nuclear factor 1-alpha
T123	Hepatocyte nuclear factor 4-alpha
T124	Hexokinase-2
T125	High affinity immunoglobulin epsilon receptor subunit beta
T126	Homeobox protein Nkx-3.1

Table S3 (to be continued)

No.	Candidate Target
T127	Hyaluronan synthase 2
T128	Hypoxia-inducible factor 1-alpha
T129	Ig gamma-1 chain C region
T130	Induced myeloid leukemia cell differentiation protein Mcl-1
T131	Inhibitor of nuclear factor kappa-B kinase subunit alpha
T132	Insulin
T133	Insulin receptor
T134	Insulin-like growth factor 1 receptor
T135	Insulin-like growth factor II
T136	Insulin-like growth factor-binding protein 3
T137	Integrin beta-2
T138	Intercellular adhesion molecule 1
T139	Interferon gamma
T140	Interferon regulatory factor 1
T141	Interleukin-1 alpha
T142	Interleukin-1 beta
T143	Interleukin-10
T144	Interleukin-13
T145	Interleukin-2
T146	Interleukin-4
T147	Interleukin-6
T148	Interleukin-8
T149	Interstitial collagenase
T150	Intestinal-type alkaline phosphatase
T151	Kinetochore protein Nuf2
T152	Leukotriene A-4 hydrolase
T153	Low affinity immunoglobulin epsilon Fc receptor
T154	Lysozyme
T155	Maltase-glucoamylase, intestinal
T156	Matrix metalloproteinase-9
T157	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial
T158	Methylglutaconyl-CoA hydratase, mitochondrial
T159	Microtubule-associated protein 2
T160	Mineralocorticoid receptor
T161	Mitochondrial uncoupling protein 2
T162	Mitogen-activated protein kinase 1
T163	mRNA of PKA Catalytic Subunit C-alpha
T164	Muscarinic acetylcholine receptor M1
T165	Muscarinic acetylcholine receptor M2
T166	Muscarinic acetylcholine receptor M3
T167	Muscarinic acetylcholine receptor M4
T168	Muscarinic acetylcholine receptor M5

Table S3 (to be continued)

No.	Candidate Target
T169	Mu-type opioid receptor
T170	Myc proto-oncogene protein
T171	Myeloperoxidase
T172	NAD(P)H dehydrogenase [quinone] 1
T173	NADPH oxidase 1
T174	NADPH oxidase 3
T175	NADPH--cytochrome P450 reductase
T176	Neuronal acetylcholine receptor protein, alpha-7 chain
T177	Neuronal acetylcholine receptor subunit alpha-2
T178	Neutrophil cytosol factor 1
T179	NF-kappa-B essential modulator
T180	NF-kappa-B inhibitor alpha
T181	Nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase
T182	Nitric oxide synthase, endothelial
T183	Nitric oxide synthase, inducible
T184	Nuclear factor erythroid 2-related factor 2
T185	Nuclear receptor coactivator 1
T186	Nuclear receptor coactivator 2
T187	Nuclear receptor subfamily 1 group I member 2
T188	Nuclear receptor subfamily 1 group I member 3
T189	Ornithine decarboxylase
T190	Osteopontin
T191	Peroxidase C1A
T192	Peroxisomal acyl-coenzyme A oxidase 1
T193	Peroxisomal bifunctional enzyme
T194	Peroxisome proliferator activated receptor gamma
T195	Peroxisome proliferator-activated receptor alpha
T196	Peroxisome proliferator-activated receptor delta
T197	Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN
T198	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, gamma isoform
T199	Plasminogen activator inhibitor 1
T200	Poly [ADP-ribose] polymerase 1
T201	Potassium voltage-gated channel subfamily H member 2
T202	Probable E3 ubiquitin-protein ligase HERC5
T203	Procollagen C-endopeptidase enhancer 1
T204	Pro-epidermal growth factor
T205	Progesterone receptor
T206	Proliferating cell nuclear antigen
T207	Prostaglandin E synthase
T208	Prostaglandin E2 receptor EP3 subtype
T209	Prostaglandin G/H synthase 1

Table S3 (to be continued)

No.	Candidate Target
T210	Prostaglandin G/H synthase 2
T211	Prostatic acid phosphatase
T212	Proteasome activator complex subunit 3
T213	Protein CBFA2T1
T214	Protein kinase C alpha type
T215	Protein kinase C beta type
T216	Proto-oncogene c-Fos
T217	Proto-oncogene serine/threonine-protein kinase Pim-1
T218	Puromycin-sensitive aminopeptidase
T219	Putative beta-glucuronidase-like protein SMA3
T220	Queuine tRNA-ribosyltransferase
T221	RAC-alpha serine/threonine-protein kinase
T222	RAF proto-oncogene serine/threonine-protein kinase
T223	Ras association domain-containing protein 1
T224	Ras GTPase-activating protein 1
T225	Receptor tyrosine-protein kinase erbB-2
T226	Retinoblastoma-associated protein
T227	Retinoic acid receptor RXR-alpha
T228	Retinoic acid receptor RXR-gamma
T229	Rhodopsin
T230	Runt-related transcription factor 2
T231	Serine/threonine-protein kinase Chk1
T232	Serum albumin
T233	Serum paraoxonase/arylesterase 1
T234	Signal transducer and activator of transcription 1-alpha/beta
T235	Sodium channel protein type 5 subunit alpha
T236	Sodium/iodide cotransporter
T237	Sodium/potassium-transporting ATPase subunit gamma
T238	Sodium-dependent dopamine transporter
T239	Sodium-dependent noradrenaline transporter
T240	Sodium-dependent serotonin transporter
T241	Solute carrier family 2, facilitated glucose transporter member 4
T242	Solute carrier family 22 member 5
T243	Sterol regulatory element-binding protein 1
T244	Stromelysin-1
T245	Superoxide dismutase [Cu-Zn]
T246	Thrombin
T247	Thrombomodulin
T248	Thromboxane A2 receptor
T249	Tissue factor
T250	Tissue-type plasminogen activator
T251	Transcription factor AP-1

Table S3 (to be continued)

No.	Candidate Target
T252	Transcription factor E2F1
T253	Transcription factor E2F2
T254	Transcription factor p65
T255	Transforming growth factor beta-1
T256	Transient receptor potential cation channel subfamily M member 2
T257	Transient receptor potential cation channel subfamily V member 1
T258	Trifunctional enzyme subunit beta, mitochondrial
T259	Tripartite motif-containing protein 26
T260	Trypsin-1
T261	Trypsin-3
T262	Tumor necrosis factor
T263	Type I iodothyronine deiodinase
T264	Tyrosinase
T265	UDP-glucuronosyltransferase 1-1
T266	Urokinase-type plasminogen activator
T267	Vascular cell adhesion protein 1
T268	Vascular endothelial growth factor A
T269	Vascular endothelial growth factor receptor 2
T270	Voltage-dependent L-type calcium channel subunit alpha-1S
T271	Xanthine dehydrogenase/oxidase
T272	Amine oxidase [flavin-containing] B
T273	Baculoviral IAP repeat-containing protein 5
T274	Bcl-2-like protein 1
T275	Receptor tyrosine-protein kinase erbB-3
T276	Serine/threonine-protein kinase Chk2
T277	Collagen alpha-1(III) chain
T278	G1/S-specific cyclin-D2
T279	Gamma-aminobutyric-acid receptor alpha-3 subunit
T280	Protein kinase C zeta type