Good Practice in Traditional Chinese Medicine Research in the Post-Genomic Era

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Report on analysis of existing protein databases and software for *In-Silico* studies of phytocomplexes

Document description	
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Abstract	Computer databases and software available for *in-silico* studies of Chinese Herbal Medicines (CHM) are catalogued, and a review presented of the recent literature where such studies have been reported.
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1 REPORT ON EXISTING PROTEIN DATABASES AND SOFTWARE FOR *IN SILICO* STUDIES OF PHYTOCOMPLEXES

1.1 Introduction

In establishing the guidelines for good practice in *in-silico* research into traditional Chinese medicines (TCM), it was deemed appropriate first of all to draw up a catalogue of the various resources currently available, and to review the use of such resources in the *in-silico* TCM research reported in the literature to date.

The computing resources to be considered included the various databases currently available and the software that has been or might be used in analyses of these data.

The various different kinds of databases identified as relevant included those holding ethnobotanical and/or chemical and/or pharmacological and/or toxicological data on the herbs used in Chinese medicine, as well as those that hold data on known or potential molecular targets for the herbal constituents.

The software tools considered as relevant included programs that provide for (a) virtual screening of natural product libraries and chemical libraries, (b) pattern recognition, (c) proteomics/genomics/metabolomics data visualisation and analysis, and (d) text mining.

1.2 Catalogue of existing databases

A catalogue of the existing databases relevant to computational studies of Chinese Herbal Medicines (CHM) was drawn up using the knowledge and expertise of individual WP4 members together with information presented in a recent literature review covering this area[1]. The databases considered included those holding (a) botanical information on TCM herbs, and the composition of TCM formulae and their usage, (b) information on the phytochemical constituents of plants (including those used in TCM and others), and (c) information on known and potential protein targets of the phytochemical constituents of herbs used in TCM.

The databases containing category (a) and/or category (b) data are shown in Table 1; those containing data in category (c) are shown in Table 2.

Table 1 – A catalogue of databases holding (a) botanical information on TCM herbs, and the composition of TCM formulae and their use(s), and/or (b) information on the phytochemical constituents of plants (including those used in TCM and others)

DATABASE	CONTENT	SOURCE	URL
TCM Assistant	TCM herbs, herbal formulas, diseases and patent prescriptions. No structures	-	http://www.tcmassistant.com
Dictionary of Chinese Herbs	TCM herbal formulas, specificity, toxicity and side effects. No structures.	-	http://Alternativehealing.org/Chinese_herbs_dictionary.htm
Dictionary of Natural Products (DNP)	Major source of chemical information on natural products, including some biological sources, and pharmacological and toxicological data. Full set of structures.	-	http://dnp.chemnetbase.com
China Natural Products Database (CNPD)	Information on Chinese natural products including >40,000 structures. Full set of structures.	Shen *et al.*[2]	http://www.neotrident.com
3D Structure Database of Components from Chinese Herbs	3D structures (>10,000) from Chinese herbs (>2,000), with descriptors and data on clinical uses. Full set of structures.	Qiao *et al.*[3]	-
Traditional Chinese Medicines Database (TCMD)	Information and structures for >10,000 compounds from >4,500 species. Full set of structures.	Yan *et al.*[4]	http://www.cambridgesoft.com
TCM Knowledge Based Grid	TCM herb database, literature database, traditional Tibetan herb database. No structures.	-	http://www.cintcm.com

Chinese herbal constituents database (CHCD) and Bioactive plant compounds database (BPCD)	Information and structures for >13,000 constituents of ≈300 commonly used herbs. >2,500 compounds active against ≈80 targets. Full set of structures.	Ehrman *et al.*[5]	-
Dr. Duke's Phytochemical and Ethnobotanical Databases	Information on phytochemicals from >1000 plants, including Chinese herbs. No structures.	Duke[6]	http://www.ars-grin.gov/duke/
PhytochemDB	Chemical composition of 1,278 taxa (>19,000 constituents), including Chinese herbs. No structures.	-	http://ukcrop.net/perl/ace/search/PhytochemDB
Ethnopharmacological database (GPNDB™)	100,000 natural products (3D structures), biological activities, ethnopharmacological data. In-house database of Greenpharma S.A.	Do & Bernard[7]	http://www.greenpharma.com
Comprehensive Herbal Medicine Information System for Cancer (CHMIS-C)	Integrated information on cancer molecular targets, Chinese herbal recipes and phytochemical constituents. Some structures.	Fang *et al.*[8]	http://sw16.im.med.umich.edu/chmis-c/
Traditional Chinese Medicine Information Database (TCM-ID)	Information on 1197 formulas, 1098 herbs and 9852 constituents in relation to TCM diagnosis and prescription. Some structures.	Chen *et al.*[9]	http://tcm.cz3.nus.edu.sg/group/tcm-id/tcmid.asp
Traditional Chinese Medicine Database System	Bibliographic database (TCMLARS), and Chinese herb database (TCDBASE) in addition to other data. No structures.	-	http://www.cintcm.com

TCMGeneDIT	Information on relations between TCM and gene regulation, protein-protein interactions and biological pathways. No structures	Fang *et al.*[10]	http://tcm.lifescience.ntu.edu.tw/
TCM Database@Taiwan	Chinese medicine database that contains 3-D structural information of TCM constituents - ready for molecular docking simulation (database currently holds 37,170 (32,364 non-duplicate) TCM compounds from 352 TCM)	Chen[11]	http://tcm.cmu.edu.tw/review.php?menuid=3

Table 2 – A catalogue of databases holding information on the structures of known and potential protein targets of the phytochemical constituents of herbs used in TCM

DATABASE	CONTENT	SOURCE	URL
Therapeutic Target Database (TTD)	Information on 1,894 targets, 5,028 drugs, diseases, and pathways.	Zhu *et al.*[12]	http://xin.cz3.nus.edu.sg/group/ttd/ttd.asp
Potential Drug Target Database (PDTD)	Information on 830 targets, protein and active site structures, biological functions, diseases and pathways.	Gao *et al.*[13]	http://www.dddc.ac.cn/pdtd/
Protein Data Bank	Information of 70,000+ protein structures determined by single crystal X-ray diffraction or high filed ^{1}H-NMR studies	Berman *et al.*[14]	http://www.rcsb.org/pdb

Of the TCM and natural product databases that have been constructed that provide information relevant to *in-silico* studies of CHM (Table 1), the majority (10/16) appear to contain either limited or no chemical data on the phytochemical constituents of the herbs, and would clearly, therefore, have limited utility in any bioinformatics/cheminformatics research.

As regards the protein/target databases holding information relevant to *in-silico* CHM studies (Table 2), the principle resource – since it contains details of ALL publicly available protein 3D structures - is provided by the Protein Data Bank (PDB)[14]. The Therapeutic Target Database (TTD)[12] and Potential Drug Target Database (PDTD)[13] are more focused resources, providing details of proteins of specific interest in development of new drug therapies and/or studies of mechanisms of drug/CHM phytochemical action.

1.3 Types of software for use in computational studies of CHM

As for the review of databases (above), examples of software tools relevant to computational studies of Chinese Herbal Medicines (CHM) were catalogued using the knowledge and expertise of individual WP4 members together with information presented in the review by Ehrman *et al.* (2010)[1].

Several categories of software were deemed relevant:

1. Ligand based screening programs Pre-requisite(s) for use: knowledge of compounds with known activity; use: to identify putatively active compounds; prediction methods employed: Classification/regression trees (including Random Forest), Linear discriminants analysis, artificial neural networks, support vector machines.

2. Pharmacophore programs Can be either ligand-based (LB), or target-based (TB) (the latter being superior/preferable); pre-requisite(s) for use: 3D structures of known ligands to chosen targets (LB), or known 3D structures of target protein(s), and ideally known 3D structure(s) of known complex(es)) (TB). Only one program known in this class: LigandScout[15]

3. Docking programs Pre-requisites: known 3D structure(s) of target proteins; use: to 'dock' potential small molecule ligands into protein active sites, optimising their topographical and chemical complementarity, and scoring their interaction. Examples include: FlexX[16], Gold[17], Dock[18], Glide[19], MolDock[20], AutoDock[21], and LigandFit[22].

4. Pattern recognition Multi-purpose programs for post-screening analyses; algorithms employed (for the purposes of dimensionality reduction) include: principle components analysis, multi-dimensional scaling, self-organising maps, and various forms of cluster analysis.

5. Proteomics and/or genomics data visualisation and analysis Application specific programs for statistical processing and visualisation of data output from DNA micro-array experiments, MS proteomics experiments etc.

6. Text mining Use: to scour digital literature sources (PDF files, web sites etc) to extract (textual) information on CHM botanical / chemical / target / use(s). Such programs were deemed of marginal relevance here.

1.4 Reports of target-oriented *in-silico* screening of bioactive material in CHM

A comprehensive review of the reported research dealing with *in-silico* studies of bioactive material in CHM was recently presented by Ehrman *et al.*[1], covering the literature over the period 2000-2010. The report presented below represents a digest of the discussions and critique from this review that are pertinent to the WP4 considerations here.

1.4.1 Virtual screening of Chinese herbs for drug discovery

A number of studies dealing with the virtual screening (VS) of the phytochemical constituents of CHM, mostly published within the last five years, have focused on the use of computational techniques to identify potential ligands for various targets. The methods employed in screening include pharmacophore search, docking, and screening based on molecular descriptors and fingerprints.

There are a number of cases reported where compounds found in Chinese herbs have been identified as candidate inhibitors of specific enzymes or receptors by VS, and these activities have subsequently been confirmed by experiment.

In the first of the studies summarised below, it is noteworthy that significant information leading to selection of appropriate plant material came from consideration of TCM usage. This indicates the importance of ethnopharmacological data to virtual and other forms of screening.

1 Sieboldigenin, a spirostane sterol, is found in a number of species of *Smilax,* of which *S. glabra* (tufuling) is found in TCM as a 'heat clearing' herb, employed largely for arthritic joint pain and skin disease. Molecular docking showed that the compound binds to the active site of soybean lipoxygenase (SLOX), and it was later found to inhibit SLOX with an IC_{50} of 38 μM, as well as reducing carageenan-induced paw oedema[23].

2 Likewise, leucovorin was discovered as a potential anti-HIV agent by screening natural products from Chinese sources, using a molecular fingerprint derived from the HIV protease

inhibitor, saquinavir. Favourable binding to the protease active site was subsequently confirmed through molecular dynamics simulations[24].

3 Molecular fingerprints, followed by docking, were also responsible for the discovery of aurantiamide acetate, from *Artemisia annua* (qinghao), as an inhibitor of severe acute respiratory syndrome coronavirus main proteinase (SARS-CoV M^{pro})[25].

4 VS of quorum sensing inhibitors of *Pseudomonas aeruginosa*, was performed in a search for ways of preventing biofilm formation on surfaces[26]. In this study, 51 TCM compounds with known antibacterial activity were docked into the active site of the transcription activation factor TraR. Subsequent *in-vitro* screening of eight high scoring compounds identified baicalein as possessing synergistic inhibition (with ampicillin) of *P. aeruginosa* growth.

5 Scopoletin and sanggenons were identified as inhibitors of acetylcholinesterase and cyclo-oxygenase, respectively, using protein-based pharmacophores, and these activities were subsequently confirmed through *in-vitro* studies[27,28].

Other VS studies on CHM consitituents have been performed – and either involve no use of TCM in guiding the screening, or else yielded potential ligands that were not subsequently proved as active experimentally.

6 Given the use of *Epimedium* spp as tonics for 'yang invigoration' in TCM, there was interest in in the ability of its constituents to mimic the inhibitory effect of sildenafil on phosphodiesterase 5 (PDE5), and using a number of computational techniques, including pharmacophores, docking and QSAR, a variety of candidates were duly discovered as potential inhibitors of PDE5, of which some, similar in structure to ES-03b, showed docking scores comparable to another inhibitor taladafil, with hydrogen bonding to Asn663 playing a key role[29].

7 In another study[29] on aromatase inhibitors from Chinese herbs, three flavonoids – myricetin, gossypetin and liquiritigenin – were first identified by VS based on molecular descriptors, using known phytochemical inhibitors in training. These were then docked into the active site of human aromatase, where liquiritigenin was shown to have a lower binding energy than the other two. Subsequent *in vitro* experiment confirmed the docking results, liquiritigenin showing the highest potency with an IC_{50} of 0.34 μM, a tenfold increase over the first generation inhibitor, aminoglutethimide.

8 In recent years, protein kinases have become a central focus for drug discovery, and are particularly important as potential targets in the development of anti-cancer therapies. In the last two years, three studies on VS of Chinese herbs for compounds active against kinases involved in various aspects of cancer biology have been reported. These comprise aurora-A kinase, a regulator of centrosome function in mitosis[31], polo-like kinases (PLK) again implicated in mitosis[32], and KDR kinase, closely associated with vegetative endothelial growth factor (VEGF) and involved in angiogenesis of solid tumours[33]. In all cases, a similar procedure was employed, using pharmacophores generated from a set of known inhibitors.. These were then used to screen the CNPD[2] (in the case of the first two) and the TCMD[4] (for the KDR kinase pharmacophore). Subsequently, hits were filtered on the basis of Lipinski rules, QSAR regression predictions of IC_{50}, and docking to relevant PDB receptors. In the case of KDR kinase, a pterocarpan glucoside was identified from the TCMD, and subsequently found by experiment to show a dissociation constant (K_d) of 30 μM.

1.4.2 Searching for multiple target ligands in Chinese herbs

VS studies have also been conducted in the search for inhibitors of multiple functionally related targets, or components of pathways involved in specific pathologies. Such studies either involve searches for single compounds which inhibit a variety of targets, or for 'cocktails' of ligands, each of which inhibits a series of (pathologically-related) targets of interest.

Huang *et al.*[34] report multiple components of the Chinese formula *Xuefu zhuyu tang* along with their possible targets in the treatment of cardiovascular disease. In this study, 'drug-like' compounds with acceptable ADME profiles were first identified by Lipinski filtering. These were then docked to a number of targets known to play an important role in cardiovascular disease, such as rennin, angiotensin-converting enzyme (ACE), VEGFR, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) and P-glycoprotein (Pgp). 283 hits were obtained as possible inhibitors of the full set of targets. Of the 11 herbs investigated, most were identified as possible inhibitors of more than one target (10 herbs containing constituents potentially inhibiting two or more targets, and half of these containing some potentially inhibiting five targets).

Ehrman *et al.*[35], have made use of multiple decision trees to fish for compounds in Chinese herbs on the basis of structural similarity to known phytochemical inhibitors of targets involved in several disease processes, including inflammation, HIV and diabetes. In this study, Random Forest was employed to discriminate phytochemical inhibitors from over 8,000 compounds found in 240 herbs. If RF failed to distinguish a target compound satisfactorily from the relevant set of inhibitors on the basis of three sets of molecular descriptors, then that compound was flagged as a potential inhibitor. The distribution of hits was then analysed.

Despite relatively stringent selection criteria, approximately 62% of botanical species were hit at least once, with half of these predicted to contain compounds active against two or more targets. A literature search was performed to provide corroborative evidence, in which 29% of the predicted classes within these herbs were supported by recent data, with about 80% of publications providing new information distinct from that which had been used to construct the models in the first place.

Further work by Ehrman *et al.*[36] utilised protein-based screening, employing multiple PDB ligand-receptor complexes, to identify possible ligands of four major targets in inflammation – COX, p38 MAP kinase (p38 MAPK), c-Jun terminal NH_2 kinase (JNK), and phosphodiesterase 4 (PDE4). A conformational database of Chinese herbal constituents was first screened with LigandScout pharmacophores from approximately 60 PDB entries. The resulting hits were then docked to the relevant receptors, and docking scores compared to the median binding energy of all crystal ligands for that target. A comparison of cumulative scores for compounds from distinct phytochemical classes within each herb resulted in the choice of 100 herbs as being most likely to yield mimics comparable in potency to the PDB ligands used. Subsequent analysis revealed that those Chinese formulaic categories in which these herbs were most concentrated, are frequently used in the treatment of disease with a strong inflammatory component.

1.4.3 Inverse docking and target fishing

Inverse docking and target fishing are VS techniques used to identify targets 'from scratch', and they thus have a particularly important role to play in the case of phytochemicals. Indeed, two programs have been developed to aid in elucidating the targets of herbal constituents, particularly with those from Chinese medicine in mind. The first of these is INVDOCK[37], similar in terms of its docking methodology to the program DOCK[18] and making use of a database of protein cavities derived from PDB entries, against which individual compounds are tested. The algorithm employs a multi-conformer shape matching alignment of the compound to the protein cavity, followed by torsion optimization and energy minimization using the AMBER forcefield. Protein cavities were defined using overlapping spheres following the approach of Kuntz *et al.*[18], the database now numbering about 9,000 entries. Validation studies, using a number of phytochemicals for which some targets are well established, including ginsenoside Rg1, baicalin, quercetin, emodin, catchin and allicin, identified about 50% of the targets for which relevant experimental data exist[38,39]. INVDOCK has also been used to identify possible targets of the cytotoxic compound ganoderic acid D from the Chinese fungus *Ganoderma lucidum* (lingzhi), following investigation into its effects on expression of 21 proteins. The results suggested that it may be able to bind to eight of these receptors directly[40].

Another inverse docking program, TarFisDock[41], makes use of a similar procedure, involving the construction of protein receptor database from the PDB (each entry comprising those residues within 6.5 Å of the ligand), with the active site defined by spheres as above. This is followed by docking, again based on DOCK[18]. TarFisDock is designed to be used with the PDTD[13] and currently contains over 1,100 entries covering approximately 830 known or potential drug targets, with each target linked to information on biological function and disease. Query compounds can be uploaded, and the database searched for candidate targets via a web interface available at http://www.dddc.ac.cn/pdtd/. To date, this approach has been successful in finding a *Helicobacter pylori* target for the natural product N-*trans*-caffeoyltyramine in the enzyme peptide deformylase. Subsequent *in vitro* screening revealed that this compound, and one if its derivatives, were potent inhibitors with IC_{50} values of 10.8 and 1.25 μM respectively[42].

A similar approach, based again upon docking in combination with an in-house ethnopharmacological database, is Greenpharma's 'reverse pharmacognosy'[43]. Whereas pharmacognosy seeks to identify bioactive compounds from plants, based upon extraction and assay, reverse pharmacognosy seeks to screen targets and diseases from individual compounds, in part by the use of compound and target databases linked by the docking program *Selnergy*, and thence to identify botanical sources rich in these metabolites. Among other examples, ε-viniferin, a stilbene found in various species of *Sophora* (used in TCM) as well as grapes, was discovered in this way to be an inhibitor of phosphodiesterase 4.

Docking is not the only method by which phytochemical targets may be sought. As far as information derived from the PDB is concerned, one alternative concerns the use of pharmacophore models of PDB ligands, against which the compound is screened. Pharmacophore screening is quicker than docking, and filters out those compounds which are not direct mimics of the ligand from which the pharmacophore has been generated. The principal drawback is that compounds showing alternative binding modes will not then be hit, though this can equally have its advantages in that the results are more directly related to experiment than many docking simulations.

The first use of PDB-based pharmacophores for target fishing of medicinal plant constituents is reported by Rollinger *et al.*[44]. In this study, low energy conformers of 16 constituents of *Ruta graveolens* were screened against a database containing 2,208 pharmacophores. *In-vitro* screening against three targets – AChE, HRV coat protein and cannabinoid receptor type-2 (CB_2) – demonstrated a close degree of congruity between the best hits and their respective IC_{50} values.

1.5 References

[1] Ehrman, T.M., Barlow, D.J. and Hylands, P.J. (2010) *Phytochemical Informatics & Virtual Screening of Herbs used in Chinese Medicine.* Current Pharmaceutical Design, **16**, 1785-1798.

[2] Shen JH, Xu XY, Cheng F, Liu H, Luo XM, Shen JK, Chen KX, Zhao WM, Shen X, Jiang HL. (2003) Virtual Screening on Natural Products for Discovering Active Compounds and Target Information. Curr. Med. Chem. 10:1241-1253.

[3] Qiao X, Hou TJ, Zhang W, Guo SL, Xu XJ. A 3D structure database of components from Chinese traditional medicinal herbs. (2002) J. Chem. Inf. Comput. Sci. 42:481-489.

[4] Yan X, Zhou J, Xie, G. *Traditional Chinese Medicines: Molecular structures, natural sources, and applications*; Milne, G.W.A., Ed..; Ashgate; UK, 1999.

[5] Ehrman, T.M., Barlow, D.J., Hylands, P.J. (2007) *Phytochemical Databases of Chinese Herbal Constituents and Bioactive Plant Compounds with Known Target Specificities*. J. Chem. Inf. Mol. Modelling, **47**, 254-263.

[6] Duke JA. *Handbook of Phytochemical Constituents of GRAS Herbs and Other Economic Plants.* CRC Press; Boca Raton, 1992.

[7] Do QT, Bernard P. Reverse pharmacognosy: a new concept for accelerating natural drug discovery. In Khan MTH, Ather A Eds *Lead molecules from natural products*. Elsevier, 2006, pp 1-20.

[8] Fang X, Shao L, Zhang H, Wang S. (2005) CHMIS-C; a comprehensive herbal medicine information system for cancer. J. Med. Chem. 48:1481-1488.

[9] Chen X, Zhou H, Liu YB, Wang JF, Li H, Ung CY, Han LY, Cao ZW, Chen YZ. (2006) Database of traditional Chinese medicine and its application to studies of mechanism and to prescription validation. Br. J. Pharmacol. 149:1092-1103.

[10] Fang YC, Huang HC, Chen HH, Juan HF. (2008) TCMGeneDIT: a database for associated traditional Chinese medicine, gene and disease information using text mining. BMC Complement. Altern. Med. 14:8-18.

[11] Chen, CY-C (2011) TCM Database@Taiwan: The World's Largest Traditional Chinese Medicine Database for Drug Screening In Silico. PLoS ONE 6(1):e15939. doi:10.1371/journal.pone.0015939

[12] Zhu F, Han B, Kumar P, Liu X, Ma X, Wei X, Huang L, Guo Y, Han L, Zheng C, Chen Y. (2010) Update of TTD: therapeutic target database. Nucl. Acids Res. 38 (Database issue):D787-791

[13] Gao Z, Li H, Zhang H, Liu X, Kang L, Luo X, Zhu W, Chen K, Wang X, Jiang H. (2008) PDTD: a web-accessible protein database for drug target identification. BMC Bioinformatics 9:104-110.

[14] Berman, HM, Westbrook, J, Feng, Z, Gilliland, G, Bhat, TN, Weissig, H, Shindyalov, IN, Bourne, PE (2000) The Protein Data Bank Nucleic Acids Research, 28: 235-242.

[15] Langer, T Wolber, G (2004) Virtual combinatorial chemistry and in silico screening: efficient tools for lead discovery? Pure Appl Chem, 76: 991-996.

[16] Rarey, M, Wefing, S, Lengauer, T (1996) Placement of medium-sized molecular fragments in active sites of proteins. J. Comp.-aided Mol. Design 10: 41-54.

[17] Jones, G, Willett, P, Glen, RC, Leach, AR, Taylor, R (1997) Development and validation of a genetic algorithm for flexible docking. J. Mol. Biol. 267: 727-748.

[18] Shoichet, BK, Kuntz, ID (1993) Matching chemistry and shape in molecular docking. Prot. Eng. 6: 723-732.

[19] Halgren, TA, Murphy, RB, Friesner, RA, Beard, HS, Frye, LL, Pollard, WT, Banks, JL (2004) Glide: a new approach for rapid, accurate docking and scoring: 1. Method assessment of docking accuracy. J. Med. Chem. 47: 1739-1749.#

[20] Thomsen, R, Christensen, MH (2006) MolDock: a new technique for high-accuracy molecular docking. J. Med. Chem. 49: 3315-3321.

[21] Goodsell, DS, Morris, GM, Olson AJ (1996) Automated docking of flexible ligands: applications of AutoDock. J. Mol. Recogn. 9: 1-5.

[22] Venkatachalam, CM, Jiang, X, Oldfield, T, Waldman, M (2003) LigandFit: a novel method for the shape-directed rapid docking of ligands to protein active sites. J. Mol Graphics Model. 21: 289-307.

[23] Khan I, Nisar M, Ebad F, Nadeem S, Saeed M, Khan H, Ullah S, Khuda F, Karim N, Ahmad Z. (2009) Anti-inflammatory activities of sieboldigenin from *Smilax china* Linn.: Experimental and computational studies. J. Ethnopharmacol. 121:175-177.

[24] Gao WN, Li Y, Zhang R, Gao H, Xu WR, Li AX, Du QS, Zhang X, Wei DQ. (2007) Screening of new HIV inhibitors based on the database of traditional Chinese medicine. Yaoxue Xuebao 2006; 41:241-246. Wang SQ, Du QS, Zhao K, Li AX, Wei DQ, Chou KC. Virtual screening for finding natural inhibitor against cathepsin-L for SARS therapy. Amino Acids 33:129-137.

[25] Wang SQ, Du QS, Zhao K, Li AX, Wei DQ, Chou KC. (2008) Virtual screening for finding natural inhibitor against cathepsin-L for SARS therapy. Amino Acids 2007; 33:129-137.

[26] Zeng ZR, Qian L, Cao LX, Tan HM, Huang YL, Xue XL, Shen Y, Zhou SN. Virtual screening for novel quorum sensing inhibitors to eradicate biofilm formation of *Pseudomonas aeruginosa*. Appl. Microbiol. Biotechnol. 79:119-126.

[27] Rollinger JM, Hornick A, Langer T, Stuppner H, Prast H. (2004) Acetylcholinesterase inhibitory activity of scoploin and scopoletin discovered by virtual screening of natural products. J. Med. Chem. 47:6248-6254.

[28] Rollinger JM, Bodensieck A, Seger C, Ellmerer EP, Bauer R, Langer T, Stuppner H. Discovering COX-inhibiting constituents of *Morus* root bark: Activity-guided versus computer-aided methods. Planta Med. 2005; 71:399-405.

[29] Chen CY, Chang YH, Bau DT, Huang HJ, Tsai FJ, Tsai CH, Chen CYC. (2009) Discovery of potent inhibitors for phosphodiesterase 5 by virtual screening and pharmacophore analysis. Acta Pharm. Sin. 30:1186-1194.

[30] Paoletta S, Steventon GB, Wildeboer D, Ehrman TM, Hylands PJ, Barlow DJ. (2008) Screening of herbal constituents for aromatase inhibitory activity. Bioorg. Med. Chem. 16:8466-8470.

[31] Deng XQ, Wang HY, Zhao YL, Xiang ML, Jiang PD, Cao ZX, Zheng YZ, Luo SD, Yu LT, Wei YQ, Yang SY. (2008) Pharmacophore modelling and virtual screening for identification of new Aurora-A kinase inhibitors. Chem. Biol. Drug Des. 71:533-539.

[32] Wang HY, Cao ZX, Li LL, Jiang PD, Zhao YL, Luo SD, Yang L, Wei YQ, Yang SY. (2008) Pharmacophore modeling and virtual screening for designing potential PLK1 inhibitors. Bioorg. Med. Chem. Lett. 18:4972-4977.

[33] Yu H, Wang ZL, Zhang LR, Zhang JF, Huang Q. (2007) Pharmaophore modeling and in silico screening for new KDR kinase inhibitors. Bioorg. Med. Chem. Lett. 17:2126-2133.

[34] Huang Q, Qiao XB, Xu XJ. (2007) Potential synergism and inhibitors to multiple target enzymes of Xuefu Zhuyu decocotion in cardiac disease therapeutics: A computational approach. Bioorg. Med. Chem. Lett. 17:1779-1783.

[35] Ehrman TM, Barlow DJ, Hylands PJ. (2007) Virtual screening of Chinese herbs with Random Forest. J. Chem. Inf. Model. 47:264-278.

[36] Ehrman TM, Barlow DJ, Hylands PJ. (2010) In-silico search for multi-target anti-inflammatories in Chinese herbs and formulas. Bio-org. Med. Chem. 18: 2204-2218.

[37] Chen YZ, Zhi DG. (2001) Ligand-protein inverse docking and its potential use in the computer search of protein targets of a small molecule. Proteins 43: 217-226.

[38] Chen X, Ung CY, Chen YZ. (2003) Can an *in silico* drug-target search method be used to probe potential mechanisms of medicinal plant ingredients? Nat. Prod. Rep. 20: 432-444.

[39] Chen YZ, Ung CY. (2002) Computer automated prediction of potential therapeutic and toxicity protein targets of bioactive compounds from Chinese medicinal plants. Am. J. Chin. Med. 30: 139-154.

[40] Yue QX, Cao ZW, Guan SH, Liu XH, Tao L, Wu WY, Li YX, Yang PY, Liu X, Guo DA. (2008) Proteomics characterization of the cytotoxicity of mechanism of ganoderic acid D and computer-automated estimation of the possible drug target network. Mol. Cell Proteomics 7: 949-961.

[41] Li HL, Gao ZT, Kang L, Zhang HL, Yang K, Yu KQ, Luo XM, Zhu WL, Chen KX, Shen JH, Wang XC, Jiang HL. (2006) TarFisDock: a web server for identifying drug targets with docking approach. Nucl. Acids Res. 34:219-224.

[42] Cai JH, Han C, Hu TC, Zhang J, Wu DL, Wang FD, Liu YQ, Ding JP, Chen KX, Yue JM, Shen X, Jiang HL. (2006) Peptide deformylase is a potential target for anti-*Helicobacter*

pylori drugs: Reverse docking, enzymatic assay, and X-ray crystallography validation. Protein Sci. 15:2071-2081.

[43] Do QT, Bernard P. Reverse pharmacognosy: a new concept for accelerating natural drug discovery. In Khan MTH, Ather A Eds *Lead molecules from natural products*. Elsevier, 2006, pp 1-20.

[44] Rollinger JM, Schuster D, Danzl B, Schwaiger S, Markt P, Schmidtke M, Gertsch J, Raduner S, Wolber G, Langer T, Stuppner H. (2009) *In silico* target fishing for rationalized ligand discovery exemplified on constituents of *Ruta graveolens*. Planta Med. 75:195-204.