Bioorganic & Medicinal Chemistry 18 (2010) 2204–2218

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

In silico search for multi-target anti-inflammatories in Chinese herbs and formulas

Thomas M. Ehrman [a], David J. Barlow [a,*], Peter J. Hylands [b]

[a] Pharmaceutical Sciences Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK
[b] Centre for Natural Medicines Research, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK

ARTICLE INFO

Article history:
Received 13 August 2009
Revised 21 January 2010
Accepted 29 January 2010
Available online 8 February 2010

Keywords:
Chinese herbs
c-Jun terminal-NH_2 kinase
COX
Docking
JNK
LigandScout
MolDock
p38 MAP kinase
PDB
Pharmacophore
Phosphodiesterase

ABSTRACT

Chinese herbs were screened for compounds which may be active against four targets involved in inflammation, using pharmacophore-assisted docking. Multiple LigandScout (LS) pharmacophores built from ligand–receptor complexes in the protein databank (PDB) were first employed to select compounds. These compounds were then docked using LS-derived templates and ranked according to docking score. The targets comprised cyclo-oxygenases 1 & 2 (COX), p38 MAP kinase (p38), c-Jun terminal-NH_2 kinase (JNK) and type 4 cAMP-specific phosphodiesterase (PDE4). The results revealed that multi-target inhibitors are likely to be relatively common in Chinese herbs. Details of their distribution are given, in addition to experimental evidence supporting these results. Examples of compounds predicted to be active against at least three targets are presented, and their features outlined. The distribution of herbs containing predicted inhibitors was also analysed in relation to 192 Chinese formulas from over 50 herbal categories. Among those found to contain a high proportion of these herbs were formulas traditionally used to treat fever, headache, rheumatoid arthritis, inflammatory bowel disorders, skin disease, cancer, and traumatic injury. Relationships between multi-target drug discovery and Chinese medicine are discussed.

1. Introduction

The paradoxical situation in which the pharmaceutical industry now finds itself, where breakthroughs in technology and an increasing volume of chemical and biological information have been matched by a puzzling decrease in the emergence of new drug entities, has led many to wonder whether a return to nature may not offer some attractions in rediscovering the 'sweet spot' in drug discovery.[1]

Yet, due in part to the relative neglect of natural products over recent decades, both the volume and quality of information necessary to evaluate fully the potential of new chemical entities, particularly in the initial stages of selection, is often less than satisfactory. This is particularly true of phytochemicals compared to natural products of microbial origin. Thus, only a single monograph exists so far which attempts to bring together data on the molecular targets of plant compounds,[2] and though information on the chemical constituents of herbs has seen an almost exponential increase over recent decades, it is perhaps doubtful whether such progress can be sustained in view of the alarming rate at which species of potential medicinal value may be disappearing.[3]

Clearly, other information has to be exploited as effectively as possible, and in this respect virtual screening offers many avenues of exploration. In particular, it can harness the large amount of data on compounds of synthetic origin, or on macromolecular complexes such as those found in the protein databank (PDB), to construct models which can then be used to screen natural products for compounds expected to show similar activities.

Opportunities to use the PDB for purposes of virtual screening have received considerable impetus in recent years, not only from advances in docking technology, but also in the appearance of automated methods for the construction of 3D pharmacophores from ligand–receptor complexes, in which the relevant features are identified from experimentally determined patterns of hydrogen-bonding, ionic and hydrophobic interactions.[4] Such models can be used both to filter conformational databases of target compounds, and to direct docking experiments with pharmacophore-derived templates. This can result in an increase in the speed with which a target database can be screened compared to conventional docking protocols, can make screening using multiple models (parallel screening) against multiple targets a realistic goal,[5] and may also increase the accuracy of prediction, in that hits are likely, on the basis of pharmacophore features, to bind to the same receptor residues as crystal ligands.

* Corresponding author. Tel.: +44 207 848 4827; fax: +44 207 848 4800.
E-mail address: dave.barlow@kcl.ac.uk (D.J. Barlow).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.01.070

The targets selected in this study, cyclo-oxygenases 1 & 2 (COX), p38 MAP kinase (p38), c-Jun terminal-NH_2 kinase (JNK) and type 4 cAMP-specific phosphodiesterase (PDE4), were chosen for a variety of reasons. First and foremost, though inflammation is not the only therapeutic niche that all of them share, it is nevertheless much the most important one. The constitutively expressed COX-1 and inducible COX-2 are the best known targets of non-steroidal anti-inflammatory drugs to date,[6] while p38 plays a central role in inflammation due to its involvement in the production of tumour necrosis factor (TNF)-α and other cytokines, induction of pro-inflammatory enzymes such as COX-2, inducible nitric oxide synthase (iNOS) and matrix metalloproteinases, and adhesion molecule expression.[7] As such, it is a preferred target for the development of drugs to treat rheumatoid arthritis. More recently both p38 and JNK have been found to play a role in chronic obstructive pulmonary disease (COPD), another condition with a strong inflammatory component.[8]

JNK, closely related to p38, is a more recent addition to the list of inflammation targets, and has elicited great interest since its discovery in the 1990s. The presence and activity of the JNK pathway in multiple cell types involved in the inflammatory process has drawn attention to the development of JNK inhibitors as potential immuno-modulatory agents with applications to the treatment of arthritis, asthma, cancer and neurodegenerative disease.[9]

Meanwhile, PDE4, long considered a promising target for the treatment of asthma, COPD[10] and depression,[11] has more recently been suggested as a potential target in the treatment of inflammatory bowel disease,[12] and may be of more general interest in the development of new anti-inflammatory drugs.[13] The close relationships between PDE4, p38 and JNK have been highlighted by the discovery that roflumilast, a potent and selective PDE4 inhibitor, prevents phosphorylation of both p38 and JNK, thus blocking production of inflammatory mediators such as TNF-α, and interleukin (IL)-1β, and induction of COX-2.[14] This in turn suggests that compounds with the ability to inhibit more than one of these targets, or alternatively, combinations of compounds which inhibit each, may have greater potential in combating pathologies with an inflammatory component than selective inhibitors against only a single target.

A further factor in choosing these four enzymes was the fact that, by comparison to many targets of therapeutic interest, they are well represented by PDB entries, with ligands from a variety of chemical classes. Almost all of these ligands are inhibitors, many having a high degree of potency. Among them are: (1) *COX*—indomethacin and derivatives, diclofenac, flurbiprofen, fatty acids, and the selective COX-2 inhibitor SC-558; (2) *JNK*—imidazole–pyrimidines, phenanthrolines, pyridine–carboxamide inhibitors, pyrazoloquinolones, aminopyridines, 4-anilinopyrimidines, cyanobenzothienamides, and pyrazolylpyrrole inhibitors; (3) *p38*—pyridinylimidazole and derivatives, 4-anilinoquinazolines, diarylurea inhibitors, dihydroquinazolinones, pyridol-pyrimidines, 4-azaindoles, various pyridine and indole-derived inhibitors, diphenylether derivatives, triazolopyridine, and pyrazolourea and pyrazoloamine compounds; (4) *PDE4*—rolipram, mesopram, roflumilast and related inhibitors, sildenafil and vardenafil, and pyrazole carboxylic esters. References to these crystal ligands are indicated in superscript in column 1 of Table 1 (see below), and may be found in the Supplementary data. While high pharmacophore diversity is theoretically achievable using only a single scaffold (provided substituents are sufficiently varied), utilizing multiple chemical classes will greatly enhance diversity and rate of hits in most cases.

Previous ligand-based virtual screening of Chinese herbs suggested that a large number of compounds might inhibit multiple targets.[15] Furthermore, it was found that compounds from different classes within the same plant might show activity against the same target, possibly due in part to motifs conserved across a variety of chemical scaffolds. Protein-based parallel screening against a range of targets, which may be used together in the treatment of a particular condition such as inflammation, can therefore highlight those herbs which might be expected to show particular promise as a source of multi-target inhibitors, or on herbal combinations which achieve similar effects.

Here, multiple pharmacophore models have been built with LigandScout (LS)[4] to screen our Chinese herbal constituents database[†] (CHCD)[16] for compounds expected to show some degree of binding affinity for the four molecular targets outlined above. The resulting hits were then docked with MolDock,[17] incorporating LS features as constraints, and ranked according to docking score. Their distribution among Chinese herbs was analysed, and relations with those Chinese formulas used in the treatment of inflammatory conditions further explored.

2. Data and methods

2.1. PDB entries used in search and docking

For each target, every non-redundant entry in the PDB was used where possible. Redundant entries refer to those entries where both the ligand and receptor subtype (e.g., JNK1 or JNK3, PDE4B or PDE4D) are identical and hydrogen bond contacts between ligand and protein involve the same residues. A small number of entries were omitted in cases where information on crystal coordinates or other pertinent details were missing.

The number of entries selected for each target, and its subtypes, were: (1) COX-1 = 7, COX-2 = 3; (2) p38α = 29; (3) JNK-1 = 4, JNK-3 = 7; (4) PDE4B = 6, PDE4D = 8. All entries, with the exception of COX, are of human origin. For COX, sheep (COX-1) and mouse (COX-2) variants were used.

2.2. Phytochemical conformational database from Chinese herbs

2.2.1. Lipinski filtering

All 'druglike' compounds were first selected from the CHCD.[†] For a compound to be considered druglike it had to violate no more than 1 out of 4 rules from Lipinski's 'rule-of-five', which predicts passive intestinal absorption of a chemical compound. The rules are: (1) molecular weight <500; (2) number of hydrogen bond acceptors $\leqslant$5; (3) number of hydrogen bond donors $\leqslant$10; (4) calculated log P <5. Log P was calculated by an atomic contribution method.[18] The number of compounds selected in this way numbered 5978.

For the purposes of this study, prior filtering using the 'rule-of-five' served to remove a variety of compounds, such as a number of highly glycosylated saponins, which are either likely to be poorly absorbed, or else are likely to undergo extensive modification prior to absorption.

2.2.2. Conformer generation

For all druglike compounds, a maximum of fifty 3D low energy conformers were generated in MOE (Chemical Computing Group, Montreal, Quebec). Though 50 conformers might be considered a relatively small number in the case of some drugs and natural products, which have a large number of rotatable bonds, for the great majority of phytochemicals (with the exception of long chain aliphatics and aromatics) it is sufficient, as these compounds are frequently fairly rigid. In most instances, MOE failed to generate

[†] The CHCD is an in-house database (details for which have been published in Ref. 16), but it will hopefully be made publically available in 2010.

Table 1
Details of PDB ligands used in pharmacophore search and docking

PDB ID	Isoform	Ligand	IC_{50} (nM)	MW	LS pharmacophore features	H-bond contacts	MolDock Score (kcal/mol)	RMSD (Å)	DPI (Å)
COX									
1cqe[2,4]	1	Flurbiprofen	500	243.2	4 hyd, 2 acc, 1 neg	Arg120, Tyr355	−84.34	1.581	1.01*
1pge[1,2]	1	Iodosuprofen	1000	385.2	4 hyd, 2 acc, 1 neg	Tyr355, Ser530	−98.06	0.183	1.43
1pgg[1,2]	1	Iodoindomethacin	780	448.2	4 hyd, 1 acc, 1 neg	Tyr355	−102.25	0.360	3.44
1q4g[3]	1	α-Methyl-4-biphenyl-acetic acid	110	225.2	3 hyd, 2 acc, 1 neg	Arg120, Tyr355	−87.84	0.138	0.35
1diy[5,6]	1	Arachidonic acid	—	303.4	5 hyd, 1 acc, 1 neg	Arg120, Tyr355	−9.06	3.342	1.24*
1fe2[5,6]	1	Dihomo-γ-linoleic acid	—	305.4	6 hyd, 1 acc, 1 neg	Arg120, Tyr355	−18.36	1.416	1.20
1igx[5,6]	1	Eicosapentanoic acid	—	301.4	7 hyd, 1 acc, 1 neg	Arg120, Tyr355	−58.92	8.853	1.43*
1igz[5,6]	1	Linoleic acid	—	279.4	6 hyd, 1 acc, 1 neg	Arg120, Tyr355	−52.42	2.229	1.02*
3pgh[2,4]	2	Flurbiprofen	500	243.2	4 hyd, 1 acc, 1 neg	Tyr355	−87.41	0.235	0.91
4cox[1,2]	2	Indomethacin	68	356.7	4 hyd, 3 acc, 1 neg	Tyr355, Ser530	−120.41	0.212	1.29
6cox[2]	2	SC-558 (selective inhibitor)	9.3	446.2	4 hyd, 2 acc, 1 don	Arg120, Gln192, Phe518	−119.21	0.290	0.97
JNK									
1pmn[7]	3	Imidazole–pyrimidine inhibitor (I)	7.1	580.2	6 hyd, 1 aro, 1 acc	Val78	−121.94	0.430	0.47
1pmq[7]	3	Imidazole–pyrimidine inhibitor (II)	1.6	516.5	6 hyd, 1 aro, 1 acc	Met149	−125.01	1.352	0.48
1pmu[7]	3	Phenanthroline inhibitor	590	272.3	3 hyd, 3 acc	Gln75, Met149, Gln155	−105.02	0.087	0.88
2exc[8]	3	Pyridine–carboxamide inhibitor (I)	—	420.4	5 hyd, 1 acc, 1 don	Met149	−112.47	0.237	0.24
2g01[9]	1	Pyrazoloquinolone inhibitor	1220	262.6	3 hyd, 1 acc	Met111	−60.72	5.91	2.39*
2gmx[10]	1	Aminopyridine inhibitor	77	435.2	3 hyd, 2 acc, 1 don	Lys55, Met108, Glu109, Met111	−92.33	2.805	2.23
2h96[8]	1	Pyridine–carboxamide inhibitor (II)	2300	343.3	3 hyd, 1 acc	Met111	−87.34	0.173	1.14
2no3[11]	1	4-Anilino-pyrimidine inhibitor	—	321.3	3 hyd, 1 acc, 1 don	Leu110, Met111	−82.25	0.232	1.27
2o0u[12]	3	Cyanobenzo-thienamide inhibitor (I)	—	473.6	3 hyd, 2 aro, 1acc, 1 don	Met146, Met149	−136.70	3.047	0.45*
2o2u[12]	3	Cyanobenzo-thienamide inhibitor (II)	—	300.3	3 hyd, 1 aro, 1 acc, 1 don	Met146, Met149	−101.06	0.175	0.66
2ok1[13]	3	Pyrazolylpyrrole inhibitor	—	376.8	3 hyd, 1 acc, 1 don	Glu147, Met149	−110.29	1.454	0.66
p38									
1a9u[14]	α	Pyridinyl-imidazole inhibitor (SB203580)	48	377.4	4 hyd, 2 aro, 2 acc	Lys53, Met109	−87.79	0.208	0.59
1bl6[14]	α	Pyridinyl-imidazole inhibitor (SB216995)	160	293.3	4 hyd, 1 aro, 1 acc	Met109	−63.05	0.156	0.59
1bl7[14]	α	Pyridinyl-imidazole inhibitor (SB220025)	19	339.3	3 hyd, 1 aro, 2 acc, 1 don, 1 pos	Lys53, Met109, Asp168	−92.09	2.048	0.70*
1bmk[14]	α	Pyridinyl-imidazole inhibitor (SB218655)	25	309.3	4 hyd, 1 aro, 2 acc	Lys53, Met109	−81.64	0.308	0.51
1di9[15]	α	4-Anilino-quinazoline inhibitor	5000	327.4	3 hyd, 1 aro, 1 acc	Met109	−75.81	0.268	0.68
1kv1[16]	α	Diarylurea inhibitor (I)	1160	306.7	3 hyd, 1 acc, 2 don	Glu71, Asp168	−87.98	0.225	0.74
1kv2[16]	α	Diarylurea inhibitor (II)	0.1	528.7	5 hyd, 1 acc, 2 don	Glu71, Asp168	−151.47	0.238	1.14
1m7q[17,18]	α	Dihydro-quinazolinone inhibitor	2.6	488.3	3 hyd, 1 aro, 1 acc, 1 pos	Met109	−96.67	0.158	0.53

1ouk[14]	α	Pyridinyl-imidazole inhibitor (SB084501)	0.13	507.5	5 hyd, 1 aro, 1 acc, 1 pos	Met109	−125.61	0.339	0.59
1ouy[18]	α	Pyridol-pyrimidine inhibitor (SB094501)	4.3	518.3	6 hyd, 2 acc, 1 pos	Met109, Gly110	−109.09	0.136	0.57
1ove[17,18]	α	Dihydro-quinolinone	0.74	488.3	6 hyd, 1 aro, 1 acc	Met109, Gly110	−123.54	0.131	0.34
1oz1[19]	α	4-Azaindole inhibitor	—	305.3	3 hyd, 1 aro, 2 acc, 1 pos	Lys53, Met109	−74.84	0.124	0.42
1w7h[20]	α	3-(Benzyloxy)-pyridin-2-amine	100,000	200.2	2 hyd, 1 aro, 1 acc, 1 don	Thr106, His107, Met109	−68.66	0.207	0.40
1w82[16]	α	Diarylurea inhibitor (III)	196	368.8	4 hyd, 2 aro, 1 acc, 1 don	Glu71, Asp168	−133.15	0.663	0.41
1w83[20]	α	Pyridine-derived inhibitor (I)	65	441.8	5 hyd, 1 aro, 2 acc, 1 don	Glu71, Met109, Asp168	−116.47	0.502	0.73
1w84[20]	α	Indole-derived inhibitor (I)	35,000	222.2	3 hyd, 2 aro, 1 acc, 1 don	Ala51, Met109	−76.51	0.199	0.41
1wbn[20]	α	Pyridine-derived inhibitor (II)	350	399.8	4 hyd, 2 aro, 2 acc, 1 don	Glu71, Met109, Asp168	−121.69	0.239	0.51
1wbs[20]	α	Indole-derived inhibitor (II)	630	444.5	5 hyd, 2 aro, 2 acc, 2 don	Ala51, Glu71, Met109, Asp168	−155.00	0.262	0.25
1wbt[20]	α	Indole-derived inhibitor (III)	340	444.5	6 hyd, 2 aro, 1 acc, 1 don	Glu71, Asp168	−147.18	0.380	0.37
1wbv[20]	α	Indole-derived inhibitor (IV)	162,000	339.3	3 hyd, 2 aro, 1 acc, 1 don	Glu71, Asp168	−116.34	0.171	0.31
1wbw[20]	α	Pyridine-derived inhibitor (III)	44,000	250.3	3 hyd, 1 aro, 1 acc, 1 don	His107, Met109	−83.34	0.263	0.68
1yqj[20]	α	Pyridazine inhibitor	7.3	458.5	4 hyd, 2 aro, 1 acc, 1 don, 1 pos	Met109, Ser154	−139.45	0.126	0.35
1zyj[21]	α	Diphenylether derivate (I)	1500	304.3	3 hyd, 1 aro, 2 acc	Met109, Gly110	−88.90	0.282	0.39
1zz2[21]	α	Diphenylether derivative (II)	6000	467.5	4 hyd, 1 aro, 1 acc, 3 don, 1 pos	Asp150, Asn155, Asp168	−134.43	0.204	0.44
1zzl[22]	α	Triazolo-pyridine	5	322.3	4 hyd, 1 aro, 3 acc	Lys53, Met109, Gly110	−118.82	0.214	0.17
2baj[23]	α	Pyrazolourea compound (I)	4	408.3	4 hyd, 1 acc, 1 don, 2 pos	Glu71, Asp168	−130.25	0.197	0.59
2bak[23]	α	Pyrazolourea compound (II)	37	577.6	4 hyd, 1 aro, 2 acc	Met109, Asp168	−20.12	6.122	0.49*
2bal[23]	α	Pyrazoloamine compound (I)	490	376.3	3 hyd, 1 aro, 1 acc, 1 don	Thr106, Met109	−95.76	0.193	0.45
2baq[23]	α	Pyrazoloamine compound (II)	—	371.3	3 hyd, 1 acc, 1 don	Thr106, His107, Met109	−106.11	6.959	1.07*
PDE4									
1oyn[24,25]	D	[R,S]-Rolipram	550	275.3	3 hyd, 2 acc	Gln369	−84.87	0.381	0.43
1tbb[24,25]	D	Rolipram	—	192.2	3 hyd, 2 acc	Gln369	−86.24	0.412	0.20
1xlx[26]	B	Cilomilast	25	342.4	3 hyd, 2 acc, 1 neg	Gln369	−94.03	0.346	0.52
1xlz[26]	B	Filaminast	960	294.3	2 hyd, 2 acc, 1 pos	Gln369	−99.76	2.074	0.35*
1xm4[26]	B	Piclamilast	0.041	382.2	4 hyd, 1 aro, 2 acc	Gln369	−98.36	0.411	0.53
1xm6[26]	B	[R]-Mesopram	420	265.3	3 hyd, 2 acc	Gln369	−96.48	0.125	0.28
1xmu[26]	B	Roflumilast	0.84	404.2	5 hyd, 2 aro, 2 acc	Gln369	−110.34	0.245	0.51
1xom[26]	D	Cilomilast	11	342.4	3 hyd, 2 acc, 1 neg	Gln369	−120.17	1.672	0.15*
1xon[26]	D	Piclamilast	0.021	382.2	4 hyd, 2 aro, 2 acc	Gln369	−98.54	0.446	0.19
1xor[26]	D	Zardaverine	390	268.2	3 hyd, 2 aro, 4 acc, 1 don	Tyr159, Asp318, Gln369	−91.07	0.353	0.14
1xos[26]	B	Sildenafil (Viagra)	20,000	476.6	4 hyd, 1 acc, 1 pos	Tyr233	−102.69	0.498	0.49
1y2b[27]	D	Pyrazole carboxylic ester inhibitor (I)	82,000	168.2	3 hyd, 1 acc	Gln369	−58.49	0.209	0.11
1y2c[27]	D	Pyrazole carboxylic ester inhibitor (II)	270	244.3	4 hyd, 1 aro, 1 acc	Gln369	−73.15	0.339	0.17
1y2d[27]	D	Pyrazole carboxylic ester inhibitor (III)	2000	274.3	4 hyd, 1 acc	Gln369	−61.97	0.361	0.17
1y2e[27]	D	Pyrazole carboxylic ester inhibitor (IV)	160	259.3	4 hyd, 1 aro, 1 acc	Gln369	−74.76	0.150	0.34

PDB ID = four character PDB identifier representing each entry—references in superscript may be found in the Supplementary data; Isoform = receptor subtype (e.g., COX-1, PDE4D, p38α or JNK3;) Ligand = name or class of ligand; IC_{50} (nM) = the nanomolar concentration needed to effect 50% inhibition; MW = molecular weight; LS pharmacophore features = number of LigandScout pharmacophore features (aro = aromatic ring; hyd = hydrophobic sphere; don = H-bond donor; acc = H-bond acceptor; pos = positively charged group; neg = negatively charged group); H-bond contacts = residues with which ligand makes hydrogen bond contacts; MolDock score = MolDock re-ranking score (kcal mol^{-1}); RMSD = RMSD value between crystal coordinates and lowest energy (highest ranking) pose]; DPI = diffraction coordinate-precision index. An asterisk (*) indicates a failure (see text).

even this number, given the RMSD constraints imposed for two conformers to be considered identical (RMSD $\leqslant$0.15 Å).

For details of the stochastic conformational search algorithm employed, the reader is referred to the MOE manual ('conformation import'). Briefly, the algorithm employs a parallelized fragment-based approach, in which molecules are subdivided into overlapping fragments each of which is subjected to stochastic conformational search. The resulting fragment conformers are minimized and then assembled by superposing common atoms. Only fragments with a strain energy of $\leqslant$7 kcal mol^{-1} were retained after search and minimization, and only resulting whole molecule conformers with a strain energy of less than 4 kcal mol^{-1} were accepted. Fragments were minimized using the MMFF94 forcefield.[19] The total number of conformers generated for the 5978 compounds, which remained after Lipinski filtering, was 131,278, giving an average of approximately 22 conformers per compound.

All acidic and basic groups were ionized, both in LS search and subsequent docking, and, where known, chiral constraints were imposed on conformers, according to the information in the CHCD, using the skeletal numbering system in the *Dictionary of Natural Products*.[20] It should be noted, however, that for a number of compounds this information does not exist. In such cases, conformers were generated in which stereogenic centres were not constrained according to the information on chirality in the CHCD. As is clear from the information currently found in the CHCD, however, the better a herb has been characterised phytochemically (as judged by the number of entries for that herb), the more enantiomers have been found. Since the analysis reported here focuses on phytochemical classes within herbs, as opposed to individual compounds (see below), it is likely that even where information on chirality is lacking, enantiomers within the herb (which may not yet have been identified) will reflect the 3D information encoded in those conformers which have been built without these constraints.

2.3. Pharmacophore interpretation and search

LS may be used to construct pharmacophores of varying degrees of sophistication, suitable for export to different programs. In this analysis, MOE-compatible pharmacophores were first built from the ligand of each entry in Table 1. LS defaults were used for all parameters except exclusion volumes, where distances of up to 8 Å from the nearest ligand atom (as opposed to the default of 4 Å, which failed in some instances to cover all alpha carbons in the vicinity of the ligand) were used to identify these. Ionized forms of crystal ligands were used in all cases where ionizable groups exist.

Pharmacophores were then exported to MOE for conformational screening. Prior to screening, it was necessary to make a number of adjustments, since feature interpretation differs slightly between the two programs. Those aromatic rings which LS classified simply as hydrophobic groups were classified as either aromatic or hydrophobic in MOE, using the PCHD scheme (which incorporates directionality of hydrogen bond donors and acceptors, and orientation of aromatic rings). In this way compounds with *either* an aromatic ring (provided it is not heterocyclic, and thus not able to form hydrogen bonds) *or* hydrophobic group, at the appropriate position, will be hit. This is of particular relevance to phytochemical screening, in that terpenoids for the most part lack aromatic rings, while, conversely, rings in phenolic compounds, with the exception of sugars, are almost always aromatic. In this way, an LS pharmacophore in which an aromatic ring is not directly classified as such (due to lack of detection of π–π stacking or cation–π interactions) but rather as a set of hydrophobic atoms, can be interpreted in MOE in a manner which is useful to selection of both phenolic and terpenoid compounds.

Search utilized partial pharmacophores on the basis of the proportion of compounds hit for each feature left out. For each model the number of features left out was determined by the requirement that at least 0.5% of compounds had to be hit. If fewer hits than this were observed, then a subsequent search was run in which one less feature was required. However, no fewer than five features were used. The latter requirement was relaxed in the case of two small JNK inhibitors (2h96 and 2g01) for which LS identified only four features. In the case of these two entries, only the full complement of features was used.

Details of LS parameters used in pharmacophore interpretation may be found in the Supplementary data.

2.4. Docking

All hits were subsequently docked to the receptor of the relevant PDB entry using MolDock (Molegro ApS, Aarhus). This uses evolutionary programming based upon guided differential evolution to find optimal poses of candidate ligands,[21] and tends to produce high accuracy solutions, as judged by validation results, though at some cost to speed.[17]

In cases where a compound was hit by multiple pharmacophores for each target, the PDB entry was selected for docking with the pharmacophore of which it shared the greatest number of features. Though LS does not consider side chain orientation in placing exclusion volumes, rigid receptor docking was in all cases imposed, and water molecules were not included, resulting in greater speed. Of the 58 PDB entries containing details of water molecules, no evidence was found that any act as ligand–receptor hydrogen bond bridges (using hydrogen bond detection in MOE), though hydrogen bonds between ligand and water molecules are commonly found in the case of PDE4, and may influence binding to some extent.

A total of 10 simulations per compound were run. In the great majority of instances, this has been found to be sufficient to identify the lowest energy pose using the MolDock algorithm, and is an important consideration in high-throughput work. Prior to docking, MolDock's cavity detection algorithm was implemented, which has been found to further increase the accuracy of docking and reduce the number of runs required.[17]

For each run a single pose was chosen on the basis of tabu clustering in which a penalty is imposed on any solution less than a specified RMSD threshold (using the default setting of 2.00 Å) from a preceding one. For each compound the lowest energy pose was then selected from these 10 solutions.

During docking, the algorithm was guided by the MolDock scoring procedure which combines ligand–protein and intra-ligand energy terms, based upon the PLP scoring function of Yang and Chen.[22] The Grid version of the MolDock scoring function was employed since this is faster and more suitable for high-throughput screening.

LS-derived constraints were imposed during docking using MolDock's template scoring function, in which poses are rewarded on the basis of similarity to patterns specified by template groups representing pharmacophore features. In this procedure, if an atom matches a group definition (such as a hydrogen bond acceptor) it will be rewarded according to its proximity to the group center using a Gaussian distance measure. For each atom in the ligand, score contributions from centers of all template groups are taken into account, and the overall score is normalized by that of a perfectly fitting ligand (i.e., one containing only the specified template groups in their exact positions). Equal weights were assigned to all features.

After docking, a number of further scores were calculated, including binding affinity and re-ranking score. The latter utilizes a more advanced scoring scheme than that used during docking, and is often more useful for accurate ranking of poses (see Section 3).

In order to assess the efficacy of MolDock in finding low energy solutions for those PDB entries included in this study, crystal ligands were also docked to their respective receptors, the top ranking score recorded, and the RMSD of that pose from the corresponding crystal co-ordinates computed. Subsequent analysis, using the diffraction coordinate-precision index (DPI), is discussed in Results, Section 3.1.

Further details on MolDock scoring functions may be found in the Supplementary data.

3. Results

3.1. Characteristics and potencies of crystal ligands used in pharmacophore search and modeling

The crystallographic data used in LigandScout pharmacophore search and the subsequent (MolDock) docking studies are summarized in Table 1. The PDB entries for each target are listed separately, and the following information included for each: the PDB ID (e.g., 1cqe, 1xom) and reference, the enzyme isoform, the ligand name or chemical class, the ligand's molecular weight, and (where available) its IC_{50} (in most cases sourced from the PDBBind database,[23] but for the COX ligands taken from Sutherland et al.[24]). Table 1 also enumerates the LS pharmacophore features for each ligand, and the receptor residues with which the ligand forms hydrogen bonds.

As a means to provide a *pre hoc* evaluation of the reliability of the MolDock screening of the CHCD, a series of cognate re-dockings were first performed for all crystal ligands—calculating the root mean square difference in atomic co-ordinates (RMSD, in Å) following a least squares superposition of the observed and the first ranked docked ligand poses. In each of these comparisons, the self-docking was taken to be *unsuccessful* if (a) the calculated RMSD was greater than 1.5 Å *and* (b) if the calculated RMSD was greater than the uncertainty in the crystal structure atomic co-ordinates. The latter was quantified as $\sqrt{2}\cdot$DPI, where DPI is the diffraction coordinate-precision index (in Å) computed (for each PDB entry) according to a modified version[25] of the formula due to Blow:[26]

$$\mathrm{DPI} = 0.31(1+s)^{1/2}\cdot V_{\mathrm{M}}^{-1/2}\cdot C^{5/6}\cdot R_{\mathrm{Free}}\cdot d_{\mathrm{min}}^{5/2}$$

where s is the fraction of solvent present in the crystal, V_{M} is the asymmetric unit volume per unit molecular weight (in $Å^3$), C is the completeness of the data, R_{Free} is the free R factor, and d_{min} is the resolution of the crystal structure determination (in Å).

The MolDock re-ranking scores obtained in the self-docking experiments (see Section 2), together with the corresponding RMSD and DPI values are given in Table 1; the self-dockings which proved unsuccessful are flagged with an asterisk. It will be noted that only 11 of the 65 self-dockings are considered as failures, which means that 83% of the docked poses were very close to their corresponding crystal pose. Given the rather stringent criteria employed here, therefore, it is clear that the MolDock scoring scheme used in the subsequent virtual screening of the CHCD provides a reliable means to identify candidate ligands for the various inflammatory targets.

3.2. Enrichment of crystal ligands

It is unwise to place too much reliance on the docking scores achieved by compounds with unknown activity, in the absence of some measure of enrichment for known actives. Enrichment attempts to quantify, in various ways, the proportion of active compounds found near the top of the list when docking scores for all compounds tested are ranked in order of decreasing binding affinity.

A number of metrics have been proposed, such as the area under the receiver operating characteristic curve (ROC), average rank of actives, Z-score, analysis of variance (ANOVA), enrichment factor (EF), robust initial enhancement (RIE), and Boltzmann-enhanced discrimination of receiver operating characteristic (BEDROC).[27] Of these, EF is one of the best known and most widely used, as well as being one of the simpler measures. Since none of the other methods offers any clear-cut advantage over this in many instances, we therefore chose to use EF as a measure of the relative proportion of crystal ligands (known actives) to phytochemical compounds in different fractions of ranked compounds, ranging from 1% to 100%.

Our definition of EF follows that of Pearlman and Charifson,[28] which is described by the following equation:

$$\mathrm{EF} = \frac{\mathrm{Actives}_{\mathrm{sampled}}/N_{\mathrm{sampled}}}{\mathrm{Actives}_{\mathrm{total}}/N_{\mathrm{total}}}$$

where, in this case, actives = crystal ligands, N = number of compounds (comprising both actives and compounds from the CHCD), and the term 'sampled' refers to the number of compounds found within a particular fraction (1%, 10%, etc.) of the top ranking scores.

The maximum EF value for any fraction of the list depends on the number of compounds, and the ratio of the total number of actives to number of compounds sampled in each fraction. Thus, if only the top 10% of the ranked compounds needs to be sampled in order to recover all the active compounds included in docking, then the corresponding EF is 10. If only the top 1% need be sampled, then the corresponding value is 100, However, this only holds where $\mathrm{Actives}_{\mathrm{total}} < N_{\mathrm{sampled}}$. This was the case here, with the following numbers of crystal ligands and phytochemicals for each target: *COX*—11, 2782; *JNK*—11, 3295; *p38*—29, 2911, and *PDE4*—15, 2679. An EF of 1 (for any percentage of compounds sampled) indicates that the number of actives recovered is no better than random expectation, whereas a value below 1 shows that the docking procedure actually discriminated against active compounds.

Performing EF calculations for different percentages gives a better idea, than a single measure, of the extent to which actives cluster near the top of the list. If actives are largely found near the top and gradually fall off as one goes down the list, then this will be reflected in a negative exponential curve.

This was found to be the case here, with actives (crystal ligands) heavily concentrated near the top of the list for each target, particularly for JNK, p38 and PDE4. For these targets, EFs of crystal ligands in the top 1% of each list were 46.4, 32.1 and 29.4, respectively. The results for COX are less good, though the EF for the top 1% is still 14.2.

It was also observed that a high proportion of the most potent inhibitors for each target were identified among the top 1% of scores in each list. In the case of COX, the two compounds showing the lowest IC_{50} values in Table 1, the selective COX-2 inhibitor SC-558 (*6cox*) and indomethacin (*4cox*), ranked 1 and 2, respectively. For PDE4, two highly potent inhibitors, roflumilast (*1xmu*) and piclamilast (*1xon*), ranked 1 and 19, with the slightly weaker inhibitor cilomilast (*1xlx*) being 7 from the top. In the case of JNK, the strongest inhibitor (*1pmq*), was also found in the top 1%. Of the five p38 ligands found in the top 1%, four (*1ouy*, *1yqj*, *1zzl* and *2baj*) were among the most potent, with an average IC_{50} of 5.3 nM.

3.3. Examples of crystal ligands and phytochemical mimics

LigandScout (InteLigand GmbH, Vienna) provides an interface for automatic detection of pharmacophore features which define binding interactions between a ligand and its receptor. PDB entries are first imported, and those features interacting with receptor residues identified. All other features are ignored. Thus, an LS phar-

macophore constitutes a functionally relevant subset of the total number of possible features characterizing the ligand.

As with conventional pharmacophores, LS features are assigned to one of six types—hydrogen bond donor, hydrogen bond acceptor, anion, cation, aromatic ring, and hydrophobic group. Before defining an aromatic ring, however, LS identifies π–π or cation–π interactions. In cases where these are not found, the aromatic ring may either remain undefined or alternatively some or all of its atoms may be classified as a hydrophobic group. Exclusion volumes are also included, placed on alpha carbons of the protein backbone, to filter out compounds which clash sterically with the receptor. An example is shown in Figure 1 (exclusion volumes are not shown).

Figure 2 contains images of the crystal structures of crystal ligands for each target superimposed with docked poses of phytochemical mimics showing similar pharmacophore features and binding characteristics. In the image on the left is the ligand showing the nature and disposition of LS features, while each case on the right shows the same inhibitor overlaid with the top ranking pose of a Chinese herbal compound (shown in red) taken from among the 10 highest ranking compounds against that target. These compounds contained five or more LS features, and achieved a docking score, after appropriate pharmacophore-derived constraints had been imposed, commensurate with the ligands in Table 1.

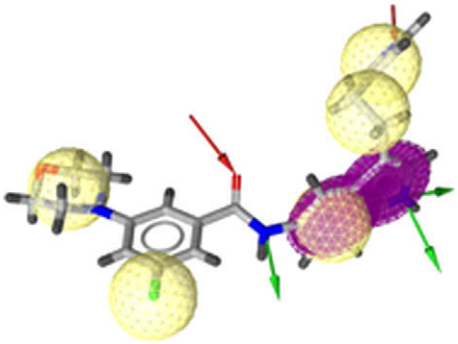

Figure 1. Example of a LigandScout pharmacophore for PDB entry 1WBS. [The ligand is a p38 MAP kinase inhibitor. Red arrow = H-bond acceptor; green arrow = H-bond donor; yellow sphere = hydrophobic sphere, and purple disc = aromatic ring. Note that, in the case of the latter, only those aromatic rings which are involved in π–π or cation–π interactions with the receptor are identified as such.]

For each example, the docking energies (kcal mol^{-1}) for crystal ligand and relevant phytochemical were as follows: (1) *4cox*: {−120.417, −114.874}; (2) *1yqj*: {−139.457, −97.224}; (3) *1pmn*: {−121.948, −120.668}; (4) *1xmu*: {−110.341, −107.092}.

With the exception of *1yqj*, docking scores are very similar between crystal ligands and their mimics. These are not isolated examples, many of the top ranking phytochemicals achieving comparable scores in each case.

3.4. Distribution of predicted inhibitors in Chinese herbs

For each target, median MolDock re-ranking scores were computed for all compounds in each phytochemical class within each herb. Thus, for example, in the case of licorice, which is characterised by oleanane triterpene saponins, isoflavonoids, and a smaller number of compounds from other classes, median values were computed from all compounds within each of these classes. Each median score thus characterizes an herb/class group as opposed to an entire herb.

The following classes were covered: aliphatics, alkaloids, small phenolics, lignans, quinones, flavonoids, tannins, mono-, sesqui-, di- and triterpenes, and sterols. Flavonoids, mono- sesqui- and triterpenes contain distinctive subclasses (well represented in Chinese herbs), namely isoflavonoids, iridoids, sesquiterpene lactones and sterols, which were treated separately. For each herb,

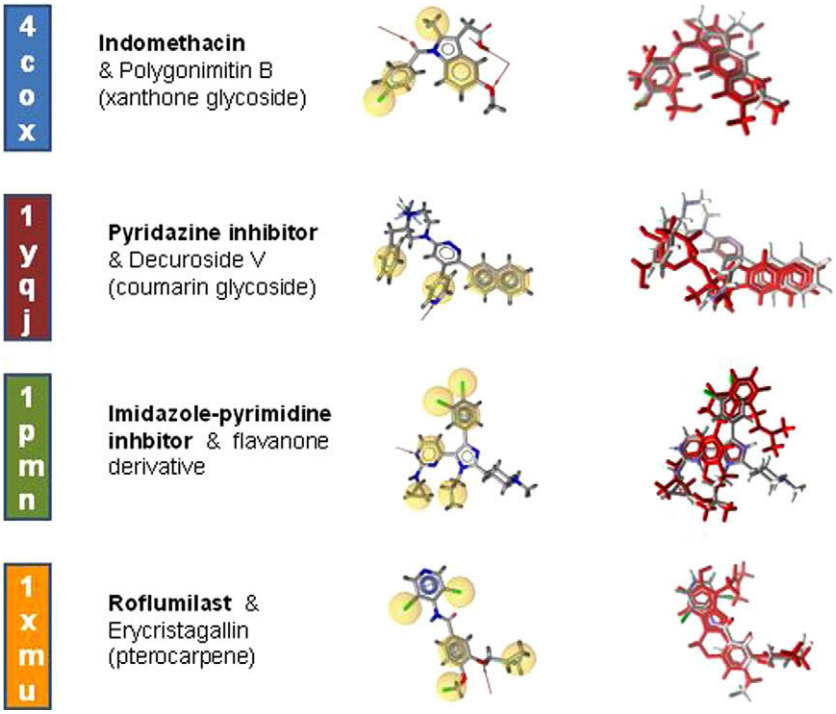

Figure 2. Crystal ligands, LS pharmacophores and low energy poses of phytochemical mimics for one PDB entries per target. [Red arrow = H-bond acceptor; green arrow = H-bond donor; yellow sphere = hydrophobic feature; purple disc = aromatic ring. Structures shown in red in the right hand column represent docked poses of phytochemical mimics of the corresponding compound (bold type) in the left-hand column.] = COX; = p38; = JNK; = PDE4.

it is generally the case that all compounds within each of these relatively broad categories are structurally very similar and usually derived from the same scaffold. In cases where this was not the case then each of these classes was further split into homogeneous sets of compounds, and median scores computed separately for each. However, this was seldom observed.

Only classes for which three or more compounds have been recorded so far in that herb were considered. This helps to eliminate minor constituents which are less likely to contribute towards the activity of an herb. The reason that median as opposed to average scores were chosen concerns the fact that the latter are more easily influenced by outliers, which in turn may reflect suspect docking scores for the compounds in question.

Herb/class groups were ranked in order of increasing binding energy, low energy poses ranking above higher energy ones. Further analysis was restricted to the top 50 herb/class groups for each target. A number of factors need to be considered in making this choice. Firstly, in seeking to identify relationships between the results presented here and data relating to the clinical practice of Chinese medicine (see below), it is meaningless to consider only a handful of compounds, though that might be reasonable if the aim was simply to limit search to highly potent phytochemicals for purposes of drug discovery. Where broader issues need to be addressed, however, a larger number of herbs and classes must be included.

To determine the optimal number of herb/class groups to include in further analysis, a comparison was made between the cumulative distributions of compounds with 'good' docking scores compared to those with 'poor' scores as herb/class groups were sequentially added in order of rank. A compound was defined as 'good' if its score was lower than the median value of all the crystal ligands (in Table 1) for that target, whereas a compound was classified as 'poor' if its score was higher than the highest energy score of all the crystal ligands. If a compound failed to satisfy either of these two criteria (i.e., its score lay between the median and highest scores of the crystal ligands), it remained uncounted. Median and highest scores of crystal ligands for each target were as follows: (1) COX: median = −87.33, highest = 9.21; (2) JNK: median = −86.98, highest = 0; (3) p38: median = −89.21, highest = 10.76; (4) PDE4: median = −86.91, highest = 11.90. In the case of JNK the highest score was, in fact, 130.26, indicating very weak binding, so 0 was taken as the cut-off instead.

Cumulative counts of 'good' and 'poor' compounds found in the 100 top ranking herb/class groups, for each target, and the differential between them, are shown in Figure 3.

For all targets, the cumulative distributions are fairly similar with a net balance in favour of 'good' compounds in up to approximately 50 pooled herb/class groups. As more groups are added, the number of 'poor' compounds grows rapidly beyond this point. If the differential counts, that is, counts of 'poor' compounds subtracted from that of 'good' compounds, are calculated for each point on the x-axis, and the average taken (bottom chart), it is observed that this crosses the abscissa (indicating parity between 'good' and 'poor' compounds) at about 50.

It appears therefore that taking the top ranking 50 herb/class groups per target is optimal, not only in providing sufficient data for analysis in relation to the herbal categories of Chinese medicine (see below), but also in capturing the highest proportion of 'good' compounds, while simultaneously minimizing the number of 'poor' compounds included.

Table 2 shows the class and target distribution for all 100 herbs identified according to the above selection procedure. All herbs are listed by both botanical and equivalent Chinese nomenclature, employing the pinyin system of romanization for Chinese characters. For each target, the class(es) within which predicted inhibitors are found is given in shorthand (see legend of Table 2 for details). References from the literature are given in the column on the extreme right. These refer to papers which provided evidence to support the predictions. Bold italics refer to those papers which provided direct experimental evidence for one or more of the classes listed for each herb (or related species) against one or more targets, while normal type indicates those papers which report anti-inflammatory activity of the herb in question, without details of the class or mechanism involved. These references are listed in the Supplementary data.

The table reveals that many herbs are expected to contain inhibitors for two or more targets (48/100, or 48%), and that, for individual targets, inhibitors from more than one phytochemical class may be found in a single herb (14% of herbs). In many herbs the same class is predicted to be active against more than one target, accounting for 37% of herbs. In other words, for a large proportion of herbs we may expect that the same class will provide anti-inflammatory compounds against multiple targets.

If one considers the distribution of classes in relation to different targets (Fig. 4), we note that the larger terpenoids (diterpenes, triterpenes and sterols), in particular, are very sparsely represented. This is not primarily due to LS and Lipinski filtering (though the larger, more highly glycosylated saponins were excluded at this stage) but rather to the fact that these compounds tended to perform poorly in docking simulations. Smaller terpenoids (sesqui- and monoterpenes, including iridoids), by contrast, are better represented, particularly in the case of COX.

The major phenolic classes of interest comprise smaller phenolics (phenylpropanoids, diarylalkyls, xanthones, etc.), coumarins (and related benzopyrans), flavonoids (excluding isoflavonoids), and lignans. Though the smaller phenolics are well represented for all targets, they are especially so in the case of PDE4. Flavonoid inhibitors are predicted to occur more frequently in the case of p38, and to a lesser extent for JNK and PDE4, while lignans are predominant in the case of JNK.

Aliphatics rarely featured, despite the fact that a number of the COX pharmacophores were derived from fatty acids, and that structurally similar acetylenes (which are characteristic of a number of Chinese herbs) are known to inhibit COX.[29,30] However, these were not highlighted by LigandScout, possibly due to the fact that most do not possess the carboxyl groups which play a role in the binding of fatty acids, and may therefore rely on other features which were not present among the crystal ligands used.

The occurrence of alkaloids, structurally the most diverse class, is sporadic, with a number of skeletal types implicated. These include quinoline, quinazoline, isobutylamide, β-carboline, tryptamine, protoberberine and aporphine types.

Botanically, the herbs in Table 2 belong to a total of 49 families in all. However, only 16 of these families contained more than one herb. Those with the largest numbers of herbs were Asteraceae containing 13 herbs, Fabaceae (11), Apiaceae (7), Lamiaceae (6), Rutaceae (4), Magnoliaceae (3), Polygonaceae (3) and Rubiaceae (3). In the case of the Asteraceae, sesquiterpenes were identified in eight of these herbs as potential inhibitors of all targets, though other classes such as monoterpenes, coumarins, flavonoids and small phenolics were also found. For the Fabaceae, it was the flavonoids (and isoflavonoids—the only family in which these were identified with any frequency) which constituted the major class. Meanwhile, in the case of the Apicaeae it was coumarins which were most often implicated, while alkaloids played the same role in the Rutaceae, and quinones in the Rubiaceae. The associations between these phytochemical classes and their respective families are quite well-known,[20] and it is likely that similar compounds from other plants in these families may show comparable patterns of activity, and act as multi-target anti-inflammatories of varying degrees of potency.

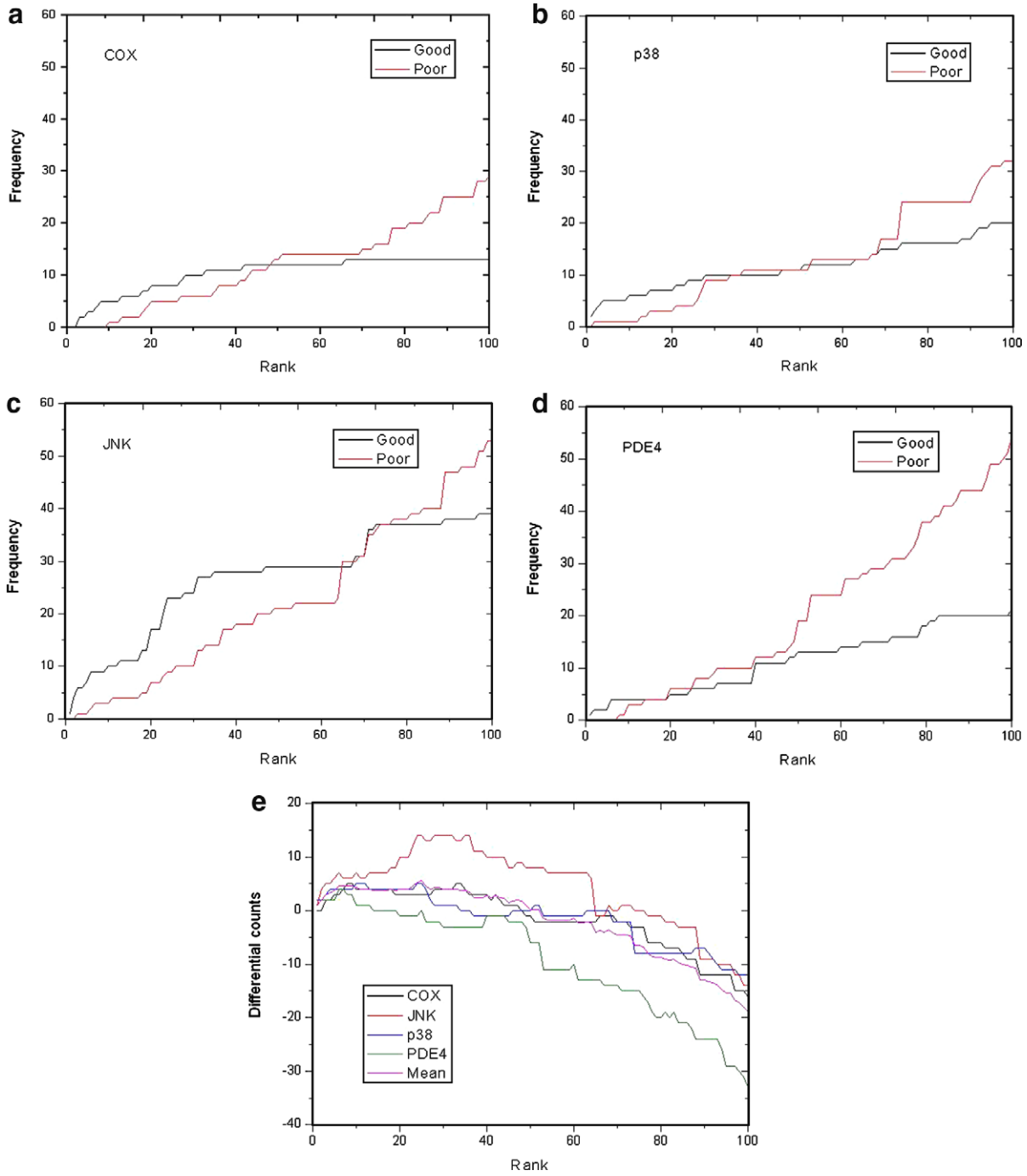

Figure 3. Cumulative distribution of 'good' and 'poor' scores for compounds within the top 100 herb/class groups ranked according to median docking scores. [Cumulative number of herb/class groups is shown on the *x*-axis, while cumulative number of compounds is shown on the *y*-axis. Differential counts for all targets, and their average, are shown in the bottom chart.]

Concerning previously published material which might support these predictions, the right hand column of Table 2 reveals that for an appreciable number of herbs (35) no information could be found. As regards the others, strong support (as defined above) was found in the case of 18 herbs, while for the other 47 herbs the only information available concerned general anti-inflammatory properties, without details as to the mechanisms involved. It is worth noting that much of this information is very recent. Over 80% of relevant papers have been published since the start of 2000, with over 50% published since 2005 (see Supplementary data). This demonstrates that research on the anti-inflammatory potential of many Chinese herbs is still at an early stage, but also that interest has rapidly gained momentum over recent years.

3.5. Examples and characteristics of predicted multi-target inhibitors

As mentioned above, the same phytochemical class is implicated as providing multi-target inhibitors in the case of 37% of the herbs listed in Table 2. These classes are structurally varied, including aliphatics, alkaloids, monoterpenes and iridoids, sesqui- and diterpenes, lignans, quinones and a variety of small phenolics.

Table 2
Patterns of predicted activity of herb/class groups against COX, p38, JNK and PDE4

Species	Herb (pinyin)	COX	p38	JNK	PDE4	Refs.
Abrus precatorius	Ji Gu Cao		F	F	F	1
Acorus gramineus	Shi Chang Pu	SP			SP	—
Ailanthus altissima	Chun Pi			X	X	2
Albizzia julibrissin	He Huan Pi		F			—
Aloe sp.	Lu Hui			B		3, 4
Andrographis paniculata	Chuan Xin Lian	D		D	D	5, 6, 7
Angelica pubescens	Du Huo				B	***8***
Angelica sinensis	Dang Gui				B, SP	8
Aquilaria agallocha	Chen Xiang	B,S				—
Arctium lappa	Niu Bang Zi	A				9
Aristolochia debilis	Qing Mu Xiang	S				—
Artemisia annua	Qing Hao	S				10
Artemisia capillaris	Yin Chen Hao	SP		B, F, SP	B, F, SP	11
Artemisia vulgaris	Ai Ye	S		F		—
Asarum sieboldii	Xi Xin				M	12
Aster tataricus	Zi Wan		M	M		—
Astragalus membranaceus	Huang Qi	iF				13
Belamcanda chinensis	She Gan	iF				14, 15
Bidens pilosa	Xian Feng Cao		SP			16, ***17***, 18, 19
Biota orientalis	Bai Zi Ren		F			—
Bletilla striata	Bai Ji			SP		—
Caesalpinia sappan	Su Mu		iF		SP	—
Carthamus tinctorius	Hong Hua	M	X	X		—
Cassia obtusifolia	Jue Ming Zi	Q				—
Cassia senna	Fan Xie Ye			Q		—
Chrysanthemum indicum	Ye Ju Hua	S				20, 21
Chrysanthemum morifolium	Ju Hua			S	S	20, 21
Cinnamomum cassia	Gui Zhi	F			SP	22, 23, ***24***
Cistanche deserticola	Rou Cong Rong		I	SP		—
Cnidium monnieri	She Chuang Zi	M	B, M		M	25
Codonopsis pilosula	Dang Shen	A, SP	A		A, SP	***26***
Commiphora spp.	Mo Yao	S		L	S	27, ***28***
Coriandrum sativum	Yan Sui Zi	M			B	—
Curculigo orchioides	Xian Mao			SP		—
Curcuma longa	Yu Jin	S		L		29, 30
Curcuma zedoaria	E Zhu		SP			29, 30
Cuscuta chinensis	Tu Si Zi			L		—
Cynanchum atratum	Bai Wei		St			31, 32
Cyperus rotundus	Xiang Fu	S				—
Dalbergia odorifera	Jiang Xiang	iF	F, iF			33
Dictamnus dasycarpus	Bai Xian Pi	X			X	***34***
Dioscorea opposita	Shan Yao				SP	35
Dracaena cinnabari	Xue Jie	D		F	F	36
Epimedium spp.	Yin Yang Huo	SP	L	B, F, L	B, SP	37, ***38***, 39
Erythrina variegata	Hai Tong Pi				F	—
Eucommia ulmoides	Du Zhong		I, L, SP	L	SP	40
Eupatorium chinense	Tu Niu Xi		S		S	41, 42
Evodia rutaecarpa	Wu Zhu Yu		X		X	43
Foeniculum vulgare	Xiao Hui Xiang	SP			SP	44
Forsythia suspensa	Lian Qiao	A	L	A, L, SP		45, 46, ***47***
Fritillaria thunbergii	Zhe Bei Mu				SP	48
Gardenia jasminoides	Zhi Zi		I, M		I	49, 50, ***51, 52***
Gastrodia elata	Tian Ma		SP			53
Ginkgo biloba	Bai Guo Ye	D	F			54, 55
Glycyrrhiza uralensis	Gan Cao	SP	SP	B, SP		56, 57
Hypericum japonicum	Tian Ji Huang			B		58
Imperata cylindrica	Bai Mao Gen		L			—
Inula britannica	Xuan Fu Hua			S	B, F, SP	59, 60
Jasminum officinale	Mo Li Hua	M			SP	61
Juncus effusus	Deng Xin Cao			T		—
Ligusticum chuanxiong	Chuan Xiong	B				—
Lilium brownii	Bai He	SP	SP		SP	***62***
Lippia nodiflora	Peng Lai Cao	F		F		63
Lithospermum erythrorhizon	Zi Cao	Q	Q	Q	Q	64, 65, 66, 67
Lonicera japonica	Jin Yin Hua	I	I	I		***68***, 69, 70
Magnolia biondii	Xin Yi Hua	S			X	***71***
Magnolia officinalis	Hou Po	L	L, SP	L, SP	L, X	***72***
Mentha haplocalyx	Bo He		F			61, ***73, 74***
Morinda officinalis	Ba Ji Tian	Q			Q	75
Morus alba	Sang Zhi		F		F	76, 77
Myristica fragrans	Rou Dou Kou				SP	—
Nandina domestica	Tan Zhu Zi	X				—
Notopterygium incisum	Qiang Huo			B		—

(*continued on next page*)

Table 2 (*continued*)

Species	Herb (pinyin)	COX	p38	JNK	PDE4	Refs.
Paeonia suffruticosa	Mu Dan Pi			SP		78, 79, 80
Patrinia villosa	Bai Jiang			I		—
Perilla frutescens	Zi Su Ye	M	SP		M	81, 82, ***83***, 84, 85
Phellodendron amurense	Huang Bai	X				86
Pinus massoniana	Song Jie	D		L		—
Piper nigrum	Hu Jiao			L, X		—
Pogostemon heyneanus	Guang Huo Xiang		SP			—
Polygala spp.	Yuan Zhi	SP			SP	***87***
Polygonum multiflorum	He Shou Wu		F	SP		***88, 89***
Psoralea corylifolia	Bu Gu Zhi		F	F		—
Pueraria lobata	Ge Gen		iF			90, ***91***
Rheum palmatum	Da Huang		B, SP	SP		92, 93, 94
Rosa laevigata	Jin Ying Zi		T			95, 96, 97
Rubia cordifolia	Qian Cao Gen				Q	—
Santalum album	Tan Xiang				SP	—
Saussurea lappa	Mu Xiang	X, S		S		98, 99
Schisandra chinensis	Wu Wei Zi	M	M			—
Schizonepeta tenuifolia	Jing Jie	M	F, M			100, 101
Scrophularia ningpoensis	Xuan Shen			I		102, 103
Scutellaria rivularis	Ban Zhi Lian				F	***104***, 105
Sophora subprostrata	Shan Dou Gen			F		—
Syzygium aromaticum	Ding Xiang		T			106
Tribulus terrestris	Bai Ji Li			X		—
Vitex spp.	Man Jing Zi				I	107
Xanthium strumarium	Cang Er Zi	S		S	S	108, 109
Zanthoxylum spp.	Chuan Jiao	M	L, X			110
Zea mays	Yu Mi Xu		F			—

Class: A = aliphatic; B = benzopyran/coumarin; D = diterpene; F = flavonoid; iF = isoflavonoid; I = iridoid; L = lignan; M = monoterpene; Q = quinone; S = sesquiterpene; SP = small phenolic; St = sterol; T = triterpene; X = alkaloid. Refs = references to papers providing supporting evidence, found in the supplementary data. Bold italics indicate papers providing direct experimental evidence to support target predictions, while normal type indicates papers giving evidence of anti-inflammatory activity without details of the class or mechanism involved.

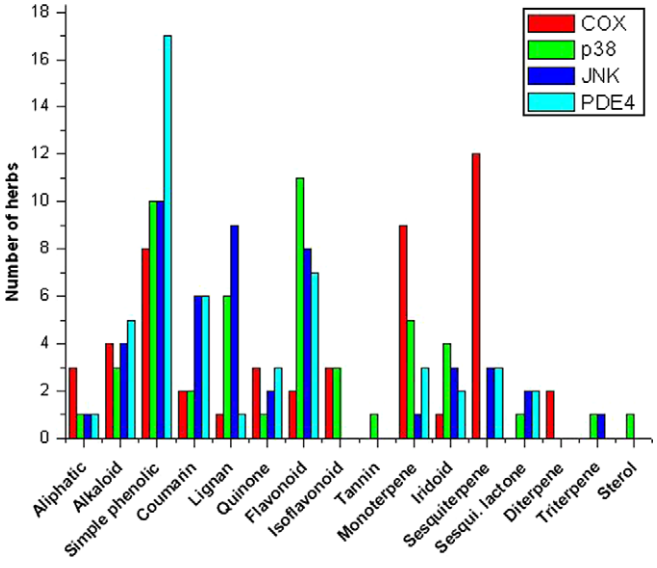

Figure 4. Distribution of phytochemical classes from herbs in Table 2 containing predicted inhibitors against COX, p38, JNK and PDE4.

Twelve examples are shown in Figure 5. These were selected from each herb/class group in Table 2 predicted to be active against 3 or more targets. The exception is the alkaloid in the bottom right hand corner for which only two targets are relevant but which was included as one of the few representatives of its class.

Despite the fact that a large number of classes are included, closer inspection reveals that there are a number of recurring features in such compounds. One is the presence of prenyl groups in the case of capillartemisin B and gancaonin R. Other compounds which include substituents similar to prenyl groups are xanthumanol (with a substituted butyl group) and cnidioside C (with a substituted prenyl group).

Search of all compounds from herb/class groups in Table 2 for prenyl substituents found that 118 out of 860 such compounds (13.7%) shared this characteristic. These substituents were only found for 4.3% of other compounds in the CHCD (5118) thus giving an enrichment of 3.19.

Glycosides are also characteristic of predicted multi-target inhibitors, the examples in Figure 5 being loganin, precatorin I, 3-hexenyl glucoside, cnidioside C and regaloside A. Small glycosides of this type, unlike the larger saponins, do not violate Lipinski rules, and in many cases are likely to be easily absorbed. Such compounds account for 21.9% of the 860 representing Table 2. Their molecular weight in all cases was under 550. By contrast, glycosides having a weight of less than 550 in the remainder of the CHCD accounted for only 8%, giving an enrichment of 2.74. The possible significance of both these features is discussed below.

3.6. Relations with Chinese herbal prescriptions

With rare exceptions, such as the use of single herb 'tonics' (ginseng, for example), Chinese herbal medicine is built around the idea of herbal combinations or formulas, each of which is constructed to address specific disorders. Many of the most famous doctors of antiquity were renowned for their ability to construct elegant, balanced formulas, often incorporating herbs with different properties, and it is perhaps this feature, more than any other, which gives Chinese herbal medicine its unique characteristics.[31] As with single herbs, herbal formulas are grouped into categories (just over 50), all formulas in each category being considered suitable to address a range of related pathologies, though individually they have slightly different emphases.[32] In considering specific therapeutic applications of Chinese herbal medicine, significantly more can therefore be learnt from an analysis of herbal formulas than from considering herbs in isolation.

In seeking to identify multi-target anti-inflammatory Chinese prescriptions, an analysis was made of 192 of the best known formulas in Chinese medicine,[32] identifying those which contained

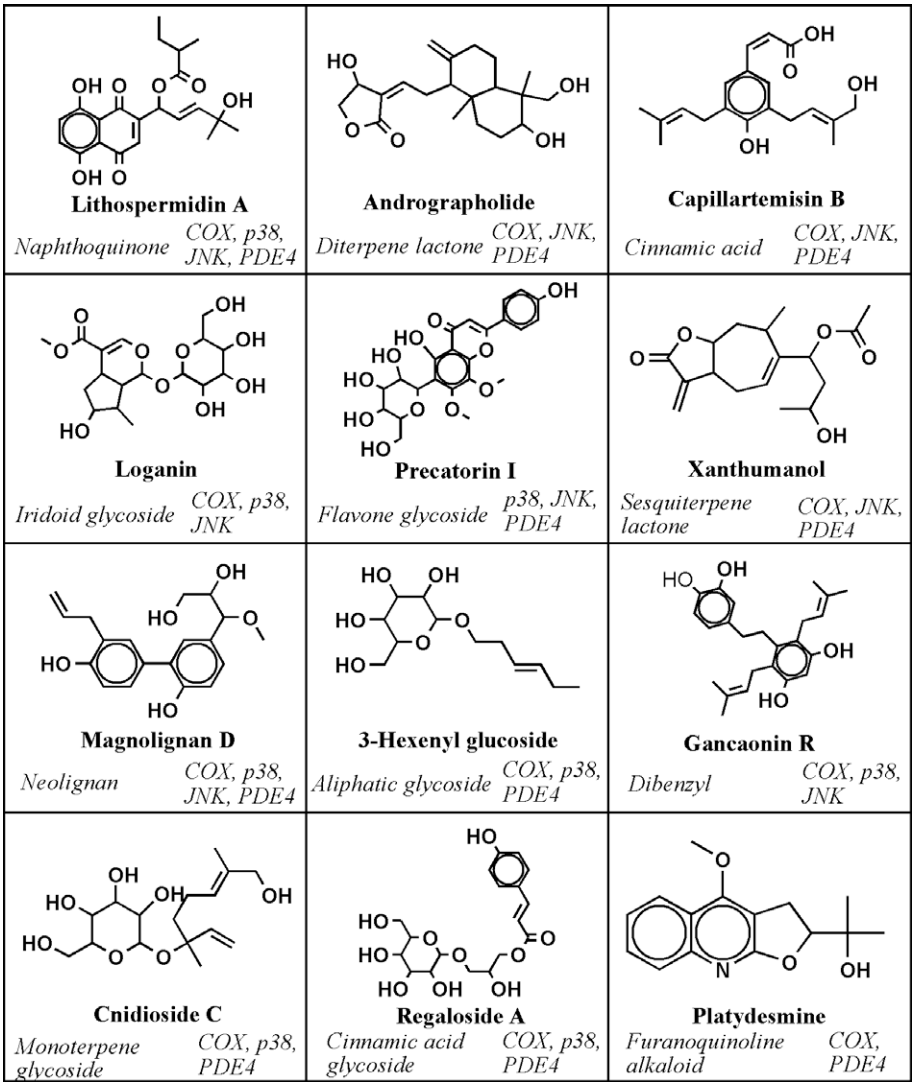

Figure 5. Twelve examples of predicted multi-target inhibitors from Chinese herbs. [Phytochemical class is given in the bottom left-hand corner of each panel, while targets are in the bottom right.]

three or more herbs from Table 2. This number was used as a cut-off value since analysis of all formulas yielded a median value of three target-related herbs per formula. The median value of target-related herbs in all the formulas satisfying this requirement for each category was then used to rank the categories. Of the categories characterized by formulas with the highest number of target-related herbs, those most obviously involved in the treatment of disease with a strong inflammatory component are shown in Table 3. In keeping with modern western TCM practice, Chinese medical terms such as 'Heat' or 'Wind' are given an initial capital letter to distinguish them from these terms in normal usage.

Space does not permit a full discussion of these categories, but some of the major signs and symptoms, which would prompt use of the formulas in each, are given in column 5. The number of formulas containing three or more target-related herbs are given in column 3, while the median value for these is shown in column 4. It should be appreciated that each category also includes formulas which contain few if any of the herbs from Table 2, and in such cases one cannot predict their likely mode of action. Examples of representative formulas are given for each category, along with the major herbs from Table 2 found in these formulas (excepting *Glycyrrhiza* (licorice) due to its ubiquitous presence in Chinese herbal prescriptions).

Categories have been assigned to five groups, as shown in column 2. This classification is based on the similarities between indications for each category, constituent herbs within formulas, and the systems which they address, as follows:

(1) The largest group includes a number of closely related categories, all of which 'Release the Exterior', which is characterized predominantly by fever brought on by 'Wind Heat'. Other symptoms include stiff neck, red sore eyes, headache and a stuffy nose. There may also be a skin rash, and these formulas or modified versions are frequently used in the treatment of skin disease.[32] In Western medicine the most common causes of these, however, are infectious diseases which give rise to elevated temperature and headache.
(2) 'Dispel Wind Damp' formulas comprise the second group. Wind Damp is closely associated with rheumatic conditions in Chinese medicine, which are still the major focus of non-steroidal anti-inflammatory therapy in the West.
(3) Formulas which either 'Restrain Leakage of the Intestines' or 'Reduce Food Stagnation' comprise the fourth group. Both are characterized by abdominal pain and associated symptoms, frequently due to inflammation of the GI tract. The

Table 3
Ranking of Chinese formulaic categories according to frequency of target-related herbs from Table 2 in component formulas

Formula category	Gp	NF ⩾3	MTH	Traditional indications	Representative herbs (from Table 2)
Release exterior heat	1	4	5	Fever, headache, stiff neck, thirst, sore eyes	*Mentha haplocalyx, Pueraria lobata, Forsythia suspensa, Arctium lappa, Schizonopeta tenuifolium*
Dispel wind-damp	2	4	5	Rheumatic conditions, pain, numbness	*Ligusticum chuanxiong, Angelica pubescens, Cinnamomum cassia, Notopterygium incisum, Angelica sinensis*
Restrain leakage from intestines	3	2	5	Diarrhoea, irritable bowel syndrome, abdominal pain, lethargy	*Myristica fragrans, Psoralea corylifolia, Codonopsis pilosula, Saussurea lappa, Cinnamomum cassia*
Release exterior with head and neck symptoms	1	3	4	Fever, stiff neck, headache, nasal discharge	*Xanthium sibiricum, Ligusticum chuanxiong, Asarum sieboldii, Magnolia biondii, Notopterygium incisum*
Release exterior–interior excess	1	2	4	Fever, red sore eyes, nasal discharge, constipation	*Ligusticum chuanxiong, Angelica sinsensis, Magnolia officinalis, Rheum palmatum*
Clear heat, relieve toxicity	4	5	4	Fever, nosebleed, rash, sores, swelling, pain	*Lonicera japonica, Forsythia suspensa, Scrophularia ningpoensis, Paeonia suffruticosa, Gardenia jasminoides*
Invigorate blood, dispel stasis	5	4	4	Severe pain, abscess, ulcer	*Ligusticum chuanxiong, Angelica sinensis, Carthamus tinctorium, Commiphora* spp., *Notopterygium incisum*
Release wind from skin and channels	4	1	4	Weeping, itchy red skin lesions	*Schizonopeta tenuifolia, Arctium lappa, Angelica sinensis*
Reduce food stagnation	3	3	4	Abdominal pain, nausea, diarrhoea or constipation	*Saussurea lappa, Codonopsis pilosula, Myristica fragrans, Cyperus rotundus*
Release exterior with interior deficiency	1	4	3.5	Fever, chills, pain and stiffness, headache, dry cough	*Notopterygium incisum, Angelica pubescens, Cynanchum atratum, Mentha haplocalyx, Astragalus membranaceus, Asarum sieboldii*
Invigorate blood, treat traumatic injury	5	3	3.5	Severe pain and swelling from traumatic injury	*Ligusticum chuanxiong, Angelica sinensis, Carthamus tinctorium, Commiphora* spp., *Dracaena cinnabari*

Gp = group to which each category (column 1) belongs (see text for details of the five groups to which categories were assigned); NF ⩾3 = no. of formulas with three or more target-related herbs; MTH = median no. of target-related herbs in these formulas; Traditional indications = signs and symptoms used in Chinese medicine to select a formula from the relevant category; Representative herbs for each formulaic category are taken from Table 2.

recent suggestion that PDE4 inhibitors may prove useful in the treatment of inflammatory bowel disease is of interest in this respect.[12]

(4) Prescriptions used to treat skin disease and cancer are found in various categories of which two listed in Table 3, 'Clear Heat, Relieve Toxicity' and 'Release Wind from Skin and Channels' are among the more important, in addition to group 1 categories above. Formulas from the former are used in particular to treat rash and swelling, as well as various forms of cancer, while those from the latter are specific for weeping, itchy red lesions.[32] Evidence exists that COX-2 is involved in inflammation-related skin cancers,[33] and is upregulated in psoriasis.[34] Cytokines and MAP kinases have also been suggested as potential targets for inflammatory skin disease.[35] Meanwhile, PDE4 inhibitors have shown promise in the treatment of allergic dermatitis and psoriasis, due in part to their suppression of Th1 and Th2 cytokines.[36]

(5) Two categories, 'Invigorate Blood, Dispel Stasis' and 'Invigorate Blood, Treat Traumatic Injury', are used in the treatment of severe pain and swelling, frequently the result of abscess, cancer or traumatic injury. These symptoms have a strong inflammatory component so their inclusion comes as no surprise.

The evidence from Table 3 therefore suggests that many of the categories in Chinese herbal medicine, in which inflammatory processes play a prominent role, are well represented by formulas involving herbs likely to contain inhibitors of major inflammatory targets.

4. Discussion

4.1. Protocols for PDB mining

Using the PDB for purposes of data mining poses a number of challenges, as well as opportunities, and highlights various issues which have to be addressed in applying protocols which can exploit its full potential, while at the same time minimizing the risk of false prediction from data of variable quality.

As far as pharmacophores are concerned, LigandScout introduces several methods to interpret ligands in PDB entries, including Figueras' ring perception algorithm, the use of geometry templates to determine valence of bonds between heavy atoms, and Kekulé patterns to detect aromaticity.[4] This ensures that subsequent pharmacophore detection is more dependable than it might be using original data.

Meanwhile, the procedure outlined in Section 3.1 of Results, whereby the quality of the self-docked pose in relation to its crystal co-ordinates is judged both on the basis of the corresponding RMSD (which should be less than 1.5 Å) and the imprecision associated with the crystal structure, attempts to minimize possible pitfalls in evaluating the quality of a particular scoring scheme (in this instance, MolDock re-ranking). The DPI gives a more exact measure of the uncertainty associated with atomic coordinates of a crystal structure than does the more commonly used atomic resolution, and though generally correlated with the latter, admits of many exceptions.[25]

Use of the MolDock re-ranking score also achieved acceptable results for enrichment of known actives in this study, particularly in the case of JNK, p38 and PDE4. In the case of COX, exclusion of scores for arachidonic acid (a substrate) and analogous fatty acids, all of which showed relatively weak binding, did not result in a noticeable improvement in EFs.

Docking and pharmacophores are often seen as alternatives, and their respective characteristics and advantages sometimes enumerated. LS pharmacophores provide an attractive alternative to docking, though it is not always clear how they may best be used. For example, restricting hits to those matching the full complement of features, though very rapid, can unduly limit the number of compounds identified, particularly when the number of features is quite large (e.g., above six), since, for obvious reasons, more complex pharmacophores will pick up a much more restricted set of compounds than those with fewer features.

Partial pharmacophore search, as used here, can increase both the number and diversity of compounds selected, but the resulting hit list, which will contain both strong and weak ligands, cannot be satisfactorily ranked simply on the basis of LS features. Docking provides the most obvious solution to this problem, and is likely to give better results if LS features can be simultaneously incorporated. This will result in identification of compounds which both satisfy the requirements of LS filtering, and achieve low docking scores when subject to the same constraints.

In this study, only those compounds satisfying three tests were identified as potential inhibitors, and considered for further analysis. These tests comprised (1) Lipinski 'rule-of-five' filtering, to identify compounds likely to be show favourable absorption, (2) LS pharmacophore screening, to identify possible mimics of PDB ligands (which in almost all cases display moderate to strong inhibition of their respective targets in vitro), and, finally, (3) docking (using LS constraints), to further restrict the list of hits to those with acceptable binding energies. Though docking without constraints might reveal different binding energies for some compounds, the interest in this instance was to see how well compounds could dock in the presence of those pharmacophore-derived constraints which had been responsible for their identification in the first place.

4.2. Scaffolds and substituents of predicted inhibitors

Table 2 indicates that a large number of phytochemical classes are likely to be involved in inhibition of inflammatory targets. Particularly prominent were phenolics, including lignans and flavonoids, and the smaller terpenoids, such as monoterpenes, iridoids and sesquiterpenes.

The larger triterpenes, among the most numerous constituents in Chinese herbs, were by contrast seldom identified as inhibitors of any of the targets investigated. As previously mentioned, they were frequently hit during LS filtering but failed, quite comprehensively, to dock to their respective receptors with appropriate scores. This may be due to their relatively large size and rigid structure. A number of triterpenes from Chinese herbs are nevertheless known to inhibit COX (e.g., boswellic acids which are active against COX-1,[37] and a number of oleanane triterpenes from *Picrorhiza kurroa* against COX-2[38]). In the case of esculentoside A and its derivatives, however, it has been demonstrated that the introduction of an aromatic ring greatly enhanced activity,[39] which suggests that particular patterns of substitution (presumably lacking from the data used here) may be necessary to ensure good binding. As far as p38 and JNK are concerned, there is very limited evidence so far that triterpenes act as inhibitors, though ginsenoside Re has recently been reported to inhibit JNK,[40] while for PDE4 a number of studies indicate that some, including ginsenoside Rg1, inhibit activity though usually only mildly.[41] There appear to be only limited experimental data, therefore, to indicate that they should have played a more prominent role in virtual screening.

Concerning the nature of typical substituents that were observed among high ranking hits, the fact that both prenylated compounds and small glycosides were moderately enriched is of some significance. The former have received an increasing amount of attention in recent years, as prenyl groups increase lipophilicity of a compound, as well as its affinity both for biological membranes, and also protein receptors, which are generally characterised by hydrophobic pockets.[42] Flavonoids, particularly from the Fabaceae and Moraceae, show a particularly high diversity of such compounds, though they are also found in other phenolic classes and some alkaloids. A number of SARs indicate that prenyl groups often increase potency, including activity against COX and LOX.[43] The results presented here suggest that they may confer the ability to bind with greater affinity to other anti-inflammatory targets as well. Other structurally similar hydrophobic sidechains, such as butenyl groups, may be equally significant.

The role of small glycosides, which include monoterpenes, iridoids and single ring phenolics, is of further interest. While prenyl groups may increase the lipophilicity of a molecule and enable it to bind within a receptor pocket, sugars confer greater capacity for hydrogen bonding. While less well explored, pharmacologically, than other glycosides such as flavonoid glycosides or triterpene saponins, these compounds characterize a number of very important herbs in Chinese medicine, such as *Rehmannia glutinosa*, *Paeonia* spp., *Codonopsis pilosula*, *Eucommia ulmoides* and others. Many of these are regarded as relatively mild in effect and are consequently often included in 'tonic' formulas.[32] The findings presented here suggest that it is the possession of a simple sugar, rather than the nature of the parent aglycone (provided it is of low molecular weight) which determines the ability of these compounds to bind to multiple protein targets. This may consequently yield clues as to the mode of action of a variety of Chinese tonic herbs at the molecular level.

4.3. Multi-target phytochemicals and Chinese medicine

The 'magic bullet' paradigm of drug discovery relies on the concept of compounds which bind specifically to a single target and show a high degree of potency. Of recent interest, however, is the suggestion that it is non-specific and relatively weak patterns of activity, by contrast, which may ultimately prove of great importance in drug discovery.[44] Though research on multi-target drugs is still at an early stage, studies have indicated that certain antipsychotic drugs, for instance, appear to be more effective when several types of receptor are hit.[45] Further advantages of this approach may include a reduced risk of adverse effects. An example concerns memantine, and other low affinity NMDA receptor antagonists, which are associated with a lower prevalence of side-effects than high-affinity single target drugs.[46]

What of the role that phytochemicals may play in this? Recent studies point to multi-target activity of a number of well known plant compounds. These include triterpene gymnemic acids which are active against targets implicated in hyperglycemia, the sesquiterpene β-eudesmol as a multifactorial anti-angiogenic agent, and the benzopyran nipradilol which appears to inactivate a variety of processes involved in glaucoma.[47] Both Random Forest analysis based on phytochemical ligands,[15] and LS-assisted docking, suggest that herbal constituents, by virtue of their apparent ability to bind to a number of functionally related targets, may be ideally suited to provide the type of weak to moderate multi-target inhibitors which are of particular interest to this emerging paradigm of drug discovery.

Mapping the ligand–receptor space of Chinese and other herbs in this fashion could thus provide a valuable resource for research in both orthodox and complementary medicine, but the question still remains as to how such a resource might best be utilised for therapeutic purposes. One answer concerns the use of information contained in Chinese herbal formulas, which represent perhaps the most comprehensive repository of knowledge available today on the synergistic effects of phytochemicals, and their relations with human disease. Provided that major targets of the herb/class entities present in each formula can be identified with some precision, then information on the clinical usage of formulas, containing the herbs in question, can be applied to establish relationships between molecular targets and the range of conditions for which those formulas are employed.

Previous work on predicted COX inhibitors from European herbs has also demonstrated that ethnopharmacological data from ancient herbal sources can be combined with pharmacophore-based screening results to give greater enrichment of potential

inhibitors, as well as providing information on clinical uses.[48] Other recent developments, which are of particular significance to identifying the molecular targets of Chinese (and other) herbal constituents, concern the appearance of two inverse docking programs—INVDOCK,[49] and TarFisDock.[50] Inverse docking, which aims to identify targets of a particular ligand as opposed to the ligands of a single target and other forms of 'target fishing', though of general relevance, are of special interest in this endeavour.

The present study, though preliminary and small-scale in relation to such goals, nevertheless identified herbs which might be expected to show activity against multiple targets involved in inflammation. Furthermore, the TCM categories in which formulas containing these herbs were most concentrated, were frequently found to be used for conditions which are either treated with anti-inflammatories in Western medicine, or where the targets in question have been proposed as appropriate to their treatment. This in turn implies that further exploration of the ligand–receptor space of Chinese herbs may prove amenable to interpretation according to the insights of Chinese medicine, and may cast light on the combined effects of chemical classes in the development of multi-target medicines.

Acknowledgements

We gratefully acknowledge the generosity of InteLigand GmbH in making LigandScout freely available to us for the duration of this work, and the help and support of Gerhard Wolber (LigandScout) and René Thomsen (MolDock).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.01.070.

References and notes

1. Rollinger, J. M.; Stuppner, H.; Langer, T. *Prog. Drug Res.* **2008**, *65*, 213.
2. Polya, G. *Biochemical Targets of Plant Bioactive Compounds: A Pharmacological Reference Guide to Sites of Action and Biological Effects*; Taylor & Francis: London, 2003.
3. Hawkins, B. *Plants for Life: Medicinal Plant Conservation and Botanic Gardens*; Botanic Gardens Conservation International: Richmond, UK, 2008. p 6.
4. Wolber, G.; Langer, T. *J. Chem. Inf. Model.* **2004**, *45*, 160.
5. Steindl, T. M.; Schuster, D.; Laggner, C.; Langer, T. *J. Chem. Inf. Model.* **2006**, *46*, 2146.
6. Süleyman, H.; Demircan, B.; Karagöz, Y. *Pharmacol. Rep.* **2007**, *59*, 247.
7. Schieven, G. L. *Curr. Top. Med. Chem.* **2005**, *5*, 921.
8. Mercer, B. A.; D'Armiento, J. M. *Int. J. Chron. Obstruct. Pulmon. Dis.* **2006**, *1*, 137.
9. Manning, A. M.; Davis, R. J. *Nat. Rev. Drug Disc.* **2003**, *2*, 554.
10. Spina, D. *Drugs* **2003**, *63*, 2575.
11. Renau, T. E. *Curr. Opin. Invest. Drugs* **2004**, *5*, 34.
12. Banner, K. H.; Trevethick, M. A. *Trends Pharmacol. Sci.* **2004**, *8*, 430.
13. Dastidar, S. G.; Rajagopal, D.; Ray, A. *Curr. Opin. Invest. Drugs* **2007**, *8*, 364.
14. Kwak, H. J.; Song, J. S.; Heo, J. Y.; Yang, S. D.; Nam, J. Y.; Cheon, H. G. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 1188.
15. Ehrman, T. M.; Barlow, D. J.; Hylands, P. J. *J. Chem. Inf. Model.* **2007**, *47*, 264.
16. Ehrman, T. M.; Barlow, D. J.; Hylands, P. J. *J. Chem. Inf. Model.* **2007**, *47*, 254.
17. Thomsen, R.; Christensen, M. H. *J. Med. Chem.* **2006**, *49*, 3315.
18. Wildman, S. A.; Crippen, G. M. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 868.
19. Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490.
20. *Dictionary of Natural Products (CD-ROM versions)*, Chapman & Hall/CRC, Boca Raton, FL, USA, 2008.
21. Storn, R.; Price, K. *Differential Evolution—A Simple and Efficient Adaptive Scheme for Global Optimization over Continuous Spaces*. Tech-report, International Computer Science Institute, Berkeley, 1995.
22. Yang, J.-M.; Chen, C.-C. *Proteins* **2004**, *55*, 288.
23. Wang, R.; Fang, X.; Lu, Y.; Wang, S. *J. Med. Chem.* **2004**, *47*, 2977.
24. Sutherland, J. L.; O'Brien, L. A.; Weaver, D. F. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 1906.
25. Hawkins, P. C. D.; Warren, G. L.; Skillman, A. G.; Nicholls, A. *J. Comput. Aided Mol. Des.* **2008**, *22*, 179.
26. Blow, D. M. *Acta Crystallogr., Sect. D* **2002**, *58*, 792.
27. Truchon, J.-F.; Bayly, C. I. *J. Chem. Inf. Model.* **2007**, *47*, 488.
28. Pearlman, D. A.; Charifson, P. S. *J. Med. Chem.* **2001**, *44*, 502.
29. Liu, J. H.; Zschocke, S.; Reininger, E.; Bauer, R. *Planta Med.* **1998**, *64*, 525.
30. Resch, M.; Heilmann, J.; Steigel, A.; Bauer, R. *Planta Med.* **2001**, *67*, 437.
31. Unschuld, P. *Medicine in China: A History of Pharmaceutics*; University of California: Berkeley, 1986. pp 17–83.
32. Bensky, D.; Barolet, R. *Chinese Herbal Medicine: Formulas and Strategies*; Eastland Press: Seattle, 1990.
33. Rundhaug, J. E.; Fischer, S. M. *Photochem. Photobiol.* **2008**, *84*, 322.
34. Koczan, D.; Guthke, R.; Thiesen, H. J.; Ibrahim, S. M.; Kundt, G.; Krentz, H.; Gross, G.; Kunz, M. *Eur. J. Dermatol.* **2005**, *15*, 251.
35. Numeroff, R. P.; Dinarello, C. A.; Asadullah, K. *Eur. Cytokine Netw.* **2005**, *16*, 101.
36. Bäumer, W.; Hoppmann, J.; Rundfeldt, C.; Kietzmann, M. *Inflamm. Allergy Drug Targets* **2007**, *6*, 17.
37. Siemoneit, U.; Hofmann, B.; Kather, N.; Lamkemeyer, T.; Madlung, J.; Franke, L.; Schneider, G.; Jauch, J.; Poeckel, D.; Werz, O. *Biochem. Pharmacol.* **2008**, *75*, 503.
38. Zhang, Y.; Dewitt, D. L.; Murugesan, S.; Nair, M. G. *Life Sci.* **2005**, *77*, 3222.
39. Wu, F.; Yi, Y.; Sun, P.; Zhang, D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6430.
40. Zhang, Z. G.; Li, X. Y.; Lv, W. S.; Yang, Y. S.; Gao, H.; Yang, J.; Shen, Y.; Ning, G. *Mol. Endocrinol.* **2008**, *22*, 186.
41. Stancheva, S. L.; Alova, L. G. *Gen. Pharmacol.* **1993**, *24*, 1459.
42. Botta, B.; Vitali, A.; Menendez, P.; Misiti, D.; Delle Monache, G. *Curr. Med. Chem.* **2005**, *12*, 713.
43. Chi, Y. S.; Jong, H. G.; Son, K. H.; Chang, H. W.; Kang, S. S.; Kim, H. P. *Biochem. Pharmacol.* **2001**, *62*, 1185.
44. Csermely, P.; Ágoston, V.; Pongor, S. *Trends Pharm. Sci.* **2006**, *26*, 178.
45. Roth, B. L.; Sheffler, D. J.; Kroeze, W. K. *Nat. Rev. Drug Disc.* **2004**, *3*, 353.
46. Rogawaki, M. A. *Amino Acids* **2000**, *19*, 133.
47. Kimura, I. *Yakugaku Zasshi* **2006**, *126*, 133.
48. Rollinger, J. M.; Haupt, S.; Stuppner, H.; Langer, T. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 480.
49. Chen, X.; Ung, C. Y.; Chen, Y. *Nat. Prod. Rep.* **2003**, *20*, 432.
50. Li, H.; Gao, Z.; Kang, L.; Zhang, H.; Yang, K.; Yu, K.; Luo, X.; Zhu, W.; Chen, K.; Shen, J.; Wang, X.; Jiang, H. *Nucleic Acids Res.* **2006**, *34*, 219.