Anti-influenza agents from Traditional Chinese Medicine

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After new human transmissible H1N1 (swine flu) viruses were reported in Mexico and the United States in April 2009, the World Health Organization (WHO) announced the emergence of a novel influenza A virus. Most governments in the world have been alerted and are monitoring the situation closely. As one of the official responses to the H1N1 pandemic, the Chinese government has released three editions of a document entitled "Recommended Schemes for Pandemic Influenza A Diagnoses and Treatments". The third edition recommended the use of not only two targeted anti-flu drugs, oseltamivir and zanamivir, but also four anti-flu TCM (Traditional Chinese Medicine) prescriptions. Since then, TCM has played a significant role in fighting the pandemic. TCM drugs comprise multiple compounds regulating multiple targets for a given class of medical indications, and are tunable to the symptoms of the individual. This review summarizes anti-influenza agents from TCM, including compounds, herbs, and TCM prescriptions, and suggests that, by further investigating TCM theory and mining TCM databases, a better drug discovery paradigm may arise – one that can be beneficial to both TCM and modern medicine.

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1 Introduction

Influenza, commonly referred to as the flu, is an acute respiratory infectious disease caused by influenza viruses. These viruses have a negative single-stranded RNA with eight gene segments, namely PB1, PB2, PA, HA, NP, NA, M and NS.[1] Its subtype is determined by the antigenicity of two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Influenza viruses are RNA viruses made up three of the five genera of the family Orthomyxoviridae:[2] influenza A, B and C. Of the three types of influenza virus, type A infects a wide range of avian and mammalian species. It can be further classified into subtypes according to the serological reactivity of HA and NA. Sixteen serotypes of HA (H1 to H16) and 9 of NA (N1 to N9)[3] circulate in avian and mammalian hosts. The structure of an influenza virus is depicted in Fig. 1.

Due to antigenic drift and antigenic shift, influenza viruses can result in periodic epidemics (sometimes pandemics),[1] causing high morbidity and mortality.[4] Each year in the United States, more than 200,000 patients are admitted to hospitals because of influenza, and there are approximately 36,000 influenza-related deaths.[5] Three influenza pandemics occurred in the 20th century, each of which was caused by the appearance of a new strain of the virus in humans. These pandemics killed tens of millions of people. Often, these new strains appear when an existing flu virus spreads to humans from other animal species, or when an existing human strain picks up new genes from a virus that usually infects birds or pigs.[6]

Influenza A virus subtype H5N1, also known as 'bird flu', is a virulent influenza in birds, and may cause viral disease in many species, including humans.[7] A bird-adapted strain of H5N1, called HPAI A (H5N1)† is highly pathogenic, and has made the likelihood of a human influenza pandemic and the possible socioeconomic impact a major worldwide concern.[8–13] However,

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† HPAI – Highly Pathogenic Avian Influenza.

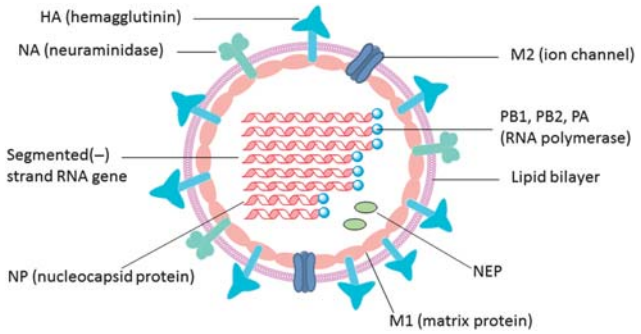

Fig. 1 The structure of an influenza virus virion.

the culprit of the pandemic that erupted in spring 2009 was actually another subtype of influenza A virus – H1N1. This virus, isolated from patients in the United States, was found to be made up of genetic elements from four different flu viruses – North American swine influenza, North American avian influenza, human influenza, and a swine influenza virus typically found in Asia and Europe. This strain, which is often called 'swine flu' by the public media, appears to be a result of re-assortment of human, avian and swine influenza viruses (in all, four different strains of subtype H1N1), with swine as a 'mixing vessel'.[14–17]

Although much has been learned about influenza viruses, key questions still remain unanswered. These include such factors as: determining interspecies transmission, re-assortment, and human-to-human transmission. In spite of its relative mildness compared to the 1918 pandemic swine influenza virus (H1N1), which killed as many as 50 million people,[18] the pandemic 2009 (H1N1) virus is still a threat. It is hard to predict the possibility for the current virus to acquire resistance to clinical drugs through re-assortment with uniformly resistant seasonal influenza virus strains. Also, the current virus may increase its virulence by antigenic drift, as seen in the Spanish flu and the re-assortment involved in seasonal swine flu.[19] Therefore, efforts to improve understanding of the factors that determine viral pathogenicity or transmissibility are critical, and developing improved and new antiviral drugs and vaccines will be crucial for controlling future influenza outbreaks.[20]

Vaccines play an important role in combating influenza, and as the swine flu pandemic picked up steam, researchers raced to develop vaccinations against it. Several strategies for meeting the threat of avian influenza by production of vaccines have been developed recently.[21–23] However, vaccination has provided only limited scope for control because of the tendency of the virus to mutate to escape the immune system. In addition, the supply of vaccine was far from sufficient, and mistrust of the vaccine was significant.[24]

Although the question of virus antigenic 'drift' or 'shift' is of crucial importance for future vaccine development, it has much less bearing on antiviral drug design, as the vaccine should be relevant to all variants of influenza A virus.[25] In the face of the persistent threat of human influenza, there is much concern about the shortage in both the number and supply of effective anti-influenza virus agents.[26–28]

The number of anti-influenza virus agents has increased in the past few years, and many chemical and biological anti-influenza agents have been investigated. The adamantane-based M2 ion channel protein inhibitors rimantidine and amantadine were the first drugs available for the treatment of influenza in history.[29,30] Although both can be effective against influenza virus A infection (influenza B and C viruses are not included as they have no M2 ion channel protein), they have been reported to cause central nervous system side-effects, and have given rise to the rapid emergence of drug-resistant viral strains.[30–32] In light of current threats, the development of novel anti-influenza drugs remains a high priority. Table 1 lists currently available anti-influenza drugs, while Table 2 lists anti-influenza vaccines and drugs in development.[2]

Natural products and their derivatives have historically been invaluable as a source of therapeutic agents. Recent technological advances, coupled with unrealized expectations from current lead-generation strategies, have led to renewed interest in natural products in drug discovery.[34] This is also the case in the field of anti-influenza research.

Oseltamivir (Tamiflu®) is the first orally active neuraminidase inhibitor commercially developed and widely used in the treatment of influenza. According to Roche, the major bottleneck in oseltamivir production is the lack of availability of shikimic acid, which cannot be synthesized economically. However, the main resource for shikimic acid is a TCM herb, star anise. The application of structure-based drug design approaches to the

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Table 1 Currently available anti-influenza drugs

Drug	Originator/Licensee	Year (country) of first launch
Amantadine hydrochloride (Symmetrel®)	Endo	1964
Rimantadine hydrochloride (Flumadine®)	Forest	1987 (France)
Zanamivir (Relenza®)	Biota/GlaxoSmithKline	1999 (Australia)
Oseltamivir phosphate (Tamiflu®)	Gilead/Roche	1999 (Switzerland)
Peramivir (Rapiacta®)	BioCryst/Shionogi	2010 (Japan)

Table 2 Anti-influenza vaccines and drugs in development (data source: Thomson Reuters Life Sciences group)[33]

Name/Code	Organization	Target/Type	Status
PUR-003	Pulmatrix	Cationic airway lining modulator	Phase II
JVRS-100	Juvaris	Cationic lipid/DNA complexes	Phase II
F-10	Xoma	Disinfectants	Preclinical
FluNhance	Protein Sciences	Efficacy-enhancing additive to influenza vaccines	Phase II
EV-075	Evolva	Immunomodulator	Preclinical
Rintatolimod	HemispheRx	Immunomodulatory double-stranded RNA	Preclinical
Terameprocol	Erimos	Inhibitor of prostaglandins, inflammatory cytokines and chemokines	Preclinical
Human leukocyte interferon alpha	HemispheRx	Interferon	Phase II
Nitazoxanide	Romark	Maturation inhibition of viral hemagglutinin	Phase II
CR-6261	Crucell	Monoclonal antibody	Preclinical
FluCide	NanoViricides	Nanoviricide	Preclinical
FluCide-HP	NanoViricides	Nanoviricide	Preclinical
FluCide-I	NanoViricides	Nanoviricide	Preclinical
Laninamivir octanoate	Daiichi Sankyo	Neuraminidase inhibitor	Pre-registered
684069	VaxInnate	NF-κB	Preclinical
ANX-201	Adventrx Pharmaceuticals	Reverse transcriptase inhibitor	Preclinical
Favipiravir	Toyama	RNA viral infections inhibitor	Phase III
STP-702	Sirnaomics	RNAi	Preclinical
DAS-181	NexBio	Sialidase Fusion Protein	Phase II
ALN-FLU01	Novartis/Alnylam Pharmaceuticals	siRNAs	Preclinical
Factor 5A1 siRNA	Senesco	siRNAs	Preclinical
StatC	Canopus BioPharma	Statin/caffeine combination	Preclinical
QS-21	Acambis	Stimulon adjuvant	Phase I
MCT-465	MultiCell Technologies	Synthetic dsRNA therapeutic	Preclinical
TCAD	Adamas	Triple combination therapy	Phase II
BTL-TML-001	Beech Tree Labs	—	IND-filed
TG-21	Obio Pharmaceutical	—	Preclinical
ATI-0655	Arisyn Therapeutics	—	Preclinical
394549	Sanofi Pasteur	Vaccine	Phase II
420795	DelSite	Vaccine	Preclinical
423250	Baylor College of Medicine (BCM)	Vaccine	Phase II
424477	Vaxine	Vaccine	Phase II
426173	Solvay/Norwood Immunology	Vaccine	Phase II
426415	CytoGenix	Vaccine	Preclinical
427488	Generex	Vaccine	Phase I
430974	Novavax	Vaccine	Phase II
434372	Novavax	Vaccine	Phase II
448976	MedImmune	Vaccine	Preclinical
452409	LigoCyte	Vaccine	Preclinical
460762	Sinovac	Vaccine	Phase II
463263	Intercell USA	Vaccine	Phase II
463293	Medicago	Vaccine	Phase I
468374	US Dept. of Health & Human Services	Vaccine	Phase I
468744	Juvaris	Vaccine	Preclinical
473074	Nat. Inst. Allergy & Infectious Dis.	Vaccine	Phase II
4SC-301	4SC	Vaccine	Preclinical
628652	Vaxart	Vaccine	Preclinical
639397	iBioPharma	Vaccine	Preclinical
646010	Sanofi Pasteur	Vaccine	Phase I
648869	Vivaldi	Vaccine	Preclinical
649281	Baxter	Vaccine	Phase I
649740	iBioPharma	Vaccine	Preclinical
654231	LigoCyte	Vaccine	Preclinical
654232	LigoCyte	Vaccine	Preclinical
655089	University of Bergen	Vaccine	Phase I
656331	Novavax	Vaccine	Preclinical
660702	Novavax	Vaccine	Phase III
663747	Vical	Vaccine	Preclinical
665334	Pevion	Vaccine	Preclinical
665575	Vaxine	Vaccine	Phase II
665813	AlphaVax	Vaccine	Preclinical
673657	Hualan Biological Bacterin	Vaccine	Registered
677378	Nat. Inst. Allergy & Infectious Dis.	Vaccine	Preclinical
677762	NasVax	Vaccine	Preclinical
678336	Sanofi-Aventis	Vaccine	Phase II

Table 2 (*Contd.*)

Name/Code	Organization	Target/Type	Status
680072	Jiangsu Yanshen Biological	Vaccine	Registered
681702	NanoBio	Vaccine	Preclinical
683625	Eurocine	Vaccine	Phase II
Ad4-H5-Vtn	PaxVax	Vaccine	Phase I
Adimflu-S(A/H1N1)	ADImmune	Vaccine	Phase I
ADnVN1203/04.H5	Vaxin	Vaccine	Phase I
Agrippal-IC31	Novartis/Intercell	Vaccine	Phase I
AP205-M2	Cytos Biotechnology	Vaccine	Preclinical
AVX-502	AlphaVax	Vaccine	Phase II
BVX-M001	BiondVax Pharmaceuticals	Vaccine	Phase II
CEL-1000	Cel-Sci	Vaccine	Preclinical
Celvapan	Baxter	Vaccine	Registered
CSL-401	CSL	Vaccine	Registered
CSL-412	CSL	Vaccine	Phase II
EP-1400	Affitech	Vaccine	Preclinical
F10-IgG1	Dana-Farber Cancer Institute	Vaccine	Preclinical
Flagellin.HuHa/flagellin.AvHA	VaxInnate	Vaccine	Phase I
Flagellin.HuM2e	VaxInnate	Vaccine	Phase II
Fluad-H5N1	Novartis Vaccines and Diagnostics	Vaccine	Registered
FluCell	Sanofi Pasteur/Crucell	Vaccine	Phase II
FLU-v	PepTcell	Vaccine	Preclinical
Fluvacc	Avir Green Hills Biotechnology	Vaccine	Phase I
Fluval H5N1	OMNINVEST	Vaccine	Registered
Fluzone/JVRS-100	Juvaris	Vaccine	Phase II
Fluzone/Vaxfectin	Vical	Vaccine	Phase I
FP-01	Immune Targeting Systems (ITS)	Vaccine	Preclinical
GBH-04L1	Avir Green Hills Biotechnology	Vaccine	Phase I
GC-501	Green Cross	Vaccine	Phase III
GelVac	DelSite	Vaccine	IND-filed
GHB-01L1	Avir Green Hills Biotechnology	Vaccine	Phase I
GHB-11L1	Avir Green Hills Biotechnology	Vaccine	Phase II
GI-8000	GlobeImmune	Vaccine	Preclinical
Grippol Plus	Solvay	Vaccine	Registered
GSK-1119711A	GlaxoSmithKline	Vaccine	Registered
GSK-1557484A	GlaxoSmithKline	Vaccine	Phase III
GSK-2115160A	GlaxoSmithKline	Vaccine	Phase I
GSK-2186877A	GlaxoSmithKline	Vaccine	Phase III
GSK-2321138A	GlaxoSmithKline	Vaccine	Phase II
GSK-2340269A	GlaxoSmithKline	Vaccine	Phase III
GSK-2340272A	GlaxoSmithKline	Vaccine	Phase III
GSK-2340273A	GlaxoSmithKline	Vaccine	Phase III
GSK-2340274A	GlaxoSmithKline	Vaccine	Phase III
GSK-576389A	GlaxoSmithKline	Vaccine	Phase III
H5N1 pandemic influenza vaccine	Biken/Kitasato Institute/Denka	Vaccine	Pre-registered
H6N1-Teal-HK 97/AA	Nat. Inst. Allergy & Infectious Dis.	Vaccine	Phase I
H7N3 BC 2004/AA *ca*	Nat. Inst. Allergy & Infectious Dis./ MedImmune	Vaccine	Phase I
H9N2 avian influenza vaccine	Novartis Vaccines and Diagnostics	Vaccine	Pre-registered
HN-VAC	Bharat Biotech	Vaccine	Phase I
Homspera	University of Pittsburgh	Vaccine	Preclinical
Humenza	Sanofi pasteur	Vaccine	Recommended approval
Influenza A (H1N1) monovalent vaccine	GlaxoSmithKline	Vaccine	Registered
Influvac TC	Solvay	Vaccine	Registered
Intradermal seasonal flu vaccine	Sanofi Pasteur	Vaccine	Registered
LEAPS-H1N1	Cel-Sci	Vaccine	Phase I
LG-611	Lentigen	Vaccine	Preclinical
L-M2eA	Molecular Express	Vaccine	Preclinical
M2e/NP-ISS	Dynavax	Vaccine	Preclinical
M2e-HBc	Sanofi-Aventis/Acambis	Vaccine	Phase I
MEDI-3250	MedImmune	Vaccine	Phase III
MEDI-8662	MedImmune	Vaccine	Phase III
MG-1109	Green Cross/Mogam Biotechnology Research Institute	Vaccine	Preclinical
MVA-NP + M1	University of Oxford	Vaccine	Phase II
NB-006	NanoBio	Vaccine	Preclinical
NB-1008	NanoBio	Vaccine	Phase I
NP-ISS/M2e-ISS	Dynavax	Vaccine	Preclinical
Pandemrix (H5N1)	GlaxoSmithKline	Vaccine	Registered

Table 2 (*Contd.*)

Name/Code	Organization	Target/Type	Status
Panflu (H5N1)	Sinovac	Vaccine	Registered
PER.C-flu	Sanofi Pasteur/Crucell	Vaccine	Phase I
Prepandemic influenza vaccine (H5N1)	GlaxoSmithKline	Vaccine	Registered
Pre-pandemic MF59-adjuvanted H5N1 vaccine	Novartis Vaccines and Diagnostics	Vaccine	Phase III
REP-2031	REPLICor	Vaccine	Preclinical
Replikins H1N1 swine flu vaccine	Replikins	Vaccine	Preclinical
Replikins H5N1 bird flu vaccine	Replikins	Vaccine	Preclinical
Replikins PanFlu vaccine	Replikins	Vaccine	Preclinical
Replikins Transflu	Replikins	Vaccine	Preclinical
SB-001	StormBio	Vaccine	Preclinical
SB-002	StormBio	Vaccine	Preclinical
SB-003	StormBio	Vaccine	Preclinical
SCH-900795	BioDiem/Nobilon	Vaccine	Phase I
SynCom universal flu vaccine	Inovio Biomedical	Vaccine	Preclinical
SynCon H1N1	Inovio Biomedical	Vaccine	Preclinical
UMN-0501	UMN Pharma	Vaccine	Phase II
UMN-0502	Protein Sciences	Vaccine	Pre-registered
V-512	Merck & Co.	Vaccine	Phase I
VAX-125	VaxInnate	Vaccine	Phase II
VaxiSome-Influenza	NasVax	Vaccine	Phase II
VCIV	Baxter	Vaccine	Phase III
VCL-IPM1	Vical	Vaccine	Phase I
VCL-IPT1	Vical	Vaccine	Phase I
VGX-3400	VGX Pharmaceuticals	Vaccine	IND-filed
VLI-03A	VectorLogics	Vaccine	Preclinical
VRC-AVIDNA036-00- VP	Nat. Inst. Allergy & Infectious Dis.	Vaccine	Phase I
VRC-FLUDDNA056-00VP	Nat. Inst. Allergy & Infectious Dis.	Vaccine	Phase I
VRC-FLUDDNA057-00-VP	Nat. Inst. Allergy & Infectious Dis.	Vaccine	Phase I
VRC-FLUDNA047-00- VP	Nat. Inst. Allergy & Infectious Dis.	Vaccine	Phase I

design of anti-influenza agents targeted at NA, HA, and other viral surface glycoproteins has been reviewed by Wade.[35] This review focuses on summarizing anti-flu compounds, herbs, and combined herbs (recipes) from TCM in order to emphasise the differences and similarities between modern drugs and TCM drugs. By comparing TCM drugs and modern drugs, we hope to reveal a new drug discovery paradigm.

2 Hemagglutinin

Ten years have passed since the mechanism of cellular entry influenza viruses was uncovered.[36] The viral envelope glycoprotein hemagglutinin (HA), which contains the receptor-binding site for initial attachment to the sialylated cellular receptors, and governs the receptor specificity of human *versus* avian influenza virus subtypes,[37,38] is critical for binding to cellular receptors and fusion of the viral and endosomal membranes. Two mechanisms that are not mutually exclusive, re-assortment and interspecies transmission, result in the introduction of viruses with new HA subtypes into human populations.

Of the 16 known serotypes of HA, six have been isolated from humans at the molecular level, namely, H1, H2, H3, H5, H7, and H9; H1–3 have been involved in past pandemics.[39,40] The 16 subtypes of HA form five clades and further segregate into two groups[41–43] (Fig. 2 and Fig. 3). Comparison of the available HA structures indicate that they differ on a group-specific basis in regions that are prominent in the changes required for membrane fusion.[44,45] The fact that some of the fusion inhibitors work only on certain subtypes of HA suggests that they may bind in one of these regions.

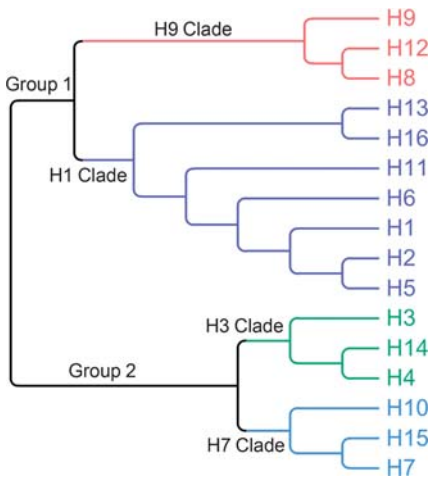


Fig. 2 The hierarchical clusters of HA subtypes.[44] Drawn using TreeGraph 2.[163]

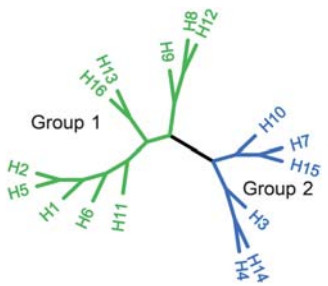


Fig. 3 The phylogenetic tree of influenza A HAs.[43] Drawn using TreeIllustrator.[164]

The first structure for the HA was published in 1981 by Wilson and colleagues.[46] Since then, other crystal structures have been continuously appearing, including three principal molecular conformations assumed by the proteins during the virus life cycle: the precursor structure, the structure of the cleaved native HA present on the surface of infectious virions, and the structure of HA following the conformational changes involved in membrane fusion.[47]

Research into the structure of the HA ectodomain alone[46,48,49] and on complexes with sialic acid and its receptor analogs[50–56] has provided abundant information on the receptor binding site, located at the receptor binding domain shown in Fig. 4. The specificity of the receptor binding site for sialic acid and the way it is linked to a vicinal galactose residue is responsible for host range-restriction. The major obstacle that influenza viruses must overcome to adapt themselves to human hosts is the difference between α2–3 linkages (avian)/α2–6 linkages (human) and sialic acids connected to galactose.[57,58]

Among avian viruses, residues within the HA receptor binding site are highly conserved. These residues are different from human-adapted viruses,[59] and this can affect receptor-binding specificity and antigenicity. RBS and fusion domains (Fig. 5) can be used for inhibitor design.[36,43,60–62] Several small-molecule inhibitors that prevent the conformational change of the HA at low pH, have been reported.[43,63,64] However, the lower activity against some human influenza virus subtypes, rapid drug resistance, and unsatisfactory outcome in animal models, have slowed their development.[65]

3 Neuraminidase

Neuraminidase (NA) is a glycohydrolase that catalyzes the cleavage of terminal α-ketosidically linked sialic acids from a large variety of glycoproteins, glycolipids, and oligosaccharides.[66,67] It facilitates progeny virus release and spread of the infection to neighboring cells by removing sialic acid from infected cell surfaces and newly formed virions during the final stages of influenza virus infection.[68,69] It also helps the virus to go through the ciliated epithelium of the human airway by removing the decoy receptors on mucins, cilia, and the cellular glycocalyx.[70]

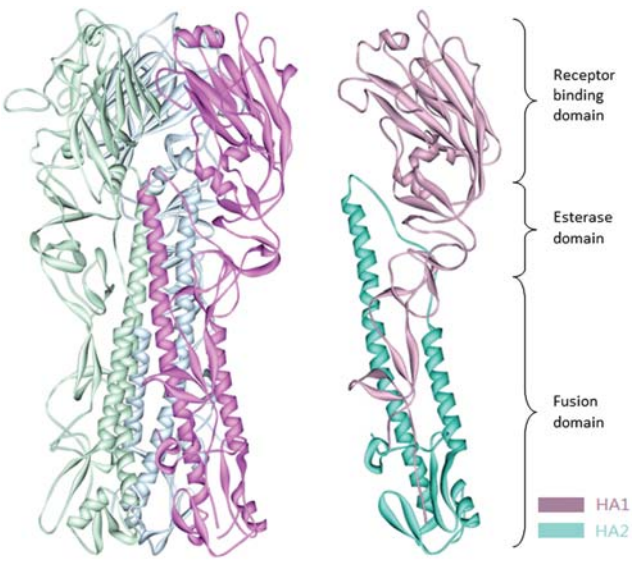


Fig. 4 Hemagglutinin trimer (left) and monomer (right).

Neuraminidase structure-oriented inhibitor design programs have resulted in three new drugs, zanamivir (Relenza®)[71] oseltamivir (Tamiflu®)[72] and peramivir (Rapiacta®).[73] The structure of the NA active site is highly conserved across all nine NA subtypes from influenza A and influenza B. This fact makes NA inhibitors good anti-influenza agents.

The NA active site is very polar, and consisting of amino acids with polar side chains, such as, Arg, Asp, and Glu (Fig. 6). This suggests that electrostatic interactions play critical roles for binding.[74]

In order to identify the 'hot' residues in the binding site, the NA–inhibitor co-crystal structures from the RCSB Protein Data Bank have been aligned and superimposed. The frequencies of the residues that have most interactions with ligands are depicted in Fig. 7, showing that residues 118, 119, 151, 152, 292 and 371 are the 'hot' residues for ligand binding.

In 2006, Russell and coworkers divided influenza A virus NA proteins into two subtypes: subtype 1 (N1, N4, N5 and N8) and subtype 2 (N2, N3, N6, N7 and N9). X-ray crystallography revealed that the proteins of subtype 1 have additional cavities connected to the active sites next to ligand-binding pockets (but that proteins of subtype 2 do not have additional cavities).[75] These additional cavities provide new opportunities for novel selective inhibitors (Fig. 8).

Taylor and von Itzstein have proposed a four-step enzymatic catalytic mechanism for influenza virus neuraminidase[76] (Fig. 9). The first step involves the distortion of the α-sialoside from a $^2C_5$ chair conformer to a boat conformer when the sialoside binds to the sialidase. The second step leads to an oxocarbocation ion intermediate, the sialosyl cation. The third step is the formation of the transition state of the substrate, and the last step affords α-Neu5Ac, which then mutarotates to the more favourable anomer β-Neu5Ac.

4 M2 and other protein targets

The M2 protein from influenza A virus is a low-pH-activated proton channel that mediates acidification of the interior of viral particles trapped in endosomes.[77] The structure–function relationship of the M2 protein was summarized by Pinto and Lamb in 2006. The available biochemical and solid-state NMR data indicated that the transmembrane domain of the M2 protein is comprised of a four-helix bundle with a tilt of about 25°. These experiments showed that residues Val27, Ala30, Gly34, His37 and Trp41 line the aqueous pore, that His37 forms a barrier to large molecules, and that Trp41 functions as a gate that closes with high pH. Functional studies indicate that the portion of the cytoplasmic domain nearest the membrane is important for normal ion channel function, and solid-state NMR studies indicate that the structure of this domain brings it into close proximity to the inner membrane leaflet. The most remarkable aspect of this channel is the observation that much of its functionality is provided for by the His37 and Trp41 residues found in one turn of the transmembrane helical bundle.[78] A number of structures of truncated forms of M2 have been reported (PDB codes: 3LBW, 2KQT, 2KAD, 3C9J, 3BKD, 1NYJ and 1MP6).

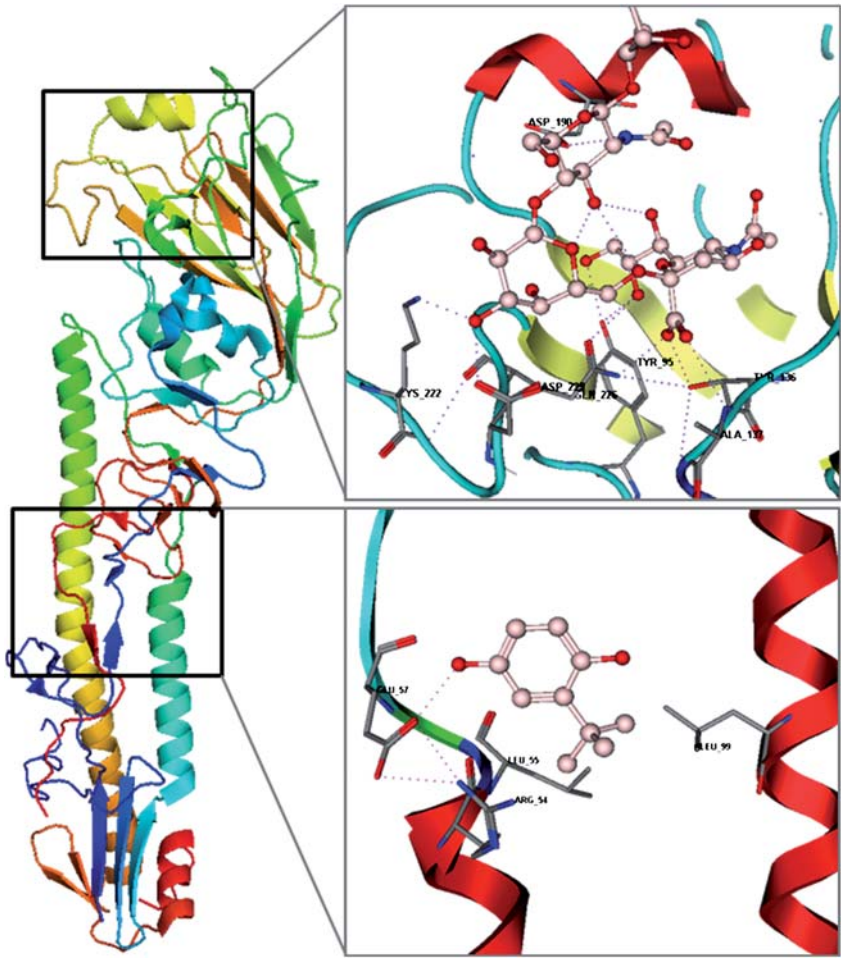


Fig. 5 The binding site for HA group-specific inhibition by RBS inhibitors and membrane fusion inhibitors. The enlarged figure on the top right shows the receptor binding site, while the one below shows the binding site of membrane fusion (PDB codes: 2WRG, 3EYK).

The known inhibitors of M2 are amantadine and rimantadine, which are adamantane derivatives. They bind to the transmembrane region (albeit in different sites) and stop the protons from entering the virion, which then does not disintegrate (Fig. 10).

Recently, resistance to these drugs in humans, birds and pigs has reached more than 90%, and lot of attention has been paid to the mechanism of drug resistance. Schnell and Chou[79] reported the structure of the tetrameric M2 channel in complex with rimantadine, determined by NMR. In the closed state, four tightly packed transmembrane helices define a narrow channel, in which a 'tryptophan gate' is locked by intermolecular interactions with aspartic acid. A carboxy-terminated amphipathic helix oriented nearly perpendicular to the transmembrane helix forms an inward-facing base. Lowering the pH destabilizes the transmembrane helical packing and unlocks the gate, admitting water to conduct protons, whereas the C-terminal base remains intact, preventing dissociation of the tetramer. Rimantadine binds at four equivalent sites near the gate on the lipid-facing side of the channel and stabilizes the closed conformation of the pore. Drug resistance mutations are predicted to counter the effect of drug binding by either increasing the hydrophilicity of the pore or weakening helix–helix packing, thus facilitating channel opening. Cady and colleagues[80] reported the NMR structure of the tetrameric M2 channel in complex with amantadine. They show by solid-state NMR spectroscopy that two amantadine-binding sites exist in M2 in phospholipid bilayers. The high-affinity site, occupied by a single amantadine, is located in the N-terminal channel lumen, surrounded by residues mutated in amantadine-resistant viruses. Quantification of the protein–amantadine distances resulted in a 0.3 Å-resolution structure of the high-affinity binding site. The second, low-affinity, site was observed on the C-terminal protein surface, but only when the drug reaches high concentrations in the bilayer. The orientation and dynamics of the drug are distinct in the two sites. Amantadine physically occludes the M2 channel, thus paving the way for developing new antiviral drugs against influenza viruses.

This study demonstrates the ability of solid-state NMR to elucidate small-molecule interactions with membrane proteins and determine high-resolution structures of their complexes. These NMR structures are used for structure based M2 inhibitor design.[81] Rosenberg and Casarotto[82] conducted a series of surface plasmon resonance experiments designed to accurately measure the affinity of amantadine and rimantadine to M2 ion channels embedded in 1,2-dimyristoyl-*sn*-glycero-phosphocholine (DMPC) liposomes. They find that this class of drug is

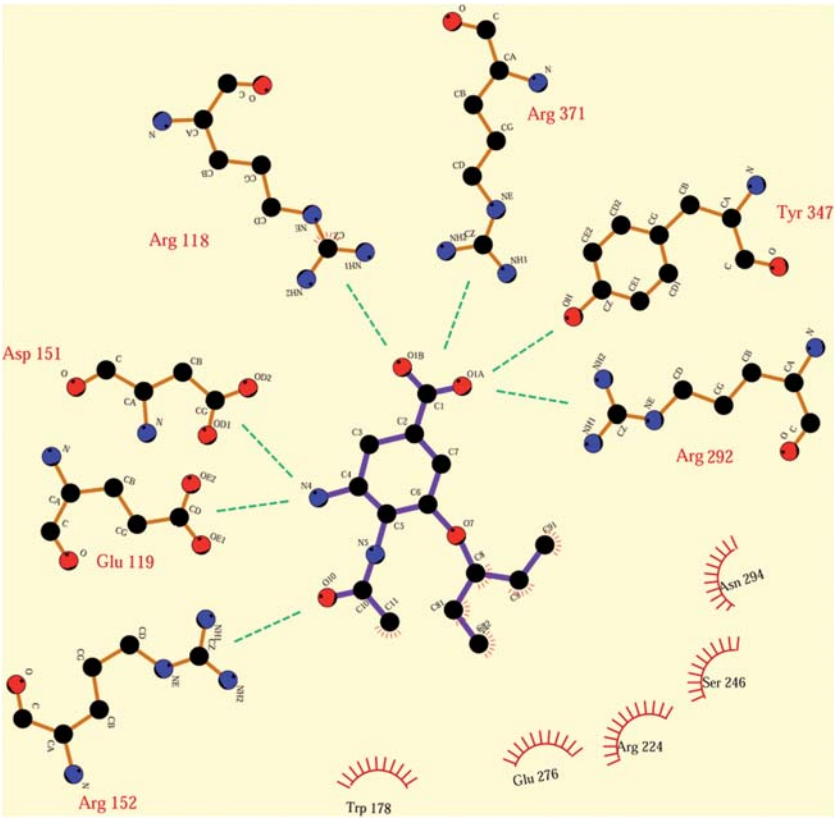


Fig. 6 A diagram illustrating an NA inhibitor (oseltamivir) inside the binding pocket. The green dashed lines indicate the hydrogen-bonding interactions.

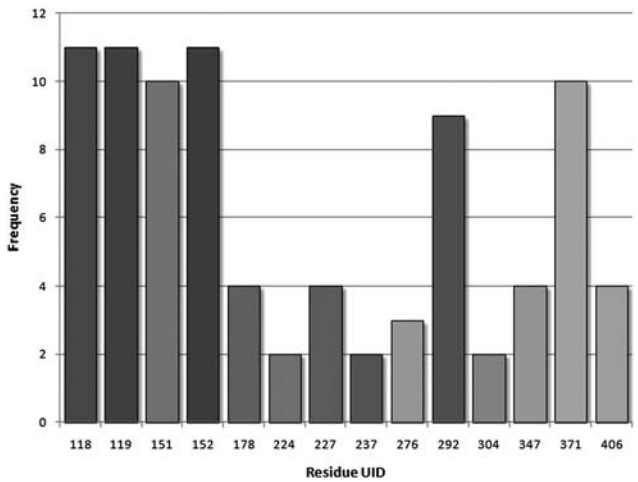


Fig. 7 NA 'hot' residues for ligand binding. All co-crystal structures are aligned (referenced) against the structure of the NA with PDB code 2HU4.

capable of binding M2 with two different affinities (in the order of 10^{-4} and 10^{-7} M), suggesting that both proposed binding sites are feasible. By examining drug binding to M2 mutant constructs (V27A, S31N and D44A), it was possible to probe the location of the two binding sites. They show that a high-affinity binding site corresponds to the M2 ion channel pore, whereas the secondary, low-affinity binding site can be attributed to the lipid face of the pore. Through computational simulation, Phongphanphanee and colleagues[83] have proposed a new mechanism of proton transfer through the gating region of the channel. A hydronium ion passes a proton to a non-protonated histidine *via* a hydrogen bond, and then the other protonated histidine releases a proton to a water molecule *via* a hydrogen bond – a process that transfers a proton effectively from one water molecule to another.

Other interesting protein targets, such as nucleoprotein, viral polymerase, and non-structural protein NS1A, have recently been well reviewed by Das and colleagues.[84] Small molecular ligands against these targets have not yet been identified.

5 Hemagglutinin inhibitors

Kamitakahara and colleagues reported a lysoganglioside/poly-L-glutamic acid conjugate as a picomolar inhibitor of influenza hemagglutinin.[85]

Only a few hemagglutinin inhibitors have been discovered from herbs related to TCM. Roschek and coworkers isolated two anti-influenza flavonoids (**1** and **2**) from an optimized elderberry (*Sambucus nigra*) fruit extract.[86] The molecular mode-of-action was determined through the direct binding assay technology and DART (Direct Analysis in Real Time) TOF-MS. Flavonoids bind to H1N1 virions and stop viruses infecting host cells. This mode-of-action was further verified by synthesizing **1**, racemic dihydromyricetin (**3**), and the 3-hydroxyflavonone of **2** (Fig. 11).

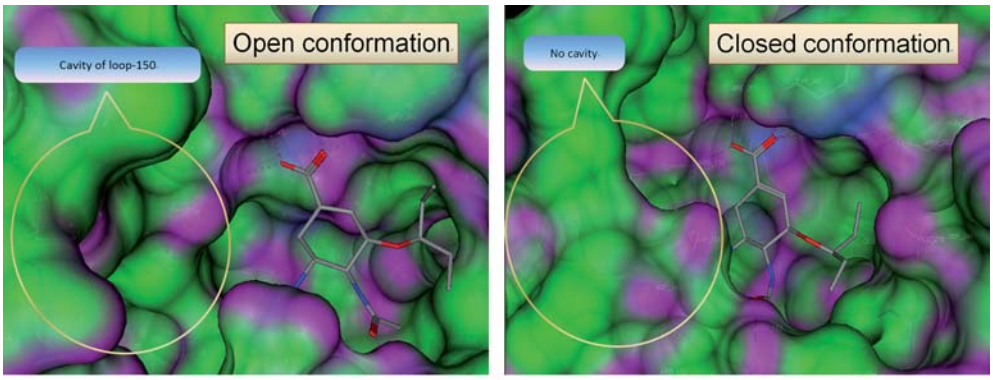


Fig. 8 Proteins of subtype 1 have additional cavities (shown open in the left-hand panel, and closed in the right-hand panel), but those of subtype 2 lack such cavities.

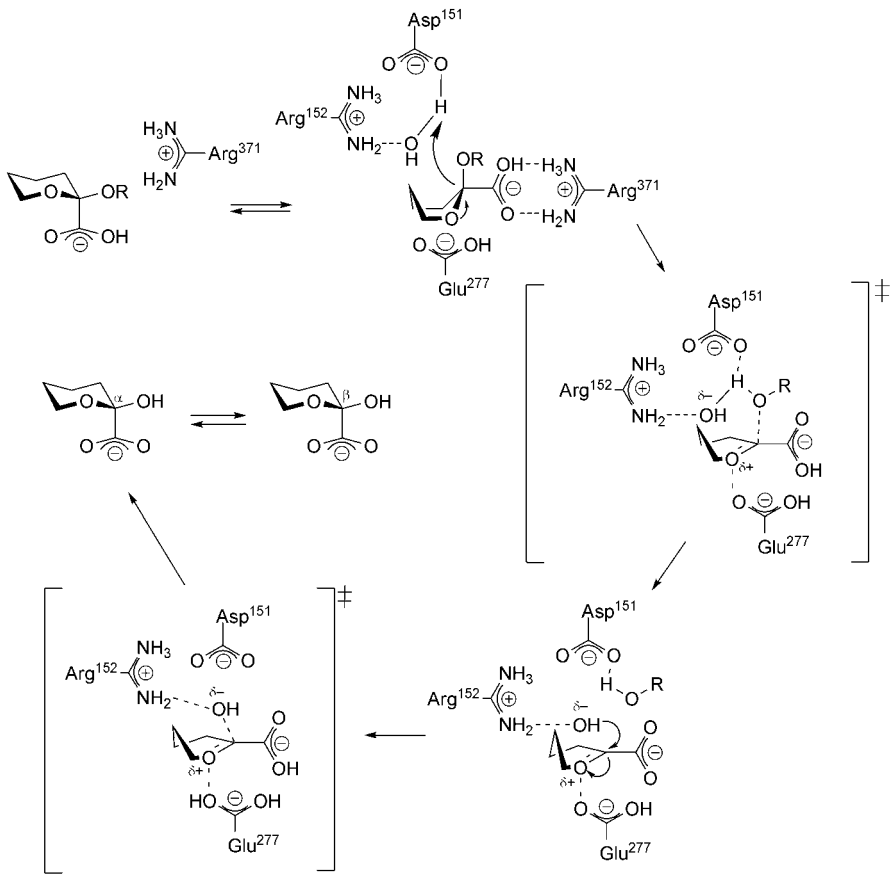


Fig. 9 Proposed catalytic mechanism of influenza virus neuraminidase.[76]

Compound **1** gave an IC_{50} of 0.13 μg/mL (0.36 μM) for H1N1 infection inhibition, while dihydromyricetin (**3**) had an IC_{50} of 2.8 μg/mL (8.7 μM). These values compare favorably to the known anti-influenza activities of oseltamivir (Tamiflu®; 0.32 μM) and amantadine (27 μM),[78] as is shown in Table 3.

Modeling analyses indicate that the A and B rings of compounds **1** and **2** form an axis with inter-phenolic ring distances of 10.5 Å and 10.9 Å, respectively (Fig. 12). This is the favored distance for forming hydrogen bonds within the hemagglutinin (HA) binding pocket (14–15 Å in size).[87] It is believed that these compounds inhibit H1N1 infection through binding to the viral envelope, which is responsible for binding and recognizing the host cells.

A commercial standardized extract of the widely used herb *Echinacea purpurea* (‘*Zichuju*’, Echinaforce®, EF) has become a very popular herbal ‘remedy’ for the symptoms of colds and flu. Sharma and colleagues studied have EF anti-influenza activity, and revealed that H1N1, H3N2, H5N1 and H7N7 were

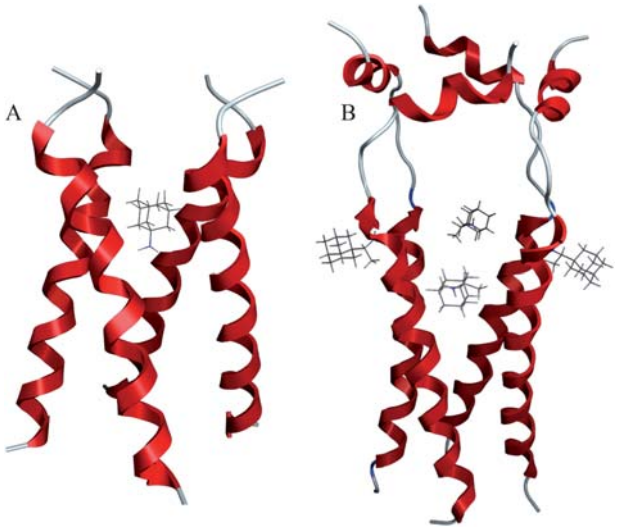


Fig. 10 A: Adamantane binding (PDB: 2KQT). B: Rimantadine binding (PDB: 2RLF).

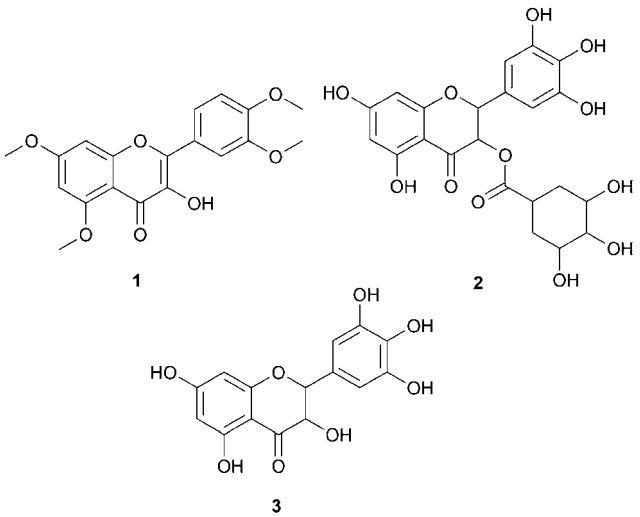


Fig. 11 Structures of compounds **1**, **2** and **3**.

Table 3 H1N1 infection inhibitions of oseltamivir, amantadine, and compounds **1**, **3**

Compound	IC_{50} (μg/mL)	IC_{50} (μM)
5,7,3′,4′-Tetra-*O*-methylquercetin (**1**)	0.15	0.36
(±)-Dihydromyricetin (**3**)	2.8	8.7
Oseltamivir (neuraminidase inhibitor)	0.1	0.32
Amantadine (M2 inhibitor)	4.1	27

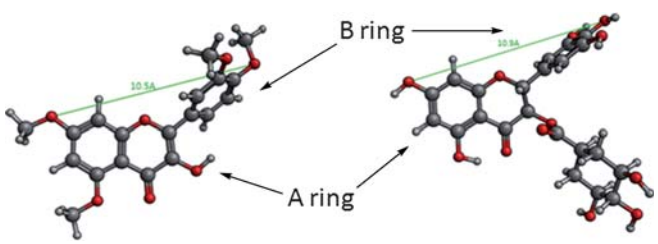


Fig. 12 The favored conformations of compounds **1** and **2** for binding to hemagglutinin's binding pockets.

inactivated in cell culture assays with the EF extract at concentrations ranging from the recommended dose for oral consumption to dosages several orders of magnitude lower. The extract also effectively reversed virus-induced pro-inflammatory responses in cultured epithelial cells.[88]

Detailed studies indicate that maximum inhibition in virus replication can be reached if EF and the virus come into contact directly prior to the infection. Hemagglutination assays showed that the extract inhibited the receptor binding activity of the virus, suggesting that the extract interferes with the viral entry into cells. In addition, the Tamiflu®-resistant virus was just as susceptible to EF as the wild-type virus.[89]

The fruit of *Chaenomeles sinensis* ('*Mugua*'), which is widely used as a TCM to treat throat diseases, is available from plants widely distributed throughout China and Japan. In 2005, Hamauzu and co-workers suggested that *C. sinensis* has antiviral activity, having found that the phenolic extract of *C. sinensis* inhibits the hemagglutination activity of the influenza virus, and stops the first step of the infection by disabling the virus's ability to adhere to host cells.[90]

In 2008, Sawai and colleagues examined the anti-influenza virus activity of *C. sinensis* more precisely.[91] They evaluated the anti-influenza virus activity of a 50% ethanol extract of the fruit of *C. sinensis*. Treatment with the extract at concentrations greater than 5 mg/mL reduced the plaque titers of both influenza A and B viruses to less than 10% of those of untreated viruses. When the 50 mg/mL extract was added to the culture medium after inoculation of the virus, viral NS2 protein (non-structural protein) synthesis was selectively inhibited, and progeny virus was not detected in the infected cell medium. As a result, they concluded that high molecular weight polyphenols in the fruits of *C. sinensis* neutralize influenza virus by inhibiting hemagglutination activity and by suppressing NS2 protein synthesis.

6 Neuraminidase inhibitors

Structure-based drug design is widely applied in the design of NA inhibitors. Based upon the co-crystal structures of NA, sialic acid and DANA (analogs of neuraminic acid known to inhibit NA *in vitro*[92,93]), von Itzstein discovered two potent NA inhibitors, a 4-guanidino analog of DANA and zanamivir.[71,76,94] Zanamivir was approved by FDA in 1999, and is effective against influenza A and influenza B. Due to its highly polar nature, it is administrated by oral inhalation.

Oseltamivir carboxylate, reported by Kim and colleagues, was approved by the FDA in 1999.[72] It is effective against neuraminidases from influenzas A and B, and is orally active. It is the most commonly used antiviral drug, and works by preventing the release of viral particles from infected cells. However, it has been reported that it can cause vomiting and nausea, and the number of reports of oseltamivir resistance is increasing.[95–101] Oseltamivir itself is not from TCM, but its starting material, shikimic acid, is a TCM herb. The limited availability of shikimic acid has recently become a concern.

A number of aromatic compounds have also been designed to inhibit neuraminidase, but none of these compounds was potent enough to proceed to clinical trials.[102–105] Abbott Laboratories have designed five-membered pyrrolidine derivatives,[106–108] while Chand and co-workers have synthesised cyclopentane

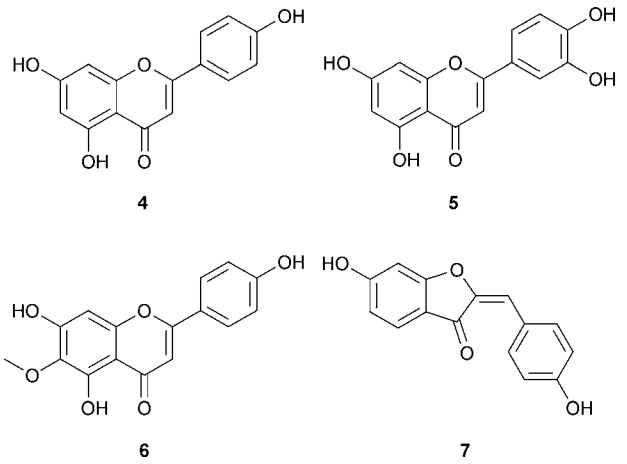


Fig. 13 Flavonoids **4–7**.

derivatives[68,99–102] as potent neuraminidase inhibitors. One of these cyclopentane derivatives, peramivir (RWJ-270201, BCX-1812), is highly potent against a wide range of strains of influenza virus, and has been approved and launched in Japan. Due to low oral bioavailability, it can only be formulated for intravenous infusion, and therefore, more attention has been paid to seeking neuraminidase inhibitors from natural products or TCM.

Flavonoids are polyphenolic compounds that are frequently found from TCM herbs. As early as 1990, 5,7,4′-trihydroxy-8-methoxyflavone (F36) was found to be an influenza virus sialidase inhibitory compound.[109] In 2008, Liu and co-workers studied the structure–activity relationship (SAR) of 25 flavonoids on their neuraminidase (NA) activity of influenza virus. The NA inhibition activities are in the following order: aurones > flavones/flavonols > isoflavones > flavanones/flavanonols and flavanes/flavanols.

The SAR analyses of flavonoids on influenza virus NAs show that the 4′-OH, 7-OH, C4═O and C2═C3 groups are essential for NA inhibition, and that the glycosyl group can reduce the inhibition significantly. As flavonoid derivatives, apigenin (**4**), luteolin (**5**), dinatin (**6**) and 2-((*E*)-4-hydroxyphenylidene)-6-hydroxy-2,3-dihydrobenzofuran-3-one (**7**) (Fig. 13) have been discovered to be highly potent against influenza virus, with IC_{50} values of 4.74–24.7 μM in a cytopathic effect inhibition assay.[110]

Examination of the TCM herb *Elsholtzia rugulosa* (‘*Yebazi*’), which is widely used in the treatment of cold and fever, produced

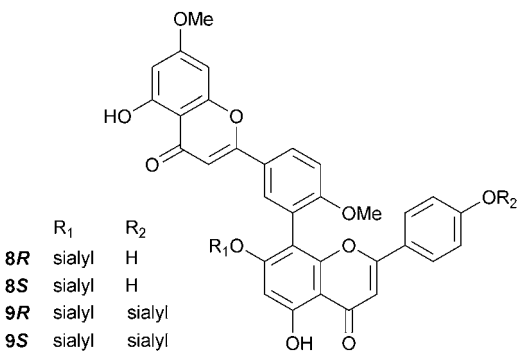
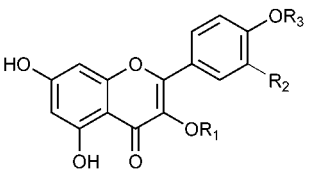
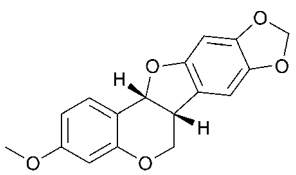


Fig. 14 Biflavonoid derivatives **8R**, **8S**, **9R** and **9S**.



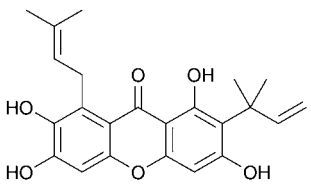
	R_1	R_2	R_3
10	β-xylopyranosyl(1→2)-α-thamnopyranosyl(1→6)-β-glucopyranosyl	β-glucopyranosyloxy	H
11	β-xylopyranosyl(1→2)-β-glucopyranosyl	β-glucopyranosyloxy	CH_3
12	β-xylopyranosyl(1→2)-β-glucopyranosyl	β-glucopyranosyloxy	H
13	β-xylopyranosyl(1→2)-β-glucopyranosyl	OH	H
14	β-glucopyranosyl	OH	H
15	β-xylopyranosyl(1→2)-β-glucopyranosyl	H	H
16	β-xylopyranosyl(1→2)-α-thamnopyranosyl(1→6)-β-glucopyranosyl	H	H
17	β-glucopyranosyl	H	H
18	β-glucopyranosyl(1→4)-α-rhamnopyranosyl	H	H
19	β-galactopyranosyl	H	H

Fig. 15 Flavanoid derivatives **10–19**.



20

Fig. 16 A pterocarpan derivative (**20**).



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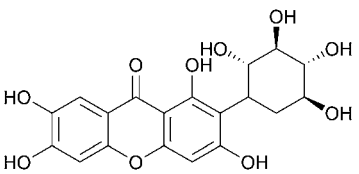

mangiferin

Fig. 17 A xanthone derivative (**21**).

apigenin (**4**) and luteolin (**5**) (in addition to three other constituents), and these were found to have IC_{50} values against the influenza A and B virus of 1.43 μg/mL and 2.06 μg/mL, respectively.[111]

Biflavonoids, such as amentoflavone derivatives, have also been reported to have antiviral activities,[112] and consequently, many biflavonoids have been screened for their inhibitory activities against influenza virus. Ginkgetin isolated from *Ginkgo biloba* ('*Yinxing*') and *Cephalotaxus harringtonia* ('*Cufei*') were found to have potent inhibitory activity against influenza virus sialidase. Ginkgetin–sialic acid conjugates **8R**, **8S**, **9R** and **9S**, which were prepared by the conjugation of the biflavonoid ginkgetin from a plant source and sialic acid of animal origin, showed a significant survival effect in influenza-virus-infected mice (Fig. 14). Acid–aglycone conjugation of ginkgetin lowered the cytotoxicity and enhanced the inhibitory activity against influenza virus sialidase.[113]

Another bioassay-guided fractionation of an ethanol extract of the seeds of *Aesculus chinensis* ('*Qiyeshu*') (a TCM herb) led to the isolation of two new flavanoids, **10** and **11**, along with eight known ones, **12–19** (Fig. 15). The antiviral activity was measured by a cytopathic effect inhibition assay,[114–116] and **18** demonstrated significant antiviral activity against influenza A (H1N1), with an IC_{50} of 24.5 μg/mL and a SI (selectivity index) of 16.0.[117]

To develop novel NA inhibitors, Ryu and colleagues have isolated pterocarpans (Fig. 16) and flavanones from well-known TCM herbs through a bioassay-guided fractionation approach.[118] The pterocarpan derivative **20** was found to have the most potent NA inhibitory activity, with an IC_{50} value of 1.4 μM. The docking studies indicate that pterocarpan derivatives may bind to the binding pocket adjacent to the active site, as shown in Fig. 8.

Flavanone and xanthone were also found to have NA inhibitory activities.[119,120] These compounds are from *Cudrania tricuspidata* ('*Zheshu*'), which is used in China as a TCM herb for traumatic injuries. Compound **21** displays nanomolar activity (IC_{50} = 0.08 ± 0.01 μM), and is 200-fold more potent than the first reported xanthone derivative neuraminidase inhibitor, mangiferin (IC_{50} = 16.2 ± 4.2 μM)[121] (Fig. 17).

Another compound, the flavanone **22** (Fig. 18), has potency against neuraminidase, with an IC_{50} of 380 nM. This compound has the same scaffold as xanthone and mangiferin – a frequently occurring motif of the active components in TCM.

Similarly, gossypetin (**23**) and kaempferol (**24**) (Fig. 19) extracted from the root of *Rhodiola rosea* ('*Hongjingtian*'), exhibited significant NA inhibitory activities, with an IC_{50} of 1.25 μg/mL for both H1N1 and H9N2, in Madin–Darby canine kidney cells.[122] Gossypetin exhibited the most potent inhibitory activity, with IC_{50} values of 0.8 and 2.6 μM towards neuraminidases from *Clostridium perfringens* and recombinant influenza virus A (rvH1N1), respectively. In contrast, kaempferol exhibited the highest activity against influenza viruses H1N1 and H9N2, with EC_{50} values of 30.2 and 18.5 μM, respectively. The activity was found to depend on the position and number of hydroxy groups on the flavonoid backbone.

Recently, polyphenol compounds isolated from the roots of *Glycyrrhiza uralensis* ('*Gancao*') was reported for neuraminidase inhibition,[123] and 18 derivatives were screened against NA. Of these, isoliquiritigenin (**25**) and glycyrol (**26**) (Fig. 20) exhibited the most potent NA inhibitory activity, with IC_{50} values of 9.0 μM and 3.1 μM, respectively. Interestingly, the scaffolds of these compounds are resveratrol-like – a motif that has a broad distribution in compounds from TCM herbs.

7 Anti-flu herbs from TCM

A number of anti-flu agents have been discovered from TCM herbs, although the mechanisms of action have not yet been elucidated. *Hypericum perforatum* ('*Guanyelianqiao*' or St John's wort) is a TCM herb for arresting bleeding, anti-bacteria, and defervescence. Pu and colleagues screened *H. perforatum* extract against influenza A virus (H1N1) *in vitro* and *in vivo* using the cytopathic effect and Neutral Red dye uptake,[124] and found that it could inhibit the virus *in vitro* with an EC_{50} of 40 μg/mL. The extract's CC_{50} (50% cytotoxic concentration) in Madin–Darby canine kidney cells was 1.5 mg/mL. Ribavirin was run in parallel in this experiment, and found to have an EC_{50} value of 5.0 μg/mL, with a mean CC_{50} of 520 μg/mL.

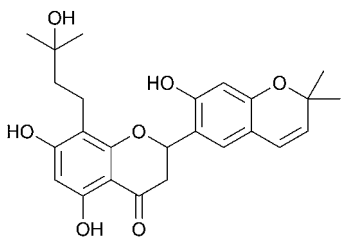

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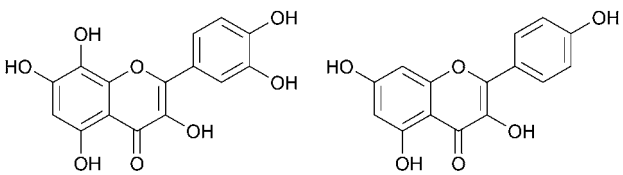
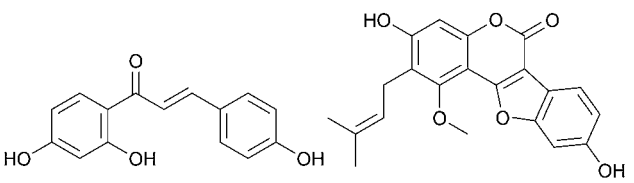
Fig. 18 The flavanone **22**.



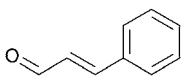
23 **24**

Fig. 19 Gossypetin (**23**) and kaempferol (**24**).



25 **26**

Fig. 20 Isoliquiritigenin (**25**) and glycyrol (**26**).



27

Fig. 21 *trans*-Cinnamaldehyde.

TCM herbal extracts from *Forsythia suspensa* ('*Lianqiao*'), *Andrographis paniculata* ('*Chuanxinlian*') and *Glycyrrhiza uralensis* ('*Gancao*') were screened by Ko and co-workers. It was found that these extracts suppressed influenza A virus-induced RANTES secretion by human bronchial epithelial cells. Therefore, it was suggested that these TCM herbs could be beneficial for the treatment of chronic inflammatory conditions followed by viral infection.[125]

Mantani and co-workers reported that the extract of *Ephedra* spp. (Ephedrae Herba, '*Mahuang*'), a key TCM herb for inducing sweat, dispelling 'exogenous evils', and relieving cough and asthma, inhibited acidification of endosomes and lysosomes dose-dependently (100–400 μg/mL) with the disappearance of acidified endosomes/lysosomes. Moreover, the growth of influenza A/PR/8/34 (H1N1) (PR8) virus was inhibited when the cells were treated with the extract for 1 h immediately after infection, or as early as 5–10 min after infection. The inhibitory effect of Ephedrae Herba was completely or partially reversed by $FeCl_3$, a tannin-reactive agent, strongly suggesting that tannin is one of the active components in the extract.[126]

Chinese cinnamon ('*Rougui*') is a TCM herb for eliminating cold to stop pain, and activating the blood to promote menstruation. One of the components, *trans*-cinnamaldehyde (**27**) (Fig. 21) was studied by Hayashi and colleagues, who found that it could inhibit the growth of influenza A/PR/8 virus *in vitro* and *in vivo*. It also inhibited the growth of influenza PR8 virus in Madin–Darby canine kidney cells dose-dependently without cytotoxicity, which was confirmed by an MTT assay. *trans*-Cinnamaldehyde targeted the mid-stage of virus growth, in sharp contrast to 'Ephedrae Herba', which targets the early stage of virus growth.[126] RT-PCR and SDS-PAGE assays clearly demonstrated that *trans*-cinnamaldehyde inhibited viral protein synthesis at the post-transcription level, based on the fact that mRNA is synthesized in drug-treated cells at almost the same level as in drug-untreated cells.[127]

The stem of *Clematis montana* ('*Xiuqiuteng*') is a frequently used TCM herb for inducing diuresis for treating stranguria, and for hemagogic and lactagogic treatments. *Clematis montana* lectin had antiviral activity against various viruses in cell culture. Moreover, it inhibited influenza A subtype H1N1, influenza A subtype H3N2 and influenza B, with EC_{50} values of 33, 44 and 45 μg/mL, respectively.[128]

Alkaloids exist widely in TCM and natural products. The antiviral activity of the total alkaloid extract from *Commelina communis* ('*Yazhicao*') against influenza virus A/PR/8/34 (H1N1) has been investigated *in vitro* and *in vivo*. This extract exhibited inhibitory action on the growth of influenza virus in Madin–Darby canine kidney cells when added before or after viral infection. The results obtained in mice infected with influenza virus also suggest that this extract has a pronounced protective effect against infection by the influenza A virus.[129]

As major components of TCM herbs and natural products, sugars and derivatives are known to inhibit virus multiplications. High concentrated 2-deoxy-D-glucose and D-glucosamine can impair glycosylation of viral glycoproteins.[130–133] 1-Deoxynojirimycin (an alkaloid found in mulberry leaves ('*Sangye*'), a TCM herb) and castanospermine inhibit glycosylation by trimming enzymes to stop the synthesis of sugar chains on viral surface.[134–138] In other instances, it was found that these sugars impaired the viral protein biosynthesis and destabilization of the proteins.[139,140]

Huang and co-workers have screened a number of natural and synthetic sugar analogues for antiviral activities (anti-influenza included), and found that introducing a benzyl group to these sugars could endow them with anti-influenza activity, whereas sugars substituted with methyl, acetyl, uridyl, thiocyanyl, azido, isopropylidene or benzylidene groups had no anti-flu activity. In addition, all the sugars containing a 2-deoxy-2-acetamido group were inactive as anti-flu agents.

Ferula assa-foetida ('*Awei*') is a TCM herb for expelling parasites and food stagnancy. Lee and co-workers reported that about 30 compounds from the herb extract have been screened against flu viruses and cancer cells.[141] Compounds **28–36** (Fig. 22) showed impressive potency against influenza A virus

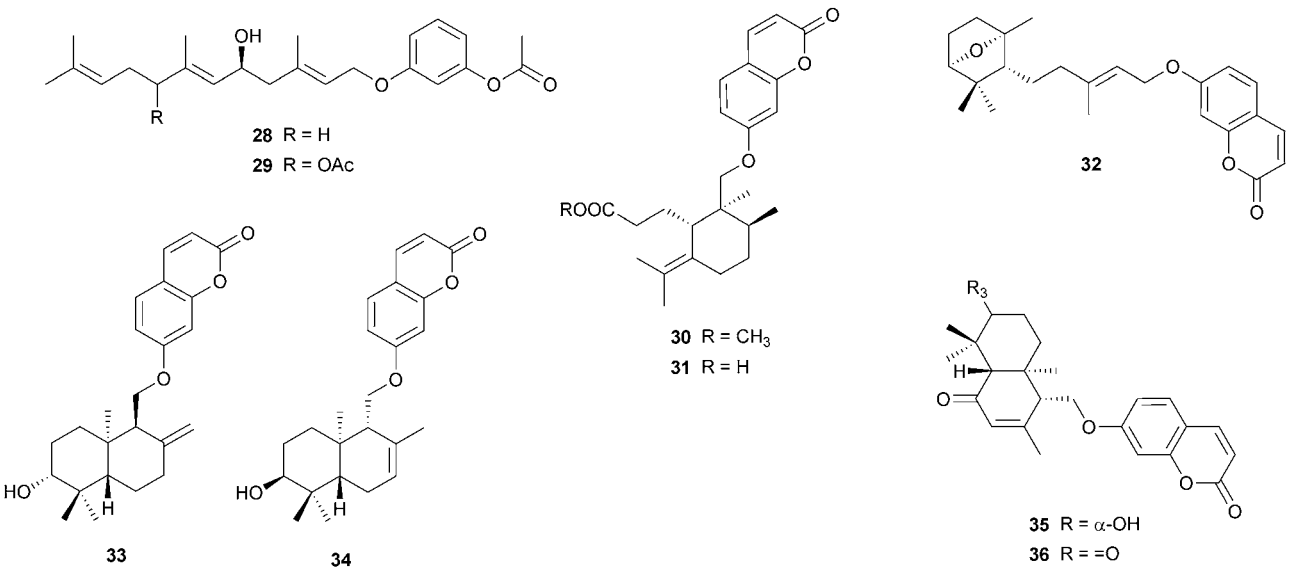


Fig. 22 Compounds **28–36** from *Ferula assa-foetida*.

Table 4 TCM herbs with anti-flu activities with non-specified or unconfirmed targets

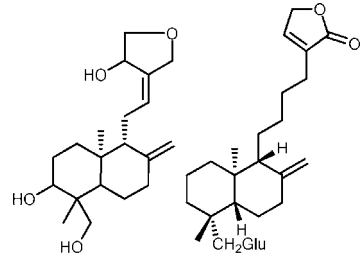
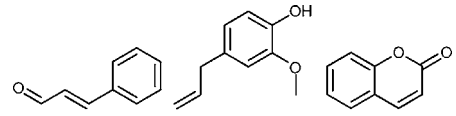
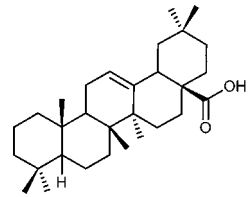
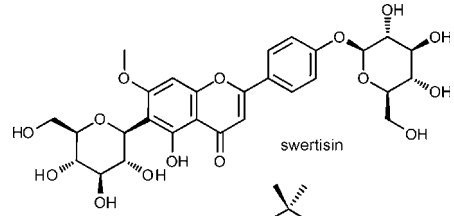
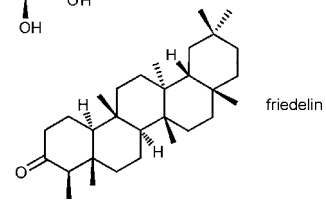
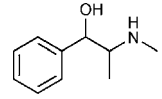
Scientific name	Herb name	Plant part	Screen protocol	Activities	Representative components
Andrographis paniculata	'*Chuanxinlian*' (Herba Andrographitis)	Whole plant	Suppressing effect on RANTES secretion by H1N1-infected A549 bronchial epithelial cells	$IC_{50} = 1.2 \pm 0.4$ μg/mL	 andrographolide neoandrographolide
Cinnamomum verum or *Cinnamomum cassia*	'*Rougui*' (Cortex Cinnamomi)	Bark	MTT (tetrazolium hydroxide salt), reverse transcriptase PCR and SDS-PAGE assays	Inhibits the growth of influenza PR8 virus in a concentration-dependent fashion (20–200 μM) without cytotoxicity	 cinnamaldehyde eugenol coumarin
Clematis montana	'*Xiuqiuteng*'	Stem	Exposure to cell cultures infected by influenza A H1N1 and H3N2, and influenza B	Activity towards influenza A H1N1, H3N2 and influenza B with EC_{50} values of 34, 45 and 45 μg/mL (visual cytopathic effect score). MTS values 37.2, 51.0 and 27.6, respectively	 deoxyoleanolic acid
Commelina communis	'*Yazhicao*'	Leaf stalk	Inhibitory effect on the growth of influenza virus A/PR/8/34 (H1N1) in Madin–Darby canine kidney cells	$EC_{50} = 160$ μg/mL; $CC_{50} = 1880$ μg/mL	 swertisin  friedelin
Ephedra spp.	'*Mahuang*' (Ephedrae Herba)	Whole plant	Effect on acidification of intracellular compartments by fluorescence microscopy; effect on growth of influenza A/PR/8/34 virus	Concentration-dependent inhibition (100–400 μg/mL)	 pseudoephedrine

Table 4 (*Contd.*)

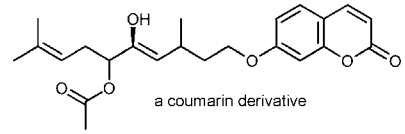
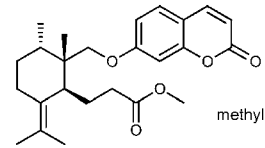
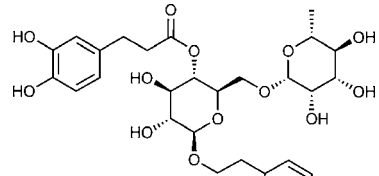
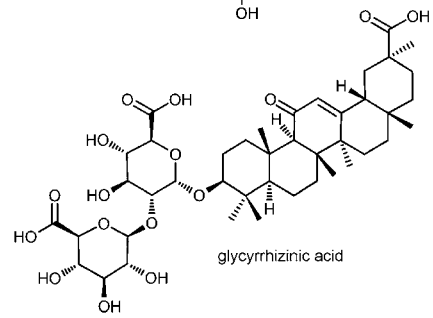
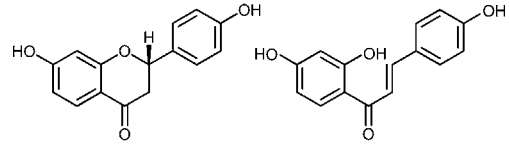
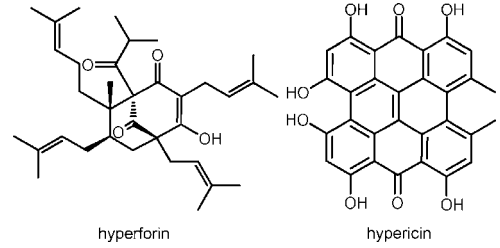
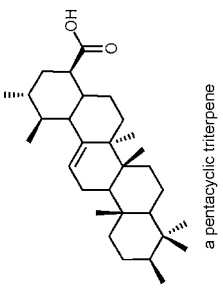
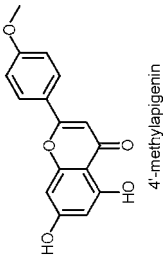
Scientific name	Herb name	Plant part	Screen protocol	Activities	Representative components
Ferula assa-foetida	'*Awei*'	Root	Activity against influenza A virus (H1N1) evaluated by the XTT method in Madin–Darby canine kidney cells	IC_{50} = 0.26–0.86 μg/mL	 a coumarin derivative  methyl galbanate
Forsythia suspensa	'*Lianqiao*' (Fructus Forsythiae)	Fruit	Suppressing effect on RANTES secretion by H1N1-infected A549 bronchial epithelial cells	IC_{50} = 35–48 μg/mL	 forsythiaside
				IC_{50} = 35–48 μg/mL	 glycyrrhizinic acid
Glycyrrhiza uralensis	'*Gancao*' (Radix Glycyrrhiza)	Root	Suppressing effect on RANTES secretion by H1N1-infected A549 bronchial epithelial cells		 liquiritigenin isoliquiritigenin
Hypericum perforatum	'*Guanyelianqiao*'	Whole plant	Cytopathic effect and Neutral Red dye uptake	EC_{50} = 40 μg/mL; CC_{50} = 1.5 mg/mL	 hyperforin hypericin

Table 4 (*Contd.*)

Scientific name	Herb name	Plant part	Screen protocol	Activities	Representative components
Mosla scabra	'*Shijining*'	Whole plant	Evaluation of anti-influenza activity in embryonated eggs and in a mouse infection model	IC_{50} = 0.15 mg/mL	 a pentacyclic triterpene  4′-methylapigenin

(H_1N_1) (IC_{50} 0.26–0.86 μg/mL), as evaluated by the XTT method.

Mosla scabra ('*Shijining*') is a TCM herb, and a widely used species in southeastern China for antipyretic and antiviral drugs for the treatment of colds, fever, inflammation and chronic bronchitis. Yu and co-workers screened its aqueous extract for anti-influenza virus activities in embryonated eggs and in a mouse infection model. The results showed that the efficacy against influenza A virus was similar to that of ribavirin.[142]

Propolis has been used worldwide as a traditional medicine for thousands of years and as a dietary supplement to maintain or improve human health.[143–145] The anti-influenza virus activity of propolis was screened in a plaque reduction assay by Shimizu and colleagues, in which four ethanol extracts of propolis, with anti-influenza virus activity *in vitro*, were further examined for anti-influenza efficacy in a murine influenza virus infection model.[146]

The above-mentioned TCM herbs have been found to be anti-flu agents, but their mechanisms of action have not yet been elucidated. The components of these herbs are listed in Table 4.

8 TCM anti-flu prescriptions

The key difference between TCM and modern medicine is that TCM prescriptions consist of single or combined herbs, but modern drugs consist of single or combined compounds.

TCM prescriptions have been practiced for thousands of years, and a rich clinical knowledge has been accumulated. In 2009, as one of the official responses to the H1N1 pandemic, the Chinese government announced three editions of a document entitled "Recommended Schemes for Pandemic Influenza A Diagnoses and Treatments". The third edition of the document recommended not only two targeted anti-flu drugs, oseltamivir and zanamivir, but also four anti-flu TCM prescriptions (Table 5).[147] Since then, TCM has played an important role in fighting the pandemic in China.

It is important to realize that the key TCM principle is to make the diagnosis and treatment based on an overall analysis of the illness and the patient's condition. In Table 5, it can be seen that patients are classified into two types: those with light symptoms and those with severe symptoms. Each type is divided into subtypes. For each subtype, the basic TCM prescription will be modified by adding or removing herbs in order to personalize the treatment.

A number of TCM databases have been created in recent decades,[148–155] The TCM Structure Database created by Zhou and co-workers,[156] the World Traditional Medicine Patent Database (WTM),[157] and the Saphron TCM Database[158] are examples. Zhou's TCM Structure Database has more than 30,000 structures with the annotations of herb material data, bioactivity data, disease data, and many other references, and is one of the best TCM structure databases for virtual screening or providing inspiration to medicinal chemists. The Saphron TCM database also contains more than 30,000 structures, with annotated information on 1400 diseases, 180,000 non-patented TCM prescriptions, 9000 TCM herb materials, and 120,000 bioactivities. Finally, WTM is the world's first traditional medicine patent database with integration of multinational patent resources. WTM provides search capabilities and analytical tools

for natural medicine research and drug development, and covers all patents involving natural products with pharmaceutical, nutraceutical, cosmetic and agrochemical applications. It covers traditional medicine patents from 1985 onwards from 22 countries and two international organizations (including those with country codes CN, JP, KR, IN, US, SU, BZ, DE, FR, RO, CA, AU, GB, WO and EP), and has over 110,000 basic patents of traditional medicine with about 200,000 family patents. WTM is well indexed, enabling many types of search option, including chemical structure searching.

Based on the searches of the WTM and Saphron databases, we summarize in Table 6 the TCM herbs used to treat respiratory tract infections.

Combing the search results from the Saphron and WTM databases, the 30 most frequently used TCM herbs for treating respiratory tract infections are indicated in Fig. 23.

The top 10 herbs from Fig. 23 contain approximately 800 main components. Scaffold-based analyses[159] show that these compounds can be divided into four main families (Fig. 24). Many molecules from Family I are lysoganglioside-like compounds, which are potential hemagglutinin inhibitors. Compounds from Family III are potential hemagglutinin inhibitors, based upon the current reports.[86,87] Compounds from Family II are likely sialic acid mimics, which are potential neuraminidase inhibitors. Compounds from family IV are steroid-like compounds, which may be responsible for reducing flu symptoms.

Table 5 Recommended schemes for pandemic influenza A diagnoses and treatments

TCM classification		Symptoms	TCM therapy	Basic TCM prescription
Feng-re-fan-wei	Wind-heat affecting the defense system – light symptoms	Early onset, fever or no fever, red throat, light cough, little phlegm, no sweat. Tongue: red, thin coating or greasy, floating pulse	Dispersing wind and clearing away heat	Flos Lonicerae (‘*Jinyinhua*’), Fructus Forsythiae (‘*Lianqiao*’), Folium Mori (‘*Sangye*’), Flos Chrysanthemi (‘*Yejuhua*’), Radix Platycodi (‘*Jiegeng*’), Fructus Arctii (‘*Niubangzi*’), Herba Lophatheri (‘*Danzhuye*’), Rhizoma Phragmitis (‘*Lugen*’), Herba Menthae (‘*Behe*’), Radix Glycyrrhiza (‘*Gancao*’)
Re-du-xi-fei	Pyretic toxicity attacking the lungs – light symptoms	High fever, cough, sticky phlegm, thirst, sore throat, red eyes. Tongue: red, yellow coating or greasy, floating pulse	Clearing away the lung-heat, detoxification	Herbal Ephedrae (‘*Mahuang*’), Semen Armeniacae Amarum (‘*Kuxingren*’), Radix Glycyrrhiza (‘*Gancao*’), Gypsum Fibrosuum (‘*Shigao*’), Rhizoma Anemarrhenae (‘*Zhimu*’), Bulbus Fritillariae Thunbergii (‘*Zhebei*’), Radix Platycodi (‘*Jiegeng*’), Radix Scutellariae (‘*Huangqin*’), Radix Bupleuri (‘*Chaihu*’)
Re-du-yong-fei	Pyretic toxicity obstructing the lungs – severe symptoms	High fever, cough, yellow sticky phlegm, breathing difficult, palpitations, dysphoria, dark purple lips. Tongue: red, brown or gray coating, floating pulse.	Clearing heat and purging the lungs, detoxification	Herbal Ephedrae (‘*Mahuang*’), Gypsum Fibrosuum (‘*Shigao*’), Semen Armeniacae Amarum (‘*Kuxingren*’), Rhizoma Anemarrhenae (‘*Zhimu*’), Herba Houttuyniae (‘*Yuxingcao*’), Semen Lepidi (‘*Tinglizi*’), Rhizoma Fagopyri Cymosi (‘*Jinqiaomai*’), Radix Scutellariae (‘*Huangqin*’), Bulbus Fritillariae Thunbergii (‘*Zhebei*’), Radix et Rhizoma Rhei (‘*Dahuang*’), Cortex Moutan (‘*Mudanpi*’), Herba Artemisiae (‘*Qinghao*’)
Qi-ying-liang-fan	Overburning of both *qifen* (vital energy) and *yingfen* (nourishment) – severe symptoms	High fever, thirst, dysphoria or coma and delirium, cough or emptysis, choking, shortness of breath. Tongue: red, brown or gray coating, floating pulse.	Clearing *qifen* (vital energy) and cooling *yingfen* (nourishment)	Cornu Bubali (‘*Shuiniujiao*’), Radix Rehmanniae (‘*Shudihuang*’), Radix Paeoniae Rubra (‘*Chishao*’), Flos Lonicerae (‘*Jinyinhua*’), Radix Salviae Miltiorrhizae (‘*Danshen*’), Fructus Forsythiae (‘*Lianqiao*’), Radix Ophiopogonis (‘*Maidong*’), Herba Lophatheri (‘*Danzhuye*’), Gypsum Fibrosuum (‘*Shigao*’), Fructus Gardeniae (‘*Jiaozhizi*’)

Table 6 TCM classifications and the associated herbs for treating respiratory tract infections

TCM classification	Associated herbs
TCMs commonly used to treat colds	Herba Pogostemonis (‘*Guanghuoxiang*’), Folium Isatidis (‘*Daqingye*’), Herbal Ephedrae (‘*Mahuang*’), Herba Schizonepetae (‘*Jingjiesui*’), Herba Menthae (‘*Behe*’), Folium Perillae (‘*Zisuye*’), Fructus Forsythiae (‘*Lianqiao*’), Ramulus Cinnamomi (‘*Guizhi*’), Radix Astragali (‘*Huangqi*’), Radix Isatidis (‘*Banlangen*’), Herba Eupatorii (‘*Peilan*’), Herba Houttuyniae (‘*Yuxingcao*’)
Antiviral TCMs belonging to the ‘lung meridian’	Flos Lonicerae (‘*Jinyinhua*’), Flos Chrysanthemi Indici (‘*Yejuhua*’), Rhizoma Polygonati (‘*Huangjing*’), Fructus Arctii (‘*Niubangzi*’), Fructus Schisandrae (‘*Wuweizi*’), Radix Scutellariae (‘*Huangqin*’), Herba Andrographitis (‘*Chuanxinlian*’), Radix Trichosanthis (‘*Tianhuafen*’), Radix Glycyrrhiza (‘*Gancao*’), Folium Perillae (‘*Zisuye*’), Folium Pyrrosiae (‘*Shiwei*’), Herba Schizonepetae (‘*Jingjiesui*’), Rhizome Cimicifugae (‘*Shenma*’), Fructus Forsythiae (‘*Lianqiao*’), Herbal Ephedrae (‘*Mahuang*’), Rhizoma Anemarrhenae (‘*Zhimu*’), Herba Menthae Haplocalycis (‘*Behe*’), Herba Pogostemonis (‘*Guanghuoxiang*’), Rhizoma Belamcandae (‘*Shegan*’), Radix Sophorae Flavescentis (‘*Kushen*’), Herba Houttuyniae (‘*Yuxingcao*’), Radix Astragali (‘*Huangqi*’), Ramulus Cinnamomi (‘*Guizhi*’), Folium Isatidis (‘*Daqingye*’), Cortex Lycii (‘*Digupi*’), Caulis Lonicerae (‘*Rendongteng*’)
Antiviral TCMs commonly used to treat lung diseases	Radix Scutellariae (‘*Huangqin*’), Spica Prunellae (‘*Xiakucao*’), Flos Lonicerae (‘*Jinyinhua*’), Radix Glycyrrhiza (‘*Gancao*’), Herba Houttuyniae (‘*Yuxingcao*’), Fructus Forsythiae (‘*Lianqiao*’), Cortex Lycii (‘*Digupi*’), Folium Pyrrosiae (‘*Shiwei*’), Folium Isatidis (‘*Daqingye*’)
Antiviral TCMs commonly used to treat pyreticosis	Rhizoma Polygoni Cuspidati (‘*Huzhang*’), Rhizoma Coptidis (‘*Huanglian*’), Radix Scutellariae (‘*Huangqin*’), Radix Sophorae Flavescentis (‘*Kushen*’), Semen Raphani (‘*Lafuzi*’), Radix Isatidis (‘*Banlangen*’), Radix Bupleuri (‘*Chaihu*’), Fructus Forsythiae (‘*Lianqiao*’), Herba Houttuyniae (‘*Yuxingcao*’), Radix Stemonae (‘*Baibu*’)
Antibiotic and antiviral TCMs	Herba Schizonepetae (‘*Jingjiesui*’), Herba Menthae Haplocalycis (‘*Behe*’), Folium Perillae (‘*Zisuye*’), Fructus Forsythiae (‘*Lianqiao*’), Ramulus Cinnamomi (‘*Guizhi*’), Herbal Ephedrae (‘*Mahuang*’), Herba Pogostemonis (‘*Guanghuoxiang*’), Radix Astragali (‘*Huangqi*’)
Antiviral TCMs with immune regulatory function	Herba Houttuyniae (‘*Yuxingcao*’), Flos Lonicerae (‘*Jinyinhua*’), Fructus Forsythiae (‘*Lianqiao*’), Rhizoma Polygonati (‘*Huangjing*’), Radix Astragali (‘*Huangqi*’), Radix Trichosanthis (‘*Tianhuafen*’), Fructus Schisandrae (‘*Wuweizi*’), Herba Epimedii (‘*Yinyanghuo*’), Radix Scutellariae (‘*Huangqin*’), Radix Sophorae Flavescentis (‘*Kushen*’), Semen Lablab Album (‘*Baibiandou*’), Herba Schizonepetae (‘*Jingjiesui*’), Radix Bupleuri (‘*Chaihu*’), Cortex Moutan (‘*Mudanpi*’), Radix Glycyrrhiza (‘*Gancao*’), Radix Paeoniae Alba (‘*Baishao*’), Rhizoma Cimicifugae (‘*Shengma*’), Herba Artemisiae (‘*Qinghao*’), Folium Perillae (‘*Zisuye*’), Radix Isatidis (‘*Banlangen*’)

9 Concluding thoughts

Modern drugs are pure compounds or combinations of them, with well-elucidated mechanisms of actions at the molecular level. A central tenet of modern medicine is that a drug should be a pure compound that selectively interacts with one target in order to cure one disease; an ideal modern drug should work for all patients.

In contrast, TCM drugs are mixtures of raw herbs, which may be pre-processed or not. An underlying theme of TCM is that herbs are classified based on traditional Chinese pharmacological concepts‡. A TCM prescription is composed on the spot, based upon the recognition of a patient’s symptoms, which indicates which herbs to combine – it is therefore true ‘personalized medicine’. This fact supports the idea of polyvalence, or a form of synergy in medical treatment, a topic that has been the subject of recent publications.[160,161]

Although modern medicine and TCM may seem very different, they should have a common foundation, as herbs of a given TCM class have a common structural scaffold (Fig. 25). Most anti-flu TCM herbs belong to the heat-clearing and detoxifying class, which comprises the four structural families shown in Fig. 24. It is interesting that some compounds from this class have scaffolds formed from more than one structural family (Fig. 26).

These facts may inspire medicinal chemists to seek new ways of optimizing drug leads. For example, as shown in Fig. 25, natural metabolic systems can join together two biphenols (scaffold Family II) with a disaccharide fragment.

‡ Examples of properties that TCM herbs may possess are: diaphoretic, heat-clearing and detoxifying, lapactic, expelling wind-damp, interior-warming, *qi*-regulating, aiding digestion, helminthic, blood-regulating, reducing phlegm, stopping coughing, relieving asthma, sedating and tranquilizing, calming the liver, arresting wind, inducing resuscitation, tonifying (supplementing), lowering swelling, emetic, and for external use.

On the surface of the H1N1 virus, hemagglutinins, neuraminidases, M1 (matrix) proteins, and M2 (ion channel) proteins could all be anti-flu targets. If a small molecule could inhibit all of them, it would be a powerful antiviral agent, but even if one existed, it would probably be very toxic due to the lack of selectivity. This may be why current drug discovery pursues one compound against one target for one indication. However, this paradigm has the disadvantage of a high failure rate, and therefore high cost.

TCM prescriptions follow a different paradigm. TCM uses mixtures of compounds, divided into several families, each family attacking/regulating one or more targets. Some TCM

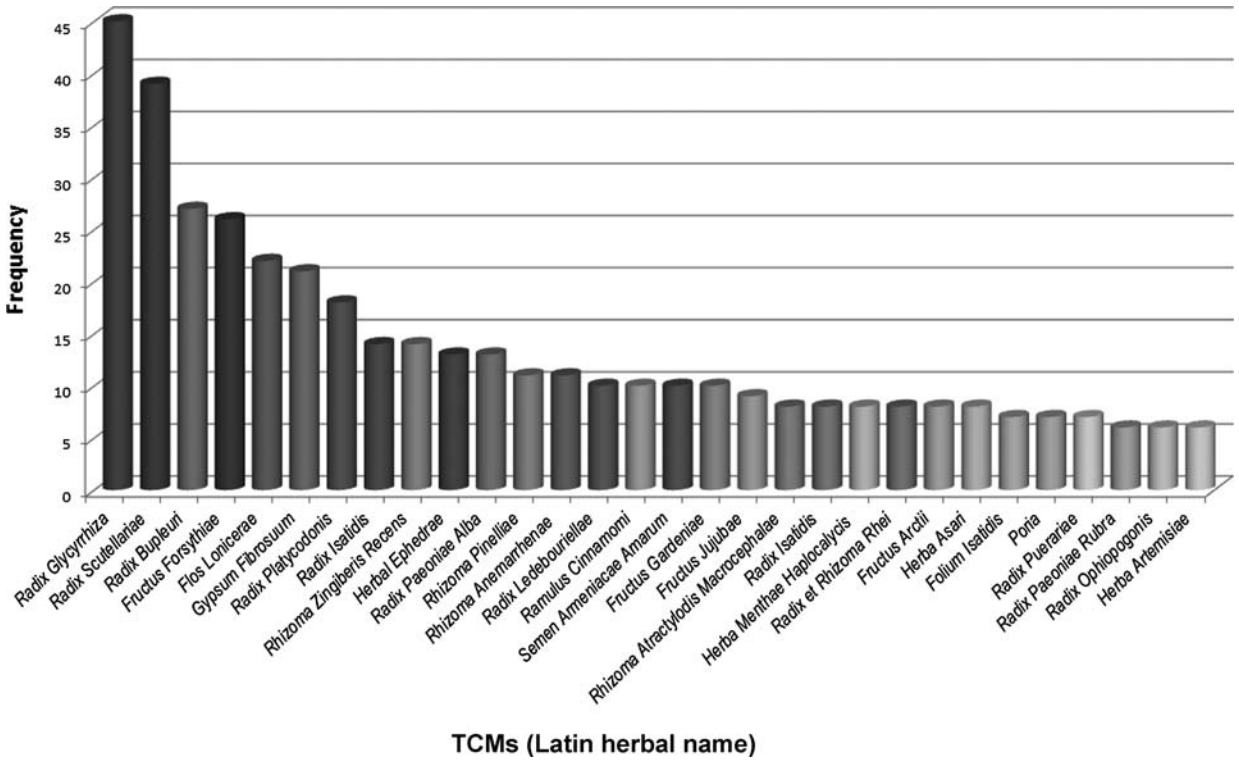


Fig. 23 The thirty most frequently used TCM herbs for treating respiratory tract infections.

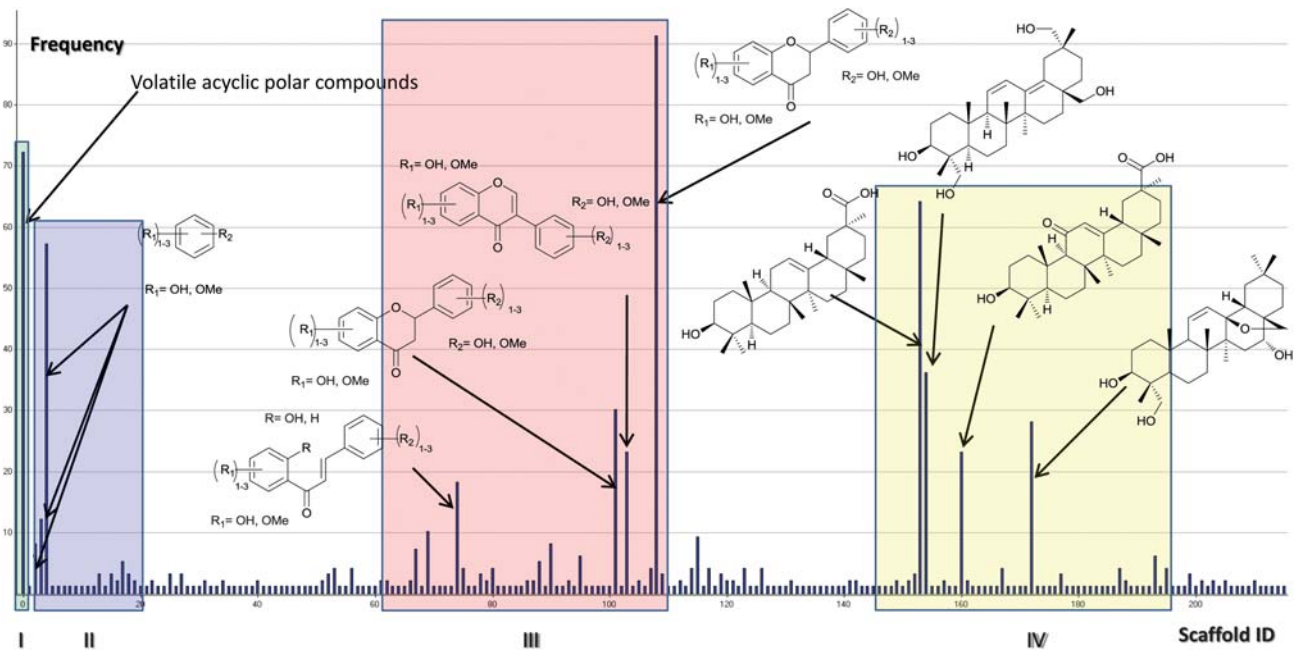


Fig. 24 The four structural families contained in the top 10 TCM anti-flu herbs. Family I: volatile acyclic polar/bipolar compounds; Family II: monocyclic polyphenols; Family III: tricyclic or 'pre-tricyclic' polyphenols; Family IV: pentacyclic compounds.

components are for killing the virus directly, and these are named the 'King' components. Others may be responsible for reducing the symptoms, and they are named 'Minister', 'Assistant', and 'Messenger' components. In case of TCMs to combat H1N1 influenza, the herbs containing components primarily from Families I–III (Fig. 24) may be 'Kings', while those herbs containing components primarily from Family IV may be 'Ministers', 'Assistants', or 'Messengers'.

In summary, TCM drugs comprise multiple compounds regulating multiple targets for a class of medical indications, and are tunable for the symptoms of an individual. By further investigating TCM theory and practice, as outlined in this

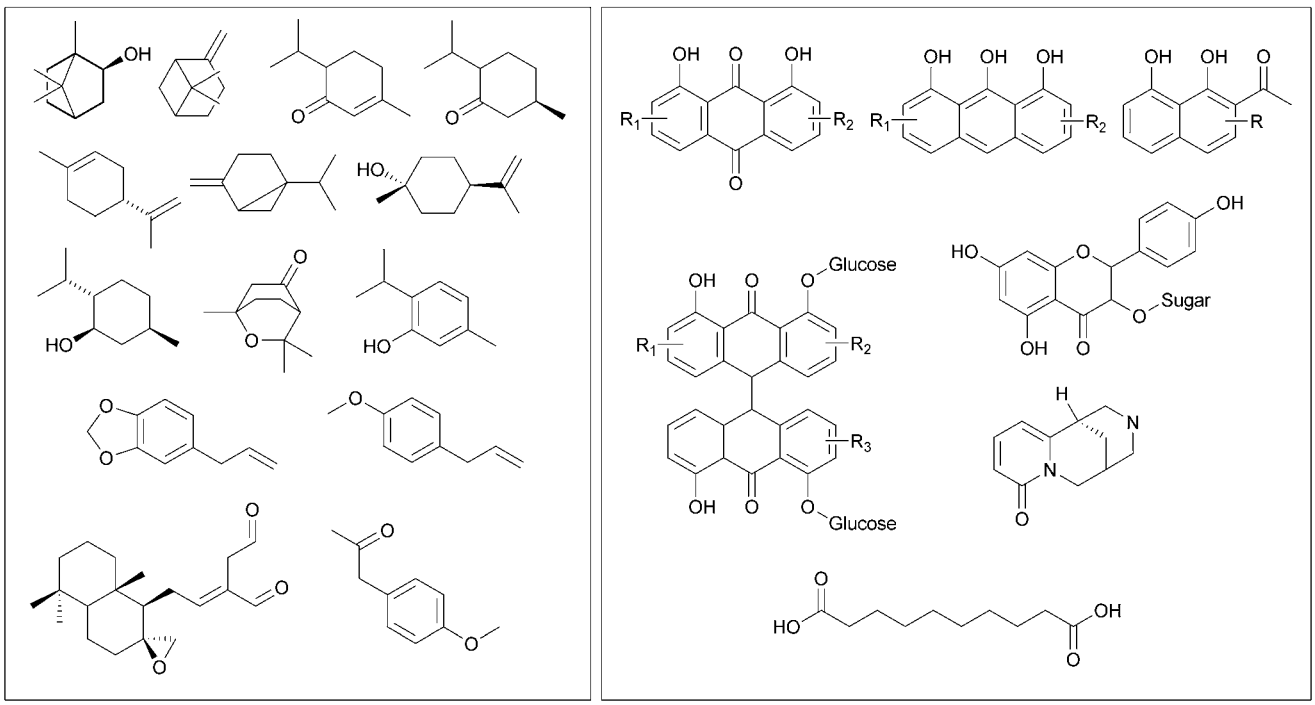


Fig. 25 Herbs of the same TCM class (left: 'relieve exterior'; right: 'discharging') have a common structural scaffold.[162]

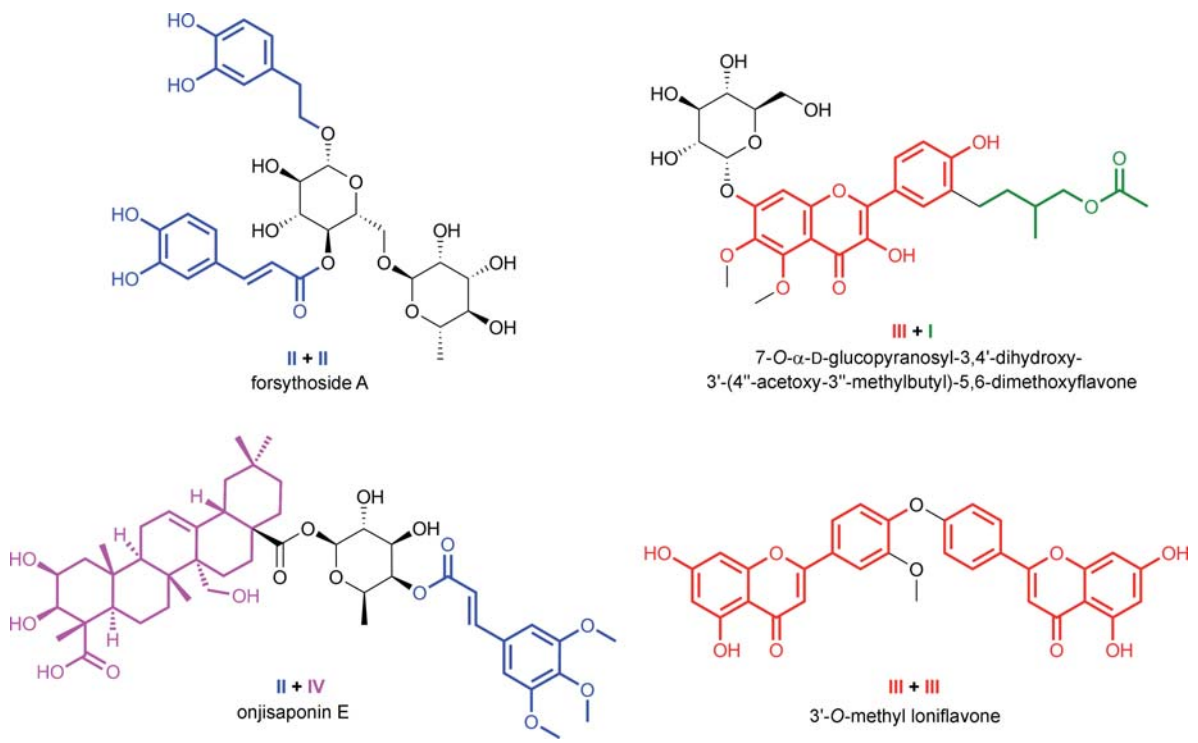


Fig. 26 Compounds constructed from more than one natural scaffold. The labels I–IV refer to the groupings shown in Fig. 24.

review, a better drug discovery paradigm may arise – a paradigm that could be beneficial to both TCM and modern medicine.

10 Acknowledgements

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