Natural products against acute respiratory infections: Strategies and lessons learned

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Abstract

Ethnopharmacological relevance: A wide variety of traditional herbal remedies have been used throughout history for the treatment of symptoms related to acute respiratory infections (ARIs).

Aim of the review: The present work provides a timely overview of natural products affecting the most common pathogens involved in ARIs, in particular influenza viruses and rhinoviruses as well as bacteria involved in co-infections, their molecular targets, their role in drug discovery, and the current portfolio of available naturally derived anti-ARI drugs.

Materials and Methods: Literature of the last ten years was evaluated for natural products active against influenza viruses and rhinoviruses. The collected bioactive agents were further investigated for reported activities against ARI-relevant bacteria, and analysed for the chemical space they cover in relation to currently known natural products and approved drugs.

Results: An overview of (i) natural compounds active in target-based and/or phenotypic assays relevant to ARIs, (ii) extracts, and (iii) *in vivo* data are provided, offering not only a starting point for further in-depth phytochemical and antimicrobial studies, but also revealing insights into the most relevant anti-ARI scaffolds and compound classes. Investigations of the chemical space of bioactive natural products based on principal component analysis show that many of these compounds are drug-like. However, some bioactive natural products are substantially larger and have more polar groups than most approved drugs. A workflow with various strategies for the discovery of novel antiviral agents is suggested, thereby evaluating the merit of *in silico* techniques, the use of complementary assays, and the relevance of ethnopharmacological knowledge on the exploration of the therapeutic potential of natural products.

Conclusions: The longstanding ethnopharmacological tradition of natural remedies against ARIs highlights their therapeutic impact and remains a highly valuable selection criterion for

natural materials to be investigated in the search for novel anti-ARI acting concepts. We observe a tendency towards assaying for broad-spectrum antivirals and antibacterials mainly discovered in interdisciplinary academic settings, and ascertain a clear demand for more translational studies to strengthen efforts for the development of effective and safe therapeutic agents for patients suffering from ARIs.

Keywords

antivirals, antibacterials, co-infection, influenza, rhinovirus, chemoinformatics

Abbreviations

ARIs	acute respiratory infections
CC_{50}	50% cytotoxic concentration
CPE	cytopathic effect
HTS	high throughput screening
IC_{50}	50% inhibitory concentration
IV	influenza virus
MIC	minimum inhibitory concentration
mRNA	messenger ribonucleic acid
MTS	medium throughput screening
RNA	ribonucleic acid
RV	rhinovirus

Contents

1. Acute respiratory infections (ARIs): Pathogens and relevant targets

Acute respiratory infections (ARIs) with mild (*e.g.* common cold) to severe (influenza and influenza-like illness) symptoms affect the life of millions of people worldwide each year. Among these infections, lower respiratory tract infections are the fourth most common cause of death globally and the primary cause in low-income countries (WHO, 2018).

Improved diagnostic tests, such as the introduction of multiplex polymerase chain reaction diagnosis, allow close-to-real-time surveillance of a broad range of respiratory viruses and bacteria (single as well as co-infections) in ambulant and clinical specimens (Biancardi et al., 2016; Visseaux et al., 2017). The results of these surveillance programs are summarized in national, European, and worldwide reports and are available in "real-time" databases, e.g. at the Robert-Koch-Institute in Berlin, Germany (Influenza, 2019), and the Clinical Virology Network (CVN, 2019).

Influenza viruses A and B, together with enteroviruses such as the rhinoviruses A, B, and C, account for the majority of ARIs (Heikkinen and Jarvinen, 2003; Monto, 2002; Visseaux et al., 2017). Therefore, this review will exclusively focus on influenza viruses and rhinoviruses. Both pathogens are known to boost secondary bacterial infections (co-infections). Concerning influenza viruses, the M2 ion channel protein, the enzymes neuraminidase and viral polymerase represent established targets of approved anti-influenza drugs (Tab. 1) (De Clercq and Li, 2016; Furuta et al., 2017; Hayden et al., 2018). For influenza prevention, vaccines are available but poorly accepted (Nguyen et al., 2011). In contrast to influenza, no drugs are approved for the treatment of rhinovirus infections today. Previous attempts to develop an anti-rhinoviral vaccine failed due to the high number (159) of circulating serotypes and insufficient cross-protective immunity between serotypes (Stepanova et al., 2019). Although rhinoviruses cause a mild contagious disease, they may trigger bacterial otitis media, sinusitis, and pneumonia (Jacobs et al., 2013).

The co-infection of influenza viruses and bacteria (e.g. *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Haemophilus influenzae*) contributes significantly to the mortality rates of seasonal influenza epidemics as well as pandemics and is therefore called lethal synergism (Brundage and Shanks, 2008; McCullers, 2014). Table 1 provides an overview of the main viral pathogens involved in ARIs including their molecular targets as well as approved drugs (natural product based or synthetic).

Table 1. Overview of the molecular targets and approved drugs of influenza and rhinoviruses.

Viruses involved in ARIs	Description	Potential drug targets	Approved drugs
Influenza viruses A and B	***Orthomyxoviridae*, enveloped, negative-sense single-stranded RNA viruses, segmented genome**	hemagglutinin (= surface glycoprotein)	---
		nucleoprotein	---
		viral polymerase	favipiravir (**8**) (Avigan®), baloxavir marboxil (Xofluza®)
		M2 ion channel protein (only active against influenza A viruses)	amantadine (Symmetrel®), rimantadine (Flumadine®)
		neuraminidase (= surface glycoprotein)	oseltamivir (**1**) (Tamiflu®), zanamivir (**2**) (Relenza® – inhalative; Dectova® - intavenously), peramivir (**6**) (e.g. Rapivab®, Alpivab®), laninamivir (**7**) (Inavir®)
Rhinoviruses	***Picornaviridae*, non-enveloped, positive-sense, single-stranded RNA viruses**	viral proteins: e.g. protease 3C, the viral polymerase, and a small hydrophobic pocket in the capsid protein VP1	---

2. Approved small-molecule drugs against ARIs from or inspired by nature

To date there is only one class of natural product-derived drugs approved for the treatment of virus-induced ARIs: influenza neuraminidase inhibitors (Fig. 1). All presently known neuraminidase inhibitors are natural product derivatives and/or substances mimicking the transition state of N-acetyl-neuraminic acid, the endogenous substrate of viral neuramindase (Newman and Cragg, 2016; von Itzstein, 2007). The development of neuraminidase inhibitors

has been guided by structure-based molecular design. In 1999, the first two neuraminidase inhibitors, oseltamivir (**1**) (Tamiflu®) and zanamivir (**2**) (Relenza®), were approved as drugs by the FDA. Zanamivir is commonly applied via inhalation, a requirement related to its high polarity and low bioavailability. In addition, zanamivir was approved for intravenous application. Its ethyl ester derivative, oseltamivir, is a prodrug designed for improved bioavailability, and is the first approved, orally bioavailable neuraminidase inhibitor (Kim et al., 1997).

Synthesis of oseltamivir starts from either quinic acid (**3**) or shikimic acid (**4**) (Fig. 1). Both metabolites are widespread in nature, whereof the latter one is obtained in high yields (3-7%) from star anise pods, i.e. the star-like fruits of *Illicium verum* (Ghosh et al., 2012; Nguyen et al., 2006). It can also be produced by fermentation of genetically modified *E. coli* (Johansson et al., 2005; Krämer et al., 2003). Several natural derivatives of quinic acid, such as chlorogenic acid (**5**), have been probed for anti-influenza activity. In particular, the catechol group from the caffeic acid moiety of chlorogenic acid derivatives showed to be responsible for the inhibition of neuraminidases, although they might not reach the viral target *in vivo* due to their transformation in the gut (Gamaleldin Elsadig Karar et al., 2016).

In 2010, two further neuraminidase-inhibiting N-acetyl-neuraminic acid derivatives have been launched for the treatment of influenza: peramivir (**6**) (Rapivab®) and laninamivir (**7**) (Inavir®). Peramivir has been approved as a drug in Japan, South Korea, the US, and Europe. Laninamivir, which is approved in Japan only, is a long-acting zanamivir derivative that is applied via inhalation.

In 2014, the viral polymerase inhibitor favipiravir (**8**) (Avigan®) was approved in Japan for stockpiling against influenza pandemics (Furuta et al., 2017). Although favipiravir is a synthetic compound, its pyrazine carboxamide is based on a natural-product-like nucleoside scaffold. Favipiravir is a prodrug, which, after oral administration, is metabolized to the bioactive favipiravir-ribofuranosyl-5′-triphosphate. This metabolite acts against RNA viruses via

selective binding to PB1 inhibiting the viral polymerase (Jin et al., 2013). Favipiravir's efficacy in influenza treatment has lately been doubted because of a lack of efficacy in primary human airway cells (Yoon et al., 2018).

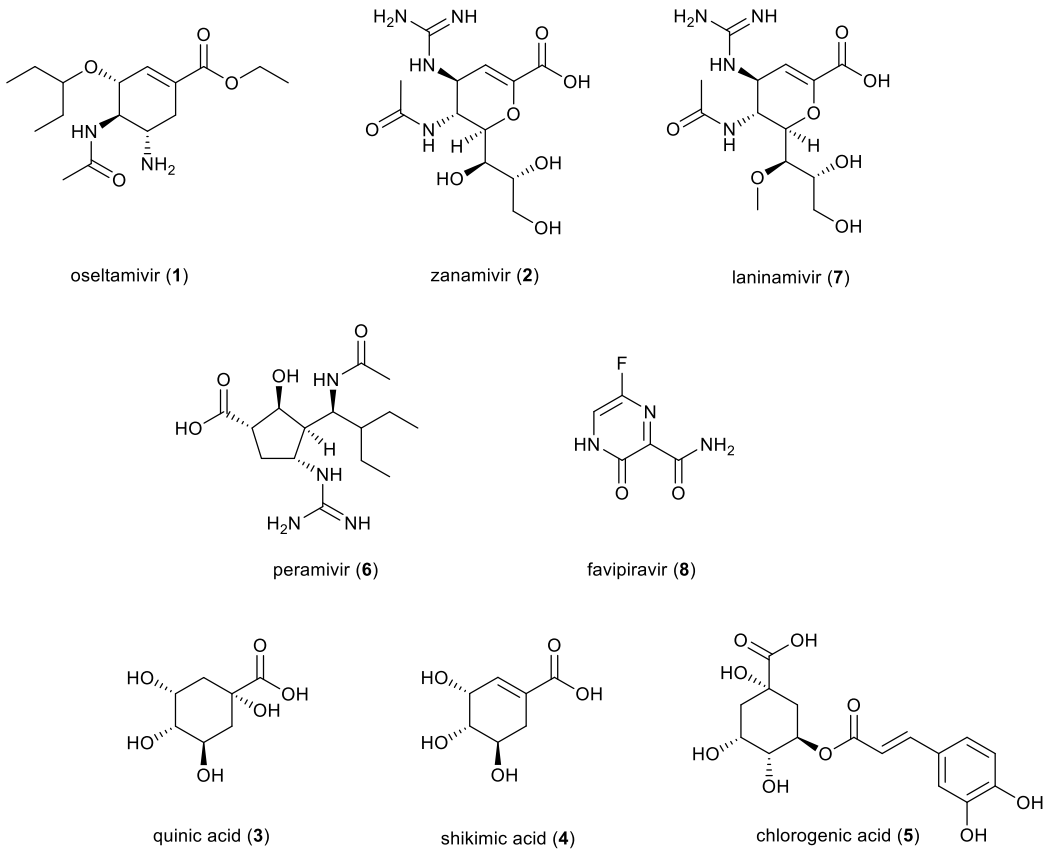

Fig. 1. Chemical structures of approved anti-influenza drugs inspired by nature (**1**, **2**, **6**-**8**), their natural precursor molecules (**3** and **4**), and chlorogenic acid (**5**).

3. Natural products with reported activities relevant to the treatment of ARIs

Nature is still the primary source of healthcare for people in developing countries. According to the WHO, in Africa the ratio of traditional healers to population is 1:500, whereas the ratio of medical doctors to population is 1:40.000, which is related to the lack of availability and accessibility of conventional medicines (WHO, 2013).

The most frequently used remedies for the management of ARIs, especially in children, are natural-based agents (mainly from botanical sources) due to easy access, low cost (Lucas et al.,

2018) and lack of specific antiviral drugs. Plants and microorganisms are a rich source of pharmacologically relevant small molecules because they have no immune system and, in consequence, are forced to defend themselves against enemies with potent natural products (Jones and Dangl, 2006). Compounds from nature have been used for the treatment of microbial (viral and bacterial) infections throughout history, and it is estimated that two-thirds of all of today's approved antibacterial drugs are derived from natural products (Martinez et al., 2015; Newman and Cragg, 2016).

However, challenges involved in the evaluation and comparison of outcomes from clinical studies have limited the number of botanicals approved by regulatory agencies for medical use (Kellogg et al., 2019). From 1981 to 2014 neither an antibacterial nor an antiviral botanical drug has been approved by the FDA. However, 11 out of 140 drugs introduced to the markets during this period are genuine natural products with antibacterial activity, whereas no genuine natural product with antiviral activity has been launched as new drug. Comparing the numbers of newly approved small chemical entities, only 22% (i.e. 14 out of 64) are entirely synthetic antiviral drugs leaving, a quite high portion of 78% for drug substances derived from or inspired by natural products (Newman and Cragg, 2016).

3.1. Methods for the extraction of literature data

We used SciFinder® to search for any literature published between January 2009 and June 2019 that is relevant to the research of natural products for the prevention and treatment of ARIs, in particular those caused by influenza or rhinoviruses. More specifically, we searched for any "journal", "letter", and "review" matching the research topic "natural products" in combination with any of the following keywords: "acute respiratory infection", "influenza" and "neuraminidase" or "rhinovirus". Documents in languages other than English and publications reporting on active extracts but lacking information on the origin of natural products were not considered. Furthermore, natural products relevant in ARIs identified by these searches were

manually screened for information on additional antibacterial activity, thereby considering the following species: *Streptococcus pneumoniae* and *S. pyogenes*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

3.2. Data evaluation and aspects to consider in the search for natural products against ARIs

An overview of bioactive pure compounds identified during our survey is provided in Table 2; results from extract testing are reported in Table 3 (including information on the solvents used for extract preparation); observations from *in vivo* studies (preclinical animal models or clinical studies) are presented in Table 4.

For better comparability of the biological data presented in Table 2, activity data (50% inhibition concentration: IC_{50} values; % inhibition at a certain concentration) are presented together with the corresponding positive controls. In the case of cell-based assays, information on the cytotoxicity of compounds (50% cytotoxic concentration: CC_{50}) is also provided.

A major challenge for the evaluation of natural products with reported activities against ARIs is the diversity of viral and bacterial strains. There is a large body of literature reporting on drug resistance related to the exchange of amino acids in viral proteins, for example, influenza virus neuraminidase (Abed and Boivin, 2017; Hoffmann et al., 2016). This fact underlines the limited comparability of activity data of different viral strains involved in ARIs. Comparability of activity data is further hampered by the fact that in many publications (i) the positive control (known inhibitor or drug) is missing, (ii) activity data are not reported as numbers but provided only as part of figures without any supplementary material, and (iii) activity at only one concentration is given, thus missing dose-dependency.

Moreover, comparability of activity data may also strongly be impacted by the used assay under investigation: target- or cell-based assays are usually the first access to bioactivity. The choice of respective assays depends on the level of available target information as well as the aims of

the study. For example, target-based assays are often used in the search for novel neuraminidase inhibitors that overcome resistance of influenza viruses to established drugs (Ding et al., 2017; Grienke et al., 2014; Kirchmair et al., 2011; Sriwilaijaroen et al., 2012). Previously coined "invalid metabolic panaceas" ascribed to natural compounds showing manifold bioactivities revealed a high prevalence of compounds to interfere in particular with the target-based neuraminidase inhibition assays (Bisson et al., 2016). This phenomenon raises concerns about the validity of natural products as lead compounds for neuraminidase inhibitors. In general, the reliability of target-based neuraminidase inhibition assays using fluorescence (FL), chemiluminescence (CL), and colorimetric readouts can be hampered by self-FL, signal quenching or the color of the samples (Chamni and De-Eknamkul, 2013; Kongkamnerd et al., 2011; Richter et al., 2015). To avoid assay interference pitfalls when dealing with self-FL and CL- or FL-quenching compounds (Henrich and Beutler, 2013), complementary assays have been established in our group (Richter et al., 2015). However, the test results of target-based assays do not necessarily correspond well with those of cell-based assays (e.g. virus yield reduction assay, cytopathic effect inhibition assay, plaque reduction assay) that capture cell permeability and full infection pathways rather than single targets (e.g. neuraminidase or receptor inhibition) (Martinez et al., 2015). In cell-based assays, an activity value alone (often expressed as the 50% effective or inhibitory concentration) has little validity, but is to be set in proportion to a control value (50% cytotoxic concentration to calculate the selectivity index (SI), positive control, vehicle control) for significance. Both target- und cell-based assays lack any kind of holistic effect on an organism such as metabolic processes and interactions with the immune response.

As apparent from Table 2, flavonoids, including their glycosides and chlorogenic acid derivatives, represent the most important class of natural products for which anti-influenza or anti-rhinovirus activities have been reported (Fig. 2). Further relevant compound classes include diarylheptanoids, iridoidglycosides, lignans and their glycosides, phenanthrenes,

phenolic compounds (including tannins), triterpenoids (including their glycosides), and xanthones.

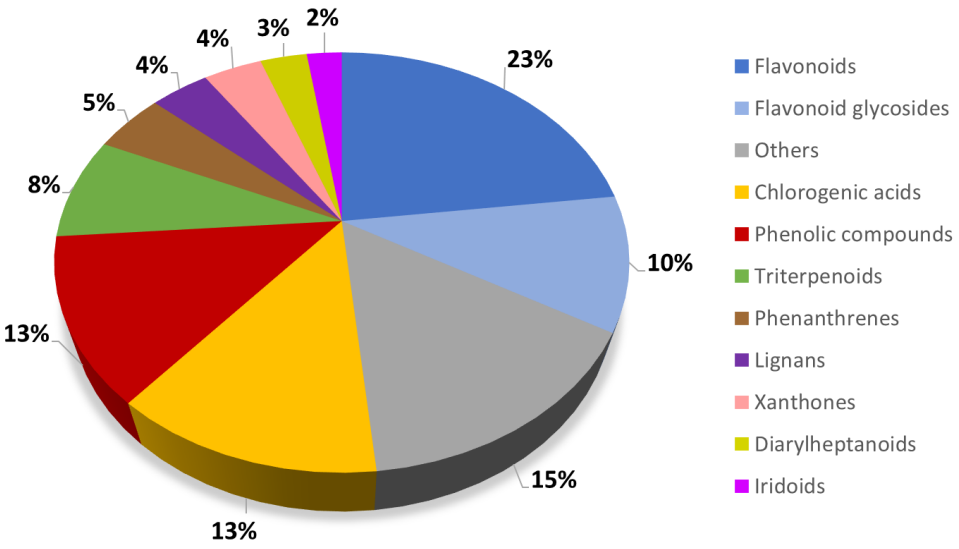

Fig. 2. Percentages of natural product compound classes listed in Tab. 2.

Using principal component analysis, we compared the chemical space of all bioactive natural products listed in Table 2 ($IC_{50} < 70\ \mu M$) with that of a large set of known natural products (201,761 compounds compiled previously) and approved drugs. The set of known natural products consists of 201,761 unique compounds that we compiled previously (Chen et al., 2019); the set of approved drugs was retrieved from DrugBank (Wishart et al., 2017). Fifteen key physicochemical properties (e.g. molecular weight and log *P*) were used to describe the molecules in a technical approach identical to the one described in Chen et al., 2019. As shown in Fig. 3A, many of the natural products active in ARI-relevant phenotypic and target-based assays populate areas in chemical space that are densely populated by approved drugs. Taking the loadings into consideration (Figure 3B), several natural products active against IVs and RVs are observed to be heavier (and larger) and to consist of more hydrogen bond donors and acceptors than most approved drugs.

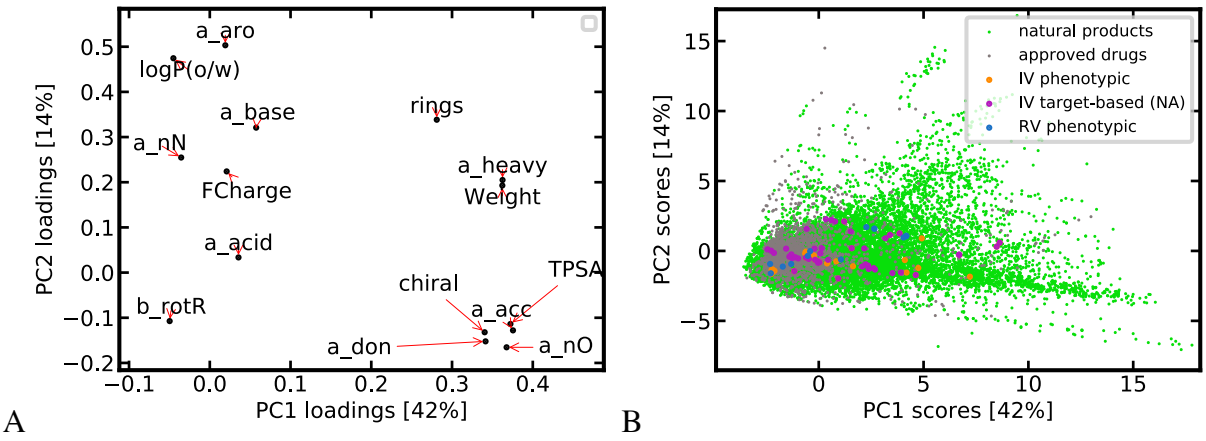

Fig. 3. PCA of the of the chemical space of natural products active in biological assays relevant to ARIs, known natural products and approved drugs. (A) PCA loadings plot. (B) PCA score plot. For the sake of clarity, only 10% of the 201,761 compounds of the known natural products data set are depicted. The PCA is based on 15 important physicochemical properties: molecular weight (Weight), log *P* (log *P* (o/w)), topological polar surface area (TPSA), number of hydrogen bond acceptors (a_acc), number of hydrogen bond donors (a_don), number of heavy atoms (a_heavy), fraction of rotatable bonds (b_rotR), number of nitrogen atoms (a_nN), number of oxygen atoms (a_nO), number of acidic atoms (a_acid), number of basic atoms (a_base), sum of formal charges (FCharge), number of aromatic atoms (a_aro) and number of chiral centers (chiral), and number of rings (rings). The percentage of the total variance explained by the first two principal components (PC1, PC2) is reported in the respective axis labels.

Table 2. Natural compounds with reported activities against ARIs: Anti-influenza virus, anti-rhinovirus, and dual antiviral and antibacterial compounds.

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
Chlorogenic acids								
n.g.	1,4-dicaffeoylquinic acid	IV, H1N1 A/PR/8/1934	NAI - CL	17.0 µM	oseltamivir: 0.0002 µM		n.r.	(Kirchmair et al., 2011)
n.g.	1,5-di-O-caffeoylquinic acid	CP NA	NAI - FL	23.0 fold at 10 µM	oseltamivir at 10 µM		n.r.	(Gamaleldin Elsadig Karar et al., 2016)
n.g.	3,4,5-tri-O-caffeoylquinic acid	CP NA	NAI - FL	20.0 fold at 10 µM	oseltamivir at 10 µM		n.r.	
		IV, rH5N1 (N-His)-Tag	NAI - FL	20.0 fold at 10 µM	oseltamivir at 100 µM			
n.g.	3,4-di-O-caffeoylquinic acid	CP NA	NAI - FL	25.0 fold at 10 µM	oseltamivir at 10 µM		n.r.	
		IV, rH5N1 (N-His)-Tag	NAI - FL	24.0 fold at 10 µM	oseltamivir at 100 µM			
n.g.	3,5-di-O-caffeoylquinic acid	CP NA	NAI - FL	28.0 fold at 10 µM	oseltamivir at 10 µM		n.r.	
		IV, H5N1	NAI - FL	58.0 fold at 100 µM	oseltamivir at 100 µM			
		IV, rH5N1 (N-His)-Tag	NAI - FL	22.0 fold at 10 µM	oseltamivir at 100 µM			
n.g.	3-O-caffeoylglucose	IV, CP NA	NAI - FL	20.0 fold at 10 µM	oseltamivir at 10 µM		n.r.	
n.g.	4,5-di-O-caffeoylquinic acid	CP NA	NAI - FL	25.0 fold at 10 µM	oseltamivir at 10 µM		n.r.	
		IV, rH5N1 (N-His)-Tag	NAI - FL	28.0 fold at 10 µM	oseltamivir at 100 µM			
n.g.	5-O-caffeoylquinic acid	CP NA	NAI - FL	24.0 fold at 10 µM	oseltamivir at 10 µM		n.r.	

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
n.g.	caffeic acid	IV, rH5N1 (N-His)-Tag	NAI - FL	63.0 fold at 100 µM	oseltamivir at 100 µM		PA	(Gamaleldin Elsadig Karar et al., 2016; Perumal et al., 2015)
n.g.	methyl-3,4-di-O-caffeoylquinate	CP NA	NAI - FL	38.0 fold at 10 µM	oseltamivir at 10 µM		SA	(Gamaleldin Elsadig Karar et al., 2016; Zhang et al., 2013)
Ilex asprella (Hook. et Arn.) Champ. ex Benth.	3,4,5-trimethoxyphenol b-D-5-O-caffeoyl-apiofuranosyl-(16)-b-D-glucopyranoside	IV, H1N1 A/PR/8/1934	NAI - FL	1.7 µM	oseltamivir: 0.9 µM		n.r.	(Peng et al., 2016)
Lonicera japonica Thunb.	3,4-di-O-caffeoylquinic acid	CP NA	NAI - FL	68.3 µM	oseltamivir: 11.82 µM		n.r.	(Zhao et al., 2018)
	3,5-di-O-caffeoylquinic acid	CP NA	NAI - FL	61.2 µM	oseltamivir: 11.82 µM		SA	(Xiong et al., 2013; Zhao et al., 2018)
	chlorogenic acid	IV, H1N1 A/FM1/1/1947	CPE	39.4 µM	n.g.	364.3 µM in MDCK cells	n.r.	(Ding et al., 2017)
		IV, H1N1 A/Jinnan/15/2009	CPE	54.8 µM	n.g.	364.3 µM in MDCK cells		
		IV, H1N1 A/PR/8/1934	CPE	44.9 µM	oseltamivir: ~ 60% at 2 µM	364.3 µM in MDCK cells		
		IV, H1N1 A/PR/8/1934	NAI - FL	22.1 µM	n.g.			
		IV, H3N2 A/Beijing/32/1992	CPE	62.3 µM	oseltamivir: ~ 60% at 2 µM	364.3 µM in MDCK cells		
		IV, H3N2 A/Beijing/32/1992	NAI - FL	59.1 µM	n.g.			
		IV, H3N2 A/Hubei/3/2005	CPE	51.2 µM	n.g.	364.3 µM in MDCK cells		
		IV, H3N2 A/Zhuhui/1222/2010	CPE	71.9 µM	n.g.	364.3 µM in MDCK cells		
		IV, H1N1 A/PR/8/1934	NAI - FL	84.7 µM	oseltamivir: 0.007 µM		n.r.	

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
Moringa oleifera Lam.	5-O-caffeoyl quinic acid	IV, H1N1 A/PR/8/1934	NAI - FL	78.5 µM	oseltamivir: 0.007 µM		n.r.	(Kashiwada et al., 2012)
Polygonum chinense L.	caffeic acid	IV, B/Lee/1940	CPE	81.6 µM	oseltamivir: 0.21 µM	> 1,665.2 µM in MDCK cells	PA	(Perumal et al., 2015; Tran et al., 2017)
		IV, H1N1 A/PR/8/1934	CPE	209.8 µM	oseltamivir: < 0.005 µM	> 1,665.2 µM in MDCK cells		
		IV, H3N2 A/HK/2/1968	CPE	178.2 µM	oseltamivir: < 0.07 µM	> 1,665.2 µM in MDCK cells		
Diarylheptanoids								
Alpinia katsumadai Hayata	(E,E)-1,7-diphenyl-4,6-heptadien-3-one	IV, H1N1 A/PR/8/1934	NAI - CL	6.1 µM	oseltamivir: 0.0001 µM		n.r.	(Grienke et al., 2010)
	(E,E)-5-hydroxy-1,7-diphenyl-4,6-heptadien-3-one	IV, H1N1 A/PR/8/1934	NAI - CL	4.7 µM	oseltamivir: 0.0001 µM		n.r.	
	(S)-1,7-diphenyl-6(E)-hepten-3-ol	IV, H1N1 A/PR/8/1934	NAI - CL	4.1 µM	oseltamivir: 0.0001 µM		n.r.	
	katsumadain A	CP NA	NAI - CL	0.1 µM	oseltamivir: 43.5 µM		SP	(Richter et al., 2015)
		CP NA	NAI - FL	2.8 µM	oseltamivir: 61.3 µM			
		CP NA	NAI - lectin-based HA	2.4 µM	oseltamivir: 100 µM			
		IV, H1N1 A/342/2009	NAI - CL	0.6 µM	oseltamivir: > 0.03 µM			(Kirchmair et al., 2011; Walther et al., 2016)
		IV, H1N1 A/Belzig/2/2001a	NAI - CL	0.6 µM	oseltamivir: 0.0002 µM			(Grienke et al., 2010; Walther et al., 2016)
		IV, H1N1 A/Brest/IDT7490/2008	NAI - CL	1.6 µM	oseltamivir: 0.0002 µM			
		IV, H1N1 A/Horneburg/IDT7489/2008	NAI - CL	1.1 µM	oseltamivir: 0.0001 µM			

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
		IV, H1N1 A/Jena/5258/2009	NAI - CL	0.4 µM	oseltamivir: 0.0002 µM			(Richter et al., 2015)
		IV, H1N1 A/Jena/525820/09	NAI - FL	48.4 µM	oseltamivir: 0.0005 µM			
		IV, H1N1 A/Jena/5528/2009	NAI - CL	0.2 µM	oseltamivir: 0.0001 µM			(Kirchmair et al., 2011; Walther et al., 2016)
		IV, H1N1 A/Jena/5555/2009	NAI - CL	0.3 µM	oseltamivir: 0.0001 µM			
		IV, H1N1 A/Jena/8178/2009+rNanA	plaque reduction	28.1% at 20 µM	oseltamivir at 1 µM			
		IV, H1N1 A/Jena/8178/2009+rNanB	plaque reduction	21.0% at 20 µM	oseltamivir at 1 µM			
		IV, H1N1 A/Potsdam/15/1981a	NAI - CL	0.7 µM	oseltamivir: 0.0002 µM			(Grienke et al., 2010; Walther et al., 2016)
		IV, H1N1 A/PR/8/1934	NAI - CL	1.1 µM	oseltamivir: 0.0001 µM			
		SP CJ9400	NAI - lectin-based HA	0.7 µM	oseltamivir: 0.3 µM			(Walther et al., 2015)
		SP D39	NAI - lectin-based HA	1.0 µM	oseltamivir: 10.0 µM			
		SP DSM20566	NAI - CL	0.9 µM	oseltamivir: 0.6 µM			(Richter et al., 2015)
		SP DSM20566	NAI - FL	13.4 µM	oseltamivir: 1.1 µM			
		SP DSM20566	NAI - lectin-based HA	3.2 µM	oseltamivir: 2.1 µM			(Walther et al., 2015)
		SP DSM20566 rNanA	NAI - lectin-based HA	3.2 µM	oseltamivir: 3.2 µM			
		SP DSM20566 rNanB	NAI - lectin-based HA	5.4 µM	oseltamivir: 31.6 µM			

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
		VC NA	NAI - CL	0.4 µM	zanamivir: 20.6 µM			(Richter et al., 2015)
		VC NA	NAI - FL	15.0 µM	zanamivir: 42.5 µM			
		VC NA	NAI - lectin-based HA	1.7 µM	zanamivir: 54.4 µM			
Flavonoids								
Euphorbia ebracetolata Hayata	ent-(13S)-13-hydroxyatis-16-ene-3,14-dione	RV, B3	Cell titer-Glo Lumninescent Cell Viability	25.3 µM	n.g.		n.r.	(Wang et al., 2018)
	ent-(3β,13S)-3,13-dihydroxyatis-16-en-14-one	RV, B3	Cell titer-Glo Lumninescent Cell Viability	49.3 µM	n.g.		n.r.	
	ent-13(R)-hydroxy-3,14-dioxo-16-atisene	RV, B3	Cell titer-Glo Lumninescent Cell Viability	80.1 µM	n.g.		n.r.	
	ebracetone B	RV, B3	Cell titer-Glo Lumninescent Cell Viability	90.4 µM	n.g.		n.r.	
n.g.	4'-O-methylochnaflavone	IV, H1N1 A/342/2009	NAI - CL	40.7 µM	oseltamivir: > 0.03 µM		n.r.	(Kirchmair et al., 2011)
		IV, H1N1 A/Jena/5528/2009	NAI - CL	3.5 µM	oseltamivir: 0.0001 µM			
		IV, H1N1 A/Jena/5555/2009	NAI - CL	2.0 µM	oseltamivir: 0.0001 µM			
		IV, H1N1 A/PR/8/1934	NAI - CL	2.1 µM	oseltamivir: 0.0002 µM			
n.g.	gossypetin	IV, H1N1 A/PR/8/1934	CPE	43.0 µM	oseltamivir: 8.3 µM	> 283.0 µM in MDCK cells	n.r.	(Jeong et al., 2009)
		IV, H9N2 A/Chicken/Korea/MS96/1996	CPE	36.3 µM	oseltamivir: 6.3 µM	> 283.0 µM in MDCK cells		
n.g.	quercetin	IV, H1N1 A/PR/8/1934	CPE	43.1 µM	oseltamivir: 8.3 µM	> 253.8 µM in MDCK cells	n.r.	

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n.g.	quercetin-5,7,3',4'-tetramethylether	IV, H1N1 A/342/2009	NAI - CL	14.8 µM	oseltamivir: > 0.03 µM		n.r.	(Kirchmair et al., 2011)
		IV, H1N1 A/Jena/5528/2009	NAI - CL	0.4 µM	oseltamivir: 0.0001 µM			
		IV, H1N1 A/Jena/5555/2009	NAI - CL	1.0 µM	oseltamivir: 0.0001 µM			
		IV, H1N1 A/PR/8/34	NAI - CL	1.1 µM	oseltamivir: 0.0002 µM			
Artocarpus sp.	artocarpin	IV, H1N1 A/342/2009	NAI - CL	0.6 µM	oseltamivir: > 0.03 µM		SP	(Kirchmair et al., 2011)
		IV, H1N1 A/Jena/5528/2009	NAI - CL	0.2 µM	oseltamivir: 0.0001 µM			
		IV, H1N1 A/Jena/5555/2009	NAI - CL	0.3 µM	oseltamivir: 0.0001 µM			
		IV, H1N1 A/Jena/8178/2009+rNanA	Plaque reduction	44.3% at 20 µM	oseltamivir at 1 µM			(Walther et al., 2016)
		IV, H1N1 A/Jena/8178/2009+rNanB	Plaque reduction	77.1% at 20 µM	oseltamivir at 1 µM			
		IV, H1N1 A/PR/8/1934	NAI - CL	0.2 µM	oseltamivir: 0.0002 µM			(Kirchmair et al., 2011)
		SP DSM20566	NAI - lectin-based HA	7.7 µM	oseltamivir: 2.1 µM			(Walther et al., 2015)
		SP DSM20566 rNanA	NAI - FL	10.0 µM	oseltamivir: 2.9 µM			(Walther et al., 2016)
		SP DSM20566 rNanB	NAI - lectin-based HA	10.0 µM	oseltamivir: 31.6 µM			
		SP DSM20566r NanA	NAI - lectin-based HA	10.0 µM	oseltamivir: 3.2 µM			
Glycyrrhiza glabra L.	(E)-1-[2,4-dihydroxy-3-(3-methyl-2-butenyl)-	IV, H1N1 A/Jena/8178/2009	CPE	29.7% at 50 µM	oseltamivir: 0.03 µM	135.0 µM in MDCK cells	n.r.	(Grienke et al., 2014)

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
	phenyl]-3-(8-hydroxy-2,2-dimethyl-2H-1-benzopyran-6-yl)-2-propen-1-one							
		IV, H3N2 A/HK/1968	CPE	48.1% at 50 µM	oseltamivir: 0.004 µM	135.0 µM in MDCK cells		
	biochanin B	IV, H1N1 A/Jena/8178/2009	CPE	38.2% at 50 µM	oseltamivir: 0.03 µM	123.0 µM in MDCK cells	n.r.	
		IV, H3N2 A/HK/1968	CPE	42.6% at 50 µM	oseltamivir: 0.004 µM	123.0 µM in MDCK cells		
	glabrone	IV, H1N1 A/Jena/8178/2009	CPE	34.7% at 50 µM	oseltamivir: 0.03 µM	90.8 µM in MDCK cells	n.r.	
		IV, H3N2 A/HK/1968	CPE	24.2% at 50 µM	oseltamivir: 0.004 µM	90.8 µM in MDCK cells		
	licoflavone B	IV, H1N1 A/Jena/8178/2009	CPE	34.2% at 50 µM	oseltamivir: 0.03 µM	79.7 µM in MDCK cells	n.r.	
Glycyrrhiza inflata Batalin	isoliquiritigenin	IV, H1N1	NAI - FL	32.8 µM	oseltamivir: 0.13 µM		n.r.	(Dao et al., 2011)
		IV, H1N1 (H274Y)	NAI - FL	13.3 µM	oseltamivir: 16.4 µM			
		IV, H9N2	NAI - FL	37.9 µM	oseltamivir: 0.016 µM			
Lonicera japonica Thunb.	luteolin	CP NA	NAI - FL	53.2 µM	oseltamivir: 11.82 µM		n.r.	(Zhao et al., 2018)
Morus alba L.	kuwanon L	SP DSM20566	NAI - FL	31.6 µM	oseltamivir: 2.8 µM		SP	(Grienke et al., 2016)
	sanggenol A	IV, H1N1 A/Jena/8178/2009	NAI - FL	50.2 µM	oseltamivir: 0.004 µM		SP	
		SP DSM20566	NAI - FL	31.6 µM	oseltamivir: 2.08 µM			

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	sanggenol B	SP DSM20566	NAI - FL	31.6 µM	oseltamivir: 2.08 µM		SP	
	sanggenon C	IV, H1N1 A/Jena/8178/2009	CPE	8.3 µM	n.g.	51.7 µM in MDCK cells	SP	
		IV, H1N1 A/Jena/8178/2009	NAI - FL	50.6 µM	oseltamivir: 0.004 µM			
	sanggenon D	SP DSM20566	NAI - FL	31.6 µM	oseltamivir: 2.08 µM		SP	
	sanggenon G	IV, H1N1 A/Jena/8178/2009	CPE	8.8 µM	n.g.	> 100 µM in MDCK cells	SP	
		IV, H1N1 A/Jena/8178/2009	NAI - FL	30.9 µM	oseltamivir: 0.004 µM			
		SP DSM20566	NAI - FL	5.4 µM	oseltamivir: 2.08 µM			
Pithecellobium clypearia (Jack) Benth.	7-O-galloyltricetiflavan	IV, H1N1 A/PR/8/34	NAI - FL	36.9 µM	zanamivir: 0.00009 µM		n.r.	(Kang et al., 2014)
Polygonum chinense L.	quercetin	IV, B/Lee/1940	CPE	49.7 µM	oseltamivir: 0.21 µM	992.6 µM in MDCK cells	SA	(Alvarez et al., 2008; Tran et al., 2017)
		IV, H1N1 A/PR/8/1934	CPE	41.7 µM	oseltamivir: 0.07 µM	992.6 µM in MDCK cells		
		IV, H3N2 A/HK/2/1968	CPE	43.3 µM	oseltamivir: 0.005 µM	992.6 µM in MDCK cells		
Rhodiola rosea L.	herbacetin	IV, H1N1 A/PR/8/1934	CPE	35.0 µM	oseltamivir: 8.3 µM	293.7 µM in MDCK cells	n.r.	(Jeong et al., 2009)
		IV, H9N2 A/Chicken/Korea/MS96/1996	CPE	23.0 µM	oseltamivir: 6.3 µM	293.7 µM in MDCK cells		
		IV, H1N1 A/Bervig_Mission/1/1918	NAI - FL	8.9 µM	oseltamivir: 0.0016 µM			
	kaempferol	IV, H1N1 A/PR/8/1934	CPE	30.2 µM	oseltamivir: 8.3 µM	> 300 µM in MDCK cells	n.r.	

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		IV, H9N2 A/Chicken/Korea/MS96/1996	CPE	18.5 µM	oseltamivir: 6.3 µM	> 300 µM in MDCK cells		
		IV, H1N1 A/Bervig_Mission/1/1918	NAI - FL	11.2 µM	oseltamivir: 0.0016 µM			
	rhodiolinin	IV, H1N1 A/PR/8/1934	CPE	41.7 µM	oseltamivir: 8.3 µM	> 300 µM in MDCK cells	n.r.	
		IV, H9N2 A/Chicken/Korea/MS96/1996	CPE	29.3 µM	oseltamivir: 6.3 µM	> 300 µM in MDCK cells		
		IV, H1N1 A/Bervig_Mission/1/1918	NAI - FL	10.3 µM	oseltamivir: 0.0016 µM			
Salvia plebeia R. Br.	hispidulin	IV, H1N1 A/PR/8/1934	CPE	22.6 µM	oseltamivir: 0.55 µM	> 200 µM in MDCK cells	n.r.	(Bang et al., 2016)
		IV, H1N1 A/PR/8/1934	NAI - FL	19.8 µM	oseltamivir: 0.1 µM			
	luteolin	IV, H1N1 A/PR/8/1934	NAI - FL	18.0 µM	oseltamivir: 0.1 µM		n.r.	
	nepetin	IV, H1N1 A/PR/8/1934	CPE	17.5 µM	oseltamivir: 0.55 µM	> 200 µM in MDCK cells	n.r.	
		IV, H1N1 A/PR/8/1934	NAI - FL	11.2 µM	oseltamivir: 0.1 µM			
Flavonoid glycosides								
n.g.	cosmosiin	IV, H1N1 A/PR/8/1934	CPE	40.0 µM	oseltamivir: 8.3 µM	> 300 µM in MDCK cells	n.r.	(Jeong et al., 2009)
	nicotiflorin	IV, H1N1 A/PR/8/1934	CPE	40.1 µM	oseltamivir: 8.3 µM	> 300 µM in MDCK cells	n.r.	
Castanea crenata Siebold & Zucc.	kaempferol-3-O-[2″,6″-di-O-E-p-coumaroyl]-β-D-glucopyranoside	RV, 1B	CPE	1.2 µM	rupintrivir: < 0.04 µM	> 50 µM in HeLa cells	n.r.	(Kim et al., 2019)

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	kaempferol-3-O-[3″-acetyl-2″,6″-di-E-p-coumaroyl]-β-D-glucopyranoside	RV, 1B	CPE	5.5 µM	rupintrivir: < 0.04 µM	> 50 µM in HeLa cells	n.r.	
	kaempferol-3-O-[4″-acetyl-2″,6″-di-E-p-coumaroyl]-β-D-glucopyranoside	RV, 1B	CPE	7.5 µM	rupintrivir: < 0.04 µM	> 50 µM in HeLa cells	n.r.	
Cleistocalyx operculatus (Roxb.) Merr. and Perry	myricetin-3′,5′-dimethylether 3-O-β-D-galactopyranoside	IV, H1N1 A/PR/8/1934	NAI - FL	8.7 µM	oseltamivir: 0.1058 µM		n.r.	(Ha et al., 2016)
		IV, H1N1 A/PR/8/1934 (H274Y)	NAI - FL	9.3 µM	oseltamivir: 7.42 µM			
		IV, H9N2 A/Chicken/Korea/O1 310/2001	NAI - FL	6.5 µM	oseltamivir: 0.0129 µM			
Glycyrrhiza glabra L.	prunin	IV, H1N1 A/Jena/8178/2009	CPE	49.6% at 50 µM	oseltamivir: 0.03 µM	> 126.0 µM in MDCK cells	n.r.	(Grienke et al., 2014)
Lonicera japonica Thunb.	luteolin-7-O-ß-glucoside	IV, CP NA	NAI - FL	76.5 µM	oseltamivir: 11.82 µM		SA	(Xiong et al., 2013; Zhao et al., 2018)
Matteuccia struthiopteris (L.) Tod.	matteflavoside G	IV, H1N1 A/PR/8/1934	NAI - FL	6.9 µM	ribavirin: 19.7 µM		n.r.	(Li et al., 2015)
Moringa oleifera Lam.	quercetin 3-O-b-D-(6"-O-malonyl)-glucoside	IV, H1N1 A/PR/8/1934	NAI - FL	46.0 µM	oseltamivir: 0.007 µM		n.r.	(Kashiwada et al., 2012)
Rhodiola rosea L.	rhodionin	IV, rH1N1 A/Bervig_Mission/1/ 1918	NAI - FL	32.2 µM	oseltamivir: 0.0016 µM		n.r.	(Jeong et al., 2009)
	rhodiosin	IV, H9N2 A/Chicken/Korea/M S96/1996	CPE	35.1 µM	oseltamivir: 6.3 µM	297.3 µM in MDCK cells	n.r.	
		IV, rH1N1 A/Bervig_Mission/1/ 1918	NAI - FL	56.5 µM	oseltamivir: 0.0016 µM			
Syzygium aromaticum (L.) Merr. et Perry	isorhamnetin-3-O-b-D-glucopyranoside	IV, H1N1 A/PR/8/1934	NAI - FL	23.8 µM	zanamivir: 0.004 µM		n.r.	(He et al., 2017)

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Iridoids								
Gardenia jasminoides J.Ellis	geniposide	IV, H1N1 A/jiangsu/1/2009	CPE	87.7 µM	peramivir: n.g.	1,040.0 µM in MDCK cells	n.r.	(Zhang et al., 2017)
Lonicera japonica Thunb.	dimethylsecologloganoside	IV, H1N1 A/PR/8/1934	Plaque reduction	49.3% at 100 µg/mL	oseltamivir at 0.1 µg/mL		n.r.	(Kashiwada et al., 2013)
	secoxyloganin	IV, H1N1 A/PR/8/1934	Plaque reduction	53.1% at 100 µg/mL	oseltamivir at 0.1 µg/mL		SA	(Kashiwada et al., 2013; Xiong et al., 2013)
Lignans								
Forsythia viridissima Lindl.	conicaol A	RV, A1B	CPE	13.0 µM	rupuntrivir: n.g.	> 50 µM in HeLa cells	n.r.	(Huh et al., 2019)
	matairesinol	RV, A1B	CPE	42.2 µM	rupuntrivir: n.g.	> 50 µM in HeLa cells	n.r.	
	viridissimaol A	RV, A1B	CPE	45.7 µM	rupuntrivir: n.g.	> 50 µM in HeLa cells	n.r.	
	viridissimaol B	RV, A1B	CPE	47.5 µM	rupuntrivir: n.g.	> 50 µM in HeLa cells	n.r.	
Isatis indigotica Fortune ex Lindl.	clemastanin B	IV, H1N1 A/Guangzhou/GRID 07/2009	CPE	253.0 µM	ribavirin: 49.1 µM	21,808.7 µM in MDCK cells	n.r.	(Yang et al., 2013)
		IV, H7N3 A/Duck/Guangdong/ 1994	CPE	255.1 µM	ribavirin: 57.3 µM	21,808.7 µM in MDCK cells		
Others								
n.g.	1-(5-hydroxyl-2,2,-dimethyl-2H-1-benzopyran-6-yl)-2-phenyl-ethanone	IV, H1N1 A/Jena/5528/2009	NAI - CL	2.0 µM	oseltamivir: 0.0001 µM		n.r.	(Kirchmair et al., 2011)
		IV, H1N1 A/Jena/5555/2009	NAI - CL	2.2 µM	oseltamivir: 0.0001 µM			

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		IV, H1N1 A/PR/8/1934	NAI - CL	1.3 µM	oseltamivir: 0.0002 µM			
n.g.	9-deoxythysanone	RV	3C protease/solid-phase fluorescent	20.3 µM	n.g.		n.r.	(Young Jeong et al., 2014)
Thysanophora penicilloides (Roum.) W.B. Kendr.	thysanone		3C protease/solid-phase fluorescent	51.8 µM	n.g.		n.r.	
Aspergillus terreus Thom	pulvic acid	IV, H1N1 A/PR/8/1934	CPE	94.4 µM	zanamivir: 0.085 µM	> 811.0 µM in MDCK cells	n.r.	(Gao et al., 2013)
Cleistocalyx operculatus (Roxb.) Merr. and Perry	2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone	IV, H1N1 A/PR/8/1934	NAI - FL	8.2 µM	oseltamivir: 0.1058 µM		n.r.	(Ha et al., 2016)
		IV, H1N1 A/PR/8/1934 (H274Y)	NAI - FL	8.8 µM	oseltamivir: 7.42 µM			
		IV, H9N2 A/Chicken/Korea/O1 310/2001	NAI - FL	5.1 µM	oseltamivir: 0.0129 µM			
Glycyrrhiza glabra L.	3,4-dihydro-8,8-dimethyl-2H,8H-benzo[1,2-b:3,4-b′]dipyran-3-ol	IV, H1N1 A/Jena/8178/2009	CPE	36.1% at 50 µM	oseltamivir: 0.03 µM	336.0 µM in MDCK cells	n.r.	(Grienke et al., 2014)
	hispaglabridin B	IV, H1N1 A/Jena/8178/2009	CPE	48.4% at 50 µM	oseltamivir: 0.03 µM	39.2 µM in MDCK cells	n.r.	
		IV, H3N2 A/HK/1968	CPE	31.2% at 50 µM	oseltamivir: 0.004 µM	39.2 µM in MDCK cells		
Streptomyces sp. SMU03	(4S)-4-hydroxy-10-methyl-11-oxo-dodec-2-en-1,4-olide	IV, H1N1 A/FM1/1/1947	CPE	27.2 µM	umifenovir: 11.7 µM	170.1 µM in MDCK cells	n.r.	(Li et al., 2018a)
		IV, H1N1 A/PR/8/1934	CPE	1.4 µM	umifenovir: 0.94 µM	170.1 µM in MDCK cells		
		IV, H1N1 A/PR/8/1934(H274Y)	CPE	16.1 µM	umifenovir: n.g.	170.1 µM in MDCK cells		

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		IV, H3N2 A/Aichi/2/1968	CPE	33.9 µM	umifenovir: 20.9 µM	170.1 µM in MDCK cells		
Glycyrrhiza inflata Batalin	echinantin	IV, H1N1	NAI - FL	21.5 µM	oseltamivir: 0.13 µM		n.r.	(Dao et al., 2011)
		IV, H1N1 (H274Y)	NAI - FL	8.1 µM	oseltamivir: 16.4 µM			
		IV, H9N2	NAI - FL	21.1 µM	oseltamivir: 0.016 µM			
Tolypocladium inflatum W. Gams	cyclosporin A	IV, B/Brisbane/60/2008 (Victoria)	Plaque reduction	3.2 µM	oseltamivir: n.g.	15.2 µM in MDCK cells	n.r.	(Ma et al., 2016)
		IV, B/Phuket/3073/2013	Plaque reduction	1.0 µM	oseltamivir: n.g.	15.2 µM in MDCK cells		
		IV, H1N1 A/California/07/2009	Plaque reduction	11.7 µM	oseltamivir: n.g.	15.2 µM in MDCK cells		
		IV, H1N1 A/Texas/04/2009	Plaque reduction	2.3 µM	oseltamivir: n.g.	15.2 µM in MDCK cells		
		IV, H1N1 A/WSN/1933	Plaque reduction	2.1 µM	oseltamivir: n.g.	15.2 µM in MDCK cells		
		IV, H3N2 A/Switzerland/9715293/2013	Plaque reduction	0.4 µM	oseltamivir: n.g.	15.2 µM in MDCK cells		
		IV, H3N2 A/Udorn/1972	Plaque reduction	2.6 µM	oseltamivir: n.g.	15.2 µM in MDCK cells		
n.g.	camphecene	IV, B/Lee/1940	Hemolysis	~63 µM	rimantadine 80% at 100 µM		n.r.	(Zarubaev et al., 2015)
		IV, B/Lee/1940	Yield reduction	52.7 µM	rimantadine: 3 µM	5.7 µM in MDCK cells		
		IV, H1N1 A/California/07/09	Yield reduction	3.6 µM	rimantadine: 55.6 µM	701.4 µM in MDCK cells		
		IV, H1N1 A/PR/8/1934	Yield reduction	8.3 µM	rimantadine: 6 µM	1.1 µM in MDCK cells		

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		IV, H3N2 A/Aichi/2/1968	Yield reduction	83.8 µM	rimantadine: 41 µM	9.5 µM in MDCK cells		
		IV, H5N2 A/mallardPennsylvania/10218/84	Yield reduction	79.8 µM	rimantadine: 59 µM	4.9 µM in MDCK cells		
		IV, subtype A	Hemolysis	~63.0 µM	rimantadine 70% at 100 µM			
Penicillium simplicissimum (Oudem.) Thom MA-332	simpterpenoid A	IV	NAI - FL	0.0081 µM	oseltamivir: 0.0032 µM		n.r.	(Li et al., 2018b)
Neorhodomela aculeata (L.P. Perestenko) Masuda.	2,2′,3-tribromo-3′,4,4′,5-tetrahydroxy-6′-methoxymethyldiphenylmethane	RV, A2	CPE	13.9 µM	ribavirin: 8.8 µM	> 39.1 µM in HeLa cells	n.r.	(Park et al., 2012)
		RV, B3	CPE	9.2 µM	ribavirin: 20.8 µM	> 39.1 µM in HeLa cells		
	lanosol	RV, A2	CPE	8.4 µM	ribavirin: 8.8 µM	> 67.1 µM in HeLa cells	n.r.	
Bupleurum fructicosum L.	(E)-3-(3,4-dimethoxy-phenyl)-2-propen-1-yl (Z)-2-[(Z)-2-methyl-2-butenoyloxymethyl) butenoate	RV, A39	CPE	2.4 µM	pleconaril: 0.1 µM	> 20.3 µM in HeLa cells	n.r.	(Fois et al., 2017)
	4-O-methylcinnamyl angelic acid ester	RV, A39	CPE	30.9 µM	pleconaril: 0.1 µM	> 248.0 µM in HeLa cells	n.r.	
	cis-9,17-octadecadiene-12,14-diyne-1,16-diol	RV, A39	CPE	1.8 µM	pleconaril: 0.1 µM	> 14.6 µM in HeLa cells	n.r.	
Phellinus ignarius (L.) Quél	3-hydroxy-2-methyl-4-pyrone	IV, H5N1	CPE	3.2 µM	zanamivir: 15 µM	> 435.1 µM in MDCK cells	n.r.	(Song et al., 2014)
Chaetomium coarctatum Kuntze ex Fries	aureonitol	IV, B/MEMPHIS/20/1996	Hemagglutination	0.4 µM	n.g.		n.r.	(Sacramento et al., 2015)

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
		IV, B/MEMPHIS/20/1996	Yield reduction	2.0 µM	oseltamivir: 0.052 µM	1,429.0 µM in MDCK cells		
		IV, H1N1 A/RJ/512/2009	Hemagglutination	0.1 µM	n.g.			
		IV, H1N1 /RJ/512/2009	Yield reduction	0.4 µM	oseltamivir: 0.012 µM	1,428.0 µM in MDCK cells		
		IV, H3N2 A/ENG/42/1972	Hemagglutination	0.1 µM	n.g.			
		IV, H3N2 A/ENG/42/1972	Yield reduction	0.1 µM	oseltamivir: 0.03 µM	1,426.0 µM in MDCK cells		
		IV, H3N2 A/WA/01/2007	Hemagglutination	0.1 µM	n.g.			
		IV, H3N2 A/WA/01/2007	Yield reduction	0.3 µM	oseltamivir: 0.03 µM	1,427.0 µM in MDCK cells		
Phenanthrenes								
Bletilla striata (Thunb.) Rchb.f.	2,2′,7′-trihydroxy-3′,4,5′,7-tetramethoxy-9′,10′-dihydro-1,1′-di-phenanthrene	IV, H1N1 A/jiangsu/1/2012	NAI - FL	16.8 µM	oseltamivir: 0.3 µM		n.r.	(Shi et al., 2017)
	2,2′–dyhydroxyl-4,4′,7,7′-9′,10′-dihydro-1,6′-di-phenanthrene	IV, H1N1 A/jiangsu/1/2016	NAI - FL	57.6 µM	oseltamivir: 0.3 µM		n.r.	
	2,7-dyhydroxyl-4-methoxy-9,10-dihydro-phenanthrene	IV, H1N1 A/jiangsu/1/2011	NAI - FL	72.6 µM	oseltamivir: 0.3 µM		n.r.	
	2-hydroxyl-4,7-dimethoxyphenanthrene	IV, H1N1 A/jiangsu/1/2015	NAI - FL	87.5 µM	oseltamivir: 0.3 µM		n.r.	
	4,4′,7,7′-tetrahydroxy-2,2′,8,8′-	IV, H1N1 A/jiangsu/1/2010	CPE	14.6 µM	oseltamivir: 4.9 µM	80.0 µM in MDCK cells	n.r.	

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
	tetramethoxy-1,1′-di-phenanthrene							
		IV, H1N1 A/jiangsu/1/2013	NAI - FL	21.7 µM	oseltamivir: 0.3 µM			
	4,4′,7,7′-tetrahydroxy-2,2′-dimethoxy-1,1′-di-phenanthrene	IV, H1N1 A/jiangsu/1/2014	NAI - FL	16.1 µM	oseltamivir: 0.3 µM		n.r.	
Phenolic compounds								
Phellinus ignarius (L.) Quél	1-(3,4-dihydroxyphenyl) ethanone	IV, H5N1	CPE	9.8 µM	zanamivir: 15 µM	258.3 µM in MDCK cells	n.r.	(Song et al., 2014)
	1,2-benzenediol	IV, H5N1	CPE	30.7 µM	zanamivir: 15 µM	602.2 µM in MDCK cells	n.r.	
	4-methyl-1,2-benzenediol	IV, H5N1	CPE	12.4 µM	zanamivir: 15 µM	363.0 µM in MDCK cells	n.r.	
	eudesm-1b,6a, 11-triol	IV, H5N1	CPE	0.1 µM	zanamivir: 15 µM	85.4 µM	n.r.	
		IV, H5N1	NAI - FL	0.7 µM	zanamivir: 0.0035 µM			
Salvia plebeia R. Br.	rosmarinic acid methyl ester	IV, H1N1 A/PR/8/1934	CPE	22.6 µM	oseltamivir: 0.55 µM	> 200 µM in MDCK cells	SP	(Aziz et al., 2014; Bang et al., 2016)
		IV, H1N1 A/PR/8/1934	NAI - FL	16.7 µM	oseltamivir: 0.1 µM			
Pogostemon cablin Benth.	patchouli alcohol	IV, H1N1 A/PR/8/1934	Plaque forming	75% at 2 µg/mL	zanamivir at 1 µg/mL		n.r.	(Kiyohara et al., 2012)
Lagerstroemia speciosa (L.) Pers.	ellagic acid	RV, A2	CPE	125.7 µM	ribavirin: 286.6 µM	> 330.9 µM in HeLa cells	KP	(Dey et al., 2015; Park et al., 2014)
		RV, B3	CPE	102.6 µM	ribavirin: 290.7 µM	> 330.9 µM in HeLa cells		
		RV, B4	CPE	96.0 µM	ribavirin: 258.0 µM	> 330.9 µM in HeLa cells		

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
Pithecellobium clypearia Benth	7-O-galloylplumbocatechin A	IV, B/Jiangsu/10/2003	NAI - FL	78.7 µM	zanamivir: 0.0009 µM		n.r.	(Kang et al., 2014)
		IV, H1N1 A/PR/819/34	NAI - FL	59.8 µM	zanamivir: 0.0001 µM			
		IV, H3N2 A/Sydney/5/97	NAI - FL	64.6 µM	zanamivir: 0.0006 µM			
Polygonum chinense L.	gallic acid	IV, H1N1 A/PR/8/1934	CPE	122.3 µM	oseltamivir: 0.07 µM	653.1 µM in MDCK cells	n.r.	(Tran et al., 2017)
		IV, H3N2 A/HK/2/1968	CPE	102.9 µM	oseltamivir: < 0.005 µM	653.1 µM in MDCK cells		
	methyl gallate	IV, B/Lee/1940	CPE	79.8 µM	oseltamivir: 0.21 µM	> 1,629.1 µM in MDCK cells	KP	(Noundou et al., 2016; Tran et al., 2017)
		IV, H1N1 A/PR/8/1934	CPE	98.3 µM	oseltamivir: 0.07 µM	> 1,629.1 µM in MDCK cells		
		IV, H3N2 A/HK/2/68	CPE	92.9 µM	oseltamivir: < 0.005 µM	> 1,629.1 µM in MDCK cells		
Punica granatum L.	punicalagin	IV, H3N2 A/HK/2/1968	Hemagglutination	9.2 µM	n.g.		n.r.	(Haidari et al., 2009)
Syzygium aromaticum (L.) Merr. et Perry	1,2,3-tri-O-galloylglucose	IV, H1N1 A/PR/8/1934	CPE	5.3 µM	ribavirin: 46.7 µM	651.4 µM in MDCK cells	n.r.	(He et al., 2017)
	1,3-di-O-galloyl-4,6-HHDP-glucose	IV, H1N1 A/PR/8/1934	NAI - FL	11.2 µM	zanamivir: 0.004 µM		n.r.	
	casuarictin	IV, H1N1 A/PR/8/1934	CPE	14.2 µM	ribavirin: 46.7 µM	> 534.2 µM in MDCK cells	n.r.	
		IV, H1N1 A/PR/8/1934	NAI - FL	19.1 µM	zanamivir: 0.004 µM			
	eugeniin	IV, H1N1 A/PR/8/1934	CPE	4.6 µM	ribavirin: 46.7 µM	374.3 µM in MDCK cells	n.r.	
		IV, H1N1 A/PR/8/1934	NAI - FL	8.4 µM	zanamivir: 0.004 µM			

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
	tellimagrandin I	IV, H1N1 A/PR/8/1934	CPE	3.9 µM	ribavirin: 46.7 µM	101.0 µM in MDCK cells	SA	(He et al., 2017; Shiota et al., 2004)
		IV, H1N1 A/PR/8/1934	NAI - FL	23.5 µM	zanamivir: 0.004 µM			
Triterpenoids								
Ilex asprella (Hook. et Arn.) Champ. ex Benth.	asprellcoside A	IV, H1N1 A/PR/8/1934	CPE	4.1 µM	oseltamivir: 0.9 µM	> 100 µM in A549 cells	n.r.	(Peng et al., 2016)
Castanea crenata Siebold & Zucc.	castaartancrenoic acid D	RV, A1B	CPE	6.3 µM	rupintrivir: < 0.04 mM	> 50 µM in HeLa cells	n.r.	(Kim et al., 2019)
	castaartancrenoic acid E	RV, A1B	CPE	5.6 µM	rupintrivir: < 0.04 mM	> 50 µM in HeLa cells	n.r.	
Ganoderma lingzhi S.H. Wu, Y. Cao & Y.C. Dai	ganoderic acid T-Q	IV, H1N1 A/California/04/2009	NAI - FL	81.7% at 200 µM	n.g.		n.r.	(Zhu et al., 2015)
		IV, H1N1 A/California/04/2009(N295S)	NAI - FL	62.7% at 200 µM	n.g.			
		IV, H3N2 A/Babol/36/2005(E119V)	NAI - FL	55.4% at 200 µM	n.g.			
		IV, H5N1 A/Hubei/1/2011	NAI - FL	94.4% at 200 µM	n.g.			
	ganoderic acid TR	IV, H1N1 A/California/04/2009	NAI - FL	87.4% at 200 µM	n.g.		n.r.	
		IV, H1N1 A/California/04/2009(N295S)	NAI - FL	57.7% at 200 µM	n.g.			
		IV, H3N2 A/Babol/36/2005(E119V)	NAI - FL	59.2% at 200 µM	n.g.			
		IV, H5N1 A/Hubei/1/2011	NAI - FL	96.5% at 200 µM	n.g.			

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
Gloeophyllum odoratum (Wulfen) Imazeki	21-hydroxylanosterol	IV, H1N1 A/Jena/8178/2010	CPE	34.5 µM	oseltamivir: 0.076 µM	> 100 µM in MDCK cells	n.r.	(Grienke et al., 2019)
		IV, H3N2 A/HK/1969	CPE	9.0 µM	oseltamivir: 0.004 µM	> 100 µM in MDCK cells		
	eburicodiol	IV, H1N1 A/Jena/8178/2009	CPE	31.2 µM	oseltamivir: 0.076 µM	> 100 µM in MDCK cells	n.r.	
		IV, H3N2 A/HK/1968	CPE	15.4 µM	oseltamivir: 0.004 µM	> 100 µM in MDCK cells		
	gloeophyllin K	IV, H1N1 A/Jena/8178/2009	CPE	46.4 µM	oseltamivir: 0.076 µM	> 100 µM in MDCK cells	n.r.	
	trametenolic acid B	IV, H1N1 A/Jena/8178/2009	CPE	11.3 µM	oseltamivir: 0.076 µM	> 100 µM in MDCK cells	n.r.	
		IV, H3N2 A/HK/1968	CPE	14.1 µM	oseltamivir: 0.004 µM	> 100 µM in MDCK cells		
n.g.	O-[2-O-(1-methyl-N-acetylneuraminyl)]ethyl 3β-hydroxy-lup-20(29)-en-28-oate	IV, H1N1 A/WSN/1933	CPE	41.2 µM	oseltamivir: 46.5 µM	> 500 µM in MDCK cells	n.r.	(Han et al., 2016)
Xanthones								
Garcinia × *mangostana* L.	garcinone C	IV, H5N1	NAI - FL	95.5 µM	oseltamivir: 0.0048 µM		n.r.	(Ikram et al., 2015)
	rubraxanthone	IV, H5N1	NAI - FL	89.7 µM	oseltamivir: 0.0048 µM		n.r.	
	α-mangostin	IV, H5N1	NAI - FL	92.0 µM	oseltamivir: 0.0048 µM		SA, PA	(Ikram et al., 2015; Narasimhan et al., 2017)
Polygala karensium Kurz	1,3, 7-trihydroxyxanthone	IV, H1N1 A/PR/8/1934	NAI - FL	109.7 µM	oseltamivir: 0.13 µM		n.r.	(Dao et al., 2012)
		IV, H1N1 A/PR/8/34(H274Y)	NAI - FL	37.3 µM	oseltamivir: 16.3 µM			

Natural source	Compound name	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
		IV, H9N2	NAI - FL	101.6 µM	oseltamivir: 0.016 µM			
	1,7-dihydroxy-4-methoxyxanthone	IV, H1N1 A/PR/8/1934	NAI - FL	110.0 µM	oseltamivir: 0.13 µM		SA	(Dao et al., 2012; Joseph et al., 2006)
		IV, H1N1 A/PR/8/1934(H274Y)	NAI - FL	49.6 µM	oseltamivir: 16.3 µM			
		IV, H9N2	NAI - FL	99.1 µM	oseltamivir: 0.016 µM			

Abbreviations: CP = *Clostridium perfringens,* CPE = cytopathic effect, CL = chemiluminescence, FL = fluorescence, HA = hemagglutination, HI = *Haemophilus influenzae*, IV = influenza virus, KP = *Klebsiella pneumoniae*, MDCK = Madin-Darby canine kidney cells, MIC = minimum inhibitory concentration, MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTS = 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, NA = neuraminidase, NAI = neuraminidase inhibitor, n.g. = not given, n.r. = not reported, PA = *Pseudomonas aeroguinosa*, RV = rhinovirus, SA = *Staphylococcus aureus, SP = Streptococcus pneumoniae,* SPy = *Streptococcus pyogenes,* VC = *Vibrio cholerae*.

Table 3. Examples of extracts with reported activities related to ARIs: Anti-influenza virus, anti-rhinovirus, dual antiviral and antibacterial actives.

Natural source	Organ	Type of extract	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
Alpinia zerumbet (Pers.) B.L.Burtt & R.M.Sm.	leaf	W	CP NA	NAI - FL	43.0 µg/mL	quercetin: 34.7 µg/mL		n.r.	(Upadhyay et al., 2011)
	root		CP NA	NAI - FL	57.0 µg/mL	quercetin: 34.7 µg/mL		n.r.	
Camellia sinensis (L.) Kuntze	leaf	W	IV, H1N1 A/Kitakyushu/10/2006	NAI - FL	195 µg/mL	oseltamivir: 1.42 µM		n.r.	(Sriwilaijaroen et al., 2012)
			IV, H1N1 A/Narita/1/2009	NAI - FL	22.1 µg/mL	oseltamivir: 0.0026 µM			
			IV, H1N1 A/Yamaguchi/20/2006	NAI - FL	152 µg/mL	oseltamivir: 0.0029 µM			
Clinacanthus siamensis Bremek.	leaf	E	IV, B/Ibaraki/2/1985	NAI - FL	21.3% at 100 µg/mL	oseltamivir 99.7% at 10 µg/mL		n.r.	(Wirotesangthong et al., 2009)
			IV, H1N1 A/PR/8/1934	NAI - FL	26.6% at 100 µg/mL	oseltamivir 97.9% at 10 µg/mL			
			IV, H3N2 A/Guizhou/54/1989	NAI - FL	31.2% at 100 µg/mL	oseltamivir 99.7% at 10 µg/mL			
Curcuma longa L.	rhizome	E	IV, B/Ibaraki/2/1985	NAI - FL	43.4% at 100 µg/mL	oseltamivir 99.7% at 10 µg/mL		n.r.	(Wirotesangthong et al., 2009)
			IV, H1N1 A/PR/8/1934	NAI - FL	63.2% at 100 µg/mL	oseltamivir 97.9% at 10 µg/mL			
			IV, H3N2 A/Guizhou/54/1989	NAI - FL	51.8% at 100 µg/mL	oseltamivir 99.7% at 10 µg/mL			
Echinacea purpurea (L.) Moench	arial part + root	65% E	IV, H3N2 A/Vicotria + HI NTHi	HI adherence (CFU/100 cells)	Extract dilution 1:200: 0.55-fold decresion	control: 3.08-fold increase		HI	(Vimalanathan et al., 2017)
			IV, H3N2 A/Vicotria + SA ATCC 25923	SA adherence (CFU/100 cells)	Extract dilution 1:200: 0.86-fold decresion	control: 1.70-fold increase		SA	
Ficus religiosa L.	bark	M	RV, 1A	CPE	5.5 µg/mL	n.g.	66.5 µg/mL in HeLa cells	n.r.	(Cagno et al., 2015)

Natural source	Organ	Type of extract	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
Gloeophyllum odoratum (Wulfen) Imazeki (strain 23)	fruit body	E	IV, H3N2 A/HK/1969	CPE	13.0 µg/mL	oseltamivir: n.g.	> 100 µg/mL in MDCK cells	n.r.	(Grienke et al., 2018)
Gloeophyllum odoratum (Wulfen) Imazeki (strain 28)	fruit body	E	IV, H3N2 A/HK/1969	CPE	9.4 µg/mL	oseltamivir: n.g.	> 100 µg/mL in MDCK cells	n.r.	
Gloeophyllum odoratum (Wulfen) Imazeki (strain 54)	fruit body	E	IV, H3N2 A/HK/1969	CPE	15.0 µg/mL	oseltamivir: n.g.	> 100 µg/mL in MDCK celsl	n.r.	
			RV, A2	CPE	16.0 µg/mL	oseltamivir: n.g.	> 100 µg/mL in HeLa cells		
Garcinia × *mangostana* L.	hull	M	IV, H5N1	NAI - FL	83.0% at 250 µg/mL	n.g.		n.r.	(Ikram et al., 2015)
Glycyrrhiza glabra L.	root	Aglycone-enriched fraction	IV, H3N2 A/HK/1968	NAI-CL	0.3 µg/mL	oseltamivir: 0.0003 µM		n.r.	(Grienke et al., 2014)
	root	M	IV, H3N2 A/HK/1968	NAI-CL	1.7 µg/mL	oseltamivir: 0.0003 µM		n.r.	
Morus alba L.	root bark	M	IV, H1N1 A/Jena/8178/2009	CPE	9.3 µg/mL	n.g.	75.20 µg/mL in MDCK cells	SP	(Grienke et al., 2016)
Neorhodomela aculeata (L.P. Perestenko) Masuda.	red alga	M	RV, B2	CPE	17.6 µg/mL	ribavirin: 17.4 µg/ml	> 20 µg/mL in HeLa cells	n.r.	(Park et al., 2012)
			RV, A3	CPE	18.3 µg/mL	ribavirin: 14.3 µg/ml	> 20 µg/mL in HeLa cells		
Nephelium lappaceum L.	pericarp	E	IV, B/Ibaraki/2/1985	NAI - FL	39.3% at 100 µg/mL	oseltamivir 99.7% at 10 µg/mL		PA	(Sulistiyaningsih et al., 2018; Wirotesangthong et al., 2009)
Pelargonium sidoides DC.	root	11% E	IV, H1N1 A/New Caledonia/20/1999	CPE	9.5 µg/mL	n.g.	> 100 µg/mL in MDCK cells	n.r.	(Michaelis et al., 2011)
			IV, H3N2 A/California/7/2004	CPE	8.7 µg/mL	n.g.	> 100 µg/mL in MDCK cells		
Polygonum chinense L.	leaf	EAc	IV, B/Lee/1940	CPE	50.8 µg/mL	oseltamivir: 1.2 µM	> 300 µg/mL in MDCK cells	n.r.	(Tran et al., 2017)

Natural source	Organ	Type of extract	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
		M	IV, B/Lee/1940	CPE	55.5 µg/mL	oseltamivir: 1.2 µM	> 300 µg/mL in MDCK cells	n.r.	
		B	IV, H1N1 A/PuertoRico/8/1934	CPE	45.9 µg/mL	oseltamivir: 0.38 µM	> 300 µg/mL in MDCK cells	n.r.	
		EA	IV, H1N1 A/PuertoRico/8/1934	CPE	46.9 µg/mL	oseltamivir: 0.38 µM	> 300 µg/mL in MDCK cells	n.r.	
		M	IV, H1N1 A/PuertoRico/8/1934	CPE	55.0 µg/mL	oseltamivir: 0.38 µM	> 300 µg/mL in MDCK cells	n.r.	
		B	IV, H3N2 A/Hong Kong/2/1968	CPE	18.3 µg/mL	oseltamivir: 20.5 µM	> 300 µg/mL in MDCK cells	n.r.	
		EA	IV, H3N2 A/Hong Kong/2/1968	CPE	23.2 µg/mL	oseltamivir: 20.5 µM	> 300 µg/mL in MDCK cells	n.r.	
		M	IV, H3N2 A/Hong Kong/2/1968	CPE	38.4 µg/mL	oseltamivir: 20.5 µM	> 300 µg/mL in MDCK cells	n.r.	
Poncirus trifoliata L.	seed	E	IV, H1N1 A/PuertoRico/8/1934	CPE	2.5 µg/mL	oseltamivir: 3.7 µM	1,250 µg/mL in MDCK cells	n.r.	(Heo et al., 2018)
			IV, H1N1 A/PuertoRico/8/1934 NA mutant	CPE	3.9 µg/mL	oseltamivir: 31.3 µM	1,250 µg/mL in MDCK cells		
Psidium guajava L.	leaf	W	IV, H1N1 A/Kitakyushu/10/2006	NAI - FL	75 µg/mL	oseltamivir: 1.42 µM		KP, PA, SA, SP	(Morais-Braga et al., 2016; Sriwilaijaroen et al., 2012)
			IV, H1N1 A/Narita/1/2009	NAI - FL	4.4 µg/ml	oseltamivir: 0.0026 µM			
			IV, H1N1 A/Yamaguchi/20/2006	NAI - FL	68.3 µg/mL	oseltamivir: 0.0029 µM			
Punica granatum L.	fruit	W	IV, H3N2 A/Hong Kong/2/1968	Hemagglutination	1.25 µg/mL	n.g.		KP	(Dey et al., 2015; Haidari et al., 2009)
Rhodiola rosea L.	rhizome	W	IV, H1N1 A/PR/8/1934	CPE	78.5 µg/mL	oseltamivir: 8.3 µM	> 500 µg/mL in MDCK cells	n.r.	(Jeong et al., 2009)
Sclerocarya birrea (A.Rich.) Hochst.	bark	D	IV, H3N2 A/HK/1969	CPE	7.9 µg/mL	n.g.	> 100 µg/mL in MDCK cells	n.r.	(Grienke et al., 2018)

Natural source	Organ	Type of extract	Subtype, strain/isolate or target	Inhibitory assay	Activity in comparison to positive control or 50% inhibition concentration (IC_{50})	Positive control: used at a certain concentration or 50% inhibition concentration (IC_{50})	50% Cytotoxic concentration (CC_{50})	Antibacterial activity	Reference(s)
		E	IV, H3N2 A/HK/1969	CPE	26.0 µg/mL	n.g.	> 100 µg/mL in MDCK cells	n.r.	
			IV, H3N2 A/HK/1969	CPE	29.0 µg/mL	n.g.	> 100 µg/mL in MDCK cells		
Syzygium aromaticum (L.) Merr. et Perry	flower bud	M	IV, H1N1 A/PR/8/1934	NAI - FL	9.1 µg/mL	zanamivir: 0.004 µg/mL		SA	(He et al., 2017; Perumal et al., 2017)
Thunbergia laurifolia Lindl.	leaf	E	IV, H3N2 A/Guizhou/54/1989	NAI - FL	38.3% at 100 µg/mL	oseltamivir 99.7% at 10 µg/mL		n.r.	(Wirotesangthong et al., 2009)
Sinupret®	-	51% E	IV, H1N1 A/California/07/2009	CPE	43.4 µg/mL	amantadine: 6 µg/ml	> 500 µg/mL in MDCK cells	n.r.	(Glatthaar-Saalmuller et al., 2011)
			IV, H1N1 A/Chile 1/1983	CPE	124.8 µg/mL	amantadine: 5 µg/ml	> 500 µg/mL in MDCK cells		
			RV, A14	CPE	50.5 µg/mL	n.g.	> 500 µg/mL in HeLa cells		

Abbreviations: CP = *Clostridium perfringens,* CPE = cytopathic effect, CL = chemiluminescence, FL = fluorescence, HI = *Haemophilus influenzae*, IV = influenza virus, KP = *Klebsiella pneumoniae*, MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, NA = neuraminidase, NAI = neuraminidase inhibitor, n.g. = not given, n.r. = not reported, PA = *Pseudomonas aeroguinosa*, RV = rhinovirus, SA = *Staphylococcus aureus, SP = Streptococcus pneumoniae,* SPy = *Streptococcus pyogenes*.

Extraction solvents: B = butanol, D = dichloromethane, E = ethanol, EA = ethyl acetate, M = methanol, W = water.

3.3. Strategies to identify natural products against ARIs

During the evaluation of literature data on natural products targeting ARIs we found that ethnopharmacological knowledge is the main criterion for selecting natural starting materials for further investigations. Extract screening followed by bioassay-guided fractionation yielded the majority of bioactive compounds summarized in Table 2. As presented in Table 3, numerous studies identified extracts with pronounced anti-influenza virus and anti-rhinovirus activities, still lying idle to be further investigated for their antiviral constituents and for unraveling their molecular mechanism. In the following chapters, different strategies to identify these compounds are discussed giving selected outstanding examples.

3.3.1. Extract screening

An important tool in modern drug discovery is high or medium throughput screening (HTS, MTS). In the field of natural products however, the number of bioactive compounds discovered using this approach is lower than 1% (Henrich and Beutler, 2013; Thornburg et al., 2018). This may be related to the scarcity and preciousness of natural product isolates. In the case of an extract screening, this approach implies that further labor- and equipment-intense phytochemical work is necessary to finally isolate and identify the bioactive constituents.

In a recently performed phenotypic CPE-based MTS, some 160 extracts have been screened for the identification of anti-influenza virus, anti-rhinovirus and anti-coxsackie natural material (Grienke et al., 2018). Among these extracts different strains of the polypore fungus *Gloeophyllum odoratum* were found to show significant inhibition of influenza A viruses (H3N2). Further mycochemical investigation led to the isolation of trametenolic acid B showing IC_{50} values of 11.3 μM and 14.1 μM on two different H3N2 influenza A virus strains in a CPE assay (Grienke et al., 2019).

Another type of MTS deals with the concept of bioaffinity chromatography (Zhao et al., 2018). Zhao et al. used magnetic beads coated with immobilized influenza virus neuraminidase for compound fishing in natural extracts or pure compound libraries. The authors first tested the

system with an artificial model mixture containing known neuraminidase inhibitors such as oseltamivir as well as known inactive natural products such as the tetracycloquinolizidine alkaloid matrine. As a proof-of-concept, this ligand fishing strategy was applied to the complex extract of the flowers of a *Lonicera* species. With this approach, combined with further chromatographic and MS/MS techniques, flavonoid and phenolic acid derivatives, i.e. luteolin, luteolin-7-O-β-D-glucoside, 3,5-di-O-caffeoylquinic acid, and 3,4-di-O-caffeoylquinic acid, were identified as neuraminidase inhibitors (Zhao et al., 2018). Moreover, a good reusability of the neuraminidase-magnetic beads was demonstrated. Although in this example only moderately active compounds were discovered (IC_{50}s between 53 and 77 μM), this approach seems to have a great potential to identify minor active components which are often overlooked in a conventional bio-guided fractionation set-up.

3.3.2. Bioassay-guided fractionation

The classic and also most common strategy for identifying bioactive natural products is bioassay-guided fractionation. Initially starting from a bioactive crude extract, this may lead to bioactive fractions and, via iterative testing, to the pure compound(s) responsible for the observed activity. Many research groups have successfully applied this concept to isolate antiviral constituents from a natural starting material. In the case of a dichloromethane extract of *Bupleurum fruticosum* leaves this resulted in the isolation of two potent anti-rhinovirus agents, i.e. a polyacetylene and a phenylpropenol derivative. These compounds were active against human rhinovirus A39 with IC_{50} values measured in a CPE reduction assay of 1.8 μM and 2.4 μM (SI = 8.1 and 8.5), respectively (Fois et al., 2017). As another example of bioassay-guided fractionation using an *in vitro* fluorescence-based neuraminidase inhibition assay, He et al. discovered anti-influenza polyphenols from Flos Caryophylli with IC_{50}s between 8.4 μM and 94.1 μM (He et al., 2017).

However, bioassay-guided fractionation faces many pitfalls. For instance, due to assay-interfering components present in the fractions, isolation efforts might be guided towards inactive (or false positive) constituents and minor active constituents are easily overlooked.

3.3.3. Computational approaches

Computational methods are established as an important pillar of natural products-based drug discovery, in particular also in the context of antiviral research. One of the most well-known examples of the application of *in silico* methods is the successful design of zanamivir based on experimental structures of the viral enzyme co-crystallized with sialic acid and analogues thereof (von Itzstein et al., 1993).

As of 2017, the molecular structures of more than 250,000 unique natural products have been deposited in virtual libraries (Chen et al., 2017), most of which are freely accessible. These resources can be used, among many other applications, for virtual screening for promising natural products. Also, the amount of published structural data on viral proteins has been steeply increasing throughout the last decade. As of 2018, high-quality structures of more than 2,000 complexes of natural products bound to biomacromolecules have been deposited in the Protein Data Bank (PDB) (Chen et al., 2018).

NP-Scout is a machine learning model for the identification of natural products (Chen et al., 2019). According to predictions with NP-Scout, close to 25% of all (unique) small molecules reported in high-quality co-crystals with biomacromolecules in the PDB (PDB subset taken from (Chen et al., 2018)) have a likelihood of being a natural product of greater than 0.8 (Fig. 4B). In other words, approximately one-quarter of all co-crystallized small molecules are either genuine natural products or natural product-like. This corroborates the relevance of structural data to natural product-based drug discovery. For comparison, in Fig. 4A the same type of distribution is reported for a dataset of more than 230,000 natural products (Chen et al., 2018). Of this dataset, NP-Scout correctly identifies more than 95% of all compounds as natural product-like.

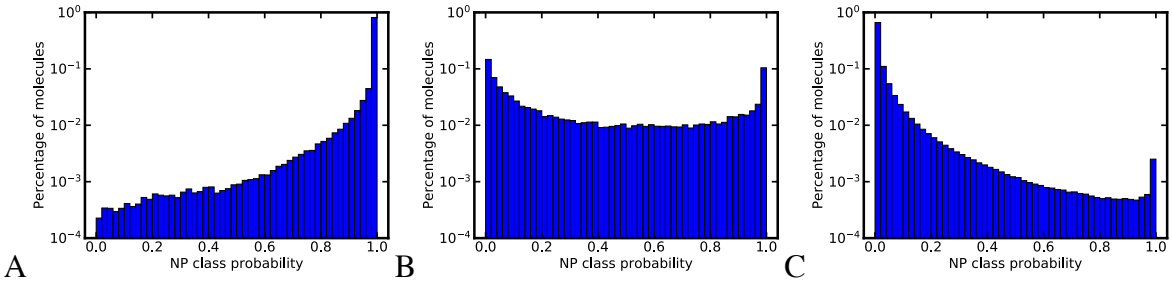

Fig. 4. Predicted natural product class probability distributions for (A) a set of more than 230,000 natural products, (B) a comprehensive set of small-molecule ligands observed in high-quality co-crystals in the PDB and (C) the "in-stock" subset of ZINC. A compound is predicted as natural product if the class probability is greater than 0.5; below this value it is considered to be of synthetic origin. Note that the y-axis is in logarithmic scale.

Virtual screening approaches can also be employed for prioritizing plant material for extraction, chromatographic work-up, and pharmacological studies. For example, Ikram et al. recently employed a docking approach to identify plant materials enriched with natural products likely to be active against influenza neuraminidase. Some of the compounds isolated from the selected materials, such as the xanthone α-mangostin, showed (moderate) activity against the viral enzyme (Ikram et al., 2015).

In general, the bottleneck of virtual screening is not technology but the limited availability of material for testing. Only an estimated 10% of the above-mentioned 250,000 natural products registered in virtual databases are readily obtainable from public and commercial sources (Chen et al., 2017). However, this number increases substantially when looking at natural-product-like compounds rather than genuine natural products only.

The "in-stock" subset of the ZINC database (Sterling and Irwin, 2015) lists more than nine million compounds that are readily purchasable. Among those, NP-Scout assigns a natural product class probability of 0.8 to approximately 70,000 compounds (less than 1%), meaning that these compounds are either genuine natural products or have a substantial amount of structural features characteristic to natural products (Fig. 4C).

Advanced homology modelling techniques and molecular dynamics simulations expand the applicability of structure-based methods well beyond measured structures of biomacromolecules. For example, molecular dynamics simulations were instrumental in the representation of the active site flexibility of influenza virus neuraminidase and the derivation of the possible binding mode of katsumadain A, a diarylheptanoid inhibitor from *Alpinia katsumadai* with measured IC_{50} values between 0.59 μM and 1.64 μM against the viral enzyme of several porcine H1N1 isolates (Grienke et al., 2010). Molecular dynamics simulations have also been employed to explain the dependency of the catalytic activity of influenza neuraminidase on its assembly state (von Grafenstein et al., 2015).

Although data on measured biological activities of natural products remain sparse in comparison to that of synthetic compounds, also methods for *in silico* target prediction are becoming increasingly relevant to natural products-based drug discovery (Fang et al., 2017; Rollinger et al., 2009).

Concerning rhinoviruses, a pharmacophore-based VS approach using the target rhinovirus A2 coat protein, revealed two antiviral compounds isolated from the gum resin of *Ferula asafetida*, namely farnesiferol B (IC_{50} = 1.0 μM) and farnesiferol C (IC_{50} = 0.96 μM) with selective anti-rhinovirus activity in a CPE inhibition assay (Rollinger et al., 2008).

The value of computational methods in natural products-based drug discovery extends to the prediction of absorption, distribution, metabolism, excretion (ADME), toxicity and further properties. One example where computational methods are widely applicable to natural products is the prediction of metabolically labile atom positions (Kirchmair et al., 2015), most of these predictors being machine learning models (Tyzack and Kirchmair, 2019).

Of particular relevance to natural products-based drug discovery are *in silico* methods for the identification of compounds prone to causing assay interference. Recently, we established a web service (Stork et al., 2019) that allows the prediction of pan-assay interference compounds (Baell and Holloway, 2010), frequent hitters (Roche et al., 2002), aggregators (McGovern et

al., 2002; Reker et al., 2019) and compounds with undesirable chemical and pharmacological properties.

3.3.4. Host targeting

In the search for broad-spectrum antivirals, host targeting is a strategy as a therapy regimen in ARIs (Martinez et al., 2015). Here, host cell proteins involved in e.g. viral replication, signalling or immunresponse, serve as targets for agents to combat ARIs. For instance, DAS181 (Fludase), cleaves off sialic acids from the host cell surface and thus prevents influenza virus attachment, entry, and replication (Koszalka et al., 2017; Marjuki et al., 2014). Another example for host-targeting as well as drug repurposing is the antiparasitic drug nitazoxanide (NTZ), a compound preventing the exit of newly built influenza viruses from the host cell by interfering with the assembly of viral hemagglutinin (Koszalka et al., 2017).

The concept of host targeting has been exemplified by the herbal drug *Andrographis paniculata*. Extracts of *A. paniculata* are reported to significantly improve the overall symptoms of ARIs compared to placebo (randomized controlled trials, n = 596), but the results have to be considered critically due to the heterogeneity of data and often missing manufacturing or quality control details (Hu et al., 2017).

3.3.5. Drug repurposing

In the context of drug repurposing, Medina-Franco et al. have followed a multitarget approach to systematically identify potential additional targets of existing or virtual chemical compounds (Medina-Franco et al., 2013). For example, the immunosuppressant drug cyclosporine A was found to exhibit a broad-spectrum antiviral activity against several influenza virus strains. Dealing with the imminent issue of the emergence of resitstant strains, cyclosporine A was subjected to serial viral passage experiments resulting in a high *in vitro* genetic barrier of drug resistance. Moreover, mechanistic studies revealed the antiviral activity at the intermediate step of viral replication after the entrance of the virus to the host cell (Ma et al., 2016).

Traditionally used to treat gastrointestinal disorders, Heo et al. recently discovered a *Poncirus trifoliata* orange seed extract to significantly inhibit oseltamivir-sensitive as well as -resistant influenza viruses on the endocytosis pathway. This novel mode of antiviral activity renders the *P. trifoliata* extract a highly potential remedy to fight resistant strains. Noteworthy, this extract competitively outrules the synthetic and single-target molecule oseltamivir phosphate by its multi-target anti-influenza activity (Heo et al., 2018).

3.3.6. Combined approaches

In an attempt to identify the best strategy for finding new nature-derived ARI therapeutics, we found that following different approaches led to promising results. Thus, the most fruitful concept seemed to be the combination of several strategies. This has been further developed by Nothias et al., who worked on bioactivity-based molecular networking. New drug leads were discovered by tandem mass spectrometry and bioactivity score prediction. Relative abundance of a molecule in a fraction subsequently is associated to bioactivity, which led to the identification of antiviral compounds of an extract of *Euphorbia dendroides* that were not discovered by classical bioactivity-guided fractionation (Nothias et al., 2018). In one of our recently conduced studies (Grienke et al., 2018), ethnopharmacological knowledge was interlinked with phenotypic screening technologies and computational methods to prioritise promising extracts, and at the same time to get clues about their virtually predicted hits. This combined approach enables the rapid and target-oriented identification of putatively bioactive consituents, while also providing insight into their molecular mechanism. This combinatory approach enabled to rapidly identify, for example, neuraminidase-inhibiting constituents of licorice (Grienke et al., 2014).

3.4. Does knowledge from traditional medicine matter?

Empirical knowledge and ethnopharmacological hints about multicomponent herbal remedies with yet undisclosed mechanisms of action are valuable selection criteria to identify antimicrobials from nature. The Chinese medicinal herb *Morus alba* root bark (sāng bái pí),

traditionally used against symptoms related to influenza and pneumonia, was recently in the focus of our investigations. The isolated prenylated flavonoids, among them sanggenon G and sanggenol A, not only showed significant inhibitory activities against influenza and pneumococcal neuraminidases, but also an inhibition of planktonic pneumococcal growth and biofilm formation observed by scanning electron microscopy (Grienke et al., 2016).

Another traditional Chinese medicinal plant, i.e. *Lonicera japonica*, was found to be rich in chlorogenic acids. The antiviral properties of this ubiquitous compound class were systematically investigated *in vitro* (CPE, time-of-addition experiment, nucleoprotein localization, neuraminidase inhibtion) as well as *in vivo* (H1N1 influenza A virus infected mice). Chlorogenic acid was shown to significantly inhibit growth of different influenza A virus strains (H1N1 and H3N2) with IC_{50} values ranging from 22.1 μM to 71.9 μM in a CPE inhibition assay. Chlorogenic acid was reported to interfere in the late stage of the infectious cycle due to down-regulation of nucleoprotein expression and neuraminidase inhibition. *In vivo,* chlorogenic acid was administered i.v. (100 mg/kg/d), leading to a survival rate of 60%, whereas 100% died in the placebo group. The histological investigation of lung tissue evidenced reduced virus titers and alleviated inflammation (Ding et al., 2017).

Although ethnopharmacological references are a valuable incentive to investigate a specific herbal drug, one has to be aware that they only give us hints for benefits in the treatment of symptoms, but not on its causative pathogen or involved targets. Additionally, the translation of ethnopharmacological knowledge not only points to a putative antimicrobial activity, but can also (or exclusively) refer to an anti-inflammatory or immune-stimulating activity as e.g. demonstrated by the elucidated activities of extracts and constituents from *Echinacea purpurea* (Sharma et al., 2006; Vimalanathan et al., 2017) and *Andrographis paniculata* (Coon and Ernst, 2004; Hu et al., 2017).

The *materia medica* of many cultures favors curative agents consisting of mixtures of herbal (and animal) drugs, thus being a composition of complex mixtures by themselves, so-called

composita (as e.g. in ancient Roman and Egyptian recipes) or formulations (as e.g. in Ayurveda, traditional Chinese and Kampo medicine). In modern pharmacognostic research with its simplified aim to track down the overall effect to one or a few (co)effectors, this habit multiplies researchers' difficulties to unravel the complexity in terms of bioactive constituents and involved molecular mechanisms as well as additive or even synergistic effects (see Hochu-ekki-to, Sinupret®, Esberitox®).

Ethnopharmacology converts traditional cultural and cross-cultural knowledge of medicinal plants and their therapeutic applicability into a helpful tool in drug discovery (Leonti et al., 2017). Going back centuries in the history of traditional medicine, *Echinacea purpurea* has been one of the most prominent examples for the treatment of ARIs (Barrett, 2003). A standardized 65% ethanolic extract of *Echinacea purpurea* significantly reduced the adhesion of *Haemophilus influenzae* and *Staphylococcus aureus* to bronchial epithelial cells infected with influenza virus (Vimalanathan et al., 2017). Echinacea extracts (ethanolic root extract and pressed juice of aerial parts) have been shown to reverse the rhinovirus induced release of pro-inflammatory cytokines and chemokines (Sharma et al., 2006). Oral administration of an aqueous-ethanolic extract of *Thuja occidentialis*, *Baptisia tinctoria*, *E. purpurea* and *E. pallida* (Esberitox®) resulted in a beneficial effect on influenza virus infected BALB/c mice compared to placebo (Bodinet et al., 2002). These data point out why this plant has stood the test of time: the beneficial effect of *E. purpurea* against influenza virus infections and its lethal synergism with bacterial superinfections is obvious (Vimalanathan et al., 2017), however there is no evidence for a significant effect on rhinovirus infection (Rollinger and Schmidtke, 2011). Even though this plant has been intensively investigated, we neither know the one compound responsible for bioactivity, nor the exact mechanism of action (Senica et al., 2018).

Hochu-ekki-to is a mixture of ten herbs used in Japanese traditional medicine. It was shown to reduce the amount of rhinovirus B14-RNA after 120 h from 100% in DMSO (0.2%) to 75% when tracheal epithelial cells were treated with 0.1 mg/mL with Hochu-ekki-to. Inhibition of

baseline intercellular cell adhesion molecule-1 mRNA expression at an extent of more than 50% compared to 0.2% DMSO and a reduced number of acidic endosomes pointed towards a distinct viral entry blockage. The decreased release of cytokines (IL-1β, IL-6 and TNFα) three days post infection also suggested a modulation of airway inflammation after RV14 infection. Glycyrrhizin as a main constituent of Hochu-ekki-to was proposed to contribute to the anti-rhinovirus effect, however the potency of the mixture is higher (Yamaya et al., 2007).

One of the few examples for approved anti-ARI herbal medicinal product is derived from a well-defined *Pelargonium sidoides* extract (Eps 7630®). With the indication for the treatment of acute bronchitis this extract significantly suppresses the replication of influenza virus strains (H1N1 and H3N2) *in vitro* with IC_{50}s of 9.5 μg/mL and 8.7 μg/mL, respectively (Table 3) (Michaelis et al., 2011).

Further, as given in Table 4, *in vivo* experiments underline the beneficial effect by reduction of cough frequency in cough models and enhancement of bronchosecretolysis. The antitussive effect was measured in an ammonia-induced cough model decreasing the number of coughs from 34.6 in the untreated mice to 4.9 when mice were treated with 120 mg/kg/d *P. sidoides* extract (Bao et al., 2015). Beyond influenza virus, also anti-rhinovirus effects of this extract were investigated in human bronchial epithelial cells indicating inhibitory effects by down-regulation of cell membrane docking proteins and up-regulation of host defence proteins (Roth et al., 2019).

Notwithstanding the importance of ethnopharmacological knowledge as incentive to explore the large reservoir of chemical space in natural products, it is imperative to also exploit the biosynthetic machinery of fungi and bacteria besides traditional source organisms like plants (Pye et al., 2017).

3.5. Translatability from *in vitro* to *in vivo* studies and beyond

During preclinical drug development multiple *in vitro* and *in vivo* studies need to be performed to classify synthetic compounds or natural products as potential drug candidates. The *in vitro*

studies using target- and cell-based assays alone or in combination allow for the identification of bioactive extracts, hit compounds thereof, their target, their mechanism of action, and their antimicrobial spectrum. Cell-based assays also give first hints on the compatibility of identifed inhibitors for cells (Grienke et al., 2018). Furthermore, the target- and cell-based assays can be used in structure-activity-relationship studies aiming to enhance the inhibitory activity and to identify a lead compound for drug development (Grienke et al., 2010). Co-cell-culture models e.g. comprising human or murine lung epithelial cell lines as well as immune cell lines (monocytes/macrophages, dendritic cells) additionally mimic selected parameters of the *in vivo* situation e.g. receptor expression (important for viral infection and spread), pattern recognition receptors, and innate immune response by reflecting the interplay between these epithial, endothelial, and immune cells (Mosig et al., 2017). Furthermore, pro-inflammatory mediators produced by infected cells and contributing to the severity of symptoms can be studied. For example, a humane triple co-culture model consisting of a humane bronchial epithelial cell line, macrophages and dendritic cells was established (Blom et al., 2016). Noteworthy, a translation of results from target- and cell-culture-based assays is not always given. The reasons are manifold. For example, neuraminidase inhibition activity in cell culture depends on receptor expression as well as the functional balance of the influenza virus hemagglutinin and neuraminidase (Barnett et al., 2000; Bauer et al., 2012; Mishin et al., 2005). A further reason for discrepancies between target and cell-based assay is that higher inhibitor concentrations can commonly be tested in target-based assays because their readout is not hampered by cytotoxicity.

To better mimic the *in vivo* conditions, lung *ex vivo* models were established and used, for example, to analyse the course of influenza virus infection (Chan et al., 2016; Hocke et al., 2017; Weinheimer et al., 2012) and anti-influenza virus activity (Nicholas et al., 2015) as well as rhinovirus infection (Bochkov et al., 2011). The availibilty of *ex vivo* models is limited by the access to organ material and high costs. Moreover, *ex vivo* models are not mimicking the

systemic effects of ARI infections like cytokine networks, inflammation, adaptive immune response etc. Neither (co-)cell culture nor *ex vivo* models do fully reflect the complex *in vivo* situation, where the adsorption, distribution, metabolism, excretion, and toxicity of the identified inhibitors but also the complex pathogen-host interactions can impact the efficacy of inhibitory activity. This is the reason why the results obtained with (co-)cell-culture and *ex vivo* models are not directly transferable to *in vivo* or human studies. However, the application of such models can help to preselect inhibitors for *in vivo* studies and thereaby to reduce the number of animal experiments.

During preclinical development of inhibitors, animal models are important to confirm the efficacy of potential antimicrobials as well as for drug resistance studies. For example mice (Gluck et al., 2013), ferrets (Frise et al., 2016; Oh et al., 2018; Roosenhoff et al., 2018), and pigs (Duerrwald et al., 2013) are applied in anti-influenza virus studies. In addition, embryonated egg models were successfully applied for anti-influenza virus studies (Sauerbrei et al., 2006; Shi et al., 2017). In contrast, there are no good *in vivo* models mimicking rhinovirus infection. Generally, the proven compatibility and strong efficacy of an inhibitor *in vitro* represent an absolute prerequisite of *in vivo* studies. Therefore, only a small portion of the initially *in vitro* identified antimicrobial active natural products summarized in Tables 2 and 3 proceeded to *in vivo* studies as summarized in Table 4 where they are grouped according to the used infection model (viral, bacterial or co-infection model) as well as the respective activity read-out in comparison to the control (positive or negative).

As a substitute for a missing suitable animal model for anti-rhinovirus studies, human rhinovirus challenge models were used in preclinical studies to prove the antiviral effect of potential drug candidates e.g. pirodavir, pleconaril, and rupintrivir (Hayden et al., 1992; Hayden et al., 2003; Lambkin-Williams et al., 2018; Turner et al., 1993). Although some drug candidates were well tolerated and effective (reduction in viral load and symptoms) in

rhinovirus challenge models, side effects and limited treatment effects were recorded in clinical studies. To the best of our knowledge, no natural products were studied by this manner.

However, randomized clinical studies were performed with plant extracts or constituents therof concerning safety and efficacy. The meta-analysis of six clinical studies with ethanolic extracts from *Echinacea* revealed a reduced risk of respiratory infections (Schapowal et al., 2015). Another randomized trial with *Cistus* monitored a stronger symptom reduction over the course of treatment with *Cistus* extract compared to green tea extract (Kalus et al., 2010). In addition, the results of a placebo-controlled, randomized trial with a poly-furanosyl-pyranosyl-saccharide-based extract of *Panax quinquefolius* (CVT-E002) demonstrated that it is well tolerated and reduces moderate to severe ARI and sore throat (High et al., 2012). According to publications in the Cochrane Database of Systematic Reviews (i) *Pelargonium sidoides* did not show serious side effects, whereas a low evidence for reduction of chronic bronchitis and sinusitis was found (Timmer et al., 2013), (ii) no serious side effects but also no effects on acute sinusitis were induced by *Cyclamen europaeum* (Zalmanovici Trestioreanu et al., 2018) and (iii) the effect of garlic for the common cold remains unclear (Lissiman et al., 2012). According the Cochrane authors, the study quality needs to be improved. This is also relevant for the publised clinical studies with Chinese medicinal herbs for influenza, sore throat, and acute bronchitis (Huang et al., 2012; Jiang et al., 2013; Jiang et al., 2012). The insufficient quality of data did not allow for drawing conclusions about the benefits of Chinese herbs.

Table 4. Natural products (extracts and pure compounds) with reported *in vivo* activities related to ARIs: Anti-influenza virus, anti-rhinovirus and dual antiviral and antibacterial actives.

Natural source	Type of extract	Compound name	*In vivo* model	Pathogen	Study parameter	Activity	Control	Reference
n.g.	-	camphecene	BALB/c mice	IV, H1N1 A/California/07/2009	survival	survival rate: 60% (50 mg/kg/d p.o.)	survival rate: oseltamivir: 80% (20 mg/kg/d p.o.)	(Zarubaev et al., 2015)
				IV, H1N1 A/California/07/2009	survival	survival rate: 70% (100 mg/kg/d p.o.)	survival rate: oseltamivir: 80% (20 mg/kg/d p.o.)	
				IV, B/Lee/1940	survival	survival rate: 10% (50 mg/kg/d p.o.)	survival rate: oseltamivir: 90% (10 mg/kg/d p.o.)	
				IV, B/Lee/1940	survival	survival rate: 90% (100 mg/kg/d p.o.)	survival rate: oseltamivir: 90% (10 mg/kg/d p.o.)	
Artemisia vestita Wall. ex Besser	essential oil	-	swiss albino mice	SPy ATCC 12344	lung tissue (Log10 CFU/g of organ p.i.)	0.1 mg/mouse 2x/day	ciprofloxacin 0.1 mg/mouse	(Yang et al., 2015)
				SPy ATCC 12344	lung tissue (Log10 CFU/g of organ p.i.)	day 3 p.i.: 4.13 CFU	day 3 p.i.: 3.32 CFU	
				SPy ATCC 12344	lung tissue (Log10 CFU/g of organ p.i.)	day 6 p.i.: 3.92 CFU	day 6 p.i.: 3.52 CFU	
				SPy ATCC 12344	lung tissue (Log10 CFU/g of organ p.i.)	day 9 p.i.: 4.12 CFU	day 9 p.i.: 3.38 CFU	
	-	grandisol		SPy ATCC 12344	lung tissue (Log10 CFU/g of organ p.i.)	0.135 mg/mouse 2x/day	negative control	
				SPy ATCC 12344	lung tissue (Log10 CFU/g of organ p.i.)	day 3 p.i.: 4.92 CFU	day 3 p.i.: 7.22 CFU	
				SPy ATCC 12344	lung tissue (Log10 CFU/g of organ p.i.)	day 6 p.i.: 4.52 CFU	day 6 p.i.: 7.10 CFU	
				SPy ATCC 12344	lung tissue (Log10 CFU/g of organ p.i.)	day 9 p.i.: 4.88 CFU	day 9 p.i.: 7.30 CFU	
Bergenia purpurascens (Hook.f. & Thomson) Engl.	M	-	neonatal rats	SA	survival	survival rate: 48.57% (50 mg/kg/d)	positive control without infection: 80% survival	(Liu et al., 2018)
				SA	survival	survival rate: 60.0% (100 mg/kg/d)	negative control: 34% survival	
Bletilla striata (Thunb.) Rchb.f.	-	2,2,7′-trihydroxy-4,4′,7-trimethoxy-9′,10′-dihydro-1,1′-diphenanthrene	dmbryonated hen eggs	IV, H1N1 A/Jiangsu/1/2016	IC_{50} in embryonated eggs model	IC_{50} at 0.08 mmol/egg: 79.3%	oseltamivir at 0.01 mmol/egg: 100%	(Shi et al., 2017)
		2,2′,7′-trihydroxy-3′,4,5′,7-tetramethoxy-9′,10′-dihydro-		IV, H1N1 A/Jiangsu/1/2016	IC_{50} in embryonated eggs model	IC_{50} at 0.08 mmol/egg: 17.2%	oseltamivir at 0.01 mmol/egg: 100%	

Natural source	Type of extract	Compound name	*In vivo* model	Pathogen	Study parameter	Activity	Control	Reference
		1,1′-di-phenanthrene						
		2,2′–dyhydroxyl-4,4′,7,7′-9′,10′-dihydro-1,6′-di-phenanthrene		IV, H1N1 A/Jiangsu/1/2016	IC_{50} in embryonated eggs model	IC_{50} at(0.08 mmol/egg: 75.9%	oseltamivir at 0.01 mmol/egg: 100%	
		2,7-dyhydroxyl-4-methoxy-9,10-dihydro-phenanthrene		IV, H1N1 A/Jiangsu/1/2016	IC_{50} in embryonated eggs model	IC_{50} at 0.08 mmol/egg: 20.7%	oseltamivir at 0.01 mmol/egg: 100%	
		2,7-dyhydroxyl-4-methoxyphenanthrene		IV, H1N1 A/Jiangsu/1/2016	IC_{50} in embryonated eggs model	IC_{50} at 0.08 mmol/egg: 34.5%	oseltamivir at 0.01 mmol/egg: 100%	
		4,4′,7,7′-tetrahydroxy-2,2′,8,8′-tetramethoxy-1,1′-di-phenanthrene		IV, H1N1 A/Jiangsu/1/2016	IC_{50} in embryonated eggs model	IC_{50} at 0.08 mmol/egg: 34.5%	oseltamivir at 0.01 mmol/egg: 100%	
		4,4′,7,7′-tetrahydroxy-2,2′-dimethoxy-1,1′-di-phenanthrene		IV, H1N1 A/Jiangsu/1/2016	IC_{50} in embryonated eggs model	IC_{50} at 0.08 mmol/egg: 34. %	oseltamivir at 0.01mmol/egg: 100%	
		4,5-dyhydroxyl-2-methoxy-9,10-dihydro-phenanthrene		IV, H1N1 A/Jiangsu/1/2016	IC_{50} in embryonated eggs model	IC_{50} at 0.08 mmol/egg: 34.5%	oseltamivir at 0.01 mmol/egg: 100%	
Cistus x. *incanus* L.	Cystus052®	-	humans	viral or/and bacterial infection (throat swabs samples)	severe fever	day 0: 40% (~260 mg polyphenols/d)	green tea day 0: 50% (~480 mg polyphenols/d)	(Kalus et al., 2010)
				viral or/and bacterial infection (throat swabs samples)	severe fever	day 3-4: <10% (~260 mg polyphenols/d)	green tea day 3-4: 39% (~480 mg polyphenols/d)	
Clinacanthus siamensis Bremek.	E	-	BALB/c mice	IV, H3N2 A/Guizhou/54/1989	IgG1 and IgA in broncheo-alveolar wash (up to 20 days p.i.)	induction of humoral immune response (day 19) (100 mg/kg/d p.o.)	oseltamivir (0.1 mg/kg/d p.o.): no induction of humoral activity	(Wirotesangthong et al., 2009)
Gardenia jasminoides J.Ellis	-	geniposide	ICR mice	IV, H1N1 A/Jiangsu/1/2009	survival	survival rate: 90% (20 mg/kg) 8 days p.i.	peramivir survival rate: 90% (30 mg/kg) 8 days p.i.	(Zhang et al., 2017)
Lonicera japonica Thunb.	-	chlorogenic acid	BALB/c mice	IV, H1N1 A/PuertoRico/8/1934	survival	survival rate: 60% (100 mg/kg/d i.v.)	oseltamivir: 70% (100 mg/kg/d)	(Ding et al., 2017)
				IV, H3N2 A/Beijing/32/1992	survival	survival rate: 50% (100 mg/kg/d i.v.)	oseltamivir: 70% (100 mg/kg/d)	

Natural source	Type of extract	Compound name	*In vivo* model	Pathogen	Study parameter	Activity	Control	Reference
				IV, H1N1 A/PuertoRico/8/1934	virus titre (5 days p.i.)	3.77 Log10CCID50/g	5.52 Log10CCID50/g in placebo group	
Panax quinquefolius L.	CVT-E002™	-	humans (early-stage untreated chronic lymphocytic leukemia)	n.g.	sore throat (moderate-severe, study period: 3 months)	10% at 200 mg/2x/d	placebo: ~23%	(High et al., 2012)
Pelargonium sidoides DC	11% E	-	guinea pigs	-	citric acid-induced cough model	10 mg/kg: number of coughs: 6.1	negative control: number of coughs: 20.0 *Radix glycyrrhizae* 5.5 ml/kg: 6.7	(Bao et al., 2015)
					citric acid-induced cough model	20 mg/kg: number of coughs: 7.5		
					citric acid-induced cough model	45 mg/kg: number of coughs: 5.5		
			ICR miceSPE-class	-	ammonia-induced coughing	20 mg/kg: number of coughs: 9.8	Negative control: number of coughs: 34.6 *Radix glycyrrhizae* 5.5 ml/kg: 12.0	
					ammonia-induced coughing	40 mg/kg: number of coughs: 5.5		
					ammonia-induced coughing	120 mg/kg: number of coughs: 4.9		
					bronchosecretolytic effect (phenol red secretion)	20 mg/kg: phenol red: c = 349.1 µg/ml	phenol red: c = 274.3 µg/ml *Radix glycyrrhizae* 5.5 ml/kg: phenol red: c = 401.6 µg/ml	
					bronchosecretolytic effect (phenol red secretion)	40 mg/kg: phenol red: c = 414.1 µg/ml		
					bronchosecretolytic effect (phenol red secretion)	120 mg/kg: phenol red: c = 474.5 µg/ml		
Zuccagnia punctata Cav.	E	-	infant swiss albino mice	SP AV6	lung tissue, blood (Log10 CFU/g of organ)	1 mg/mouse p.o. 2x/day	Amoxicillin: 2 mg/mouse	(Zampini et al., 2012)
				SP AV6	lung tissue, blood (Log10 CFU/g of organ)	day 3 p.i.: 4.64 CFU	day 3 p.i.: 4.34 CFU	
				SP AV6	lung tissue, blood (Log10 CFU/g of organ)	day 5 p.i.: 4.09 CFU	day 5 p.i.: 3.67 CFU	
				SP AV6	lung tissue, blood (Log10 CFU/g of organ)	day 7 p.i.: 4.30 CFU	day 7 p.i.: 3.41 CFU	
	-	7-hydroxyflavanone		SP AV6	lung tissue, blood (Log10 CFU/g of organ)	1 mg/mouse p.o. 2x/day	negative control	
				SP AV6	lung tissue, blood (Log10 CFU/g of organ)	day 3 p.i.: 4.43 CFU	day 3 p.i.: 5.49 CFU	
				SP AV6	lung tissue, blood (Log10 CFU/g of organ)	day 5 p.i.: 4.13 CFU	day 5 p.i.: 5.29 CFU	
				SP AV6	lung tissue, blood (Log10 CFU/g of organ)	day 7 p.i.: 4.35 CFU	day 7 p.i.: 5.36 CFU	

Abbreviations: c = concentration, CFU = colony forming units, i.v. = intravenous, IV = influenza virus, n.g. = not given. p.i. = post infection, p.o. = peroral, SA = *Staphylococcus aureus*, SP = *Streptococcus pneumoniae*, SPy = *Streptococcus pyogenes*

Extraction solvents: E = ethanol, M = methanol

4. Conclusion and future perspectives

In many cultures all over the world, herbal remedies have a longstanding tradition as a preferred choice to treat ARIs. The applied herbal remedies are complex multicomponent mixtures, where individual constituents can exert their effects through interactions with multiple viral and bacterial targets (multi-targeting) in a multi-functional (pleiotropic) way.

Despite the fact that the identification and development of novel innovative anti-ARI agents from natural sources are of utmost importance, promising lead candidates and clinical evidence are largely missing. To fill this gap, an arsenal of sophisticated strategies is required to investigate antimicrobial natural products more comprehensively with straightforward protocols and assays for the assessment of their value within drug discovery initiatives.

Evaluating the impact of natural products to combat ARIs, this review critically addresses the relevance of traditional knowledge as a main criterion for the biased selection of starting materials and the strategies which have been pursued.

Regarding the overall influence of natural products on ARIs within the last ten years we encountered a vast amount of literature data. The majority consists of *in vitro* studies, where the "one compound-one target" paradigm is strongly represented, since pure compounds were mainly tested only against one target pathogen and/or one target. As ARIs in many cases are characterized by a complex interplay of more than one pathogen (McCullers, 2014; Visseaux et al., 2017), testing natural products against one distinct virus or bacterium represents only a part of the puzzle. Hence, an interpretation of the significance of the published results for the treatment of ARIs is difficult unless accompanied with meaningful *in vivo* experiments. Co-infection models *in vitro* as well as *in vivo* might be an advanced approach mimicking the complex infectious condition. However, in the current literature such multi-targeting approaches are rather the exceptions than the rule.

Concerning targets of distinct pathogens involved in ARIs, influenza neuraminidase has evolved as the most popular druggable motive for natural products (as well as synthetic compounds). Due to well-established and easily available neuraminidase inhibition assay kits, which however are prone to assay interferences, extensive screening campaigns have resulted in an accumulation of a vast amount of *in vitro* data contemplating the largest group of anti-ARI natural product lead candidates. Although other anti-influenza virus and anti-rhinovirus targets are known (e.g. hemagglutinin, nucleoprotein), the degree of their experimental advance and the knowledge about their druggability is in its infancy. On the phenotypic/cell-based level, the evaluation of the inhibition of the viral cytopathic effect has evolved as the most commonly applied assay, giving insights into general antiviral activity.

Comparative analysis of the chemical space of all bioactive natural products discussed in this work shows that many of these compounds are drug-like but also that there are several bioactive natural products which are substantially larger and have more hydrogen bond donors and acceptors than most approved drugs.

To discover anti-influenza virus natural compounds with drug-like properties, we broadly applied the cytopathic effect inhibition assay in a recently accomplished 5-years project from the Austrian Science Fund (FWF P24587). In this project, based on the knowledge of antiviral herbal remedies from traditional medicine, starting materials from plants and fungi were selected for the generation of 162 extracts. Intriguingly, defining an antiviral activity threshold with an IC_{50} value of ≤ 50 μg/mL, the sample set revealed 20% and 11% active extracts against influenza virus A/Hong Kong/1968 and rhinovirus A2, respectively (Grienke et al., 2018). These data underline the importance of ethnopharmacological knowledge in the selection of plant materials to achieve a high yield of "hit extracts" for further investigation. In most cases, data from the phenotypic antiviral screening in combination with information from virtually predicted hits guided the analytical and phytochemical investigations for the identification of novel antiviral lead structures from nature. During this project, an assay protocol for the

straightforward identification of anti-influenza molecular mechanisms and a standard procedure for ruling out false positives at an early stage have been established (Fig. 5).

Taking together the data from our research and available literature data from the last ten years, there is a clear tendency towards assaying for more broadspectrum antiviral and antibacterial effects bearing a large potential for further investigations in this interdisciplinary field.

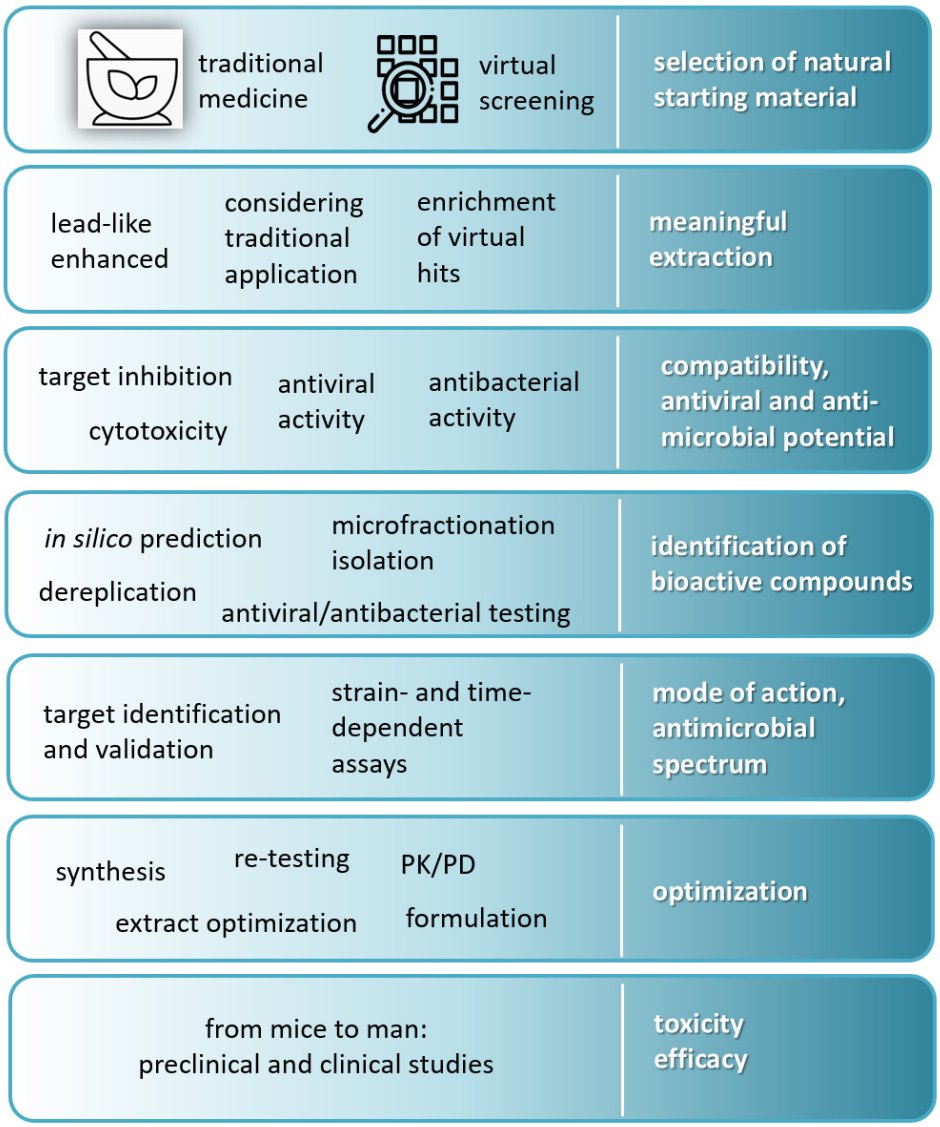

Fig. 5. Workflow for the selection, extraction and identification of natural products against ARIs.

Author contributions

UG, MS, and JMR conceived the study. JL extracted the literature data and drafted the manuscript. JK and YC contributed to the chemical space analysis and discussion of computational approaches. All authors discussed the results and contributed to the final manuscript.

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