

Review

A perspective on picornavirus inhibitors and concrete evolution of WIN compounds

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Abstract: The family Picornaviridae is one of the largest families of human viral pathogens, causing an extensive range of clinical manifestations from mild fever, common cold to serious paralytic poliomyelitis, COPD, etc., some of which can even be life-threatening. Picornaviruses also cause zoonotic epidemics that result in dramatic social and economical losses. Although no efficient antiviral agent for prophylaxis or treatment of picornavirus infections has been officially approved yet, a large number of anti-picornavirus compounds with potent activity have been developed and investigated, through which further information about picornavirus has been revealed as well. Viral mRNA translation, viral mRNA replication and especially the viral capsid are the three main targets of these compounds having been extensively studied. The typical one is the WIN series of compounds that bind to the viral capsid and inhibit viral attachment or uncoating. Herein, a perspective on picornavirus inhibitors and a concrete evolution of WIN compounds will be presented in this paper.

Keywords: Picornavirus; Antiviral agent; Capsid binding inhibitor; WIN compound; Pleconaril

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1. Introduction

1.1. Classification and pathopoiesis

Picornaviruses are a type of non-enveloped single-positive stranded RNA viruses with a small icosahedral

protein capsid outside. The family Picornaviridae is one of the largest medically important families of human and animal viral pathogens. The Picornaviridae family consists of 13 genera, including the Aphthovirus, Avihepatovirus, Cardiovirus, Cosavirus, Enterovirus, Erbovirus, Hepatovirus, Kobuvirus, Parechovirus, Sapelovirus, Senecavirus, Teschovirus and Tremovirus, and the 13 genera contain at least 285 different picornavirus types^[1-3]. These viruses can cause an extensive range of clinical manifestations in human beings as well as in animals, some of which are mild while the others are more serious and even life-threatening^[3]. Cardiovirus, Enterovirus, Hepatovirus, Kobuvirus, Parechovirus and a proposed genus Cosavirus are the six genera that harbor human pathogens^[2] (Table 1).

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Table 1. The six genera of picornaviruses pathogenic to human

Genus	Species	Groups	Clinical manifestations
Cardiovirus	Theiler's murine encephalomyelitis virus (TMEV, 12 types)		Myocarditis, acute & chronic encephalitis, respiratory diseases and gastroenteritis
	Encephalomyocarditis virus (EMCV, 1 type)		Myocarditis, encephalitis and lesions in pancreas
Enterovirus	Human rhinovirus A (HRV-A, 74 types)	2 groups--- A and B	Common cold, patients asthma, congestive heart failure, bronchiectasis, cystic fibrosis and even otitis
	Human rhinovirus B (HRV-B, 25 types)		
	Human rhinovirus C (HRV-C, 10 types)		
	Bovine enterovirus (BEV, 2 types)		
	Human enterovirus A (HEV-A, 21 types including some Cocksackie A viruses)	4 groups--- PV; EchV; CAV (CAV, CBVs); Numbered HEV	Hand, foot and mouth disease (HFMD), paralytic poliomyelitis, hemorrhagic conjunctivitis, herpangina, meningitis, encephalitis, myocarditis, pancreatitis, acute paralysis, neonatal sepsis and chronic fatigue
	Human enterovirus B (HEV-B, 59 types including Cocksackie B viruses and Echoviruses)		
	Human enterovirus C (HEV-C, 19 types including Polioviruses and the other Cocksackie A viruses)		
	Human enterovirus D (HEV-D, 3 types)		
	Porcine enterovirus B (PEV, 2 types)		
	Simian enterovirus A (SEV, 1 type)		
Hepatovirus	Hepatitis A virus (HAV, 1 type)		Hepatitis
Kobuvirus	Aichi virus (1 type)		Acute gastroenteritis
	Bovine kobuvirus (1 type)		
	Porcine kobuvirus (1 type)		
Parechovirus	Parechovirus (HPeV, 14 types)		Respiratory tract or gastrointestinal illness, severe sequelae
Cosavirus	Human Cosavirus (HcoSV A–E, 5 types)		AFP and acute diarrhea

Theiler's murine encephalomyelitis virus (TMEV) and encephalomyocarditis virus (EMCV) are the two species of Cardiovirus identified so far^[2]. Two types out of twelve of TMEV, Vilyuisk human encephalomyelitis virus and Saffold virus infect humans, usually causing a persistent infection in the central nervous system (CNS) such as myocarditis and acute/chronic encephalitis, as well as respiratory diseases and gastroenteritis^[4]. EMCV contracts both humans and other mammalian species with rodents being its natural reservoir^[2]. Human EMCV infection may be associated with myocarditis and encephalitis and even pancreatic lesions^[5].

The Enterovirus includes 219 virus types and is the largest genus of the family Picornaviridae. It has been divided into 10 species^[2], among which three are the known human rhinoviruses (HRVs), the HRV-A, B and C. The rest seven species are enteroviruses (EVs) that infect both humans and animals, including Bovine enterovirus (BEV, 2 types), Human enterovirus A (HEV-A, 21 types including some Cocksackie A viruses), Human enterovirus B (HEV-B, 59 types including Cocksackie B viruses and Echoviruses), Human enterovirus C (HEV-C, 19 types including Polioviruses and the other Cocksackie A viruses), Human enterovirus D (HEV-D, 3 types), Porcine enterovirus B (PEV, 2 types) and Simian enterovirus A (SEV, 1 type)^[6].

HRVs (over 100 virus serotypes) are also divided into two groups (group A and B) according to their susceptibility to cellular capsid-binding receptors. The majority of HRVs interact with Intercellular Adhesion Molecule 1 (ICAM-1) and the rest of the HRVs bind low-density lipoprotein receptor^[7]. HRVs are a major causative agent of upper respiratory tract symptoms and HRV infections

are often symptomatic and are responsible for more than 50% of the nasopharyngeal syndrome (the "common cold") in the general population at all ages^[2,6,8]. Although HRV infections are self-limited, there is increasing evidence linking HRV infection with asthma, bronchiectasis, congestive heart failure, cystic fibrosis and even otitis^[8]. EVs, on the basis of their clinical importance, are usually classified into four groups, including the Polioviruses (PVs, 3 serotypes), Echoviruses (EchV, 28 serotypes), Cocksackieviruses (CAVs, 23 serotypes; CBVs, 6 serotypes) and numbered enteroviruses^[1]. Human infections caused by EVs are often asymptomatic or mild and are diagnosed as non-specific viral syndromes. Clinical manifestations of EV infections range from fever alone or specific syndromes such as hand, foot and mouth disease (HFMD), Hemorrhagic Conjunctivitis and herpangina. In certain cases EVs can cause severe and life-threatening infections such as meningitis, encephalitis, myocarditis, pancreatitis, acute paralysis, neonatal sepsis and chronic fatigue^[9]. The prototype of the EV, poliovirus, once caused paralytic poliomyelitis or infantile paralysis and threatened millions of lives worldwide. Poliovirus infections were put under controlled upon the establishment of the live attenuated oral PV vaccine and inactivated PV vaccine in the 1960s^[10]. Large outbreaks of aseptic meningitis caused by Echovirus have occurred in different continents, and annual virus outbreaks are frequently reported^[11]. HFMD, a common infectious illness that spreads in young children and often complicated with encephalitis or myocarditis, is caused by EV-71 and CAV-16 and has become a rampant disease among different Asian countries in recent years^[12].

The Hepatitis A virus (HAV) remains to be the only

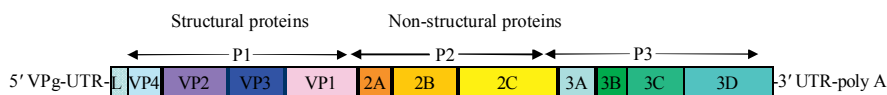


Figure 1. Protein unit regions in picornavirus genomes. The actual genome organization may deviate in some picornaviruses^[2].

species in the genus Hepatovirus up to now. HAV, is a virus that infects both humans and primates and is characterized by clinical hepatitis and jaundice^[13]. The Aichi virus, Bovine kobuvirus and Porcine kobuvirus are the three species comprise the genus Kobuvirus. Aichi virus is the prototype virus of Kobuvirus and has been shown to be associated with acute gastroenteritis in humans^[14]. A class of picornavirus was recognized in early 1990s and consequently reassigned to a new genus designated the Parechovirus^[15]. In this genus there are 14 types of parechoviruses, including HPeV 1–14 that infect humans, and some newly emerged or recently re-identified virus types^[2,16]. HPeV infections mainly occurred among young children, and most of them are reported subclinical or mild and cause respiratory tract or gastrointestinal illness. However, more severe sequelae involving AFP, myocarditis, encephalitis, aseptic meningitis, neonatal sepsis, and Reye syndrome have also been reported to be associated with HPeV infections^[17]. The human Cosaviruse (HCoSV) consists of five genomic groups, HCoSV A–E, and is a Picornavirus genus newly isolated from healthy and unhealthy children with AFP in Pakistan and from a child with acute diarrhea in Australia^[18].

A fact that we can't ignore is that new or unknown picornaviruses are always emerging through spontaneous mutations or recombinations and most of the new viruses are confined to animals. However, it is entirely possible that these viruses will evolve to infect humans or other mammalian species and potentially cause new or more serious human diseases^[19]. Regardless of the extensive harm to humans, zoonotic transmissions of picornaviruses like foot-and-mouth disease virus (FMDV), swine vesicular virus (SVDV) etc, also result in dramatic social economically losses^[20]. Unfortunately, up to now, no single efficient antiviral agent for prophylaxis or treatment of picornavirus infections has been developed, and the therapies are mainly only targeting symptoms caused by the infection. The vaccines established are against only PV, HAV and FMDV^[3,21]. Therefore the continuation of the search for a potent broad-spectrum anti-picornaviral agent is required and even imperative.

1.2. Viral genome, proteome and life circle

Picornavirions are non-enveloped small icosahedrally spherical particles. The viral particles are around 30–40 nm in diameter and are encapsulating a single-stranded, positive sense (messenger-active) RNA as their genome in the protein capsides. Picornaviridae family shares a similar genomic organization that consists of approximately 6700–8800 nucleotides and only varies in some regions of the RNA^[2] (Fig. 1). A small protein named VPg involved in the initiation of viral RNA replication is

linked covalently to the 5'-end of the viral RNA chain. The 3'-terminus of the viral RNA is polyadenylated and called the poly-A tract or tail^[22]. Both ends of the genome RNA are non-coding regions (the NCR) or untranslated regions (the UTR) and account for approximately 10%–12% of the RNA in size^[23]. Within the long and highly structured 5'-NCR there is a cloverleaf-like structure that is essential for the negative-stranded RNA synthesis and contains an internal ribosome entry site (IRES), which regulates the ribosomal internal initiation of the translation^[24]. The 3'-NCR is relatively short but also harbors knot-like secondary structures that interact with cellular factors and facilitate the proliferation of viruses in the cytoplasm of eukaryotic cells^[25]. The vast area between the two NCRs is the open reading frame (the ORF), which encodes for a single large poly-protein of about 2200 amino-acid residues long and contains all protein units the virus needs for its replication in exact stoichiometry^[2,26].

Picornaviral proteome is a single poly-protein that contains protein precursors (intermediates) or mature proteins (final protein units) in a relatively conserved order. The poly-protein then subsequently undergoes a self-cleavage event via a *cis*-acting autoproteolytic mechanism to generate the three precursors P1, P2 and P3^[2] (Fig. 1). In the same subset rank of the P1, P2 and P3, a polypeptide called L protein that is only present in Cardioviruses and Aphotoviruses, is located immediately preceding the P1 region at the *N*-terminus^[4,27]. The P1 precursor is further cleaved into four viral structure proteins VP4, VP2, VP3 and VP1 sequentially. The P2 and P3 precursor is further processed into seven non-structure proteins. P2 is processed into protease 2A, viroporin 2B and NTPase 2C, while P3 is processed into NTPase 3A, RNA replication primer 3B (uridylated VPg), protease 3C and RNA polymerase 3D^[28]. Dimer precursors also have unique functions. For example, the precursor 3CD is believed to cleave other viral peptide precursors, and the intermediate 3AB binds to the viral RNA polymerase 3D and stimulates its activity^[29]. It's likely that after the production of precursors and mature proteins, the majority of cleavage of the junctions in the poly-protein and intermediates is catalyzed by viral protease 2A, 3C and 3CD in enteroviruses^[30]. Viral proteins also play indispensable roles in the pathogenesis and interactions with drugs in addition to their contributions to the viral life circle.

The life circle of picornaviruses can generally be divided into five stages, including invasion (exposure of host), attachment or absorption, endocytosis and uncoating, replication and assembly and apoptosis and shedding (Fig. 2). The capsids of picornaviruses are constructed by units called protomers, and each protomer contains one

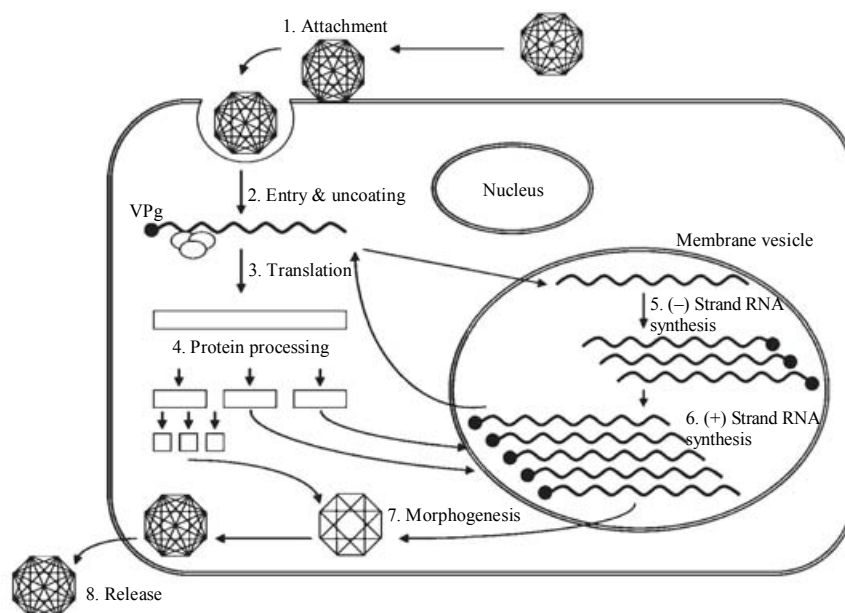


Figure 2. General model of life cycle of picornavirus^[3].

copy of each of the four viral structure proteins VP1, VP2, VP3 and VP4^[8]. There are many formed deep depressions (“canyon”) scattered on the surface of the viral capsid, which serve as the cellular receptor-binding sites^[31,32]. After invasion into human body (invasion stage), the viruses infect the normal host cells by binding to their intercellular adhesion molecules on cytomembrane, such as the intercellular adhesion molecule-1 (ICAM-1), CD54 and VCAM-1 and direct these molecules into the canyon on the viral surface (attachment or absorption stage)^[33]. The accomplishment of attachment triggers a change in the conformation of the capsid, causing the release of the VP4 component and the exposure of the VP1 component, which then facilitates membrane interaction and thus prepares the viruses for uncoating^[23]. Often the virus enters the cytoplasm of the host cell through endocytosis via a protein-mediated process after the attachment, during which the viral uncoating occurs (uncoating stage). The cytoplasm is where the main course of viral life cycle, the viral replication occurs. Noticeably, the viral genome RNA replication needs to occur inside membranous vesicles in the cytoplasm^[34,35].

During the replication that employs the host machinery, picornaviruses discontinue the translation to host cellular proteins once the mature viral proteome is produced, utilizing the common strategy of cleaving the eukaryotic cell translation initiation factor eIF4GI (a cap-binding protein) by viral proteases such as 2A^{pro}, 3C^{pro}, and even the L protease in some genera. The cleavage of eIF4GI then prevents the formation of the cap-dependent mRNA translation initiation complex^[36]. While this discontinuity doesn’t have any impact upon viral translation since it depends on viral IRES and does not need the involvement of eIF4GI. Through the cleavage of certain eukaryotic cellular transcription initiation actors and activators, the host gene expression is further hindered by picornaviruses^[37]. Besides

the production of viral proteome mentioned above, the viral RNA genome must also be copied to fully replicate as well. First, the VPg leader protein is uridylylated by the 3D polymerase using the orf1 cis-acting RNA element (in Polio, is a cloverleaf in the 2C region of the genome RNA) and then serves as a primer for picornavirus negative-strand RNA synthesis based on the positive strand template. The negative strands conduct conversely as the templates to produce the new RNA genome primed with uridylylated VPg (3B) by 3D polymerase, which are then packaged into the newly assembled virions from the 4 structure proteins to accomplish the assembly step (assembly stage)^[23]. The signaling cascades leading to host cell death are activated in the meantime while viral replication occurring. Other than the blocking to the host RNA translation and gene transcription by viral proteases mentioned above, the viral protein 2B also contributes to the extensive distress to the host cell, which destabilizes ion gradients, degrades the membrane and facilitates the release of mature virions (the final stage)^[38].

Picornaviruses possess highly conserved genome and proteome and strikingly similar life circles across various genera, which provides the basis for the developments of broad-spectrum antiviral agents. Theoretically, every functional molecule involved in the viral life cycle can be a potential target for antiviral agent development. So far, however, previously reported antiviral targets are generally distributed in viral replication and assembly, as well as viral attachment and uncoating. To date, the majority of numerous anti-picornavirus compounds with significant in vitro activity against main picornaviral genera do bind to the viral capsid and inhibit either viral attachment or subsequent uncoating. Among these anti-picornavirus compounds, the ‘WIN’ compounds are the most extensively studied^[14,39]. Therefore, a general perspective of picornavirus inhibitors functioning after uncoating (reviewed in detail by De Palma^[3], Shih^[8], Yang^[23], Liang^[91], et al.) and a

detailed review of the developments of the picornaviral capsid-binding inhibitors is provided here.

2. A general perspective of picornavirus inhibitors working after uncoating

2.1. During the viral mRNA translation

Please see the summary in Table 2 and Figure 3.

2.2. During the viral mRNA replication

Please see the summary in Figure 3 and Table 3.

2.3. During assembly of virus

Hydantoin (against PV and CAV) **32** (Fig. 3). Inhibit the encapsidation of viral RNA^[77].

Table 2. Picornavirus inhibitors working during the viral mRNA translation

Target		Inhibitor	Introductory note
Targeting 2A protease		Thiol alkylating agents (against PV)	Among these agents, iodoacetamide 1 and <i>N</i> -ethylmaleimide 2 proved most potent ^[40]
		Elastase-specific inhibitors (against PV and HRV)	Methoxysuccinyl-Ala-Ala-Pro-Val-chloromethylketone (MCPK) and elastatinal are reported to inhibit picornaviral 2A protease ^[42]
		Homophthalimides (against HRV)	Originally designed inhibiting 3Cpro also demonstrated possessing HRV-14 2Apro inhibitory activity ^[43]
		Fluoromethyl ketones-derivatized peptides (against HRV)	Commercially available as inhibitors of caspases and also proved inactivating HRV 2Apro ^[44]
Targeting 3C protease	Peptidic inhibitors	Peptide aldehydes (against HRV), eg. 3	Containing an aldehydized Gln serving as an electrophilic anchoring group and were shown to form reversible covalent adduct with 3Cpro (3 as a representative) ^[45]
		<i>S</i> -nitrothiols (against HRV), eg. 4	Serving as a NO donor through an <i>S</i> -transnitrosylation process ^[46]
		Michael acceptor analogues (against HRV), eg. 5	An electron-withdrawing group irreversibly binding to the active site cysteine of 3Cpro ^[47]
		Rupintrivir/AG7088 and derivatives (against HRV, HEV and CVB3) ^[48] , eg. 6	AP1-lactam-containing peptidic inhibitors highly specific for picornaviral 3Cpro and inhibiting the production of IL-6 and IL-8 in human bronchial epithelial cell line (6 , AG7088) ^[49]
			Unable to exert significant clinical efficiency, further clinical development of rupintrivir was halted
			Aiming at the natural cleavage site of the HRV-14 3Cpro ^[50]
		Diazomethyl ketones (DMK) (against HRV), eg. 7	
		Azapeptides (against HAV), eg. 8	Irreversibly alkylate the active site cysteine of viral protease ^[51]
		Keto-glutamine analogues (against HAV), eg. 9	Contain a phthalhydrazido group α to the ketone moiety affording these compounds inhibit HAV 3Cpro reversibly ^[52]
		Peptidyl monofluoromethylketones (against HAV), eg. 10	Overcome the metabolic degradation of peptidic aldehydes and efficient in vivo ^[53]
	Non-peptidic inhibitors	Peptidyl <i>N</i> -iodoacetamides (against HAV, HRV and PV), eg. 11	Inhibite cysteine protease irreversibly with iodoacetamide moiety ^[54]
		Tripeptidyl α -ketoamides (against HRV), eg. 12	A novel peptidic series exhibiting impressive protease inhibitory activity ^[55]
		Isatins (against HRV), eg. 13	A series of 2,3-dioxindoles containing a reactive α -ketoamide group and being direct against the protease pocket ^[56]
		Quinone analogues (against HRV), eg. 14	Disrupt the activity of cysteine protease by their attackable Michael acceptor moiety ^[57]
		Heteroaromatic esters (against HRV), eg. 15	A π -stacking interaction could be formed with histidine (His40) by the heteroaromatic groups ^[58]
		2-Pyridone-containing peptidomimetics (against HRV), eg. 16	Irreversible inhibitors with high oral bioavailability in animals ^[59]
		Substituted Benzamide Inhibitors (against CV), eg. 17	Possessing a Michael acceptor and a crystal structure mimicking 3Cpro substrate ^[60]
		Homophthalimides (against HRV), eg. 18	Display antiviral activities correlating well with the 3Cpro inhibition data ^[61]
		β -Lactones (against HAV), eg. 19	Irreversibly inhibit the protease ^[62]
	Pseudoxazolones (against HAV and HRV), eg. 20	The imine position on these reagents reacts with the active site of the protease ^[63]	
	Microbial extracts (against HRV), eg. 21	Four microbial fermentation extracts containing either naphtoquinone-lactol, quinine-like citrinin, radicinin or triterpene sulfates ^[64]	

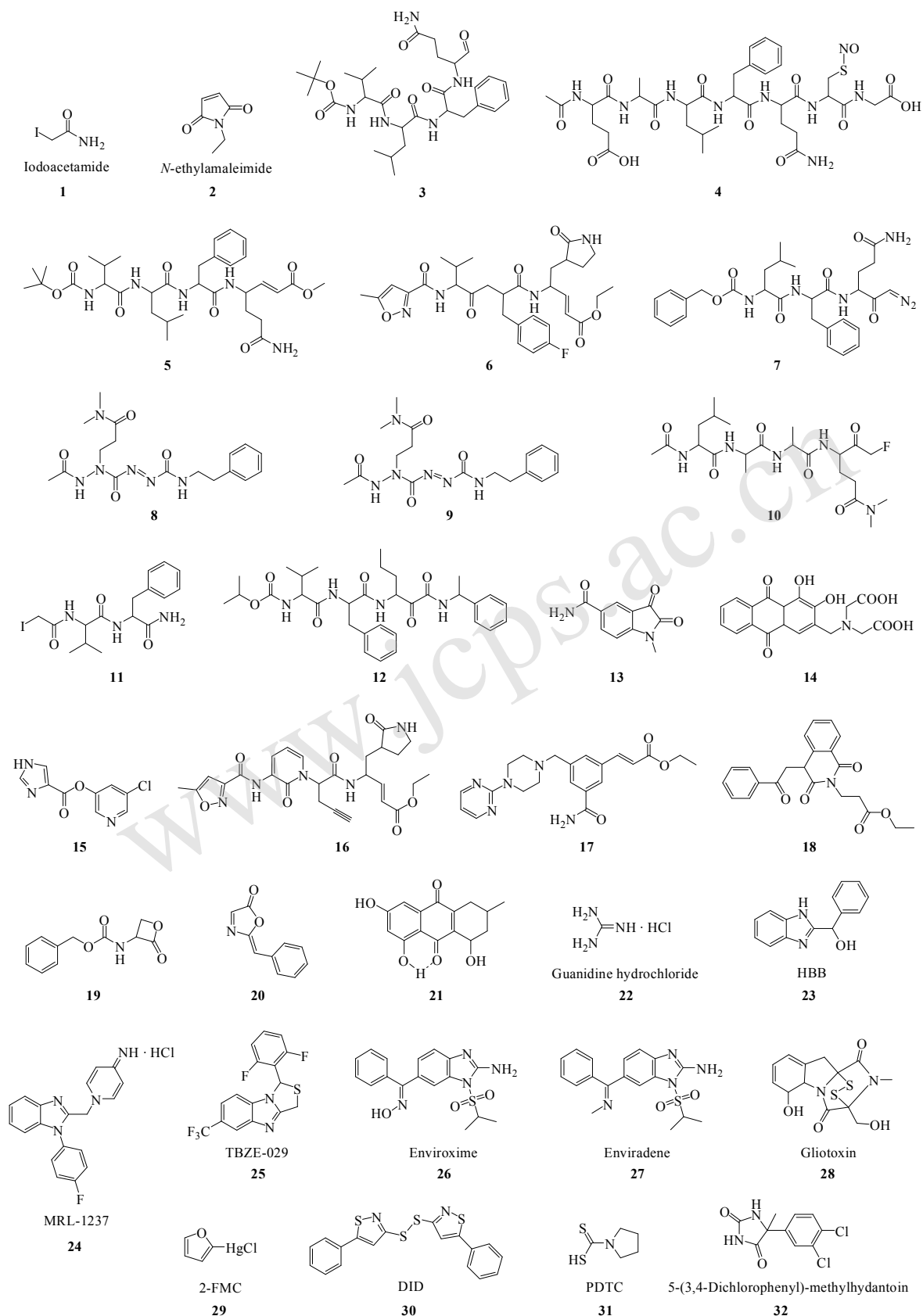


Figure 3. Some picornavirus inhibitors working after uncoating.

2.4. RNAi/Targeting directly the mRNA

Another strategy to inhibit picornaviral that has been widely studied over the last few years is the RNA interference (RNAi) strategy. The RNAi strategy is based on specific gene silencing, by utilizing designed short interfering RNAs (siRNAs). siRNAs are produced from the cleavage of a long double-stranded RNA (dsRNA) by the dicer complex and are subsequently incorporated into a multi-protein RNA-induced silencing complex (RISC). siRNAs then guide RISC to the homologous target mRNA, which is subsequently endonucleolytic cleaved and silenced^[78]. In order to inhibit viral RNA replication by targeting several extremely conserved regions in the viral genome, siRNA sequence Stealth-996, 2570 and 3020, have been synthesized by Jin et al. against HEV^[79]. The VP1 and 3D regions of picornaviral genome have also been chosen as siRNA targets to inhibit the production of viral structure protein VP1 and non-structure protein 3D. A series of siRNAs, including VP1-siRNAs and si-3Ds have been designed against FMDV and EV70^[78,80]. 5'-NCR of picornaviral RNA plays a key role during the RNA replication because of its interaction with 3D^{pol}, thus the 5'-NCR has become a target of siRNA containing locked nucleic acids (LNAs) to hinder the function of 3D^{pol} achieving a high antiviral potency against CBV^[81], or even a target of the DNA antisense oligonucleotides (DNAASOs) to prevent RNase H recruitment in translation^[23].

3. The developments in the research of the picornaviral capsid-binding inhibitors

3.1. Viral capsid and inhibitory mechanism

Picornavirus virions are icosahedrally shaped particles

with 60 protomers, each composed of the four viral structure proteins, VP1, VP2 and VP3, around 30 kD each, lie on the external surface of the capsid, while the relatively smaller VP4 lies inward and is presumably in contact with the viral genome^[82]. On each icosahedral face of the capsid a large cleft are revealed to be present and five of the clefts form a star-shaped plateau by surrounding each of the 12 entamer axes on the virus surface (Fig. 4). The cleft is mostly called “the canyon” and was suggested to be the binding site of host cell receptors. The rim of the canyon in HRV 14 has been reported to be the immunogenic neutralizing sites with considerable sequence variability. The most conserved region on the virus surface is the attachment site that is hidden in the canyon, thus protecting the conserved residues that might be required for host cell receptor recognition

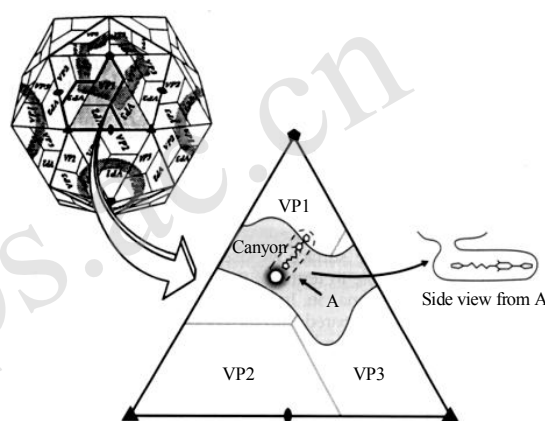


Figure 4. Diagrammatic view of picornavirus with enlargement of one icosahedral asymmetric unit showing the outline of the canyon and the entrance to the WIN pocket^[82].

Table 3. Picornavirus inhibitors working during the viral mRNA replication

Target	Inhibitor	Introductory note
Targeting non-structure protein 2C	Guanidine hydrochloride 22 (against PV, CV, EchV and FMDV)	An extensively studied picornavirus inhibitors which inactivated 2C protein ^[65,66]
	2-(α -Hydroxybenzyl)-benzimidazole (HBB) 23 (against PV, CV and EchV)	Likely interacts with 2C protein but still needs further determination ^[67]
	MRL-1237 24 (against PV and CBV) ^[68]	
	TBZE-029 25 (against HEV, CV and EchV)	A thiazolobenzimidazole derivative that initially discovered as an inhibitor of HIV-1 reported a selective inhibitor to picornaviruses ^[69]
Targeting non-structure protein 3A	Enviroxime and analogues (against HRV and HEV), eg. 26 , 27	A benzimidazole derivative which proved to be highly active against the replication of rhino and enteroviruses in vitro ^[70]
Targeting 3D protein/ RNA-dependent RNA polymerase (RdRp)	Gliotoxin 28 (against PV)	The first inhibitor of this enzyme and effective in animal model ^[71]
Other mRNA replication inhibitors	2-Furylmercury chloride (2-FMC) 29 (against HRV)	A mercury containing compound inhibiting the HRV-2 positive-strand RNA synthesis ^[72]
	Isothiazole Derivatives, eg. DID 30 (against PV)	Prevent viral RNA chain elongation via inhibition of replicase activity ^[73]
	Flavonoids (against PV, HRV and CV)	Interfer with some process of viral replication ^[74]
	Nucleoside analogues (against FMDV, PV and CBV)	Inhibit the viral RNA-dependent RNA polymerase by 2'-C-methylcytidine ^[75]
	Pyrrolidine Dithiocarbamate (PDTTC) 31 (against PV, CBV and HRV)	Interfer with viral RNA and protein synthesis and viral polyprotein processing as well ^[76]

from the host's immune system^[83]. As for the four viral structure proteins, the sequence of VP4 is the most conserved, while VP1 exhibits the greatest sequence variability, which is consistent with several viral life movements, including receptor attachment, viral uncoating and even immunogenic neutralizing^[84]. In the major genera of picornaviruses hydrophobic pockets, an "open" conformation formed by the beta-barrel of VP1, was found just underneath the receptor binding sites, namely the canyon. The hydrophobic pockets usually contain the so-called "pocket factors", which refer mostly to fatty acids with alkyl chains of eight or more carbon atoms long^[82].

HRV is the major causative agents of the common cold and is divided into three receptor groups based on their binding selectivity to three different host cell receptors^[85]. ICAM-1 is the major host receptor of these picornaviruses^[86] (Fig. 5). In the process of viral attachment there is a competition between the binding of the pocket factor to the pocket and the binding of the receptor to the receptor binding sites. As a result, pocket factors usually are repelled out during viral attachment, because the receptor binds better than the pocket factor. And only when the pocket is temporarily empty and allowable for the canyon floor to be deformed downwards can be receptor seat itself into the canyon, thus initiating the endocytosis process and destabilizing the virus as required for uncoating^[82]. Filling the internal hydrophobic cavity, pocket factors increase the thermal stability of the virus, which is required to stabilize the virus in transit from one cell to the next. Therefore, the pocket factor is comparatively a reversible stabilizing factor that is required for regulating rival properties at different stages of their life cycle. Furthermore, the pH of the environment is also an important dimension to the viral life cycle. HEVs are acid stable and retain their infectivity at pH lower than 3.0, while HRV are labile at pH lower than 6.0. In fact, acid pH decreases viral stability and reduces the amount of binding metal ions that appear to participate in regulating the assembly and disassembly of virions^[82,160].

Like the pocket factor, many antiviral compounds can also bind into the hydrophobic pocket, displacing the pocket factor and inhibiting viral uncoating. Among these

compounds, the series of WIN compounds are the most extensively studied. Structural studies of several WIN compounds in complex with HRV-14 have revealed the WIN binding site to be underneath the floor of the canyon^[87,88]. However, for some inhibiting compounds, the binds to the binding site are weaker than that of ICAM-1, and little inhibitory activity is presented. Therefore the affinity of an antiviral compound for the pocket needs to be higher than that of ICAM-1 for its binding site^[82]. The binding of the inhibitory compounds induces the consequent drastic conformational changes in the floor of the canyon, thus the virus attachment is prevented as a consequence (Fig. 5). As the amino acid sequence constituting the hydrophobic pocket in VP1 correlates strongly with the virus susceptibility, sometimes the antiviral agents only displace the pocket factor and cause minor conformational changes in the virus structure that are not sufficient to inhibit viral attachment^[89]. Despite of these minor changes, one or three additional interprotomer hydrogen bonds are formed, causing a potential loss in the flexibility of the capsid. Thereby, the pentamer channel through which the infectious RNA was delivered into the cytoplasm is constricted or stiffened and has lost its flexibility required for uncoating. The viral uncoating is thus halted^[90]. In summary, WIN compounds prevent viral attachment through causing drastic conformational changes in the floor of the canyon, or hamper viral uncoating via causing a potential loss in flexibility of the pentamer channel.

In addition, studies on HRV-14 revealed that shorter, more hydrophilic WIN fragments (WIN 52452 and WIN 58768) can bind into the pocket and mimic the cellular cofactors observed in the hydrophobic pocket for some picornaviruses, but are unlikely to inhibit viral attachment^[91]. However, The binding of WIN compounds increases the capsid stability and also the degree of the binding metal ions, which is the opposite effect of acidic pH and breaks the viral life regulation. Drug-resistant or -dependent variants of VP1 cleft generated for WIN compounds in HRV-14, 16, poliovirus type 3 (PV-3) and coxsackievirus B3 (CBV-3) also provide additional evidence for the interaction between WIN compounds and the viral capsid^[92,93].

3.2. Evolution of the WIN compounds

3.2.1. Isoxazols

WIN compounds refer to a class of viral capsid-binding inhibitors. Research on the WIN compounds was initiated by the Sterling Winthrop Pharmaceuticals Research Division and other pharmaceutical companies about three decades ago. These compounds are often known as a serial number prefixed with the abbreviation "WIN" from "Winthrop", and are characterized with the ability to bind the hydrophobic pocket within VP1, located beneath the canyon^[82]. The program directed towards the discovery of WIN compounds was initiated from Arildone (**34**, Fig. 6), a compound derived from benzodioxol diketone intermediate (**33**) for ajuvenile hormone mimetics. The compound not only exhibited in vitro activity against herpesvirus^[94], but was also found to selectively inhibit poliovirus replication

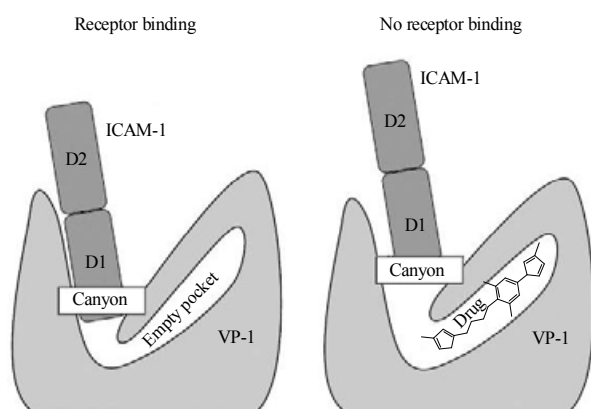


Figure 5. Mechanism of the inhibitory activities of WIN compounds^[3].

in vitro. It was further demonstrated that the compound efficiently inhibited the virus in vivo in mice infected intracerebrally with polio-2 virus in 1982^[95]. Then a series of related compounds have been synthesized and screened by Diana et al. for an agent with broad spectrum activity against picornaviruses. As a result, it was discovered that replacing the diketone moiety with a methylated isoxazole ring in the structure of Arildones generated a compounds possessing in vitro activity against both HRV-2 and polio-2, among which compounds **35** and **36** exhibited the highest efficiency^[96]. Compound **35** not only showed higher inhibitory activity than 4',6-dichloroflavan against HRV-2 and polio-2, but also was efficient against polio-3,9 and Echo-11, while **36** was the most efficient isoxazoles compound against HRV-2 with an MIC of 0.01 $\mu\text{g/mL}$. However, these isoxazoles, especially **36**, is metabolically highly unstable, as there was a carbethoxyl group on its benzene ring. As a result, this compound failed to exert any observable effect in infected mice, most likely due to the metabolic hydrolysis of the functional group.

3.2.2. Disoxaril

To develop an orally effective anti-picornaviruses agent, several modifications to the ester group were introduced to the original WIN compound. The compound oxazoline was prepared as a cyclic variation of the ethyl ester for its similar space-filling requirements. The oxazolines possess outstanding anti-viral activities among various homologues after one oxygen atom in the molecule was replaced with a nitrogen atom^[97]. This was the WIN 51711 (**37**, Fig. 7), also known as Disoxaril, showing an anti-polio-2 activity of 0.004–0.1 $\mu\text{g/mL}$ in MIC and an anti-HRV-2 activity of 0.1 $\mu\text{g/mL}$ ^[98]. The five-, six- and seven-carbon chain homologues exhibited comparable activities while the four- and eight-carbon chain homologues were considerably less effective. In addition, the effect of substitution of the oxazoline ring on antipicornavirus activity was studied to find that substitution on the oxazoline ring generally leads to a reduction in activity against polio-2, but the one (**38**, Fig. 7) with a methyl group on the 4-position, shown a relative increase in its activity against HRV-2. Disoxaril was evaluated in vivo and it was found that an oral dose

of 4 mg/kg led to significantly reduced mortality in mice infected intracerebrally with HRV-2. Disoxaril was also shown to be effective in preventing paralysis when administered intraperitoneally to mice infected subcutaneously with a lethal dose of EchV-9. Disoxaril is the first compound to be clinically evaluated among the WIN compounds^[96,99]. However, it was found that high dose of disoxaril induces crystalluria in patients, leading to the removal of disoxaril from clinical studies^[100]. X-ray crystallographic studies of disoxaril and the other 4-methyloxazoline analogues bound to rhinovirus-14 clearly demonstrated that these compounds bind in a specific hydrophobic pocket within viral capsid protein 1 (VP1) and thus hinder viral uncoating^[96].

3.2.3. 2,6-Dichloro analogue (WIN 54954)

Since binding of the WIN compounds to the virion is dependent upon the amino acids in the binding pocket, any changes in this region could accordingly affect the binding and, consequently, the antiviral activity of the compounds. Studies have shown that compared to the unsubstituted disoxaril analogues, substituents at the 2-position of the phenyl ring greatly improved the compound's anti-rhinovirus activity and were prompt to broaden the antiviral spectrum as well. Notably, a five-carbon chain was required for optimum activity in the new series of compounds. In the analysis of the mono-substituted analogues in this series, good correlations between MIC and molecular weight (MW), LogP and σ_m (an electronic descriptor) were found. However, when the QSAR research was conducted on the disubstituted compound series, no correlation between MIC and σ_m or MW was found, although a better correlation between LogP and MIC ($R = 0.93$) was observed. Since lipophilicity of either the monosubstituted or disubstituted analogues still correlated with their antiviral activity, the drug-binding pockets in the virus were predicted to be hydrophobic in nature. This study also revealed that there were spatial constraints in these pockets as they restricted the entry of compounds with bulk substitute groups, like C_2H_5 and $t\text{-Bu}$ (**39** and **40**, Fig. 8), and prevented them from exhibiting significant antiviral activity. The apparent curve at the end of the line generated by $\log [1/\text{MIC}]$ vs $\log P$ might also be a reflection of these limitations^[101].

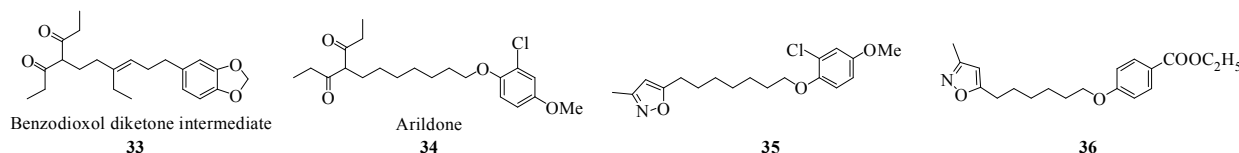


Figure 6. Structures of isoxazol compounds.

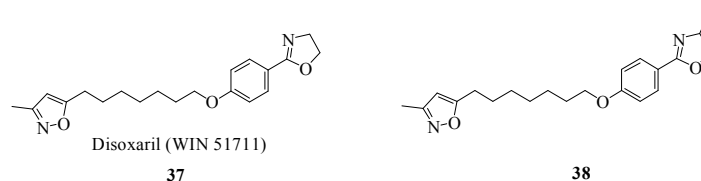


Figure 7. Structures of compounds **37** and **38**.

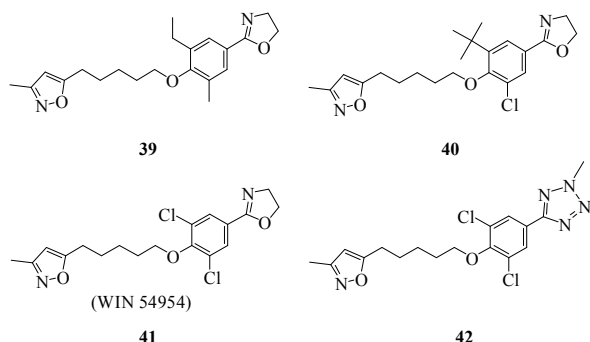


Figure 8. Structures of 2,6-dichloro analogues.

Among the WIN compound series studied above, the 2,6-dichloro analogue WIN 54954 (**41**, Fig. 8) demonstrated the highest level of activity against 15 HRV serotypes with an MIC_{80} of 0.33 μM . It also showed potent anti-viral activities against several enteroviruses. Echovirus-12 (EchV-12) and coxsackievirus A-9 (CAV-9) are particularly sensitive to WIN 54954 with MICs of 0.008 and 0.05 μM respectively. WIN 54954 exhibited a broader spectrum of activity and greater viral sensitivity than disoxaril. WIN 54954 has inhibitory activity against 80% of the HRV serotypes at a concentration of 0.25 $\mu g/mL$ or less, while disoxaril only inhibits 12% of the HRV serotypes^[101]. WIN 54954 was also demonstrated to be effective in murine models infected with strains of CBV-4 and CAV-9^[102]. WIN 54954 was also tolerated in phase I clinical trial in humans infected with CAV-21, but lacked the activity against HRV-23 and 29^[103]. However, WIN 54954 was metabolized quickly and induced reversible hepatitis, making it an unpromising candidate for further studies^[104]. In 1992, a variety of heterocyclic derivatives of WIN 54954, in which the oxazoline ring was changed to the benzene ring or was replaced with different heterocyclic rings, were synthesized and tested for their antiviral activity against HRV-14. A QSAR research was carried out in order to evaluate the antiviral activities of the compound series. However, no meaningful correlation was observed between their antiviral activities and any of the physicochemical parameters. Fortunately, the 2-methyltetrazole derivative (**40**, Fig. 8) in this series exhibited the highest level of activity comparable to WIN 54954^[105].

3.2.4. 2-Methyltetrazoles (WIN 61605)

WIN 54954 is easily metabolized mainly because the oxazoline ring suffers from acid lability and the three hydrolysis products are completely devoid of anti-rhinovirus activity. As reported, one of the most promising replacements for the oxazoline ring is 2-methyltetrazole, which is acid stable while maintaining potent broad-spectrum activity^[105]. Consequently, a series of analogues of **42** (mentioned above) were prepared. It was found in this series that the dimethylphenyl analogues exhibit greater bioavailability than the corresponding dichlorophenyl counterparts, maintaining the potent activity comparable to **42**. Among the dimethylphenyl analogues, the compounds with a three

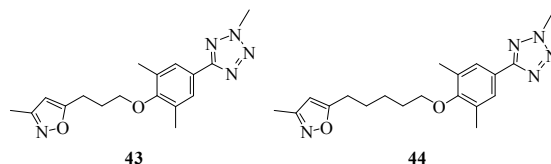


Figure 9. Structures of 2-methyltetrazole compounds.

and five-carbon linkage between the ether oxygen atom and the isoxazole ring showed similar antiviral activity, respectively (**43** and **44**, Fig. 9). For the compound with a five-carbon linkage, changing the methyl group on the tetrazole ring to longer alkyl substituents resulted in an activity reduction against both HRV-14 and HRV-1A. Studies suggested that the antiviral activity correlates with compounds' ability to optimally occupy space of the binding site, particularly in the pore area near the entry of the pocket. Also the sizes of the HRV-1A and HRV-14 binding sites are different. The binding pocket of HRV-1A is shorter and wider, suggesting that a shorter molecule with more flexibility would be desirable for broad-spectrum activity. Thereafter, the side chain on the isoxazole ring of **43** was extended to generate the propyl homologue WIN 61605 (**45**, Fig. 10), which showed a substantial improvement in its potency against HRV-14 while retaining activity against HRV-1A. WIN 61605 also demonstrated a broader spectrum of activity with an MIC_{80} of 0.2 μM for 15 HRV serotypes. The X-ray structure of WIN 61605 bound to HRV-16 showed that the propyl side chain extends into the pore of the binding site with a possible hydrophobic interaction in this area, while the phenyltetrazole portion participates to form a stacking conformation with Tyr¹²⁸ and Tyr¹⁵² residues in the same orientation as WIN 54954 in the pocket^[106].

3.2.5. Oxadiazoles (WIN 61893)

WIN 61605 resulted from this study, was considered a clinical candidate for the treatment of rhino- and enteroviral infections. Unfortunately, administration of this compound to beagles induced an increase in the liver production of cytochrome P450. The hepatotoxic effects were assumed to result from the tetrazole ring or its metabolic product, which prevented WIN 61605 from undergoing further clinical trial. At this point, other bioisosteres with both metabolic stability and biosecurity became the best option. Oxadiazoles, which possess muscarinic agonist activity^[107] and partial benzodiazepine receptor agonist activity^[108], were first proposed as ester bioisosteres. Thereby, a series of 1,2,4-oxadiazoles with a variety of substituents at 5-position of the oxadiazole ring were prepared. The methyl homologue WIN 61893 (**46**, Fig. 10) exhibited excellent activity against the 15 HRV serotypes tested. Increasing the length of the side chain attached to the oxadiazole ring resulted in a reduction in its activity. If any hydrophilic group were introduced in this position, the compounds would become inactive. However, removal of this methyl group entirely rendered the compound weakly active. The methyl group on the isoxazole ring can be replaced by short alkyl chains

or alkoxyalkyl groups without much increase in activity, whereas introduction of a hydroxyl group to the side chain led to a notable decrease in activity. These results were also in agreement with conclusions obtained from volume map and CoMFA studies^[109] on related series of compounds bound to HRV-14 where the binding site was naturally hydrophobic. Two other isomeric oxadiazoles were synthesized and evaluated as well. And the result shows that they are both of lower potency than WIN 61893^[100].

3.2.6. Pleconaril (WIN 63843)

WIN 61893 would make a promising anti-picornaviral agent except for the fact that its metabolic rate is not markedly different from WIN 54954, which is not sufficient to reach the criteria for further studies. The examination of the metabolic stability in vitro was actualized by monkey liver microsomal assay, which most closely approximated the metabolism in human body. An approach to design an analogue with comparable or higher activity and enhanced metabolic stability is to determine the nature of the metabolites, ascertain the major metabolic sites and then block them. In the monkey liver microsome assay, two major products, which are monohydroxylated at each of the terminal methyl groups of WIN 61893 (3:1 in ratio), were observed. The majority of the metabolic products were monohydroxylated at the methyl group attached to the isoxazole ring. Hence a trifluoromethyl group was initially introduced at this position to replace the methyl group (47, Fig. 11). Three major metabolites were obtained from the new compound and the major product was hydroxylated at the methyl group attached to the oxadiazole. However, this modification did not appear to have enhanced the overall metabolic stability of the molecule. Thus, another attempt to stabilize the molecule was made hydroxylating the methyl group on the oxadiazole ring, resulting in WIN 63843 (48, Fig. 11). This is the compound later called “pleconaril”. Approximately 96% of this compound was recovered unchanged in the monkey liver microsome assay and the principle metabolic product was a monohydroxylated derivative. The percentage of the unchanged compound revealed a phenomenon called the “global protective effect”, which was further demonstrated by the result of the monkey liver microsome assay on analogues with various substituents on the isoxazole ring and on several derivatives with replacements of the isoxazole ring with other heterocycles. In addition, the trifluoromethyl analogues displayed significantly greater stability when compared to the methyloxadiazoles. Actually the global protective effect was not restricted to the trifluoromethyl group. The incorporation of cyclopropyl, difluoromethyl, carboxamide and ethoxy substituents into the 5-position of the oxadiazole ring also provided protection against P-450 metabolism. The mechanism can be proposed as the overall change of the electrostatic potential of the molecule induced by the trifluoromethylated isoxazole, which made the binding to the P-450 more difficult and caused the resistance to metabolism^[104].

Intravenously administered pleconaril showed prolonged half-life and significantly decreased in the plasma clearance

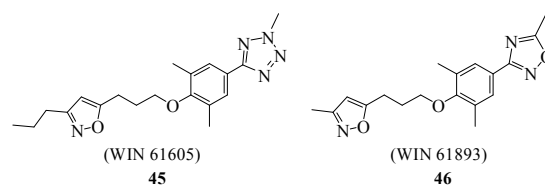


Figure 10. Structures of oxadiazole compounds.

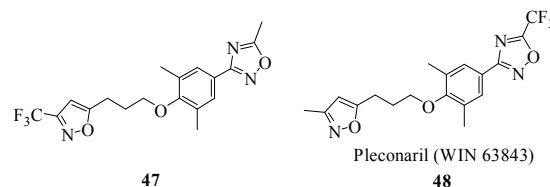


Figure 11. Structures of compounds 47 and 48.

value in beagles^[104]. Pleconaril was licensed to ViroPharma in 1997 and later a broad antiviral spectrum of this agent against enteroviruses was found^[110]. Pleconaril sailed through clinical trials testing its pharmacodynamics and pharmacokinetics via applying it to treating a series of severe infections caused by various HEVs^[111]. However, in a double blind, placebo-controlled trial of pleconaril in infants suffered from enterovirus meningitis, several adverse effects of the drug were discovered^[112]. In a phase III clinical trial, pleconaril was proven to reduce the duration and the severity of common cold symptoms^[113]. Unfortunately, based on drug interactions resulting from the activation of cytochrome P-450 3A enzymes^[114] and marginal treatment effects^[115], the US FDA rejected pleconaril as a curative for the common cold. In 2003, ViroPharma licensed pleconaril to Schering-Plough. And in 2007, a phase II trial of pleconaril nasal spray on common cold symptoms and asthma exacerbations caused by HRV exposure was completed without the result reported.

3.3. Other picornaviral capsid-binding inhibitors

3.3.1. Pirodavir and BTA compounds

Pirodavir (R77975) (Fig. 12) is a compound containing a pyridazine moiety^[116]. This compound exhibited an antiviral activity against 80% of HRV serotypes at the concentration of 0.064 $\mu\text{g/mL}$ or less, rendering them non-infectious by directly contacting and neutralizing them. The compound is believed to possess a direct interaction with the viral capsid^[117]. This mechanism was further demonstrated by drug-resistant mutants, which exhibited cross-resistance to WIN compounds. The crystallographic study on the binding complex of predecessor R61837 and the capsid protein revealed that the compound binds to the same hydrophobic pocket as the WIN agents^[118]. In experimentally induced HRV infection, Pirodavir showed high clinical efficacy and hindered the viruses from shedding. However, in a trial against naturally occurring rhinovirus colds, no therapeutic efficacy was found of intranasally administered pirodavir^[119]. This was later explained by the rapid hydrolysis of the ester moiety on its structure. Thus two series of bioisosteric analogues of

pirodavir were synthesized, one was the oxime ethers and the other was the 2-ethoxybenzoxazoles. In the first series, the compound designated as BTA-188 (Fig. 12) and its analogue BTA-39 (Fig. 12) inhibited 59 HRV strains with the EC_{50} values ranging from 0.5 nM to 6.7 nM^[120]. The second series have a 2-ethoxybenzoxazole moiety in the place of the benzaldehyde oxime ether moiety in BTA-188 and showed an equivalent activity to BTA-188. This compound is also approximately 10 times more active than pleconaril, has greater hydrolytic stability, longer half-life and oral bioavailability^[121]. The oral bioavailable BTA-798 (Fig. 6) in this series was developed for patients with COPD and asthma against HRV infections. BTA-798 also showed a potent activity against HEV-71^[122].

3.3.2. Pyridyl imidazolidinones

Pleconaril was observed to be unable to neutralize the cytopathic effect (CPE) in cultured cells caused by HEV isolates from the 1998 outbreak in Taiwan, China^[123]. To develop agents against this serotype of HEV, pleconaril and its analogues were used as templates for computer assisted drug design (CADD). As a result, imidazolidinones emerged and was shown to have significant antiviral activity. A *para*-position substituted phenoxy ring and a pyridine connected with imidazolidinone are essential for the activity against HEV-71^[123]. The compounds aim to inhibit virus attachment or uncoating as revealed by the antiviral mechanism study of BPR0Z-194 (Fig. 13). This was further confirmed by sequence analysis of the drug-resistant viruses, revealing a mutation at the position 192 of VP1^[124]. New series of pyridyl imidazolidinones (**54**, Fig. 13) with an oxime ether moiety as the substituent of phenoxy ring exerted a high specificity for HEV, particularly HEV-71 with an EC_{50} value as low as 1.0 nM^[125]. It was found that the inhibitory activity of these compounds could be dramatically improved with a substituent of oxadiazole or tetrazole ring at the *para*-position of the phenoxy group and a methylation at the 2 or 3-position of the link chain.

3.3.3. Biphenyl derivatives

As the CBV3 Nancy strain is resistant to pleconaril, a series of [(biphenyloxy)propyl]isoxazole derivatives of pleconaril was synthesized (**55**, Fig. 14). These compounds showed excellent activity against HRV-2, and moderate activity towards the pleconaril-resistant CVB3 Nancy strain^[126].

3.3.4. 4',6-Dichloroflavan (BW 683C) and 3(2H)-isoflavene

As an anti-rhinovirus compound, 4',6-dichloroflavan (BW 683C) (Fig. 15) was developed and evaluated. It has an EC_{50} value ranging from 7 nM to 170 nM, but is only active against a limited number of HRV types^[127]. The mechanism of antiviral action of BW 683C was studied. It was shown to bind the hydrophobic pocket to inhibit viral uncoating^[127,128]. This compound possesses good oral bioavailability, but failed to protect healthy volunteers from experimental rhinovirus infection in clinical trials^[129]. 3(2H)-Isoflavene (Fig. 15), another analogue of the flavans, was reported to exhibit a much broader spectrum against picornaviruses than BW 683C^[130]. Analysis of drug-resistant viruses carrying mutations in VP1 confirmed its uncoating inhibiting activity^[131].

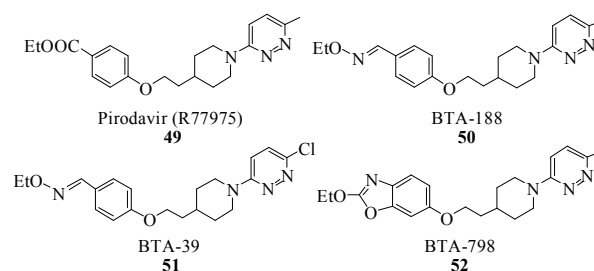


Figure 12. Structures of pirodavir and BTA compounds.

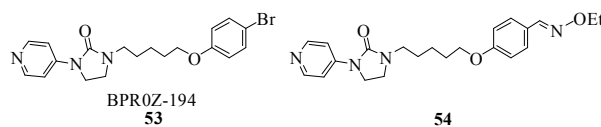


Figure 13. Structures of pyridyl imidazolidinones.

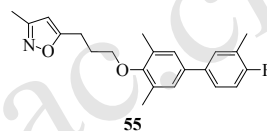


Figure 14. Representative of biphenyl derivatives.

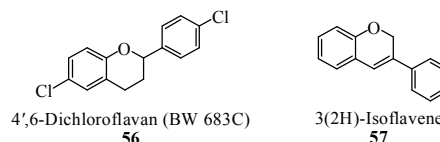


Figure 15. Structures of 4',6-dichloroflavan (BW 683C) and 3(2H)-isoflavene.

3.3.5. MDL compounds, phenoxybenzenes and phenoxy-pyridines

From a phenylpyranopyridine parental structure, a series of MDL compounds, referred to as the 6 position-substituted 2-(3',4'-dichlorophenoxy)-2H-pyrano[2,3-β]pyridine, were prepared by Marion Merrell Dow. This series of compounds inhibit the replication of 32 serotypes of HRV with an EC_{50} values as low as 0.006 μg/mL against^[132]. These compounds bind to the capsid and inhibited viral uncoating as showed by mechanism studies with MDL 20610 in this series (Fig. 16)^[133]. At the same company, a series of phenoxybenzenes were also synthesized. The most active compound in this series, 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile (MDL-860) (Fig. 16)^[134], possesses broad-spectrum anti-picornavirus activity and inhibites 80% of the HRV and HEV strains tested at a low concentration of 1 μg/mL^[241]. CBV-3 and CAV-21 were also susceptible to MDL-860 in murine models, confirming the *in vivo* activity of this compound^[134]. To minimize the toxicity associated with nitril group and maintain the optimal structural skeleton, a series of phenoxy-pyridine carbonitriles were synthesized,

among which 6-(3,4-dichlorophenoxy)-3-(ethylthio)-2-pyridinecarbonitrile (DEPC) showed high in vitro and in vivo activities and protected mice from lethal CAV-21 infection via oral administration of 37.5 mg/kg/d^[135]. The mechanism studies confirmed that DEPC targets viral uncoating^[136].

3.3.6. SCH compounds and SDZ compounds

SCH compounds refer to a class of compounds developed by Schering-Plough. The compound with a phenoxy imidazole structure (SCH38057) (Fig. 17) showed an inhibitory activity against several HEVs and HRVs in vitro and against CBV-3 or EchV-9 in vivo^[137]. Crystallographic studies revealed that the compound binds at the innermost end of the hydrophobic pocket^[138]. Another compound SCH 47802 (Fig. 11) exerts strong activity against PV, several HEVs and CVs^[139]. SDZ compounds are another class of anti-rhinoviral compounds sharing the piperazine-ring motif developed by the Sandoz Forschungs institute. In this class, SDZ 880-061 inhibited 85% of HRV serotypes tested at concentrations lower than 3 µg/mL^[140]. Structural studies of SDZ 880-061 bound to HRV-14 revealed that this compound binds in the same hydrophobic pocket, but leaves the innermost portion of the pocket vacant, which is different from the action of SCH38057^[140]. Comparably, SDZ 35-682 (Fig. 17) was shown to fill the entire hydrophobic pocket, but with a restricted spectrum^[141].

3.3.7. Chalcones

At Roche, 40-ethoxy-20-hydroxy-4,60-dimethoxychalcone (Ro 09-0410) (Fig. 18) was identified as a potent inhibitor of rhinoviruses. This compound has a specific binding pattern to the rhinovirus capsid, has showed no activity against other picornaviruses tested. Its amide analogues were 10 times more active than Ro 09-0410 against HRVs^[142].

3.3.8. Rhodanine

Rhodanine (Fig. 18), 2-thio-4-oxothiazolidine, is a selective inhibitor of EchV-12 without inhibitory effect against other viruses. It was confirmed to be an uncoating inhibitor as virions exposed to rhodanine were inactive to heat or alkaline degradation^[143].

3.3.9. Dibenzofuran and dibenzosuberol derivatives

In HRV-14- and HRV-16-induced CPE (chronic pulmonary emphysema) reduction assays, 2-hydroxy-3-dibenzofuran carboxylic acid and dibenzosuberone (Fig. 19), from a series of dibenzofuran and dibenzosuberol derivatives, respectively, were revealed to have inhibitory activity against HRV replication. Exposure to the compounds after viral absorption led to the loss of their antiviral activities, suggesting that they act as capsid binding inhibiting agents^[144].

4. Summary

The number of picornaviruses is enormous. They consist of 13 genera and cause an extensive range of clinical manifestations of different severeness among human beings

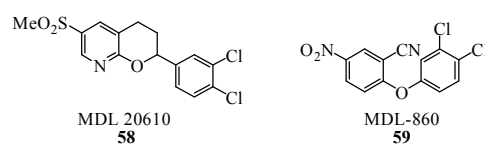


Figure 16. Structures of MDL compounds.

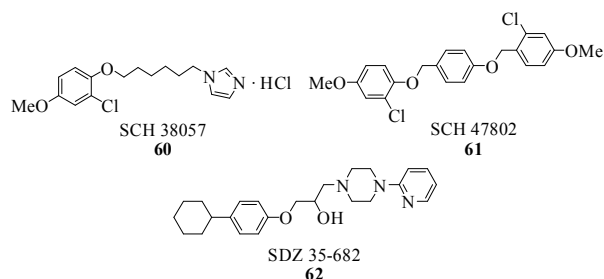


Figure 17. Structures of SCH and SDZ compounds.

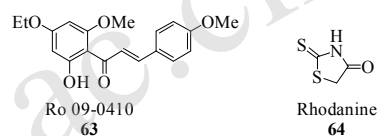


Figure 18. Structures of chalcones and rhodanine.

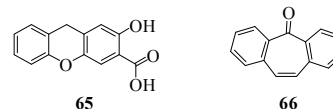


Figure 19. Structures of dibenzofuran and dibenzosuberol derivatives.

as well as animals, some of which can even be life-threatening. Classification of picornaviruses is rather complicated, but the viruses infecting humans are commonly found in six genera, especially the largest enterovirus genus including 219 virus types. This genus has been widely investigated and chosen as the major flora for antiviral drug developments, because this genus embraced various main pathogens to human, such as PVs, HRVs, CVs and other numbered HEVs. They possess highly conserved genome and proteome and very similar life circles, which lays the foundation for the developments of broad-spectrum antiviral agents.

The majority of numerous anti-picornavirus compounds target the viral capsid and inhibit either viral attachment or subsequent uncoating. The hydrophobic pockets on viral capsid, which is an “open” conformation formed by the beta-barrel of VP1, were found just underneath the host cell receptor binding sites. Viral capsid-binding inhibitors bind into the hydrophobic pockets and induce conformation changes in the pocket or stiffen the pentamer channel, thus preventing viral attachment or uncoating. Among these inhibitors, the WIN compounds are the most extensively studied. Evolution of the WIN compounds

progressed successively through isoxazols, disoxaril, 2,6-dichloro analogue (WIN 54954), 2-methyltetrazoles (WIN 61605), oxadiazoles (WIN 61893), and finally the pleconaril (WIN 63843). In 2007, a phase II trial of pleconaril nasal spray on common cold symptoms and asthma exacerbations caused by HRV exposure was completed. Other picornaviral capsid-binding inhibitors, such as pirodavir (BTA compound), pyridyl imidazolidinones and MDL compounds, were also studied and found to have potent inhibitory activity. Other anti-picornavirus agents mainly target 2A and 3C protease during viral mRNA translation, or target non-structure protein 2C and 3A and 3D protein/the RdRp during viral mRNA replication. However, the exact mechanism of the anti-viral activities of these drugs still remains to be elucidated. Over the last few years, the RNA interference (RNAi) strategy has been widely studied as a promising picornaviral inhibitory strategy.

Acknowledgments

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小核糖核酸病毒抑制剂研究及WIN系列抗病毒化合物的发展

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摘要: 小核糖核酸病毒科是人类病毒性病原体中最大的家族之一。它可以导致一系列不同程度的临床症状, 从轻微的发热、普通感冒到严重的瘫痪型脊髓灰质炎、慢性阻塞性肺病等, 其中有一些极具致命性。小核糖核酸病毒还会引起动物性大流行, 给人类社会带来巨大的经济损失。虽然到目前为止, 还没有正式批准的能够有效预防或治疗小核糖核酸病毒感染的药物上市, 但是大量具有很好的抗小核糖核酸病毒活性的化合物已经被研发出来。通过对这些物质的研究, 也揭示出许多有关小核糖核酸病毒的信息。病毒信使RNA的翻译过程, 复制过程和病毒衣壳蛋白集中了这些被广泛研究的抗病毒物质的主要靶点。其中一类通过键合病毒衣壳, 抑制病毒吸附和脱壳的WIN系列化合物最为典型。因此本文将介绍抗小核糖核酸病毒化合物研究的总体概况并对WIN系列化合物的具体演进过程进行讨论。

关键词: 小核糖核酸病毒; 抗病毒物质; 衣壳键合抑制剂; WIN系列化合物; 普来可那立

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