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Back to the Future: Advances in Development of Broad-Spectrum Capsid-Binding Inhibitors of Enteroviruses

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Abstract

The hydrophobic pocket within viral capsid protein 1 is a target to combat the rhino- and enteroviruses (RV and EV) using small molecules. The highly conserved amino acids lining this pocket enable the development of antivirals with broad-spectrum of activity against numerous RVs and EVs. Inhibitor binding blocks: the attachment of the virion to the host cell membrane, viral uncoating, and/or production of infectious virus particles. Syntheses and biological studies of the most well-known antipicornaviral capsid binders have been reviewed and we propose next steps in this research.

Keywords

antivirals; drug design/discovery; common cold; pleconaril; rhinovirus; enterovirus

Introduction

Respiratory enteroviruses (EVs) and rhinoviruses (RVs) belonging to the family *Picornaviridae* represent the leading cause of upper respiratory tract infections leading to millions of lost schools and working days [1, 2, 3, 4]. In addition, their role in lower respiratory tract infections is increasingly reported [4, 5]. RVs also exacerbate asthma [6, 7] and chronic obstructive pulmonary disease [8]. EV may additionally result in a wide range of other acute and chronic diseases, e.g. foot-hand-and mouth-disease, poliomyelitis, heart disease, encephalitis, meningitis, severe bronchiolitis and pneumonia [9]. Vaccine development for these viruses is complicated by the multiplicity of serotypes. About 100 human EV serotypes (species A-D) and more than 160 RV (species A-C) serotypes exist with most having epidemiological significance. Today, there are only vaccines to prevent poliomyelitis caused by polioviruses (PV) [1–3, 10] and two inactivated enterovirus A 71 (EV-A71) vaccines in China for prevention of hand-foot-and-mouth disease [11]. Due to the

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lack of effective drugs, current treatment of EV and RV infections aims to reduce and shorten symptoms (e.g. fever and pain, fatigue, and nasal blockage in the case of the common cold). Because of problems with vaccination, development of effective antivirals is the only alternative. Considering the huge number of serotypes, effort has focused on the discovery of drugs with broad spectrum activity inhibiting various EV and RV serotypes. Cellular proteins e.g. cellular receptors such as ICAM-1 (inter-cellular adhesion molecule 1) for human rhinoviruses and viral proteins that are highly conserved among serotypes (e.g. protease, polymerase, hydrophobic pocket in VP1) might represent potential targets for inhibitors acting against multiple serotypes and thus having broad-spectrum activity. Consistently compounds binding to the viral protease, polymerase or into a small hydrophobic pocket in capsid protein 1 (VP1) have been developed that inhibit multiple serotypes *in vitro* [12]. The most promising results have thus far been obtained with the capsid-binding inhibitors pleconaril and vapendavir both inhibiting various EVs and RVs of species and B (broad-spectrum inhibitors) [13, 14]. In 2002, pleconaril (Picovir) failed to win approval from the FDA for the treatment of the common cold due to limited efficacy, resistant viral strains, and the interference with metabolic pathways of other drugs leading to significant adverse effects (such as headache or menstrual dysfunction etc) [15]. To date, this was the closest a drug has come as a potential treatment for these viruses. The most recent clinical failure of note in this class of compounds is vapendavir (BTA789; Avigen) which failed in Phase IIb for treatment of rhinoviral infections in patients with asthma in February 2017 [16]. Hence further effort is needed to overcome the known shortcomings of these molecules (such as interference with metabolic pathways of other drugs, drug resistance etc.) in order to obtain a drug that can ultimately be approved by regulators and reach patients to be used alone or in combination to combat EV and RV infections. A further challenge represents the RV species C, discovered in 2006, that are not targeted by these broad-spectrum capsid-binding inhibitors [17].

This review aims to summarize our current knowledge about the synthesis, structure-activity relationships and issues in the development of capsid-binding inhibitors as potent broad-spectrum anti-enteroviral drugs. We will first provide a short overview of the virus structure and replication cycle focusing on viral capsid proteins and their function, in particular VP1. Second, we will summarize the discovery of synthetic hit compounds from different chemical classes binding to VP1 of EVs and RVs. We provide an overview of synthesis and biological evaluation of WIN- and WIN-like-compounds with capsid-binding mechanism of action and outline synthesis and biological evaluation of inhibitors from other chemical classes with the same mechanism of action.

We hope that the complex review of synthetic pathways and activity of WIN-compounds will help medicinal chemists to develop their own strategy for future discovery of more potent antiviral agents. The syntheses that are described of other significant capsid-binding inhibitors will help researchers quickly understand the structure activity relationships and molecular features which they can use in their own future next generation compounds.

1. Structure and specific function of VP1 in the viral replication cycle

VP1 is one of the four viral capsid proteins (VP1–4; each having 60 copies) composing the icosahedral capsid of picornaviruses (about 30 nm in diameter) (Fig. 1). Together with VP2 and VP3, VP1 is found on the external surface of the capsid, while VP4 is located inside the capsid. The capsid proteins pack the viral genome, a single-stranded RNA of positive polarity (mRNA-like structure), via interactions with VP4.

The external localization of VP1–3 predisposes their interaction with surface molecules of immune and host cells (VP1–3). Their loops act as antigenic sites. The canyon enables binding to one or multiple cell surface molecules (e.g. sialic acid, ICAM, LDL, DAF, or CAR) for enteroviral attachment to susceptible host cells [10]. Beneath the canyon, there is a small hydrophobic pocket built by amino acids of VP1 that are conserved among EVs and RVs leading to similarities in the pocket structure [18–20]. In the majority of EVs and RVs this pocket is filled with a fatty acid (so-called pocket factor). In contrast, the hydrophobic pocket in VP1 of RV-C is filled with multiple bulky residues, likely making this impossible for currently known inhibitors to bind. Receptor binding and/or pH changes in endosomes induce structural changes in the viral capsid triggering the release of the pocket factor and/or subsequently capsid destabilization as reviewed recently by Baggen *et al.* [10]. The viruses penetrate into the host cell and release their genomic RNA into the cytoplasm (uncoating) where protein synthesis, genome replication, and virion assembly of picornaviruses takes place (Fig 2).

Theoretically, each stage of the viral life cycle is a separate potential target for blockage of viral replication. Inhibition of attachment of the virus to the host cell wall and/or uncoating are seen as the most preferable strategy in our opinion. Whereas the use of soluble receptor-fragments, antibodies that bind to receptors or receptor-binding motifs on the viral capsid is limited to viruses sharing a common receptor [10], binding of specific, so called capsid-binding inhibitors into the hydrophobic pocket in VP1 was shown to have the potential to block multiple EVs and RVs (with the exception of RV-C, as described earlier). The development of such broad-spectrum inhibitors acting across EV and RV will be reviewed here. As a result of their binding to the viral capsid, EV and RV cannot be “addressed” (attached) to the host cells and/or “undressed” (uncoated). The viral replication cycle is therefore blocked at its first steps.

2. Capsid-binding inhibitors

2.1. Synthesis and biological study of WIN-compounds and their derivatives with the capsid-binding mechanism of action

The development of antipicornaviral compounds with the capsid-binding mechanism of action began over 40 years ago with the synthesis of compounds by the Sterling-Winthrop Company, widely known as WIN-compounds. It was discovered that some β-diketones from a synthetic library were active against equine rhinovirus during the course of screening for new antivirals [21–24]. Several modifications of the structure were performed with a view to improving antiviral activity and resulted in the formation of arildone – WIN 38020 (Fig. 3). Arildone specifically inhibited replication of poliomyelitis virus *In vitro* through prevention

of virion uncoating, while *in vivo* it prevented poliovirus-induced paralysis and death in mice [25–27].

Further attempts to increase the spectrum of arildone activity, as well as address chemical and metabolic instability of the *β*-diketone fragment resulted in development of 3,4,5-trisubstituted isoxazoles prepared from the corresponding diketones according to Scheme I [28].

This article also describes the method of synthesis of 3,5-disubstituted isoxazoles (Scheme 2). Treatment of 3,5-dimetylisoxazole with *n*-butyllitium in THF at −70°C followed by alkylation with bromide **2** gave product **5** in 65% yield. Esters **9**, with the exception of the tert-butyl ester, could be prepared by direct alkylation due to formation of by-products because of interaction of the ester group the carbanion of dimethylisoxazole. The hydrolysis of the nitrile **6** gave the acid **7** which were esterified or amidated.

The compounds were tested for activity against rhinovirus-A2 (RV-A2) and poliovirus-2 (PV-2). The highest antiviral activity was demonstrated by 3,5-disubstituted isoxazoles **5a** and **9a**. Despite the high level of activity in relation to RV-2, compound **9a** did not demonstrate activity against PV-2, echovirus-3 and echovirus-11 viruses, while compound **5a** occurs to be active in relation to all five viruses (IC_{50} 0.04–0.15 μM). It was also subjected to further screening against 27 rhinovirus serotypes and found to be active against 24 of them (IC_{50} range of 0.01–5.8 μM).

In follow up research, Guy D. Diana's group studied a series of [[(4,5-dihydro-2-oxasolyl)phenoxy]alkyl]-isoxazoles for their antipicornaviral activity [29]. Compounds were synthesized according to Schemes 3 and 4. 4-((6-Bromohexyl)oxy)benzonitrile **12** was prepared by reaction of cyanophenol **11** with dibromohexane, using potassium carbonate in acetone (this synthesis was described in [22]). Reaction of 3,5-dimetylisoxazole with LDA at −70°C followed by treatment with 1**2** gave **6**. The nitrile **6** was converted to the imino ester **13** under standard conditions (hydrochloric acid in ethanol and diethyl ether). Compound **13** was converted to its free base and then heated with various aminoethanols to give the corresponding oxazoline **14a-f**.

The effect of substitution in the oxazoline ring was tested on two representatives of *Picornaviridae* viruses: RV-A2 and PV-2. Compound **14a** without substituents in the oxazoline ring exhibited the highest antiviral activity in this series of compounds. When compared to **14a** (IC_{50} = 0.004 μM), none of the compounds was more effective against PV-2. The presence of lipophilic (methyl) groups in the oxazoline ring (**14b** and **d**) significantly reduces the effect of compounds against poliovirus-2 (IC_{50} ~ 0.12–1.10 μM), but at the same time slightly increases activity against rhinovirus-2 (IC_{50} ~ 0.04–0.06 μM). Introduction of hydrophilic (hydroxymethyl) groups (**14c** and **e**) significantly reduces antiviral activity towards RV-A2, as well as PV-2 (IC_{50} ~ 0.90–2.60 μM or inactive). The 5-methoxymethyl compound, **14f**, was devoid of activity against polio-2.

In parallel, these researchers varied the length of aliphatic chain in the most active compound from the series, specifically compound **14a** with non-substituted oxazoline, in

order to better study the structure-activity relationship (Scheme 4). Methyl 4-hydroxybenzoate **15** was heated with the appropriate amino alcohols to give amides **16** which on treatment with thionyl chloride in isopropyl acetate at room temperature converted into compounds **17** in high yields. Alkylation of **17** with the (bromoalkyl)isoxazoles gave the respective oxazolines **18a-e**.

These compounds were then screened in the plaque-reduction assay. The five- and seven-carbon chain homologues exhibited the highest activity against PV-2 (IC_{50} = 0.003 and 0.004 μM, respectively) while the six-carbon homolog exhibited slightly less activity (IC_{50} = 0.008 μM). All three compounds, however, were equipotent against RV-A2 with IC_{50} = 0.10–0.12 μM. The four- and eight-carbon homologues were less effective against both viruses

Thus, compound **18d** (WIN 51711), also known as disoxaril [30–32], was the most active compound in the series. *In vitro* disoxaril inhibited a number of rhino- and enteroviruses with IC_{50} of 0.004 to 6.2 and 0.004 to 0.17 μM, respectively. *In vivo* this compound prevented paralysis and death in mice infected with PV-2 and echovirus-9 when dosed twice a day at 15.6 mg/kg [33, 34]. However, despite its *in vivo* and *in vitro* activity, the compound lacked activity against a wide range of other picornaviruses, was poorly bioavailable (15%) and induced crystalurea in phase I clinical trials.

Development of the more potent and bioavailable aromatic substituted derivative of disoxaril became the next stage of development of this antipicornaviral drug with broad-spectrum of activity. This compound was a 2,6-dichloro analogue of disoxaril, WIN 54954, synthesized according to Scheme 5 [35]. The methyl ether of 3,5-dichloro-4-hydroxybenzoic acid **19** was heated with ethanolamine to give the respective amide **20**. Treatment of **20** with thionyl chloride in isopropyl acetate produces oxazoline **21**. The compound was subjected to O-alkylation with 5-(5-bromopentyl)-3-methylisoxazole using potassium carbonate in acetonitrile, giving **22** or WIN 54954 in 56% yield.

WIN 54954 reduced viral replication and exercised a noticeable influence on the inflammatory effect of the pancreas in a mouse infected with the diabetogenic strain of the virus at a dose of 5.0 mg/kg / day [36] and decreased spreading of the virus in a mouse with virus-induced myocarditis; but it caused neurotoxicity at high doses [37]. A phase I clinical trial was successfully completed, but this compound was insufficiently active against a wide spectrum of enteroviruses likely due to existence of a rather long carbon chain (n = 5) [38]. Moreover, WIN 54954 was rapidly metabolized due to lability in the acid media of the oxazoline [39].

Derivatives of WIN 54954 where the oxazoline ring was replaced with a variety of heterocycles were synthesized by six different methods [40].

According to Scheme 6, acylation of amine **23** with the freshly made chloroanhydride **24** in dichloromethane and presence of triethylamine gave amide **25** in 74% yield. Heating this compound in decaline to 183°C during 4.5 h provided the isoxazole derivative of 2,6-dichloro analogue of disoxaril **26** in 85% yield.

To produce the remaining desired heterocyclic derivatives, convergent synthesis was required. E.g., thiophene derivatives are synthesized according to Scheme 7. The heterobiaryl Ni-catalyzed cross-coupling between p-iodoanisole **27** and the Grignard reagent **28** gave the respective anisole **29**. Following deprotection by potassium ethanthiolate in DMF and alkylation by 5-(5-bromopentyl)-3-methylisoxazole provided **30** in 12% yield.

An increase of the total yield was achieved by use of the modified Ullmann's reaction. The 4-(2-furyl)phenol **33** was produced by reaction of trimethylsilyl protected 4-iodophenol **31** and 2-furylcopper **32** in 28% yield. Alkylation of phenol **33** by the bromopentylmethylisoxazole gave the furan derivative **34** in 60% yield (Scheme 8).

The necessity for asymmetrical heterobiaryl synthesis led to development of a novel Pd^0-catalyzed cross-coupling where a number of compounds **36** were prepared in high yield from substituted aryl halides **35** and 2-(trialkylstannyl) heterocycles (Scheme 9).

The application of this cross-coupling procedure in convergent syntheses of target antiviral agents required formation of the corresponding aryl iodides and heteroarylstannates (Scheme 10). The aryl iodides were synthesized by alkylation of 4-iodophenol **37** with 5-(5-bromopentyl)-3-methylisoxazole giving ether **38** in 94% yield. Heteroarylstannates were produced either by direct metalation or by halogen-metal exchange followed by alkylation with trimethylstannyl chloride. Aryiodide **38** was reacted with c 1.1 eq. of heteroarylstannate in refluxing THF using $PdCl_2(PPh_3)_2$ as a catalyst to produce **39** in 73% total yield.

Compound **42** was prepared by Scheme 11. Alkylation of 3,5-dichloro-4-hydroxybenzonitrile **40** with 5-(5-bromopentyl)-3-methylisoxazole **17** produced the respective nitrile **41** in 48% yield. Treatment of **41** with sodium azide in DMF gave tetrazole **42** which was alkylated with methyl iodide in DMF to provide 2-methyltetrazole analogue of WIN-54954 **43** in 62% yield.

All of the heterocyclic derivatives of WIN 54954 were then tested for antirhinoviral activity. 2-Furyl, 3-pyridyl analogues and WIN 54954 exhibited comparable activity against rhinovirus-14 (RV-B14) (IC_{50} = 0.71, 0.97 and 1.2 μM, respectively). Substitution of the oxazoline with 2-oxazole slightly decreases activity (IC_{50} = 1.83 μM), while replacement with 5-oxazole significantly reduced activity (IC_{50} = 12.76 μM). Replacing the 2-furyl with a 2-thiophene ring **39** resulted in a 10-fold reduction in activity (IC_{50} = 7.32 μM), and in both examples, the corresponding 3-substituted heterocycles were inactive. Compounds with the 5-substituted furan ring were active against RV-B14 at IC_{50} of 2.00 μM for 5-chloro- and 2.64 μM for 5-methylfuran, the same 5-substituted thiophene ring exhibited much lower activity (IC_{50} = 5.3 μM for 5-chloro- and 5.2 μM for 5-methylthiophene). The 2-methyltetrazole analogue **43** (or WIN 61605) is the most active compound of the series. It is interesting to note that the 1-positional isomer demonstrates incomparably lower activity against this type of virus.

Compound **43** was also tested against 14 serotypes of rhinoviruses and, compared to WIN 54954, it exhibited the most potent inhibitory ability. Unfortunately, this compound was

hepatotoxic likely due to the presence of the tetrazole ring in the structure or because of a metabolic product [41].

A series of 1,2,4-oxadiazole analogues were developed in the course of further research to identify an acceptable substituting agent instead the oxazoline ring. The most potent antiviral agent, a 5-methylioxadiazole analogue (WIN 61893), was synthesized according to Scheme 12 [35]. The (chloropropyl)isoxazole **44** was treated with the benzonitrile **40** which gave the nitrile **45**. Treatment of **45** with hydroxylamine hydrochloride and potassium carbonate in ethanol gave amidoxime **46**. This compound was acylated with the appropriate acid chloride to give 3-(3,5-dimethyl-4-(3-(3-methylisoxazole-5-yl)propoxy)phenyl)-5-methyl-1,2,4-oxadiazole or WIN 61893 **47**.

Compound **47** was found to be one of the most active compounds in the series, but had a very short half-life due to the acid lability of the oxazoline ring [42]. The group of Diana *et al.*, then went on to study biphenyl analogues of WIN 54954. They proposed that this replacement removes the chemical and metabolic instability issues associated with predecessors in this series [41].

Two common methods, the Ullmann's reaction and Pd-Sn coupling, have been utilized for the preparation of biaryls according to Scheme 13. Phenol **49** was O-alkylated with (bromopropyl)isoxazole **48** in presence of potassium carbonate and potassium iodide in *N*-methylpyrrolydone to yield aryl bromide **50**. Bromide-lithium exchange of **50** in THF at −78°C followed by addition of tributylstannyl chloride provided the arylstannane **5** in high yield. Pd-Sn coupling of **51** with aryl iodides conducted under the typical conditions, such as DMF, 80°C and $PdCl_2(PPh_3)_2$ as a catalyst, lead to biphenyl analogs of WIN 54954 **52a-s** which were synthesized in good yields (Table 6).

The compounds were tested against 10 typical rhinovirus serotypes (RV-3,-B5, -A9, -A16, -A18, -A38, -A66, -A67, and -A75) in a TCID50 – tissue culture infectious dose. All compounds from this series were inactive (IC_{50} > 3 μM) against RVs-B3 and -B5, regardless of the nature of the substituting groups or their position in the compound. 4-Chloro- **52k**, 2-fluoro **52q**, 4-fluoro **52s** and 4-methyl **52c** analogues were the most active compounds with IC_{50} = 0.11 or 0.12 μM. The 3-hydroxy **54f** and 3-nitro **52l** analogs (IC_{50} = 0.44 μM and 0.35 μM, respectively) exhibited improved activity over their 4-substituted counterparts (IC_{50} = 0.77 μM for 4-hydroxy and IC_{50} = 0.76 μM for 4-nitro analogues). Among compounds with the substitution in position 4, compounds with 4-trifluoromethyl **52n**, 4-hydroxy **52g**, 4-nitro **52m** and 3-cyano **52o** were the least active, while the remainder of the compounds of this series were of comparable activity and all them demonstrated greater efficiency than WIN 54954.

The most promising analogues were tested for their metabolic stability using a monkey liver microsomal assay. It was found that these analogues showed significantly greater stability than WIN 54954, $t_{1/2}$ > 200 min vs 27 min respectively. The most potent compound, 3-(3-(2,6-dimethyl-4-(4-fluorophenyl)-phenoxypropyl]-3-methyloxazole (**52s**), was tested against 94 picornavirus serotypes. It was found to be active against only 64 of these

serotypes, while WIN 54954 was more active towards the various studied viruses (87 serotypes). It was then decided to halt further study of biphenyl analogs.

In an effort to improve the structure of WIN 61893, these researchers tried to understand the impact of the isoxazole fragment on antiviral activity, so 2-acetylfuran analogues were prepared (Fig. 4) [43].

However, these analogs exhibited poor pharmacokinetic profiles, thus it was decided to substitute the 3-methyloxazole for pyridine (Fig. 5), but these synthesized compounds did not demonstrate significant activity as compared to the isoxazole derivative **47** [44].

Analysis of the metabolic products of WIN 61893 indicated that the methyl group in the oxadiazole ring tends to be rapidly hydroxylated. Substitution of this group with a trifluoromethyl group led to a compound (WIN63843 or pleconaril **55**) whose rate of metabolism was reduced. A trifluoromethyl group has a protective effect on the oxadiazole ring by inhibiting hydroxylation in this position. Compound WIN63843 was synthesized according to Scheme 14 [44, 45].

The amidoxime **46** was prepared by the procedure outlined in previous article [41]. Compound **46** was treated with TFAA to give the appropriate oxadiazole, pleconaril **55**.

Pleconaril **55** was subsequently tested on 215 clinical isolates of enterovirus serotypes and demonstrated activity at IC_{50} concentration ≤0.03 μM and CC_{90} inhibition ≤ 0.18 μM with all of them [46]. It should be noted that the compound has no effect on EV71. Different clinical trials of this compound were undertaken and found to be quite ambiguous, despite the promising broad- spectrum antiviral activity in cell culture. Pleconaril proved useful in 2 of 3 neonates with severe enteroviral hepatitis [47]. Also, pleconaril resulted in a clinical response in 78% of the patients with chronic meningoencephalitis [48]. However, a double-blind placebo-controlled trial on infants with EV meningitis found no significant effect of pleconaril [49]. Pleconaril at a dose of 400 mg given thrice a day over five days was shown to have no statistically significant effect on the treatment of the common cold. Headache and menstrual dysfunction (in women) were the most common adverse effects. It caused some women to bleed between menstrual periods and interfered with hormonal birth control. Indeed, two women in a trial became pregnant while taking it [50]. Subsequent studies suggested that pleconaril induces CYP3A4 [51, 52] and this enzyme is well known to be primarily responsible for the metabolism of birth control medications [53, 54]. Viruses with resistance to pleconaril were also found in 10.7% of pleconaril-treated patients [55]. The pharmaceutical company ViroPharma halted development of this potential broad-spectrum antipicornaviral drug due to all the above mitigating factors and in 2003 they licensed the drug to Schering-Plough (now Merck), and ViroPharma was in turn purchased by Shire in 2013. It appears Merck also discontinued their work on this drug after a further Phase II clinical trial [56]. Pleconaril can be purchased from the Swedish company Apodemus AB who are also studying the therapeutic effect against the ljungan virus-and related diseases. Despite the clinical failure of this compound, the pleconaril scaffold serves as a foundation for future drug design and development of other capsid-binding inhibitors by many researchers.

Pleconaril derivatives: novel antiviral biphenylderivatives—Novel antiviral biphenylderivatives of pleconaril with various substituents and substitution patterns at the terminal benzene ring were developed in our laboratory [57]. Mono-, di- and trisubstituted [(biphenyloxy)propyl]isoxazoles were synthesized according to Scheme 15.

Bromophenole **49** was O-alkylated with chloropentyne in the presence of potassium carbonate and potassium iodide in *N*-methylpyrrolidone to yield 5-bromo-1,3-dimethyl-2-(4-pentynyloxy)benzene **56**. The condensation of compound **56** with acetaldoxime in the presence of triethylamine in DMF resulted in the 3-methylisoxazole derivative **57**. The reaction of compound **57** with various phenylboronic acids by means of tetrakis(triphenylphosphine)palladium(0) as a catalyst provided the corresponding biphenyl derivatives **58** in good yields.

These compounds were tested on a number of picornaviruses: pleconaril-resistant coxsackievirus B3 (CVB3, Nancy strain), RV-A2 and RV-B14. The 4-fluoro-substituted derivative, the most active compound, inhibits CVB3 and RV-A2 at IC_{50} 1.34 μM and 0.009 μM, respectively. However, the compound was inactive against RV-14.

Continuing our research, novel analogs with different substituents in the central phenyl ring were synthesized to study their effect on antipicornaviral activity.

It was discovered that the amino acid (isoleucine, leucine or methionine) of the CVB3 capsid protein VP1 at position 1092 (AA1092) is located in close vicinity to the central phenyl group of pleconaril and derivatives. While isoleucine is associated with capsid-binding inhibitor receptivity, leucine and methionine confer resistance to pleconaril and pleconaril derivatives [47].

Further pleconaril derivatives were synthesized (Scheme 16) and [(biphenyloxy)propyl]isoxazole (Scheme not shown) with various substituting groups in the central phenoxy ring which were also tested for their antiviral activity against enteroviruses (CVB3 with different mutations in the hydrophobic pocket) and rhinoviruses (RV-A2, -A8, -A23, -A25, -A29 and -A98) [59]. The pleconaril derivatives were synthesized by the method outlined in a previous article [40].

Pleconaril resistance of CVB3 based on Leu1092 or Met1092 was overcome by monosubstitution with 3-methyl **63b** as well as 3-bromine **63d** in the central phenyl. The 3-methoxy substitution (**63c**) resulted in a significant reduction in activity. The analogues with two methyl groups in the central ring (**63e**) as well as the external rings (**63f**) at the meta position were inactive against most of the tested CVB3 variants. The tested rhinoviruses differed in susceptibility with regard to the pleconaril analogs. The lowest antiviral activity was exhibited by the analog without any substitution in the central phenoxy ring (**63a**). Compounds with 3-methyl (**63b**), 3-bromine (**63d**) and the 3-methoxy analog (**63c**) reduced the antiviral activity against RV-A2, RV-B14, RV-A23, and RV-A25 in comparison to pleconaril. It is important to note that the four pleconaril analogues were inactive against two rhinovirus serotypes (RV-A8 and RV-A98) regardless of the nature of the substitutions. Testing of the derivatives on other entero- and rhinovirus serotypes showed ambiguous

results: compounds with the same substituting group could inhibit one serotype and not inhibit another.

Another group, Shia *et al.*, has worked on novel pleconaril-based compounds [60]. A series of pyridylimidazolidinones was designed by means of computer-based drug design, using pleconaril and other WIN compounds as a scaffold (general structure in Fig. 6). These compounds possess potent activity *in silico* against EV-71. EV-71 causes one of the most wide-spread children's disease, namely hand-foot-and-mouth disease, and the researchers wanted to develop an effective antiviral against this particular infection.

It was suggested that pyridine and the para-position of the phenyl ring in the antiviral molecules was the most critical location for realizing anti-EV-71 activity. Derivatives **64** and **65** inhibited EV-71 genotypes (A, B and C) with IC_{50} values from 0.3 to 1.6 μM. They were also tested against coxsackievirus A9 and A24 (IC_{50}~ 0.47–0.55 μM), Echovirus-9 (IC_{50} ~ 2.6 μM) and EV-D68 (IC_{50} ~ 2.13 μM), exhibiting activity against all of them. At the same time, these compounds were ineffective against a half of further picornavirus serotypes and, moreover, were extremely sensitive to mutations in a VP1 amino acid at the 1092 position [61].

Chern *et al.*, continued this work by replacing the p-substituted phenoxyl group in pyridylimidazolidinones by various oxime ethers. Among this series of compounds, the pyridylimidazolidinone **66** with an ethyl oxime ether group located at the para-position of the phenoxyl ring was the most potent antiviral compound against EV-71 with an EC_{50} of 0.001 μM [62]. Moreover, this compound showed broad-spectrum activity against most tested enterovirus serotypes. In addition, it was shown that the chain with five carbon atoms is optimal for demonstrating antipicornaviral activity. This research group also studied possible substitutions of the carbon atom in the middle of the chain for nitrogen or oxygen, as well as insertions of different functional groups in the same position [63]. However, these experiments were unsuccessful, and derivative **67** with the methyl group in the middle of the chain was found to be the most active compound of the series [64]. The same group repeated the research substituting the phenoxyl ring, which resulted in synthesis of oxadiazole derivatives with the methyl/ethyl group **68** that showed the best activity towards EV-71 (EC_{50} ~ 0.0009 and 0.0005 μM, respectively). The imidazolidinone derivatives have also shown good *in vitro* and *in vivo* activity against EV-71, and this makes their further development attractive for creation of effective anti-enteroviral drugs [65, 66].

2.2. Review of several other significant capsid-binding inhibitors

2.2.1. Vapendavir, pirodavir and oximes ethers—The history of development of vapendavir began 30 years ago at the Janssen Research Center from the synthesis of R61837 **69**, a substituted phenyl-pyridazinamine (Fig. 7), inhibiting 80 % of 100 tested rhinovirus serotypes at a concentration of 32 μM or less [67]. The subsequent drug design resulted in development of pirodavir (R77975) **70**. Compared to R61837, this compound was 500-fold more potent as an antiviral, inhibiting the same percentage of rhinovirus serotypes (about 0.064 μM). Moreover, pirodavir inhibited a broad spectrum of rhinoviruses of both groups (A and B), while R61837 acts only against rhinoviruses of group B rhinovirus serotype [68].

In a series of clinical trials, intranasal pirodavir was shown to be efficient when administered 6 times a day for 5 days in experimentally induced rhinovirus infection; however, in a series of double-blind placebo-controlled trials this compound demonstrated no clinical benefit in naturally occurring rhinovirus colds. The failure of clinical efficacy of pirodavir can be explained by its poor pharmacokinetic properties as rapid hydrolysis of the ester group gave an inactive acid [69].

To solve the problem of bioavailability, the ester group in pirodavir was substituted for the oxime ether BTA-188 **71** (Fig. 6) [70]. This compound had good oral bioavailability (62–63% in rats and 21–28% in dogs) and inhibits 56 RV laboratory strains at EC_{50} ~ 0.5–6.7 μM [71].

BTA-188 was further modified by replacing the phenyl ring for several substituted bicyclic systems to increase the half-life and oral bioavailability. Vapendavir (BTA−798) **72** is the most potent analog among the synthesized benzoxazole and benzothiazole derivatives. The compound was synthesized according to Scheme 17. Initially, 3-(4-(2-chloroethyl)piperidin-1-yl)-6-methylpyridazine **76** was prepared in three steps. Chloro-methylpyridazine was coupled with piperidinyl-ethanol to obtain intermediate **75**, which was then treated with thionyl chloride to make compound **76**. Benzoxazole-2-thiol **77** was S-alkylated with iodoethane to yield ethylthio-benzoxazole **78**. This compound was treated with boron tribromide to obtain 2-(ethylthio)benzoxazol-5-ol **79**. The condensation of **79** with **76** in the presence of potassium carbonate in DMF results in intermediate **80**, which was then reacted with sodium ethoxide to synthesize vapendavir **72**.

Vapendavir **72** was 10 times more active than pleconaril **55** and equipotent to pirodavir; moreover, it had a longer half-life and oral bioavailability due to the hydrolytic stability of the 2-ethoxybenzoxazole group compared with an ether group [72].

Vapendavir successfully completed Phase I and the first stage of Phase II clinical trials in treating of rhinoviral infections in patients with asthma or chronic obstructive lung disease, and it started the second stage of a Phase II trial in 2015 [14]. Unfortunately, in February 2017 it was reported that vapendavir failed versus placebo in the Phase IIb trial for treating asthma patients with rhinovirus infection [16].

2.2.2. SCH and pocapavir—Researchers at Schering-Plough decided to employ another scaffold for development of an antipicornaviral drug. The compound SCH 38057 **81** was developed, which is a phenoxylimidiazole salt and it was found by Rozhon *et al.*, to inhibit several entero- and rhinoviruses at EC_{50} 10.2–29.1 μM (Fig. 8) [73].

At the subsequent step the imidazole fragment was rejected; five compounds with various linkers between two o-chloro-p-metoxyphenols were synthesized. SCH 47802 **82** which contains the 1,4-oxymethylbenzene group is the most potent compound of the series (EC_{50} ~ 0.01 μM). Pocapavir (SCH 48973) **83** was developed by varying substituting groups in the second o-chloro-p-metoxyphenol fragment. Pocapavir is synthesized in two steps according to the Scheme 18. 2-Chloro-4-methoxyphenol **84** was reacted with 1,4-bis(bromomethyl)benzene **85** in the presence of potassium hydroxide in DMF to obtain

compound **86**, which was then treated with 2,6-dichlorophenol in the conditions described above to synthesize pocapavir **83** with a good yield.

Pocapavir exhibited similar activity to its predecessor and inhibited a wider range of picornaviruses (EC_{50} ~ 0.9 μM for 80% of 154 clinically isolated human enteroviruses, including poliovirus 2). However, it was decided to halt the development this drug, because it did not have efficacy against rhinoviruses [74, 75]. In 2015, a case study was reported in which pocapavir was used to treat a female infant with severe enteroviral sepsis. Despite multiple factors, including high risk mortality, the patient was discharged with minimal residual sequelae of neonatal enteroviral infection. However, it is not possible to establish the clinical antiviral effect of pocapavir from this data [76].

It is very interesting to note that pocapavir has potent and broad-spectrum antipoliovirus activity [77]. This compound inhibited all 45 polioviruses tested in a virus-induced cytopathic effect protection assay with EC_{50} values ranging from 0.003 to 0.126 μM; and 90% of the polioviruses tested were inhibited at EC_{50} of <0.076 μM.

In a randomized, blinded, placebo-controlled study involving 141 poliovirus-infected patients, treatment with pocapavir was shown to reduce the duration (5.5 days in pocapavir-treated subjects vs 13 days in placebos) and the severity of poliovirus infection. But during the clinical studies very large resistant strains of enterovirus infections were observed, with 44% in the group treated by pocapavir and 10% in group treated by placebo. Unfortunately, the authors did not describe if this resistance came during pocapavir treatment or was present before [78].

2.2.3. Pyrazolopyrimidines—Work by our group led to the investigation of a novel class of pyrazolo[3,4-d]pyrimidines as compounds with antipicornaviral broad-spectrum activity. Synthesis of about 80 derivatives, along with their physicochemical characteristics, structure-activity relationships, anti-enteroviral activities, and mechanism of activity were reported [79]. It was found that 3-(4-trifluoromethylphenyl)amino-6-phenylpyrazolo[3,4-d]pyrimidine-4-amine **96** (Scheme 19) exhibited strong activity against picornaviruses (CVB3, RV-A2, -B5, -A8, -B42 and -B48) with IC_{50} values of 0.04 to 0.64 μM. This compound was synthesized according to Scheme 20. 1-Isothiocyanato-4-(trifluoromethyl)benzene **93** was prepared by reaction of 4-(trifluoromethyl)aniline **92** with dimethylcarbamothioic chloride in refluxing toluene. Treatment of **93** with malononitrile in the presence of sodium hydride in DMF produces 2-((methylthio)((4-(trifluoromethyl)phenyl)amino)methylene)malononitrile **94**. Pyrazole **95** was produced by cyclization with malononitrile **94** by means of hydrazine in refluxing ethanol. Finally, reaction of pyrazole **95** with benzimidamide in butanol led to the target compound **96**.

Compound **96** is metabolically stable and neither an inducer nor an inhibitor of cytochrome P450 isoforms. The *in vivo* results indicate that this molecule could become a suitable drug candidate in future after further optimization [79].

2.2.4. Benzothiophenes and their derivatives—Kim *et al.* recently identified an original compound with potential inhibition activity against both RV-A and RV-B strains by

high throughput screening and developed a novel series of benzothiophenes and their derivatives (Fig. 9) [80].

Compound **100**, the most active compound of this series, inhibited RV-B14, -A21, and -A71 with EC_{50} values of 0.083, 0.078, and 0.015 μM, respectively. The synthetic route for this compound is described on Scheme 20. The 6-substituted ethyl 3-methylbenzo[b]thiophene-2-carboxylate **102** was prepared from ethyl mercaptoacetate and 1-(2-fluoro-4-methoxyphenyl)ethenone **101**. Demethylation of 6-methoxy group from **102** with BBr_3 formed **103**. 6-Hydroxy-3-methylbenzo[b]thiophene-2-carboxylic acid **104** was prepared by hydrolysis of the 2-carboxylic ester group in **103**. The 6-hydroxy group in **104** was reacted with 4-chloro-N-methylpicolinamide **105** in DMSO with Cs_2CO_3 as the base to generate product of their condensation **106**. The target isopropyl ester **100** was obtained by reacting **106** with isopropanol, 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (EDCI·HCl) and 4-dimethylaminopyridine (DMAP) in CH_2Cl_2.

The results of mutation analysis suggested that **100** inhibited viral uncoating by binding in the viral capsid in VP1, similar to pleconaril, but final evidence was not provided.

2.2.5. 4,5-Dimethoxybenzene derivatives—Roche *et al.* synthesized 99 4,5-dimethoxybenzene derivatives through Tetrakis(DimethylAmino)Ethylene (TDAE) methodology and evaluated their antirhinoviral activity. 4-[1-hydroxy-2-(4,5-dimethoxy-2-nitrophenyl)-1-hydroxyethyl]benzonitrile **101** (Fig. 10) possesses the most potent and selective activity against RVs in this series (EC_{50} ~ 2 ± 0.6 μM for RV-B14), but the compound does not outperform pleconaril nor possess broad-spectrum antirhinoviral inhibition [81]. Through studying the interactions of **101** within the drug-binding pocket it was found the open end of the pocket is left empty. In the author's opinion, more bulky compounds for filling the viral pocket were required.

Several chemists have synthesized series of 4,5-dimethoxybenzene derivatives with biphenyl groups to optimize such interactions. 2-(4,5-Dimethoxy-2-nitrophenyl)-1-(4-(pyridin-4-yl)phenyl)ethanol **102** (Fig. 10) exhibited similar activity with the previous compound **101** (EC_{50} ~ 3.4±1.0 μM for RV-B14), but the CC_{50} is much higher than that of compound **101** (101: CC_{50} = 104.0 ± 22.2 μM; 102: CC_{50} > 263 μM) [82]. This group focused on 4,5-dimethoxybenzene derivatives with five- and six-membered heterocycles. Pyrazole analogue **103** exhibits activity against RV-B14 slightly less than that of pleconaril (**103**: EC_{50} ~ 0.76 μM; pleconaril: EC_{50} ~ 0.3 μM) and lower cytotoxicity (**103**: CC_{50} > 271 μM; pleconaril: CC_{50} > 131 μM) [83].

A series of the 4-pyridinyl, 1H-pyrazol-1-yl and 1H-triazol-1-yl analogues were evaluated against RV-B14. Compounds **104** and **105** were synthesized according to Scheme 21. The 2-nitro-4-bromo-benzyl chloride **107** was prepared by reduction of the corresponding carboxylic acid **106** with a stabilized BH_3-THF solution and following chlorination with $SOCl_2$. Subsequent reaction of **107** with (4-(1*H*-pyrazol-1-yl)benzaldehyde and 4-(1*H*-1,2,4-triazol-1-yl)benzaldehyde) in DMF resulted in pyrazole and triazole derivatives **108** and **109**. The final compounds **104** and **105** were prepared as a result of palladium-catalyzed coupling ($Pd(dppf)Cl_2$ as a catalyst) with 1-methylpyrazole-4-boronic acid pinacol ester.

Compound (*S*)-**104** and (*S*)-**105** exhibited potent anti-RV-B14 (**104**: EC_{50} ~ 0.035 μM; **105**: EC_{50} ~ 0.06 μM) and anti-RV-B70 (**104**: EC_{50} ~ 0.017 μM; **105**: EC_{50} ~ 0.05 μM) activities, but were not active against RVs type A (except RV-A89) [84].

3. Future steps for the improvement of the drug-like properties of the capsid binding inhibitors

The development of effective capsid-binding inhibitors with broad-spectrum activity is an essential, ambitious and an important problem that has yet to be completely solved as there is currently no small molecule drug approved in this class by any regulatory authority. Our review of over 40 years of work in this field demonstrates that the significant time and investment already put into this area may be recouped if we can suggest improvements to these failed compounds that may ultimately fix some of their limitations in terms of efficacy and side effects.

For example, Braun *et al* identified and characterized two new mutations of I1207 in CVB3, I1207K and I1207R, which can confer resistance to pleconaril [58]. The amino acid 1207 does not belong to the moieties lining the drug-binding pocket. It is located on the GH loop of VP1 that plays an important role in capsid function. However, neither I1207K nor I1207R affected viral replication in HeLa cells.

The drug resistance mechanism of the I1207K and I1207R variants was studied using molecular dynamics simulations. R1095 was identified as a key residue responsible for the loss of efficacy of pleconaril in the mutants. This conformational change induced by the mutations brings its positively charged side chain close to methylisoxazole moiety of pleconaril and the βH strand. The highly polar group repels the hydrophobic moiety of the drug. At the same time, it destabilizes the βH strand and pushes it away from the βC strand. This leads to an opening of the hydrophobic pocket and a loss of hydrophobic contacts between the ligand and the βH strand. Also, the water-mediated interaction network between pleconaril and the protein environment is impaired by the mutations, which adds to the destabilization of the protein ligand complex and, together with the reorientation of R1095, induces the shift of the βC strand.

Based on the experimental and computational data it can be concluded that a highly specific drug resistance mechanism does not affect the virus replication rates. Substitutions of I1207 by lysine and arginine induce a complex cascade of structural rearrangements that abolish the affinity of the drug molecule to the binding site while the function of the pocket factor is maintained. This is plausible, as it is known from crystal structures that the carboxylic acid moiety of the pocket factor is interacting with R1095 and would not be repelled by its approach [85].

The insights from this study demonstrate the vulnerability of the therapeutic efficacy of antivirals targeting the hydrophobic pocket of picornaviruses with emerging resistant virus variants. As shown by experimental data, resistance-causing mutations are only observed for a few specific amino acid positions as a result of an evolutionary optimization of drug-resistance and virus replication rates, in this case I1092 and I1207.

Modification of the methylisoxazole moiety of pleconaril analogues for compatibility with an approaching charge of R1095 would render these molecules more likely to maintain efficacy in I1207K and I1207R mutants. For example, a negatively charged group such as a carbonic acid function could be introduced into the ligand, which instead of being repelled by the R1095 side chain could serve as a ligand anchor by forming a salt bridge. Carboxylic acid groups may lead to unfavorable absorption, distribution, metabolism and excretion (ADME) profiles for antivirals, which could be addressed by a prodrug concept. Introduction of bioisosteric groups such as a tetrazole (as in the case of losartan) or an acylsylfonamide is a further possible strategy to overcome ADME issues [86] although these may also have issues [41]. Compounds with activity on strains with mutations at I1207K and I1207R may be less effective on the wild-type virus. In patients infected with the pleconaril-sensitive virus a combination with pleconaril (or analogs thereof) could prevent the incidence of these drug-resistant variants.

Efforts to avoid the induction of CYP3A with pleconaril by modifying the molecule to avoid potential features (hydrophobic and hydrogen bonding) that could be important for binding to the Pregnane X Receptor (PXR), a nuclear hormone receptor which is a transcriptional regulator of this and other genes [87–89], may also be a viable approach to develop new analogs.

Conclusion

There is hence an opportunity to learn from these previous efforts. Interestingly, this review demonstrates that there is now plentiful data in the public domain for these capsid inhibitor compounds and their many analogs that could be used to generate machine learning models [90] or other computational models which could in turn assist scientists in drug discovery and multidimensional optimization alongside ADME/Tox properties [91, 92]. There has been little ligand-based or quantitative structure activity analysis in this area to date [93, 94]. There is hence the potential to build on the decades of drug discovery efforts in this area to develop new broad-spectrum capsid binding inhibitors of enteroviruses with an increased probability of clinical success. Our own efforts in drug discovery in this area have gone back to the future, to do just this.

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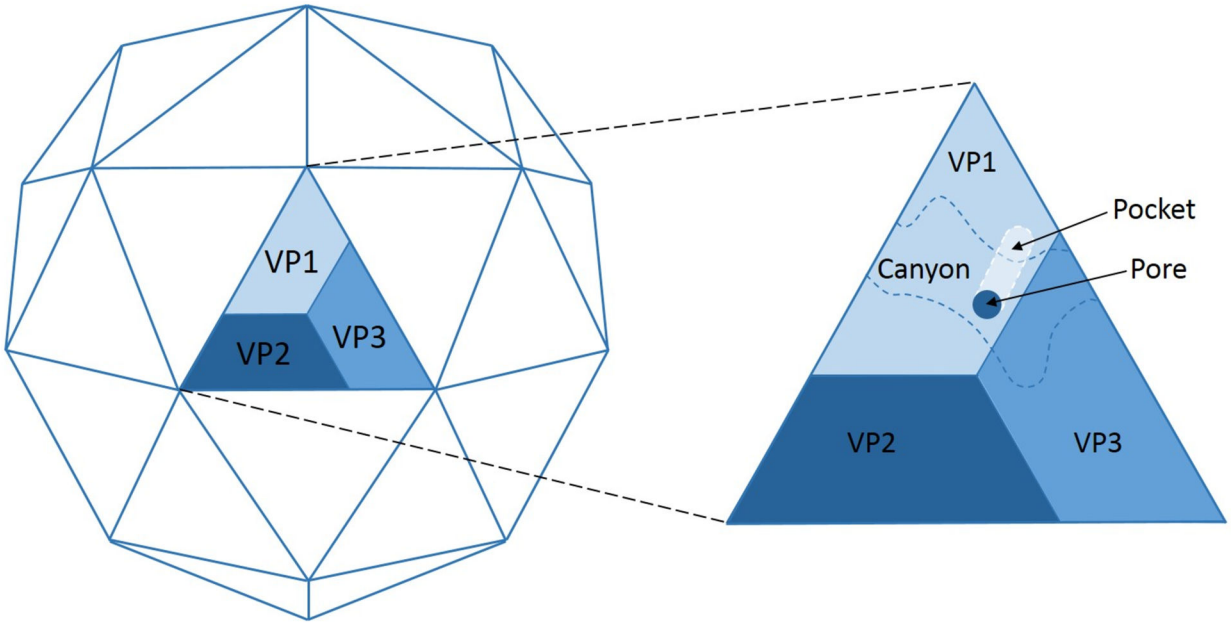


Fig. 1. Viral capsid structure and location of canyon. This model is derived and modified from De Palma et al. [12].

The three surface proteins VP1-3 do not have appreciable amino acid sequence homology. However, all three form an eight-stranded anti-parallel ß-barrel. Its wedged shape facilitates VP1, 2, and 3 assembly into an icosahedral viral capsid. The amino acid loops between the ß-strands and the N- and C-terminal sequences are structurally different and determine the morphology and antigenicity of distinct EV and RV [18]. Generally, capsid morphology is characterized by a star-shaped plateau at the 5-fold axis of capsid symmetry. It is surrounded by a deep depression (called a canyon) in most EV and RV. Just beneath the canyon floor is a hydrophobic pocket that might be empty (e.g. RV-B14) or is filled with a lipped, sphingosine or fatty acid (called a pocket factor) in the majority of EV and RV. The amino acids of the hydrophobic pocket are highly conserved across EV and also RV- A and -B, whereas at distinct amino acid positions a polymorphism may exist [19]. Another protrusion has been detected at the 3-fold axis of symmetry.

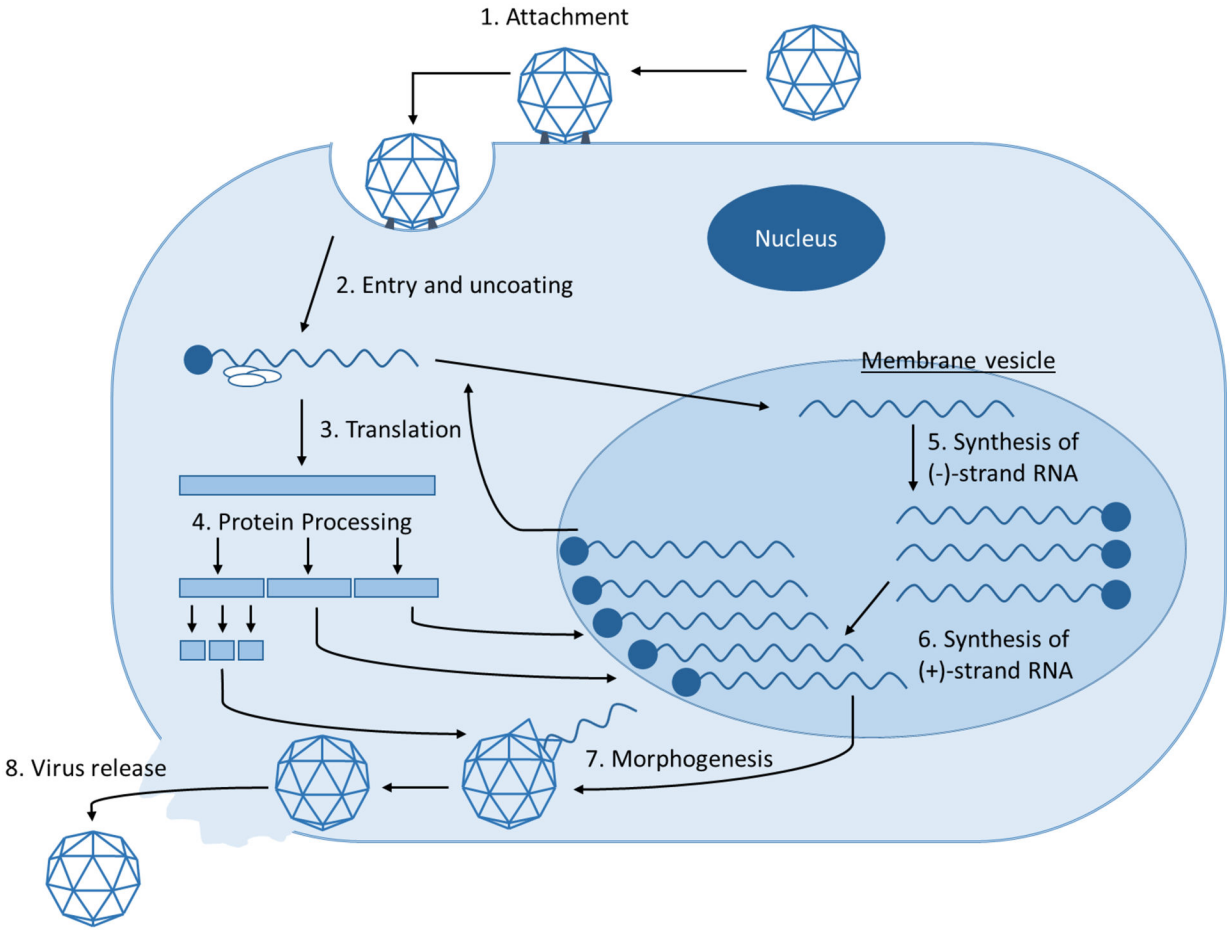


Fig. 2. Replication cycle of picornavirus. This model is derived and modified from De Palma et al. [12].

After interaction of the positive-stranded viral RNA with host cell ribosomes a polyprotein is translated and then cleaved into individual viral proteins (capsid proteins and non-structural proteins) by viral proteases 2A and 3C/3CD. The RNA-dependent polymerase synthesizes the minus strand matrix from the surface of the plus strand and uses it to replicate the genome. Viral protein synthesis and RNA replication run in parallel to produce a huge number of virus protein and RNA genome copies. Once a critical amount of viral structural proteins is reached, they begin to assemble and form protomers which further assemble into pentamers to form the capsid. After interaction of the capsid proteins with the genome, progeny virion are produced. They are released from the host cell by means of lysis [18].

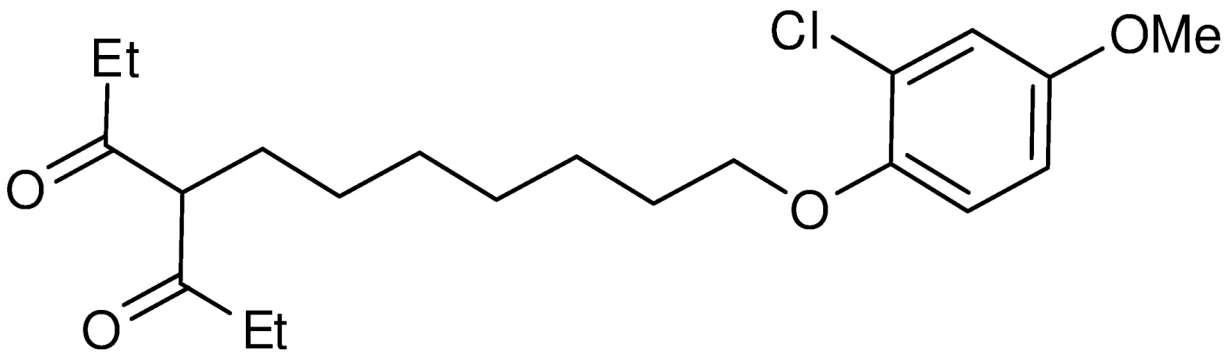
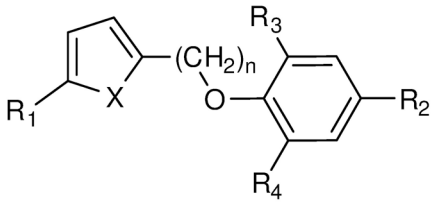


Fig. 3.
Structure of arildone



53

R_1 = CH_3, CHO, $COCH_3$
R_2 = 2-oxazoline, 2-methyltetrazole, 5-methyl-1,2,4-oxadiazole
R_3 = H, Cl, CH_3
R_4 = H, Cl, Br, CH_3
X = O, n = 3, 5

Fig. 4.
General structure of furan analogs

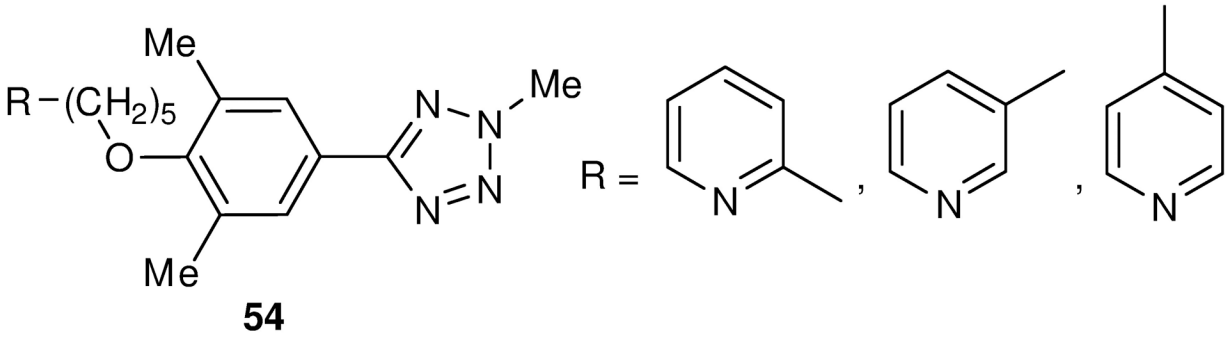


Fig. 5.
General structure of pyridine analogues of WIN 61605

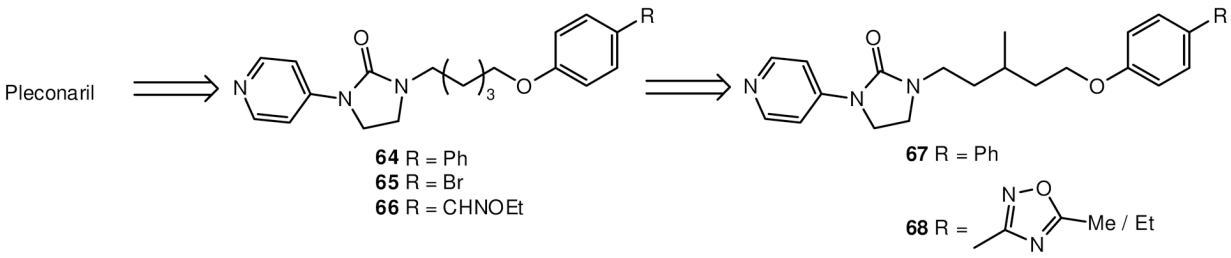


Fig. 6.
Development of pleconaril-based antivirals by Shia et al.

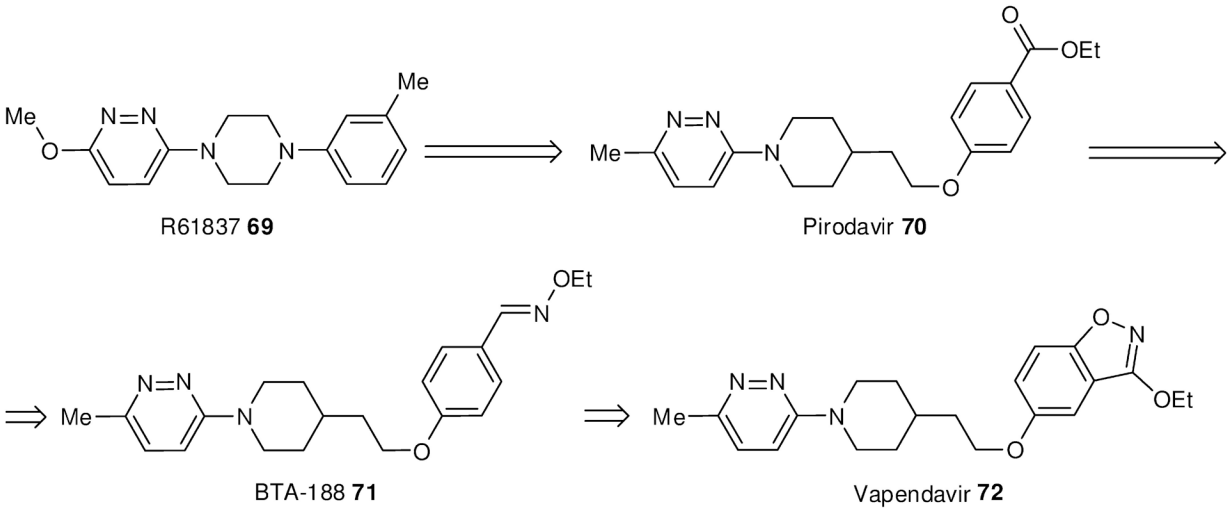


Fig. 7.
Evolution of pyridazine analogues which led to discovery of vapendavir

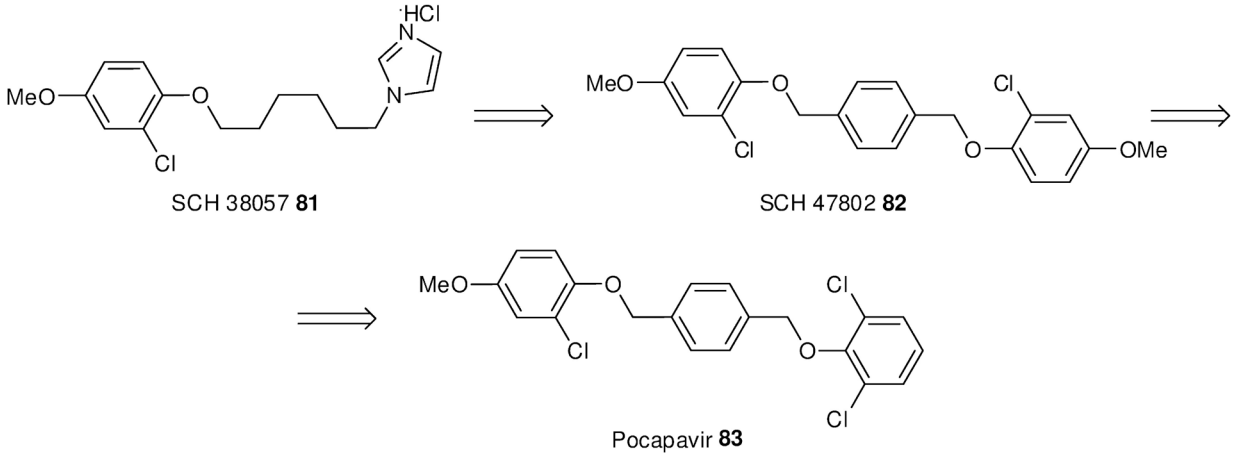


Fig. 8.
Scheme of pocapavir development

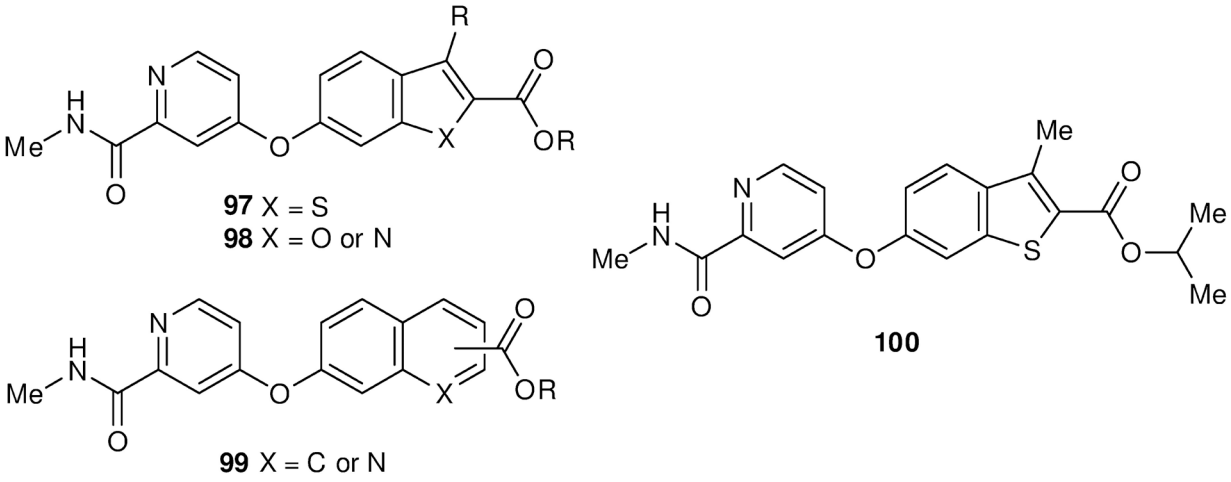


Fig. 9.

Benzothiophenes **97**, their derivatives **98**, **99** and the most active compound of this series **100**

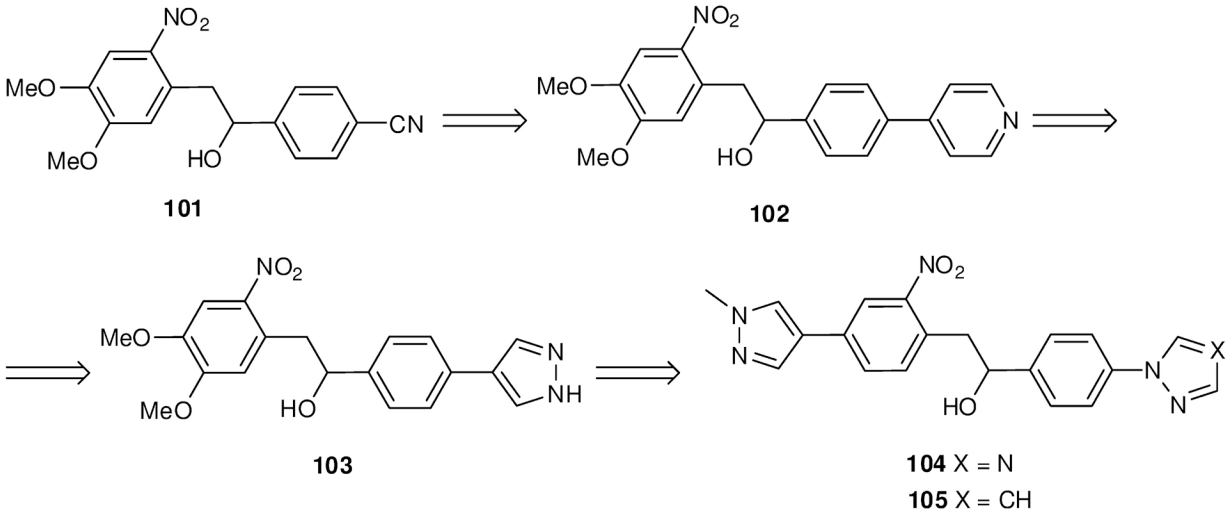
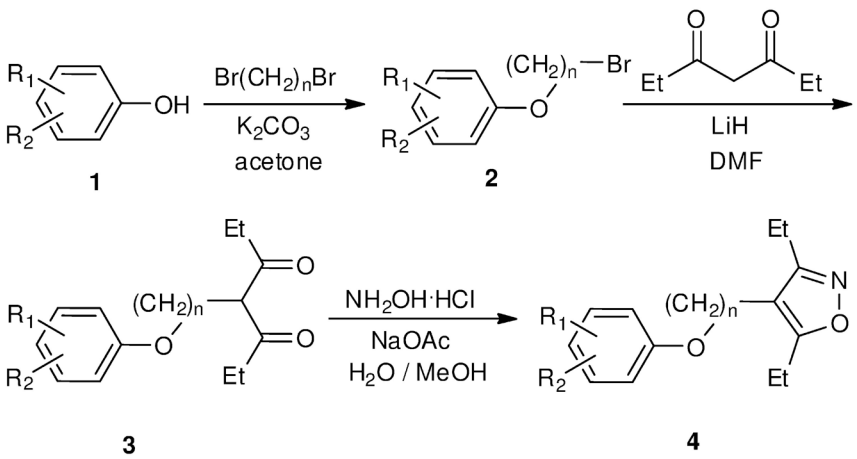
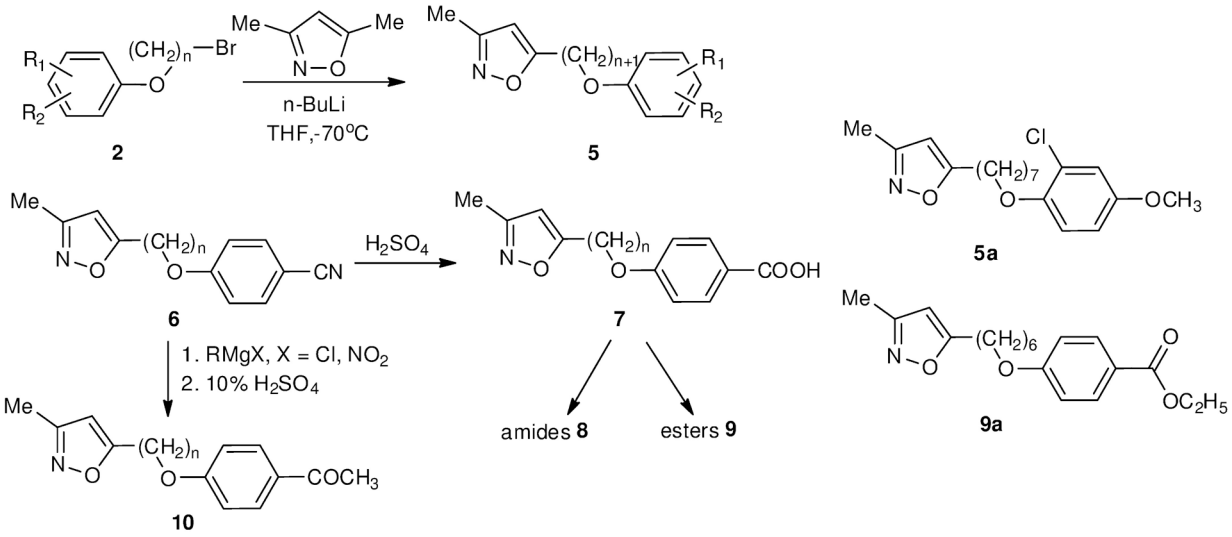


Fig. 10.
Route of 4,5-dimethoxybenzene derivatives development



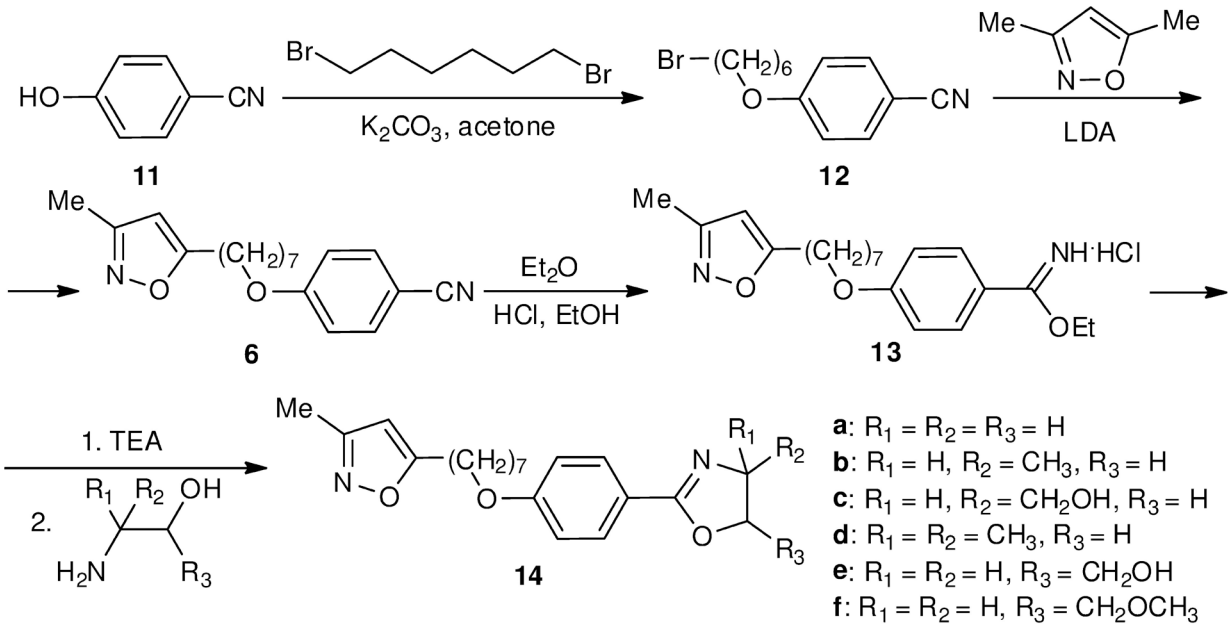
a: R_1 = 2-Cl, R_2 = 4-CH_3O, n = 6; **b**: R_1 = H, R_2 = 4-OH, n = 6; **c**: R_1 = H, R_2 = 4-CH_3S, n = 6;
d: R_1 = 2-CF_3, R_2 = H, n = 6; **e**: R_1 = 3-I, R_2 = H, n = 6; **f**: R_1 = 2-NO_2, R_2 = 4-CH_3O, n = 6;
g: R_1 = H, R_2 = 4-Br, n = 6; **h**: R_1 = 2-Cl, R_2 = 4-F, n = 6; **i**: R_1, R_2 = 3,4-OCH_2O, n = 6;
j: R_1 = 2-Cl, R_2 = 6-Cl, n = 6; **k**: R_1 = H, R_2 = 4-CH_3O, n = 6; **l**: R_1 = H, R_2 = 4-$COOC_2H_5$, n = 6;
m: R_1 = H, R_2 = 4-$COOC_2H_5$, n = 7; **n**: R_1 = H, R_2 = 4-$COOC_2H_5$, n = 8; **o**: R_1 = 2-CH_3O, R_2 = 6-CH_3O, n = 6;
p: R_1 = 2-Br, R_2 = 4-CH_3O, n = 6; **q**: R_1 = 2-$COOCH_3$, R_2 = 4-CH_3O, n = 6; **r**: R_1 = 2-COOH, R_2 = 4-CH_3O, n = 6;
s: R_1 = H, R_2 = 4-COOH, n = 6; **t**: R_1 = 2-Cl, R_2 = 4-$COOCH_3$, n = 6; **u**: R_1 = 2-Br, R_2 = 6-$COOCH_3$, n = 6

Scheme 1.
Synthetic route of 3,4,5-trisubstituted isoxazoles **4**

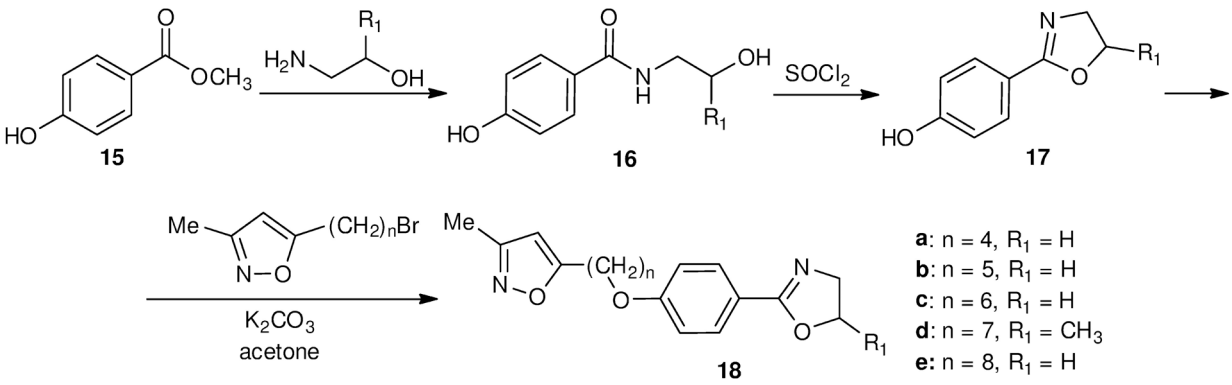


Scheme 2.

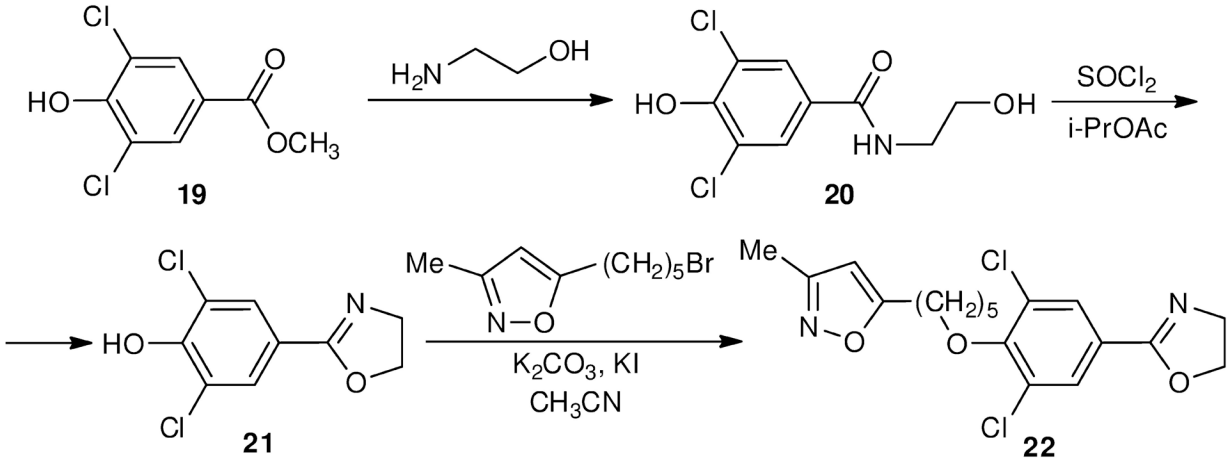
Synthesis of 3,5-disubstituted isoxazoles **5a** and **9a**



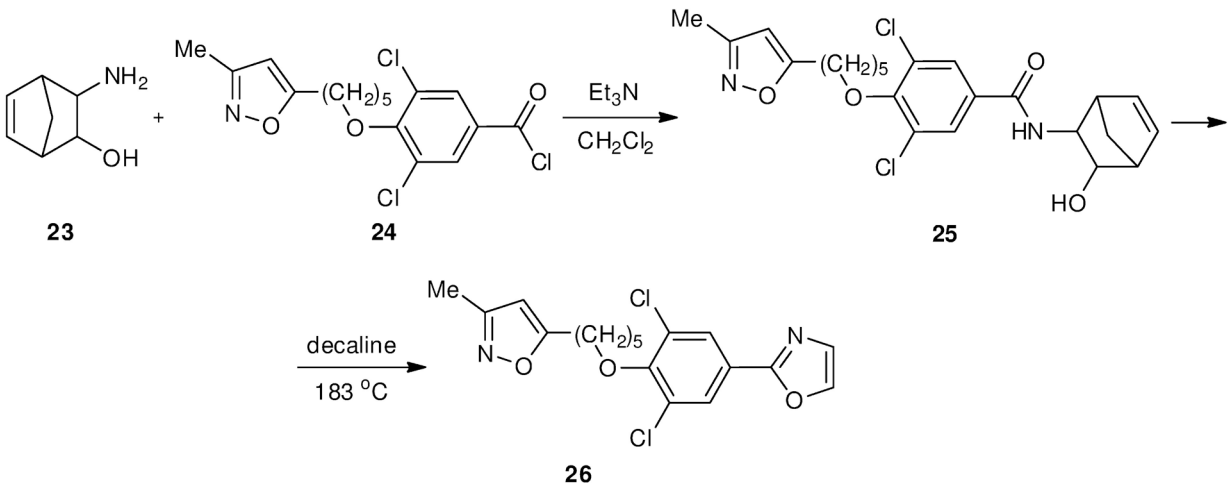
Scheme 3.
Synthesis of some [[(4,5-dihydro-2-oxasolyl)phenoxy]alkyl]-isoxazoles



Scheme 4.
Synthetic route of oxazolines 18a-e



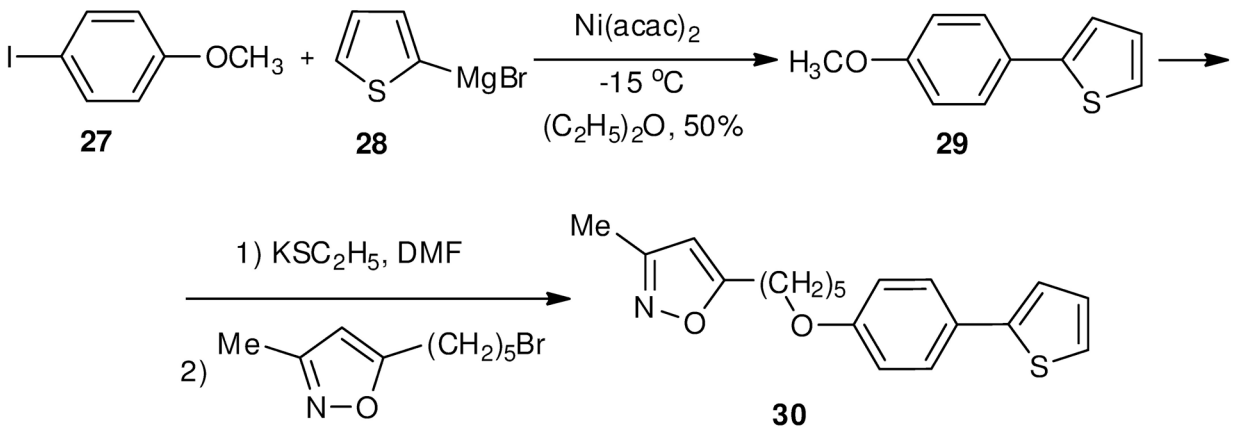
Scheme 5.
Three-step-synthesis of WIN 54954 (**22**)



23 24 25 26

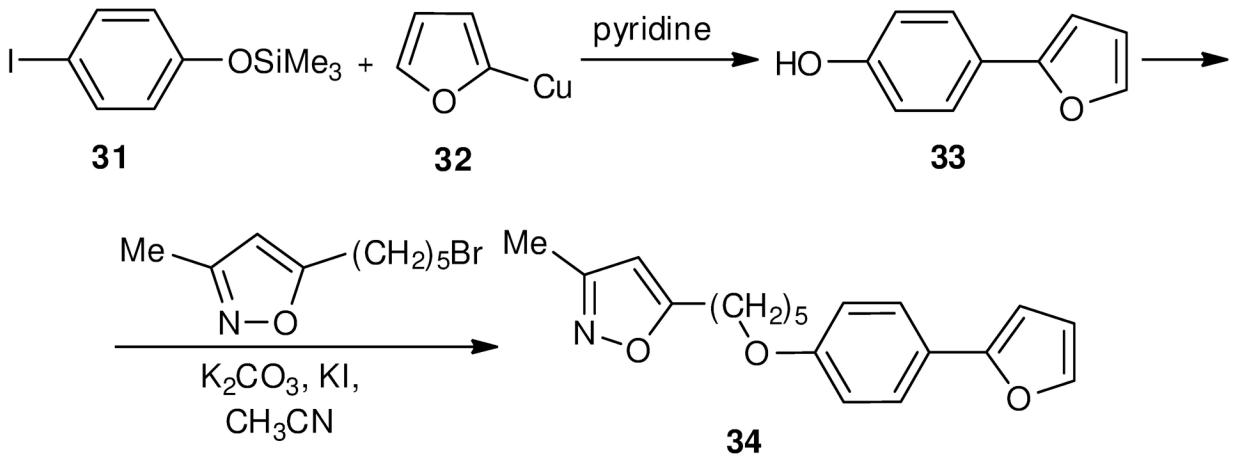
Scheme 6.

Synthesis of isoxazole derivative of 2,6-dichloro analogue of disoxaril **26**

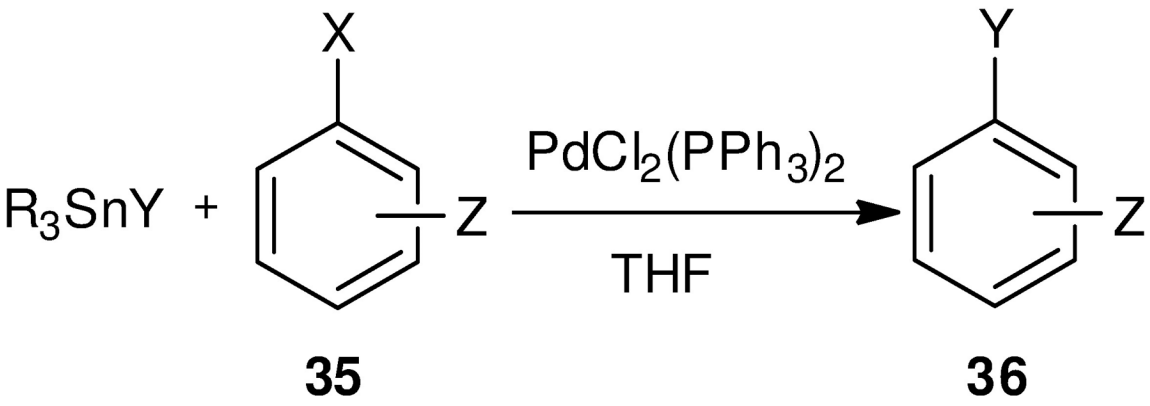


Scheme 7.

Synthetic route of thiophene derivative of WIN 54954 **30**

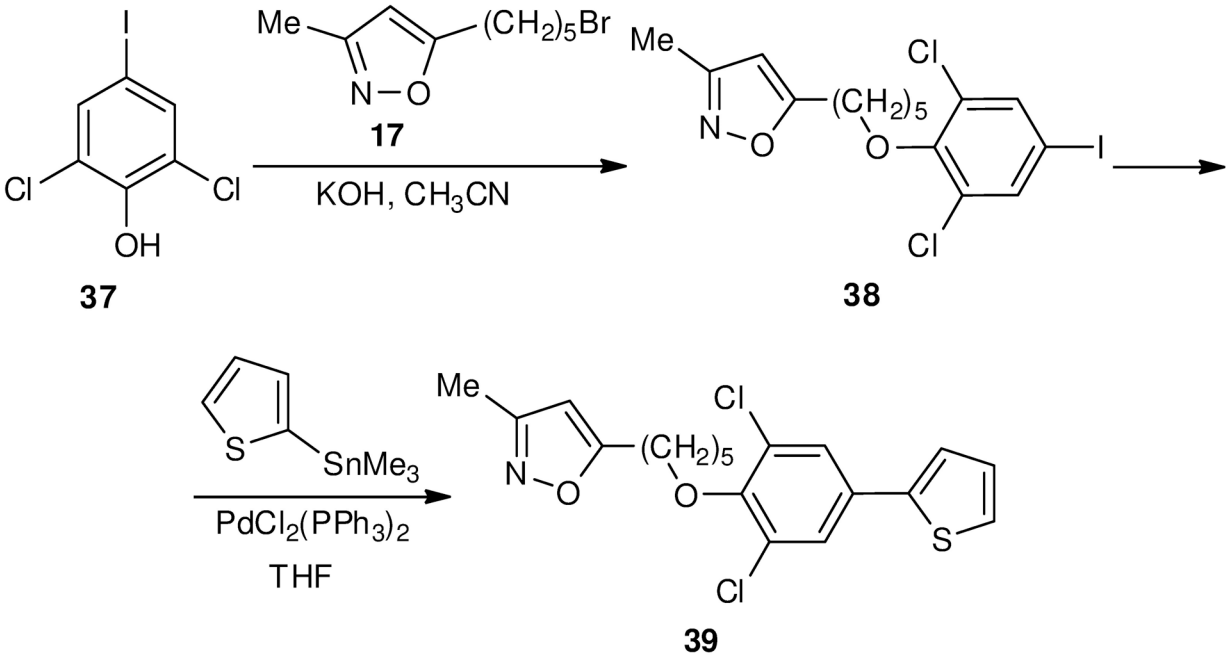


Scheme 8.
Synthetic route of furan derivative of WIN 54954 **34**

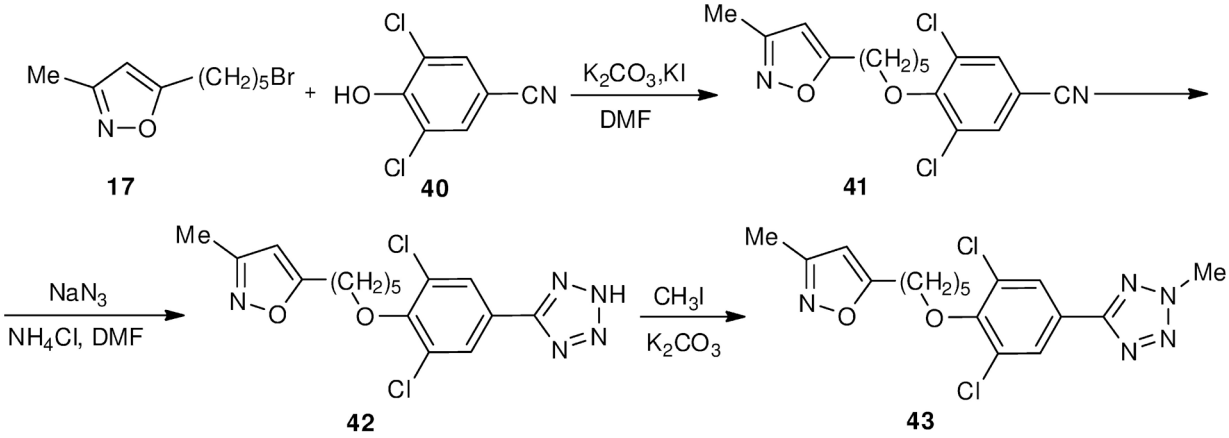


X = Br, I
Y = furyl, tienyl, pyridyl, 2-pyrryl, phenyl, imidazolyl
R = CH_3, *n*-bytil

Scheme 9.
Synthesis of compounds 36 by Pd^0-catalyzed cross-coupling

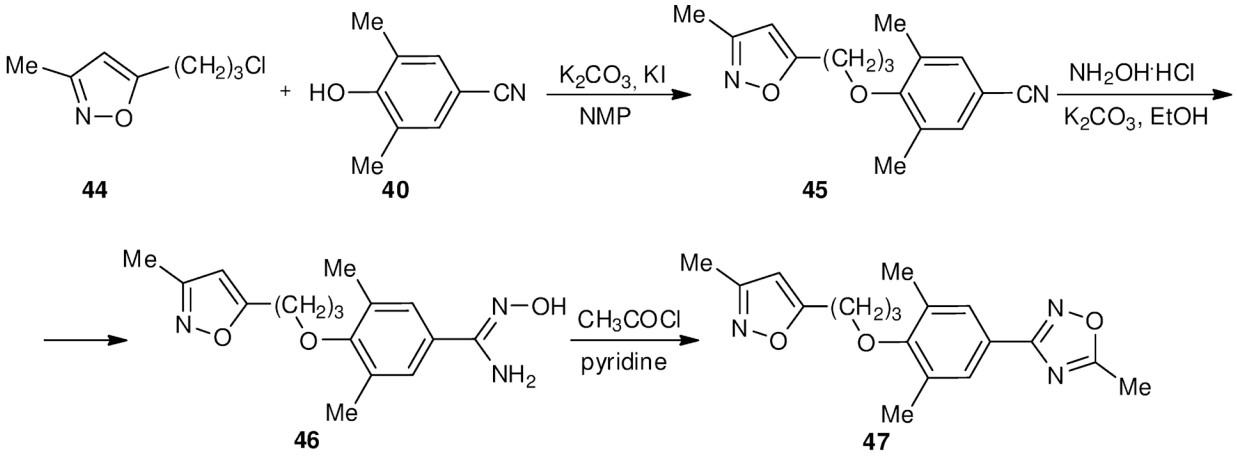


Scheme 10.
Synthetic route of thiophene derivative of WIN 54954 **39**



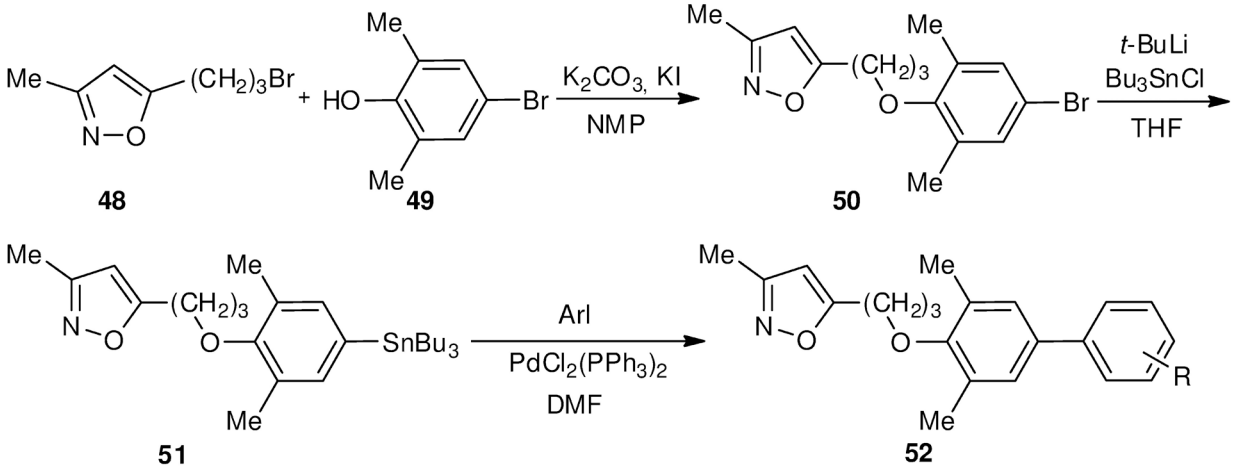
Scheme 11.

Synthesis of 2-methyltetrazole analogue of WIN-54954 **43**



Scheme 12.

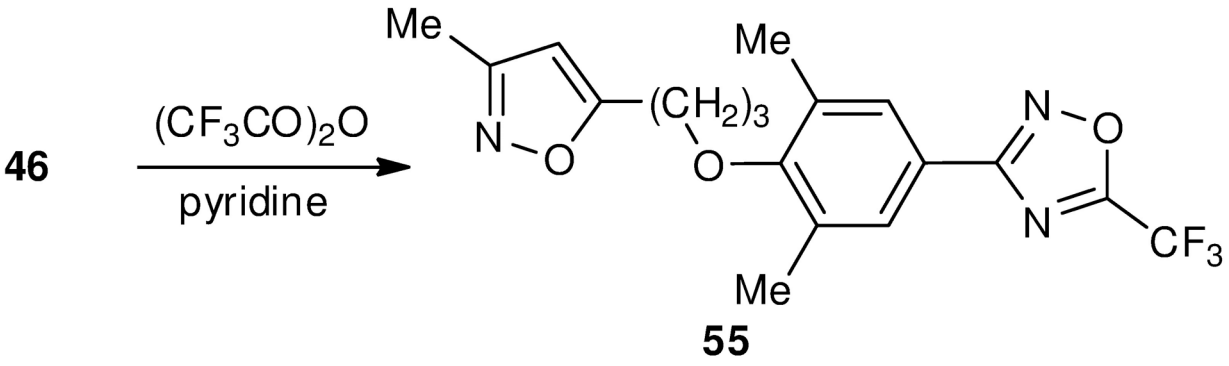
Synthesis of 3-(3,5-dimethyl-4-(3-(3-methylisoxazole-5-yl)propoxy)phenyl)-5-methyl-1,2,4-oxadiazole or WIN 61893 **47**



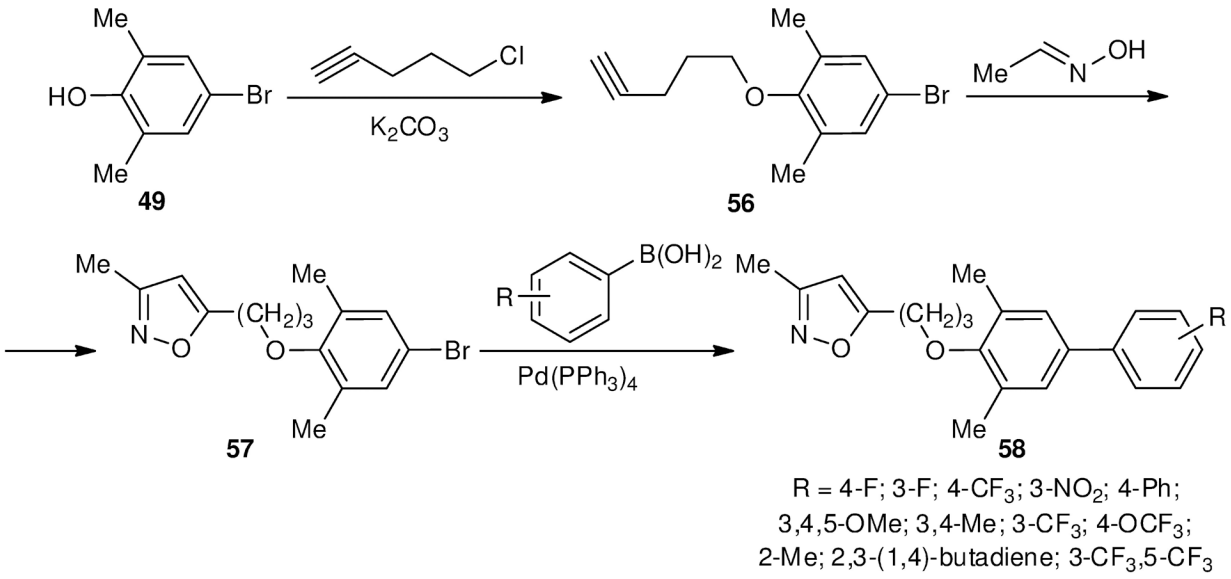
R: **a** H, **b** 3-Me, **c** 4-Me, **d** 3-OMe, **e** 4-OMe, **f** 3-OH, **g** 4-OH, **h** 3-CH_2OH, **i** 4-NMe_2, **j** 3-Cl, **k** 4-Cl, **l** 3-NO_2, **m** 4-NO_2, **n** 4-CF_3, **o** 3-CN, **p** 4-CN, **q** 2-F, **r** 3-F, **s** 4-F

Scheme 13.

Synthetic route of biphenyl analogs of WIN 54954 **52a-s**

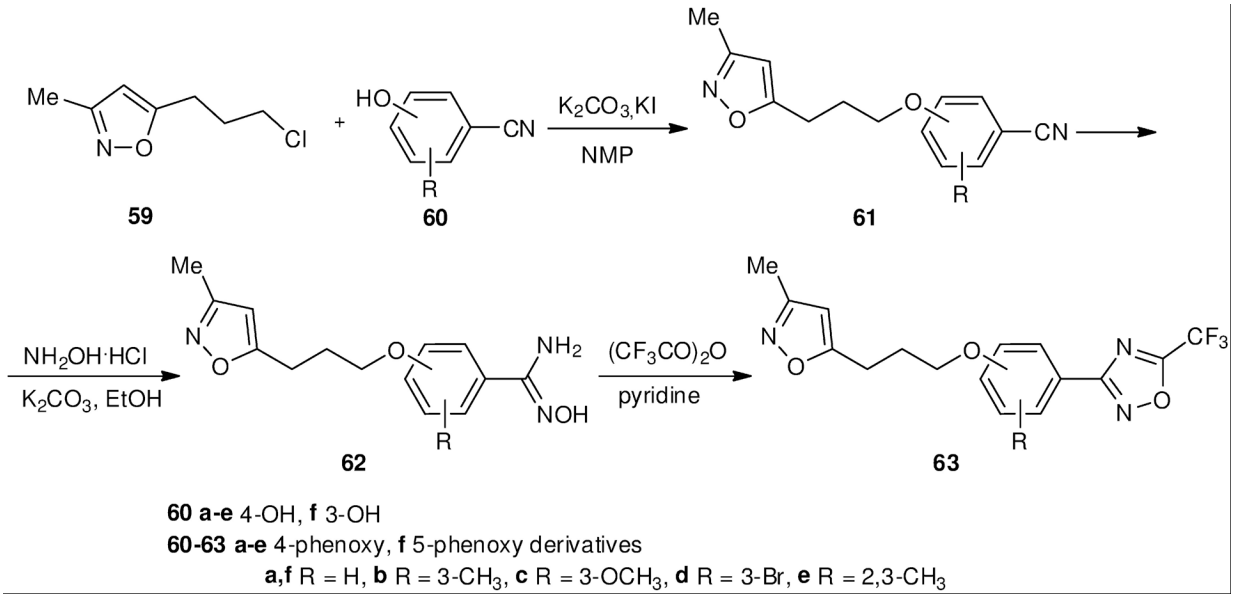


Scheme 14.
Synthesis of pleconaril **55** by treatment of amidoxime **46** with TFAA



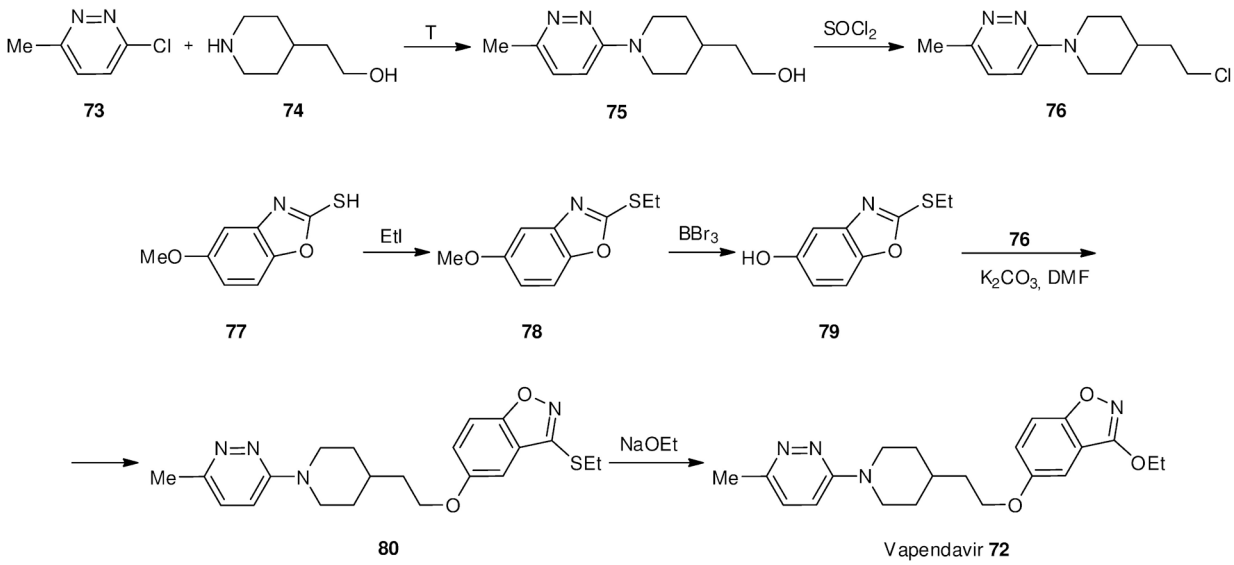
Scheme 15.

Synthetic route of novel biphenylderivatives of pleconaril **58**

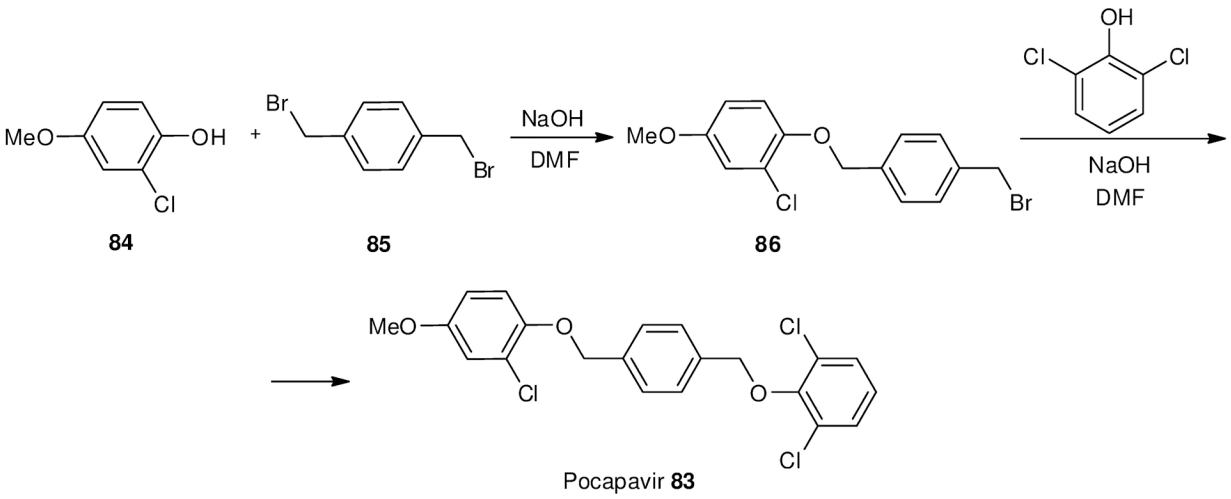


Scheme 16.

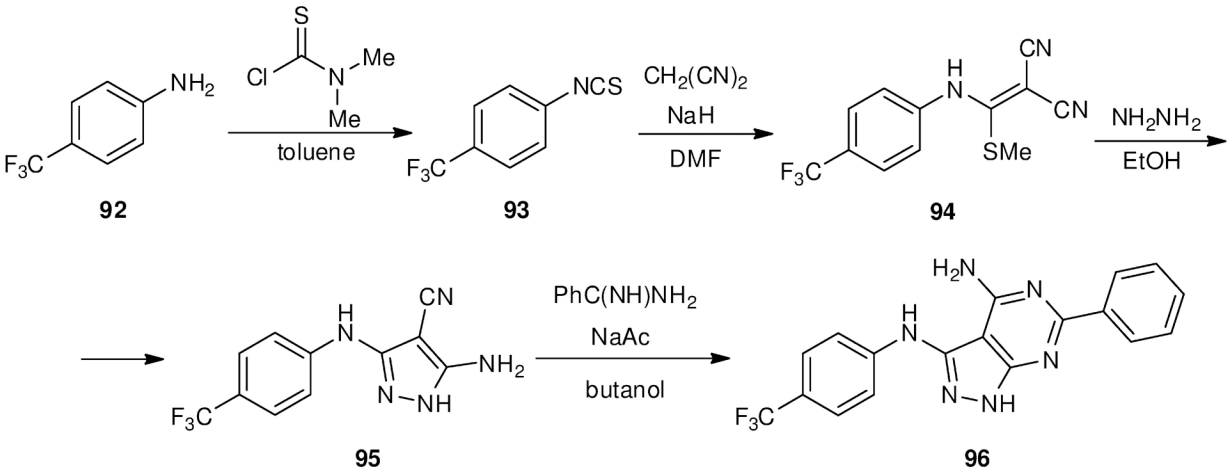
Synthesis of some pleconaril derivatives **63**



Scheme 17.
Synthesis of Vapendavir **72**

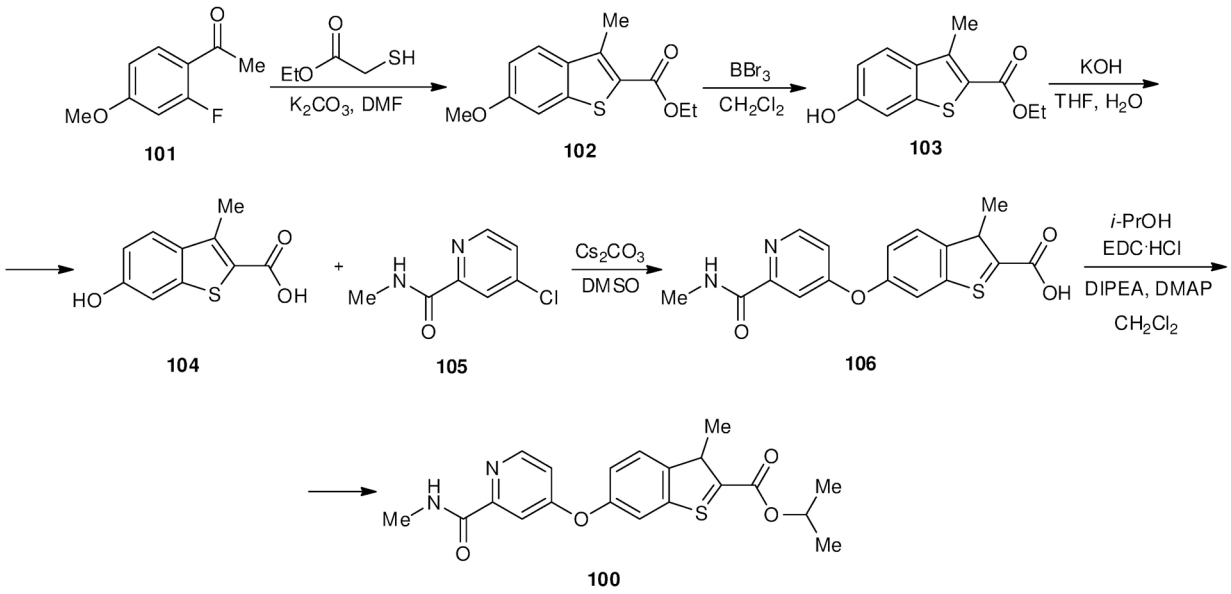


Scheme 18.
Synthesis of Pocapavir **83**



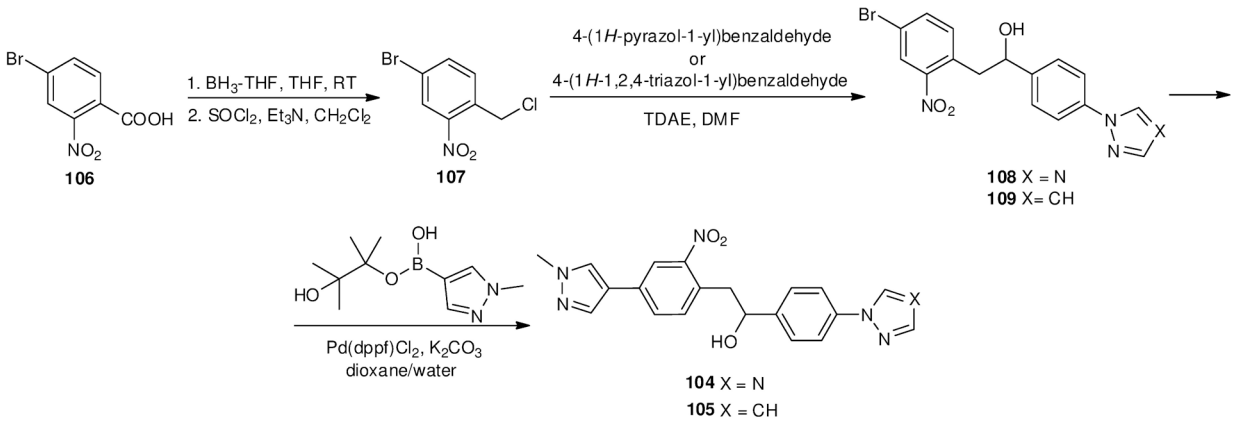
Scheme 19.

Synthetic route of 3-(4-trifluoromethylphenyl)amino-6-phenylpyrazolo[3,4-d]pyrimidine-4-amine **96**



Scheme 20.

Synthesis of benzothiophene derivative **100**



Scheme 21.

Synthesis of 1H-pyrazol-1-yl and 1H-triazol-1-yl analogues of 4,5-dimethoxybenzene **104** and **105**