**Novel [(biphenyloxy)propyl]isoxazole derivatives for inhibition of human rhinovirus 2 and coxsackievirus B3 replication**

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**Objectives:** During this study, novel biphenyl derivatives were synthesized and tested for antiviral activity.

**Methods:** A new method based on the Suzuki coupling reaction has been established for the synthesis of these polysubstituted chain systems. In parallel with cytotoxicity, the antiviral activity of biphenyl derivatives has been determined in cytopathic effect (CPE)-inhibitory assays with the pleconaril-resistant coxsackievirus B3 (CVB3) strain Nancy, human rhinovirus 2 (HRV-2) and 14 (HRV-14) and in plaque reduction assays with the pleconaril-sensitive human isolate CVB3 97-927 in HeLa cells. Based on the results from these investigations the selectivity index (SI) was determined as the ratio of the 50% cytotoxic concentration to the 50% inhibitory concentration.

**Results:** The new method based on the Suzuki coupling reaction includes the condensation of 2,6-dimethyl-4-bromophenol with pentyne chloride by means of potassium carbonate and potassium iodide in N-methylpyrrolidone-2 and yields 5-bromo-1,3-dimethyl-2-(4-pentynyloxy)benzene. Its condensation with methylacetaldoxime results in 3-methylisoxazole derivatives. The following reaction with different benzeneboronic acids by means of tetrakis(triphenylphosphine)-palladium(0) finally yields the corresponding derivatives. Several of the novel synthesized derivatives demonstrated a good antiviral activity on CVB3 (SI > 2 to > 37.5) and a strong anti-HRV-2 activity (SI > 50 to > 200). In contrast, none of the compounds inhibited the HRV-14-induced CPE.

**Conclusions:** These results indicate that [(biphenyloxy)propyl]isoxazole derivatives are potential inhibitors of HRV-2 and CVB3 replication, and make them promising agents for the specific treatment of these virus infections.

Keywords: antivirals, biphenyls, Suzuki reaction, picornaviruses, rhinoviruses, coxsackieviruses

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**Introduction**

Picornaviruses, in particular enteroviruses and rhinoviruses, are responsible for the majority of human viral disease.¹ The disease syndromes range from mild upper respiratory disease to fatal neurological or cardiac-based illnesses. Enteroviruses cause aseptic meningitis, encephalitis, febrile illness, hand, foot and mouth disease, myocarditis, and a variety of respiratory illnesses, whereas rhinoviruses are estimated to cause approximately one-third of all upper respiratory tract viral infections. Despite the fact that this virus family includes more than 200 virus species, vaccines have been developed only for polioviruses. Up to now, there is no specific antiviral therapy to treat or prevent enterovirus and rhinovirus disease.²

Picornaviruses are non-enveloped single-stranded positive-sense RNA viruses. The viral RNA genome is packaged in a capsid consisting of 60 repeating protomeric units, each one containing a copy of the four viral proteins VP1, VP2, VP3 and VP4. The structural organization of the viral capsid of several picornaviruses, e.g. human rhinovirus 14 (HRV-14), poliovirus and coxsackievirus B3 has been elucidated by crystallization...
and resolution of the three-dimensional structure. Structural differences among these picornaviruses were detected primarily on the viral surface. Interestingly, the β-sandwich structure of VP1, VP2 and VP3 was proved to be very conserved between rhinoviruses and enteroviruses. A hydrophobic pocket was found in VP1, which is occupied by a pocket factor, proposed to be a fatty acid, in most virus species.

Small antiviral compounds, the so-called ‘WIN’ compounds, may also enter this hydrophobic pocket and displace the existing pocket factor. As a result of this displacement, changes in the capsid structure that affect virus replication by preventing virus attachment to cell surfaces and/or the uncoating of viruses. The biochemical work leading to metabolically stable, highly active anti-picornavirus compounds was comprehensively reviewed by Diana. In 1995, a series of oxadiazoles was synthesized and tested for in vitro activity against isolates of human rhinoviruses and enteroviruses. Interestingly, compounds related to disoxaril (Figure 1) inhibit rhinovirus as well as poliovirus replication both in vitro and in vivo. These compounds consist of three rings, an oxadiazole, a dimethylphenol and a methylisoxazole. If the dimethylphenol or the 3-methylisoxazole cycles were substituted, the antiviral activity disappeared. After substitution of the oxadiazole by imidazole, pyrazole, tetrazole, oxazole, etc., antiviral activity was retained. By this manner, pleconaril, a new member of this class of compounds, was discovered (Figure 1). The development of pleconaril resulted from several modifications of the oxadiazole ring of disoxaril. For instance, pleconaril contains a trifluoromethyl oxadiazole that does not experience hydroxylation of the methyl group on the oxadiazole ring. By this manner pleconaril was protected from metabolism. Interestingly, this orally bioavailable and systemic-acting small-molecule inhibitor possesses a broad-spectrum activity against enteroviruses as well as rhinoviruses. Pleconaril inhibited all tested rhinovirus serotypes and 95% of non-polio enteroviruses. Interestingly, compounds related to pleconaril (Figure 1) inhibited rhinovirus at a compound concentration of 100 ng/mL or less. However, the existence of pleconaril-resistant enteroviruses, for instance coxsackievirus B3 variants, demands further optimization of the compound structure.

The aim of this study was to synthesize novel antiviral [biphenyloxy]propyl]isoxazole derivatives of pleconaril with various substituents and substitution patterns at the terminal benzene ring. For this, a new method of easy synthesis of biphenyl derivatives has been developed. The antiviral activity of these novel analogues has been determined against pleconaril-resistant as well as pleconaril-susceptible CVB3, HRV-2 and HRV-14.

Materials and methods

Chemistry

Melting points were determined according to the British Pharmacopoeia procedure and are uncorrected (Electothermal 9001; UK). If analyses are indicated only by the symbols of the elements, analytical results are within ±0.3% of the theoretical values (Carlo-Erba 5500; Italy). Nuclear magnetic resonance (NMR) spectra were determined on a Varian Unity +400 (USA). Shifts for 1H NMR are reported in ppm downfield from trimethylsilane (TMS) (δ). Mass spectra were obtained on a Finnigan SSQ-700 (USA) with direct injection. Infrared (IR) spectra were performed on a Perkin-Elmer 2000 FT-IR (USA).

5-Bromo-1,3-dimethyl-2-(4-pentynyloxy)benzene (III). A mixture of 4-bromo-2,6-dimethylphenol (5 g, 24.87 mmol), finely divided K2CO3 (6.18 g, 44.78 mmol), KI (0.4 g, 2.41 mmol), 5-chloropentane (3.95 mL, 37.26 mmol) and N-methylpyrrolidinone-2 (20 mL) was heated at 65°C for 24 h. The cooled mixture was diluted with H2O (80 mL) and EtOAc (30 mL). The layers were separated. The aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic phases were washed with H2O (50 mL), dried (Na2SO4), treated with activated carbon, filtered off, and concentrated in vacuo to an oil, which was dissolved in CHCl3, filtered though a short column of Silicagel 60, and concentrated in vacuo to 4.2 g of colourless oil (63.2%). This oil was crystallized in a refrigerator: mp 35–37°C. IR cm−1 (oil): 2118, 1625, 1588, 1577, 1472, 1435, 1350. δ (ppm) 8.26. 1H NMR (CDCl3) δ 7.13 (2H, s, 2 CH), 3.86 (2H, t, CH2O), 2.31 (2H, m, C-CH2), 2.01 (2H, m, CH2), 1.84 (1H, s, CCH). Anal. C13H15BrO, C,H,N.

5-[3-(4-Bromo-2,6-dimethylphenoxo)propyl]-3-methylisoxazole (V). Acetaldoxime (1.65 g, 28.00 mmol) in 16 mL of dimethylformamide (DMF) was added to a stirred solution of chlorosuccinimide (3.5 g, 28.08 mmol) in dried DMF (60 mL) and two drops of pyridine at 25°C. After 1 h, 5-bromo-1,3-dimethyl-2-(4-pentynyloxy)benzene (3.00 g, 11.23 mmol) in DMF (90 mL) was added. The reaction mixture was heated to 85–90°C. The solution was stirred and a solution of Et3N (3.9 mL, 27.72 mmol) in 30 mL of DMF (30 mL) was added drop by drop. The reaction was stirred for an additional 2 h, cooled and diluted with 300 mL of water and 50 mL of EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (2 × 75 mL). The combined organic phases were dried (Na2SO4), treated with activated carbon, filtered off and concentrated in vacuo to an oil. The oil was purified by column chromatography (SiO2 60, eluent: 1:1 hexane/acetone) and resulted in 3.3 g (91.6%) of 5-[3-(4-bromo-2,6-dimethylphenoxo)propyl]-3-methylisoxazole. The physicochemical data for this compound have already been described, but we obtained other data for 1H NMR spectra. mp 38–40°C (hexane). 1H NMR

Figure 1. Structures of disoxaril and pleconaril.
Antiviral [(biphenyloxy)propyl]isoxazole derivatives

(CDCl$_3$) at 7.13 (2H, s, 2CH benzene), 5.88 (1H, s, CH isoxazole), 3.84 (2H, t, OCH$_2$), 3.01 (2H, t, CH$_2$), 2.37 (3H, s, CH$_3$), 2.31 (6H, s, 2CH$_3$), 2.21 (2H, m, CH$_2$). Anal. (C$_{15}$H$_{18}$BrNO$_2$), C, H, N.

General procedure for palladium(0)-catalysed biphenyl cross-coupling according to the Suzuki–Miyaura reaction:

5-[(4-bromo-2,6-dimethyl-R-phenylphenoxy)propyl]-3-methylisoxazole (VI)

A suspension of 5-[(4-bromo-2,6-dimethylphenoxy)propyl]-3-methylisoxazole (0.60 g, 1.93 mmol), phenylboronic acid (2.10 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.09 g, 0.08 mmol) in dioxane (25 mL) was treated with a water solution (10 mL) of NaHCO$_3$ (0.78 g, 9.28 mmol) and heated at 100°C for 2 h. After addition of ethanol (10 mL), the reaction mixture was stirred and heated for an additional 2 h, cooled and diluted with 60 mL of water and 30 mL of EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (2 × 25 mL). The combined organic phases were washed twice with a 1 N solution of NaOH and then with water, dried (Na$_2$SO$_4$), treated with activated carbon, filtered and concentrated in vacuo to get the compound. Recently, compounds Vla-d were synthesized by Guiles et al. using a different method of synthesis. The physicochemical properties of compounds Vla-d described by these authors are identical with those obtained by the Suzuki reaction during preparation of this manuscript. Therefore, these compounds together with pleconaril were used as reference compounds in cytotoxicity and antiviral investigations.

Viruses and cells

Virus stocks of the pleconaril-resistant international reference strain coxsackievirus B3 Nancy (CVB3 Nancy; Institute of Poliomyelitis and Virus Encephalitis, Moscow, Russia), a pleconaril-sensitive clinical CVB3 isolate 97-927 (CVB3 97-927; kindly provided by Dr S. Diedrich, Robert-Koch Institute, Berlin, Germany), HRV-2 (kindly provided by Dr J. Seipelt, Greenhills Biotechnology Ltd, Vienna, Austria) and HRV-14 (kindly provided by Dr H.-P. Grunert, Universitätsklinikum Benjamin Franklin, Berlin, Germany) were prepared in HeLa cells (ATCC no. CCL-2), aliquotted, and stored at −80°C until use. HeLa cells were grown in Eagle’s minimal essential medium (Sigma no. M-0643) supplemented with 10% neonatal calf serum (NCS; HeLa Ohio, Greiner no. 758010; Germany), 100 U/mL penicillin and 100 μg/mL streptomycin. The test medium contains only 2% of NCS.

Determination of cytotoxicity

To determine the 50% cytotoxic concentration (CC$_{50}$), confluent HeLa cell monolayers grown in 96-well plates were incubated with serial two-fold dilutions of compounds for 72 h (37°C, 5% CO$_2$). Then, the cells were fixed and stained with a crystal violet formalin solution as described previously. After dye extraction, the optical density of individual wells was quantified spectrophotometrically at 550/630 nm with a microplate reader. Cell viability of individual compound-treated wells was evaluated as the percentage of the mean value of optical density resulting from six mock-treated cell controls, which was set as 100%. CC$_{50}$ was defined as the compound concentration, reducing the viability of untreated cell cultures by 50%.

Cytopathic effect-inhibitory assay

The cytopathic effect (CPE)-inhibitory assays were performed as described previously. Briefly, the tests were carried out using 1-day-old (HRV-2 and HRV-14) or 2-day-old (CVB3 Nancy) confluent HeLa cell monolayers growing in 96-well flat-bottomed microtitre plates (Falcon 3075). After removal of culture medium, 50 μL of drug solution and a constant amount of virus in a volume of 50 μL (0.1 and 0.01 multiplicity of infection for CVB3 and HRV-2 as well as HRV-14, respectively) were added to the cells. Six wells of non-infected cells and six wells of infected cells without the test compound served as cell and virus control, respectively, on each plate. The 50% and 100% plaque inhibitory concentrations of guanidine hydrochloride (three wells each) were included as positive controls for CVB3. Pleconaril was used as a reference compound for human rhinoviruses. Using the crystal violet uptake assay described for cytotoxic investigations, the inhibition of the virus-induced CPE was scored 24 h (CVB3) or 72 h (HRV-2 and HRV-14) post-infection when untreated infected control cells showed maximum cytopathic effect and the positive control compound-treated wells a 50% or 100% protection. The IC$_{50}$s of antiviral active compounds were determined from the mean dose–response curves of three separate experiments.

Plaque reduction assay

Confluent HeLa cells grown in 12-well plates were infected with ~50 pfu of CVB3 97-927. After 1 h of virus adsorption at 37°C in the presence of serial non-cytotoxic dilutions of test compounds, the infecting medium was removed and the cell monolayers were overlaid with test medium containing 0.4% agar-agar. After 30 h of incubation at 37°C the plates were fixed and stained with a crystal violet formalin solution to count the number of virus plaques and calculate the compound-induced plaque reduction.

Results

Synthesis and chemical characterization of new biphenyloxy derivatives

Two general methods, the Ullman and Pd-Sn coupling, have been successfully utilized for the preparation of biphenyls as novel antiviral agents. This strategy has now been further adapted to establish a highly convergent modern method for preparation of particular biphenyloxy compounds. One modification of pleconaril [(biphenyloxy)propyl]isoxazole analogue synthesis includes the primary synthesis of benzene pentine derivative with following cyclization of the isoxazole ring. Final compounds were directly synthesized from a bromo derivative with coupling according to Miyaura et al., using commercially available phenylboronic acids in the presence of tetrakis catalyst. This process results in high yields of the final product and can be easily adapted for combinatorial chemistry. In more detail, 4-bromo-2,6-dimethylphenol (I) was O-alkylated with pentyne chloride (II) in the presence of potassium carbonate and potassium iodide in methoxypropilidone yielding 5-bromo-1,3-dimethyl-2-(4-pentynyl)benzene (III) as shown in Figure 2. The condensation of 5-bromo-1,3-dimethyl-2-(4-pentynyl)benzene (III) with acetaldoxime in the presence of triethylamine in dimethylformamide results in an excellent yield of 3-methylisoxazole derivative (V). The exposure of the acetaldoxime intermediate for extended periods of time, at higher temperatures or the addition of abundance reagents, frequently caused side effects.
reaction. The reaction of 5-[3-(4-bromo-2,6-dimethylphenoxy)-propyl]-3-methylisoxazole (V) with different benzene boronic acids (VII) by means of tetrakis(triphenylphosphine)palladium(0) finally yields the corresponding [(biphenyloxy)propyl]isoxazole derivatives (VI). The tetraphenyl compound (VIII) was isolated with a yield of 5–7% as a by-product in these reactions. The physicochemical characteristics of the synthesized biphenyl compounds are shown in Table 1.

**Cytotoxicity and antiviral activity**

The CPE-inhibitory assays with the pleconaril-resistant CVB3 Nancy, HRV-2 and HRV-14, as well as the plaque reduction assays with the pleconaril-sensitive clinical isolate CVB3 97-927, were performed in HeLa cells. To exclude unspecific compound actions the cytotoxicity of compounds was determined in parallel in these cells. Based on the results the selectively index (SI) was determined as the ratio of the CC\textsubscript{50} to the IC\textsubscript{50}.

The CC\textsubscript{50} of compounds was in the range of 4.6 to >50\(\mu\)g/mL and depended on the nature of the substituent or the substitution pattern (Table 2). Whereas 4-F (VIa), 4-Ph (VIe) and 3-CF\textsubscript{3}, 5-CF\textsubscript{3} (VII) analogues were only weak or not at all cytotoxic up to the maximum test concentration of 50\(\mu\)g/mL, all other substitutions or substitution patterns led to enhanced cytotoxicity.

Most of the synthesized compounds in this series were inactive against CVB3 Nancy (data not shown) and CVB3 97–927 (Table 2). However, a weak inhibition of CVB3 Nancy-induced CPE was observed after treatment with 4-F, 3-F and 4-CF\textsubscript{3} analogues (Figure 3). The 4-F, 3-F, 2,3-(1,4) butadiene, 3- and 4-Me, and 2-Me analogues inhibited the plaque production of CVB3 97-297 with SIs of 37.3, 2.9, 2.3, 1.8 and 2.6, respectively. Whereas no inhibition of CVB3 Nancy was found after treatment with pleconaril in the non-cytotoxic dose range up to 12.5\(\mu\)g/mL (data not shown), the IC\textsubscript{50} of pleconaril against CVB3 97-297 amounted to 0.013\(\mu\)g/mL (SI 969.2).

**Table 1. Physicochemical properties of [(biphenyloxy)propyl]isoxazoles VIa-l**

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Solvent for recrystallization</th>
<th>Yield (%)</th>
<th>mp (°C)</th>
<th>Formula</th>
<th>M⁺</th>
</tr>
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<tbody>
<tr>
<td>VIa</td>
<td>4-F</td>
<td>MeOH</td>
<td>78</td>
<td>105–106</td>
<td>C\textsubscript{21}H\textsubscript{22}FNO\textsubscript{2}</td>
<td>339</td>
</tr>
<tr>
<td>VIb</td>
<td>3-F</td>
<td>a</td>
<td>69</td>
<td>43–44</td>
<td>C\textsubscript{21}H\textsubscript{23}FNO\textsubscript{2}</td>
<td>339</td>
</tr>
<tr>
<td>VIc</td>
<td>4-CF\textsubscript{3}</td>
<td>MeOH</td>
<td>67</td>
<td>93–95</td>
<td>C\textsubscript{22}H\textsubscript{22}F\textsubscript{3}NO\textsubscript{2}</td>
<td>389</td>
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<tr>
<td>VId</td>
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<td>MeOH</td>
<td>83</td>
<td>70–71</td>
<td>C\textsubscript{21}H\textsubscript{22}N\textsubscript{2}O\textsubscript{4}</td>
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<tr>
<td>VJe</td>
<td>4-Ph</td>
<td>EtOH</td>
<td>91</td>
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<tr>
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<td>MeOH</td>
<td>82</td>
<td>82–84</td>
<td>C\textsubscript{22}H\textsubscript{22}NO\textsubscript{3}</td>
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<tr>
<td>V Ig</td>
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<td>101–103</td>
<td>C\textsubscript{21}H\textsubscript{22}N\textsubscript{2}O\textsubscript{2}</td>
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<td>46</td>
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<td>C\textsubscript{22}H\textsubscript{22}F\textsubscript{3}NO\textsubscript{2}</td>
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<td>VJi</td>
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<td>99–101</td>
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<td>VJj</td>
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<td>VII</td>
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<td>56</td>
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<td>C\textsubscript{23}H\textsubscript{21}F\textsubscript{3}O\textsubscript{4}</td>
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<td>119–121</td>
<td>C\textsubscript{30}H\textsubscript{36}N\textsubscript{2}O\textsubscript{4}</td>
<td>488</td>
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\(a\) Compounds were purified by column chromatography (SiO\textsubscript{2}, eluent: chloroform).

The structures are shown in Figure 2.
The 4-F analogue exhibited the best anti-HRV-2 activity. The selectivity index of this compound was >5555. Furthermore, a strong anti-HRV-2 activity was detected for 3-F (SI 230), 3-CF3 (SI 206.9), 3-NO2 (SI 179.4), 4-CF3 (SI 112.1), and 3- and 4-Me (SI 93.9) analogues. All other compounds did moderately (SI 9.6–57.5) or not at all inhibit the HRV-2-induced cytopathic effect. In contrast, none of the compounds was active against HRV-14 regardless of the nature of the substituents or the substitution pattern (data not shown). The IC50 of the reference compound pleconaril amounted to 0.02 μg/mL for HRV-2 (SI 630) and 0.07 μg/mL for HRV-14 (SI 180).

Discussion

Drugs for successful management and control of enterovirus and rhinovirus infections have to exhibit potent antiviral activity against a broad spectrum of serotypes, and be systemically available and safe. Pleconaril, a novel capsid-binding compound blocking virus attachment and/or uncoating, meets these requirements. However, studying the resistance of enteroviruses against pleconaril, Groarke & Pevear13 detected both low and high pleconaril-resistant mutants of CVB3 consisting of single amino acid substitutions Ile-1092 → Leu or Ile-1092 → Met in the hydrophobic pocket of the viral capsid protein VP1. They suggested that these amino acid substitutions may enlarge the volume of the pocket, hinder the binding of the compound into the pocket and in this way induce pleconaril resistance. Further results from previous antiviral studies with rhinoviruses22 suggested that the oxazoline ring of pleconaril (A ring in Figure 1) may be important for the disposition of the compound in the canyon. Therefore, this part of the molecule was selected for modification. Several A ring benzene derivatives have been synthesized.24 These compounds exhibit improved metabolic stability and potency over an oxazoline derivative. During this study we synthesized and tested whether [biphenyloxy]propyl]isoxazole derivatives where the oxazoline ring of pleconaril is replaced by a phenyl ring would exhibit antiviral activity against the pleconaril-resistant CVB3 Nancy, and the pleconaril-susceptible CVB3 97-927, HRV-2 and HRV-14. Because substituted benzene derivatives allow us to vary different properties of molecules, e.g. electronic, steric and hydrophilic (or hydrophobic), properties that are extremely important for biological effects, such derivatives containing specific substituents and substitution, e.g. F, CF3 and NO2, were synthesized and studied.

The 4-F, 3-F and 4-CF3 analogues demonstrated an anti-CVB3 Nancy activity (Figure 3), whereas pleconaril did not at all. Using maximum non-cytotoxic concentrations the mean CPE inhibition reached ~50%. In addition to 4-F and 3-F analogues, the 3- and 4-Me, 2-Me and 2,3-(1,4)butadiene derivatives were active against the pleconaril-susceptible clinical isolate CVB3 97-927 (Table 2). But this antiviral activity was lower than that of pleconaril. CVB3 Nancy and CVB3 97-927 differ in the amino acid residue at position 1092 of the hydrophobic pocket in VP1. Whereas CVB3 Nancy contains a leucine at that position,23 CVB3 97-927 has an isoleucine.24 This supports the suggestion of Groarke and Pevear13 that the amino acid residue at position 1092 may affect the susceptibility of CVB3 to pleconaril. The crystal structure of a CVB3 strain containing Ile-1092

![Figure 3. Dose-dependent antiviral activity of compounds VIa-c against CVB3 Nancy. Different compound concentrations were added immediately before CVB3 infection to confluent HeLa cell monolayers. After 24 h, inhibition of viral cytopathic effect was evaluated. Each bar represents the mean ± s.d. of three independent experiments.](image-url)

Table 2. Cytotoxicity and antiviral activity of [biphenyloxy]propyl]isoxazoles VIa-l

<table>
<thead>
<tr>
<th>Compound</th>
<th>CC50 (μg/mL) HeLa cells</th>
<th>IC50 (μg/mL) CVB3 isolate 97-927</th>
<th>IC50 CVB3 isolate 97-927 SI</th>
<th>IC50 HRV-2</th>
<th>IC50 HRV-2 SI</th>
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<tr>
<td>VIa</td>
<td>&gt;50.0</td>
<td>1.34</td>
<td>&gt;37.3</td>
<td>0.009</td>
<td>&gt;5555</td>
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<tr>
<td>Vlb</td>
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<td>3.8</td>
<td>2.9</td>
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<td>Vlc</td>
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<td>&gt;12.0</td>
<td>not active</td>
<td>0.107</td>
<td>112.1</td>
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<td>Vld</td>
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<td>&gt;6.1</td>
<td>not active</td>
<td>0.034</td>
<td>179.4</td>
</tr>
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<td>Vle</td>
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<td>&gt;5.0</td>
<td>not active</td>
<td>&gt;50.0</td>
<td>not active</td>
</tr>
<tr>
<td>VIf</td>
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<td>&gt;9.9</td>
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<td>0.290</td>
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<td>VIg</td>
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<td>2.4</td>
<td>1.8</td>
<td>0.049</td>
<td>93.9</td>
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<td>not active</td>
<td>0.058</td>
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<tr>
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<td>&gt;10.8</td>
<td>not active</td>
<td>1.130</td>
<td>9.6</td>
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<td>2.6</td>
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</tr>
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</table>

*aSelectivity index calculated as the ratio of CC50 to IC50.
was published by Muckelbauer et al.\textsuperscript{4} In contrast, the crystal structure of a CVB3 having Leu-1092 is not known. Possibly, the amino acid at position 1092 has an effect on the volume of the hydrophobic pocket and in this way also on the binding of compounds into the pocket.

A series of compounds inhibited HRV-2 replication as effectively as pleconaril (Table 2). Our studies of structure–activity relationships revealed that only compounds containing small substituents such as F, CF\textsubscript{3} and Me in the benzene ring showed HRV-2 antiviral activity. The antiviral activity was reduced or lost after substitution with 4-Phe, 4-OCF\textsubscript{3}, 3-, 4- and 5-Me, as well as 2,3-(1,4)butadiene. Most active compounds have a small electron acceptor group or atom in the \textit{para} position of the phenyl ring. Derivatives with the same substituents in the \textit{meta} position or with two substitutes in the \textit{para} and \textit{meta} positions have comparable or less activity. The IC\textsubscript{50} obtained with the reference compounds, 4-F, 3-F, 3-CF\textsubscript{3}, 3-NO\textsubscript{2} analogues and pleconaril in antiviral assays with HRV-2 are in full agreement with previously published data.\textsuperscript{13} Unfortunately, all of the novel synthesized analogues were fully inactive against HRV-14. The sharp demarcation between susceptible and non-susceptible synthesized analogues were fully inactive against HRV-14. The antiviral activity was reduced or lost after substitution with 4-Phe, 4-OCF\textsubscript{3}, 3-, 4- and 5-Me, as well as 2,3-(1,4)butadiene. Most active compounds have a small electron acceptor group or atom in the \textit{para} position of the phenyl ring. Derivatives with the same substituents in the \textit{meta} position or with two substitutes in the \textit{para} and \textit{meta} positions have comparable or less activity. The IC\textsubscript{50} obtained with the reference compounds, 4-F, 3-F, 3-CF\textsubscript{3}, 3-NO\textsubscript{2} analogues and pleconaril in antiviral assays with HRV-2 are in full agreement with previously published data.\textsuperscript{13} Unfortunately, all of the novel synthesized analogues were fully inactive against HRV-14. The sharp demarcation between susceptible and non-susceptible rhinovirus serotypes has also been shown by Guiles et al.\textsuperscript{14} for HRV-3, HRV-4 and HRV-5. The reason is unclear. Interestingly, all of these insensitive serotypes belong to HRV-B viruses, also called minor group rhinoviruses, differing from HRV-A viruses, also called major group rhinoviruses, in amino acid alignment of the drug-binding pocket.\textsuperscript{15} According to Ledford et al.,\textsuperscript{15} the drug-binding pocket of HRV-14 belonging to HRV-B is significantly narrower than that of HRV-A viruses.

In summary, using a novel method of synthesis a series of [(biphenyloxy)propyl]isoxazole analogues, where the oxadiazole ring of pleconaril has been replaced with a substituted phenyl ring, showed excellent anti-HRV-2 and moderate anti-coxsackievirus B3 activity. These new biphenyl analogues offer the opportunity for the development of highly selective anti-rhinovirus agents.

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References