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2-Ethoxybenzoxazole as a bioisosteric replacement of an ethyl benzoate group in a human rhinovirus (HRV) capsid binder

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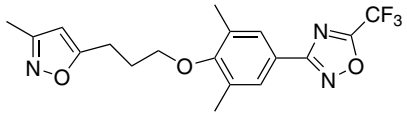
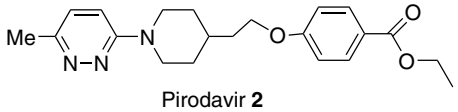
Abstract—A series of pyridazinylpiperidinyl capsid-binding compounds with novel bicyclic substituents were synthesized and screened against human rhinovirus (HRV). Several 2-alkoxy- and 2-alkylthio-benzoxazole and benzothiazole derivatives showed excellent anti-HRV activity. When tested against a panel of 16 representative HRV types the 2-ethoxybenzoxazole derivative **13** was found to have superior HRV activity (median EC_{50} 3.88 ng/mL) to known capsid-binders Pleconaril and Pirodavir. Compound **13** illustrates that a 2-alkoxybenzoxazole group can be an effective bioisostere for a benzoate ester or benzaldehyde oxime ether functionality.

Picornaviruses, particularly human rhinoviruses (HRV), cause approximately one-half of all cases of respiratory tract infection (colds)[1] and are responsible for over 25 million physician visits each year in the USA alone.[2] Although HRV infections are generally self-limiting, they are also associated with several serious upper and lower respiratory tract complications such as otitis media, chronic bronchitis and asthma.[3] No effective anti-rhinoviral agent is currently available for the control of HRV, although during the past decade three classes of active compounds have been reported including HRV capsid-binding compounds,[4] RNA synthesis inhibitors of the Enviroxime type[5] and HRV 3C protease inhibitors.[6] The orally available capsid-binder Pleconaril **1** (Fig. 1) has been shown to shorten the duration of upper respiratory illness in two large Phase III clinical studies in adults,[7] but unfortunately Pleconaril has yet to gain FDA approval for the treatment of HRV, due to safety and efficacy concerns. We now report the discovery of a promising new class of HRV capsid binders, which are significantly more active than **1**.



Pleconaril **1**



Pirodavir **2**

Figure 1. Known HRV capsid binders.

X-ray crystallography has been used to determine the capsid protein structure for several HRV types and the binding site has been determined for various capsid binders,[8] but the structural information has proved to be of limited use in the design of new inhibitors.[9] Based on reported in vitro assay results on a large set of HRV strains, the most active of the known capsid binders is the pyridazinylpiperidine derivative Pirodavir **2**, which at a concentration of 0.064 μg/mL inhibits 80% of 100 HRV strains.[10] Pirodavir undergoes facile in vivo hydrolysis of the ester functionality to the corresponding acid derivative, which is almost devoid of anti-HRV activity,[11] and therefore **2** is not suitable for oral use. When

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used intranasally six times daily against experimental HRV, **2** was found to give protection, provided administration was commenced prior to viral exposure.[12] However intranasal Pirodavir gave no statistically significant benefits in the treatment of naturally occurring HRV colds.[13] It was concluded that the lack of a clinical effect was the result of the poor pharmacokinetic properties of Pirodavir.[14] We believe that for widespread acceptance and ease of use, but also to achieve and maintain effective levels of antiviral agent in the tissues of the nasal cavity, it would be vital to develop a compound, which can be used orally. We disclose here a new family of bicyclic compounds, which are highly active against a wide range of rhinovirus serotypes in cell based in vitro assays and which have the potential for good oral availability.[15]

Previously we have reported that the oxime ether analogue **3** of Pirodavir shows excellent activity against a panel of 16 representative HRV strains (median EC_{50} 4.75 ng/mL).[16] As part of a larger structure–activity study we decided to investigate the effect on the anti-HRV activity of replacing the benzaldehyde oxime ether moiety in **3** with a bicyclic system such as a 2-ethoxybenzoxazole or 2-ethoxybenzothiazole. For the synthesis of such bicyclic isosteres the required precursors were the corresponding hydroxy compounds of types **4** and **5** (Fig. 2).

The most accessible precursor 5- or 6-hydroxy benzoazoles appeared to be the 6-hydroxy-2-alkyl-benzoxazoles **6**, which can be prepared in one step by rearrangement of the oxime of an appropriate 2,4-dihydroxyacylphenone.[17] 6-Hydroxy-2-methylmercaptobenzoxazole **7** was also readily prepared by the reaction of 4-aminoresorcinol with potassium ethyl xanthate[18] and then selective S-methylation (Scheme 1). The 6-hydroxy-benzoxazoles **6** and **7** were then coupled with either hydroxyethylpiperidine **8** or chloroethylpiperidine **9** following previously described general methods.[16] Thus the first bicyclic analogues of **3**, which we prepared were the benzoxazoles **10**–**12**, and we were sufficiently encouraged by the anti-HRV activity of compound **12** (Table 1) to go ahead with making further examples.[19] The 2-ethoxy compound **13** was prepared by simply heating the methylthio compound **12** with an excess of sodium ethoxide and we

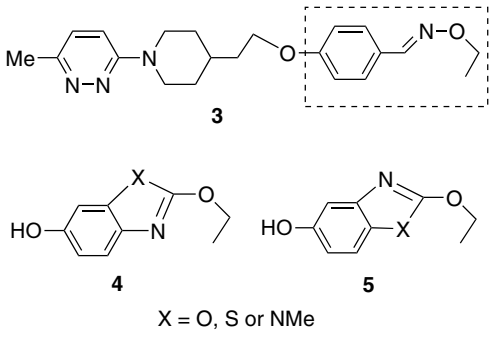
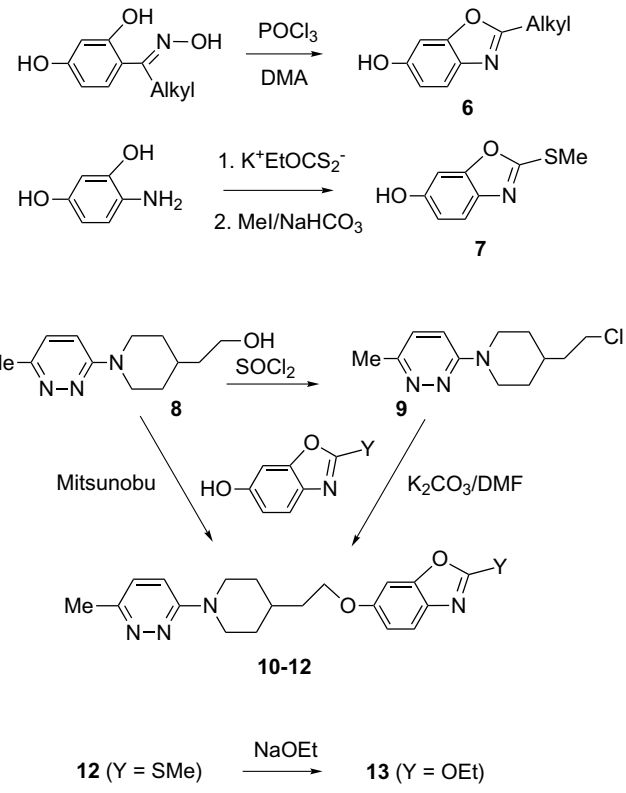


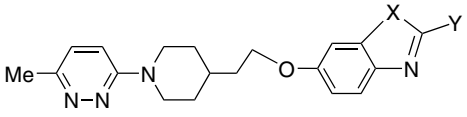
Figure 2. Oxime ether **3** and precursors for synthesis of bicyclic isosteres.



Scheme 1. Representative synthetic route to 6-linked benzoxazoles.

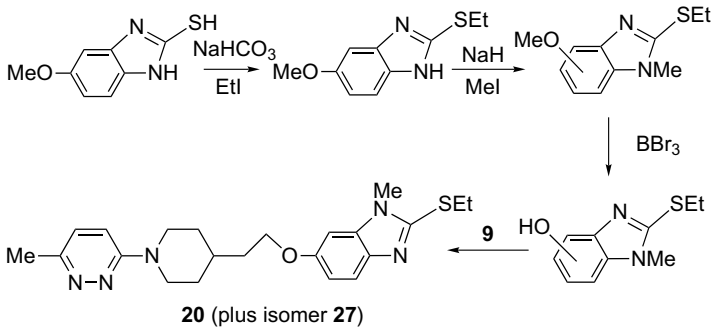
were pleased to find that **13** showed excellent anti-HRV activity, giving similar results to **3**. Using similar synthetic methods to those outlined above, more examples of 2-substituted benzoxazole systems were made and anti-viral testing established that 2-alkoxy or 2-alkylthio compounds (e.g., **13**–**14**) gave better activity than the 2-alkyl benzoxazoles (e.g., **17**–**18**) of equivalent size, whilst shorter or longer 2-alkoxy substituted com-

Table 1. Structure and anti-HRV activity of bicyclic compounds **10**–**20** compared to **3**



Compound no.	Group X	Group Y	EC_{50} (μg/mL)[a] HRV-2	EC_{50} (μg/mL)[a] HRV-14
10	O	CH_3	>0.50	>0.50
11	O	CH_2CH_3	0.144	0.130
12	O	SCH_3	0.099	0.047
13	O	OCH_2CH_3	0.003	0.007
14	O	SCH_2CH_3	0.002	0.006
15	O	$OCH_2CH_2CH_3$	0.002	0.020
16	O	OCH_3	0.159	0.099
17	O	$CH_2CH_2CH_3$	0.075	0.028
18	O	$(CH_2)_3CH_3$	0.130	0.082
19	S	OCH_2CH_3	0.007	0.006
20	NMe	SCH_2CH_3	0.102	0.169
3	O-Ethyl oxime ether		0.008	0.001

[a] None of the compounds showed cytotoxicity at the highest concentration (0.50 μg/mL).

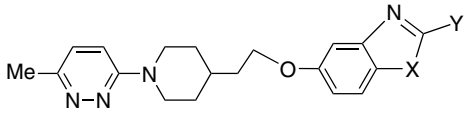


Scheme 2. Synthetic route to 5- and 6-linked *N*-methyl benzimidazoles.

pounds (**15**–**16**) were also not as active as **13**. The preparation of the benzothiazole analogue **19** of the highly active 2-ethoxybenzoxazole **13** was achieved starting from the known 2-chloro-6-hydroxybenzothiazole[20] and was found to have comparable activity. The analogous 2-ethylthio-*N*-methylbenzimidazole derivative **20** was prepared starting from commercially available 2-mercapto-5-methoxybenzimidazole following the sequence of reactions shown in Scheme 2. The product was isolated as a mixture of regioisomers **20** and **27**, which were separated by HPLC and the structures were assigned from their proton NMR spectra. Benzimidazole **20** and its regioisomer were found to be much less active than the analogous 2-ethylthio-benzoxazole **14**.

Using similar synthetic methods to those described above, a smaller set of the isomeric 5-linked bicyclic compounds **21**–**27** was also prepared and screened for activity on HRV (Table 2). For example the 5-linked 2-ethylthio-benzoxazole **23** (Scheme 3) was made starting from 2-mercapto-5-methoxybenzoxazole[21] and then the direct isomeric analogue of **13**, that is, the 2-ethoxybenzoxazole **22**, was prepared by heating **23** with excess sodium ethoxide in ethanol. Benzoxazoles **22**–**23** were found to be less active than the isomeric **13**–**14** and similarly the 5-linked benzothiazoles **24**–**26** were clearly less active than benzothiazole **19**. Given that we do not have X-ray crystal structures of any of these compounds in the complex with HRV and bearing in mind the notorious difficulty in predicting the location of capsid binders in the HRV hydrophobic pocket,[9] we do not have any convincing explanation for the superior activity of the 6-linked benzoxazole compounds compared to the 5-linked regioisomers.

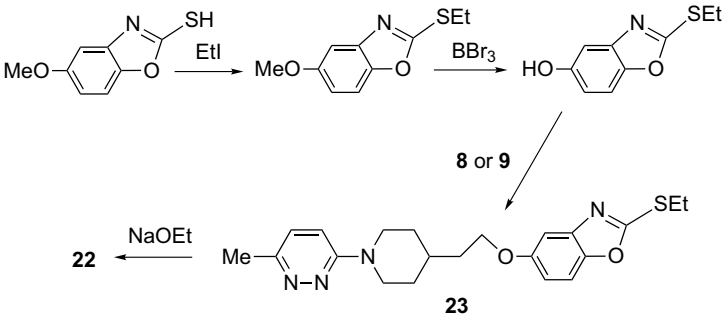
Table 2. Structure and anti-HRV activity of bicyclic compounds **21**–**27** compared to **3**



Compound no.	Group X	Group Y	EC_{50} (μg/mL)[a] HRV-2	EC_{50} (μg/mL)[a] HRV-14
21	O	CH_3	>0.50	>0.50
22	O	OCH_2CH_3	0.045	0.023[b]
23	O	SCH_2CH_3	0.004	0.050
24	S	OCH_2CH_3	0.104	0.014
25	S	$OCH_2CH_2CH_3$	0.166	0.041
26	S	SCH_2CH_3	0.165	0.049
27	NMe	SCH_2CH_3	0.086	>0.25
3	O-Ethyl oxime ether		0.006	0.001

[a] None of the compounds showed cytotoxicity at the highest concentration (0.50 μg/mL).
[b] HRV-16.

The more active bicyclic compounds were tested against a wider range of HRV subtypes and compound **13** in particular was tested against a set of 16 HRV strains, which is representative of the full spectrum of HRV serotypes.[22] The results (Table 3) show that compound **13** is approximately 10-fold more active than Pleconaril **1** and is equipotent with both Pirodavir **2** and the oxime ether **3**. Given the greater hydrolytic stability of the 2-ethoxybenzoxazole group compared with an ethyl ester, it is anticipated that compound **13** would have a much longer in vivo half life than **2** and therefore also greater oral bioavailability.[23] The bicyclic compounds reported



Scheme 3. Representative synthetic route to 5-linked benzoxazoles.

Table 3. Activity of compound **13** against a representative panel of HRV serotypes

HRV serotype	$EC_{50} \pm SD$[a] (ng/mL) for Pleconaril **1**	$EC_{50} \pm SD$[a] (ng/mL) for Pirodavir **2**	$EC_{50} \pm SD$[a] (ng/mL) for oxime ether **3**	$EC_{50} \pm SD$[a] (ng/mL) for benzoxazole **13**
2	26.47 ± 13.84	2.77 ± 2.23	9.23 ± 8.72	1.16 ± 0.81
9	37.16 ± 15.44	2.05 ± 1.05	1.09 ± 0.74	1.20 ± 1.01
14	34.31 ± 15.57	3.65 ± 1.95	1.78 ± 3.05	4.32 ± 1.27
15	69.80 ± 10.19	7.92 ± 2.92	7.77 ± 2.84	4.33 ± 1.70
16	108.03 ± 44.55	15.24 ± 6.25	11.12 ± 5.86	7.72 ± 3.53
29	45.40 ± 29.27	3.74 ± 2.59	5.99 ± 4.13	7.28 ± 7.10
39	44.88 ± 7.78	1.80 ± 1.92	2.89 ± 2.86	0.53 ± 0.023
45	1908 ± 910	>5000	>5000	>250 ($n = 3$)
51	30.40 ± 16.95	5.21 ± 4.50	2.90 ± 2.15	3.45 ± 1.13
59	387.33 ± 314.13	4.37 ± 3.17	5.08 ± 2.93	2.26 ± 1.90
63	62.30 ± 13.98	0.83 ± 0.57	2.06 ± 2.18	0.30 ± 0.11
70	53.87 ± 14.16	2.14 ± 0.71	3.13 ± 1.61	15.23 ± 9.00
72	468.70 ± 299.52	34.77 ± 6.07	21.01 ± 8.93	142.27 ± 46.62
85	34.00 ± 30.56	4.72 ± 5.59	7.90 ± 6.94	0.78 ± 0.30
86	70.04 ± 17.23	9.56 ± 6.72	4.42 ± 2.25	8.74 ± 2.71
89	29.33 ± 45.99	5.70 ± 1.90	0.83 ± 0.71	1.51 ± 0.71
Median	49.63	4.54	4.75	3.88

None of the compounds showed significant cytotoxicity at the highest concentration (1–5 μg/mL).
[a] For calculation of the EC_{50} and standard deviation (SD) all assays were run at least six times.

herein are an interesting example of the 2-alkoxy benzoazole ring system acting as a bioisosteric replacement of an aryl ester or an aryl oxime ether group.[24]

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References and notes

1. Makela, M. J.; Puhakka, T.; Ruuskanen, O.; Leinonen, M.; Saikku, P.; Kimpimaki, M.; Blomqvist, S.; Hyypia, T.; Arstila, P. *J. Clin. Microbiol.* **1998**, *36*, 539.
2. Turner, R. B. *Pediatr. Ann.* **1998**, *27*, 790.
3. Arruda, E.; Hayden, F. G. In *Antiviral Chemotherapy*; Jeffries, D. J., De Clercq, E., Eds.; John Wiley & Sons: New York, 1995; pp 321–355.
4. McKinlay, M. A.; Pevear, D. C.; Rossman, M. G. *Annu. Rev. Microbiol.* **1992**, *46*, 635.
5. Tebbe, M. J.; Spitzer, W. A.; Victor, F.; Miller, S. C.; Lee, C. C.; Sattelberg, T. R.; McKinney, E.; Tang, J. C. *J. Med. Chem.* **1997**, *40*, 3937.
6. Dragovich, P. S.; Prins, T. J.; Zhou, R.; Webber, S. E.; Marakovits, J. T.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Ford, C. E.; Burke, B. J.; Rejto, P. A.; Hendrickson, T. F.; Tuntland, T.; Brown, E. L., III; Meador, J. W., III; Ferre, R. A.; Harr, J. E. V.; Kosa, M. B.; Worland, S. T. *J. Med. Chem.* **1999**, *42*, 1213.
7. Hayden, F. G.; Herrington, D. T.; Coats, T. L.; Kim, K.; Cooper, E. C.; Villano, S. A.; Liu, S.; Hudson, S.; Pevear, D. C.; Collett, M.; McKinlay, M.Pleconaril Respiratory Infection Study Group *Clin. Infect. Dis.* **2003**, *36*, 1523.
8. For selected examples see: (a) Hadfield, A. T.; Diana, G. D.; Rossman, M. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14730; (b) Oren, D. A.; Zhang, A.; Nesvadba, H.; Rosenwirth, B. *J. Mol. Biol.* **1996**, *259*, 120; (c) Giranda, V. L.; Russo, G. R.; Felock, P. J.; Bailey, T. R.; Draper, T.; Aldous, D. J.; Guiles, J.; Dutko, F. J.; Diana, G. D.; Pevear, D. C. *Acta Crystallogr.* **1995**, *D51*, 496.
9. Diana, G.; Jaeger, E. P.; Peterson, M. L.; Treasurywala, A. M. *J. Comput. Aided Mol. Des.* **1993**, *7*, 325.
10. Andries, K.; Dewindt, B.; Snoeks, J.; Willebrords, R.; VanEemeren, K.; Stokbroekx, R.; Janssen, P. A. J. *Antimicrob. Agents Chemother.* **1992**, *36*, 100.
11. Andries, K. In *The Search for Antiviral Drugs*; Adams, J., Merluzzi, V. J., Eds.; Birkhauser: Boston, 1993; pp 179–209.
12. Hayden, F. G.; Andries, K.; Janssen, P. A. J. *Antimicrob. Agents Chemother.* **1992**, *36*, 727.
13. Hayden, F. G.; Hipskind, G. J.; Woerner, D. H.; Eisen, G. F.; Janssens, M.; Janssen, P. A. J.; Andries, K. *Antimicrob. Agents Chemother.* **1995**, *39*, 290.
14. Diana, G. D.; Pevear, D. C. *Antiviral Chem. Chemother.* **1997**, *8*, 401.
15. The HRV inhibitors described herein are the subject matter of International Patent Application PCT WO 02/50045.
16. Watson, K. G.; Brown, R. N.; Cameron, R.; Chalmers, D. K.; Hamilton, S.; Jin, B.; Krippner, G. Y.; Luttick, A.; McConnell, D. B.; Reece, P. A.; Ryan, J.; Stanislawski, P. C.; Tucker, S. P.; Wu, W.-Y.; Barnard, D. L.; Sidwell, R. W. *J. Med. Chem.* **2003**, *46*, 3181.
17. Fujita, S.; Koyama, K.; Inagaki, Y. *Synthesis* **1982**, 68.
18. Katz, L.; Cohen, M. S. *J. Org. Chem.* **1954**, *19*, 758.
19. All new compounds were initially tested on two HRV strains, generally HRV2 and HRV14, using standard cell culture cytopathic effect (CPE) based assays as described in Sidwell, R. W.; Huffman, J. H. *Appl. Microbiol.* **1976**, *22*, 797. All tests were carried out in duplicate generally using a dilution series of seven compound concentrations and EC_{50} values were determined both visually and by a dye uptake method. The variability of the results between duplicate runs and methods of determination was generally no more than one dilution.
20. Anderson, D. J. United States Patent 4,873,346.
21. Lok, R.; Leone, R. E.; Williams, A. J. *J. Org. Chem.* **1996**, *61*, 3289.

22. Andries, K.; Dewindt, B.; Snoeks, J.; Willebrords, R.; Stokbroekx, R.; Lewi, P. J. *Antiviral Res.* **1991**, *16*, 213.
23. Details of pharmacokinetic studies will be the subject of a future publication.
24. For reviews of bioisosterism see: (a) Lipinski, C. A. *Annu. Rep. Med. Chem.* **1986**, *21*, 283; (b) Burger, A. *Prog. Drug. Res.* **1991**, *37*, 287; (c) Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147.