Structure activity relationship of novel antiviral nucleosides against Enterovirus A71

Dilip K. Tosh[a], Kiran S. Toti[a], Brett L. Hurst[b], Justin G. Julander[b], Kenneth A. Jacobson[a],[*]

[a] *Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Disease, National Institutes of Health, 9000 Rockville Pike, Bethesda, MA 20892, USA*
[b] *Institute for Antiviral Research, Utah State University, 5600 Old Main Hill, VSB 305, Logan, UT 84322-5600, USA*

ABSTRACT

Various (North)-methanocarba adenosine derivatives, containing rigid bicyclo[3.1.0]hexane ribose substitution, were screened for activity against representative viruses, and inhibition was observed after treatment of Enterovirus A71 with a 2-chloro-N^6-1-cyclopropyl-2-methylpropan-1-yl derivative (**17**). μM activity was also seen when testing **17** against other enteroviruses in the *Picornaviridae* family. Based on this hit, structural congeners of **17**, containing other N^6-alkyl groups and 5′ modifications, were synthesized and tested. The structure activity relationship is relatively narrow, with most modifications of the adenine or the methanocarba ring reducing or abolishing the inhibitory potency. 4′-Truncated **31** (MRS5474), 4′-fluoromethyl **48** (MRS7704) and 4′-chloromethyl **49** nucleosides displayed EC_{50} ~3–4 μM, and **31** and **48** achieved SI ≥10. However, methanocarba analogues of ribavirin and N^6-benzyladenosine, shown previously to have anti-EV-A71 activity, were inactive. Thus, we identified methanocarba nucleosides as a new scaffold for enterovirus inhibition with a narrow structure activity relationship and no similarity to previously published anti-enteroviral nucleosides.

Human infection by members of the *Picornaviridae* family of enteroviruses is a continuing public health threat, leading to rashes and skin lesions (hand-foot-mouth disease) as well as neurological and respiratory symptoms that are often fatal.[1] There have been periodic outbreaks caused by Enterovirus A71 (EV-A71) and Enterovirus D68, especially in Eastern Asia but also in Western countries. Recently, acute flaccid myelitis (AFM) associated with Enterovirus 68 has affected hundreds of children in the US.[2] Poliovirus, which does have a vaccine, is a member of the same enterovirus family and causes poliomyelitis. The *Picornaviridae* are non-enveloped, positive-stranded RNA viruses. These viruses are usually spread by the fecal-to-mouth route and survive in the acidic gastric fluid before invading the lymphatic system. There are no approved potent antiviral small molecules for the enteroviruses.[3,4] Nevertheless, pleconaril **1** (Chart 1, Phase 3 halted), BTA-798 **2** (Phase 2b completed) and V-073 **3** (Phase 2b) that block viral entry through the viral capsid have shown promise in clinical trials. An epidithio-diketopiperazine, KCN-21, potently inhibited several members of the *Picornaviridae* family.[5,6] Also, viral enzyme inhibitors, e.g. 3C protease and RNA-dependent RNA polymerase 3D^{pol}, and viral entry inhibitors are being developed.[4,7,8]

The crystal structure of EV-A71 revealed details of a lipidic pocket factor for regulating the uncoating process bound in solvent-accessible hydrophobic canyon, and this canyon might serve as a target region for small molecule inhibitors.[9] P-selectin glycoprotein ligand-1 (PSGL-1) and scavenger receptor class B, member 2 (SCARB2) are putative cellular receptors on T cells that are associated with EV-A71 infection.[10] There is no known effective treatment for EV-A71 infection,[4] but a vaccine has shown efficacy in clinical trials.[11] Small molecules that inhibit EV-A71 replication, such as pyridyl imidazolidinones (oxadiazoles),[12] have been reported but not yet approved for clinical use.[3,13–15] Among a series of oxadiazoles, BPR0Z 074 **4**, inhibited the EV-A71 capsid protein with an IC_{50} of 0.8 nM.[15] The known antiviral drug ribavirin **5**, a nucleoside, is a weak inhibitor of EV-A71, of which resistant mutants have been identified, EV-D68 and the novel coronavirus SAR-CoV-2.[4,13,6–18] Other synthetic nucleoside derivatives were found to interact with the EV-A71 2C peptide to inhibit viral infection more potently,[19–21] but some of these nucleosides, such as N^6-benzyl-adenosine **6** (EC_{50} 5 μM), are associated with a small selectivity index (SI) for antiviral activity compared to cell toxicity. Modification of the benzyl group, as in N^6-(3-trifluoromethyl)benzyl-adenosine **7** (EC_{50} 0.068 μM), increased both the potency and SI.[21] Other N^6- and $C2′$-derivatized adenine nucleosides showed moderate potency in inhibiting EV-A71.[22,23] None of the EV-A71 antivirals or vaccines are approved clinically, and although drug repurposing efforts are

* Corresponding author at: Molecular Recognition Section, Bldg. 8A, Rm. B1A-19, NIH, NIDDK, LBC, Bethesda, MD 20892-081, USA.
  E-mail address: kennethj@niddk.nih.gov (K.A. Jacobson).

1, pleconaril

2, BTA 798

3, pocapavir, V-073

4, BPR0Z 074

5, ribavirin

6, R = H, N^6-benzyl-adenosine
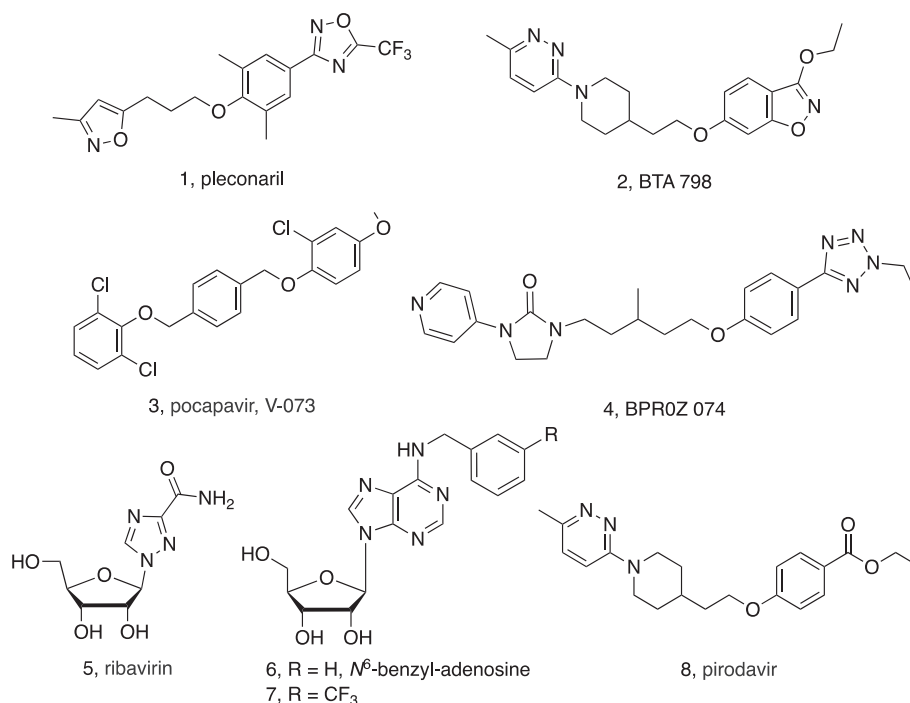**7**, R = CF$_3$

8, pirodavir

Chart 1. EV-A71 antiviral compounds.

underway, novel antiviral agents for the *Picornaviridae* family are needed.[24,25]

We have utilized a rigid bicyclic mimic of the ribose moiety for the design of nucleosides and nucleotides that bind to various G protein-coupled receptors (GPCRs) and transporters.[26–31] The methanocarba modification substitutes a bicyclo[3.1.0]hexane ring in place of the tetrahydrofuryl moiety of ribose. This modification was originally introduced by Marquez and colleagues for antiviral and anticancer therapeutics.[32] One methanocarba isomer maintains the North (N)-conformation of the pseudoribose ring, and an alternate placement of the fused cyclopropyl ring maintains a South (S)-conformation. (N)-Methanocarba nucleosides are often associated with enhanced affinity at various target proteins, compared to the corresponding ribosides.[26]

We screened adenosine derivatives from our in-house library to evaluate the applicability of the (N)-methanocarba nucleosides to viral inhibition including EV-A71 and other *Picornaviridae*. Based on the initial hit, the compounds in this study were either synthesized for this work, or samples were obtained from previous studies in our lab (Table 1).[15–19] Several parallel series were compared depending on the substitution at the 4′ position: adenosine-like CH$_2$OH (**13 – 30**), 4′-truncated (**31 – 43**), 4′-carbonyl (**44 – 47**) and substituted CH$_2$OH (**48 – 52**) derivatives. Additional methanocarba nucleoside derivatives (**53 – 59**), including 7-deazaadenosine derivatives and (S)-methanocarba-uridine, that were found to be inactive EV-A71 and other viruses are listed in Table S1 (Supplementary data). We first examined the in vitro inhibition of the replication of EV-A71 in a cell-based assay and compared the potency (EC$_{50}$) in inhibiting the Tainin/4643/98 strain with the CC$_{50}$ (compound concentration that reduces cell viability by 50%) in Vero 76 (monkey kidney epithelial) cells.[6,33] The detailed procedures for these assays are described in Smee et al.[5] Two antiviral parameters were measured: visual (virus yield reduction) and neutral red (cytopathy effect). The SI was calculated as the ratio of the toxic concentration, as CC$_{50}$ divided by the viral inhibitory concentration, as EC$_{50}$ (Table 1). Pirodavir **8** was used as a known standard antiviral drug and gave a SI ≥ 30.[33]

The synthetic routes to the novel (N)-methanocarba nucleoside analogues are shown in Schemes 1 and 2 with procedures in Supplementary data. The syntheses of other analogues are found in
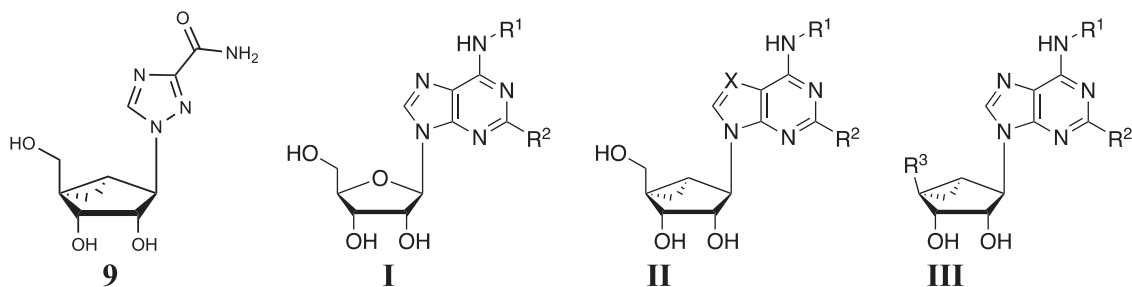
structure activity relationship (SAR) studies of adenosine receptors or 5HT$_{2B}$/5HT$_{2C}$ serotonin receptors as indicated in Table 1.[27–29,31] Additional compounds were either reported or utilized intermediates found in our earlier publications on opioid receptors or adenosine kinase inhibitors.[30,34] 2′,3′-Isopropylidene-protected (N)-methanocarba intermediates **60**, **61** and **71** were prepared as reported.[28–30] For 5′-hydroxy nucleosides (with adenine or 7-deazaadenine as nucleobase), nucleophilic substitution of 6-chloro with various amines was performed, and a 2-chloro or 2-iodo was optionally substituted with a 2-methylthio group. For adenine nucleosides having groups other than OH at the 5′ position, functional group substitution was accomplished by nucleophilic attack on a 5′-chloro **73** or 5′-mesylate **74** intermediate.

The SAR of (N)-methanocarba-adenosine derivatives as antiviral agents against Enterovirus A71 is summarized in Fig. 1. Although ribavirin **5** weakly inhibited EV-71A viral yield in infected cells (IC$_{50}$ of 65 µg/mL, 266 µM),[17] the (N)-methanocarba analogue **9** of ribavirin[31] was inactive at 50 µM and higher concentrations were not tested. However, various analogues (**15 – 19**, **21**, and **25 – 29**) in the (N)-methanocarba adenosine series containing a 5′–CH$_2$OH displayed measurable potency in inhibition of EV-A71 replication, in two assays of the cytopathic effect of the virus. In the 4′–CH$_2$OH series, compound **17** with a N^6-(R)-1-cyclopropyl-2-methylpropan-1-yl group inhibited viral replication with moderate potency as determined visually (Table 1, EC$_{90}$ = 7.7 µM), and the corresponding (S) diastereoisomer **21** displayed EC$_{50}$ values of 12 and 2.3 µM in the visual and neutral red assays, respectively. Other subtle N^6 group modifications of **17** produced EC$_{50}$ values in the range of 2 – 31 µM (neutral red assay). Other potent nucleoside inhibitors with Formula II were compounds **16**, **17** and **21**. Slight enlargement of the rings of N^6-dicyclopropylmethyl analogue **16** to N^6-dicyclobutylmethyl in **30** prevented inhibition. Other analogues of **17** modified at the 5′ carbon indicated that fluoro **48**, chloro **49**, and azido **51** substitution of the hydroxyl group led to intermediate potency, but 5′-methylthio **50** and 5′-amino **52** substitution reduced inhibition. An N^6-benzyl analogue **14** with *C*2-Cl was inactive at 100 µM in both assays. Thus, the SAR surrounding hydrophobic alkyl or aryl groups at the N^6 position had distinct steric requirements.

The 4′-truncated (N)-methanocarba-adenosine analogues were

Table 1
Activity of nucleoside derivatives in inhibition of Enterovirus 71 (Tainin/4643/98 strain), in the Vero 76 cell line. X = N, unless noted. The source of previously reported compounds is indicated by its citation. The configuration of chiral N^6 substituents appears at the lower right corner.

Compound	R^1 =	R^2 =, other	Visual (µM)[a]			Neutral red (µM)[a]		
			EC_{50}	CC_{50}	SI_{50}	EC_{50}	CC_{50}	SI_{50}
8	[Pirodavir][c]	–	0.86	> 27	> 31	0.57	17	30
9[31]	–	–	> 50	> 50	b	> 50	> 50	b
formula I								
10[31]		H	> 50	> 50	b	> 50	> 50	b
11[31]		H	> 100	> 100	b	> 100	> 100	b
12[31]		Cl	> 32	32	b	> 27	27	b
formula II (R^2 = Cl, unless noted)								
13[29,31]	H	Cl	> 50	> 50	b	> 36	36	b
14		Cl	> 100	> 100	b	> 100	> 100	b
15		Cl	32	> 100	> 3.1	32	> 100	> 3.1
16[29,31]		Cl	10	> 100	> 10	3.2	68	21
17[29]		Cl	7.7 EC_{90}	24	3.1SI_{90}	9.1	24	2.6
18		Cl, X = CH	32	> 100	> 3.1	24	86	3.6
19		I	18	100	5.6	20	46	2.3
20	(R)	SCH_3	> 100	> 100	b	66	> 100	> 1.5
21[29]	(S)	Cl	12	> 50	> 4.2	2.3	> 50	> 22
22	(S)	Cl	> 100	> 100	b	> 100	> 100	b
23	(S)	Cl, X = CH	> 100	> 100	b	> 63	63	b
24	(S)	SCH_3, X = CH	> 100	> 100	b	> 100	> 100	b
25	(R)	Cl	32	> 100	> 3.1	31	> 100	> 3.2

(*continued on next page*)

Table 1 (*continued*)

Compound	R^1 =	R^2 =, other	Visual (μM)[a]			Neutral red (μM)[a]		
			EC_{50}	CC_{50}	SI_{50}	EC_{50}	CC_{50}	SI_{50}
26[29]		Cl	16	> 50	> 3.1	2.3	> 50	> 22
27[29]		Cl	16	> 50	> 3.1	5.3	> 50	> 9.4
28[29]		Cl	16	> 50	> 3.1	8	13	1.6
29[29]		Cl	16	> 50	> 3.1	9.3	14	1.5
30[31]		Cl	16	50	3.1	> 28	28	b
formula III, R^3 = H **31**[27,28,31]		Cl	3.6	> 100	> 28	3.4	34	10
32[28]		H	> 50	> 50	b	> 50	> 50	b
33[28]		I	> 50	> 50	b	> 50	50	b
34[30]		I,X = CH	> 100	100	b	32	44	1.4
35[28]		$NHNH_2$	> 50	> 50	b	> 50	50	b
36[28]			> 50	> 50	b	> 50	50	b
37[28]			> 50	> 50	b	> 50	50	b
38[28]		Cl	16	> 50	> 3.1	12	> 50	> 4.2
39[28]		Cl	21	> 50	> 2.4	8.1	9.9	1.2
40[28]		Cl	18	> 50	> 2.8	> 7.6	7.6	b
41[28]		Cl	> 50	> 50	b	> 10	10	b
42[31]		Cl	> 16	16	b	> 17	17	b
43[27]		Cl	> 50	> 50	b	> 23	23	b
formula III, R^3 = H **44**[29]		Cl, R^3 = $CO_2CH_2CH_3$	> 50	> 50	b	> 1.5	1.5	b
45[29]		Cl, R^3 = CO_2H	> 50	> 50	b	> 50	50	b
46[29]		Cl, R^3 = $CONH(CH_2)_2NH_2$	> 50	> 50	b	> 50	50	b

(*continued on next page*)

Table 1 (continued)

Compound	R^1 =	R^2 =, other	Visual (µM)[a]			Neutral red (µM)[a]		
			EC_{50}	CC_{50}	SI_{50}	EC_{50}	CC_{50}	SI_{50}
47[29]		Cl, R^3 = CONH(CH$_2$)$_3$NH$_2$	> 50	> 50	b	> 50	50	b
48	(R)	Cl, R^3 = CH$_2$F	3.6	36	10	3.0	33	11
49	(R)	Cl, R^3 = CH$_2$Cl	4.3	32	7.4	3.9	27	6.9
50	(R)	Cl, R^3 = CH$_2$SCH$_3$	> 32	32	b	8.4	32	3.8
51	(R)	Cl, R^3 = CH$_2$N$_3$	5.3	32	6.0	4.8	32	6.7
52	(R)	Cl, R^3 = CH$_2$NH$_2$	> 32	32	b	> 32	32	b

Footnotes:

a - EC_{50} (or EC_{90} when indicated) - compound concentration that reduces viral replication by 50% (or 90%). CC_{50} - compound concentration that reduces cell viability by 50%. SI_{50} (selectivity index) = CC_{50}/EC_{50}.
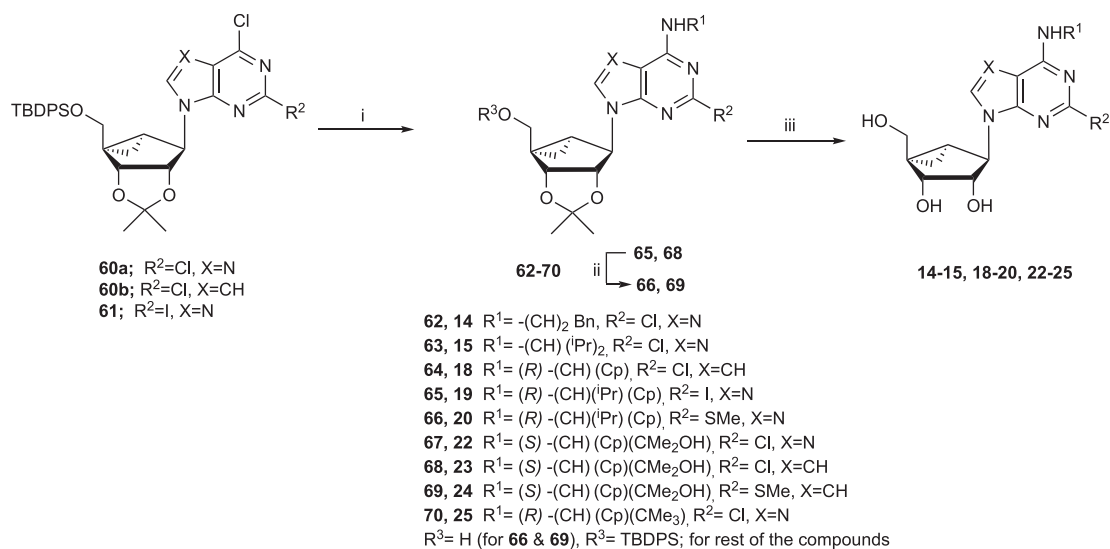
b - undetermined low value.

c - EC_{50} values of pirodavir were in the range of 0.036–0.32 (visual) and 0.056–0.22 (neutral red) µg/mL.

originally synthesized as adenosine receptor ligands.[27,28] N^6-Dicyclopropylmethyl analogue **31** (MRS5474) was moderately active in inhibiting viral replication (neutral red EC_{50} = 3.4 µM), but replacement of its 2-Cl with H prevented this activity. Thus, we examined other N^6 modified analogues in both the truncated and the 4′–CH$_2$OH series. The oxidized analogue **44** containing an ethyl ester group at the 4′ position was inactive. Homologation of **31** to the corresponding truncated N^6-dicyclobutylmethyl analogue **411** prevented activity, but the substitution with an N^6-(R)-1-cyclopropyl-2-methylpropan-1-yl group in **38** retained inhibition but with 4-fold less potency than the parent truncated compound **31**.

Several ribose-containing 2-H analogues **10** and **11** were inactive against EV-A71. However, there was indication in the 4′-truncated series that a 2-Cl substituent enhances EV-A71 inhibition (**31** vs. **32**). Therefore, a direct comparison of (N)-methanocarba 2-Cl-adenosine analogue **30** and its ribose equivalent **12** indicated that the methanocarba ring of **30** promotes the antiviral activity.

Lead compound **17** was tested against three *Picornaviridae* viruses related to EV-A71, coxsackie virus B3, enterovirus 68 and poliovirus 1, and the EC_{50} values indicated moderate inhibition (Table 2). EC_{50} values were in the range of 16 – 32 µM. In addition, compounds **31** and **48** showed $EC_{50/90}$ values of 8 – 16 µM against enterovirus 68,

60a; R^2=Cl, X=N
60b; R^2=Cl, X=CH
61; R^2=I, X=N

62-70

65, 68
66, 69

14-15, 18-20, 22-25

62, 14 R^1= -(CH)$_2$ Bn, R^2= Cl, X=N
63, 15 R^1= -(CH) (^{i}Pr)$_2$, R^2= Cl, X=N
64, 18 R^1= (R) -(CH) (Cp), R^2= Cl, X=CH
65, 19 R^1= (R) -(CH)(^{i}Pr) (Cp), R^2= I, X=N
66, 20 R^1= (R) -(CH)(^{i}Pr) (Cp), R^2= SMe, X=N
67, 22 R^1= (S) -(CH) (Cp)(CMe$_2$OH), R^2= Cl, X=N
68, 23 R^1= (S) -(CH) (Cp)(CMe$_2$OH), R^2= Cl, X=CH
69, 24 R^1= (S) -(CH) (Cp)(CMe$_2$OH), R^2= SMe, X=CH
70, 25 R^1= (R) -(CH) (Cp)(CMe$_3$), R^2= Cl, X=N
R^3= H (for **66** & **69**), R^3= TBDPS; for rest of the compounds

Scheme 1. Synthesis of 5′-hydroxy nucleosides (adenine and 7-deazaadenine). Reagents and Conditions: (i) R^1NH$_2$, DIPEA, 2-propanol, 70 °C (for X = N) or 140 °C, µwave (for X = CH); (ii) NaSMe, DMF, 90 °C; (iii) 10%TFA, MeOH, 70 °C.
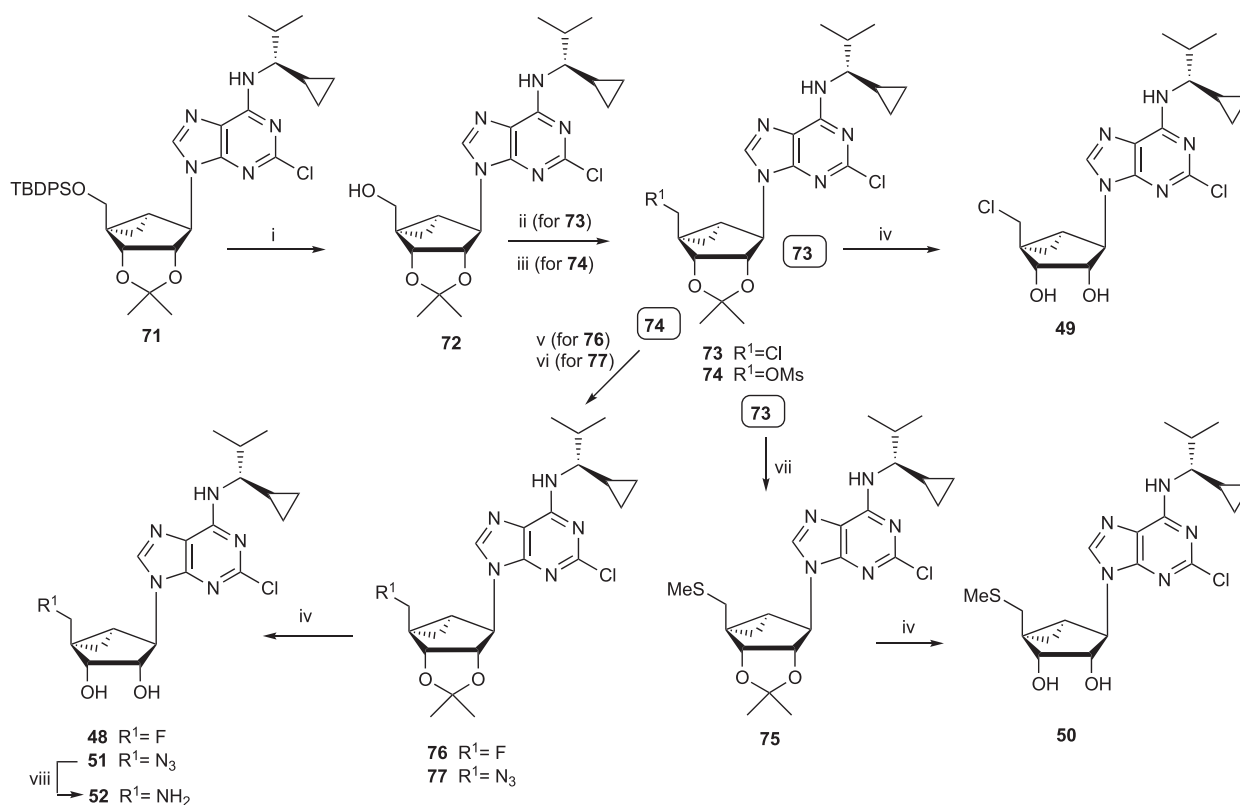
Scheme 2. Synthesis of 5′–non-hydroxy nucleosides. Reagents and Conditions: (i) TBAF, THF, rt; (ii) SOCl$_2$, pyridine, CH$_3$CN, −5 °C to rt; (iii) MsCl, pyridine, rt; (iv) 10% TFA, MeOH, 70 °C; (v) TBAF, THF, 70 °C; (vi) NaN$_3$, DMF, 60 °C; (vii) NaSMe, DMF, 0 °C; (viii) PMe$_3$, THF.

demonstrating activity as indicated by an SI > 10. Also, more structurally diverse (N)- or (S)-methanocarba nucleosides (**53 – 59**) were evaluated in inhibition of EV-A71 replication (Table S1, Supplementary data), but all were inactive. Several nucleosides showed very weak activity (Table S2) against respiratory syncytial virus (**31**), Rift Valley fever virus (**59**) and Tacaribe virus (**16**).

Off-target activities of these nucleosides are present, principally binding to adenosine and 5HT$_{2B/C}$ serotonin receptors, with affinities often in a similar range or greater than their antiviral potencies. Some of these other activities might be tolerable in antiviral agents. We previously reported that 2-Cl-adenine nucleoside **17** binds as an agonist to A$_1$, A$_{2A}$ and A$_3$ adenosine receptors (K$_i$ values 103, 2200 and

1240 nM, respectively), and as an antagonist to the 5HT$_{2B}$ and 5HT$_{2C}$ receptors (K$_i$ values 163 and 190 nM, respectively).[29] Other adenosine receptor affinities, for **13**, **16**, **21**, **26–29** and **44**,[29] for **12**,[31] and for **31–33**, **35–41** and **43**,[28] were reported to be variable, but generally K$_i$ > 0.1 μM. Truncated compound **31** was characterized as a moderately selective A$_1$ agonist,[28,31] and compounds **11** and **12** were potent at the A$_1$ receptor (K$_i$ < 10 nM).[31] Thus, there was no correlation between adenosine or serotonin receptor affinity of the reported compounds and their antiviral activity. The novel nucleosides were also tested systematically for off-target interactions at 45 different receptors, channels and transporters by the Psychoactive Drug Screening Program (PDSP, Supplementary data).[35] Representative hits were 2-iodo **19** and
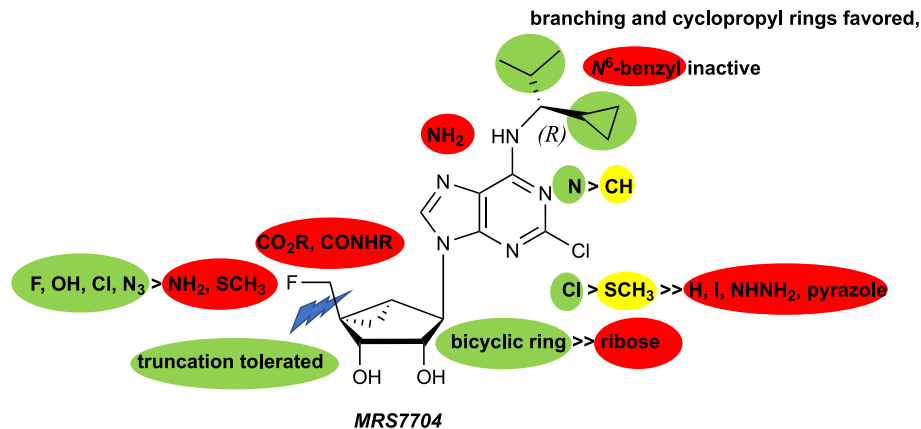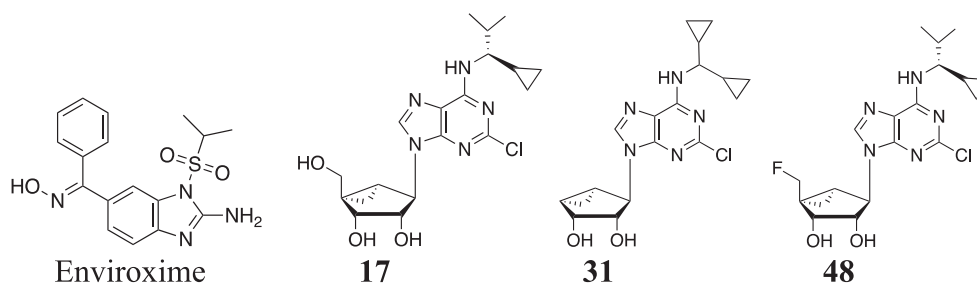

Fig. 1. SAR pattern of adenosine derivatives in antiviral activity against EV-A71, relative to 4′-fluoromethyl derivative **48**.

Table 2
Evaluation of the inhibition by selected nucleosides of three other members of the *Picornaviridae* family of enteroviruses. Enviroxime is shown as a reference inhibitor. EC_{50} (or EC_{90}, when indicated) is shown.

Compound	Visual (μM)			Neutral red (μM)		
	$EC_{50, 90}$	CC_{50}	SI_{50}	EC_{50}	CC_{50}	SI_{50}
Coxsackie virus B3 (HA 201,933 strain, Vero76 cells)						
Enviroxime	< 0.01	> 10	> 1000	< 0.01	4.5	> 450
17	16	> 50	> 3.1	16	> 50	> 3.1
Enterovirus 68 (US/KY/14–18953 strain, RD cells)						
Enviroxime	0.032	4.2	130	0.032	1.5	47
17	32	> 100	> 3.1	32	> 100	> 3.1
31	15 (EC_{90})	41	2.7	9.3	41	4.4
48	7.7 (EC_{90})	42	5.4	16	42	2.6
Poliovirus 1 (Mahoney strain, Vero76 cells)						
Enviroxime	0.015	> 10	> 670	< 0.01	4.9	> 490
17	28	> 100	> 3.6	27	> 100	> 3.7

2-methylthio **20** analogues, which bound to the 5HT$_{2B}$ and 5HT$_{2C}$ receptors with K$_i$ values in the range of 0.17 – 0.43 μM. Compound **15**, which is a bond-opened form of **17**, the 7-deaza analogue **18**, and 5′-fluoro derivative **48** bound to the 5HT$_{2B}$ receptor with K$_i$ values of 552 ± 167, 1310 ± 260 and 934 ± 66 nM, respectively.

In conclusion, we have discovered inhibition of EV-A71 by **17** and shown that other enteroviruses in the *Picornaviridae* family were similarly inhibited. We do not know the mode of interaction with the virus, and the approach must currently be through empirical SAR probing. The bicyclic ribose substitution and 2-Cl promote the viral inhibition. Thus, we have identified rigid (N)-methanocarba nucleosides as a new scaffold for enterovirus inhibition with a relatively narrow SAR. There was not a strong dependence of the viral inhibition on the N^6 group's stereochemistry. Analogues that displayed more potent inhibition than the initial hit compound **17** include 4′-truncated **31**, 4′-fluoromethyl **48** and 4′-chloromethyl **49** nucleosides that displayed EC_{50} values of ~3–4 μM. Compounds **31** and **48** achieved an SI of ≥10 by both criteria (visual and neutral red methods). Intermediate potency as was also associated with closely related analogues **16**, **21**, and **51**. Larger N^6 groups, such as benzyl, and C2 substitution other than chloro resulted in inactivity. Thus, we have identified methanocarba nucleosides as a new scaffold for enterovirus inhibition and defined its initial SAR, which did not resemble previously published anti-enteroviral nucleosides, suggesting a site of action, or mode of binding that is different than reported nucleosides. Future studies will be needed to enhance both the antiviral potency and the selectivity of these nucleosides.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127599.

References

1. Jones E, Pillay TD, Liu F, et al. *Eur J Paediatr Neurol*. 2018;22(5):763–773.
2. Messacar K, Asturias EJ, Hixon AM, et al. *Lancet Infectious Dis*. 2018;18(8):e239–e247.
3. Benschop KS, van der Avoort HG, Duizer E, Koopmans MP. *Antivir Ther*. 2015;20:121–130.
4. Bauer L, Lyoo H, van der Schaar HM, Strating JRPM, van Kuppeveld FJM. *Curr Opin Virol*. 2017;24:1–8.
5. Smee DF, Evans WJ, Nicolaou KC, Tarbet EB, Day CW. *Antivir Res*. 2016;131:61–65.
6. Nicolaou KC, Lu M, Totokotsopoulos S, et al. *J Am Chem Soc*. 2012;134:17320–17332.
7. van der Linden L, Wolthers KC, van Kuppeveld FJ. *Viruses*. 2015;7:4529–4562.
8. Martínez-Gualda B, Sun L, Martí-Marí O, et al. *J Med Chem*. 2020;63(1):349–368. https://doi.org/10.1021/acs.jmedchem.9b01737.
9. Plevka P, Perera R, Cardosa J, Kuhn RJ, Rossmann MG. *Science*. 2012;336(6086):1274. https://doi.org/10.1126/science.1218713.
10. Nishimura Y, Shimizu H. *Front Microbiol*. 2012;3:105. https://doi.org/10.3389/fmicb.2012.00105.
11. Zhu F, Xu W, Xia J, et al. *New Engl J Med*. 2014;370(9):818–828. https://doi.org/10.1056/NEJMoa1304923.
12. Shia K-S, Li W-T, Chang C-M, et al. *J Med Chem*. 2002;45(8):1644–1655.

13. H.J. Thibaut A.M. De Palma J. Neyts Biochem Pharmacol. 83 2 2012 185 192 10. 1016/j.bcp.2011.08.016.
14. Pourianfar HR, Grollo L.. *J Microbiol Immunol Infect.* 2015;48(1):1–8.
15. Shang L, Xu M, Yin Z. *Antivir. Res.* 2013;97(2):183–194.
16. Sadeghipour S, Bek EJ, McMinn PC. *J Virol.* 2013;87(3):1759–1769.
17. Li ZH, Li CM, Ling P, et al. *J Infect Dis.* 2008;197:854e7.
18. Khalili JS, Zhu H, Mak NSA, Yan Y, Zhu Y. *J Med Virol.* 2020;92:740–746. https://doi.org/10.1002/jmv.25798.
19. Drenichev MS, Oslovsky VE, Sun L, et al. *Eur J Med Chem.* 2016;111:84–94.
20. Oslovsky VE, Drenichev MS, Sun L, et al. *Molecules.* 2017;22:1219.
21. Arita M, Wakita T, Shimizu H. *J Gen Virol.* 2008;89(10):2518–2530.
22. Tararov VI, Tijsma A, Kolyachkina SV, et al. *Eur J Med Chem.* 2015;90:406–413.
23. Shang L, Wang Y, Qing J, et al. *Antivir Res.* 2014;112:47–58.
24. Sun J, Yogarajah T, Lee RCH, et al. *Sci Rep.* 2020;10:8159. https://doi.org/10.1038/s41598-020-65152-4.
25. Lin J, Kung Y, Shih S. *J Biomed Sci.* 2019;26:65. https://doi.org/10.1186/s12929-019-0560-7.
26. Jacobson KA, Tosh DK, Toti KS, Ciancetta A. *Drug Disc Today.* 2017;22:1782–1791.
27. Tosh DK, Phan K, Deflorian F, Wei Q, Gao ZG, Jacobson KA. *ACS Med Chem Lett.* 2011;2:626–631.
28. Tosh DK, Paoletta S, Deflorian F, et al. *J Med Chem.* 2012;55:8075–8090.
29. Tosh DK, Ciancetta A, Warnick E, Crane S, Gao ZG, Jacobson KA. *J Med Chem.* 2016;59:11006–11026.
30. Tosh DK, Ciancetta A, Mannes P, et al. *ACS Omega.* 2018;3:12658–12678.
31. Tosh DK, Rao H, Bitant A, et al. *J Med Chem.* 2019;62:1502–1522.
32. Marquez VE. The properties of locked methanocarba nucleosides in biochemistry, biotechnology and medicine. In Modified Nucleosides in Biochemistry, Biotechnology and Medicine (Herdewijn, P., ed.), 2008;307–341, Wiley-VCH.
33. Barnard DL, Hubbard VD, Smee DF, et al. *Antimicrob Agents Chemother.* 2004;48:1766–1772.
34. Toti KS, Osborne D, Ciancetta A, Boison D, Jacobson KA. *J Med Chem.* 2016;59:6860–6877.
35. Besnard J, Ruda GF, Setola V, et al. *Nature.* 2012;492:215–220.