9-Arylpurines as a Novel Class of Enterovirus Inhibitors

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Here we report on a novel class of enterovirus inhibitors that can be structurally described as 9-arylpurines. These compounds elicit activity against a variety of enteroviruses in the low μ M range including Coxsackie virus A16, A21, A24, Coxsackie virus B3, and echovirus 9. Structure–activity relationship (SAR) studies indicate that a chlorine or bromine atom is required at position 6 of the purine ring for antiviral activity. The most selective compounds in this series inhibited Coxsackie virus B3 replication in a dose-dependent manner with EC₅₀ values around 5–8 μ M. No toxicity on different cell lines was observed at concentrations up to 250 μ M. Moreover, no cross-resistance to TBZE-029 and TTP-8307 CVB3 resistant strains was detected.

Introduction

Enteroviruses are responsible of a broad spectrum of acute and chronic human diseases, including respiratory infections, meningitis, encephalitis, pancreatitis, myocarditis, and neonatal sepsis. Enteroviruses, belonging to the Picornaviridae family, are small nonenveloped and spherical RNA viruses, with a diameter of about 30 nm. Despite their high impact on human health, no drugs have been approved for the treatment of enterovirus infections.^{1,2} The genus Enterovirus comprises more than 60 serotypes of which Coxsackie virus type B3 (CVB3^a) can be considered as the prototype for the nonpolio enterovirus group. CVB3 is an important human pathogen that may cause acute and chronic viral myocarditis in children and young adults which eventually can progress to cardiomyopathy.^{3,4} In addition to heart infections, CVB3 also causes chronic inflammatory diseases of the pancreas and central nervous system.^{1,5,6} Several molecules have been reported to be selective inhibitors of enteroviruses, some of which have entered clinical trials⁷ but none of them has been formally approved. Therefore there is an urgent need for the discovery and development of chemical entities able to selectively inhibit enterovirus replication.

Given our interest in purine nucleosides and analogues,^{8–10} we focused our attention on 9-((3-hydroxymethyl)phenyl)purines, compounds that have been scarcely explored and that in some instances have been proposed as nucleoside surrogates. Chern et al. reported in 1993 the synthesis of the 8-aminoguanine derivative (Chart 1, X = OH, $Y = Z = NH_2$) that was found inactive against purine nucleoside phosphorylase.¹¹ Later on, Brakta et al. described the synthesis of the adenine derivative (Chart 1, $X = NH_2$, Y = Z = H) as a substrate of adenosine deaminase.¹² More recently, Ueno et al. prepared bis(hydroxymethyl)benzene residues directly connected to the nucleobases to construct a nucleic acid analogue.¹³ The synthesis of these 9-arylpurines has been carried out following the classical method described by Greenberg in 1959 that involves reaction of 5-amino-4, 6-dihalopyrimidines with anilines followed by a ring closing reaction.¹⁴ However, this strategy requires prolonged heating and yields are variable.

More recently, another synthetic approach to 9-arylpurines has been described that consist in the cross coupling reaction of the purine base with arylboronic acids catalyzed by copper salts, initially described by Ding¹⁵ and further studied by Gundersen,¹⁶ by adapting the general procedure of Chan,¹⁷ Lam,¹⁸ and Evans¹⁹ and recently reported by other laboratories.^{20,21} Making use of this approach, we synthesized a number of 9-((3-hydroxymethy)phenyl)purines that were evaluated against the replication of different DNA and RNA viruses. Interestingly, compounds with a chlorine atom at position 6 of the purine base (Chart 1, X = Cl) exerted significant activity against Coxsackie virus type B3 (CVB3) replication at nontoxic concentrations. On the basis of their low molecular weight, their structure that significantly differs from previously reported anti-CVB3 compounds¹ and their selectivity index, we initiated a program to synthesize different 9-arylpurines. Here we report on the synthesis, antiviral evaluation, and structure-activity relationships on this new family of anti CVB3 compounds.

Results and Discussion

Chemistry. Initially, the synthesis of 9-(3-(hydroxymethy)phenyl)purines was performed employing the conditions set up by Bakkestuen and Gundersen¹⁶ for the regioselective *N*9-arylation of purines employing arylboronic acids in the presence of Cu(II). Thus, the 6-chloropurine (1) (1.0 equiv) was allowed to react with 3-hydroxymethylphenylboronic acid

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^{*a*} Abbreviations: CVB3, Coxsackie virus type B3; MAOS, microwave-assisted organic synthesis; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium.

Chart 1. General Structure of 9-(3-(Hydroxymethy)phenyl)purines



Scheme 1. Synthesis of 9-(3-(Hydroxymethy)phenyl)purines from Arylboronic Acids^{*a*}



^{*a*} Reagents and conditions: (a) purine base (1,4-7, 1.0 equiv), aryl boronic acid **2** (2.0 equiv), 1,10-phenanthroline (2.0 equiv), Cu(OAc)₂ (2.0 equiv), DMF, rt, 3 days (**3**, 60% yield; **8**, 46% yield; **9**, 30% yield; **10**, 43% yield; **11**, 58% yield).

(2) (2.0 equiv) in the presence of 1,10- phenanthroline (2.0 equiv), cupric acetate (1.0 equiv) in dry dichloromethane and in the presence of activated 4 Å molecular sieves for 4 days at room temperature. Under these conditions, the desired coupling product 3 was obtained but the yield was quite low (26%). To improve this yield, different assays were performed including the employment of other bases such as triethylamine, pyridine, or other solvents such as 1,4-dioxane or DMF. Also the impact of the presence or absence of 4 Å molecular sieves was evaluated.²² In our hands, the best conditions were 1.0 equiv of 6-chloropurine, 2.0 equiv of the aryl boronic acid, 2.0 equiv of 1,10-phenanthroline, 2.0 equiv of cupric acetate in DMF, and the absence of molecular sieves. Under these modified conditions, compound 3 was isolated in 60% yield (Scheme 1). Similarly, the arylboronic acid 2 reacted with different purine bases (4-7) to yield the 9-arylpurines (8-11) (Scheme 1) in moderate to good yields (30-60%). The 6-chloropurine derivative 3 was further modified at position 6 using standard conditions in purine chemistry (Scheme 2). Thus, catalytic hydrogenation of 3 in the presence of Pd(C) and sodium acetate²³ gave the purine derivative **12** in 63% yield. Nucleophilic substitution reactions of 3 with sodium methoxide²⁴ and methylamine⁹ in methanol yielded the 6-OMe and 6-NHMe derivatives (13 and 14, respectively) in excellent yields (95%). Alternatively, reaction of 3 with 1 M HCl in refluxing dioxane²⁵ afforded the hypoxanthine 15 in 42% yield.

The antiviral evaluation of this first series of compounds revealed that only compounds **3**, **9**, and **10** showed significant antiviral activity. Thus, the presence of a chlorine or bromine at position 6 of the purine seemed to be important for the antiviral effect. Therefore the next series of compounds kept the 6-chloropurine as the base while different substituents were introduced at positions 3 and/or 4 of the phenyl moiety.

Reaction of the arylboronic acids 16-24 with 6-chloropurine (1) in the presence of Cu(OAc)₂ and 1,10-phenanthroline in DMF at room temperature for 3 days afforded the 9-arylpurines 25-33, with the yields specified in Table 1. It is Scheme 2. Substitution Reactions at Position 6 in Compound 3^a



^{*a*} Reagents and conditions: (a) H_2 , Pd/C, NaOAc, EtOH, 30 °C, 2 h (12, 63% yield); (b) MeONa, MeOH, Δ, 2 h, (13, 95% yield); (c) MeNH₂, MeOH, rt, 3 h (14, 95% yield); (d) HCl 1N, 1,4-dioxane, 90 °C, 16 h (15, 42% yield).

 Table 1. Synthesis of 6-Chloro-9-phenylpurines Differently Substituted at the Phenyl Ring

CI

N N H H	CI N N	R ¹ R ² 16-24	2 Cu(OAc) ₂ 1,10-phenanthroli DMF, rt, 3 days	$R^1 - \sqrt{R^2}$	N N N N 25-33
entry	reactive	R^1	\mathbb{R}^2	product	yield (%)
1	16	Н	CH ₂ OH	25	40
2	17	COCH ₃	Н	26	62
3	18	Н	COCH ₃	27	35
4	19	Н	COPh	28	39
5	20	COOCH ₃	Н	29	36
6	21	OCH ₃	Н	30	43
7	22	OCH ₂ CH(CH ₃) ₂	Н	31	36
8	23	Н	OCH ₂ CH(CH ₃) ₂	32	31
9	24	OCH ₂ O		33	28

worth mentioning that the reaction conditions are compatible with a variety of functional groups at the aryl moiety including ketones (entries 2, 3 and 4), esters (entry 5), or ethers (entries 6-10). Yields in general are moderate, but still this procedure has the advantage of affording the targeted compounds in a single reaction step from commercial precursors. The antiviral testing of this new series of compounds revealed that the 3-acetyl derivative **26** was more active against CVB3 than the initial hit **3**. Thus the next series of compounds were designed based on the structure of compound **26**, keeping the chlorine atom at position 6 of the purine base and the 3-acetyl group at the phenyl moiety.

To synthesize 9-arylpurines structurally related to compound **26**, a different synthetic approach was employed that consisted in a microwave assisted (MAOS) protocol starting from 4,6-dichloro-5-aminopyrimidines that reacted with anilines, followed by cyclization, as very recently described by us.²⁶ Reaction of the 4,6-dichloro-5-aminopyrimidines (34 and 35) with anilines (36) or acetamides (37) under microwave conditions afforded the 4,5-diamino-6-chloropyrimidines (39–42) (Scheme 3).²⁶ Hydrogenation of the commercially available 4-fluor-3-nitroacetophenone in the presence of Pt(S)/C^{27,28} for 2 h at room temperature afforded the aniline 38 that reacted with the dichloropyrimidine 34 to yield the diaminopyrimidine 43 in 38% yield for both steps. Compounds 40–43 were subjected to microwave irradiation with trimethylorthoformate in acetic anhydride at 120 °C for 1 h²⁶ to afford the purines 44–47. Moreover, the 4,5diaminopyrimidines 39 and 40 are versatile intermediates that have been employed to access to the 8-methylpurines

Scheme 3. Synthesis of 9-(3-Acetylphenyl)-6-chloropurines through MAOS



^a Reagents and conditions: (a) isobutyl alconol, HCl (cat.), MW, 150 °C, 10 min-1 h; (b) HC(OCH₃)₃, Ac₂O, MW, 120 °C, 1 h. The synthesis of **39–42** and **44–46** has been described in ref 26.

Scheme 4. Cyclization Reactions Starting from 4,5-Diaminopyrimidines^a



^a Reagents and conditions: (a)NaNO₂, HCl, CH₂Cl₂, rt, 30 min. The synthesis of **48–51** has been described in ref 26.

(48 and 49) and the 8-oxo compounds (50–51) as described (Scheme 4).²⁶ When these diaminopyrimidines 39 and 40 reacted with NaNO₂ in CH₂Cl₂ in the presence of HCl at room temperature for 30 min,²⁹ the triazolo derivatives 52 and 53 were obtained in 77 and 75% yield, respectively. Two of the most potent compounds (44 and 51) were transformed into the tertiary alcohols (54 and 55, Scheme 5) in 53 and 45% yield, respectively, by reaction with CH₃MgI. Finally, to increase the distance between the purine base and the aromatic substituent, the benzyl derivative 6-chloro-9-(3methoxybenzyl)purine (56) together with its N7-isomer (57) (Figure 1) were synthesized following the described procedure³⁰ and both compounds were included for antiviral evaluation.

Compounds **50** and **51** may exist as a tautomeric equilibrium between the keto and the enol form (Figure 2). Detailed ¹⁵N NMR studies performed on 8-hydroxyadenosine had led to the conclusion that this nucleoside exists predominantly as the keto form in solution.³¹ These authors even proposed to use the prefix "8-oxo" instead of

Scheme 5. Synthesis of 3-(2-Hydroxypropyl)phenyl Derivatives 54 and 55^{a}



^a Reagents and conditions: (a) CH₃MgI, THF, 0 °C, 90 min.



Figure 1. Structural formulas of the benzylic derivatives 56 and 57.



Figure 2. Keto-enol tautomerism for compound 50.

"8-hydroxy" to name this class of compounds. However, in more recent examples even involving biologically active compounds, the designation as "8-hydroxy"³² or "8-oxo"³³ is found in the literature. To determine which of the two forms is the predominant for the here described purines 50 and 51, $[{}^{1}H-{}^{15}N]$ NMR correlation experiments have been carried out for compound **50** in DMSO- d_6 . In the [¹H-¹⁵N] gHSQC spectrum, a correlation signal was observed between a proton at 12.40 ppm and the signal of a nitrogen at -250 ppm. Moreover, in the [¹H-¹⁵N] gHMBC experiments, a correlation peak was detected between this proton and a signal around -220 ppm. Thus, the signal at -250 ppm was assigned to N7 of the purine, while the signal at -220 ppm corresponds to N9. The other two signals in the $[^{1}H-^{15}N]$ gHMBC spectrum around -90 and -120 ppm, with correlations peaks with a proton at 8.42 ppm, correspond to N1 and N3 of the purine ring. The complete assignment of the ¹H and ¹³C NMR spectra was performed based on ¹H-¹³C correlation experiments (gHSQC and gHMBC) (see Supporting Information). From these experiments, it is noticeable that the signal corresponding to the C-8 of the purine appears at 151.9 ppm. These experiments point to the keto form as the predominant one under these experimental conditions because all the correlations are in agreement with this form. If the enol form should have been the predominant, the signal corresponding to N7 should appear approximately at -160 ppm. Thus for these 6-chloropurines, as previously shown for adenines, the predominant form in DMSO- d_6 is the keto form.

Antiviral Evaluation. The compounds synthesized were submitted to an antiviral screening against different DNA and RNA viruses. Significant antiviral activity was observed against Coxsackie virus type B3 (CVB3) replication in Vero cells, as shown in Table 2. Two of the most potent described CVB3 inhibitors, **58** (TBZE-029)³⁴ and **59** (enviroxime),³⁵ (Figure 3), were included as reference compounds (Table 2). The antiviral activity is expressed as the 50% effective concentration (EC₅₀) and the 90% effective concentration (EC₉₀). Cytotoxicity to noninfected cells is expressed as CC₅₀. The evaluation of the first series of compounds (**3**, **8–15**) revealed that a chlorine or bromine atom was required at position 6 of the purine in order to get antiviral activity, as shown for compounds **3**, **9**, and **10**. The nonsubstituted compound (**12**) or those with NHMe, OMe, or carbonyl at

Table 2. Antiviral Evaluation against CVB3 of the 9-Aryl
purines in Vero Cells^a

compd	$EC_{50} (\mu M)^b$	$EC_{90} (\mu M)^{c}$	CC ₅₀ (µM) ^d
3	14.2 ± 3.9	30 ± 9.2	>100
8	> 8.8		8.8 ± 1.0
9	17 ± 3	25	> 100
10	22 ± 0	29 ± 0.0	>100
11	>100		>100
12	>100		>100
13	>100		>100
14	>100		>100
15	>100		>100
25	11 ± 3	20 ± 4.6	>100
26	7.3 ± 2.3	9.1 ± 3.1	>100
27	35.3 ± 0.9	51 ± 7.9	>100
28	67 ± 9	85 ± 13	>100
29	11.1 ± 3.3	22 ± 11	>100
30	12.0 ± 5.3	22 ± 7.4	>100
31	29 ± 2	>100	>100
32	>100		>100
33	>100		>100
39	>100		>100
40	>100		>100
44	8.7 ± 3.1	14 ± 0.5	>100
45	> 57		>100
46	>100		>100
47	9.3 ± 0.5	15 ± 3.8	>100
48	12.2 ± 1.1	22 ± 4.5	>100
49	11 ± 0.0	15 ± 0.1	>100
50	9.3 ± 2.7	18 ± 5.9	>100
51	6.7 ± 2.1	10 ± 2.1	>100
52	>44		44 ± 0.35
53	>44		44 ± 0.21
54	8.0 ± 3.4	7.4 ± 2.1	>100
55	22 ± 0.1	29 ± 0.5	>100
56	53 ± 18	70 ± 23	>100
57	75 ± 3	>100	>100
58 (TBZE-029)	1.2 ± 0.4		> 100
59 (enviroxime)	0.7 ± 0.3		>100

^{*a*} All data are mean values \pm standard deviation for at least three independent experiments. ^{*b*} 50% effective concentration or concentration required to protect 50% of the cells against the cytopatic effect of the virus. ^{*c*} 90% effective concentration or concentration required to protect 90% of the cells against the cytopatic effect of the virus. ^{*d*} 50% cytotoxic concentration or concentration cytotoxic to 50% of the cells.



Figure 3. Structural formulas of well established anti-CVB3 compounds.

position 6 of the purine (compounds 13, 14, and 15, respectively) were antivirally inactive.

Keeping the 6-chloropurine as the base, different substituents were introduced at the positions 3 and/or 4 of the aryl ring (compounds 25-31). In particular, the 4-CH₂OH

derivative (25) was similar in antiviral activity to its 3-isomer (3). However, when comparing the 3- and 4-isomers with an acetyl substituent (26 and 27), it became clear that the 3-isomer (26) was 5-fold more potent than the 4-isomer. A similar trend was observed for the isobutoxy derivatives 31 and 32, showing that the 3-isomer (31) is moderately active while the 4-isomer (32) is inactive at the highest concentration tested (100 μ M). It is interesting to notice that different substituents at position 3 of the aryl moiety are compatible with a significant antiviral activity including a hydroxymethyl (3), an acetyl (26), a methylester (29), or a methoxy group (30). However, a double substitution at positions 3 and 4, as shown for the 1,3-dioxolane derivative 33, renders an inactive compound. Thus, compound 26 with a 3-acetyl substituent at the phenyl ring was even more potent that the initial hit, the hydroxymethyl derivative 3, and was chosen as the prototype for further modifications.

The structure-activity relationship studies of the latest series of compounds (39, 40, 44-57) indicated that the purine ring can incorporate a methyl group at positions 2, 8, or both, as shown for compounds 44, 48, and 49, respectively, without compromising the antiviral activity. However, the opening of the imidazole ring of the purine, as in the 4,5-diaminopyriminides 39 and 40, or the incorporation of a nitrogen at position 8 as in the triazolo derivatives 52 and 53, provide antivirally inactive compounds. Moreover, the triazolo derivatives show a certain degree of cytotoxicity at 44 μ M. If a keto group is introduced at position 8 of the purine (compounds 50 and 51), the antiviral activity is maintained or even slightly increased compared to the 8-unsubstituted analogues (26 and 44, respectively) based on the EC₅₀ and EC₉₀ values. Concerning the aryl moiety, incorporation of a methoxy group at position 6 of the phenyl ring (compounds 45 and 46), annihilates the antiviral effect. However, introduction of a fluor atom at position 4 (47) keeps the antiviral activity compared to 26, in agreement with the well documented bioisosterism fluor/hydrogen.³⁶ Furthermore, replacement of the acetyl group at position 3 of the phenyl ring in compounds 44 and 51 by a 2-hydroxypropyl (54 and 55, respectively) keeps the antiviral activity in particular for compound 54. Finally the N9-benzylic derivative 56, where a methylene unit has been introduced between the aryl moiety and the purine ring, proved to be much less potent than the corresponding N9-aryl analogue (30), pointing to the importance of the direct attachment of the phenyl ring to the purine in order to obtain a significant antiviral effect.

Most of the compounds had no or little adverse effect on Vero cells according to the CC_{50} values shown in Table 2. The antiproliferative activity of selected compounds (**3**, **26**, **51**, and **54**) was further determined in three different cell lines (Vero, HeLa, and MRC-5 cells) by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) method (Promega, Leiden, The Netherlands). Additionally, Vero cells survival was determined using the CellTiter-Glo luminescent cell viability assay (Promega). Compounds **51** and **54** did not result in any detectable toxicity at the highest concentration tested (Table 3).

The most potent congener, compound **26**, was selected for the evaluation against a panel of other enteroviruses (Table 4). Compound **26** proved to be active against Coxsackie virus A16 (CVA16) among enterovirus A. Also, in the group of enterovirus B, antiviral activity in the low μ M range

Table 3. Cellular Toxicity of Compounds **3**, **26**, **51**, and **54**, Determined in Three Different Cell Lines, As Assessed with the MTS Method and the CellTiter-Glo Luminescent Cell Viability $Assay^{a}$

		luminescence $CC_{50} (\mu M)^b$		
compd	Vero cells	HeLa cells	MRC-5 cells	Vero cells
3	158 ± 23	175 ± 7	229 ± 16	200 ± 78
26	206 ± 14	144 ± 14	238 ± 4	> 250
51	> 250	> 250	> 250	> 250
54	> 250	>250	> 250	> 250

^{*a*} Data are mean values \pm SD for at least three independent experiments. ^{*b*} 50% cytotoxic concentration.

Table 4. Antiviral Evaluation of Compound **26** against a Selected Panelof Enteroviruses^a

species	virus (strain)	$EC_{50} (\mu M)^b$
enterovirus A	Coxsackie virus A16 $(G-10)^c$	2.7 ± 0.76
	enterovirus 71 $(BrCr)^d$	>100
enterovirus B	Coxsackie virus A9 (Bozek) ^e	>100
	Coxsackie virus B3 (Nancy) ^f	7.3 ± 2.3
	echovirus 9 (Hill) ^c	3.3 ± 1.2
	echovirus 11 (Gregory) ^c	>100
enterovirus C	polio virus 1 (Sabin) ^f	>100
	Coxsackie virus A21 (Coe) c	4.5 ± 1.6
	Coxsackie virus A24 (clinical) ^c	2.7 ± 0.87
rhinovirus	rhinovirus 2 ^g	>100
	rhinovirus 14 ^g	>100

^{*a*} Data are mean values \pm SD for at least three independent experiments. ^{*b*} 50% effective concentration. ^{*c*} Cultured in MRC-5 cells at 37 °C. ^{*d*} Cultured in RD cells at 37 °C. ^{*e*} Cultured in Hela R at 37 °C. ^{*f*} Cultured in Vero cells at 37 °C. ^{*g*} Cultured in Hela R at 35 °C.

was found against CVB3 (Nancy) and echovirus 9. Coxsackie virus A21 (CVA21) and Coxsackie virus A24 (CVA24) were also inhibited by compound **26**. However, this compound was inactive against 15 selected rhinoviruses, poliovirus 1, enterovirus 71, and echovirus 11.

The here described compounds are significantly different in their structure to previously reported anti-CVB3 compounds. Therefore, it was of interest to determine the antiviral activity of the prototype compound **26** against CVB3 strains that are resistant to the picornavirus 2C targeting compound **58** (TBZE-029)³⁷ or to the picornavirus 3A targeting compound **60** (TTP-8307).³⁸ The replication of wild type CVB3 and these two mutant variants in the presence of the three compounds (**58**, **60**, and the arylpurine **26**) is represented in Figure 4. As shown in Figure 4c, compound **26** retained wild-type activity against these two drug resistant variants of CVB3, suggesting a different mode of action or a different molecular interaction with these two viral proteins 2C and 3A.

Conclusions

The present paper describes the discovery of a novel class of enterovirus replication inhibitors, particularly against Coxsackie virus type B3, with a scaffold of 9-arylpurines. The here described compounds are easily accessible by two different synthetic pathways: the coupling of purines bases with aryl boronic acids catalyzed by copper salts, and a microwave-assisted protocol from 4,6-dichloro-5-aminopyrimidines that reacted with anilines or acetamides, followed by heterocyclization. Several of the synthesized structures are selective inhibitors of in vitro CVB3 replication. Substituents at the purine moiety are crucial for the antiviral effect, particularly at position 6, where a chlorine or bromine atom



25 Compound [µM]

13

Figure 4. Replication of wild-type CVB3 and mutant viruses in the presence of 58 (TBZE-029) (graph A), 60 (TTP-8307) (graph B), and compound 26 (graph C). Wild-type replication (WT) is represented as black bars. Mutant 1 [TTP8307^r CVB3] (in white bars) is a resistant strain to TTP8307 with an I54F substitution in 3A. Mutant 2 [TBZE029^r CVB3] (in gray bars), resistant to TBZE029, is characterized by three mutations affecting viral protein 2C (A224 V + I227 V + A229 V). Each graph represents the number of plaque forming units, produced by wild-type virus or by the recombinant viruses at a given compound concentration.

50

is required. The direct linkage of the aryl moiety to the purine ring is also important for the antiviral activity. However, the aryl ring can incorporate different substituents in particular at position 3. Thus, the most active compounds (26, 44, 51, and 54) inhibit CVB3, with EC₅₀ values of $5-10 \,\mu$ M. Moreover, compound 26 remains active against CVB3 strains that are resistant to compounds such as 58 and 60, which targets the viral 2C and the 3A proteins, respectively. The mechanism of action of this novel class of compounds is currently being studied and will be the subject of further investigation. An effect on the binding of the virus to or the entry in the host cell has already been excluded. Interestingly, compound 26 has also no effect on viral RNA synthesis but yet efficiently prevents the production of infectious progeny virus. Therefore, these 9-arylpurines can be considered as a novel class of enterovirus inhibitors characterized by their simplicity in structure, low

VC

6,3

molecular weight, and selective inhibitory activity against enterovirus replication that deserve further exploration.

WT CVB3

Experimental Section

100

Chemistry Procedures. Melting points were obtained on a Reichert-Jung Kofler apparatus and are uncorrected. The elemental analysis was performed with a Heraeus CHN-O-RAPID instrument. The elemental compositions of the compounds agreed to within $\pm 0.4\%$ of the calculated values. For all the tested compounds, satisfactory elemental analysis was obtained supporting >95% purity. Electrospray mass spectra were measured on a quadrupole mass spectrometer equipped with an electrospray source (Hewlett-Packard, LC/MS HP 1100). ¹H and ¹³C NMR spectra were recorded on a Varian INNOVA 300 operating at 299 MHz (¹H) and 75 MHz (¹³C), respectively, and Varian INNOVA-400 operating at 399 MHz (¹H) and 99 MHz (¹³C), respectively.

Analytical TLC was performed on silica gel 60 F_{254} (Merck) precoated plates (0.2 mm). Spots were detected under UV light (254 nm) and/or charring with ninhydrin. Separations on silica gel were performed by preparative centrifugal circular thin-layer chromatography (CCTLC) on a Chromatotron (Kiesegel 60 PF_{254} gipshaltig (Merck)), with layer thickness of 1 and 2 mm and flow rate of 4 or 8 mL/min, respectively. Flash column chromatography was performed with silica gel 60 (230–400 mesh) (Merck).

Microwave reactions were performed using the Biotage Initiator 2.0 single-mode cavity instrument from Biotage (Uppsala). Experiments were carried out in sealed microwave process vials utilizing the standard absorbance level (400 W maximum power). The temperature was measured with an IR sensor on the outside of the reaction vessel.

General Procedure for the Synthesis of N9-Arylpurines. A mixture containing the corresponding arylboronic acid (1.0 mmol), the purine base (0.5 mmol), 1,10-phenanthroline (180 mg, 1.0 mmol), and cupric acetate (181 mg, 1.0 mmol) in DMF (5 mL) was stirred at room temperature for 3 days. Volatiles were removed and the residue was taken up in EtOAc (50 mL) and washed with a solution of EDTA (400 mg in 50 mL of water). The aqueous phase was further extracted with EtOAc (2 × 20 mL). The combined organic phases were dried on anhydrous Na₂SO₄, filtered, and evaporated. The residue was then purified as indicated for each compound.

6-Chloro-9-[3-(hydroxymethyl)phenyl]purine (3). Following the general procedure for the synthesis of *N*9-arylpurines, reaction of 6-chloropurine (1, 77 mg, 0.5 mmol) and 3-(hydroxymethyl)phenylboronic acid (2, 152 mg, 1.0 mmol) afforded a residue that was purified by CCTLC in the Chromatotron (CH₂Cl₂:MeOH, 50:1) to yield 77 mg (60%) of **3** as a white solid; mp 197–199 °C. MS (ES, positive mode): m/z 261 (M + H)⁺ showing the isotopic Cl pattern. ¹H NMR (DMSO-*d*₆) δ 4.74 (d, 2H, J = 6.0 Hz, CH₂O), 5.54 (t, 1H, J = 5.9 Hz, OH), 7.58–7.96 (m, 4H, Ar), 8.97 (s, 1H, H-8), 9.22 (s, 1H, H-2). ¹³C NMR (DMSO-*d*₆, 75 MHz) δ : 62.5 (CH₂O), 122.0, 122.6, 127.0, 130.0, 134.0, 146.6 (Ar), 131.6, (C-5), 144.4 (C-8), 150.0 (C-4), 151.7 (C-6), 152.5 (C-2). Anal. (C₁₂H₉ClN₄O) C, H, N.

The synthesis of compounds 8-11 was performed following a similar procedure and all details, and analytical and spectroscopic data are included in the Supporting Information.

9-[3-(Hydroxymethyl)phenyl)purine (12). To a solution of **3** (120 mg, 0.5 mmol) in EtOH (10 mL), Pd(C) (10%) (20 mg) and sodium acetate (113 mg, 1.4 mmol) were added. The reaction mixture was then hydrogenated at 35 psi at 30 °C overnight. Na₂SO₄·10H₂O was then added and the mixture was stirred for 2 h, filtered through celite, and the filtrate was evaporated. The residue was purified by CCTLC in the Chromatotron (CH₂Cl₂: MeOH, 30:1) to yield 65 mg (63%) of **12** as a white solid; mp 130–132 °C. MS (ES, positive mode): m/z 227 (M + H)⁺. ¹H NMR (DMSO- d_6) δ 4.62 (d, 2H, J= 3.6 Hz, CH₂O), 5.41 (t, 1H, J= 4.8 Hz, OH), 7.45 (d, 1H, J= 7.7 Hz, Ar), 7.59 (t, 1H, J= 7.8 Hz, Ar), 7.77 (d, 1H, J= 7.9 Hz, Ar), 7.88 (s, 1H, Ar), 9.02, 9.03, 9.29 (s, 3H, H-2, H-6, H-8). Anal. (C₁₂H₁₀N₄O) C, H, N.

9-[3-(Hydroxymethyl)phenyl]-6-methoxypurine (13). To a stirred solution of **3** (90 mg, 0.3 mmol) in MeOH (5 mL), MeONa (56 mg, 1.0 mmol) was added and the reaction mixture was refluxed for 2 h. After cooling to room temperature, the mixture was neutralized with AcOH and volatiles were removed. The residue was treated with CH₃CN, and the precipitate was filtered and washed with CH₃CN. The filtrate was then evaporated to obtain 82 mg (95%) of **13** as a white solid; mp 154–156 °C. MS (ES, positive mode): m/z 257 (M + H)⁺. ¹H NMR (DMSO- d_6) δ 4.12 (s, 3H, CH₃), 4.60 (s, 2H, CH₂), 5.41 (br s, 1H, OH), 7.42 (d, 1H, J=7.6 Hz, Ar), 7.55 (t, 1H, J=7.8 Hz, Ar), 7.72 (d, 1H, J=7.7 Hz, Ar), 7.83 (s, 1H, Ar), 8.60, 8.78 (s, 2H, H-2, H-8). Anal. (C₁₃H₁₂N₄O₂) C, H, N.

6-(Methylamino)-9-[3-(hydroxymethyl)phenyl]purine (14). A solution of **3** (70 mg, 0.2 mmol) in MeNH₂ in MeOH (33%)

(30 mL) was stirred at room temperature for 3 h. Volatiles were removed, and the residue was purified by CCTLC in the Chromatotron (CH₂Cl₂:MeOH, 20:1) to yield 65 mg (95%) of **14** as a white solid; mp 197–199 °C. MS (ES, positive mode): m/z 256 (M + H)⁺. ¹H NMR (DMSO- d_6) δ 3.04 (s, 3H, CH₃), 4.66 (d, 2H, J = 5.6 Hz, CH₂O), 5.45 (t, 1H, J = 5.7 Hz, OH), 7.45 (d, 1H, J = 7.6 Hz, Ar), 7.60 (t, 1H, J = 7.8 Hz, Ar), 7.79 (d, 1H, J = 8.0 Hz, Ar), 7.92 (s, 1H, Ar), 7.93 (br s, 1H, NH), 8.36, 8.62 (s, 2H, H-2, H-8). Anal. (C₁₃H₁₃N₅O) C, H, N.

9-[3-(Hydroxymethyl)phenyl]hypoxanthine (15). A solution of **3** (53 mg, 0.2 mmol) in 1,4-dioxane (5 mL) was treated with 1N HCl (5 mL) and heated at 90 °C overnight. After cooling to room temperature, the mixture was neutralized by the addition of Amberlite IRA400 (OH⁻ form), filtered and evaporated. The residue was then purified by CCTLC in the Chromatotron (CH₂Cl₂:MeOH, 10:1) to yield 20 mg (42%) of **15** as a white solid; mp 120–122 °C. MS (ES, positive mode): m/z 243 (M + H)⁺. ¹H NMR (DMSO- d_6) δ 4.58 (d, 2H, J = 5.4 Hz, CH₂O), 5.37 (t, 1H, J = 5.4 Hz, OH), 7.41–7.70 (m, 4H, Ar), 8.08 (s, 1H, H-2), 8.43 (s, 1H, H-8), 12.41 (br s, 1H, NH). Anal. (C₁₂H₁₀N₄O₂) C, H, N.

The synthesis of compounds **25–33** was performed following the general procedure for the synthesis of *N*9-arylpurines and all details and analytical and spectroscopic data are included in the Supporting Information.

 N^4 -(3'-Acetyl-4'-fluorophenyl)-6-chloropyrimidine-4,5-diamine (43). A solution of 4-fluor-3-nitroacetophenone (150 mg, 0.82 mmol) in EtOH (6 mL) was hydrogenated in the presence of Pt(S)/C (20 mg) at 30 psi at room temperature for 2 h. The mixture was then filtered through celite, washed with EtOH and MeOH, and the filtrate was evaporated. The crude, which contained 38, was allowed to react with 5-amino-4,6-dichloropyrimidine (34, 123 mg, 0.75 mmol) in the presence HCl (37% v/ v) (50 μ L) in isobutyl alcohol (4 mL) at 120 °C for 1 h in a microwave reactor. Volatiles were removed and the residue was taken up in EtOAc (20 mL) and washed with a saturated, cold NaHCO₃ solution (15 mL) and brine (15 mL). The organic phase was dried on anhydrous Na₂SO₄, filtered, and evaporated. The residue was purified by flash column chromatography (hexane: EtOAc, 2:1) to afford 80 mg (38% for the two steps) of 43 as a vellow solid. MS (ES, positive mode): $m/z 281 (M + H)^+$ showing the isotopic Cl pattern. ¹H NMR (DMSO- d_6) δ 2.57 (s, 3H, CH₃), 5.44 (br s, 2H, NH₂), 7.43 (m, 1H, Ar), 7.80 (s, 1H, H-2), 7.83 (m, 1H, Ar), 8.19 (m, 1H, Ar), 8.65 (s, 1H, NH).

9-(3-Acetyl-4-fluorophenyl)-6-chloropurine (47). A solution of 43 (75 mg, 0.27 mmol) and trimethyl orthoformate (0.75 mL) in acetic anhydride (0.75 mL) was placed in a microwave vessel. The reaction was irradiated at 120 °C for 1 h. After cooling, volatiles were evaporated and the residue was dissolved in CHCl₃ (15 mL) and washed with a saturated NaHCO₃ solution (15 mL) and brine (15 mL). The organic phase was dried on anhydrous Na₂SO₄, filtered, and evaporated. The residue was purified by CCTLC in the Chromatotron (CH₂Cl₂:EtOAc, 2:1). The UV-positive fractions were combined, evaporated, and further purified by CCTLC in Chromatotron (CH₂Cl₂:MeOH, 20:1) to afford 30 mg (39%) of 47 as a yellow solid; mp 162-164 °C. MS (ES, positive mode): m/z 291 (M + H)⁺ showing the isotopic Cl pattern. ¹H NMR (DMSO- d_6) δ 2.64 (s, 3H, CH₃), 7.77 (dd, 1H, J=9.9, 1.0 Hz, Ar), 8.25 (m, 1H, Ar), 8.43 (dd, 1H, J = 7.2, 2.2 Hz, Ar), 8.85, 9.02 (s, 1H, H-2, H-8). Anal. (C13H8ClFN4O) C, H, N.

3-(3-Acetylphenyl)-7-chlorotriazolo[4,5-*d*]pyrimidine (52). To a suspension of 39^{26} (150 mg, 0.57 mmol) in CH₂Cl₂ (2 mL) and 1 M HCl (2 mL), NaNO₂ (41 mg, 0.6 mL) was added in small portions. The mixture was stirred at room temperature for 30 min. Then, CH₂Cl₂ (10 mL) was added and the organic layer was washed with brine, dried on anhydrous Na₂SO₄, filtered, and evaporated. The final residue was purified by flash column chromatography (CH₂Cl₂:EtOAc, 2:1) to yield 120 mg (77%) of **52** as a brown solid; mp 127–129 °C. MS (ES, positive mode): m/z 274 (M + H)⁺ showing the isotopic Cl pattern. ¹H NMR (CDCl₃) δ 2.73 (s, 3H, CH₃), 7.77 (t, 1H, J=7.9 Hz, Ar), 8.12 (d, 1H, J=7.8 Hz, Ar), 8.49 (d, 1H, J=8.0 Hz, Ar), 8.88 (s, 1H, Ar), 9.07 (s, 1H, H-2). Anal. (C₁₂H₈ClN₅O) C, H, N.

3-(3-Acetylphenyl)-7-chloro-5-methyltriazolo[**4,5-***d*]**pyrimidine** (**53).** Following a procedure analogous to that described for the synthesis of **52**, a solution of **40**²⁶ (120 mg, 0.43 mmol) in CH₂Cl₂ (1.5 mL) and 1 M HCl (1.5 mL) was allowed to react with NaNO₂ (32 mg, 0.46 mmol) to afford 92 mg (75%) of **53** as a brown solid; mp 109–111 °C. MS (ES, positive mode): *m*/*z* 288 (M + H)⁺ showing the isotopic Cl pattern. ¹H NMR (CDCl₃) δ 2.72 (s, 3H, CH₃), 2.93 (s, 3H, CH₃), 7.75 (t, 1H, *J*=7.9 Hz, Ar), 8.10 (m, 1H, Ar), 8.47 (m, 1H, Ar), 8.86 (s, 1H, Ar). Anal. (C₁₃H₁₀ClN₅O) C, H, N.

6-Chloro-2-methyl-9-[3-(hydroxyprop-2-yl)phenyl]purine (54). A solution of **44**²⁶ (100 mg, 0.35 mmol) in anhydrous THF (9 mL) at 0 °C was treated with methylmagnesium iodide (175 μ L) for 90 min. The reaction mixture was quenched by the addition of a saturated NH₄Cl solution (2 mL). Ethyl acetate (10 mL) and water (10 mL) were added. The organic phase was dried on anhydrous Na₂SO₄, filtered, and evaporated. The final residue was purified by CCTLC in the Chromatotron (CH₂Cl₂:EtOAc, 2:1) to yield 56 mg (53%) of **54** as a white solid; mp 154–156 °C. MS (ES, positive mode): *m/z* 303 (M + H)⁺ showing the isotopic Cl pattern. ¹H NMR (DMSO-*d*₆) δ 1.49 (s, 6H, (CH₃)₂), 2.69 (s, 3H, 2-CH₃), 5.21 (s, 1H, OH), 7.53–7.69 (m, 3H, Ar), 7.90 (t, 1H, *J* = 7.8 Hz, Ar), 8.97 (s, 1H, H-8). Anal. (C₁₅H₁₅ClN₄O) C, H, N.

6-Chloro-9-(3-hydroxyprop-2-yl)-2-methylpurin-8-one (55). Following a procedure analogous to that described for the synthesis of **54**, a solution of **51**²⁶ (42 mg, 0.14 mmol) in anhydrous THF (3.5 mL) was treated with CH₃MgI (73 μ L, 0.21 mmol) at 0 °C for 3 h. The final residue was purified by CCTLC in the Chromatotron (CH₂Cl₂:EtOAc, 1:1) to afford 20 mg (45%) of **55** as a white solid; mp 170–172 °C. MS (ES, positive mode): m/z 319 (M + H)⁺ showing the isotopic Cl pattern. ¹H NMR (DMSO-*d*₆) δ 1.46 (s, 6H, (CH₃)₂), 2.47 (s, 3H, 2-CH₃), 7.36–7.67 (m, 4H, Ar), 12.13 (br s, 1H, NH). Anal. (C₁₅H₁₅ClN₄O₂) C, H, N.

NMR Experiments Performed for Compound 50. ¹H, ¹³C and ¹⁵N NMR spectra were recorded at 303 K, using DMSO-*d*₆ as the solvent, on a Varian SYSTEM 500 NMR spectrometer equipped with a 5 mm HCN cold probe. ¹H and ¹³C chemical shifts are reported in ppm from tetramethylsilane and ¹⁵N chemical shifts from nitromethane. All assignments have been performed on the basis of heteronuclear multiple quantum coherence experiments (gHMQC and gHMBC). Two-dimensional [¹H-¹³C] NMR experiments (gHSQC and gHMBC) were obtained using a ¹H spectral window of 4808 Hz, a ¹³C spectral windows of 30 000 Hz, 1 s of relaxation delay, 1024 data points, and 200 time increments, with a linear prediction to 512. The data were zero-filled to 4096×4096 real points. Typical numbers of transients per increment were 4 and 16, respectively. Two-dimensional [¹H⁻¹⁵N] gHSQC and [¹H⁻¹⁵N] gHMBC experiments was carried out with the same conditions, using 16 and 64 transients per increment, respectively, and a ¹⁵N spectral window of 15200 Hz.

Antiviral Activity. The antiviral activity of the selected compounds was calculated by an MTS-based CPE reduction assay, which compares the optical density of infected compoundtreated cells with uninfected compound-free cells. Briefly, serial dilutions of the compounds and 100 CCID₅₀ of virus were added to the appropriate cell line, grown to confluence in 96-well plates. After 3 days of incubation at 37 °C (until complete CPE was observed in the infected and untreated virus control (VC)), cell viability was measured using the MTS/PMS method (Promega, Leiden, The Netherlands). Briefly, the optical density of each well was quantified spectrophotometrically at 498 nm in a microplate reader. CPE values were calculated and the 50% effective concentration (EC₅₀) was defined as the concentration of compound that inhibited virus-induced cytopathic effect formation by 50% and was calculated using linear interpolation. Each experiment was repeated at least three times.

Cytotoxic Assays. Cytotoxic evaluation of the selected compounds was performed using the MTS-method or CellTiter-Glo luminescent cell viability assay (Promega) and the 50% cytotoxic concentration (CC_{50}) was calculated as the concentration of compound that inhibited cell proliferation by 50% using linear interpolation. Briefly, the same experimental setup was used as for the antiviral assay, but for cytotoxicity determination, uninfected cultures were incubated with serial dilution of compound for three days at 37 °C. Each experiment was repeated at least three times.

Viral Plaque Assays. For determination of viral plaques, Vero cells, grown to confluence in six-well plates, were infected with CVB3 at 37 °C. After 2 h, the virus was removed, the cells were washed twice with phosphate-buffered saline (PBS), and the growth medium was replaced with agar (final concentration, 0.5%) in the presence or absence of compound. After 3 to 4 days, plaques were visualized. Briefly, cells were fixed with 2 mL of a solution containing 4% formaldehyde, after which the agar was removed. A 2% Giemsa solution was used to stain the cells.

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Supporting Information Available: Synthesis and spectroscopic data of compounds 8–11 and 25–33. Elemental analysis of compounds 3, 8–15, 25–33, 47, 52–55 is included. Figure S1 represents the ¹H and ¹³C chemical shifts from compound 50 based on ¹H–¹³C NMR correlation experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

- De Palma, A. M.; Vliegen, I.; De Clercq, E.; Neyts, J. Selective inhibitors of picornavirus replication. *Med. Res. Rev.* 2008, 28, 823–884.
- (2) Chen, T. C.; Weng, K. F.; Chang, S. C.; Lin, J. Y.; Huang, P. N.; Shih, S. R. Development of antiviral agents for enteroviruses. *J. Antimicrob. Chemother.* 2008, *62*, 1169–1173.
- (3) Knowlton, K. U. CVB infection and mechanisms of viral cardiomyopathy. In *Group B Coxsackieviruses*, **2008**; Vol. 323, pp 315-335.
- (4) Kawai, C. From myocarditis to cardiomyopathy: Mechanisms of inflammation and cell death: Learning from the past for the future. *Circulation* 1999, 99, 1091–1100.
- (5) Drescher, K. M.; Tracy, S. M. The CVB and etiology of type 1 diabetes. In *Group B Coxsackieviruses*; Springer-Verlag: New York, 2008; Vol. 323, pp 259–274.
- (6) Huber, S.; Ramsingh, A. I. Coxsackievirus-induced pancreatitis. *Viral Immunol.* **2004**, *17*, 358–369.
- (7) Barnard, D. L. Current status of anti-picornavirus therapies. *Curr. Pharm. Des.* 2006, *12*, 1379–1390.
- (8) Priego, E. M.; Pérez-Pérez, M. J.; Kuenzel, J.; de Vries, H.; Ijzerman, A. P.; Camarasa, M. J.; Martín-Santamaría, S. Selective human adenosine A₃ antagonists based on pyrido[2,1-*f*]purine-2,4diones: novel features of hA₃ antagonist binding. *ChemMedChem* 2008, 3, 111–119.
- (9) Casanova, E.; Hernández, A. I.; Priego, E. M.; Liekens, S.; Camarasa, M. J.; Balzarini, J.; Pérez-Pérez, M. J. 5'-O-Tritylinosine and analogues as allosteric inhibitors of human thymidine phosphorylase. J. Med. Chem. 2006, 49, 5562–5570.

- (10) Priego, E. M.; Kuenzel, J. V.; Ijzerman, A. P.; Camarasa, M. J.; Pérez-Pérez, M. J. Pyrido[2,1-*f*]purine-2,4-dione derivatives as a novel class of highly potent human A₃ adenosine receptor antagonists. *J. Med. Chem.* **2002**, *45*, 3337–3344.
 (11) Chern, J. W.; Lee, H. Y.; Chen, C. S.; Shewach, D. S.; Daddona, P.
- (11) Chern, J. W.; Lee, H. Y.; Chen, C. S.; Shewach, D. S.; Daddona, P. E.; Townsend, L. B. Nucleosides. 5. Synthesis of guanine and formycin B derivatives as potential inhibitors of purine nucleoside phosphorylase. J. Med. Chem. 1993, 36, 1024–1031.
- (12) Brakta, M.; Murthy, D.; Ellis, L.; Phadtare, S. 9-[(Hydroxymethyl)phenyl]adenines: new aryladenine substrates of adenosine deaminase. *Bioorg. Med. Chem. Lett.* 2002, *12*, 1489–1492.
- (13) Ueno, Y.; Kato, T.; Sato, K.; Ito, Y.; Yoshida, M.; Inoue, T.; Shibata, A.; Ebihara, M.; Kitade, Y. Synthesis and properties of nucleic acid analogues consisting of a benzene-phosphate backbone. J. Org. Chem. 2005, 70, 7925–7935.
- (14) Greenberg, S. M.; Ross, L. O.; Robins, R. K. Potential purine antagonists XXI. Preparation of some 9-phenyl-6-substituted purines. J. Org. Chem. 1959, 24, 1314–1317.
- (15) Ding, S.; Gray, N. S.; Ding, Q.; Schultz, P. G. Expanding the diversity of purine libraries. *Tetrahedron Lett.* **2001**, *42*, 8751–8755.
- (16) Bakkestuen, A. K.; Gundersen, L. L. Regioselective N-9 arylation of purines employing arylboronic acids in the presence of Cu(II). *Tetrahedron Lett.* 2003, 44, 3359–3362.
- (17) Chan, D. M. T.; Monaco, K. L.; Wang, R. P.; Winters, M. P. New *N* and *O*-arylations with phenylboronic acids and cupric acetate. *Tetrahedron Lett.* **1998**, *39*, 2933–2936.
 (18) Lam, P. Y. S.; Clark, C. G.; Saubern, S.; Adams, J.; Winters, M. P.;
- (18) Lam, P. Y. S.; Clark, C. G.; Saubern, S.; Adams, J.; Winters, M. P.; Chan, D. M. T.; Combs, A. New aryl/heteroaryl C–N bond crosscoupling reactions via arylboronic acid cupric acetate arylation. *Tetrahedron Lett.* **1998**, *39*, 2941–2944.
- (19) Evans, D. A.; Katz, J. L.; West, T. R. Synthesis of diaryl ethers through the copper-promoted arylation of phenols with arylboronic acids. An expedient synthesis of thyroxine. *Tetrahedron Lett.* **1998**, *39*, 2937–2940.
- (20) Huang, H.; Liu, H.; Chen, K. X.; Jiang, H. L. Microwave-assisted rapid synthesis of 2,6,9-substituted purines. J. Comb. Chem. 2007, 9, 197–199.
- (21) Úeno, Y.; Kawamura, A.; Takasu, K.; Komatsuzaki, S.; Kato, T.; Kuboe, S.; Kitamura, Y.; Kitade, Y. Synthesis and properties of a novel molecular beacon containing a benzene-phosphate backbone at its stem moiety. Org. Biomol. Chem. 2009, 7, 2761–2769.
- (22) Ley, S. V.; Thomas, A. W. Modern synthetic methods for coppermediated C(aryl)–O, C(aryl)–N, and C(aryl)–S bond formation. *Angew. Chem., Int. Ed.* 2003, *42*, 5400–5449.
 (23) Huryn, D. M.; Sluboski, B. C.; Tam, S. Y.; Weigele, M.; Sim, I.;
- (23) Huryn, D. M.; Sluboski, B. C.; Tam, S. Y.; Weigele, M.; Sim, I.; Anderson, B. D.; Mitsuya, H.; Broder, S. Synthesis and anti-HIV activity of isonucleosides. *J. Med. Chem.* **1992**, *35*, 2347–2354.
- (24) Harnden, M. R.; Wyatt, P. G.; Boyd, M. R.; Sutton, D. Synthesis and antiviral activity of 9-alkoxypurines. 1. 9-(3-Hydroxypropoxy)purines and 9-[3-hydroxy-2-(hydroxymethyl)propoxy]purines. J. Med. Chem. 1990, 33, 187–196.
- (25) Pospisil, P.; Pilger, B. D.; Marveggio, S.; Schelling, P.; Wurth, C.; Scapozza, L.; Folkers, G.; Pongracic, M.; Mintas, M.; Malic, S. R. Synthesis, kinetics, and molecular docking of novel 9-(2-hydroxypropyl)purine nucleoside analogs as ligands of herpesviral thymidine kinases. *Helv. Chim. Acta* 2002, *85*, 3237–3250.

- (26) Aguado, L.; Camarasa, M. J.; Pérez-Pérez, M. J. Microwave-assisted synthesis of 9-arylpurines. J. Comb. Chem. 2009, 11, 210–212.
- (27) Wu, Y. J.; He, H.; Sun, L. Q.; Wu, D. D.; Gao, Q.; Li, H. Y. Synthesis of fluorinated 1-(3-morpholin-4-yl-phenyl)-ethylamines. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1725–1728.
- (28) Tamura, R.; Oda, D.; Kurokawa, H. Synthesis of alpha-amino ketone hydrochlorides via chemoselective hydrogenation of alphanitro ketones. *Tetrahedron Lett.* **1986**, *27*, 5759–5762.
- (29) Kelley, J. L.; Davis, R. G.; McLean, E. W.; Glen, R. C.; Soroko, F. E.; Cooper, B. R. Synthesis and anticonvulsant activity of *N*-benzylpyrrolo[2,3-*d*]pyrimidines, *N*-benzylpyrazolo[3,4-*d*]pyrimidines, and *N*-benzyltriazolo[4,5-*d*]pyrimidine: imidazole ringmodified analogs of 9-(2-flurobenzyl)-6-(methylamino)-9*H*-purine. J. Med. Chem. **1995**, 38, 3884–3888.
- (30) Bakkestuen, A. K.; Gundersen, L. L.; Utenova, B. T. Synthesis, biological activity, and SAR of antimycobacterial 9-aryl-, 9-arylsulfonyl-, and 9-benzyl-6-(2-furyl)purines. J. Med. Chem. 2005, 48, 2710–2723.
- (31) Cho, B. P.; Evans, F. E. Structure of oxidatively damaged nucleic acid adducts. 3. Tautomerism, ionization and protonation of 8-hydroxyadenosine studied by ¹⁵N NMR spectroscopy. *Nucleic Acids Res.* **1991**, *19*, 1041–1047.
- (32) Isobe, Y.; Kurimoto, A.; Tobe, M.; Hashimoto, K.; Nakamura, T.; Norimura, K.; Ogita, H.; Takaku, H. Synthesis and biological evaluation of novel 9-substituted-8-hydroxyadenine derivatives as potent interferon inducers. *J. Med. Chem.* 2006, 49, 2088–2095.
 (33) Weterings, J. J.; Khan, S.; van Noort, G. J. V.; Melief, C. J. M.;
- (33) Weterings, J. J.; Khan, S.; van Noort, G. J. V.; Melief, C. J. M.; Overkleeft, H. S.; van der Burg, S. H.; Ossendorp, F.; van der Marel, G. A.; Filippov, D. V. 2-Azidoalkoxy-7-hydro-8-oxoadenine derivatives as TLR7 agonists inducing dendritic cell maturation. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2249–2251.
- (34) De Palma, A. M.; Heggermont, W.; Leyssen, P.; Purstinger, G.; Wimmer, E.; De Clercq, E.; Rao, A.; Monforte, A. M.; Chimirri, A.; Neyts, J. Anti-enterovirus activity and structure-activity relationship of a series of 2,6-dihalophenyl-substituted 1H,3Hthiazolo[3,4-a]benzimidazoles. *Biochem. Biophys. Res. Commun.* 2007, 353, 628-632.
- (35) Langford, M. P.; Ball, W. A.; Ganley, J. P. Inhibition of the enteroviruses that cause acute hemorrhagic conjunctivitis (Ahc) by benzimidazoles-enviroxime (Ly-122772) and enviradone (Ly-127123). *Antiviral Res.* **1995**, *27*, 355–365.
- (36) Patani, G. A.; LaVoie, E. J. Bioisosterism: A rational approach in drug design. *Chem. Rev.* 1996, *96*, 3147–3176.
 (37) De Palma, A. M.; Heggermont, W.; Lanke, K.; Coutard, B.;
- (37) De Palma, A. M.; Heggermont, W.; Lanke, K.; Coutard, B.; Bergmann, M.; Monforte, A. M.; Canard, B.; De Clercq, E.; Chimirri, A.; Purstinger, G.; Rohayem, J.; van Kuppeveld, F.; Neyts, J. The thiazolobenzimidazole TBZE-029 inhibits enterovirus replication by targeting a short region immediately downstream from motif C in the nonstructural protein 2C. J. Virol. 2008, 82, 4720–4730.
- (38) De Palma, A. M.; Thibaut, H. J.; van der Linden, L.; Lanke, K.; Heggermont, W.; Ireland, S.; Andrews, R.; Arimilli, M.; Al-Tel, T. H.; De Clercq, E.; van Kuppeveld, F.; Neyts, J. Mutations in the Nonstructural Protein 3A Confer Resistance to the Novel Enterovirus Replication Inhibitor TTP-8307. *Antimicrob. Agents Chemother*. 2009, 53, 1850–1857.