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Design, synthesis and biological evaluation of novel aminothiazoles as antiviral compounds acting against human rhinovirus

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ABSTRACT

We describe here the design, synthesis and biological evaluation of antiviral compounds acting against human rhinovirus (HRV). A series of aminothiazoles demonstrated pan-activity against the HRV genotypes screened and productive structure–activity relationships. A comprehensive investigational library was designed and performed allowing the identification of potent compounds with lower molecular weight and improved ADME profile. **31d-1**, **31d-2**, **31f** showed good exposures in CD-1 mice. The mechanism of action was discovered to be a host target: the lipid kinase phosphatidylinositol 4-kinase III beta (PI4KIIIß). The identification of the pan-HRV active compound **31f** combined with a structurally distinct literature compound **T-00127-HEV1** allowed the assessment of target related tolerability of inhibiting this kinase for a short period of time in order to prevent HRV replication.

Human rhinovirus (HRV) is a major cause of the common cold and is associated with being a trigger for asthma and chronic obstructive pulmonary disease (COPD) exacerbations in children and adults.[1,2] There are no approved drugs for the treatment of HRV infections to date. Therefore there is a high medical need to identify a treatment for HRV infection to prevent disease exacerbation in patients with asthma and COPD. Preliminary results in a trial to prevent asthma exacerbations using the capsid-binding inhibitor BTA-798 trended towards being positive, however it has been reported that it is not effective against all HRV genotypes.[3,4]

HRV is a non-enveloped single positive-stranded RNA virus belonging to the Picornaviridae family (*Enterovirus* genus) with more than 133 genotypes, divided into three species A, B and C, based on sequencing the complete genomes.[5,6] HRV-A and HRV-C are more likely to cause moderate to severe illness than HRV-B.[7]

Identifying compounds that had broad anti-rhinoviral activity against species and genotypes was a key objective of our exploration. In order to find novel HRV pan-active inhibitors of viral replication, a phenotypic antiviral screening strategy was chosen. A subset of 98K compounds from the Boehringer Ingelheim collection was screened.[8] The hits underwent profiling and prospecting to eliminate those with demonstrated cytotoxicity,[9] lack of selectivity against other virus (HIV, CMV, HCV), lack of potential for pan-HRV genotype activity and lack of productive structure–activity relationships (SAR). A set of 20 best HRV hits was obtained from the 98K compounds screen. Preliminary mechanisms of action studies were performed mainly to rule out capsid-binding inhibitors due to lack of genotype coverage.[10] The hit triage allowed at the end to identify one hit: compound **1** (Table 1).

This compound is quite attractive from a medicinal chemistry perspective in that it is very modular and offers many potential sites at which to carry out SAR. However, it contains an aniline

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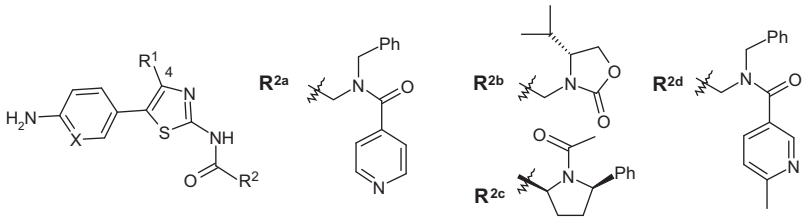
Table 1
Activity and ADME and physicochemical properties of compounds **1–6**

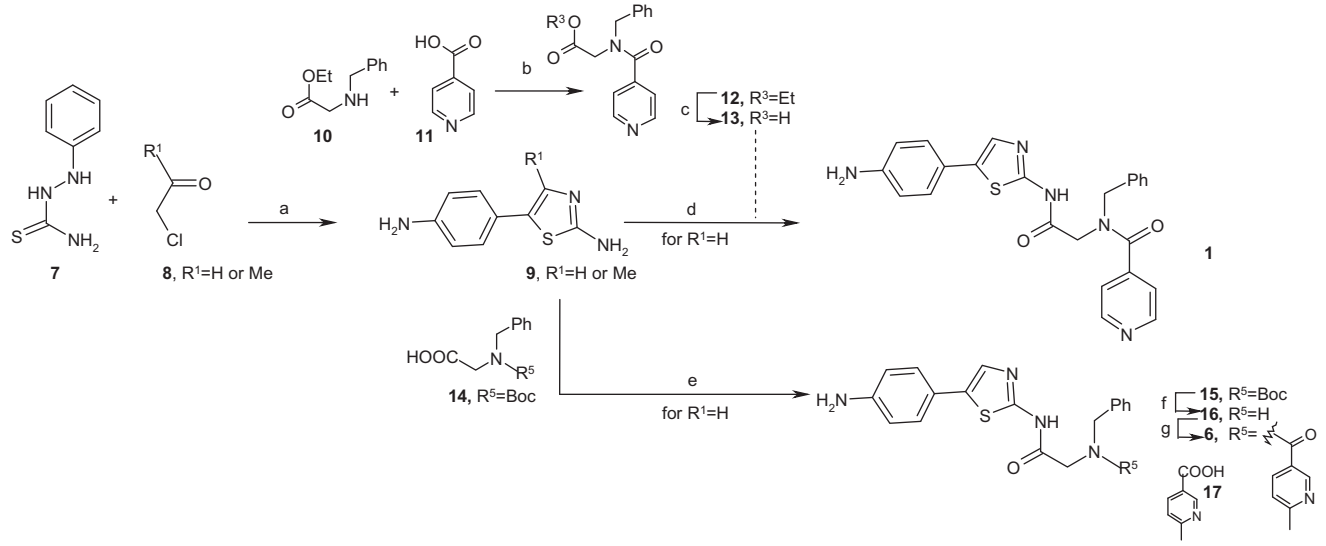


Compds	X	R1	R^2	EC_{50} [a] (nM)									CC_{50} [b] (μM)	Sol pH 2/6.8 (μg/mL)	Log *D* pH 7.4	Caco-2 [c] (10^{-6} cm/s)	HLM [d] % Q_H	CYP 2D6/3A4 (μg)
				HRV 14	HRV 16	HRV 1a	HRV 2	HRV 8	HRV 54	HRV 89	HRV 45	HRV 5						
Lead criteria								<1000			>25		>20 × EC_{50}	>25	0–5	>0.5	<75	>1
1	C	H	R^{2a}	640	630	1600	790	210	1300	790	1200	660	>65	>479/<0.2	2.9	11.3	66	na [e] /0.5
2	N	H	R^{2a}	1650	1350	1200	510	300	280	1700	630	600	>65	1000/3	2.6	2.3	45	2.6/1.8
3	N	Me	R^{2a}	410	290	960	na	260	400	610	350	430	46	>987/125	2.8	2.1	42	4.3/0.71
4	C	Me	R^{2b}	990	905	930	470	140	530	680	360	430	>65	>814/2.5	2.5	19	<25	25/>30
5	C	H	R^{2c}	220	1400	1200	915	130	395	890	430	535	>65	58/<0.1	3.2	21	74	na/0.98
6	C	H	R^{2d}	1080	1230	1240	945	590	890	1100	720	490	>64	>1000/<0.1	3.4	17	47	>30/15

[a] Anti-HRV activity is evaluated in a cell-culture based assay (viral replication assay) that measures the ability of compounds to protect from the cytopathic effect (CPE) caused by HRV replication.
[b] Cytotoxicity on H1-Hela cells, targeted window $CC_{50} > 20 \times EC_{50}$.
[c] Caco-2 A to B, permeability on a model using cultured polarized monolayer of human epithelial colorectal adenocarcinoma cells.
[d] HLM: human liver microsome: in vitro metabolic clearance expressed as % of estimated human hepatic blood flow of 21 mL/min/kg.
[e] Na: not available.

structural alert that would have to be removed.[11] As shown in Table 1, compound **1** is pan-active against the screened genotypes, and has a good window with respect to the cytotoxicity (CC_{50} > 65 μM). At the time the SAR expansion started, the mode of action of compound **1** was not known. Initial studies showed that the series acts later in the infectious cycle of HRV at the level of RNA replication, but it was found not to be active against HRV $3D^{pol}$ or HRV 3C protease.[8] Initial in vitro ADME profiling on compound **1** highlights a good Caco-2 permeability and log*D*, whereas the improvement of the metabolic stability and CYP 3A4 inhibition profile was desirable.

The objectives of this study were to identify the key pharmacophores, complete the SAR, improve potency and ADME profile, and to try to identify replacements for the aniline. The elucidation of the mode of action and determination of the biotarget is described in detail elsewhere.[8] Primary SAR studies of compound **1**, have shown that the aniline can be replaced with the aminopyridine (compound **2**). This product demonstrated pan-activity within approximatively two fold of compound **1**. Additional profiling showed significant improvement in solubility at pH 2.0 and 6.8 over the reference compound **1**. Additional benefits include lower lipophilicity and improved human liver microsome (HLM) stability.



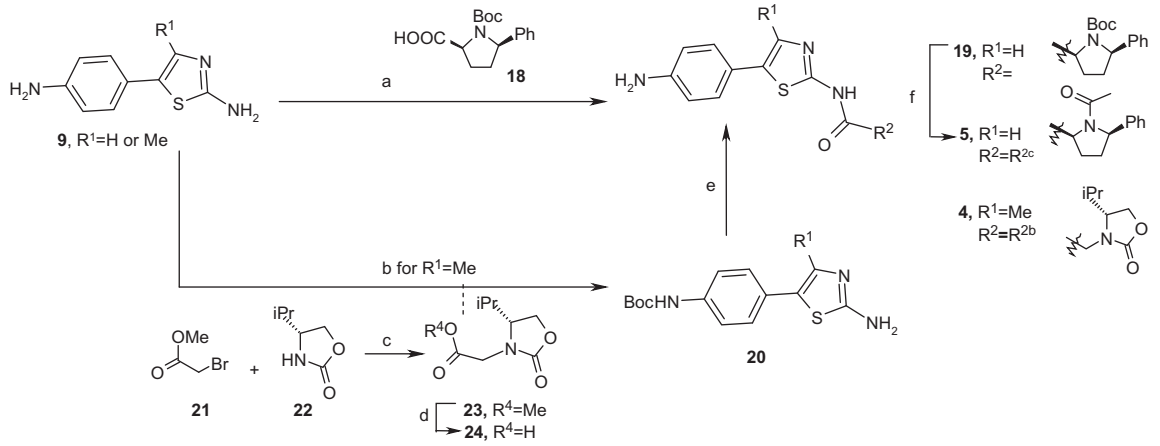
Scheme 1. Synthesis of compounds **1** and **6**. Reagents and conditions: (a) for R^1 = H, H_2O/HCl 1 N (83/17), 100 °C, overnight; for R^1 = Me, MeOH/HCl 1 N (83/17), 40 °C, overnight, both used as a crude; (b) DMF, HATU, DIPEA, 30 min, amine, room temperature, overnight, 68%; (c) DMSO, NaOH 10 N, room temperature, overnight, 40%; (d) MeCN, HATU, DIPEA, room temperature 1 h, 22%; (e) MeCN, HATU, DIPEA, used as crude; (f) DCM, TFA, overnight, 60%; (g) DMF, HATU, DIPEA, carboxylic acid **17** room temperature.

A similar CYP 3A4 inhibitory activity was observed and the Caco-2 permeability is still in the targeted window. Introduction of a methyl group R^1 in position 4 of the aminothiazole heterocycle (compound **3**) resulted in an improvement in potency on the studied genotypes' panel with good solubility, log*D* and HLM stability. Having substituents in position 4 and 5 is also a way known to prevent metabolic activation. Less conservative SAR were also explored. Compounds **4** and **5** with a simplified, rigidified right handside (RHS) maintained potency comparable to the hit compound **1**. Compound **4** addresses the CYP3A4 inhibition observed with the original hit while maintaining a very good permeability.

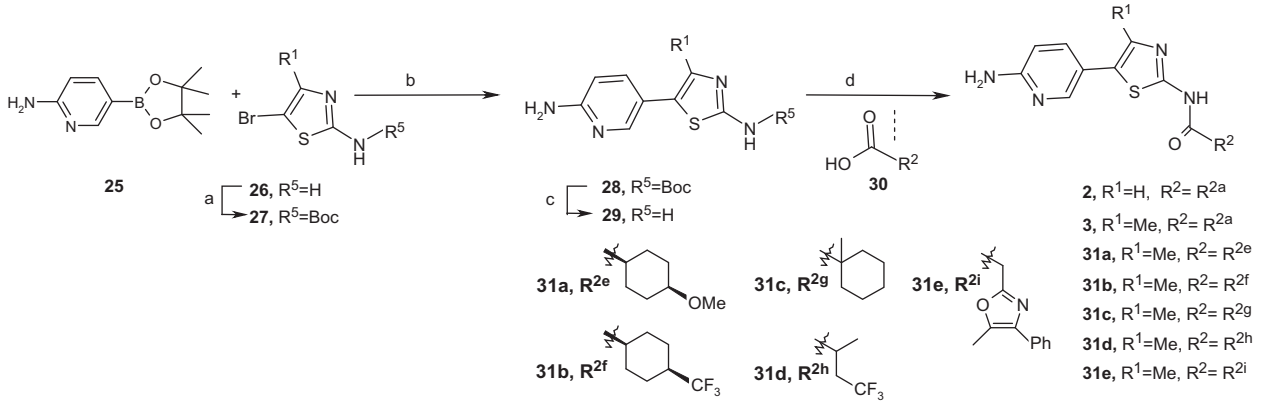
Initial SAR studies on the hit compound **1** have shown that the aniline and the aminothiazole are key pharmacophores, and that these parts are very sensitive to minor changes.[12] However, the aniline can be replaced by the aminopyridine, and introduction of the methyl in position 4 improved potency.[13] Compound **4** and **5** have shown that the RHS of the molecule tolerates significant structure modifications. This finding prompted us to search for new compounds with novel RHS structures with lower molecular weight, improved potency and ADME properties, and a comprehensive investigational library was designed and synthesized for this purpose.

The general synthesis procedure of designed compounds is illustrated in Schemes 1–3. In order to prepare the aniline compounds **1**, **4**–**6**, the key scaffold **9** was obtained by heating reagents **7** and **8** (with R^1 = Me or H) using HCl 1 N.[14] Carboxylic acid **13** was prepared by amide coupling of **10** with **11** followed by ester hydrolysis. Selective amide formation on the aminothiazole group of scaffold **9** (with R^1 = H) using carboxylic acids **13** or **14** in acetonitrile, HATU and DIPEA provided compound **1** or compound **15**. After removal of the protecting Boc group and coupling with acid **17**, compound **16** led to analog **6**. As shown in Scheme 2, selective amide formation on the aminothiazole group of **9** led to intermediate **19** using carboxylic acid **18**. Removal of the Boc group in the left-hand side and acylation provided analog **5**. For the synthesis of analog **4**, the scaffold **20**, which was protected by a Boc group on the aniline (R^1 = Me) was used as well as the carboxylic acid **24**. Nucleophilic substitution of **21** with **22** followed by ester hydrolysis provided intermediate **24**. Analog **4** was prepared from **20** by amide coupling followed by Boc deprotection of the aniline.

For the aminopyridine analogs (such as compound **2**, **3** and the ones from the library), reagent **26** is protected with a Boc group, leading to **27** (Scheme 3). A Suzuki coupling is performed between intermediate **27** and the boronate ester **25**. The key intermediate

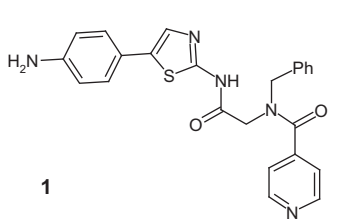


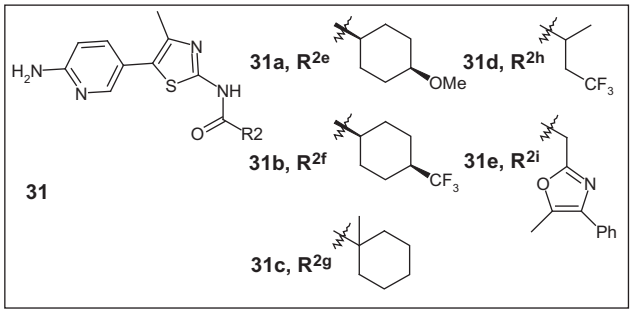
Scheme 2. Synthesis of compounds **4** and **5**. Reagents and conditions: (a) MeCN, HATU, DIPEA, room temperature 2 h, 59%; (b) DMSO, Et_3N, Boc_2O, room temperature, overnight, 42%; (c) THF, 0 °C, NaH, 0 °C for 5 min, room temp for 30 min, then electrophile, room temperature overnight, 33%; (d) THF/ MeOH (1/1), NaOH 1 N, room temperature overweekend, 53%; (e) MeCN, HATU, DIPEA, room temperature overnight then, concentration to dryness, HCl 4 N/dioxane, 1 h, concentration to dryness 49%; (f) HCl 4 N/dioxane, 1 h, concentration to dryness, then MeCN, HATU, DIPEA, CH_3CO_2H, room temperature overnight, 26%.

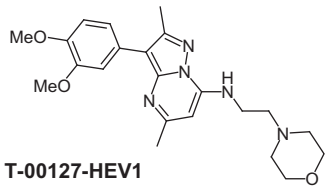


Scheme 3. Synthesis of compounds **2**, **3** and **31a** to **e**. Reagents and conditions: (a) for R^1 = H, DCM, Boc_2O, Et_3N, DMAP, room temperature, overnight, 73%; for R^1 = Me, DCM, Boc_2O, Et_3N, DMAP, room temperature, 6 h., 66%; (b) for R^1 = H, Dioxane (0.07 M), H_2O (0.5 M), **27** (1.5 equiv), $Na2CO_3$ (3 equiv), $Pd(PPh_3)_4$ (0.2 equiv), 15 min, μw-high, 115 °C, 46%; for R^1 = Me, DMF (0.06 M), H_2O (0.6 M), **27** (1.5 equiv), $Ba(OH)_2$ (3 equiv), $Pd(PPh_3)_4$ (0.2 equiv), 20 min, MW-high, 115 °C, 62%; (c) for R^1 = H, TFA/DCM(1/1) (0.17 M), room temperature, 1.5 h, 100%; for R^1 = Me, HCl 4 N/dioxane (0.2 M), 65 °C, 3 h, 97%; (d) for compound **2** and **3**, DMF, HATU, DIPEA, room temperature overnight, 34%; for compounds **31a**–**e**, DCM, HATU, DIPEA, room temperature overnight, 19–66%.

Table 2
Activity and ADME and physicochemical properties of best 3D similar and diverse compounds from the comprehensive investigational library (**31**) compared to compound **1**







Compd	EC$_{50}$[a] (nM)									PI4K IIIβ (nM)	PI4K IIIα (nM)	CC$_{50}$[b] (μM)	sol pH 2/6.8 (μg/mL)	log*D* pH 7.4	Caco-2 [c] 10^{-6} cm/s)	HLM [d]% Q_H	CYP 3A4 (μM)
	HRV-14	HRV-16	HRV-1a	HRV-2	HRV-8	HRV-54	HRV-89	HRV-45	HRV-5								
1	640	630	1600	790	210	1300	790	1200	660	100	>10,000	>64	479/<0.2	2.9	11.3	66	0.5
31a (R^{2e})	500	285	680	710	160	360	295	200	na[e]	22	>10,000	22	>1000/14	2.8	18	<24	>30
31b R^{2f}	290	140	300	295	125	235	200	135	145	53	>10,000	33	>1000/1.1	3.8	22	34	>30
31c R^{2g}	325	145	340	335	84	235	190	na	130	40	3600	39	>1000/8.7	3.8	20	76	17
31d R^{2h}	450	450	390	390	63	320	370	390	180	35	2800	24	>1000/16	2.9	20	31	>30
31e R^{2i}	730	795	360	430	190	430	330	320	490	67	4500	>64	57/<0.1	4.0	26	48	7.7

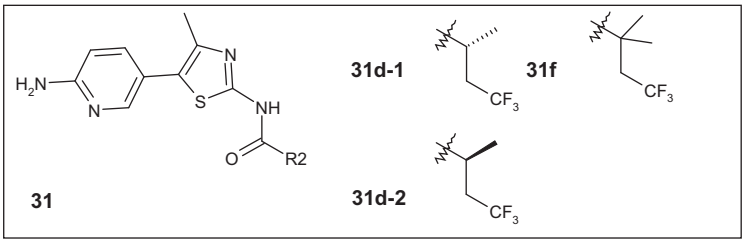
[a] Anti-HRV activity is evaluated in a cell-culture based assay (viral replication assay) that measures the ability of compounds to protect from the cytopathic effect (CPE) caused by HRV replication.
[b] Cytotoxicity on H1-Hela cells, targeted window $CC_{50} > 20 \times EC_{50}$.
[c] Caco-2 A to B, permeability on a model using cultured polarized monolayer of human epithelial colorectal adenocarcinoma cells.
[d] HLM: human liver microsome: in vitro metabolic clearance expressed as % of estimated human hepatic blood flow of 21 mL/min/kg.
[e] Na: not available.

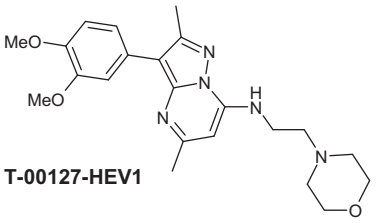
Table 3
Rat PK profile[a] of compounds **31a** and **31b** versus **T-00127-HEV1**

Compd	EC_{50} (nM) HRV-14	EC_{50} (nM) HRV-16	CC_{50} (nM)	PI4KIIIβ (nM)	C_{max} (μM)	AUC (μM × h)	%F	Cl_{tot} (mL/min/kg)	$T_{1/2}$ (h)	Vss
31a	500	285	22,000	22	1.5	2.65	77	70	0.38	1.88
31d	450	450	24,000	35	1.9	9.7	160[b]	40	1.7	4.2
T-00127-HEV1[c]	2300	1800	>65,000	48	2.0	10.0	91	18	2.8	4.4

[a] Rat PK at 5 mg/kg po and at 2 mg/kg iv.
[b] >100 due to large extrapolation of the AUC po at 5 mg/kg and from AUC iv at 2 mg/kg.
[c] For **T-00127-HEV1**, sol (at pH 2/6.8) = 1000 and 42 μg/mL; log*D* (at pH 7.4) = 2.7; Caco-2 = 27 × 10^{-6} cm/s; HLM QH < 25%; IC_{50} CYP 3A4 > 30 μM.

Table 4
Activity on enteroviruses and on a panel of kinases of compounds **31d-1**, **31d-2**, **31f** and **T-00127-HEV1**





Compds	EC_{50} (nM)			EC_{50} HRV-14[b]								PI4K IIIβ (nM)	PI4K IIIα (nM)	Lipid kinase specifi city	Protein kinase specificity
	HRV-B14 replicon[a]	HRV-A16 replicon[a]	HRV-C15 replicon[a]	HRV-14[b]	HRV-16[b]	EV-71	Echo virus	Polio	CV-B4	CV-B3	CC_{50} (μM)				
31d-1	310	115	260	995	1200	1300	450	1100	380	1100	>64	225	>10,000	0/8	2/277[c]
31d-2	83	33	45	290	360	240	170	280	61	520	>66	53	2750	3/8[d]	3/277[e]
31-f	120	44	72	470	190	170	<23	130	120	280	>66	48	>10,000	2/8[f]	2/277[g]
T-00127-HEV1	590	250	200	2300	1800	4400	860	1200	720	4900	>65	137	>10,000	0/8	0/277

[a] Replicon assay, measuring inhibition of the RNA replication of HRV via a luciferase reporter replicon. HRV-B14 and HRV-A16 are subgenomic reporter replicons and HRV-C15 is a full-length reporter replicon.
[b] Cell-culture based assay (viral replication assay) that measures the ability of compounds to protect from the cytopathic effect (CPE)caused by HRV replication.
[c] STK17A (IC_{50} = 2.9 μM); MAP4K2 (IC_{50} = 9.7 μM).
[d] PIK3C2A (IC_{50} = 4.8 μM), PIK3C2B (IC_{50} = 8.9 μM), PIK3CD (IC_{50} = 11 μM).
[e] CDK9 (IC_{50} = 5.3 μM), DYRK3 (IC_{50} = 10 μM), MAP4K4 (IC_{50} = 8.0 μM).
[f] PIK3C2A (IC_{50}=12 μM), PIK3C2B (IC_{50} = 5.6 μM).
[g] STK17A (IC_{50} = 2.1 μM), MSK2 (IC_{50} = 4.6 μM).

28 is deprotected. The scaffold **29** offers two sites for the amide formation: one at the aminothiazole and the other one at the aminopyridine. Selective amide formation on the aminothiazole group, was obtained using HATU, DIPEA and dichloromethane, solvent in which the scaffold is poorly soluble.[15]

The synthesis of the building block **28** with R^1 = Me, necessary for the comprehensive investigational library has been optimized, using for the Suzuki step a design of experiment (DOE) approach with the software MODDE. Three variables were studied: the palladium catalyst [$Pd(PPh_3)_4$ or $Pd(dppf)_2Cl_2$ or $Pd(OAc)_2$ with the DavePhos ligand], the base [Na_2CO_3, Cs_2CO_3, $Ba(OH)_2$, tBuOK or K_3PO_4] and the solvent [dioxane or DMF or a mixture DME-MeOH]. Running 18 experiments planned with the DOE software MODDE allowed us to improve the reaction yield from 42% to 62% (see Supplementary data Fig. S1 for the table with the 18 experiments of the DOE). The best results were found using barium hydroxide as

Table 5
Profiling of compounds **31d-1**, **31d-2**, **31f** and **T-00127-HEV1**: ADME assays and dose escalating PK in CD-1 mice

Compds	EC_{50} (nM)			Sol pH 2/6.8 (μg/mL)	LogD pH 7.4	Caco-2[b] (10^{-6} cm/s)	H LM[c]% Q_H	CYP 3A4 (μM)	C_{max} (μM) at 5 mg/kg 50 mg/kg 250 mg/kg	AUC_ (μM × h) at 5 mg/kg 50 mg/kg 250 mg/kg	Cl_{tot} (mL/min/kg) iv at 2 mg/kg
	HRV-B14 replicon[a]	HRV-A16 replicon[a]	HRV-C15 replicon[a]								
31d-1	310	115	260	1000/82	3.2	44	<24	>30	1.8 18 55	2.7 44 176	91
31d-2	83	33	45	>1000/51	3.2	42	48	>30	2.2 20 26	4.0 38 12	71
31-f	120	44	72	>1000/13	3.4	41	27	17	0.5 12 59	208 514 732	58
T-00127-HEV1	590	250	200	1000/2	2.7	11	25	>30	1.9 15 52	3.6 45 307	nd[d]

[a] Replicon assay, measuring inhibition of the RNA replication of HRV via a luciferase reporter replicon.
[b] Caco-2 A to B, permeability on a model using cultured polarized monolayer of human epithelial colorectal adenocarcinoma cells.
[c] HLM: human liver microsome: in vitro metabolic clearance expressed as % of estimated human hepatic blood flow of 21 mL/min/kg.
[d] Nd: not done.

a base, $Pd(PPh_3)_4$ for the catalyst, and DMF as the solvent. The reaction was performed successfully on a 7g scale.

For the design of the comprehensive investigational library of the RHS variations (Scheme 3, compounds **31**) 3D pharmacophore hypotheses and diversity calculations were employed. One part of this library (set 1) was selected based on 3D shape and chemical similarity to bioactive conformation hypotheses that were generated for compounds **4** and **6**, using a 3D alignment approach. The other part of the library (set 2) was based purely on chemical diversity and was complementary to set 1 (refer to Supplementary data S2 for details on the computational design process). The library was planned to generate ~400 analogs. The list of suitable in-house and commercially available reagents (carboxylic acids **30**) was pre-filtered according to physico-chemical properties of the enumerated compounds via Pipeline Pilot and SpotFire,[16] undesirable (toxic or reactive) groups were removed, and supplier availability was considered. This process led to 3564 acids that were prioritized with the above mentioned 3D similarity and diversity based approach. In total, 285 representative analogs were synthesized (81% chemical success rate). 46 analogs had EC_{50} values below 2 μM and 21 below 1 μM versus HRV14. The active analogs were found in both sets the similar and diverse ones.

During the design and synthesis of these potent analogs, the mode of action of this series was identified to be preventing HRV replication via inhibiting the host lipid kinase phosphatidylinositol 4-kinase III beta (PI4KIIIβ).[8] At the same time this work was being conducted, a study was published showing that the target of enviroxime-like compounds is PI4KIIIβ.[17] The compound identified in this prior study was **T-00127-HEV1**.

For the most potent analogs (**31a–e**) the bioresults, ADME and physchem properties are presented in Table 2. Compounds **31a–c** are belonging to set 1 of 3D similar structures and compounds **31d** and **31e** are belonging to the set 2 of diverse structures. Compounds **31a–e** were pan-active against the screened genotypes, had a decreased molecular weight and showed improvement for their microsome stability (HLM), their permeability (Caco-2 A to B) and their CYP 3A4 when compared with compound **1**. Compounds **31a–d** showed also an improved solubility at pH 2 and 6.8. This library allowed the identification of novel, improved RHS regarding not only potency but also physchem and ADME properties. Based on HLM and their overall profile compound **31a** and **31d** were selected for rat PK evaluation (Table 3). Compound **31d** showed an improved exposure (AUC = 9.7 μM × h) and a lower total clearance when compared to compound **31a**. For comparison, the data for compound T-**00127-HEV1** are shown in Table 3. For the most potent analogs against HRV, identified from the library (**31a–31e**), the activity against PI4KIIIß and α is reported in Table 2.

PI4KIIIß is involved in a membrane trafficking between the endoplasmic reticulum, the Golgi and the secretory pathway and is expressed widely in all tissues in the body. Hsu et al.[18] proposed that the enterovirus protein 3A promotes the recruitment of PI4-KIIIß to the Golgi membrane. The recruited PI4KIIIß is suggested to remodel the membrane through phosphorylation and this recruits $3D^{pol}$ to the membrane and thereby creating a site that promotes viral RNA synthesis. The effect of inhibiting selectively PI4KIIIß over a shorter duration was not known.

In order to identify the best compounds with a profile which would allow an in vivo risk assessment of this kinase, the two enantiomers of compound **31d** were separated by supercritical fluid chromatography (SFC) and profiled. The enantiomer **31d-2** was more potent than the enantiomer **31d-1** versus all genotypes tested. **31d-2** and **31d-1** had a similar CD-1 mouse PK at 5 mg/kg with a good exposure. The gem-dimethyl analog **31f** was also synthesized and profiled and this compound maintained the good profile (for potency and CD-1 mouse PK at 5 mg/kg) seen with the best enantiomer **31d-2**.

Compounds **31d-1**, **31d-2**, **31f** and the literature compound **T-00127-HEV1** were completely profiled in a panel of 8 lipid kinases and 277 protein kinases available at Invitrogen. An excellent specificity for PI4KIIIß versus other lipid and protein kinases was found. As shown in Table 4, the few instances where there is a minor inhibitory activity, the IC_{50} is in the order of 2000–10,000 nM (vs an IC_{50} PI4KIIIß of 53–225 nM).

In view of the limitation of the current HRV pharmacological animal models, it was proposed to test compounds from two structurally diverse classes in the related enterovirus Coxsackie virus (CV-B4) mouse pancreatitis model in order to distinguish compound related from target related toxicity. Therefore, compounds **31d-1**, **d-2**, **f** and **T-00127-HEV1** were profiled in a panel of enteroviruses including CV-B4. As shown in Table 4, they showed activity against this panel of viruses.

In order to identify compounds for the in vivo evaluation, analogs **31d-1**, **31d-2**, **31f** and **T-00127-HEV1** were evaluated in a dose escalation study in CD-1 mice at 5, 50 and 250 mg/kg (Table 5).[19] A good exposure was achieved for these compounds, but at 250 mg/kg the gem-dimethyl carrying **31f** showed a superior exposure and a higher C_{max} when compared to **31d-2**. Therefore compound **31f** was selected together with compound **T-00127-HEV1** (structurally diverse) based on their overall profile (potency, ADME properties, PK profile and selectivity). When these compounds underwent repeat-dosing in SJL mice that are used in the CVB-4 pancreatitis model, deleterious effects were seen with mortality observed at all tested doses.[8]

In summary, compounds meeting lead criteria were obtained: **31f** for example showed potency and pan-activity against the screened HRV genotypes and against other enteroviruses such as CV-B4. Compounds with selectivity for PI4KIIIß were obtained and they reached acceptable ADME and PK profiles with good exposure in CD-1 mice at 5, 50 and 250 mg/kg. This allowed us to perform repeated dosing studies in-house in SJL mice with two structurally diverse PI4KIIIß inhibitors: **31f** and **T-00127-HEV1**, and to show that short term inhibition of PI4KIIIβ is deleterious. This data raises doubts on the safety of inhibiting PI4KIIIβ in order to reduce HRV-induced asthma and COPD exacerbations.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.04.077.

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9. Cytotoxicity in H1-Hela, Huh7.5, MRC-5 and C8166.
10. There are several biotargets known for HRV: inhibitors of attachment (eg Tremacamra), capsid binders (e.g., pleconaril, pirodavir, BTA-798), replication inhibitors (e.g., enviroxime) and protein processing inhibitors (e.g. rupintrivir). Capsid binders are characterized by acting early in the time of addition experiment, by their inactivity against HRV45 and are known to protect HRV from the heat treatment by binding and stabilizing the core protein VP1.
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