Computer-aided design, synthesis and evaluation of novel non-nucleoside inhibitors of the viral polymerase as antiviral agents against Norovirus

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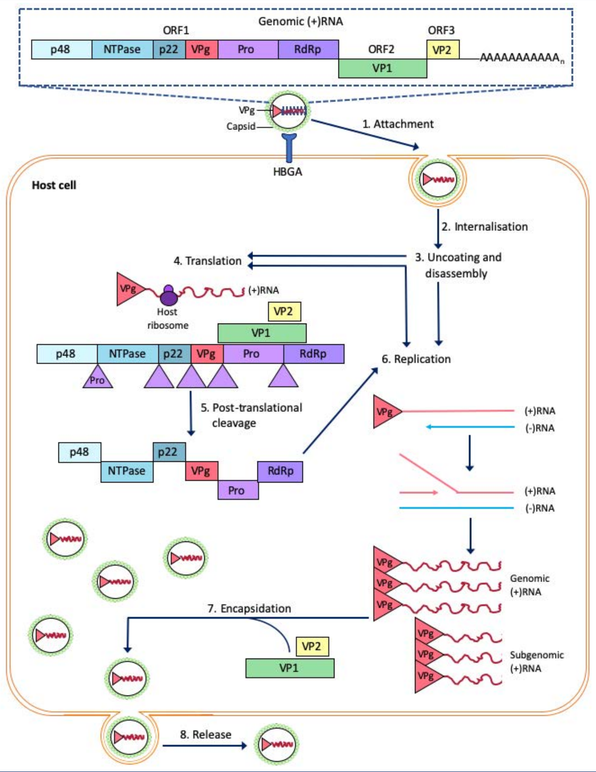
Norovirus and the RdRp

Norovirus represents the first cause of food-borne illness worldwide, leading to extensive outbreaks of gastroenteritis, a life-threatening condition in the developing countries. In the UK alone, norovirus is a major cause for the closure of hospitals and wards, with an estimated cost to the health service of £81 million per year.

No vaccines or specific antivirals are currently available for this viral infection, leading to an ample need for the development of antiviral treatments.

One promising target for the identification of anti-norovirus agents is the viral RNA-dependent RNA polymerase (RdRp), responsible for the synthesis of the viral RNA genome. Even though different inhibitors of this enzyme have been identified, most of them lack the ability to inhibit the viral replication in cell-based systems.



Our non-nucleoside RdRp inhibitors

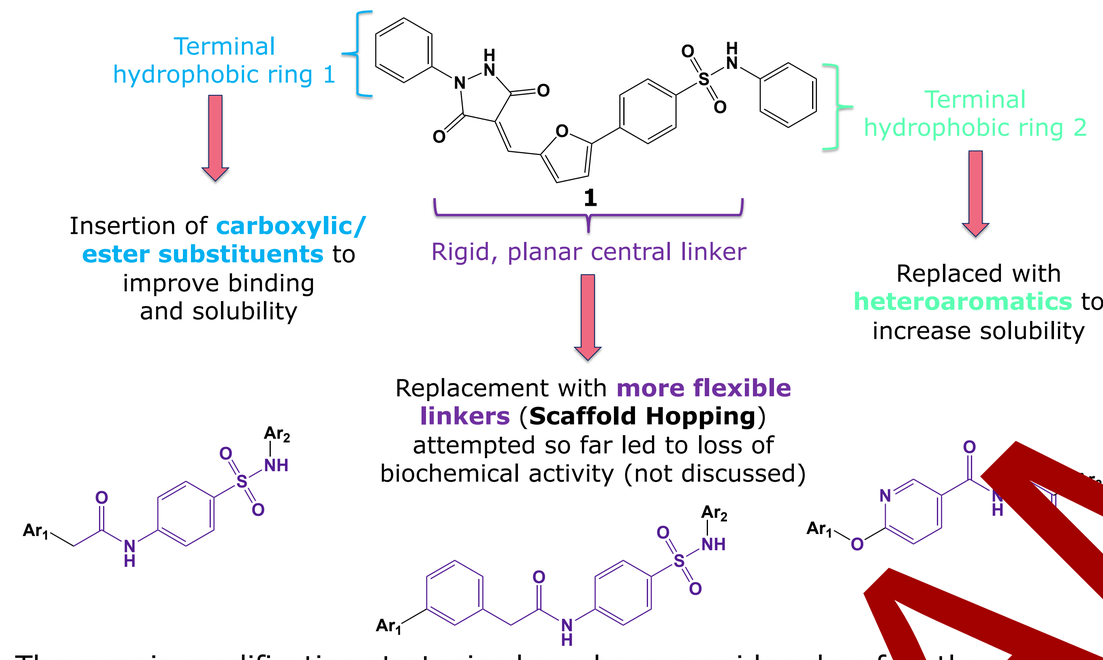
With a previous *in silico* screening of commercial compounds on the norovirus polymerase structure, four non-nucleoside small molecules were found to inhibit the human norovirus polymerase in biochemical assays. The potential of these scaffolds to arrest the viral replication was limited, mainly due to their poor solubility.[1]

Starting from these findings, the chemical scaffold of our best HNoV RdRp inhibitor, **1**, was rationally modified and assessed with a series of docking simulations, in order to prepare new analogues which maintain a good predicted binding to the viral polymerase, and show higher solubility. Improved derivatives were identified both for enzyme inhibition and for their ability to arrest the viral replication in cell-based assays. These compounds are now being further explored with new modifications to optimise their antiviral profiles.

Rational design and molecular modelling

1. Modifications on compound 1

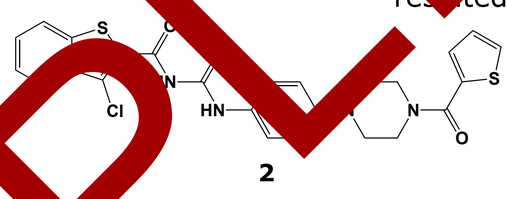
The structure of **1** is characterised by the presence of a rigid central linker and two terminal aromatic rings, which render **1** highly hydrophobic and poorly soluble.

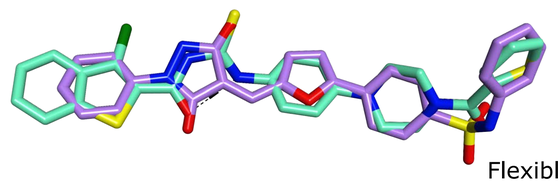


Three main modification strategies have been considered so far: the new structural analogues with aromatic modifications at ring 1 and ring 2 are predicted to enhance the compound binding to the active site of HNoV RdRp.

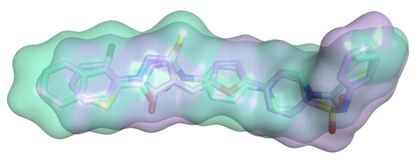
2. Flexible alignment

As an alternative strategy to identify potential central linker replacements with which to reduce the solubility issues, the structure of **1** was compared to the scaffolds of reported non-nucleoside inhibitors of viral polymerases using the Flexible Alignment tool in MOE 2018.10. From this study, compound **2**, an inhibitor of Zika virus RdRp,[2] resulted as best match for its structural overlapping with **1**.

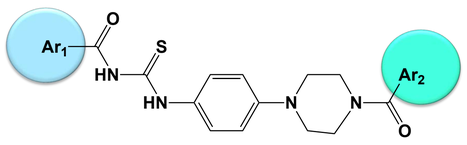




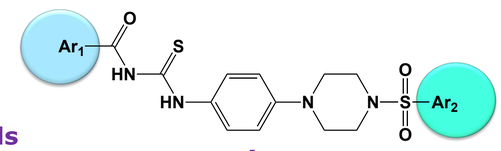
Flexible alignment results between **1** and **2**

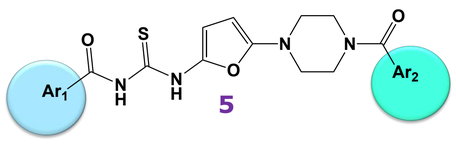


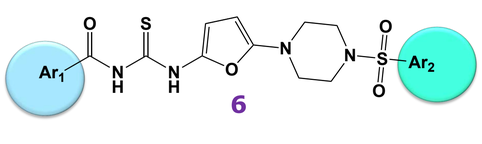
After confirming with a gel-shift based assay that **2** inhibits HNoV RdRp function at 20 μM, the structures of **1** and **2** were combined to design new structural analogues with a new, non-planar central linker. The new analogues prepared present improved solubility in comparison with **1**.



4 new scaffolds with a non-planar linker designed





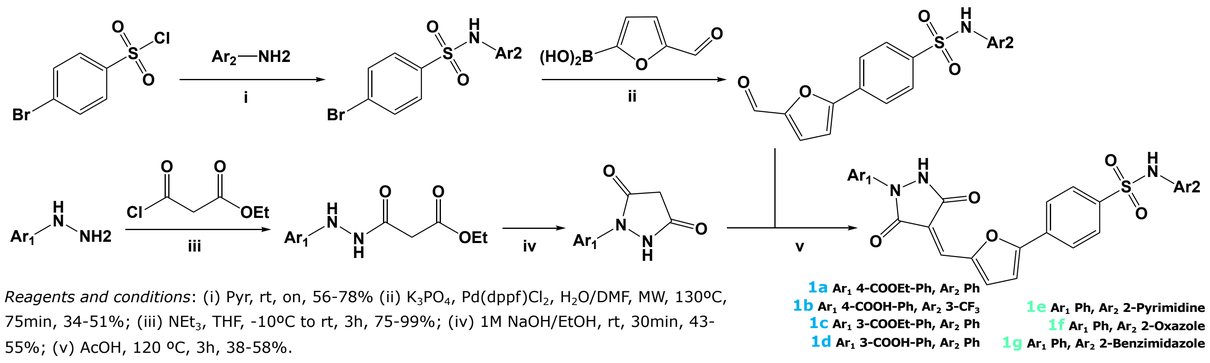


A small family of new analogues has been successfully synthesised for scaffolds **3** and **4**, as shown below, while the preparation of **5** and **6** is currently ongoing.

Chemistry

Modifications at Ar_1 and Ar_2

Six new analogues of **1** were prepared so far. For final products with a free carboxylic function (**1b**, **1d**), the carboxylate was converted to ethyl ester prior to step 1 (i), then hydrolysed from final products **1a**, **1c**.

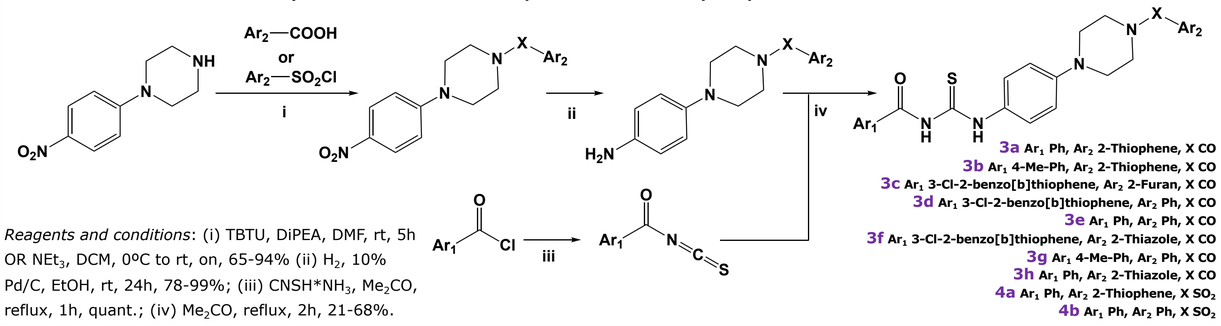


Reagents and conditions: (i) Pyr, rt, on, 56-78% (ii) K_3PO_4, $Pd(dppf)Cl_2$, H_2O/DMF, MW, 130ºC, 75min, 34-51%; (iii) NEt_3, THF, -10ºC to rt, 3h, 75-99%; (iv) 1M NaOH/EtOH, rt, 30min, 43-55%; (v) AcOH, 120 ºC, 3h, 38-58%.

1a Ar_1 4-COOEt-Ph, Ar_2 Ph
1b Ar_1 4-COOH-Ph, Ar_2 3-CF_3
1c Ar_1 3-COOEt-Ph, Ar_2 Ph
1d Ar_1 3-COOH-Ph, Ar_2 Ph
1e Ar_1 Ph, Ar_2 2-Pyrimidine
1f Ar_1 Ph, Ar_2 2-Oxazole
1g Ar_1 Ph, Ar_2 2-Benzimidazole

Non-planar linkers

An initial family of 10 new compounds was prepared for scaffolds **3** and **4**.



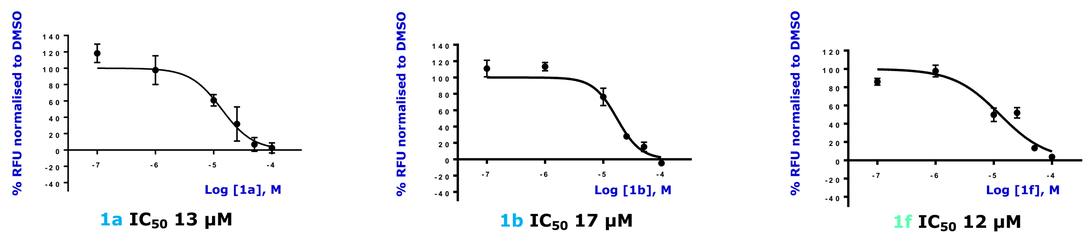
Reagents and conditions: (i) TBTU, DiPEA, DMF, rt, 5h OR NEt_3, DCM, 0ºC to rt, on, 65-94% (ii) H_2, 10% Pd/C, EtOH, rt, 24h, 78-99%; (iii) CNSH*NH_3, Me_2CO, reflux, 1h, quant.; (iv) Me_2CO, reflux, 2h, 21-68%.

3a Ar_1 Ph, Ar_2 2-Thiophene, X CO
3b Ar_1 4-Me-Ph, Ar_2 2-Thiophene, X CO
3c Ar_1 3-Cl-2-benzo[b]thiophene, Ar_2 2-Furan, X CO
3d Ar_1 3-Cl-2-benzo[b]thiophene, Ar_2 Ph, X CO
3e Ar_1 Ph, Ar_2 Ph, X CO
3f Ar_1 3-Cl-2-benzo[b]thiophene, Ar_2 2-Thiazole, X CO
3g Ar_1 4-Me-Ph, Ar_2 Ph, X CO
3h Ar_1 Ph, Ar_2 2-Thiazole, X CO
4a Ar_1 Ph, Ar_2 2-Thiophene, X SO_2
4b Ar_1 Ph, Ar_2 Ph, X SO_2

Biology

All new compounds were evaluated for their inhibition of hNoV RdRp activity and their ability to interfere with MNV and hNoV in cell-based assays. While all inhibit at least partially hNoV RdRp activity at 20 μM, the most effective in the RdRp Picogreen assay[1] are **1a** and **1f**.

Quantitative fluorescent RdRp activity assay



1a IC_{50} 13 μM
1b IC_{50} 17 μM
1f IC_{50} 12 μM

All compounds were evaluated in an initial fluorescent activity screen using human norovirus Sydney 2012 (GII.4) RdRp, at a fixed concentration of 20 μM, using PPNDS as positive control. For **1a-g**, an IC_{50} was calculated with the same Picogreen assay. **1a-g** all display IC_{50} values in the 10-20 μM range; **1a**, **1b** and **1f** are shown as representative examples. Percentage of inhibition is normalised to control DMSO. Mean values of triplicate datasets with standard error of the mean are shown. Activity for all new compounds was also confirmed in a gel-shift assay (data not shown),[1] at a fixed concentration of 100 μM.

Antiviral assays and conclusions

The new compounds were evaluated for their antiviral effect in both a MNV CPE reduction assay in RAW cells and a hNoV GI replicon assay in HGT cells. Antiviral hits were identified within the new scaffolds **3** and **4**. In particular, due to their antiviral effect in the hNoV replicon system, **3b** and **4a** represent a promising starting point for further structural optimisation.

Compound	MNV CPE EC_{50} (μM)	CC_{50} (μM)	hNoV EC_{50} (μM)
3b	45±11	>100	6±4
3f	66±12	>100	n.d.
4a	44±17	>100	17±11
4b	67±12	>100	8±2

Acknowledgements
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References
[1]Ferla, S., et al. *Sci. Rep.* 2018, 8:4129. [2]Pattnaik, A., et al. *Antivir. Res.* 2018, 151:78-86.