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Synthesis and antiviral evaluation of novel peptidomimetics as norovirus protease inhibitors

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ABSTRACT

A series of tripeptidyl transition state inhibitors with new P1 and warhead moieties were synthesized and evaluated in a GI-1 norovirus replicon system and against GII-4 and GI-1 norovirus proteases. Compound **19**, containing a 6-membered ring at the P1 position and a reactive aldehyde warhead exhibited submicromolar replicon inhibition. Retaining the same peptidyl scaffold, several reactive warheads were tested for protease inhibition and norovirus replicon inhibition. Of the six that were synthesized and tested, compounds **42**, **43**, and **45** potently inhibited the protease in biochemical assay and GI-1 norovirus replicon in the nanomolar range.

Human noroviruses are single stranded, positive sense RNA viruses from the *Caliciviridae* family. Out of the six existing genotypes, genotypes I, II and IV are highly contagious pathogens that are the primary cause of acute gastroenteritis. In the US, there is an estimated 20 million cases of gastroenteritis each year resulting in more than 50,000 hospitalizations and nearly 570–800 deaths. Even though there is no therapeutic approved for the treatment of norovirus (NoV) infections, peptidomimetic inhibitors of the NoV protease (Fig. 1) have emerged recently as a promising option.[1] Indeed, NoV 3C-like cysteine protease is essential to generate mature nonstructural proteins by cleavage of the viral polyprotein. Transition state inhibitors, such as compound **1**,[2] in which the peptidic moiety is selectively recognized by the 3CL protease while the electrophilic warhead reacts with Cys139 to form a covalent bond, can block the protease enzymatic activity (Fig. 2). The general structure of these inhibitors can be divided into 5 distinct portions: P1, P2, P3, cap and warhead (Fig. 1). Modifications of the P2 and P3 portions have been explored in the past but due to their key role in the specificity of these compounds, only minimal modifications seem to be tolerated at these positions. Chemical modification of the P1 position has only been partially explored[3] and a (*S*)-γ -lactam P1 seems to remain the group of choice. Alternatively, the warhead moiety has been largely studied and several groups such as aldehydes, α-ketoamides, α-hydroxyphosphonates, and bisulfite adducts have been successfully evaluated.[2]

Herein, we report our effort to identify more effective peptidomimetics, inhibitors of NoV protease, using the structure of reported compound **1** as a starting point (Reported anti-NoV EC_{50} = 0.04 μM).[2] More specifically, we explored the chemical space around the P1 position and evaluated new potential warheads.

Evaluation of new P1 moieties: Based on several NoV PIs in which the P1 lactam moiety was successfully replaced by acyclic amides,[3] replacement of compound **1**′s pyrrolidinone P1 moiety with an azetidine amide (compound **15a**), an azetidine carbamate (compound **15b**) or a 1,2,3-triazole (compound **15c**) was explored. In a similar manner, since (*S*)-δ -lactam segments at P1 position have been proven to be an effective replacement for (*S*)-γ-lactam groups in the case of peptidomimetic aldehydes, inhibitors of the human enterovirus protease,[4] we decided to apply the same strategy and prepared analog **19**. Amino acid precursors bearing the desired P1 moieties were prepared according to the chemistry described in Scheme 1. Compound **3** was synthesized by reacting acid derivative **2**[5] with azetidine in presence of HOBT, EDC and DIPEA while intermediate **5** was obtained from amine **4**[6] by first, reaction with *N,N*′-disuccinimidyl carbonate (DSC) followed by treatment with azetidine in presence of DIPEA. Triazole derivative **7** was obtained under classical copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) conditions by reaction of azido compound **6**[7] with trimethylsilylacetylene. Piperidinone intermediate **9** was prepared from natural glutamic acid **8** by following previously published procedures.[8]

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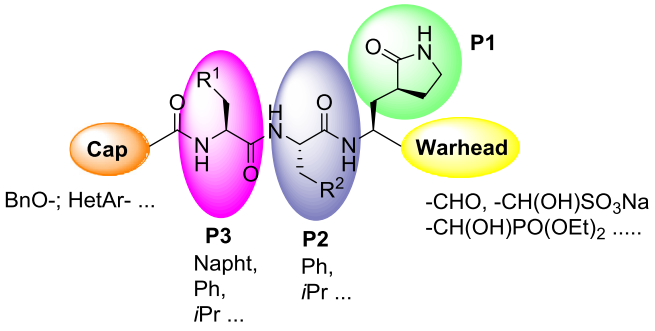


Fig. 1. General structure of known NoV cysteine protease inhibitors.

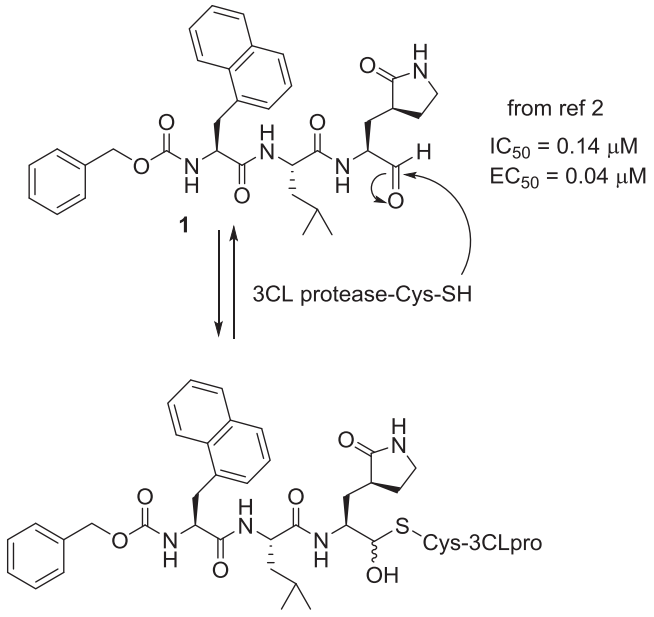
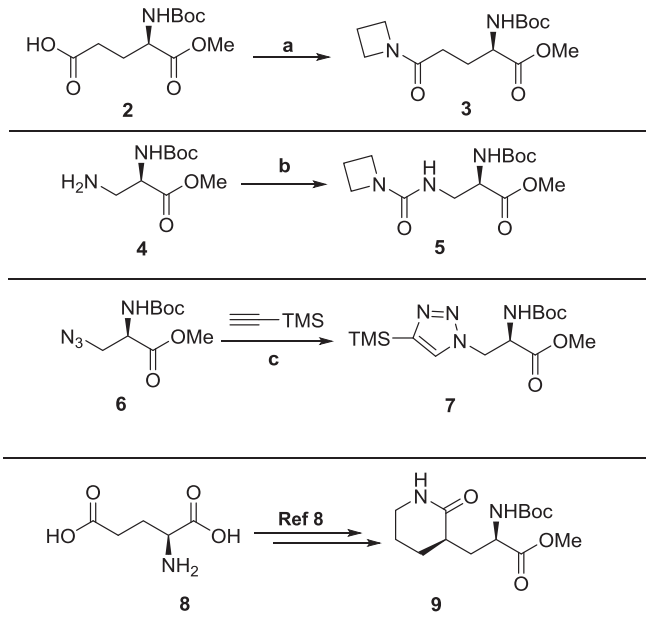


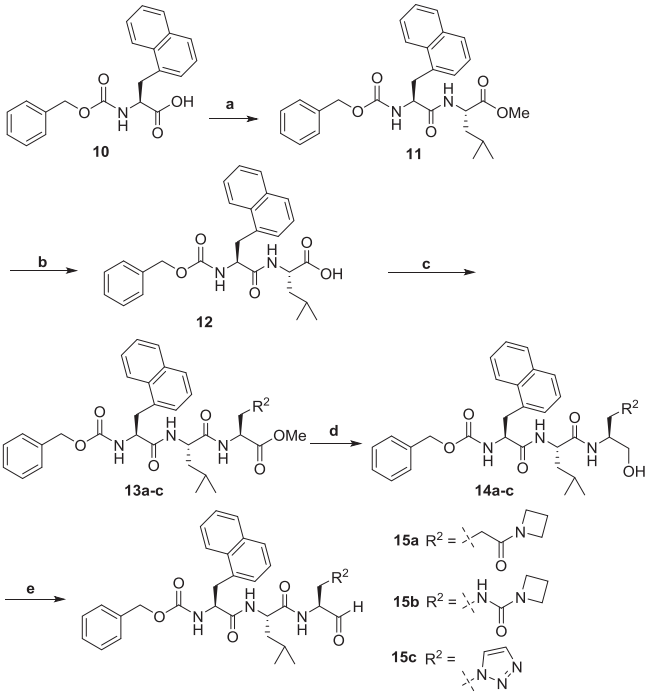
Fig. 2. Interaction of a NoV cysteine protease with known peptidyl transition state inhibitor **1**.

Targeted peptidomimetic inhibitors **15a–c** were finally obtained from commercially available amino acid **10** (Scheme 2). Coupling of **10** with L-leucine methyl ester in presence of HOBt, EDC and DIPEA gave ester **11** which was then saponified in presence of LiOH and coupled with either intermediates **3**, **5** or **7**. Final aldehyde derivatives **15a–c** were obtained through a reduction/oxidation sequence using $LiBH_4$ and a sulfur trioxide pyridine complex. On the other hand, deprotection of intermediate **9** under acidic conditions followed by coupling with Boc-L-leucine gave **16**[9] which was then deprotected and coupled with amino acid **10**. As for the other analogs, a final reduction/oxidation sequence gave access to the desired peptidomimetic **19** (Scheme 3).

Evaluation of new warheads: The chemical reactivity of the warhead is essential and if too reactive, it can lead to a lack of selectivity and undesired off target effects. Therefore, we evaluated several groups as replacement for the highly reactive aldehyde warhead moiety in reference compound **1**. These groups include electrophiles such as a vinyl aldehyde (compound **26**), a halogen (compound **46**), epoxides (compounds **30** and **41**), a trifluoromethyl ketone (Compound **45**) or an acrylonitrile-based Michael acceptor, known to react with cysteines (Compound **43**).[10] The replacement of the aldehyde warhead by a thiol in order to potentially form a disulfide bond with NoV protease Cys139 was also evaluated.



Scheme 1. Reagents and conditions: a) Azetidine, HOBt, EDC·HCl, DIPEA, DCM, rt, 12 h, 72%. b) 1). *N,N′*-Disuccinimidyl carbonate (DSC), DCM, rt, 24 h; 2). Azetidine, DIPEA, DCM, rt, overnight, 31%; c) Na Ascorbate, $CuSO_4$, H_2O/*t*-BuOH, rt, 5 h, 90%. (See above-mentioned references for further information.)



Scheme 2. Reagents and conditions: a) H-*L*-Leu-OMe·HCl, HOBt, EDC·HCl, DIPEA, DCM, rt, 52%; b) LiOH, THF-H_2O, rt, 2 h, 95%; c) 1). **3**, **5** or **7**, TFA/DCM, rt.; 2). HOBt, EDC·HCl, DIPEA, DCM, rt.; d) $LiBH_4$, THF; e) SO_3·Py, DIPEA, DCM-DMSO, rt.

Peptidomimetic **26**, containing a vinyl aldehyde warhead, was prepared from known vinyl ethyl ester derivative **20**[11] (Scheme 4). After deprotection in presence of TFA, compound **21** was coupled with Boc-L-leucine under classical peptidic coupling conditions to give intermediate **22**. **22** was then deprotected and coupled with amino acid **10** to access vinyl ester derivative **24**. Final reduction of the ester group with DIBAL-H followed by oxidation of alcohol