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Discovery and SAR studies of methionine–proline anilides as dengue virus NS2B-NS3 protease inhibitors

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ABSTRACT

A series of methionine–proline dipeptide derivatives and their analogues were designed, synthesized and assayed against the serotype 2 dengue virus NS2B-NS3 protease, and methionine–proline anilides **1** and **2** were found to be the most active DENV 2 NS2B-NS3 competitive inhibitors with K_i values of 4.9 and 10.5 μM. The structure and activity relationship and the molecular docking revealed that L-proline, L-methionine and *p*-nitroaniline in **1** and **2** are the important characters in blocking the active site of NS2B-NS3 protease. Our current results suggest that the title dipeptidic scaffold represents a promising structural core to discover a new class of active NS2B-NS3 competitive inhibitors.

Dengue viruses (DENVs) are classified as the *Flavivirus* genus[1] and are causative pathogens of mild to severe epidemic diseases like dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS).[2,3] These diseases are transmitted by *Aedes aegypti* mosquitoes and are threatening up to 2.5 billion people in more than 100 countries in tropical and subtropical regions. Until now, there is no specific treatment for dengue diseases.[4,5]

There are four distinctive, but closely related, serotypes of dengue viruses named as DENV-1, DENV-2, DENV-3 and DENV-4. Recovery from infection by one serotype of DENV provides lifelong immunity against that particular serotype, however, cross-immunity to the other serotypes after recovery is only partial and temporary, and more dangerously, subsequent infection by any of the other three serotypes will enhance the risk of developing severe dengue.[5,6] This antibody-dependent enhancement effect (ADE) of DENVs makes the vaccine development extremely difficult, which is the major reason for no effective vaccine against DENV/s up to now.[6] Therefore, targeting DENV NS2B-NS3 protease using small molecule drugs is an urgent and important antiviral therapeutic treatment.

DENV is an enveloped RNA virus with ~11 kb positive strand RNA genome encoding a single polypeptide, which is subsequently processed by the virus-encoded trypsin-like NS2B-NS3 protease to generate three structural proteins (capsid protein, precursor-membrane protein, and envelope protein) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).[7,8] The 184-residue NS3pro is the enzymatic domain with a serine protease catalytic triad (His51, Asp75, and Ser135). Optimal catalytic activity of NS3 depends on the presence of NS2B.[9] Since the NS2B-NS3 protease is essential for the maturation and pathogenesis of dengue virus, it is considered to be a promising target for antidengue virus drug development.[10,11]

Many efforts have been made to develop antidengue virus NS2B-NS3 agents.[10,12] In the light of the fact that the active site of NS3-NS2B is shallow and prefers to cleave substrates with dibasic amino acids in vitro,[9] we screened our lab's chemical library biased on compounds with multi-substituted and strained ring/s and varied potential binding sites and some hits were discovered. Among these hits, two modified dipeptides of methionine–proline

Abbreviations: SAR, structure and activity relationship; NS2B-NS3, nonstructural protein 2B and 3; DENV, dengue virus; K_i, inhibition constant; TFA, trifluoroacetic acid; EtOAc, ethyl acetate; DCM, dichloromethylene; EtOH, ethyl alcohol; DMAP, 4-(*N*,*N*′-dimethyl)-aminopyridine; DCC, dicyclohexylcarbodiimide.

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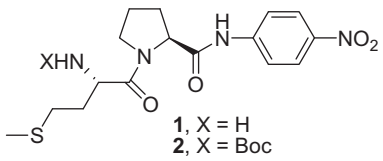


Figure 1. Structures of two dipeptide inhibitors.

anilides **1**[13] and **2**[13] (Fig. 1) were demonstrated as good NS2B-NS3 protease inhibitors with K_i values of 4.9 and 10.5 μM, respectively, and **1** with an N-terminal free amine is more active than **2** with an N-terminal carbamate.

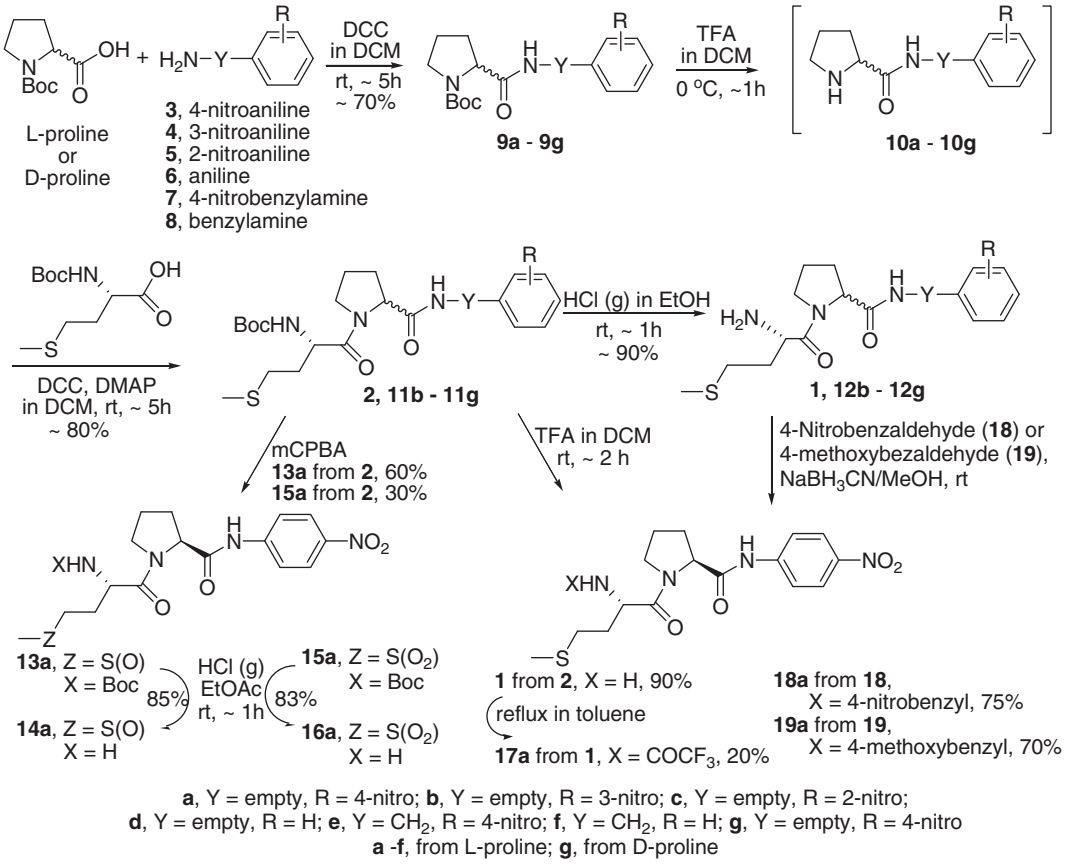
In our aims to search for small molecular entities targeting dengue virus NS2B-NS3 protease, we launched our preliminary SAR studies on these dipeptides as the first step. In this Letter, we describe the design, synthesis and evaluation of a series of compounds based on lead dipeptide compound **1** and their structure–activity relationship (SAR).

The title modified dipeptides were synthesized according to the general methodology for polypeptide synthesis and the synthesis of the desired compounds is outlined in Schemes 1 and 2. Boc-protected L- or D-proline was reacted with an amine to produce a Boc-protected amide, followed by deprotection of the Boc group to yield a free amine, later the free amine group was coupled with Boc-protected methionine to generate the title Boc-protected dipeptide, and at last the title amine of dipeptide was yielded after the deprotection of Boc group. Their inhibitory activities were tested against dengue virus type 2 NS2B-NS3 protease. The structures and their inhibitory activities were summarized in Tables 1 and 2.

Firstly, we investigated the structural requirements in **1** by the replacement of *p*-nitro-aniline with different nitro-substituted phenyl amines or benzyl amines. In order to assess the importance of the nitro group and the effect of different nitro position at the aniline on the inhibitory activity, a series of dipeptide compounds (**12b**, **12c** and **12d**) were synthesized from commercially available starting materials of *o*-nitro- and *m*-nitro-anilines (**4** and **5**) and aniline (**6**) (Scheme 1). The dipeptide derivatives **12e** and **12f** of *p*-nitro-benzylamine (**7**) and benzylamine (**8**) (Scheme 1), respectively, were also prepared to evaluate the effect of elongation on activity.

Considering that the chirality of drug isomers sometimes plays the important role in the recognition between the drug and its target/s and different stereochemistry probably results in distinctive binding capacity, we studied the contribution and potential utility of the stereochemistry of proline to the anti-NS2B-NS3 enzymatic activity after the first run. Starting from 4-nitroaniline, D-proline was used to synthesize the dipeptide **12g** (Scheme 1) as a part of our exploration.

To probe the feature of methionine in **1**, modifications on methionine moiety of **1** were employed. Sulfoxide **14a** and sulphone **16a** derivatives of **1** were prepared by mCPBA oxidation of **2** followed by HCl-deprotection of Boc group (Scheme 1). Trifluoroacetyl amide **17a** was generated by refluxing trifluoroacetic salt of **1** in toluene (Scheme 1). The N-alkylated derivatives (**18a** and **19a**) of **1** were synthesized from reductive amination of **1** (Scheme 1) using $NaBH_3CN$. In addition, compounds **21**, **22**, **24**, **25** and **26** (Scheme 2), which L-phenylalanine and L-arginine substituted for L-methionine, respectively, were designed to explore the favor of methionine for the inhibitory activity of **1**.



a, Y = empty, R = 4-nitro; **b**, Y = empty, R = 3-nitro; **c**, Y = empty, R = 2-nitro;
d, Y = empty, R = H; **e**, Y = CH_2, R = 4-nitro; **f**, Y = CH_2, R = H; **g**, Y = empty, R = 4-nitro
a -**f**, from L-proline; **g**, from D-proline

Scheme 1.