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New developments for the design, synthesis and biological evaluation of potent SARS-CoV 3CL^{pro} inhibitors

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ABSTRACT

A series of trifluoromethyl, benzothiazolyl or thiazolyl ketone-containing peptidic compounds as SARS-CoV 3CL protease inhibitors were developed and their potency was evaluated by in vitro protease inhibitory assays. Three candidates had encouraging results for the development of new anti-SARS compounds.

In November 2002, it was reported an emergence of severe acute respiratory syndrome (SARS) as a highly contagious and fatal respiratory disease infecting more than 8000 individuals of which 9.6% patients died within a few months.[1] Due to highly efficient international cooperation, two groups rapidly reported that a novel coronavirus (CoV) was the causative agent of SARS.[2,3] CoV encodes a chymotrypsin-like protease (3CL^{pro}) that plays a pivotal role in the replication of the virus.[4] 3CL^{pro}, a cysteine protease, is functionally analogous to the main picornavirus protease 3C^{pro} with a catalytic dyad (Cys-145 and His-41) in the active site. Cys acts as a nucleophile, whereas His functions as a general base.[5,6] In order to find compounds that can inhibit SARS-CoV, numerous 3CL^{pro} inhibitors have been described, including C_2-symmetric diols,[7] bifunctional aryl boronic acids,[8] keto-glutamine analogs,[9] isatin derivatives,[10] α,β-unsaturated esters,[11] anilide,[12] benzotriazole[13] as well as glutamic acid and glutamine peptides possessing a trifluoromethyl ketone group as reported by us and our collaborators since 2006[14] and recently by another group.[15] However, no effective therapy has been developed so far and it is still a matter of necessity to discover new potent structures in case the disease re-emerges.

In our previous report, two compounds (Scheme 1, **1a,b**) were found to be moderate SARS-CoV 3CL^{pro} inhibitors (K_i = 116 and 134 μM, respectively).[14a] As mentioned by Cai and co-workers in 2006, the moderate activity can be the result of the formation of a typical cyclic structure (Scheme 1, compounds **2a,b**) that is not expected to interact effectively with the active site of SARS-CoV 3CL^{pro}.[16]

Herein, we report our results on improving the inhibitory activity of these compounds, by focusing on two strategies. First, keeping the trifluoromethylketone moiety in place, we investigated chemical modifications on the side chain of Glu or Gln residue at the P1 position, in order to block the formation of the cyclic structure (Scheme 1) and modulate the hydrogen bonding ability of this P1 position toward the active site, as well as modifying the amino acid residues at the P2 and P3 positions. Second, we investigated a replacement of the chemical warhead of the inhibitor, that is, the trifluoromethyl unit, by other moieties such as electron-withdrawing thiazolyl and benzothiazolyl groups. We believe that this modification would be valuable for enhancing the reactivity of the covalent-adduct formation to the active site cysteine residue in SARS-CoV 3CL^{pro}.

From a synthetic point of view, the preparation of the target compounds was envisioned following the synthetic routes illustrated in Schemes 2–4. Compounds **8a–e** were prepared from Cbz-L-Glu-OH (**3**) that was converted to the corresponding oxazolidinone acid **4** under the conditions described by Moore et al.[17] Amides **5a–d** were next prepared by coupling compound **4** with four kinds of amines using a standard HOBt–EDC·HCl coupling method for peptides, resulting in excellent yields. Compounds **5a–d** were then converted in a one-pot reaction to the correspond-

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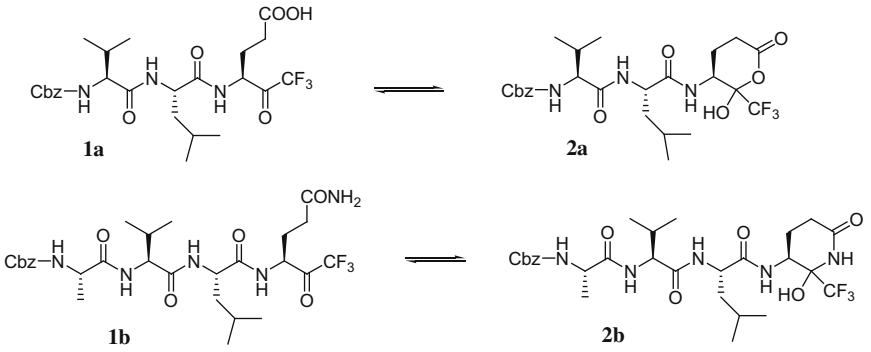
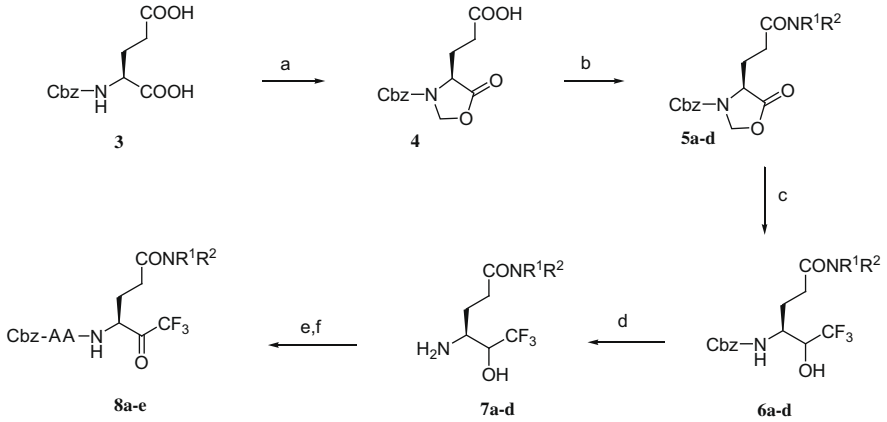
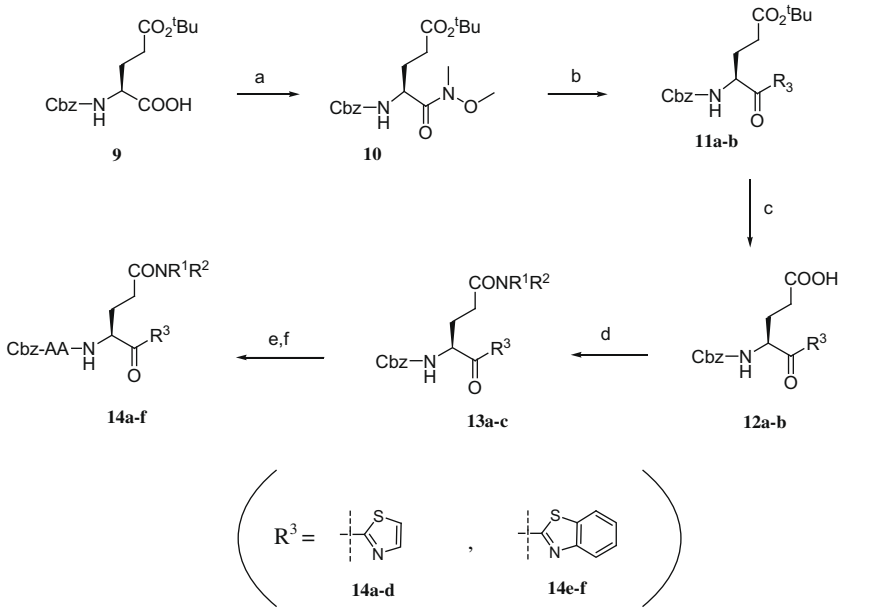


Scheme 1. Previously reported trifluoromethyl ketone-containing peptides and their corresponding cyclic non-active counterparts.



Scheme 2. Reagents and conditions: (a) paraformaldehyde, *p*-TsOH·H_2O, toluene, reflux, 2 h, 98%; (b) HNR^1R^2, HOBt, EDC·HCl, DMF, 0 °C–rt, overnight, 80–98%; (c) CsF, $CF_3Si(CH_3)_3$, THF, sonication, rt, 3 h then MeOH, rt, 30 min then $NaBH_4$, rt, overnight, 48–61%; (d) H_2, Pd/C (10%), MeOH, rt, overnight, 100%; (e) Cbz-AA-OH, HOBt, EDC·HCl, DMF, 0 °C–rt, overnight; (f) Dess–Martin periodinane, CH_2Cl_2, rt, 16 h, EtOAc then filtration through Celite followed by HPLC purification.



Scheme 3. Reagents and conditions: (a) *N*,*O*-dimethylhydroxylamine hydrochloride, EDC·HCl, HOBt, TEA, DMF, rt, 12 h, 90%; (b) thiazole or benzothiazole, *n*-BuLi, −78 °C, 2.5 h, 70%; (c) formic acid, rt, 12 h, 100%; (d) HNR^1R^2, EDC·HCl, HOBt, DMF, rt, 12 h, 90%; (e) triflic acid, DCM, rt, 5 min, 100% (f) Cbz-AA-OH, HOBt, EDC·HCl, DMF, rt, 12 h followed by HPLC purification.

ing trifluoromethylalcohols **6a–d**, whose Cbz group was de-protected after silica gel column chromatography, and the amino function in the resultant compounds **7a–d** was coupled to the appropriate peptide fragments.[14] The peptide fragments were synthesized according to known procedures.[14,18] Finally, the resulting peptides were directly engaged in the last oxidation step affording