Potent inhibitor of the human rhinovirus (HRV) 3C protease containing a backbone modified glutamine

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Based on the common cleavage site (between glutamine and glycine) of the human rhinovirus 3C protease, a series of potent inhibitors of this serine protease containing a backbone modified glutamine has been synthesized.

Human rhinoviruses (HRVs) belong to the picornavirus family and are causative agents of the common cold in humans.[1] HRVs translate their RNA genome directly into a large viral polyprotein precursor which must undergo a series of controlled proteolyses to generate the functional viral gene products.[2] The existence of virus-specific proteases such as the 3C protease, with no known cellular homologues suggests that these enzymes may be attractive targets for antiviral chemotherapeutic agents. Recent analysis has suggested that although picornavirus proteases are functionally similar to thio proteases, they are structurally related to the trypsin-like family of serine proteases.[3] It was also found that the proteolytic cleavage by the 3C protease occurs predominantly between the amino acids glutamine and glycine[4] (see Fig. 1). We have designed and synthesized molecules containing a glutamine with hydrazine modified backbone[5] as in A (Fig. 1). A representative compound containing core **A** is compound **1**, which is a potent inhibitor of the HRV 3C protease (IC_{50} = 48 nmol dm^{-3}).

Coupling of *N*-benzyloxycarbonylphenylalanine with *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide (DCC) at 0 °C provided the active ester **2** (90%), reaction of which with hydrazine in methanol at room temperature gave compound **3** in 89% yield. Michael addition of the hydrazide **3** to acrylonitrile was accomplished in refluxing ethanol over 24 h and provided the Michael addition product **4** in 66% yield. The nitrile group in compound **4** was hydrolysed to the amido group in **5** using 30% hydrogen peroxide[6] in dimethyl sulfoxide and potassium carbonate as the base. Acylation of compound **5** with bromoacetyl chloride in THF provided the final product **1** in 32% yield.

In summary, compound **1**, which is a low molecular weight and potent inhibitor of the HRV 3C protease (IC_{50} = 48 nmol dm^{-3}), has been synthesized efficiently in five steps. Compound **1** is a time-dependent irreversible inhibitor with a K_{inact}/K_i value of > 2500 mol^{-1} dm^3 s^{-1}.† Compound **1** also has definite antiviral activity in a cell-culture assay against rhinovirus type 1B in MRC-5 cell.[7]

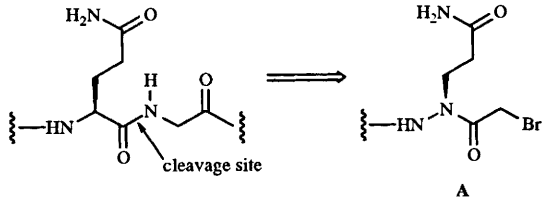
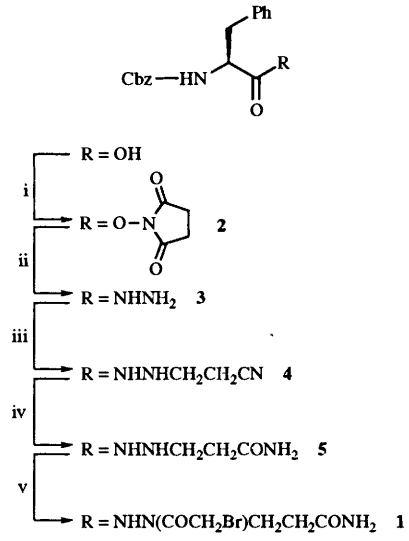


Fig. 1 The dipeptide cleavage sequence pair Gln-Gly of HRV 3C protease and the hydrazine modified backbone replacement



Scheme 1 *Reagents:* i, *N*-hydroxysuccinimide–DCC; ii, NH_2NH_2·HCl–triethylamine; iii, acrylonitrile–EtOH; iv, 30% H_2O_2–K_2CO_3–DMSO; v, bromoacetyl chloride

Experimental

Synthesis of the hydrazide 3

To a solution of hydrazine hydrochloride (0.556 g, 8.1 mmol) and triethylamine (1.3 cm^3, 9.6 mmol) in methanol (50 cm^3) was added the active ester **2** (3.0 g, 8.1 mmol). After 30 min, solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate (100 cm^3) and the solution washed successively with 10% aqueous potassium carbonate and saturated brine. It was then dried (Na_2SO_4) and concentrated under reduced pressure and the residue recrystallized (ethyl acetate–hexane) to give compound **3** (2.27 g, 89%) (Found: C, 65.2; H, 6.2; N, 13.6. $C_{17}H_{19}N_3O_3$ requires C, 65.16; H, 6.11; N, 13.41%); δ(300 MHz; $CDCl_3$) 3.05 (O, 2 H, d, *J* 7.5),‡ 3.80 (2 H, br s), 4.40 (1 H, q, *J* 7.5), 5.08 (2 H, ABq, *J* 7.5), 5.45 (1 H, br d) and 7.12–7.40 (10 H, m).

Hydrolysis of the nitrile 4

To a solution of **4** (720 mg, 1.96 mmol) in dimethyl sulfoxide (13 cm^3) was added potassium carbonate (140 mg) and 30% hydrogen peroxide (0.446 cm^3). After 7 h at 50 °C, the mixture was evaporated under reduced pressure and the crude product

† Details of the antiviral activity will be described elsewhere.

‡ *J* Values in Hz.

was purified by silica gel column chromatography (10% MeOH–CH_2Cl_2) to provide the product **5** (195 mg, 25%) (Found: C, 62.4; H, 6.2; N, 14.6. $C_{20}H_{24}N_4O_4$ requires C, 62.49; H, 6.29; N, 14.57%); δ(300 MHz; $CDCl_3$) 2.20 (2 H, m), 3.30 (4 H, m), 4.35 (1 H, q, *J* 7.5), 5.07 (2 H, s), 5.30 (1 H, br s), 5.40 (1 H, br s), 6.55 (1 H, br s) and 7.15–7.40 (10 H, m).

Synthesis of compound 1

To a solution of compound **5** (75 mg, 0.195 mmol) in tetrahydrofuran (4 cm^3) at 0 °C was added triethylamine (0.39 mmol) and bromoacetyl chloride (0.29 mmol). After removal of the cooling bath, the solution was stirred at room temperature for 30 min and then evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (5% MeOH–CH_2Cl_2) to provide compound **1** (31 mg, 32%) (Found: C, 52.4; H, 5.0; N, 11.1. $C_{22}H_{25}BrN_4O_5$ requires C, 52.28; H, 4.98; N, 11.08%); δ(300 MHz; [$^2H_6$]-DMSO) 2.20 (2 H, m), 2.95 (2 H, m), 3.65 (2 H, m), 3.90 (2 H, m), 4.20 (1 H, m), 5.00 (2 H, m), 6.85 (1 H, br s) 7.20–7.30 (10 H, m) and 7.86 (1 H, br d).

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