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ORIGINAL ARTICLE

Pseudo-peptides derived from isomannide: inhibitors of serine proteases

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Abstract In this paper, we describe the synthesis of a novel class of pseudo-peptides derived from isomannide and several oxazolones as potential inhibitors of serine proteases as well as preliminary pharmacological assays for hepatitis C. Hepatitis C, dengue and West Nile fever are among the most important flaviviruses that share one important serine protease enzyme. Serine proteases belong to the most studied class of proteolytic enzymes and are a primary target in the drug development field. Several pseudo-peptides were obtained in good yields from the reaction of isomannide and oxazolones, and their anti-HCV potential using the HCV replicon-based assay was shown.

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Introduction

The family Flaviviridae comprises more than 60 viruses, many of which are important human pathogens. Among the most important flaviviruses are the *Hepatitis C virus* (HCV), the *West Nile virus* (WN) and the *Dengue virus*. Chronic HCV infection is associated with liver cirrhosis and hepatocellular carcinoma (Bruix et al. 1989). Current therapeutic based on alpha interferon and the nucleoside analog ribavirin is only partially effective and is limited by the adverse effects of both agents (Wright et al. 2001). *Dengue virus* causes dengue fever and dengue hemorrhagic fever in millions of people each year in tropical and subtropical regions of the world. Currently, there is no vaccine or effective antiviral therapy for the four known serologically related virus types (dengue 1–4) (Yusof et al. 2000).

All flaviviruses have a positive-sense nonsegmented RNA genome that encodes a single long polyprotein processed to yield three structural proteins (C, prM and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Leung et al. 2001). A single virus-encoded protease comprising 180 amino acids of NS3 (NS3pro) is responsible for the cleavage of both in *cis* and in *trans*, which generates viral proteins that are essential for viral replication and maturation of infectious virions. The presence of a trypsin-like serine protease within the N-terminal one-third of the flavivirus NS3 protein was first proposed by (Bazan and Fletterick (1989, 1990) and (Gorbalenya et al. (1989). Their analysis of virus sequence alignments revealed that structural motifs as well as the characteristic catalytic triad (His^{51}, Asp^{75}, and Ser^{135}) of

mammalian serine proteases were conserved in all flaviviruses. As NS3pro activity is essential for viral replication, it represents a suitable target for the development of chemotherapeutic approaches for the treatment of flaviviruses. As part of our antiviral program for flaviviruses, we describe in this paper the synthesis and preliminary pharmacological assays of a series of pseudo-peptides derived from isomannide, designed as potential inhibitors of the catalytic triad of serine proteases and as analogs of the compounds previously published by us (Muri et al. 2004, 2005, 2006).

Materials and methods

General: chemistry

All solvents were purchased as reagent grade, dried, using standard conditions and stored over molecular sieves. Purification of products was carried out using silica gel flash chromatography (Whatman 60, 230–400 mesh). Routine NMR analyses were carried out on a Varian Unity Plus-300 spectrometer. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and are uncorrected. High-resolution mass spectra (HRMS) were performed on a Waters Micromass Q-Tof Micro mass spectrometer equipped with a lock spray source. The IR spectra were obtained on a Perkin-Elmer spectrometer model Spectrum One in liquid film and KBr pellets. The alpha-D measurements were done on a Perkin-Elmer 341 LC polarimeter.

1,4:3,6-Dianhydro-2,5-di-*O*-*p*-tosyl-D-mannitol (**2**). A solution of *p*-toluenesulphonyl chloride (27.36 mmol, 5.2 g) in pyridine (40 mL) was added dropwise to a solution of isomannide (13.68 mmol, 2.0 g) in dry pyridine (24 mL) and stirred at r.t overnight. The mixture was cooled and poured on ice-cold 2 N HCl. The product was extracted with ethyl acetate, dried and filtered. The crude product was recrystallized from MeOH to give a product of white solid with 90% yield. ^{1}H NMR δ ($CDCl_3$, 300 MHz): 7.80 (d, 4H, $J = 8.1$ Hz), 7.33 (d, 4H, $J = 8.1$ Hz), 4.90–4.75 (m, 2H), 4.55–4.45 (m, 2H), 3.91 (dd, 2H, $J = 6.6$, 9.3 Hz), 3.72 (dd, 2H, $J = 7.5$, 9.3 Hz), 2.44 (s, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz): 145.2 (–C), 132.9 (–C), 129.8 (–CH), 127.8 (–CH), 79.8 (–CH), 77.8 (–CH), 70.0 ($–CH_2$), 21.6 ($–CH_3$). IR (KBr) ν cm^{-1}: 3,444, 3,064, 2,956, 2,938, 2,880, 1,596, 1,371, 1,191, 1,173, 1,138, 1,113, 1,075, 1,037, 916, 881,789, 720, 669. $\alpha_D^{20} = +96$ (c, 0.1) DMSO, mp 93–94°C.

1,4:3,6-Dianhydro-2,5-diazido-2,5-dideoxy-L-iditol (**3**). NaN_3 (30.84 mmol, 2.0 g) was added to a solution of ditosylate **2** (7.71 mmol, 3.5 g) in $[bmim]^+[BF_4]^-$ (46.2 mmol, 9.3 mL), and the mixture was stirred overnight at 120°C. The mixture was cooled, water was added and the product extracted with diethyl ether. The organic layer was dried and evaporated. The crude diazide was purified by column chromatography to give a product of pale yellow liquid in 78% yield. ^{1}H NMR δ ($CDCl_3$, 300 MHz): 4.62 (s, 2H), 4.06 (dd, 2H, $J = 3.9$, 1.8 Hz), 3.98–3.60 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz): 85.8 (–CH), 71.6 ($–CH_2$), 65.5 (–CH). IR (KBr) ν cm^{-1}: 3,340, 2,953, 2,883, 2,503, 2,105, 1,471, 1,258, 1,094, 953, 914, 846. $\alpha_D^{20} = +94$ (c, 0.1) DMSO.

1,4:3,6-Dianhydro-2,5-diamino-2,5-dideoxy- L-iditol (**4**). A mixture of diazide (1.27 mmol, 0.25 g) and 10% Pd/C (0.127 mmol, 0.140 g) in EtOH (10 mL) was hydrogenated at 40 psi. After 12 h, the catalyst was filtered off and the solvent was evaporated giving a product of a hygroscopic solid with 93% yield. ^{1}HNMR δ ($CDCl_3$, 300 MHz): 5.41 (s, 2H), 4.82 (dd, 2H, $J = 9.3$, 4.8 Hz), 4.56 (dd, 2H, $J = 9.3$, 2.4 Hz), 4.34 (dd, 2H, $J = 4.5$, 2.4 Hz). ^{13} NMR ($CDCl_3$, 75 MHz): 89.9 (–CH); 75.5 ($–CH_2$); 59.2 (–CH). IR (KBr) ν cm^{-1}: 3,352, 2,952, 1,601, 1,470, 1,050. $\alpha_D^{20} = +27$ (c, 0.1) DMSO. HRMS calcd. for $C_6H_{13}N_2O_2$ 145.0977. Found 145.097.

General procedure for formation of the final products (28-48)

To a solution of amine (1.38 mmol, 0.2 g) in ethyl acetate (25 mL), was added the corresponding oxazolone (3.05 mmol). The reaction was refluxed for 24-48 h, after which the formed precipitate was filtered and washed with ethyl acetate.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(*Z*)-3,4-methylenedioxycinnamamido]-2,5-dideoxy-L-iditiol (**28**). ^{1}HNMR δ (DMSO, 300 MHz): 9.79 (s, 2H), 8.28 (d, 2H, $J = 7.2$ Hz), 7.98 (d, 4H, $J = 7.2$ Hz), 7.61–7.47 (m, 6H), 7.15 (d, 2H, $J = 1.8$ Hz), 7.10–7.05 (m, 4H), 6.91 (d, 2H, $J = 8.1$ Hz), 5.99 (s, 4H), 4.55 (s, 2H), 4.30–4.20 (m, 2H), 4.03–3.93 (m, 2H), 3.70 (dd, 2H, $J = 9.3$, 3.3 Hz). ^{13}CNMR (DMSO, 75 MHz): 165.8 (–C), 165.5 (–C), 147.7 (–C), 147.4 (–C), 133.7 (–C), 131.8 (–CH), 129.0 (–CH), 128.4 (–CH), 128.3 (–C), 127.9 (–CH), 125.0 (–CH), 108.5 (–CH), 108.5 (–CH), 108.4 (–CH), 101.4 (–C), 86.7 (–CH), 71.3 ($–CH_2$), 56.7 (–CH). IR (KBr) ν cm^{-1}: 3,233, 3,059, 2,912, 1,633, 1,580, 1,556, 1,504, 1,481, 1,447, 1,353, 1,280, 1,241, 1,035, 692. $\alpha_D^{20} = +77$ (c, 0,1) DMSO, mp 246–247°C. HRMS calcd. for $C_{40}H_{35}N_4O_{10}$ 731.2275. Found 731.3270. The product is a pale yellow solid; 70% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(*Z*)-cinnamamido]-2,5-dideoxy-L-iditiol (**29**). ^{1}HNMR δ (DMSO, 300 MHz): 9.90 (s, 2H), 8.40 (d, 2H, $J = 7.2$ Hz), 7.98 (d, 4H, $J = 6.9$ Hz), 7.60–7.48 (m, 6H), 7.40–7.20 (m, 10H), 7.11 (s, 2H), 4.59 (s, 2H), 4.30–3.90 (m, 2H), 3.98 (dd, 2H, $J = 9.3$, 6.0 Hz), 7.73 (dd, 2H, $J = 9.3$, 3.3 Hz). ^{13}CNMR

(DMSO, 75 MHz): 166.0 (–C), 165.5 (–C), 134.3 (–C), 133.7 (–C), 131.8 (–CH), 130.2 (–C), 129.,4 (–CH), 128.6 (–CH), 128.5 (–CH), 128.4 (–CH), 128.3 (–CH), 127.9 (–CH), 86.7 (–CH), 71.4 ($–CH_2$), 56.7 (–CH). IR (KBr) ν cm^{-1}: 3,270, 3,058, 2,950, 1,649, 1,579, 1,514, 1,477, 1,280, 1,206, 1,080, 692. $\alpha_D^{20} = +72$ (*c*, 0,1) DMSO, mp 169°C. HRMS calcd. for $C_{38}H_{35}N_4O_6$ 643.2478. Found 643.3465. The product is a pale yellow solid; 40% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(*Z*)-4-methoxycinnamamido]-2,5-dideoxy-L-iditiol (**30**). ^{1}HNMR δ (DMSO, 300 MHz): 9.80 (s, 2H), 8.27 (d, 2H, $J =$ 7.2 Hz), 7.99 (d, 4H, $J = 6.9$ Hz), 7.65–7.40 (m, 10H), 7.12 (s, 2H), 6.91 (d, 4H, $J = 8.7$ Hz), 4.57 (s, 2H), 4.30–4.20 (m, 2H), 3.96 (dd, 2H, $J = 9.3$, 5.7 Hz), 3.73 (s, 6H, $-CH_3$), 3.80-3.60 (m, 2H). ^{13}CNMR (DMSO, 75 MHz): 165.9 (–C), 165.7 (–C), 159.7 (–C), 133.8 (–C), 131.7 (–CH), 131.2 (–CH), 128.9 (–CH), 128.4 (–CH), 128.0 (–C), 127.9 (–CH), 126.7 (–C), 114,1 (–CH), 86.8 (–CH), 71.4 ($–CH_2$), 56.7 (–CH), 55.3 ($–CH_3$). IR (KBr) ν cm^{-1}: 3,274, 3,061, 2,962, 2,838, 1,650, 1,604, 1,578, 1,512, 1,477, 1,300, 1,255, 1,177, 1,080, 1,028, 829, 707. $\alpha_D^{20} = +86$ (*c*, 0,1) DMSO, mp 167–168°C. HRMS calcd. for $C_{40}H_{39}N_4O_8$ 703.2689. Found 703.3768. The product is a pale yellow solid; 46% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(*Z*)-3,4-dimethoxycinnamamido]-2,5-dideoxy-L-iditiol (**31**). ^{1}HNMR δ (DMSO, 300 MHz): 9.80 (s, 2H), 8.26 (d, 2H, $J = 6.9$ Hz), 8.03 (d, 4H, $J = 7.2$ Hz), 7.61–7.40 (m, 6H), 7.23–7.10 (m, 6H), 6.94 (d, 2H, $J = 8.4$ Hz), 4.57 (s, 2H), 4.30–4.20 (m, 2H), 4.10–3.90 (m, 4H), 3.73 (s, 6H, $-CH_3$), 3.50 (s, 6H, $-CH_3$). ^{13}CNMR (DMSO, 75 MHz): 165.7 (–C), 165.4 (–C), 149.3 (–C), 148.2 (–C), 133.5 (–C), 131.6 (–CH), 129.4 (–CH), 128.2 (–CH), 127.7 (–CH), 127.6 (–C), 126.7 (–C), 123.4 (–CH), 112.2 (–CH), 111.4 (–CH), 86.6 (–CH), 71.1 ($–CH_2$), 56.5 (–CH), 55.4 ($–CH_3$), 55.0 ($–CH_3$). IR (KBr) ν cm^{-1}: 3,271, 3,060, 2,960, 2,838, 1,648, 1,601, 1,579, 1,515, 1,478, 1,332, 1,263, 1,163, 1,143, 1,082, 1,023, 808, 716, 621. $\alpha_D^{20} = +72$ (*c*, 0,1) DMSO, mp 159–160°C. HRMS calcd. for $C_{42}H_{43}N_4O_{10}$ 763.2901. Found 763.4067. The product is a pale yellow solid; 55% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(*Z*)-4-fluorocinnamamido]-2,5-dideoxy-L-iditiol (**32**). ^{1}HNMR δ (DMSO, 300 MHz): 9.86 (s, 2H), 8.33 (d, 2H, $J = 6.6$ Hz), 7.98 (d, 4H, $J = 7.5$ Hz), 7.70–7.40 (m, 10H), 7.19 (t, 4H, $J = 9.0$ Hz), 7.11 (s, 2H), 4.59 (s, 2H), 4.30–4.20 (m, 2H), 3.98 (dd, 2H, $J = 9.3$, 5.7 Hz), 3.73 (dd, 2H, $J = 9.3$, 36 Hz). ^{13}CNMR (DMSO, 75 MHz): 165.2 (–C), 163.3 (–C), 160.0 (–C), 133.5 (–C), 131.3 (–C), 131.2 (–C), 130.7 (–CH), 129.8 (–CH), 128.2 (–CH), 127.7 (–CH), 115.4 (–CH), 115.1 (–CH), 86.5 (–CH), 71.1 ($–CH_2$), 56.5 (–CH). IR (KBr) ν cm^{-1}: 3,245, 3,060, 2,950, 1,646, 1,601, 1,580, 1,508, 1,476, 1,366, 1,281, 1,230, 1,159, 1,075, 1,050, 832, 700. $\alpha_D^{20} = +65$ (*c*, 0,1) DMSO, mp 152–153°C. HRMS calcd. for $C_{38}H_{33}F_2N_4O_6$ 679,2289. Found 679.3388. The product is a pale yellow solid; 37% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(*Z*)-4-chlorocinnamamido]-2,5-dideoxy-L-iditiol (**33**). ^{1}HNMR δ (DMSO, 300 MHz): 9.90 (s, 2H), 8.44 (d, 2H, $J = 6.9$ Hz), 7.97 (d, 4H, $J = 7.2$ Hz), 7.65-7.45 (m, 10H), 7.41 (d, 4H, $J = 8.7$ Hz), 7.06 (s, 2H), 4.59 (s, 2H), 4.28–4.20 (m, 2H), 3.98 (dd, 2H, $J = 9.3$, 5.7 Hz), 3.73 (dd, 2H, $J = 9.3$, 3.3 Hz). ^{13}CNMR (DMSO, 75 MHz): 165.6 (–C), 165.2 (–C), 133.4 (–C), 133.1 (–C), 132.7 (–C), 131.6 (–CH), 130.8 (–CH), 130.7 (–C), 128.3 (–CH), 128.2 (–CH), 127.7 (–CH), 126.6 (–CH), 86.5 (–CH), 71.1 ($–CH_2$), 56.5 (–CH). IR (KBr) ν cm^{-1}: 3,271, 3,061, 2,970, 1,651, 1,580, 1,514, 1,477, 1,280, 1,091, 907, 820, 709. $\alpha_D^{20} = +89$ (*c*, 0,1) DMSO, mp 166–169°C. HRMS calcd. for $C_{38}H_{33}Cl_2N_4O_6$ 711.1699. Found 711.2859. The product is a pale yellow solid; 35% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(*Z*)-4-bromocinnamamido]-2,5-dideoxy-L-iditiol (**34**). ^{1}HNMR δ (DMSO, 300 MHz): 9.89 (s, 2H), 8.44 (d, 2H, $J = 6.9$ Hz), 7.96 (d, 4H. $J = 7.5$ Hz), 7.62-7.40 (m, 14H), 7.04 (s, 2H), 4.59 (s, 2H), 4.30–4.20 (m, 2H), 4.00–3.95 (m, 2H), 3.73 (dd, 2H, $J = 9.0$, 3.0 Hz). ^{13}CNMR (DMSO, 75 MHz): 165.6 (–C), 165.1 (–C), 133.5 (–C), 133.4 (–C), 131.6 (–CH), 131.2 (–CH), 131.0 (–CH), 130.8 (–C), 128.2 (–CH), 127.7 (–CH), 126.6 (–CH), 121.4 (–C), 86.4 (–CH), 71.1 ($–CH_2$), 56.5 (–CH). IR (KBr) ν cm^{-1}: 3,266, 3,061, 2,973, 2,882, 1,650, 1,580, 1,513, 1,476, 1,280, 1,074, 1,009, 906, 816, 710. $\alpha_D^{20} = +80$ (*c*, 0,1) DMSO, mp 172–174°C. HRMS calcd. for $C_{38}H_{33}Br_2N_4O_6$ 799.0689. Found 801.1971. The product is a pale yellow solid; 40% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(*Z*)-4-trifluoromethylcinnamamido]-2,5-dideoxy-L-iditiol (**35**). ^{1}HNMR δ (DMSO, 300 MHz): 9.99 (s, 2H), 8.54 (d, 2H, $J = 6.6$ Hz), 7.97 (d, 4H, $J = 7.2$ Hz), 7.77–7.60 (m, 8H), 7.63–7.56 (m, 2H), 7.50–7.47 (m, 4H), 7.09 (s, 2H), 4.62 (s, 2H), 4.29-4.21 (m, 2H), 4.02–3.96 (m, 2H), 3.75 (dd, 2H, $J = 9.3$, 3.0 Hz). ^{13}CNMR (DMSO, 75 MHz): 165.7 (–C), 165.0 (–C), 138.5 (–C), 133.3 (–C), 132.3 (–C), 131.6 (–CH), 129.6 (–CH), 128.2 (–CH), 127.7 (–CH), 125.7 (–CH), 125.1 (–CH), 112.6 (–C), 110.2 (–C), 86.4 (–CH), 71.1 ($–CH_2$), 56.5 (–C). IR (KBr) ν cm^{-1}: 3,274, 3,065, 1,646, 1,580, 1,519, 1,477, 1,324, 1,281, 1,168, 1,126, 1,069, 830, 708. $\alpha_D^{20} = +53$ (*c*, 0,1) DMSO, mp 164–166°C. HRMS calcd. for $C_{40}H_{33}F_6N_4O_6$ 779.2226. Found 779.3478. The product is a pale yellow solid; 40% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(*Z*)-2-thiophenylacrylamido]-2,5-dideoxy-L-iditiol (**36**). ^{1}HNMR δ (DMSO, 300 MHz): 9.74 (s, 2H), 8.28 (d, 2H, $J = 6.9$ Hz), 8.06 (d, 2H, $J = 6.9$ Hz), 7.64-7.51 (m, 12H), 7.42 (d, 2H, $J = 2.7$ Hz), 7.09 (dd, 2H, $J = 4.8$, 3.6 Hz), 4.55 (s, 2H),

4.27–4.22 (m, 2H), 3.96 (dd, 2H, $J = 9.0, 5.7$ Hz), 3.70 (dd, 2H, $J = 9.0$, 3.3 Hz). ^{13}CNMR (DMSO, 75 MHz): 165.9 (–C), 164.5 (–C), 136.6 (–C), 133.7 (–C), 131.9 (–CH), 131.5 (–CH), 129.9 (–CH), 128.1 (–CH), 127.8 (–CH), 126.8 (–CH), 126.6 (–C), 125.0 (–CH), 86.6 (–CH), 71.1 (–CH_2), 56.4 (–CH). IR (KBr) ν cm^{-1}: 3,415, 3,262, 3,066, 2,965, 1,648, 1,517, 1,475, 1,282, 1,077, 1,052, 906, 706. $\alpha_D^{20} = + + 81$ (*c*, 0,1) DMSO, mp 194–196°C. HRMS calcd. for $C_{34}H_{31}N_4O_6S_2$ 655.1685. Found 655.1697. The product is a pale yellow solid; 55% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-benzamido-(Z)-3-pyridyl-acrylamido]-2,5-dideoxy-L-iditiol (**37**). ^{1}HNMR δ (DMSO, 300 MHz): 9.98 (s, 2H), 8.70 (d, 2H, $J = 1.8$ Hz), 8.50 (d, 2H, $J = 6.9$ Hz), 8.44 (dd, 2H, $J = 4.8$, 1.5 Hz), 7.98–7.91 (m, 6H), 7.61–7.48 (m, 6H), 7.37 (dd, 2H, $J = 8.1$, 5.1 Hz), 7.10 (s, 2H), 4.60 (s, 2H), 4.30–4.20 (m, 2H), 3.99 (dd, 2H, $J = 9.3$, 5.7 Hz), 3.74 (dd, 2H, $J = 9.3$, 3.3 Hz). ^{13}CNMR (DMSO, 75 MHz): 165.9 (–C), 165.7 (–C), 159.7 (–C), 133.8 (–C), 131.7 (–CH), 131.2 (–CH), 128.9 (–CH), 128.4 (–CH), 128.0 (–C), 127.9 (–CH), 126.7 (–C), 114.1 (–CH), 86.8 (–CH), 71.4 (–CH_2), 56.7 (–CH), 55.3 (–CH_3). IR (KBr) ν cm^{-1}: 3,404, 3,275, 3,065, 2,967, 1,645, 1,539, 1,515, 1,476, 1,283, 1,193, 1,083, 1,038, 908, 707. $\alpha_D^{20} = + 64$ (*c*, 0,1) DMSO, mp 167°C. HRMS calcd. for $C_{26}H_{33}N_6O_6$ 645.2462. Found 645.2468. The product is a pale yellow solid; 45% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-3,4-methylenedioxycinnamamido]-2,5-dideoxy-L-iditiol (**38**). ^{1}HNMR δ (DMSO, 300 MHz): 9.33 (s, 2H), 8.17 (d, 1H, $J =$ 6.9 Hz), 7.20–6.90 (m, 6H), 6.87 (s, 2H), 6.04 (s, 4H), 4.54 (s, 2H), 4.20–4.10 (m, 2H), 4.00–3.90 (m, 2H), 3.71–3.60 (m, 2H), 1.97 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.1 (–C), 165.3 (–C), 147.3 (–C), 147.2 (–C), 128.2 (–C), 128.0 (–C), 127.1 (–CH), 124.6 (–CH), 108.4 (–CH), 108.2 (–CH), 101.1 (–CH_2), 86.5 (–CH), 71.1 (–CH_2), 56.4 (–CH), 22.7 (–CH_3). IR (KBr) ν cm^{-1}: 3,306, 3,244, 2,982, 2,890, 1,650, 1,620, 1,532, 1,502, 1,480, 1,448, 1,373, 1,351, 1,255, 1,090, 1,071, 1,038, 929, 813, 723. $\alpha_D^{20} = + 37$ (*c*, 0,1) DMSO, mp 170–173°C. HRMS calcd. for $C_{30}H_{31}N_4O_{10}$ 607.1962. Found 607.2885. The product is a pale yellow solid; 70% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-cinnamamido]-2,5-dideoxy-L-iditiol (**39**). ^{1}HNMR δ (DMSO, 300 MHz): 9.41 (s, 2H), 8.25 (d, 2H, $J = 6.9$ Hz), 7.53 (d, 4H, $J = 7.2$ Hz), 7.42–7.20 (m, 6H), 6.88 (s, 2H), 4.57 (s, 2H), 4.23–4.16 (m, 2H), 3.96 (dd, 2H, $J = 9.3$, 5.4 Hz), 3.71 (dd, 2H, $J = 9.3$, 3.6 Hz), 1.98 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.2 (–C), 165.3 (–C), 134.1 (–C), 130.1 (–C), 129.2 (–CH), 128.4 (–CH), 128.3 (–CH), 126.5 (–CH), 86.5 (–CH), 71.2 (–CH_2), 56.4 (–CH), 22.7 (–CH_3). IR (KBr) ν cm^{-1}: 3,480, 3258, 3,056, 2,975, 2,870, 1,651, 1,537, 1,489, 1,446, 1,373, 1,287, 1,208, 1,088, 1,042, 932. $\alpha_D^{20} = + 71$ (*c*, 0,1) DMSO, mp 191–192°C. HRMS calcd. for $C_{28}H_{31}N_4O_6$ 519.2244. Found 519.2244. The product is a pale yellow solid; 95% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-4-chlorocinnamamido]-2,5-dideoxy-L-iditiol (**40**). ^{1}HNMR δ (DMSO, 300 MHz): 9.44 (s, 2H), 8.31 (d, 2H, $J = 7.2$ Hz), 7.56–7.42 (m, 8H), 6.84 (s, 2H), 4.56 (s, 2H), 4.22–4.15 (m, 2H), 3.95 (dd, 2H, $J = 9.3$, 6.0 Hz), 3.70 (dd, 2H, $J = 9.3$, 3.3 Hz), 1.97 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.1 (–C), 165.2 (–C), 133.1 (–C), 132.6 (–C), 130.8 (–CH), 130.7 (–C), 128.3 (–CH), 124.9 (–CH), 86.4 (–C), 71.1 (–CH_2), 56.5 (–C), 22.7 (–CH_3). IR (KBr) ν cm^{-1}: 3,468, 3,273, 2,977, 2,876, 1,650, 1,622, 1,537, 1,488, 1,371, 1,311, 1,280, 1,090, 1,041, 1,012, 822, 739. $\alpha_D^{20} = + 85$ (*c*, 0,1) DMSO, mp 168–170°C. HRMS calcd. for $C_{28}H_{28}N_4O_6NaCl_2$ 609.1284. Found 609.1282. The product is a pale yellow solid; 96% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-4-bromocinnamamido]-2,5-dideoxy-L-iditiol (**41**). ^{1}HNMR δ (DMSO, 300 MHz): 9.40 (s, 2H), 8.28 (d, 2H, $J = 6.6$ Hz), 7.60–7.55 (m, 4H), 7.50–7.44 (m, 4H), 6.82 (s, 2H), 4.56 (s, 2H), 4.22–4.16 (m, 2H), 3.95 (dd, 2H, $J = 9.3$, 6.0 Hz), 3.70 (dd, 2H, $J = 9.3$, 3.3 Hz), 1.97 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.0 (–C), 165.1 (–C), 133.4 (–C), 131.2 (–CH), 131.0 (–CH), 130.8 (–C), 124.8 (–CH), 121.2 (–C), 86.4 (–CH), 71.1 (–CH_2), 56.4 (–C), 22.5 (–CH_3). IR (KBr) ν cm^{-1}: 3,275, 2,974, 2,878, 1,651, 1,587, 1,537, 1,485, 1,371, 1,311, 1,278, 1,075, 1,042, 1,009, 816, 706. $\alpha_D^{20} = + 81$ (*c*, 0,1) DMSO, mp 175°C. HRMS calcd. for $C_{28}H_{28}N_4O_6NaBr_2$ 697.0273. Found 697.0306. The product is a pale yellow solid; 58% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-4-trifluoromethylcinnamamido]-2,5-dideoxy-L-iditiol (**42**). ^{1}HNMR δ (DMSO, 300 MHz): 9.52 (s, 2H), 8.38 (d, 2H, $J = 6.6$ Hz), 7.80–7.67 (m, 8H), 6.87 (s, 2H), 4.58 (s, 2H), 4.24–4.18 (m, 2H), 3.97 (dd, 2H, $J = 9.3$, 5.7 Hz), 3.72 (dd, 2H, $J =$ 9.3, 3.3 Hz), 1.98 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.1 (–C), 165.0 (–C), 138.4 (–C), 132.3 (–C), 129.6 (–CH), 125.8 (–C), 125.1 (–CH), 123.9 (–CH), 122.2 (–C), 86.4 (–CH), 71.1 (–CH_2), 56.5 (–CH), 22.7 (–CH_3). IR (KBr) ν cm^{-1}: 3,467, 3,283, 2,986, 2,883, 1,628, 1,538, 1,499, 1,374, 1,325, 1,283, 1,164, 1,125, 1,089, 1,069, 839, 729. $\alpha_D^{20} = + 51$ (*c*, 0,1) DMSO, mp: 209–210°C. HRMS calcd. for $C_{30}H_{28}N_4O_6NaF_6$ 677.1811. Found 677.1792. The product is a pale yellow solid; 50% yield.

1,4:3,6-Dianhydro-2,5-bis-n[2-acetamido-(*Z*)-4-cyanocinnamamido]-2,5-dideoxy-L-iditiol (**43**). ^{1}HNMR δ (DMSO, 300 MHz): 9.57 (s, 2H), 8.40 (d, 2H, $J = 6.6$ Hz), 7.82 (d, 4H, $J = 8.4$ Hz), 7.67 (d, 4H, $J = 8.4$ Hz), 6.83 (s, 2H), 4.58 (s, 2H), 4.23–4.17 (m, 2H), 3.96 (dd, 2H, $J = 9.3$, 5.7 Hz), 3.72 (dd, 2H, $J = 9.3$, 3.0 Hz), 1.98 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.0 (–C), 164.9 (–C), 139.2 (–C), 132.7 (–C), 132.1 (–CH), 129.6 (–CH), 123.5 (–CH), 118.6 (–C), 110.0 (–C), 86.3 (–CH), 71.1 (–CH_2), 56.5

(–CH), 22.7 (–CH_3). IR (KBr) ν cm^{-1}: 3,243, 2,980, 2,890, 2,229, 1,659, 1,534, 1,488, 1,372, 1,316, 1,267, 1,092, 1,042, 1,007, 890, 834. $\alpha_D^{20} = +106$ (*c*, 0,1) DMSO, mp 204–206°C. HRMS calcd. for $C_{30}H_{28}N_6O_6Na$ 591.1968. Found 591.1971. The product is a pale yellow solid; 56% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-4-methylcinnamamido]-2,5-dideoxy-L-iditiol (**44**). ^{1}HNMR δ (DMSO, 300 MHz): 9.33 (s, 2H), 8.19 (d, 2H, $J = 6.6$ Hz), 7.42 (d, 4H, $J = 8.4$ Hz), 7.19 (d, 4H, $J = 8.1$ Hz), 6.88 (s, 2H), 4.56 (s, 2H), 4.14 (m, 2H), 3.95 (dd, 2H, $J = 9.3$, 5.7 Hz), 3.70 (dd, 2H, $J = 9.3$, 3.3 Hz), 2.30 (s, 6H), 1.98 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.1 (–C), 165.3 (–C), 137.9 (–C), 131.2 (–C), 129.3 (–C), 129.2 (–CH), 128.9 (CH), 126.8 (–CH), 86.4 (–CH), 71.1 (–CH_2), 56.4 (–CH), 22.7 (–CH_3), 20.7 (–CH_3). IR (KBr) ν cm^{-1}: 3,243, 2,976, 2,879, 1,651, 1,531, 1,371, 1,320, 1,286, 1,206, 1,185, 1,084, 1,043, 813, 709. $\alpha_D^{20} = +83$ (*c*, 0,1) DMSO, mp 174–175°C. HRMS calcd. for $C_{30}H_{34}N_4O_6Na$ 569.2373. Found 569.2361. The product is a pale yellow solid; 52% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-2-naphthylacrylamido]-2,5-dideoxy-L-iditiol (**45**). ^{1}HNMR δ (DMSO, 300 MHz): 9.38 (s, 2H), 8.03 (s, 2H), 7.92–7.84 (m, 8H), 7.77 (d, 2H, $J = 8.1$ Hz), 7.55-7.49 (m, 4H), 7.32 (s, 2H), 4.44 (s, 2H), 3.80 (dd, 2H, $J = 9.0$, 4.8 Hz), 3.60 (dd, 2H, $J = 9.0$, 4.8 Hz), 3.42 (dd, 2H, $J = 4.8$, 2.7 Hz); 2.01 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 167.4 (–C), 167.2 (–C), 132.6 (–C), 132.5 (–C), 132.4 (–C), 131.2 (–C), 129.0 (–CH), 128.0 (–CH), 127.3 (–CH), 127.2 (–CH), 126.4 (–CH), 126.1 (–CH), 86.8 (–CH), 72.5 (–CH_2), 56.6 (–C), 22.8 (–CH_3). IR (KBr) ν cm^{-1}: 3,253, 3,054, 2,982, 2,890, 1,650, 2,153, 1,660, 1,634, 1,525, 1,380, 1,322, 1,275, 1,149, 1,050, 1,015, 811, 746, 716. $\alpha_D^{20} = +10$ (*c*, 0,1) DMSO, mp 191–193°C. The product is a pale yellow solid; 86% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-2-thiophenylacrylamido]-2,5-dideoxy-L-iditiol (**46**). ^{1}HNMR δ (DMSO, 300 MHz): 9.22 (s, 2H), 8.17 (d, 2H, $J = 7.2$ Hz), 7.69 (d, 2H, $J = 6.3$ Hz), 7.50 (s, 2H), 7.41 (d, 2H, $J = 3.9$ Hz), 7.11 (dd, 2H, $J = 4.8$, 3.3 Hz), 4.54 (s, 2H), 4.24-4.18 (m, 2H), 3.95 (dd, 2H, $J = 9.3$, 6.0 Hz), 3.70 (dd, 2H, $J = 9.3$, 3.3 Hz); 2.05 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.7 (–C), 164.5 (–C), 136.6 (–C), 131.8 (–CH), 129.8 (–CH), 126.9 (–CH), 126.6 (–C), 124.4 (–CH), 86.6 (–CH), 71.1 (–CH_2), 56.5 (–CH), 23.3 (–CH_3). IR (KBr) ν cm^{-1}: 3,309, 3,220, 2,966, 2,885, 1,651, 1,614, 1,529, 1,423, 1,367, 1,278, 1,213, 1,085, 929, 904, 853, 717. $\alpha_D^{20} = +94$ (*c*, 0,1) DMSO, mp 222–224°C. HRMS calcd. for $C_{24}H_{27}N_4O_6S_2$ 531.1372. Found 531.1385. The product is a white solid; 70% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-3,4-dimethoxycinnamamido]-2,5-dideoxy-L-iditiol (**47**). ^{1}HNMR δ (DMSO, 300 MHz): 9.33 (s, 2H), 8.13 (d, 2H, $J = 7.2$ Hz), 7.20 (d, 2H, $J = 1.5$ Hz), 7.10 (dd, 2H, $J = 8.1$, 1.5 Hz), 6.97 (d, 2H, $J = 8.7$ Hz), 6.92 (s, 2H), 4.55 (s, 2H), 4.22–4.16 (m, 2H), 3.95 (dd, 2H, $J = 9.0$, 5.7 Hz), 3.77 (s, 3H), 3.75 (s, 3H), 3.70 (dd, 2H, $J = 9.3$, 3.3 Hz); 2.00 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.2 (–C), 165.4 (–C), 149.2 (–C), 148.2 (–C), 127.9 (–C), 127.5 (–CH), 126.6 (–C), 123.0 (–CH), 112.3 (–CH), 111.5 (–CH), 86.5 (–CH), 71.2 (–CH_2), 56.5 (–CH), 55.4 (–CH_3), 55.2 (–CH_3), 22.7 (–CH_3). IR (KBr) ν cm^{-1}: 3,449, 3,289, 2,967, 2,876, 1,653, 1,619, 1,519, 1,370, 1,262, 1,145, 1,024, 905, 810. $\alpha_D^{20} = +87$ (*c*, 0,1) DMSO, mp 173–175°C. HRMS calcd. for $C_{32}H_{39}N_4O_{10}$ 639.2666. Found 639.2687. The product is a white solid; 40% yield.

1,4:3,6-Dianhydro-2,5-bis-[2-acetamido-(*Z*)-4-fluorocinnamamido]-2,5-dideoxy-L-iditiol (**48**). ^{1}HNMR δ (DMSO, 300 MHz): 9.37 (s, 2H), 8.23 (d, 2H, $J = 6.6$ Hz), 7.58 (dd, 4H, $J = 8.7$, 5.7 Hz), 7.21 (t, 4H, $J = 9.0$ Hz), 6.89 (s, 2H), 5.56 (s, 2H), 4.24-4.16 (m, 2H), 3.95 (dd, 2H, $J = 9.0$, 5.7 Hz), 3.70 (dd, 2H, $J = 9.3$, 3.3 Hz), 1.98 (s, 6H). ^{13}CNMR (DMSO, 75 MHz): 169.3 (–C), 165.3 (–C), 131.4 (–CH), 130.7 (–C), 129.8 (–C), 125.4 (–CH), 115.5 (–CH), 115.2 (–CH), 86.5 (–CH), 71.2 (–CH_2), 56.5 (–CH), 22.7 (–CH_3). IR (KBr) ν cm^{-1}: 3,468, 3,258, 2,978, 2,872, 1,647, 1,537, 1,426, 1,371, 1,281, 1,230, 1,156, 1,090, 931, 831, 706. $\alpha_D^{20} = +74$ (*c*, 0,1) DMSO, mp 228–230°C. HRMS calcd. for $C_{28}H_{29}F_2N_4O_6$ 555.2055. Found 555.2030. The product is a white solid; 72% yield.

General: biology

HCV replicon

The HCV subgenomic replicon named I389/3-3′-Luc-UbiNeo, a gift from R. Bartenschlager, was previously described (Mesaik et al. 2004). Briefly, the replicon codes for HCV NS3 through NS5B nonstructural genes under the *Encephalomyocarditis virus* (EMCV) internal ribosome entry site (IRES) and neomycin resistance under the HCV IRES. The HCV 5′ and 3′ nontranslated regions are also present. The mRNA expresses the *Firefly* luciferase (Luc) under HCV IRES. The Luc reporter is used as an indirect measurement of HCV replication and the activity of the Luc reporter is proportionally related to HCV RNA levels.

Cell culture

Huh-7 cells (Hepatoma cell line) were grown at 37°C in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Huh-7 cells were transfected with RNA transcribed from linearized pHCVreplicon plasmid (Ribomax Large Scale RNA production, Promega). Transient transfections were performed as previously

described (Muri et al. 2005). Transfected cells were selected and grown in G418 (Geneticin; Gibco) at 500 μg/ml, which was absent in all experiments. Cell growth was monitored by counting the number of viable cells with trypan blue staining. Cells were treated with recombinant human IFN (100 IU/ml in DMEM) for 18 h at 37°C, as positive control, unless stated otherwise.

IC_{50} and CC_{50} determinations using the replicon system

Briefly, 5,000 Huh-7 cells containing HCV subgenomic replicon were plated in 96-well plates in a total volume of 100 μl of growth medium in Dulbecco's modified Eagle medium containing 5% (vol/vol) fetal bovine serum without G418. Inhibitors were added 24 h post-plating in threefold dilutions at a final DMSO concentration of 1% (vol/vol). After 3 days, the cells were harvested and the *Firefly* luciferase signal was quantified using a Luciferase Assay System (Promega). The 50% inhibitory concentration (IC_{50}) values were calculated as the concentration of inhibitor at which a 50% reduction in the levels of *Firefly* luciferase signal was observed compared to untreated cells. Human interferon alpha (IFN-α) and thiophene was included in each run as a positive control. Subconfluent cultures of the Huh.7 cell line were plated out into 96-well plates and used for analysis of cell viability (cytotoxicity) or antiviral activity. The cytotoxicity of each compound was assessed as a percent of viable cells relative to untreated-cells using CellTiter-Blue (Promega), a colorimetric assay used as an indicator for cell viability.

Results and discussion

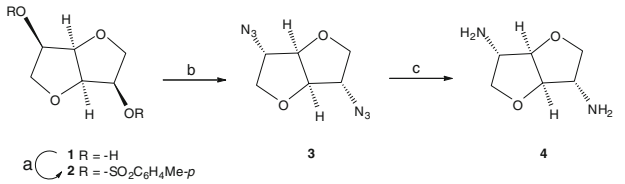
Chemistry

The peptidemimetic approach in drug research has significant potential in the design of substrate-based inhibitors. The introduction of a fused-bicyclic structure has been applied to the design of modified peptides and peptidomimetics in an effort to discover new therapeutics and gain an improved understanding of the interactions of ligands with target enzymes and receptors. Examples of such compounds include the bis-tetrahydrofuranyl, Danuravir (TMC-114), which has recently been approved for the treatment of HIV/AIDS patients (Ghosh et al. 2007), and the bicyclic proline derivative VX-950, which hass been evaluated as an inhibitor of HCV NS3 protease (Lin et al. 2004).

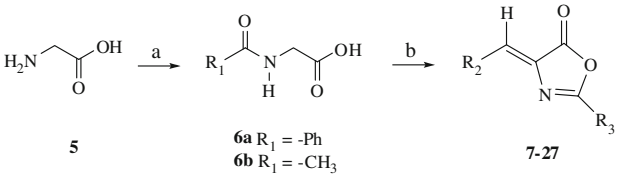
Based on our earlier results on designing and synthesis of peptides derived from isomannide, this rigid scaffold was envisaged as the center of this new family of peptidemimetic compound. The structural analogy of isomannide with cyclic rigid dipeptides and its C_2 symmetry will result in core rigid C_2 symmetric peptidemimetic compounds (Bencsik et al. 2003; Dietrich and Lubell 2003).

The pseudo-peptide compounds were prepared by the reaction of the commercial isomannide **1** and *p*-toluenesulphonyl chloride in pyridine forming the bis-sulphonated **2** with 90% yield after recrystallization with MeOH (Hockett et al. 1946; Muri et al. 2005). After which, the compound **2** reacted with sodium azide yielding the bis-azido compound **3** with inversion of configuration (Chiappe et al. 2003). Several solvents can be used in the nucleophilic substitution step such as DMF and DMSO. In our case, however, the ionic liquid, $[bmim]^+[BF_4]^-$, was shown to be superior, improving the reaction yield from 60% (using DMF) to 78%. The ionic liquid, $[bmim]^+[BF_4]^-$, was synthesized following the methodology described in literature. The ionic solvents possess several properties, such as low melting point, negligible vapor pressure, low coordinating ability and excellent thermal and chemical stability, which make them attractive alternatives to traditional solvents (Suarez et al. 1996; Lancaster et al. 2002; Earle and Seddon 2000). The reduction of diazido derivative **3** with hydrogen over palladium on carbon gave the bis-amino compound **4** a 93% yield (Scheme 1) (Archibald and Baum 1989).

Oxazolones are a class of important heterocyclic compounds, which are useful precursors for the synthesis of amino acids and peptides; so, we decided to use them to build the peptidemimetic compounds. Initially, the oxazolones (so called azalactones) (**7-27**) were synthesized from commercially available glycine (**5**) and benzoyl chloride or acetic anhydride obtaining the benzoylglycine (**6a**) with 95% yield and acetylglycine (**6b**) with 77% yield, which reacted with different aldehydes in the presence of anhydrous sodium acetate/acetic anhydride by Erlenmeyer conditions (Kitazawa et al. 1995; Mesaik et al. 2004). This methodology gives only the thermodynamically stable isomer *Z* (Scheme 2) (Rao 1976; Brocklehurst et al. 1971). The substituents, yields and melting points of the synthesized oxazolones are shown in Table 1 (Kitazawa et al. 1995; Bautista et al. 2002; Paul et al. 2004; Kuhn 1987; Slater and Somerville 1966; Wong et al. 1992; Hoshina et al. 2000; Sen and Shanker 1996; Jendralla et al. 1995; Etschenberg et al. 1979; Meiwes et al. 1997; Lisichkina et al. 1999). The last step consisted in the reaction of **4** and the respective oxazolones **7-27** yielding the final pseudo-peptide products **28-48**. Initially, MeOH, THF and DMF were tried as solvents, but the reactions showed low yields. So we decided to use AcOEt, which presented the best



Scheme 1 Synthesis of bis-amino derivative **4**. **a** TsCl, Py, r.t., overnight, 90%. **b** NaN_3, $[bmim]^+[BF_4]^-$, 12 h, 120°C, 78%. c) H_2, 10% Pd/C, EtOH, 40psi, 12 h, 93%



Scheme 2 Synthesis of Oxazolones **7-27**. **a** BzCl, 10% NaOH, HCl or Ac_2O, H_2O, 95% (**6a**) and 77% (**6b**). **b** Ac_2O, NaOAc, R_2CHO

results (Scheme 3) (Gomes et al. 2006; Valdes et al. 2007; Hernandez Valdes et al. 2004).

Biological results

Subgenomic HCV replicon cell culture systems have significantly impacted the field of HCV research and anti-HCV drug discovery (Lohmann et al. 1999, 2003; Bassit et al. 2008; Kanda et al. 2004; He et al. 2008). HCV replicon-based assay has been previously reported as sensitive and specific for anti-HCV drug screening and therefore has been considered adequate to evaluate the anti-HCV potential candidates. The anti-HCV potential of pseudo-peptides derived from isomannide **28-48** was evaluated by using the HCV replicon-based assay. The values of IC_{50} and CC_{50} of these compounds are shown in Table 2, where we can see that the methylenedioxy derivative **28** showed the most effective activity.

Conclusions

In this work, we present the synthesis of a series of pseudo-peptides derived from isomannide **28-48** and their anti-HCV potential using the HCV replicon-based assay. The compound named **28** was the most effective inhibitor compound tested with IC_{50}, near to 20 μM. However, compound **28** was also the most cytotoxic compound tested here, with CC_{50} near to 20 μM. Further investigations on the cytotoxicity of this compound should be carried out through modifications in its chemical structure in order to improve the putative selectivity on the HCV protease.

Table 1 Oxazolone derivatives **7-27** produced via Scheme 2

Compound	Names	Mp (°C)	Yield (%)
7	4-(3′,4′-Methylenedioxybenzylidene)-2-phenyl-5(4*H*)-oxazolone	200	50
8	4-Benzylidene-2-phenyl-5(4*H*)-oxazolone	169–170	61
9	4-(4′-Methoxybenzylidene)-2-phenyl-5(4*H*)-oxazolone	162–163	55
10	4-(3′,4′-Dimethoxybenzylidene)-2-phenyl-5(4*H*)-oxazolone	154–155	50
11	4-(4′-Fluorobenzylidene)-2-phenyl-5(4*H*)-oxazolone	186	71
12	4-(4′-Chlorobenzylidene)-2-phenyl-5(4*H*)-oxazolone	199–200	60
13	4-(4′-Bromobenzylidene)-2-phenyl-5(4*H*)-oxazolone	207–208	66
14	4-(4′-Trifluoromethylbenzylidene)-2-phenyl-5(4*H*)-oxazolone	174–175	78
15	4-(2′-Thiophenylmethylidene)-2-phenyl-5(4*H*)-oxazolone	176–178	84
16	4-(3'-pyridylmethylidene)-2-phenyl-5(4*H*)-oxazolone	154	40
17	4-(3′,4′-Methylenedioxybenzylidene)-2-methyl-5(4*H*)-oxazolone	183	35
18	4-Benzylidene-2-methyl-5(4*H*)-oxazolone	158	48
19	4-(4′-Chlorobenzylidene)-2-methyl-5(4*H*)-oxazolone	149	58
20	4-(4′-Bromobenzylidene)-2-methyl-5(4*H*)-oxazolone	135	66
21	4-(4'-Trifluoromethylbenzylidene)-2-methyl-5(4*H*)-oxazolone	119	55
22	4-(4′-Cyanobenzylidene)-2-methyl-5(4*H*)-oxazolone	191–192	84
23	4-(4′-Methylbenzylidene)-2-phenyl-5(4*H*)-oxazolone	136	50
24	4-(2'-Naphtylmethylidene)-2-methyl-5(4*H*)-oxazolone	225	40
25	4-(2′-Thiophenylmethylidene)-2-methyl-5(4*H*)-oxazolone	120	55
26	4-(3′,4′-Dimethoxybenzylidene)-2-methyl-5(4*H*)-oxazolone	163–165	45
27	4-(4′-Fluorobenzylidene)-2-methyl-5(4*H*)-oxazolone	149–151	64

Scheme 3 Synthesis of pseudo-peptide derivatives **28-48**. **a** AcOEt, reflux, 24 h

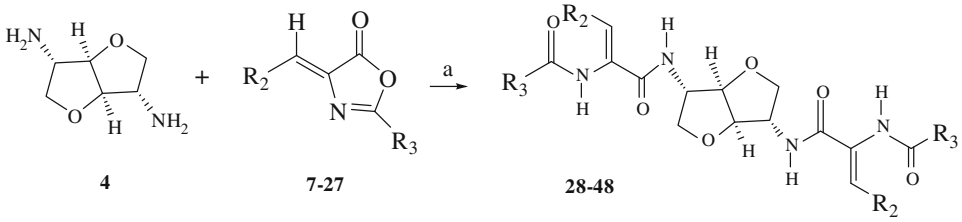


Table 2 Activity of different pseudo-peptides derived from isomannide in the HCV Luc/Ubi/Neo replicon

Compound	$CC_{50\%}$(μM)[a]	$IC_{50\%}$(μM)[b]
Thiophene	>100	5
28	>20	>20
29	NS	NS
30	>100	>100
31	NS	NS
32	NS	NS
33	NS	NS
34	>100	>100
35	>100	95
36	NT	NT
37	NS	NS
38	>100	>100
39	NS	NS
40	NS	NS
41	NS	NS
42	>100	>100
43	>100	>100
44	>100	>100
45	>100	>100
46	>100	>100
47	>100	>100
48	>100	>100

[a] Measured in Huh7 cells

[b] Measured in replicon I389/NS 3-3′-LucUbi, Neo ET

NT non-tested, *NS* non-solubility in DMEM

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