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Synthesis and evaluation of eight-membered cyclic pseudo-dipeptides

Andrew D. Abell*, Karina M. Brown, James M. Coxon**, Matthew A. Jones, Sigeru Miyamoto, Axel T. Neffe, Janna M. Nikkel, Blair G. Stuart

Department of Chemistry, University of Canterbury, Private Bag 4800, Christchurch, New Zealand

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Abstract

In the course of the development of calpain inhibitors, we report the synthesis of eight-membered cyclic pseudo dipeptides closely related to the known inhibitor SJA6017. The ring closure was effected by metathesis of the diallyl-substituted dipeptides **6** and **7**. The formation of the dipeptides under kinetic control leads to the preferential formation of the *unlike* diastereomer **7** over the *like* diastereomer **6**. The relative configuration of the diastereomers was determined by NMR and modeling studies of the related cyclic compounds **8** and **9** and their derivatives. The compounds proved not to inhibit calpain.

Keywords: Peptidomimetics; Molecular modeling; Metathesis; Cataract; Calpain

1. Introduction

The Ca^{2+} dependent cysteine proteases, known as calpains [15,21], have been connected to a number of diseases, including Alzheimer's, muscular dystrophy and some forms of cataract [1,13,19]. It is widely believed that Ca^{2+} binding induces a conformational change in calpain [12,17,22], arranging the catalytic triad and thereby activating the enzyme. The two major isoforms of calpains require different concentrations of Ca^{2+} for activation and are thereby referred to as μ- and m-calpain. Under normal circumstances, Ca^{2+} signaling of calpain leads to controlled proteolysis during cytoskeletal remodeling, signal transduction and apoptosis in mammals. Uncontrolled or high levels of Ca^{2+} within a cell can cause over-activation of calpain, and lead to tissue damage. This is thought to be the case in calpain dependent cataract formation where calpain breaks down the crystalline of the eye lens [9]. The inhibition of calpain-induced proteolysis can therefore prevent further damage in this form of cataract. A drug treatment would be an alternative to the nowadays standard of surgery and implanting an artificial lens.

There are many inhibitors described in the literature [5] that have been designed to bind the active site of calpain. Most are peptidyl or peptidomimetic competitive inhibitors that mimic the natural substrate of the enzyme. Cleavage of a natural peptide substrate is thought to occur favorably on the C-terminal side (P_1 position) of tyrosine, methionine, or arginine next to leucine or valine (P_2 position) [4,10,26,27,28]. However, many competitive inhibitors of calpain, while potent in vitro, are not good drug candidates due to lack of solubility, poor cell permeability, or high cell toxicity.

One of the most potent inhibitors known is *N*-(4-fluorophenylsulfonyl)-L valyl-L-leucinal (SJA6017) (**1**) [8,14] (Fig. 1). This compound displays potent inhibition of calcium activated m-calpain ($IC_{50} = 80$ nM [8]) and has shown to be effective against selenite induced cataract formation in rats [24] and in cataractic porcine lenses [2]. In addition it has low toxicity and is specific for cysteine pro-

Abbreviations: Boc, *tert*-butyloxycarbonyl; DCM, dichloromethane; DIBAL, diisobutyl aluminium hydride; DIEA, diisopropyl ethyl amine; EtOAc, ethyl acetate; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt, hydroxybenzotriazole; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser enhancement; RMSD, root mean square deviation; TFA, trifluoroacetic acid

* Corresponding author. Tel.: +64 3 364 2818; fax: +64 3 364 2110.
** Co-corresponding author.
E-mail addresses: andrew.abell@canterbury.ac.nz (A.D. Abell), jim.coxon@canterbury.ac.nz (J.M. Coxon).

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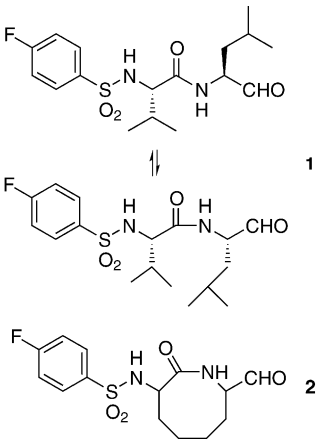

Fig. 1. The known calpain inhibitor SJA0617 **1** resembles the cyclic analogue **2** when adopting a *cis* peptide bond conformation.

teases. However, it suffers from problems of low permeability into the cell and the fact that it binds indiscriminately and readily to proteins. In vitro studies with human plasma have shown that 95.4% of **1** at concentration of 10 μM is bound to the plasma proteins. Therefore, it is necessary to make structural modifications to this compound without compromising its potency or specificity.

Our studies focused on the cyclic analogues **2** of **1** with the leucinal and valyl side chains modified and linked to form an eight-membered ring [3,7,18]. This structural modification restricts the conformational flexibility of the amide linkage. The non-natural environment of the amide linkage will alter its proteolytic stability as proteases may be less able to recognize it. Furthermore, the modification will change the hydrophilicity and thereby the ability to cross membranes, a major problem of the lead compound **1**. The restricted conformational space brought about by the eight-membered ring may also decrease the unspecific binding to other proteins. The results of the activity tests of **2**, having limited conformational flexibility, is intended to allow us to investigate further the requirements for molecular recognition in the active site of calpain. In general, most proteases are considered to preferably cleave amide bonds that are part of a beta strand secondary conformation [6,20]. The synthetic route follows Creighton and Reitz [3], but our findings correct some earlier assumptions.

2. Materials and methods

2.1. General

Proton NMR spectra were acquired on an Inova 500 spectrometer operating at 500 MHz. Carbon NMR spectra were obtained on a Varian Unity XL 300 MHz Fourier Transform spectrometer operating at 75 MHz. Spectra were obtained at a temperature of 23 °C. Chemical shifts are reported in parts per million (ppm, δ) referenced relative to tetramethylsilane (Me_4Si) (0 ppm δ). Molecular masses were detected by electron impact (EI) mass spectrometry at 4 kV and 70 eV ionization energy using a Kratos MS80 RFA spectrometer with a 250 °C source. IR spectra were obtained using a Shimadzu 8201PC series FTIR, where compounds were pressed into a KBr disk for analysis. Melting points were obtained on an electrothermal melting point apparatus and are uncalibrated. Thin layer chromatography (TLC) was performed on aluminium-backed Merck Kieselgel KG60F silica plates, and were visualized by short wavelength ultraviolet light. Flash column chromatography was carried out under positive pressure from dry nitrogen, using Merck silica gel 60 (230–400 mesh).

2.2. Synthesis

2.2.1. Synthesis of the 2-[2-tert-butoxycarbonylamino-pent-4-enoyl-(2,4-dimethoxy-benzyl)-amino]-pent-4-enoic acid methyl esters (**6**) (l) and (**7**) (u) [3]

HOBt (480 mg, 3.55 mmol) and HATU (2.32 g, 6.10 mmol) were added dropwise at room temperature to a solution of *N*-(2,4-dimethoxybenzyl)allylglycine methyl ester (**4**) (352 mg, 1.26 mmol), *N*-Boc allylglycine (**5**) (440 mg, 2.34 mmol) and DIEA (2.4 mL, 13.8 mmol) in DCM (10 mL). The mixture was stirred for 24 h, and poured into HCl (1 M aq.). The product was extracted with EtOAc (200 mL × 2), washed with saturated aq. $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent was removed in vacuo to give a brown oil, chromatographed on silica gel (10% EtOAc in petroleum ether) to give a diastereomeric mixture (*like*:*unlike* = 1:2) of **6** and **7** (571 mg, 95%) as pale yellow oil. For compound **7** IR (film) cm^{-1}: 2981, 1738, 1697, 1633, 1620, 1585, 1508, 1473, 1365; δ_H (300 MHz, $CDCl_3$): 1.40 (9H, s, O–C(CH_3)$_3$), 2.16–2.80 (4H, m, C–CH_2–C), 3.54 (3H, s, –OCH_3 *anti*), 3.56 (3H, s, OCH_3 *syn*), 3.78 (3H, s, OCH_3), 3.79 (3H, s, CO_2CH_3), 4.31 (1H, t, $^2J_{CH_2NH}$: 14.9 Hz, ArCH_2N), 4.64 (1H, t, $^2J_{CH_2NH}$: 14.4 Hz, ArCH_2N), 4.84–5.16 (6H, m, 2*x*C*H*α, 2*x*HC=CH_2), 5.4–5.86 (3H, m, 2*x*HC=CH_2, N*H*), 6.36–6.46 (2H, m, Ar*H*3,5), 7.06–7.18 (1H, m, Ar*H*6); δ_C (300 MHz, $CDCl_3$): 28.3 (C(CH_3)$_3$), 33.5 (CHCH_2CH), 37.9 (CHCH_2CH), 47.7 (CH_2N), 50.3 (*C*α), 51.9 (*C*α), 55.0 (OCH_3), 55.3 (OCH_3), 58.8 (CO_2CH_3), 79.2 (*C*Me$_3$), 98.4 (Ar–*C*3), 103.5 (Ar*C*5), 117.3 (=CH_2), 117.6 (=CH_2), 118.3 (Ar*C*1), 130.7 (Ar*C*6), 132.9 (H*C*=CH_2), 134.4 (H*C*=CH_2), 155.1 (O*C*ON), 159.1 (Ar*C*2), 161.2 (Ar*C*4), 170.8 (C*C*ON), 172.0 (*C*OOMe); TOF MS ES+: 477.2600 (*M* + H, $C_{25}H_{37}N_2O_7$ requires 477.2601).

2.2.2. Preparation of the (Z)-7-tert-butoxycarbonyl-amino-1-(2,4-dimethoxybenzyl)-8-oxo-1,2,3,6,7,8-hexahydro-azocine-2-carboxylic acid methyl esters (**8**) (l) and (**9**) (u) [3]

The mixture of **6** and **7** (235 mg, 0.49 mmol) was dissolved in DCM (250 mL) and benzylidene bis-(tricyclohexylphosphine)-dichlororuthenium (53 mg, 62 μmol) was