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POTENT FLUOROMETHYL KETONE INHIBITORS OF RECOMBINANT HUMAN CALPAIN I[+]

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Abstract. We report on a series of potent and selective dipeptide fluoromethyl ketone inhibitors of recombinant human calpain I. Compound **4f**, having a tetrahydroisoquinoline containing urea motif as N-terminus capping group, is the most potent member (k_{obs}/I = 276,000 M^{-1} s^{-1}) of this class. This compound was shown to prefer calpain I by >36-fold and approximately 4-fold over the related cysteine proteases, cathepsin B and cathepsin L, respectively.

Introduction. Calcium-activated neutral proteases (calpains) comprise a family of intracellular cysteine proteases which are ubiquitously expressed in mammalian tissues.[1] Two major forms of calpains have been identified: calpain I and calpain II. While calpain II is the predominant form in many tissues, calpain I is thought to be the predominant form activated during pathological conditions of nervous tissues. The calpain family of cysteine proteases has been implicated in many nervous system diseases and disorders, including stroke, Alzheimer's disease, amyotrophy, motor neuron damage and muscular dystrophy. Thus, in recent years, calpain inhibition has become an important pharmacological goal.[2] Potent peptide-based reversible (aldehyde and α-ketocarbonyl)[3] and irreversible (diazomethyl ketone, epoxysuccinate and acyloxymethyl ketone)[4] inhibitors have been reported. In 1992, Shaw et al. reported a dipeptide fluoromethyl ketone (Cbz-Leu-Tyr-CH_2F; k_{obs}/I = 17,000 M^{-1} s^{-1}) to be an inhibitor of chicken gizzard calpain II.[5] In this communication, we report on a series of potent dipeptide fluoromethyl ketones, **4a-f** (Scheme 1), and their inhibitory activities against recombinant human calpain I. Compound **4f** having a tetrahydroisoquinoline containing urea motif as N-terminus capping group, is the most potent dipeptide fluoromethyl ketone inhibitor of calpain I yet described. This compound also prefers calpain I over the related cysteine proteases cathepsin B and cathepsin L.

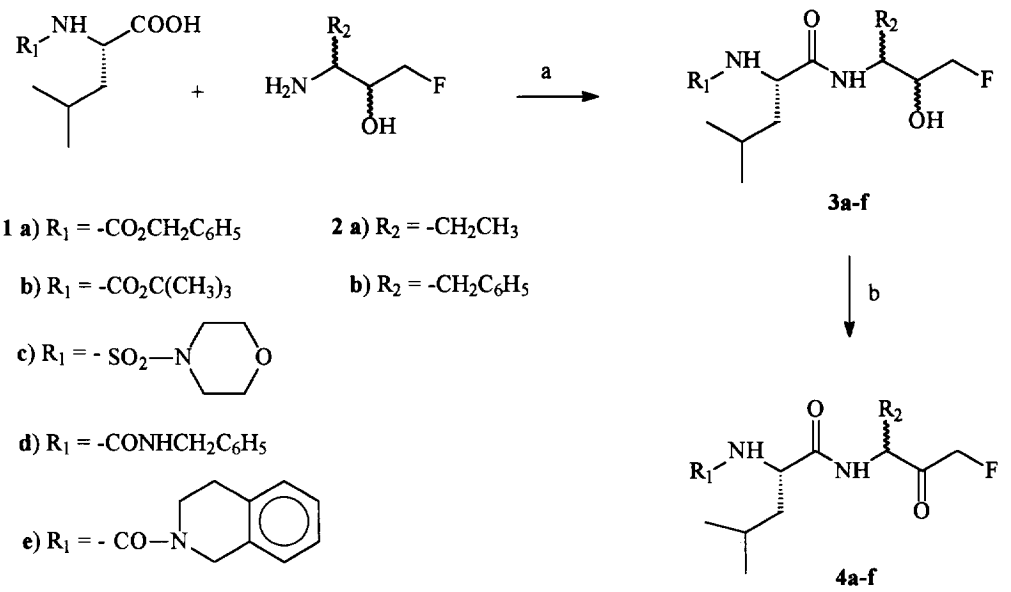
Chemistry. The syntheses of compounds **4a-f** are depicted in Scheme 1. Acylated or sulfonylated Leu (**1a-e**) was coupled with amino-fluoro-hydroxy compounds **2a-b** (prepared by following the method of Imperiali et al.,[6] modified by Revesz et al.[7]) to generate the dipeptide fluorohydroxy compounds **3a-f**. Dess-Martin oxidation of **3a-f** generated fluoromethyl ketones **4a-f**. Compounds **1a-b** are commercially available (Advanced ChemTech, Louisville, KY). Compound **1c** was synthesized by coupling leucine with morpholinosulfonyl chloride[8] (NaOH, H_2O, THF, 23 °C). Compound **1d** was obtained by reaction of benzyl isocyanate with leucine *t*-butyl ester hydrochloride salt (iPr_2EtN, CH_2Cl_2, 0 °C to 23 °C), followed by acidic hydrolysis (90% TFA, CH_2Cl_2, 23 °C). Compound **1e** was synthesized by coupling 1,2,3,4-tetrahydroisoquinoline, leucine methyl ester hydrochloride salt and triphosgene (iPr_2EtN, CH_2Cl_2, 23 °C),[9] followed by basic hydrolysis (LiOH, THF-H_2O, 23 °C). All compounds were assayed as diasteromeric mixtures, epimeric at P_1.

Biology. The inhibitory activity of the compounds **4a-f** was determined using recombinant human calpain I, prepared as described by Meyer et al.[10] Second-order rate constants for inactivation were determined by analysis of progress curves obtained in the presence of substrate (Suc-Leu-Tyr-MNA, Enzyme Systems Products, Dublin,

[+] This paper is dedicated, with affection, to Mr. Dhirendra Mohan Chatterjee on the occasion of his 75th birthday.

CA) and inhibitor.[11,12,13] Inhibition rates for **4a-f** and a reference compound, E-64, (trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane) (**5**), are shown in Table 1.

Scheme 1[a]



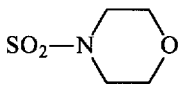
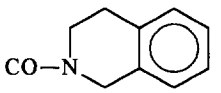
[a]Reagents: (a) BOP/HOBt/NMM/DMF, 0 °C to 23 °C; (b) Dess-Martin periodinane/CH_2Cl_2, 23 °C.

Discussion. Compounds **4a-f** exhibit good calpain inhibitory activity (cf. Shaw's result[5]). However, the P_1 sidechain has a notable effect on the potency of the compounds; thus **4b** with P_1-benzyl is >5 times more potent than compound **4a** with P_1-ethyl. Previous studies indicate that calpain prefers Leu or Val at P_2. However, the N-terminal capping group also plays a significant role in the potency of this series of compounds. Thus Cbz (**4b**) was prefered over the *t*-Boc, morpholinosulfonyl or benzyl urea substituents of **4c, d** or **4e**. Interestingly, constraining the benzyl urea motif of **4e** as part of a tetrahydroisoquinolyl moiety generated a >4 times more potent compound (**4f**). Compound **4f** (k_{obs}/I = 276,000 M^{-1} s^{-1}) is the most potent dipeptide fluoromethyl ketone inhibitor of human calpain I yet reported. It should be noted that compounds **4a-f** were also examined against two other related cysteine proteases, cathepsin B and cathepsin L, respectively.[14,15] Compound **4f** was shown to prefer calpain I by >36-fold and approximately 4-fold over cathepsin B (k_{obs}/I = 7,500 M^{-1} s^{-1}) and cathepsin L (k_{obs}/I = 72,000 M^{-1} s^{-1}), respectively.

It is interesting to note that the inactivation rates reported in this paper for a series of X-Leu-Phe-CH_2F compounds for calpain I are significantly greater than the value of 17,000 M^{-1} s^{-1} determined by Shaw et al. for Cbz-Leu-Tyr-CH_2F against chicken gizzard calpain II.[5] The basis for this discrepancy is unclear. Inactivation rates for the reference compound, E-64 (**5**), are comparable in our assay utilizing recombinant human calpain I (4,700 M^{-1} s^{-1}) and in literature reports using chicken gizzard calpain II (Pliura et al.[16] 3,700 M^{-1} s^{-1} and Parkes et

al.[17] 7,500 $M^{-1} s^{-1}$). This demonstrates the validity of our assay method and similarity between recombinant human calpain I and chicken gizzard calpain II. The conservative change in the P_1 residues of the two sequences is unlikely to account for the divergent inactivation rates.[18] The discrepancy may arise due to methodological differences in the analysis of progress curves at multiple inhibitor concentrations employed in this study and the preincubation experiments performed at a single inhibitor concentration by Shaw et al.

Table 1. Recombinant Human Calpain I Inhibitory Activity of Compounds **4a-f, 5**

Compound	R_1	R_2	k_{obs} / I $M^{-1} s^{-1}$
4a	$-CO_2CH_2C_6H_5$	$-CH_2CH_3$	24,250 (n = 3)
4b	$-CO_2CH_2C_6H_5$	$-CH_2C_6H_5$	136,300 (n = 3)
4c	$-CO_2C(CH_3)_3$	$-CH_2C_6H_5$	68,600 (n = 3)
4d		$-CH_2C_6H_5$	67,200 (n = 3)
4e	$-CONHCH_2C_6H_5$	$-CH_2C_6H_5$	67,200 (n = 3)
4f		$-CH_2C_6H_5$	276,000 (n = 6)
5 (E-64)	-	-	4,700 (n = 3)

Conclusion. We have described a series of potent and selective dipeptide fluoromethyl ketone inhibitors of recombinant human calpain I. Such inhibitors should provide useful probes for the assessment of the role of calpain in different biological functions and will be the basis of future publications from our laboratories.

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$$y = Ae^{(-kobs*t)} + B \quad (1)$$

where y is the fluorescence at time t (F_t), A is the amplitude of the reaction (F_0-F_∞), and B is the maximal amount of product formed when the enzyme is completely inactivated (F_∞). The apparent second-order rate constant for inactivation was calculated from the slope of a plot of k_{obs} versus inhibitor concentration as (k_{obs}/I)* (1+S/K_m), correcting for the effect of substrate on the inactivation rate.

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