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# THE SYNTHESIS OF KETOMETHYLENE PSEUDOPEPTIDE ANALOGUES OF DIPEPTIDE ALDEHYDE INHIBITORS OF CALPAIN

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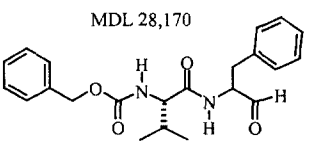
**Abstract:** The ketomethylene phenylalanal and alanal analogues of Cbz-Val-Phe-H and Cbz-Val-Ala-H have been prepared and the $K_i$ values versus chicken gizzard smooth muscle calpain were determined. The ketomethylene isosteres were significantly less potent than the corresponding dipeptide aldehydes, and this loss in activity is attributed to the absence of a critical interaction between the P1-P2 amide bond and the peptide binding region of calpain. 

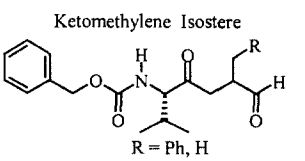
The calpains are intracellular calcium dependent neutral cysteine proteinases that are divided into 3 subtypes based on the calcium ion concentration required for activation.[1] The calpain-mediated proteolysis of architectural and regulatory proteins, which results from the ischemia-induced increase in intracellular calcium, has led to the implication of calpain in the pathogenesis of stroke.[2] The systematic evaluation of peptidyl substrate analogs containing an electrophilic carbonyl group[3] has led to the identification of Cbz-Val-Phe-H (MDL 28,170) as a potent inhibitor ($K_i$ = 10nM) which rapidly penetrates cell membranes.[4,5] Cbz-Val-Phe-H has been shown to penetrate the blood-brain barrier in a dose-dependent manner and has demonstrated efficacy in experimental models of cerebral ischemia.[6,7] For the purpose of eliminating the potential proteolysis of the P1-P2 amide bond we have synthesized the ketomethylene isosteres of Cbz-Val-Phe-H and Cbz-Val-Ala-H.

The ketomethylene isosteres were prepared utilizing α-methylene-γ-butyrolactones[8] as the key intermediates. The α-methylene-γ-butyrolactones are attractive for they allow elaboration of various P1 side chains. Using the procedures as reported by Kempf,[8] the α-methylene-γ-butyrolactones were obtained as a 1:1 mixture of diastereomers by the alkylation of Cbz-Val-H with the dianion of N-methylmethacrylamide in the presence of chlorotitanium triisoproxide, followed by lactonization in refluxing xylenes. Separation of the diastereomers **3** by flash chromatography allowed for the sodium borohydride reduction of the (5S,1'S) dihydrofuranone **4** to the diol **5**. Arylation of **4** gave the (3S,5S,1'S) dihydrofuranone **7** and sodium borohydride reduction of **7** produced diol **8**. Conversion of the diols **5** and **8** to the ketoaldehydes **6** and **9**[9] represents the first application of a bis-Swern oxidation of 1,4-diols for the preparation of γ-ketoaldehydes.

MDL 28,170



Ketomethylene Isostere



R = Ph, H

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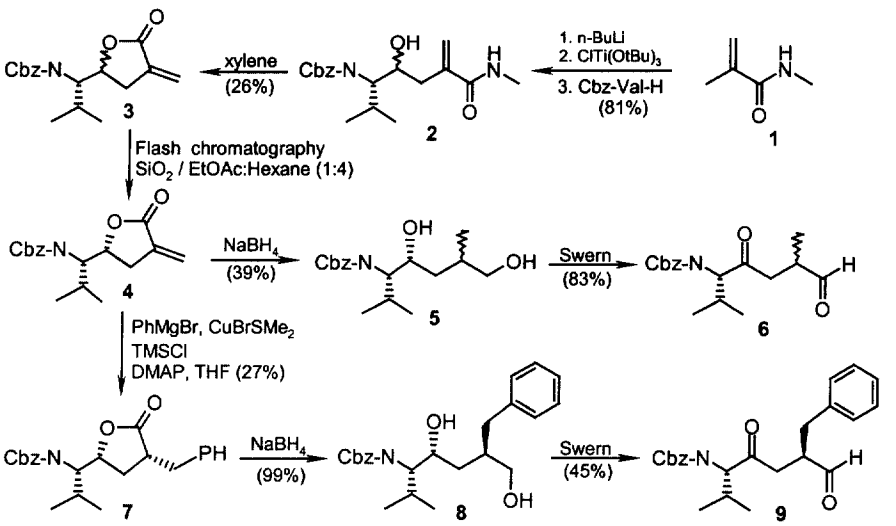
*PII:* S0960-894X(98)00704-5




Inhibition constants ($K_i$ values) for **6** and **9** were 45 μM and 2.5 μM, respectively, against chicken gizzard smooth muscle calpain.[10] Compared with MDL 28,170, the isostere **9** is 250-fold less potent. The loss in potency of **9** corresponds to the lack of a critical hydrogen bond interaction between the normally occurring NH of the P1 amide and calpain.[11,12] The lesser potency of **6** relative to **9** is consistent with the trends observed with peptide inhibitors, which indicate a preference for bulky P1 side chains by calpain.

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9. All new compounds were characterized by spectroscopic (NMR, IR, MS) data. For representative compound **9**: 1H NMR (300 MHz, CDCl3) 9.78 (s, 1H), 7.40-7.10 (series of m, 10 H), 5.29 ( br d, J = 8.5 Hz; 1H), 5.08 (s, 2 H), 4.31 (dd, J = 8.5, 4.0 Hz, 1H), 3.29 (dddd, J = 8.5, 8.5, 6.0, 4.0 Hz, 1H), 3.08 (dd, J = 13.5, 6.0 Hz, 1H), 2.98 (dd, J = 18.5, 8.5 Hz, 1H), 2.68 (dd, J = 14.0, 8.5 Hz, 1H) , 2.44 (dd, J = 18.5, 4.0 Hz, 1H), 2.25 (dqq, J = 4.0, 7.0, 7.0 Hz, 1H), 1.02 (d, J = 7.0 Hz, 3H), 0.77 (d, J = 7.0 Hz, 3H); HRMS for C23H27NO4 calc 382.2018, found 382.2023.
10. The $K_i$ values were determined as described in ref 4.
11. In the crystal structure of Cbz-Arg-Ser(OBzl)chloromethylketone in Cathepsin B, the corresponding amide NH is hydrogen-bonded to the backbone carbonyl group of G198, the sequence around which G198 (HAIRILG) is conserved in calpain (HAYSVTG).
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