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α-Ketothiazole Inhibitors of Prolyl Endopeptidase

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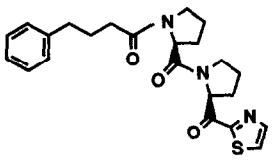
Abstract: A method is described for the preparation of a series of α-ketothiazole derivatives **1**, **2**, **3**, and **4** which were assayed for prolyl endopeptidase inhibition. The potent inhibitors **1** and **2** possess a nitrogen atom at a position β to the adjoining ketone moiety.

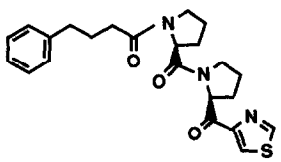
The rational design and synthesis of protease inhibitors have been an attractive field in medicinal and bioorganic chemistry.[1] A general approach has been the replacement of the scissile amide unit by an electron-deficient carbonyl group.[2] Series of aldehydes,[3] α-fluoroketones,[4] α-keto esters,[5] and α-keto amides[6] have been successfully incorporated into peptidyl protease inhibitors.

Prolyl endopeptidase[7] (PEP) (E.C.3.4.21.26) is classified as a serine protease that cleaves proline-containing peptides such as substance P, vasopressin, and bradykinin. Vasopressin is a neurotransmitter which exerts behavioral effects and it facilitates memory processes.[8] Therefore, PEP inhibitors might improve learning and memory by prolonging the half-life of neuropeptides.[9] To search for cognition enhancing drugs,[10] we introduced a novel functional group, an α-ketothiazole, in place of the aldehyde[11] and α-keto ester[12] functionalities in known PEP inhibitors.

In this paper, we report the synthesis and evaluation of α-keto thiazole derivatives **1**, **2**, and **3** and the thiophene derivative **4** as a novel type of PEP inhibitor. The new potent PEP inhibitors are dipeptides which have α-ketothiazole groups at their C-termini as shown in structures **1** and **2**.



1

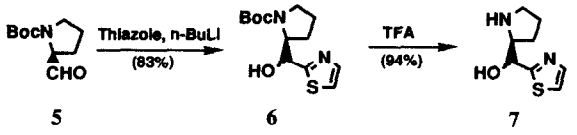


2

To investigate the structural requirements of the thiazole ring, we prepared 2-, 4-, and 5-substituted thiazoles and a thiophene derivative. Key intermediate amino alcohols **7**, **10**, and **15** were prepared as shown in Schemes I-III. Reaction with N-tert-butoxycarbonyl (Boc) -L-prolinal[13] **5** and lithiated thiazole gave the 2-

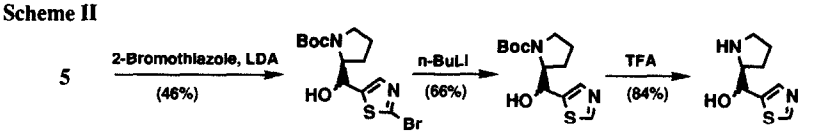
substituted thiazole **6** (Scheme I). The thiophene derivative was prepared by a similar method. Lithiation of 2-bromothiazole by LDA followed by removal of the bromine atom provided the desired 5-substituted thiazole **9** (Scheme II). The 4-substituted thiazole **15** was synthesized from the N–allyloxycarbonyl (Alloc) pyrrolidine α-hydroxy acid[12] **11** as a starting material. The O-protected α-hydroxy acid **12** was treated with ethyl chloroformate followed by diazomethane and hydrogen chloride to give chloromethyl ketone **13** (Scheme III). Cyclization of **13** with thioformamide gave the 4-substituted thiazole **14**. The N-protecting groups (Boc and Alloc) were removed by treatment with TFA and $Pd(PPh_3)_4$, respectively, to give the desired amino alcohols.

Scheme I



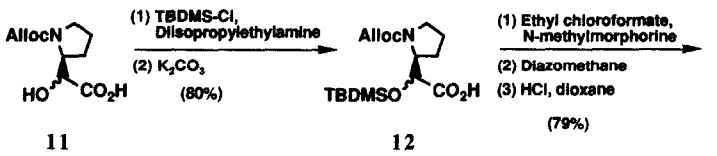
5 6 7

Scheme II

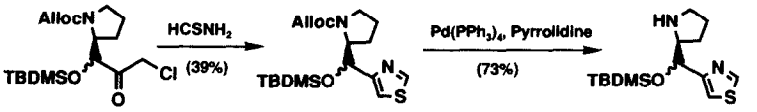


8 9 10

Scheme III

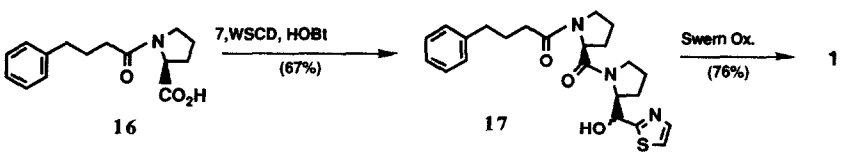


11 12



13 14 15

Condensation of the 4-phenylbutanoyl-L-proline **16** with the amino alcohol **7** using 1-ethyl-1-3-(3'-dimethylaminopropyl)carbodiimide (WSCD) afforded the dipeptide **17** as a mixture of diastereomers. In the case of the 4-substituted thiazole, the TBDMS group was removed after the coupling reaction. The desired α-keto thiazole derivative **1**[14] was prepared from the corresponding alcohol **17** via Swern oxidation. In a similar manner, the corresponding α-keto heterocycles **2**,[15] **3**,[16] and **4**[17] were synthesized.

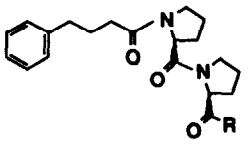


16 17

Enzyme assays for PEP inhibition were carried out by the method of Yoshimoto *et al.*[18] The results indicate that 2- and 4-substituted thiazole derivatives **1** and **2** have potency comparable to the corresponding aldehyde **5**[11] and α-keto ester derivatives[12] **6** (Table 1). In contrast to **1** and **2**, the 5-substituted thiazole derivative **3** and the thiophene derivative **4** showed negligible inhibitory activity. The nitrogen atom in the 5-substituted thiazole **3** is at a γ-position from the ketone carbonyl and the thiophene derivative **4** contains only a sulfur atom in the heterocycle. It is noteworthing that potent inhibitors **1** and **2** possess a nitrogen atom in the heterocyclic ring at a β-position from the ketone moiety. Recently, Edwards *et al.* determined by an X-ray study that a peptidyl α-ketobenzoxazole derivative was a mechanism-based elastase inhibitor in which the nitrogen atom of the benzoxazole participated in the stabilization of the tetrahedral intermediate enzyme and inhibitor complex.[19] We found that an α-ketothiazole moiety acted as a novel functional group in our PEP inhibitors and we suggest that the nitrogen atom in the thiazole ring might play a role similar to what was observed by Edwards *et al.* Furthermore, our inhibitors of PEP extend the generality of the α-heterocycle design to another serine protease.

The α-keto heterocycle derivatives were orally active and were potent brain PEP inhibitors. Details of the results will be reported elsewhere.[20]

Table 1: Inhibition of Prolyl Endopeptidase



Compound	R	IC_{50} (nM)
1		3.1
2		5.9
3		646
4		1910
5	H	8.7
6	CO_2Et	9.6

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14. Compound **1**: ^{1}H-NMR(400MHz, $CDCl_3$) δ 1.90-2.21 (m, 9H), 2.25-2.40 (m, 2H), 2.45-2.55 (m, 1H), 2.65-2.75 (m, 2H), 3.40-3.50 (m, 1H), 3.55-3.65 (m, 1H), 3.72-3.80 (m, 1H), 4.00-4.05 (m, 1H), 4.73 (dd, 1H, J=3.6, 8.0Hz), 5.75 (dd, 1H, J=4.7, 9.0Hz), 7.15-7.30 (m, 5H), 7.65 (d, 1H, J=3.1Hz), 8.00 (d, 1H, J=3.1Hz); Found:*m/z* 425.1790 Calcd for $C_{23}H_{27}N_3O_3S$: M, 425.1772.
15. Compound **2**: ^{1}H-NMR(400MHz, $CDCl_3$) δ 1.90-2.24 (m, 9H), 2.21-2.44 (m, 3H), 2.64-2.68 (m, 2H), 3.37-3.43 (m, 1H), 3.54-3.59 (m, 1H), 3.70-3.75 (m, 1H), 3.89-4.01 (m, 1H), 4.75 (dd, 1H, J=3.5, 7.8Hz), 5.71 (dd, 1H, J=4.7, 9.0Hz), 7.16-7.27 (m, 5H), 8.25 (d, 1H, J=2.0Hz), 8.88 (d, 1H, J=2.0Hz); Found:*m/z* 425.1751 Calcd for $C_{23}H_{27}N_3O_3S$: M, 425.1772.
16. Compound **3**: ^{1}H-NMR(400MHz, $CDCl_3$) δ 1.91-2.06 (m, 4H), 2.09-2.24 (m, 4H), 2.27-2.34 (m, 3H), 2.62-2.68 (m, 3H), 3.40-3.42 (m, 1H), 3.54-3.56 (m, 1H), 3.68-3.73 (m, 1H), 3.94-4.00 (m, 1H), 4.70 (dd, 1H, J=3.6, 8.0Hz), 5.33 (dd, 1H, J=4.6, 8.7Hz), 7.15-7.27 (m, 5H), 8.54 (s, 1H), 9.00 (s, 1H); Found:*m/z* 425.1717 Calcd for $C_{23}H_{27}N_3O_3S$: M, 425.1772.
17. Compound **4**: ^{1}H-NMR(400MHz, $CDCl_3$) δ 1.90-2.13 (m, 9H), 2.20-2.32 (m, 3H), 2.64-2.68 (m, 2H), 3.39-3.56 (m, 2H), 3.67-3.73 (m, 1H), 3.91-3.97 (m, 1H), 4.72 (dd, 1H, J=3.6, 7.8Hz), 5.41 (dd, 1H, J=4.2, 8.8Hz), 7.23-7.27 (m, 5H), 7.12 (dd, 1H, J=3.9, 5.0Hz), 7.63 (dd, 1H, J=1.1, 5.0Hz), 7.80 (dd, 1H, J=1.1, 3.9Hz); Found:*m/z* 424.1850 Calcd for $C_{24}H_{28}N_2O_3S$: M, 424.1819.
18. The prolyl endopeptidase from pig kidney are prepared according to the method of Yoshimoto *et al* .[21] Enzyme assays for inhibitors of prolyl endopeptidase were carried out by the following method. All assays were performed with Z-Gly-Pro-*p*-nitroanilide as substrate. The inhibitor solution (3 μL of a DMSO solution of varying concentration) was added to 180 μL of the substrate solution (0.26 mM in dioxane and 0.2 M phosphate buffer solution). To this solution, 20 μL of the enzyme solution was added and this reaction mixtures were incubated for 20 min at 37 °C. The change in absorbance was measured at 405 nm for 1 min. The IC_{50} values (n=2-4) of the test compounds **1-6** were estimated by the inhibitor concentration vs. activity curve.
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