Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 13, pp. 1541-1542, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00269-7

SYNTHESIS OF CYSTAMIDIN A (PYRROLE-3-PROPANAMIDE), A REPORTED CALPAIN INHIBITOR

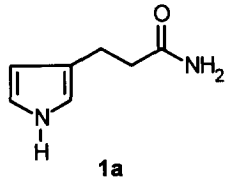
Ron Bihovsky[‡,*] and Israil Pendrak[†]

[‡]*Cephalon, Inc., 145 Brandywine Parkway, West Chester, PA 19380, U.S.A.*
[†]*SmithKline Beecham, P.O. Box 1539, King of Prussia, PA 19406, U.S.A.*

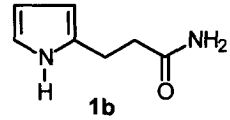
Abstract: The reported calpain inhibitor cystamidin A (**1a**, pyrrole-3-propanamide) and a regioisomer, pyrrole-2-propanamide (**1b**) have been synthesized from the corresponding pyrrolecarboxaldehydes. The previously assigned structure of cystamidin A is confirmed. However, neither synthetic cystamidin A nor regioisomer **1b** inhibits recombinant human calpain I. Copyright © 1996 Elsevier Science Ltd

Calpain (EC 3.4.22.17), an ubiquitous intracellular calcium-activated cysteine protease, plays a role in cerebral ischemia, muscular dystrophy, and various chronic neurodegenerative disorders.[1] Therefore, calpain inhibitors are of interest as potential therapeutic agents for the treatment of these disorders. Several types of calpain inhibitors have been identified, including active site directed aldehydes,[2,3] α-ketoamides,[2,4] and halomethyl ketones[2] derived from di- and tripeptides, as well as polypeptide inhibitors such as calpastatin and kininogen.[2] However, none of the existing inhibitors has properties ideal for a drug candidate. Recently, cystamidin A (**1a**, pyrrole-3-propanamide), a novel non-peptide calpain inhibitor (IC_{50} = 1.4 μM),[5] was isolated from *Streptomyces* KP-1241 culture broth.[5] The structure was assigned by spectroscopy.

In this communication, we describe a concise synthesis of cystamidin A (**1a**) capable of providing sufficient quantities for biochemical and pharmacological evaluation. In addition, the total synthesis confirms the assigned structure of cystamidin A.

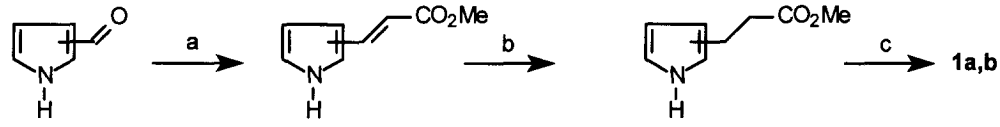


1a



1b

Cystamidin A (**1a**) and its regioisomer (**1b**) were prepared from the corresponding pyrrolecarboxaldehydes (see Scheme). Pyrrole-3-carboxaldehyde (**2a**) is readily available via Vilsmeier-Haack reaction of *N*-(triisopropylsilyl)pyrrole followed by hydrolysis (1M NaOH, 19 h) of the intermediate dimethyliminium salt.[6] Pyrrole-2-carboxaldehyde (**2b**) is commercially available. Homologation of aldehyde **2a** by a Knoevanagel condensation with monomethyl malonate,[7] and homologation of **2b** by a Wittig reaction, afforded pyrrolepropenoate esters **3a** and **3b**, respectively. Hydrogenation produced the corresponding pyrrolepropanoate esters (**4a** and **4b**),[8] which reacted slowly with saturated methanolic ammonia and catalytic NaCN[9] to give cystamidin A (**1a**) and its regioisomer (**1b**),[8] respectively.



2a,b **3a,b** **4a,b**

(a) **3a**: $MeO_2CCH_2CO_2H$, pyridine, piperidine 65 °C, 21 h, 89%; **3b**: $Ph_3P{=}CHCO_2Me$ toluene 45 °C, 3 h, 67%; (b) H_2, 10% Pd-C; MeOH, 16 h, **4a**: 97%, **4b**: 93%; (c) NH_3, NaCN (10 mol %), MeOH, 45 °C, 4 days, **1a**: 93%, **1b**: 70%.

Synthetic cystamidin A (**1a**) is spectroscopically identical (^{1}H NMR, ^{13}C NMR, IR, and MS) to the natural product.[10] In contrast, the spectral characteristics of pyrrole-2-propanamide (**1b**) readily distinguish it from cystamidin A.[11] Therefore, the structure of cystamidin A is confirmed to be as reported.[5] Surprisingly, however, neither synthetic cystamidin A nor regioisomer **1b** inhibits human calpain I under our assay conditions,[12] suggesting the presence of a potent inhibitory contaminant in isolated cystamidin A.

Acknowledgments: We thank Donna Bozyczko-Coyne, Shobha Senadhi, and Mark Ator for measuring calpain inhibition.

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10. **1a**: ^{1}H NMR (DMSO d_6) δ 2.25 (2H, t, J = 8 Hz), 2.59 (1H, t, J = 8 Hz), 5.89 (1H, br. q, J = 2 Hz), 6.50 (1H, br. q, J = 2 Hz), 6.60 (1H, dd, J = 2 Hz), 6.70 (1H, br. s), 7.24 (1H, br. s) 10.41 (br. s); ^{13}C NMR (DMSO d_6) δ 22.7, 37.0, 107.4, 114.5, 117.2, 121.7, 174.1; IR (KBr) 3400, 3240, 3150, 1640, 1400, 1260, 1160, 1070 cm^{-1}; electron spray mass spectrum *m/e* 139 ($M+H^+$), 161 ($M+Na^+$). Anal. calcd for $C_7H_{10}N_2O$: C, 60.85; H, 7.30; N, 20.28; found: C, 60.77; H, 7.34; N, 20.30. Interestingly, the melting point of synthetic cystamidin A (recrystallized from chloroform, mp 76-8 °C) differs from that reported for the natural product (mp 146-8 °C), suggesting polymorphous crystal forms.
11. **1b**: ^{1}H NMR (DMSO d_6) δ 2.33 (2H, t, J = 8 Hz), 2.71 (1H, t, J = 8 Hz), 5.71 (1H, s), 5.85 (dd, J = 2 Hz), 6.54 (1H, dd, J = 2 Hz), 6.74 (1H, br. s), 7.26 (1H, br. s), 10.43 (br. s); ^{13}C NMR (DMSO d_6) δ 23.0, 35.2, 104.1, 107.0, 115.9, 131.0, 173.7; electron spray mass spectrum *m/e* 139 ($M+H^+$), 161 ($M+Na^+$). Anal. calcd for $C_7H_{10}N_2O$: C, 60.85; H, 7.30; N, 20.28; found: C, 60.98; H, 6.75; N, 19.97. mp 97-8 °C.
12. **1a** or **1b** (10 μM) did not inhibit hydrolysis of succinyl-Leu-Tyr-MNA by recombinant human calpain I, under conditions described in Meyer, S. L.; Bozyczko-Coyne, D.; Mallya, S. K.; Spais, C. M.; Bihovsky, R.; Kawooya, J. K.; Lang, D. M.; Scott, R. W.; Siman, R. *Biochem. J.* **1996**, *314*, 511. Various known calpain inhibitors gave IC_{50}s consistent with literature values, for example: Ac-Leu-Leu-Nle-H, 7 nM; Z-Leu-Phe-CONHEt, 11 nM; human calpastatin domain I, 7 nM.

(*Received in USA* 14 *May* 1996; *accepted* 6 *June* 1996)