Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 3, pp. 287-290, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00003-6

NONPEPTIDIC INHIBITORS OF RECOMBINANT HUMAN CALPAIN I†

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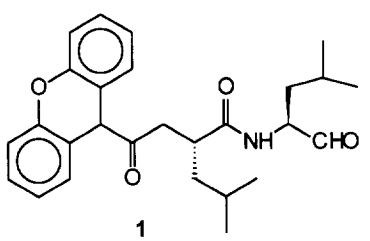
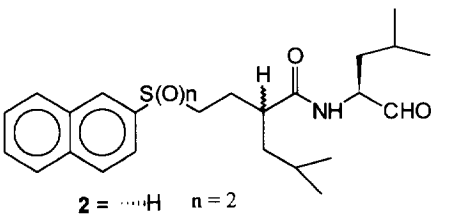
Abstract. The syntheses and biological activities of novel nonpeptidic calpain inhibitors **2-3, 12,** and **14,** derived from 2-naphthalenethiol, are described.

Calpains (I and II) are calcium-activated neutral proteases belonging to a family of intracellular cysteine proteases.[1] The possible role of calpains in the pathology of a variety of nervous system disorders including stroke, Alzheimer's disease, muscular dystrophy, and epilepsy has been suggested; thus, in recent years, calpain inhibition has become an important pharmacological goal.[2] Our involvement in inhibiting calpains emerged from our interest in new therapeutics[3] to treat stroke, one of the leading causes of mortality in the western hemisphere. Potent peptide-based reversible aldehyde and α-ketocarbonyl,[4] and irreversible halomethyl ketone, diazomethyl ketone, epoxysuccinate, and acyloxymethyl ketone[5] inhibitors of calpains have been reported. In all of these inhibitors, calpain tolerated a range of amino acids at P_1. However, the P_2-amino acid was always either L-Leu or -Val. Recently we disclosed compound **1** (IC_{50} 25 nM), a potent nonpeptidic ketomethylene containing calpain inhibitor, derived from xanthene.[6] Our work revealed that the NH at the P_2 site of a potent dipeptide inhibitor can effectively be replaced by a CH_2, provided an aromatic moiety is employed in the P_3 region. In designing our target molecule, we decided to replace the P_3-spanning xanthene with an aromatic moiety attached by a spacer to the P_2-site. Naphthalene-$S(O)_n$-CH_2CH_2 appeared to be the desired motif. We now report on this new series of nonpeptidic calpain inhibitors **2-3, 12,** and **14**, derived from 2-naphthalenethiol.



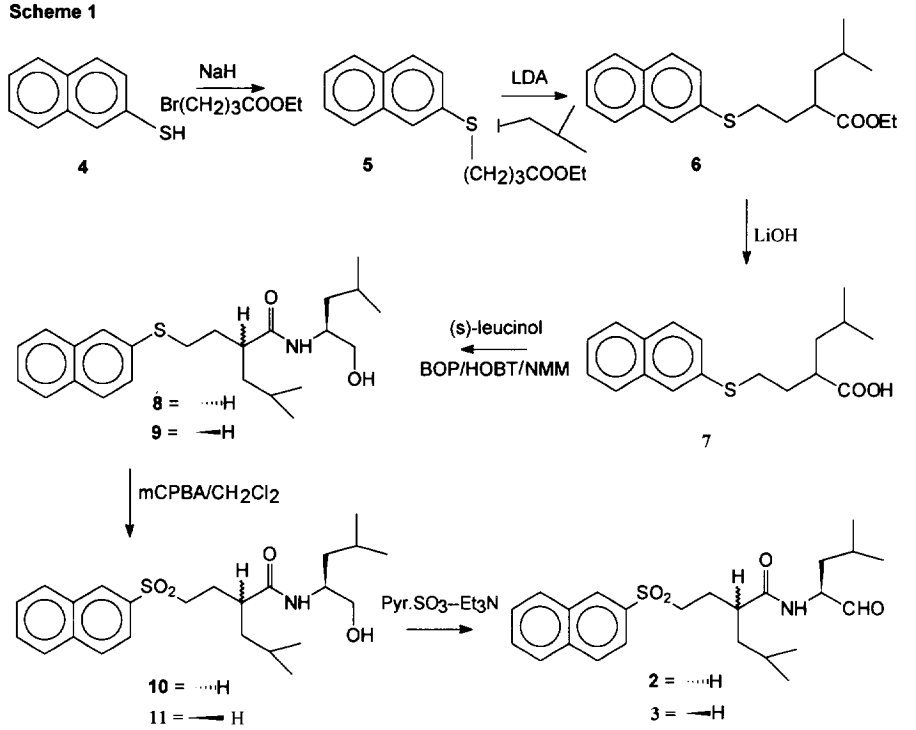
1



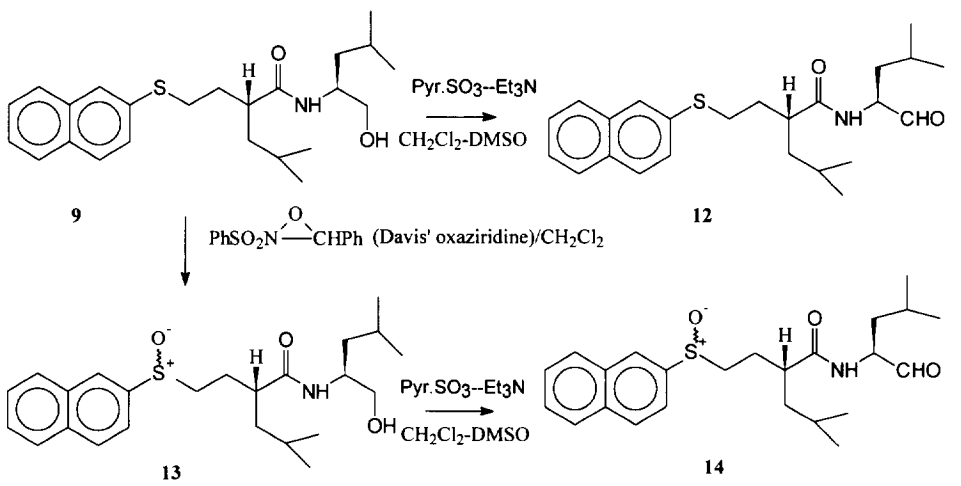
2 = ····H n = 2
3 = —H n = 2
12 = —H n = 0
14 = —H n = 1

† This paper is dedicated to Prof. Franklin A. Davis in recognition of his outstanding contributions to oxaziridine chemistry.

Scheme 1



Scheme 2



The syntheses of **2-3** are depicted in Scheme 1. Commercially available 2-naphthalenethiol (**4**) was treated with sodium hydride, followed by ethyl 4-bromobutyrate to generate the ester **5**. Ester **5** was deprotonated with LDA at -78 °C and treated with 1-iodo-2-methylpropane to produce the corresponding racemic ester **6**. Basic hydrolysis of **6** yielded the racemic acid **7** that was coupled with (*S*)-leucinol to produce the diastereomeric compounds **8** and **9**, which were easily separated by silica gel column chromatography (**8** being the faster-moving isomer). Oxidation of **8** by m-chloroperbenzoic acid generated the sulfonyl compound **10**, which on further oxidation by sulfur trioxide-pyridine complex in DMSO-CH_2Cl_2 produced the aldehyde **2**. Similar two-step transformation of **9** gave **3** via **11**. The stereochemistry around the pseudo-P_2 site in **2** and **3** was tentatively assigned as (*S*) and (*R*), respectively, based on comparison of the calpain inhibitory activity of **2-3** with that of a reference dipeptidyl aldehyde of known absolute configuration (see below). In order to examine whether different oxidation states of sulfur have any effect on inhibitory properties of this class of molecules, we also synthesized corresponding sulfide and sulfoxide analogs of compound **3**. Thus oxidation of compound **9** by sulfur trioxide-pyridine complex in DMSO-CH_2Cl_2 produced the aldehyde **12** (Scheme 2), while oxidation of **9** by Davis' oxaziridine[7] generated corresponding sulfoxide **13** (diastereomeric mixture, epimeric at sulfoxide center) which was subjected to further oxidation by sulfur trioxide-pyridine complex in DMSO-CH_2Cl_2 to produce the aldehyde **14**.

The biological activities of the compounds were determined using recombinant human calpain I, prepared as described by Meyer et al.[8] with Suc-Leu-Tyr-MNA (Enzyme System Products, Dublin, CA) as substrate.[6] Inhibitory activities of the compounds **2-3, 12, 14** (diastereomeric mixture, epimeric at sulfoxide center), and a reference dipeptidyl aldehyde Cbz-Val-Phe-H (**15**)[9] are shown in Table 1.

Table 1. Inhibitory Activities of the Compounds **2-3, 12, 14,** and **15**[a]

Compound	Calpain I (IC_{50} nM)	Cathepsin B (IC_{50} nM)	Thrombin (% inh at 10 μM)	α-Chymotrypsin (% inh at 10 μM)
2	500	4500	2	12
3	50	150	1	11
12	75	60	5	11
14	30	60	2	18
15	11	-	-	-

[a] $n \geq 3$ in all cases.

As shown in Table 1, compound **14** (IC_{50} 30 nM) compares favorably with a reference dipeptidyl aldehyde Cbz-Val-Phe-H (**15**, IC_{50} 11 nM in this assay). Interestingly, in the diastereomeric pair, inhibitor **3** is 10 times more potent than inhibitor **2** indicating the strict stereochemical requirement of calpain for the pseudo P_2 site of

this class of inhibitor. It should be noted that compound **14** is >1.5 and 2.5 times more potent than compounds **3** and **12**, respectively. Table 1 also displays the inhibitory activity of these compounds against cathepsin B, a related cysteine protease (substrate used: Cbz-Phe-Arg-AMC) and thrombin (substrate used: Cbz-Phe-Val-Arg-AMC) and α-chymotrypsin (substrate used: Succ-Ala-Ala-Pro-Phe-AMC), two serine proteases.

In conclusion, compounds **3**, **12**, and **14** represent novel additions to a growing class of potent nonpeptidic inhibitors of human calpain I. Work is currently underway to determine the cellular activities of these compounds and will be reported in due course.

Acknowledgment: We thank Dr. Jeffry Vaught for his support and encouragement, Drs. James C. Kauer, Ron Bihovsky, Mark A. Ator, Mohamed Iqbal, Rabindranath Tripathy, Gregory Wells, and Satish Mallya for many helpful discussions, and Dr. Ming Tao for supplying us with Davis' oxaziridine. We are pleased to acknowledge SmithKline Beecham Pharmaceuticals for partial support of this research.

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(*Received in USA* 2 *October* 1996; *accepted* 19 *December* 1996)