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1,2-Benzothiazine 1,1-dioxide α-ketoamide analogues as potent calpain I inhibitors

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Abstract—A series of potent 1,2-benzothiazine 1,1-dioxide α-ketoamide inhibitors of calpain I is described.

Calpains comprise a family of nonlysosomal, calcium-activated cysteine proteases that are virtually ubiquitous throughout the animal kingdom. Present in most mammalian cells, including neurons, they have been implicated in a host of neurodegenerative disorders including stroke, traumatic brain injury, spinal cord trauma, Alzheimer's disease, Parkinson's disease, multiple sclerosis, motor neuron damage, and muscular dystrophy.[1] The two major forms, calpain I and calpain II, have identical catalytic domains and are present in the cytoplasm of neurons but are activated by varying concentrations of calcium ion, the former by low micromolar and the latter by low millimolar concentrations. Consequently, it is believed calpain I is the predominant form activated by elevated calcium levels associated with neurodegenerative processes.

Previously, we reported on the discovery and synthesis of a series of 3,4-dihydro-1,2-benzothiazine-3-carboxylate 1,1-dioxide aldehydes as potent reversible calpain I inhibitors.[2] This novel class of peptide mimetics displayed comparable potency to previously reported di- and tripeptide aldehydes (e.g., *Z*-Val-Phe-H, $IC_{50} = 11$ nM),[3,4] and compared favorably as well to another novel class of peptide mimetics discovered in this laboratory, *N*-alkanesulfonyl dipeptide aldehydes and α-ketoamides possessing D-amino acid residues in the P_2 region,[5] from which they were designed (Fig. 1).

In an effort to improve on the inherent liabilities generally observed with aldehydes (e.g., rapid metabolism, lack of selectivity, potential toxicity), we embarked on a program to replace this functionality with one intrinsically more stable and amenable to drug development. Since our experience with several previously reported classes of irreversible inhibitors (e.g., halomethyl-, acyloxymethyl-, and benzotriazolyloxymethyl ketones; phosphorous-based oxymethyl ketones) was generally

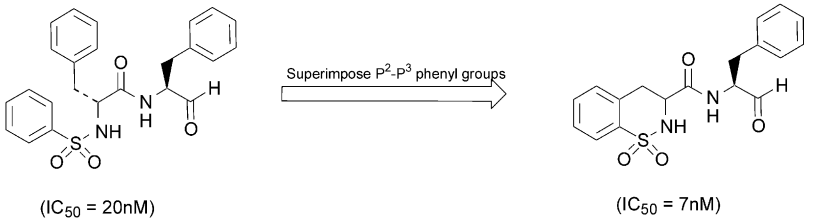

Figure 1. Conceptual design of 3,4-dihydro-1,2-benzothiazine 1,1-dioxide ring system from *N*-benzenesulfonyl D-phenylalanyl-L-phenylalanal.

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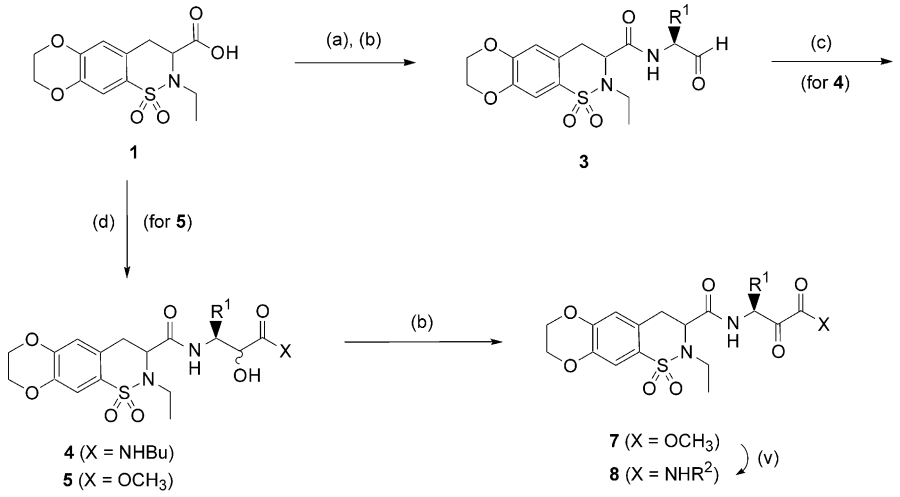
unfavorable,[6] we chose to incorporate an α-ketoamide group as one more likely to impart more desirable physicochemical and biological properties. This and related α-dicarbonyl derivatives have previously been shown to inhibit cysteine and serine proteinases reversibly with good potency and selectivity.[4,7] The synthesis and biological activity of a series of such 3,4-dihydro-1,2-benzothiazine 1,1-dioxide α-ketoamides are described in this report.

The synthesis of the 3,4-dihydro-1,2-benzothiazine-2-carboxylic acid 1,1-dioxide ring system has been described previously.[2,8] Since our earlier work in the aldehyde series showed comparable in-vitro potency for both 6-chloro and 6,7-ethylenedioxy substituents on the benzene ring, we decided to focus on 6,7-ethylenedioxy analogues due to their easier synthetic accessibility. Furthermore, as discussed below, the ketoamides were more sensitive to structural variation than their corresponding aldehyde congeners and we found that the 6,7-ethylenedioxy substituents provided significantly more active α-ketoamides than the 6-chloro substituent. The synthesis of α-ketoamides **8a–d** is shown in Scheme 1. Standard peptide coupling (HOBt/BOP/NMM) of **1** with β-aminoalcohols (**2**) followed by oxidation with Dess–Martin periodinane gave aldehydes **3,** which were submitted to Passerini-like conditions[9] with butyl isocyanide to give α-hydroxyamides **4**. Subsequent Dess–Martin oxidation gave the targets. Alternatively, coupling of **1** with α-hydroxyesters **6**[10] followed by Dess–Martin oxidation gave α-ketoesters **7**. Treatment of these esters with primary amines at ambient temperature in the absence of solvent gave α-ketoamides **8e–q**.

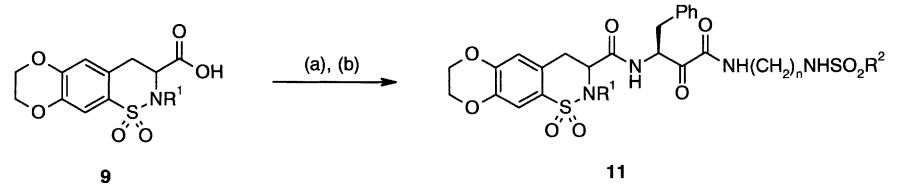
Also prepared were a series of P′-extended sulfonamides as shown by Scheme 2. Thus, HOBt/BOP coupling of **9** with **10**[11] followed by Dess–Martin oxidation gave inhibitors **11a–k**.

All compounds were prepared as an equimolar mixture of diasteromers, being racemic at the methine carbon on the benzothiazine ring and with the (*S*)-configuration at P^1. Compounds **8a–d** were separated into their individual diastereomers by conventional flash chromatography on silica gel. In our previous work with related aldehydes, the more active diastereomer was shown to have the (*S*)-configuration at the chiral methine carbon, as demonstrated by a stereoselective synthesis of the ring system via L-DOPA.[2] Presumably, this same relative configuration is responsible for the more active isomers with α-ketoamides as well.

In-vitro assays were performed with recombinant human calpain I, as previously described.[12] Several analogues display good in-vitro activity and suggest that a medium length *n*-alkyl group like butyl (**8d**, 50 nM) or phenethyl (**8k**, 63 nM) is favored over shorter, more extended, or branched aliphatic groups (see Table 1). Various heterocyclic substituents in this P′-region (**8m–q**, 170–5000 nM) were also disfavored. Notably, in Molt-4 cells (intact human T-cell leukemia cell line, a measure of cell permeability) **8c** was fairly potent



Scheme 1. Synthesis of compounds of Table 1. Reagents: (a) $H_2NCH(R^1)CH_2OH$ (2), HOBt, BOP, NMM, DMF, 0-20 °C, 4–18 h; (b) Dess–Martin periodinane, CH_2Cl_2, 0–20 °C, 1–24 h; (c) BuNC, $TiCl_4$, CH_2Cl_2, 0 °C, 4–8 h; (d) $HCl–H_2NCH(CH_2Ph)CH(OH)CO_2CH_3$ (**6**), HOBt, BOP, NMM, DMF, 0–20 °C, 4–18 h; (v) R_2NH_2 (neat), rt.



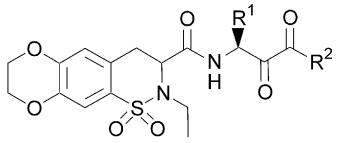
Scheme 2. Synthesis of compounds of Table 2. Reagents: (a) $HCl–H_2NCH(CH_2Ph)CH(OH)CONH(CH_2)_nNHSO_2R_2$ (**10**), HOBt, BOP, NMM, DMF, rt; (b) Dess–Martin periodinane, CH_2Cl_2, *t*-BuOH, 0–20 °C, 1–24 h.

(IC_{50} = 2.1 μM) and was detected at levels up to 1 μM in rat brain following a 1-h infusion iv at 20 mg/kg.[13]

Earlier work in these laboratories reported on the improved potency of α-ketoamides possessing P′-extended arylsulfonamides over simpler alkyl groups,[11] prompting the synthesis of the analogues presented in Table 2. Indeed, various arylsulfonamidoethylene- and propylene- derived analogues showed generally higher potency (e.g., **11i**, 29 nM) than most non-sulfonamides studied. In particular, the pyridylthiophene analogue produced the most active inhibitor (**11k**, 20 nM) and showed the best potency in Molt-4 cells as well (IC_{50} = 1.3 μM).

As we reported earlier for the corresponding aldehydes, we found the selectivity of the α-ketoamides also to be excellent against related cysteine and serine proteases.

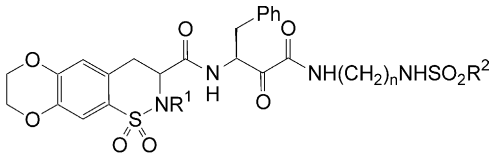
Table 1. Inhibitory activity of dihydrobenzothiazine α-ketoamide analogues



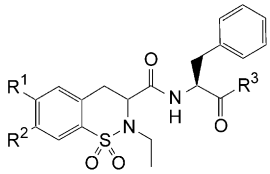
Compd	R^1	R^2	IC_{50} (nM)
7	CH_2Ph	OCH_3	1000
8a	*i*-Bu	NHBu	1000[a]
8b	*i*-Bu	NHBu	500[a]
8c	CH_2Ph	NHBu	300[a]
8d	CH_2Ph	NHBu	50[a]
8e	CH_2Ph	NEt	340
8f	CH_2Ph	$NHCH_2CH_2OCH_3$	200
8g	CH_2Ph	NH-*i*-Pr	205
8h	CH_2Ph	$NHCH_2$-cyclopropyl	286
8i	CH_2Ph	$NH(CH_2)_4CH_3$	150
8j	CH_2Ph	$NHCH_2Ph$	81
8k	CH_2Ph	$NHCH_2CH_2Ph$	63
8l	CH_2Ph	$NHCH_2CH{=}CH_2$	200
8m	CH_2Ph	$NH(CH_2)_3$-(imidazol-1-yl)	5000
8n	CH_2Ph	$NH(CH_2)_3$-(2-ketopyrrolidin-1-yl)	500
8o	CH_2Ph	$NH(CH_2)_3$-(morpholin-4-yl)	195
8p	CH_2Ph	$NHCH_2$(pyridin-2-yl)	170
8q	CH_2Ph	$NHCH_2$(pyridin-4-yl)	240

[a] Single diastereomer.

Table 2. Activity of P′-extended sulfonamide analogues

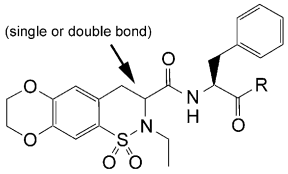


Compd	R^1	n	R^2	IC_{50} (nM)	Molt-4 Cells (μM)
11a	Et	2	CH_3	89	N.D.
11b	H	2	Ph	76	N.D.
11c	Et	2	Ph	40	5.0
11d	Et	3	Ph	35	2.7
11e	Et	2	4-NO_2–Ph	47	4.2
11f	Et	3	4-NO_2–Ph	50	3.6
11g	Et	2	3,4-Cl_2–Ph	56	N.D.
11h	Et	3	3,4-Cl_2–Ph	56	N.D.
11i	Et	2	4-F–Ph	29	2.0
11j	Et	3	4-F–Ph	50	2.4
11k	Et	2	5-(2-Pyridinyl)-thiophenyl-2-yl	20	1.3



R^1	R^2	R^3	IC_{50} (nM)
Cl	H	H	5[a]
-OCH_2CH_2O-		H	7[a]
Cl	H	CONHEt	1000
-OCH_2CH_2O-		CONHEt	340

[a]Ref. 2.



R	Bond type	IC_{50} (nM)
H	Single	7[a]
H	Double	8[a]
CONHBu	Single	50
CONHBu	Double	155

Figure 2. SAR of representative analogues illustrating enhanced sensitivity of α-ketoamide versus aldehydes to structural changes.

For example, **8c**, **11c** and **11k** were essentially inactive against cathepsin B and chymotrypsin (IC_{50} = 1–10 μM).

It should be noted that we as well as others have reported on the generally lower potency and relatively narrower structural constraints of α-ketoamides versus their aldehyde counterparts, and benzothiazines are no exception in this regard. As shown in Figure 2, both chloro and alkoxy substituents on the benzene ring, or the presence of either a single or double bond on the thiazine ring, were quite potent in the aldehyde series whereas their α-ketoamide congeners were significantly less potent and more sensitive to such changes. It is possible that the generally higher electrophilicity of aldehydes over α-dicarbonyl groups, along with the apparent strict P'_1–P'_3 structural requirements for efficient ligation in this region, conspire to make α-dicarbonyl analogues a significantly greater challenge to achieving comparable in-vitro potency.

In this paper, we have described a series of potent 3,4-dihydro-1,2-benzothiazine 1,1-dioxide α-ketoamide calpain I inhibitors. Further exploration of P′-spanning sulfonamide groups resulted in analogues rivaling the potency observed with their aldehyde counterparts. Molt-4 cell activity and brain levels of key analogues were comparable to other peptides and mimetics studied previously. Future progress in this area will hinge on advances in improving solubility and consequent formulatability.

Acknowledgements

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