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Synthesis and Biological Evaluation of Novel Piperidine Carboxamide Derived Calpain Inhibitors

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Abstract—Calpain inhibitors which are derived from piperidine carboxamides in the P^2 region were prepared and evaluated for μ-calpain inhibition. In particular, the keto amides **11f** and **11j** have K_i of 30 and 9 nM and display a more than 100-fold selectivity over the closely related cysteine protease cathepsin B. Furthermore, these compounds inhibit NMDA induced convulsions in mice indicating that calpain inhibition in brain results in some anticonvulsive properties.

Calpains represent a class of intracellular cysteine proteases of which in most mammalian cells, at least one individual member is found.[1] Calpains are proposed to be involved in many intracellular processes and their enhanced activation would result in serious cellular damage or even cell death.[2] In a number of pathological conditions, excessive calpain activities have been observed and calpains have been suggested to be involved in the progress of certain diseases.[3] Indeed, calpain inhibition has shown to have beneficial effects in experimental models, for example stroke,[4] myocardial infarction,[5] brain trauma[6] and muscular dystrophy.[7] Consequently, calpain inhibitors have attracted some attention in drug research and development with the purpose of proving their therapeutic benefits in the clinic.

A number of reversible and irreversible calpain inhibitors have been discovered such as MDL 28170 **1** and the proline derivative **2**.[8] But most of them have problems regarding selectivity, water-solubility, metabolic stability in vivo or cellular penetration.[9] Recently, several novel potent nonpeptidic calpain inhibitors have been reported.[10] We have also addressed some efforts to obtain novel calpain inhibitors, in particular inhibitors for calpain I or μ-calpain. Our initial efforts focused on replacing an amino acid moiety within the peptidic inhibitors by residues which may improve stability. Some variations are tolerated within the P^2 region and even cyclic amines such as proline **2** are potent calpain inhibitors.[11] We incorporated piperidine 4-carboxylates as building blocks into the P^2–P^3 region which led to the piperidine keto amides **3** as novel calpain inhibitors (Scheme 1).

The calpain inhibitors **11** were synthesized using two different routes outlined in Scheme 2. Route A corresponds to a method by Li et al.[12] which includes a Dakin–West-type reaction (steps c and d) to build up the keto ester moiety. Several examples could be prepared according to this route but only with low or moderate yields. The amino alcohol **9** was synthesized from **8** by a multi-step procedure.[13] Compound **9** was coupled to the piperidine carboxylate **5** using the convenient EDC/HOBT method to obtain **10** which subsequently was converted into the envisaged keto amide **11** by oxidation with either DMSO/pyr·SO_3 or DMSO/EDC. Since **9** and **10** were employed as single diastereomers, an enantiomeric keto amide **11** should be obtained after oxidation. In all cases **11** had been isolated as racemate which may be attributed to immediate racemization in solution.[13] Route A gave the racemic compounds in any case since the Dakin–West reaction destroyed the chirality in the amino acid derivative **4**.[12]

The biological activities of the prepared compounds **11** were evaluated in a common enzyme assay[14] using human μ-calpain isolated from erythrocytes and Suc-Leu-Tyr-AMC as fluorogenic substrate. The inhibition of cathepsin B was tested in a corresponding assay using human cathepsin B.[15] The results are shown in Table 1.

Several potent calpain inhibitors have been discovered within this piperidine set. The most potent derivatives, the naphthalene **11f** and the benzothiophene **11j**, show

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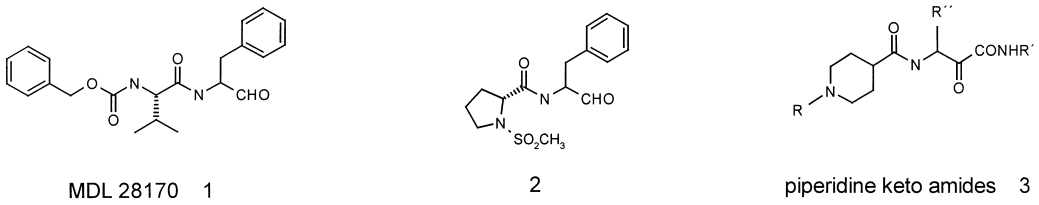
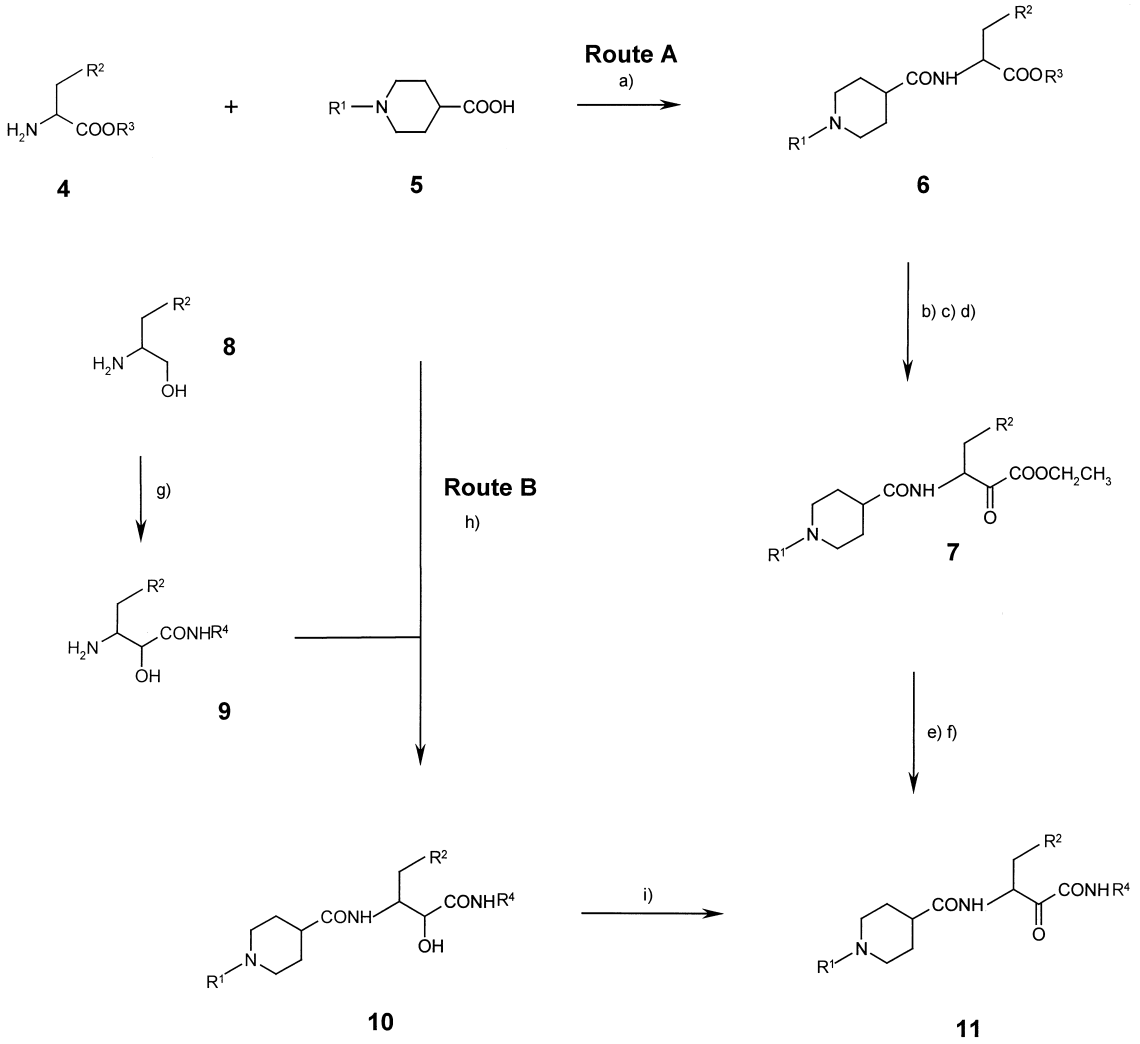


Scheme 1.



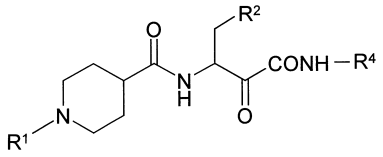
Scheme 2. (a) EDC, HOBT; (b) LiOH, rt; (c) EtOOC-COCl, THF, reflux; (d) EtONa, EtOH, rt; (e) $(CH_2SH)_2$, $BF_3 \times Et_2O$, CH_2Cl_2, rt; (f) R^4-NH_2, rt; (g) 1. DMSO, py × SO_3, rt; 2. KCN; 3. HCl, Et_2O, reflux; (h) EDC, HOBT; (i) DMSO, py × SO_3, rt.

potency comparable to that of the aldehyde derived inhibitor MDL 28170 **1**, which is one of the most potent calpain inhibitors known. We focused our interests on keto amides which we expected to show improved in vivo stability compared to aldehydes. Nevertheless, several naphthalene derivatives of **11** had been prepared as aldehydes which show potencies in calpain inhibition comparable to that of the keto amides (data not shown).

The set of the synthesized derivatives indicate a structure–activity relationship (SAR) of the piperidines. The nature of the lipophilic side chain R^2 may have considerable importance since this P^1 region represents the recognition site of calpain substrates.[16] In the present series, we limited the R^2 variations to hydrocarbon residues. In the cinnamoylic set **11b**, **11c** and **11d**, in which the methyl group of **11b** was replaced by isopropyl or phenyl, the potency increases 20- and 70-fold, respectively, demonstrating the beneficial effects of larger residues in R^2.

A simple benzoyl moiety attached to the piperidino group in the P^3 region results in only a moderate activity **11a** ($K_i = 0.56\,\mu M$). Incorporation of an ethylene bridge as spacer between phenyl ring and amide bond has no influence on potency, which may indicate that there is an extended liphophilic pocket at the enzyme binding site that is not optimally occupied by these residues.

Table 1. Synthesized novel calpain inhibitors and the results of testing calpain inhibition



11

Compound	R^1	R^2	R^4	Synthetic route[a]	Calpain inhibition K_i (μM)[b]
11a	Phenyl-CO-	Ph	H	B	0.56
11b	E-Ph-CH=CH-CO-	CH_3	H	A	33
11c	E-Ph-CH=CH-CO-	$CH(CH_3)_2$	H	A	1.35
11d	E-Ph-CH=CH-CO-	Ph	H	A	0.46
11e	E-(4-Py)-CH=CH-CO-	Ph	H	B	13.5
11f	2-Naphthyl-CO-	Ph	H	B	0.030
11g	2-Naphthyl-CH_2-	Ph	H	B	13.6
11h	3-Quinolyl-CO	Ph	H	B	0.67
11i	6-Quinolyl-CO-	Ph	H	B	0.16
11j	2-Benzothienyl-CO-	Ph	H	B	0.009
11k	2-Naphthyl-CO-	Ph	$(CH_2)_3$-1-Morpholine	A	0.96
11l	2-Naphthyl-SO_2-	Ph	H	A	0.45
11m	2-Naphthyl-SO_2-	Ph	CH_2CH_3	A	800
11n	2-Naphthyl-SO_2-	Ph	$(CH_2)_3$-1-Morpholine	A	0.81
MDL 28170 **1**					0.015

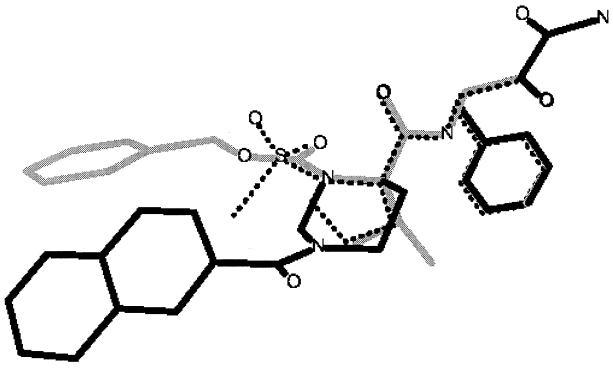
[a]See Scheme 2.
[b]The values of the inhibition constants K_i are mean values for two or more independent experiments.

Likewise, the replacement of the benzoyl residue by naphthalene (**11f**) caused an 18-fold improvement of the K_i as compared to **11a**. Also, the benzothiophen derivative **11j** has a K_i of 9 nM, which is the most potent inhibitor shown in this study. Incorporation of *N*-heterocycles such as a pyridine (**11e**) and quinolines (**11h**, **11i**) has no advantage, indicating that lipophilic residues are preferred in this region.

The amido group in P^2/P^3 is supposed to enter a hydrogen bond interaction with enzyme residues. The importance of this amido group is demonstrated by the sulfonamide **11l** and amino methyl derivative **11g** which are >10- and >100-fold less active, respectively.

Furthermore, to explore the importance of substitution at the keto amido group we selected residues which had been demonstrated to be beneficial in peptidic inhibitors.[12,17] We examined an alkyl morpholine (**11k** and **11n**) as well as an ethyl residue (**11m**), but all derivatives have no advantage over the unsubstituted compounds in respect to enzyme inhibition (Table 1).

The above results indicate important differences in the SAR of the peptidic inhibitors and the piperidines, which may be attributed to distinct orientations of the inhibitors within the competitive binding site at the enzyme. To elucidate this we modeled three inhibitors, MDL 28170 **1**, the proline derivative **2** and the naphthalene **11f**. The result is shown in Scheme 3. For the piperidine we considered several conformations and only the most favored chair conformation is depicted in Scheme 3. The superimposition was performed to obtain an optimal overlap of the active carbonyl group and the structural fragment in P^1. Scheme 3 discloses the different orientations of the naphthalene residue in **11f** compared



Scheme 3. Calpain inhibitors MDL 28170 **1** (grey line), the proline aldehyde **2** (black dotted line) and the piperidine keto amide **11e** (black bold line) have been built up by *Corina*. Finally all three compounds were superimposed to give an optimal overlap of the P^1 region.

with the phenyl ring in **1**. Both carbonyl groups, attached either to the naphthalene in **11f** and incorporated in the urethane moiety of **1**, are also distinctly orientated. It is not excluded that rotations, which are quite possible, may enable hydrogen bond interaction between the carbonyl groups and the enzyme. Nevertheless, the beneficial effects of the naphthalene ring or even more the benzothienyl residue in **11j** suggest a further lipophilic hole at the enzyme peptide backbone which can be utilized for designing novel inhibitors.

It is well known that MDL 28170 **1** and many other aldehydes derived calpain inhibitors also inhibit closely related cysteine proteases such as cathepsin B.[18] Therefore, the above-mentioned novel inhibitors were tested for their potency to inhibit cathepsin B. The results are shown in Table 2. Both inhibitors, **11f** and **11j**, were only moderate inhibitors of cathepsin B with K_i values

Table 2.

	Calpain[a] K_i/μM	Cathepsin B[a] K_i/μM	pp60src[b] IC_{50}/μM	NMDA convulsions[c] ED_{50}/mg/kg
11f	0.030	7.300	1.8	1.3
11j	0.009	3.200	nt[d]	1.0
MDL 28170 **1**	0.015	0.025[18]	0.7	nt

[a]The values of the inhibition constants K_i are mean values of two or more independent experiments.
[b]Inhibition of the tyrosine kinase pp60src degradation.
[c]Inhibition of the lethal convulsions induced by NMDA in mice.
[d]Not tested.

in the μM range. Thus, **11f** and **11j** represent potent calpain inhibitors which show a >100-fold selectivity over a closely related protease.

Since calpain is an intracellular enzyme, inhibitors have to be evaluated for their ability to penetrate into cells. This could be estimated in cellular assays in which inhibitors block the intracellular calpain mediated protein degradation. In platelets, calpain which was activated by the calcium ionophore A23187 cleaves the tyrosine kinase pp60src in a specific manner.[19] Calpain inhibitors block this degradation and, indeed, MDL 28170 and the tested piperidine **11f** inhibit this degradation with IC_{50}s of 0.7 and 1.8 μM, respectively, indicating that both compounds penetrate into cells and have comparable potencies (Table 2).

It had been reported that calpain inhibitors attentuate cellular toxicity induced by glutamate receptors agonists such as AMPA and glutamate.[20] This has raised the question whether calpain inhibitors also modify the glutamate receptor mediated effects in vivo. Therefore, we have evaluated the ability of calpain inhibitors to suppress convulsion induced by the glutamate receptor subtype agonist *N*-methyl-D-aspartic acid (NMDA) in vivo, a model which is generally used to characterize glutamate receptor antagonists.[21] Both piperidines **11f** and **11g** were tested in this model and, remarkably, both compounds exert potent anticonvulsive properties (see Table 2).[22] Their ED_{50}s were determined to be 1.3 mg/kg and 1.0 mg/kg which are rather low dosages in this model even compared with many NMDA antagonists. Nevertheless, whether calpain inhibition is responsible for this anticonvulsive property is not conclusive but, so far tested, there are no hints that these compounds act via other molecular targets such as glutamate receptors (data not shown). On the other hand, this result may indicate that these calpain inhibitors reached sufficient levels in brain even when administered systemically which makes these compounds suitable as tools for further testing.

In summary, we have outlined the synthesis and a brief SAR of novel piperidines as μ-calpain inhibitors. In particular, the piperidines **11f** and **11j** both derived from ketoamides are potent calpain inhibitors and disclose high selectivity versus the closely related cysteine protease cathepsin B. It was also shown that **11f** is effective in inhibiting the calpain mediated degradation of the tyrosine kinase pp60src. Finally, we have shown that two calpain inhibitors are able to suppress seizures induced by NMDA which also indicates that these inhibitors penetrate into brain when administered systemically. Altogether, the piperidines represent novel calpain inhibitors which are suitable tools to investigate the importance of calpain in animals.

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22. In this model, severe convulsions were induced in unanesthetized mice by intracerebroventricular injection of 10 μL of a 0.035% NMDA solution resulting in severe convulsions and death of animals. The calpain inhibitors were administered intraperitoneally (ip) 120 min prior to NMDA and the ED_{50} values were calculated as the dose which protected 50% of the animals.