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Derivatives of 3-*sec*-Butyl-1-oxo-2,3-dihydroisoquinoline as Inhibitors of μ-Calpain**

Roberto Chicharro,[a] Mercedes Alonso,[a] M. Teresa Mazo,[a] Vicente J. Arán,[b] and Bernardo Herradón*[a]

The calpains (E.C. 3.4.22.17) are a family of cysteine proteases and consist of about 20 isoenzymes.[1] Although most of these isoenzymes are present in only particular tissues or species, two isoenzymes, calpain I (or μ-calpain) and calpain II (or *m*-calpain), are ubiquitous. A distinctive mechanistic feature of calpain is that the enzyme is activated by Ca^{2+}; the in vitro Ca^{2+} concentration required for half-maximal activity of μ-calpain is 2–75 μM and for *m*-calpain, 200–1000 μM. As these concentrations are exceptionally high for in vivo activity, several alternative mechanisms for calpain activation have been proposed.[2] Both μ-calpain and *m*-calpain are reported to be involved in a wide variety of metabolic and physiological processes.[3] The following are some points about the biochemistry of calpain: 1) many proteins are partially hydrolyzed by the action of calpain,[4] 2) no natural specific substrates have been identified,[5] and 3) calpain is activated in cases of cellular stress.[6] These facts indicate that calpain is likely an unspecific protease without any precise physiological function,[7] and that it is activated under conditions of stress to hydrolyze many proteins, thus contributing to processes of cell death.[8] Owing to its role as a "protein destroyer", overly active calpain is involved in several degenerative diseases.[9] Therefore, calpain is a potential therapeutic target, and the search for inhibitors is an important pursuit. Nevertheless, this area of research is still underdeveloped, and there are few reported calpain inhibitors.[10] Most inhibitors are short peptide or peptidomimetic compounds with two to four hydrophobic or aromatic amino acids, frequently equipped with additional aromatic fragments. Similar to the inhibitors of other proteases, many calpain inhibitors have reactive electrophilic functional groups such as aldehydes, α-dicarbonyl groups, and α-heterosubstituted ketones.[11]

Previously, we reported that some derivatives of isoquinoline and pyrido[1,2-*b*]isoquinoline are calpain inhibitors.[12] However, our initial experiments showed that the parent heterocycles **1**–**4** (Figure 1) are either weak inhibitors or are not inhibitors at all; the presence of peptidic fragments is necessary for calpain inactivation. Compounds **5** and **6** are examples of peptide–heterocycle hybrids that have inhibitory activity toward calpain. As compounds of high molecular weight are unlikely to be useful as pharmaceuticals, we simplified the structure of the isoquinoline derivatives. Based on preliminary qualitative structure–activity relationship studies which indicated that the presence of a bulky hydrophobic substituent at position 3 of the isoquinoline ring increases biological activity, we decided to prepare isoquinoline derivatives with a *sec*-butyl group at position 3.[13] This expectation was fulfilled through the simple isoquinoline derivative **7** (Figure 1), which is a potent inhibitor of μ-calpain. The synthesis of **7** and several derivatives as well as their activities as calpain inhibitors are reported herein.

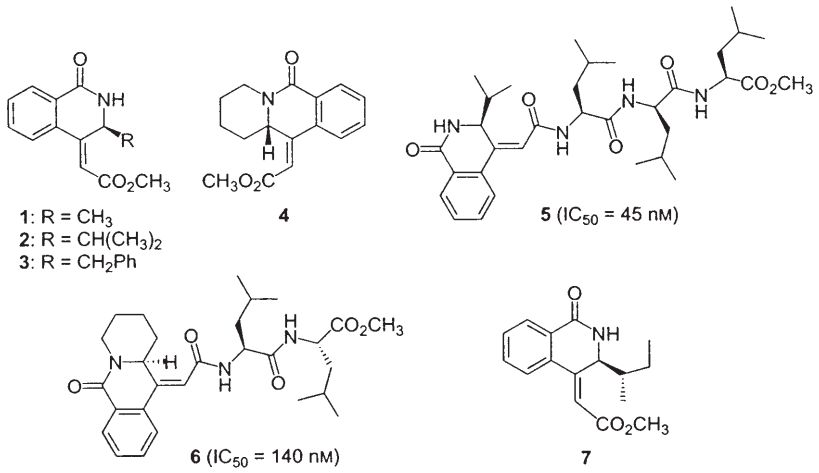

Figure 1. Heterocycles and peptide–heterocycle hybrids.

The target compound **7** was synthesized by a method similar to that previously reported for heterocycles **1**–**4**.[12,14,15] The synthesis (Scheme 1) starts from (*S*,*S*)-isoleucine methyl ester hydrochloride (**8**), which was acylated to the *N*-(2-iodobenzoyl) derivative **9**, the methoxycarbonyl group of which was chemoselectively reduced ($LiBH_4$/MeOH) to the primary alcohol **10**. This, in turn, was submitted to a sequential, one-pot Swern–Wittig reaction to give the N-acylated γ-amino-α,β-unsaturated ester **11** with *E* configuration at the double bond, as assessed by ^1H NMR (15.8 Hz coupling constant). The final step, the intramolecular cyclization of **11** to **7**, was carried out through a Heck reaction[16] under conditions similar to those reported by Overman and co-workers,[17] but in the absence of Ag^I salt[14b] to afford the target molecule **7** in good chemical yield and as a single stereoisomer and regioisomer. The synthetic sequence

[a] *R. Chicharro, M. Alonso, M. T. Mazo, Dr. B. Herradón*
Instituto de Química Orgánica General (CSIC)
Juan de la Cierva 3, 28006 Madrid (Spain)
Fax: (+34) 915 644 853
E-mail: herradon@iqog.csic.es

[b] *Dr. V. J. Arán*
Instituto de Química Médica (CSIC)
Juan de la Cierva 3, 28006 Madrid (Spain)

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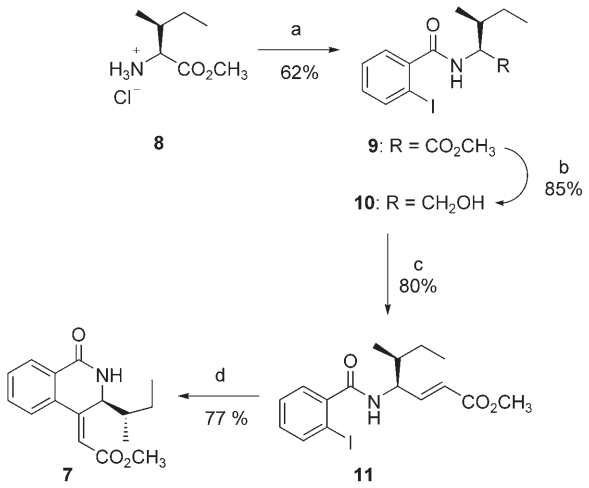


Scheme 1. Reagents and conditions: a) *o*-IC_6H_4COCl, K_2CO_3 (1 M aq.), THF, 0 °C→RT, overnight; b) $LiBH_4$, MeOH, THF, −10 °C→RT, 30 min; c) 1) DMSO/$(COCl)_2$, Et_3N, CH_2Cl_2, −78 °C, 2 h, 2) $Ph_3P{=}CHCO_2CH_3$, −78 °C→RT, overnight; d) $Pd(OAc)_2$, PPh_3, Et_3N, CH_3CN, reflux, 60 h.

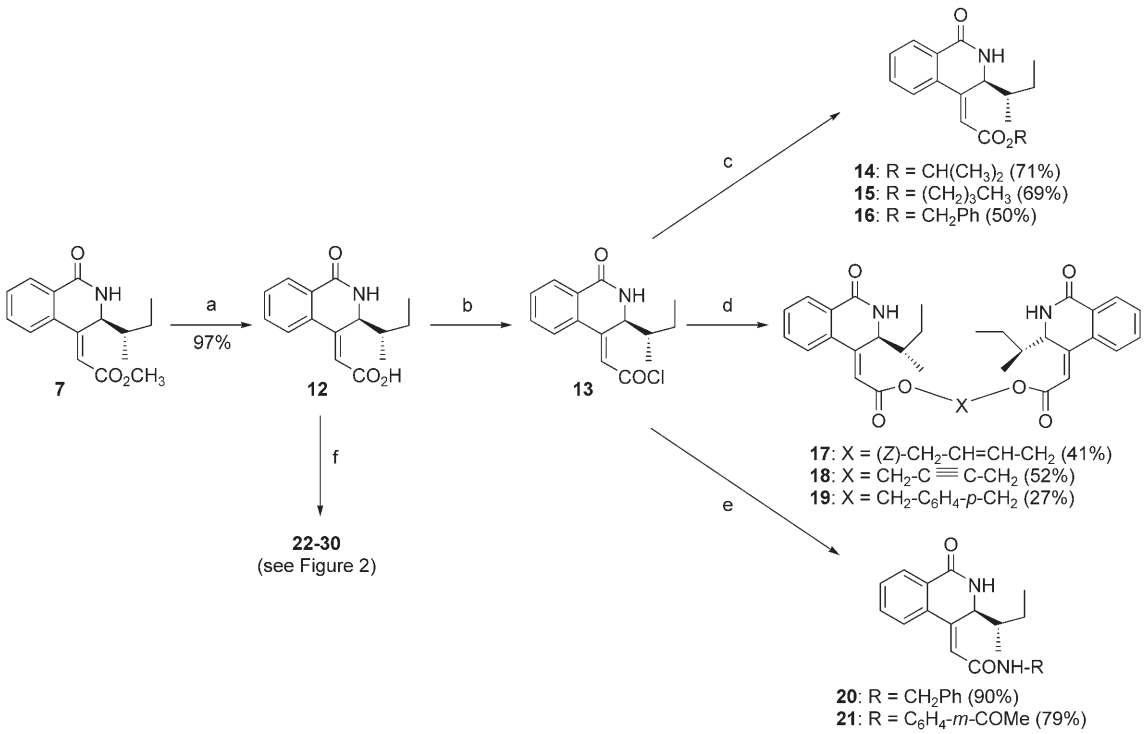
to **7** requires five reactions in four synthetic operations with good overall yield (34%) and total selectivity; furthermore, the synthesis can be carried out on the multigram scale (~10 g) with reproducible results.

With the objective to obtain derivatives of **7** with various substituents at position 4, we synthesized a variety of esters (**14**–**19**), amides (**20**–**21**), and peptide–isoquinoline hybrids (**22**–**30**) (Scheme 2). Thus, the methyl ester **7** was quantitatively hydrolyzed to the acid **12**, which, in turn, was converted into the acid chloride **13.** Without purification, **13** was allowed to react with alcohols, terminal diols, and amines to give the corresponding monoesters **14**–**16**, diesters **17**–**19**, and amides **20**–**21**, in 27–90% isolated yields from the acid (two steps). Although the yields for the preparation of the diesters **17**–**19** were moderate (27–52%), it must be noted that these were obtained in a three-step synthesis, and that there was no attempt to optimize the procedure.

The next synthetic targets were peptide–isoquinoline hybrids with a *sec*-butyl group at position 3 of the heterocycle. The rationale for the inhibitor design was discussed in a previous publication.[12] The results reported therein indicate that the active hybrids are those which have a peptide chain with two or three aliphatic, hydrophobic amino acids. Although the target molecules contain other amino acid residues, we were especially interested in the effect of isoleucine fragments toward activity.

The peptide–heterocycle hybrids **22**–**30** (Scheme 2, Figure 2) were obtained in moderate to good yields (43–83%) by reaction of the acid **12** with the corresponding peptide (**32**–**40**, Figure 3),[18] using 1-hydroxybenzotriazole (HOBT), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and 4-(dimethylamino)pyridine (DMAP) as promoters.[19] In contrast, the acid **31** (Figure 2) was prepared by hydrolysis (LiOH/MeOH/THF) of the hybrid **30**.

The isoquinolines **7** and **12**–**21**, and the peptide–isoquinoline hybrids **22**–**24**, **26**, **30**, and **31** were tested as calpain inhibitors by using a standard spectrofluorimetric method[20] with labeled casein as substrate and μ-calpain from porcine or human erythrocytes as the enzyme source.[21] The peptide al-



Scheme 2. Reagents and conditions: a) LiOH (1 M aq.), THF/H_2O, RT, overnight; b) $SOCl_2$, CH_2Cl_2, reflux, 1 h; c) ROH, CH_2Cl_2, RT overnight; d) HO-X-OH, CH_2Cl_2, RT, overnight; e) RNH_2, Et_3N, CH_2Cl_2, RT, overnight; f) HOBT, EDC, Et_3N, DMAP, peptide **32**–**40** (see Figure 3), DMF, RT, overnight.

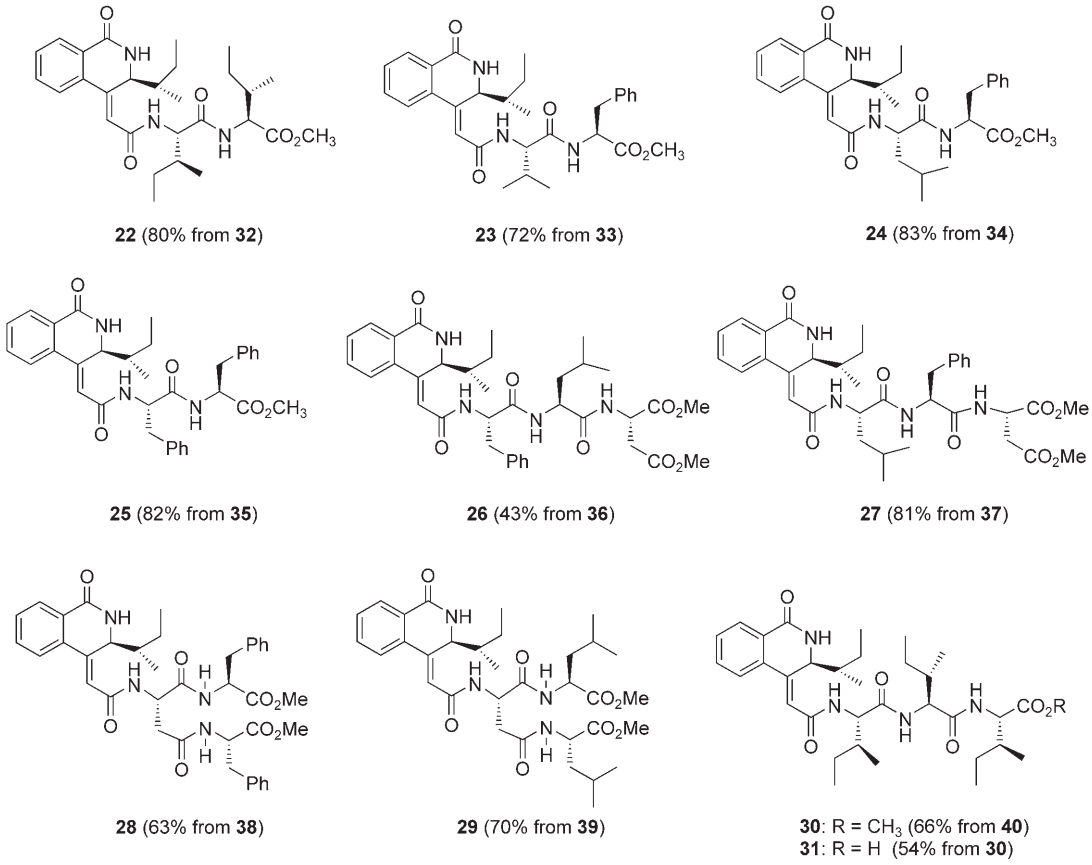


Figure 2. Peptide–isoquinoline hybrids **22**–**30** prepared from the acid **12** and the corresponding peptides **32**–**40** (see Figure 3). Values in brackets refer to isolated yields.

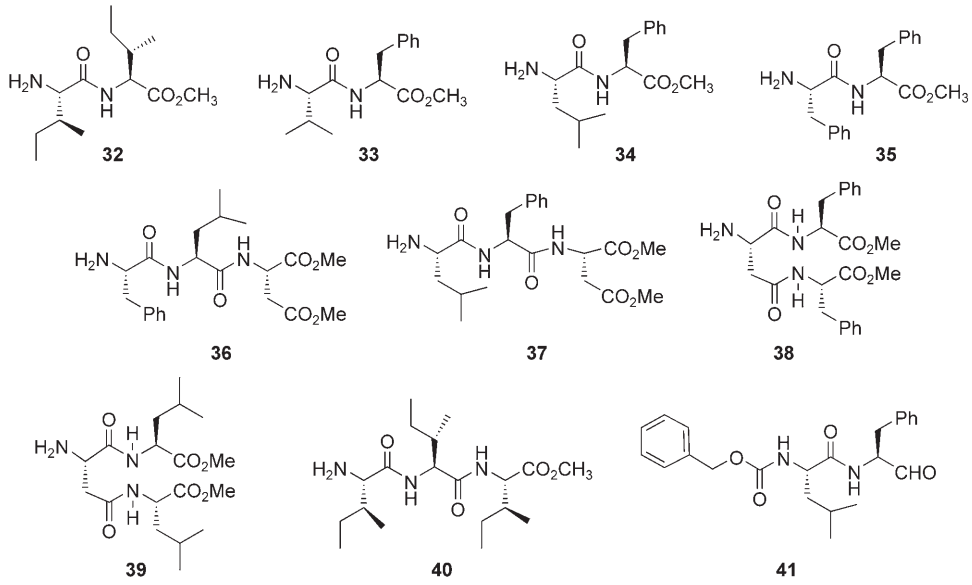


Figure 3. Peptides **32**–**40** used in the synthesis of the hybrids **22**–**30**. The structure of the reference compound **41** is also shown.

dehyde *Z*-Leu-Phe-H (**41**, Figure 3), which is one of the most potent calpain inhibitors reported to date (K_i on the order of 10 nM for human calpain, with fluorescent *N*-succinyl dipeptides as substrates),[22] was also tested under the same experimental conditions. This result allows a direct comparison of the inhibitory capacity of the isoquinoline derivatives with reported calpain inhibitors. The results (reported as IC_{50} values in Table 1) allow to some conclusions to be drawn on qualitative structure–activity relationships.

a) In our experiments, the known peptide aldehyde **41** has an IC_{50} value of 240 nM. The discrepancy with the reported result ($K_i \approx 10$ nM) is not surprising, as two different parameters and two different substrates are used to gauge the biological activity. Given that **41** is recognized as a very potent calpain inhibitor, we can conclude that the compounds with IC_{50} values lower than 240 nM are very active inhibitors.

b) The 3-*sec*-butyldihydroisoquinoline **7**, which is structurally quite simple and which can be considered a dipeptide

Table 1. Results of the inhibition of μ-calpain (porcine or human)[21] by isoquinoline derivatives and peptide–isoquinoline hybrids.

Compd	Enzyme	IC_{50} [μM]
7	porcine	0.025
12	porcine	inactive
14	porcine	inactive
15	porcine	>100
16	porcine	85
17	porcine	>100
18	porcine	59
19	human	5
20	porcine	>100
21	porcine	>100
22	porcine	0.447
23	porcine	0.626
24	human	0.066
26	human	inactive
30	porcine	0.159
31	porcine	inactive
41[a]	porcine	0.240

[a] The result for this peptide aldehyde is included for comparative purposes.

mimetic, is a highly potent calpain inhibitor; it is 200-fold more active than other isoquinolines with different substituents at position 3.[12]

c) Other 3-*sec*-butyldihydroisoquinolines, which lack amino acid residues at position 4, are either not inhibitors (acid **12** and ester **14**) or are weak inhibitors (esters **15** and **16**, diesters **17** and **18**, and amides **20** and **21**). Only the diester **19**, with three aromatic rings,[23] is a moderate inhibitor.

d) The peptide–isoquinoline hybrids show trends similar to those previously reported.[12] If the amino acid directly linked to the isoquinoline ring is aliphatic and hydrophobic (Ile, Val, or Leu), the hybrids are potent calpain inhibitors, as exemplified by compounds **22**–**24** and **30**. On the other hand, hybrids **26**, with Phe at the first position, and **31**, with a carboxy group at the C terminus, are not inhibitors.

In summary, we report the synthesis and biological evaluation of several 3-*sec*-butyldihydroisoquinolines and peptide–isoquinoline hybrids as inhibitors of μ-calpain. It was found that the relatively simple isoquinoline **7** is an excellent inhibitor of calpain, with an IC_{50} value of 25 nM. Other esters and amides related to **7** are either moderate or weak inhibitors, or are completely inactive. On the other hand, peptide–isoquinoline hybrids with an aliphatic hydrophobic amino acid at the N-terminal end of the peptide chain are very potent calpain inhibitors. Although a detailed structural study on **7** and related compounds is underway, we speculate that the mechanistic basis of these inhibitors involves reaction with the –SH group of cysteine in the active site of calpain, and the high activity of **7** arises from a conformation that causes unshielding of its electrophilic double bond.

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Keywords: calpain · inhibitors · isoquinoline · peptide–heterocycle hybrids · peptidomimetics

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