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Studies on calpain inhibitors. Synthesis of partially reduced isoquinoline-1-thione derivatives and conversion to functionalized 1-chloroisoquinolines ☆

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Abstract

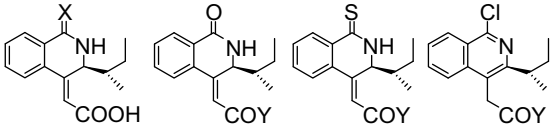
Sequential treatment of a (3-substituted-1-thioxo-1,2,3,4-tetrahydroisoquinolin-4-ylidene)acetic acid with thionyl chloride and a nucleophile did not give the expected ester or amide, but a derivative of 2-(3-substituted-1-chloroisoquinolin-4-yl)acetic acid, constituting a simple procedure for the synthesis of functionalized 1-chloroisoquinolines, which can be useful synthetic intermediates. The different reactivity between lactams and thiolactams has been computationally modelled. The activity as calpain inhibitors of both thiolactams and chloroisoquinoline has been measured, finding that some of these compounds are inhibitors in the micromolar range.

Keywords: Calpain inhibitor; Chloroisoquinoline; Lactam; Thiolactam

The calpain family of cysteine proteases catalyzes the partial hydrolysis of a huge variety of proteins,[1] constituting key regulators in a diversity of processes, such as signal transduction, apoptosis, cell cycle, neuroplasticity and cell motility.[2] Although several isoenzymes are known, the most relevant members are μ-calpain and *m*-calpain, which require, respectively, micromolar and millimolar amounts of Ca^{2+} to be fully active. The overactivation of calpain has been linked to several pathological conditions,[3] especially degenerative diseases, constituting a current pharmaceutical target.[4]

Our contribution to this area has included the design and synthesis of peptidic inhibitors derived from 3,6-dihydro-2*H*-pyrans,[5] biphenyl,[6] and nitrogenated heterocycles;[7] some of them with IC_{50} values in the picomolar range.[6a] Our previous results have shown that the presence of aromatic fragments in specific positions are essential for high activity.

Recently we have found that simple esters and amides (**A**) of the *sec*-butyl-substituted isoquinolinyl-acid **1** are potent inhibitors of μ-calpain;[8] constituting hit compounds for the development of pharmaceuticals.[9] With the objective of obtaining knowledge on structure–activity relationship, we intended to prepare thiolactams of type **B**. Since the thiocarbonyl group has different size, polarizability and capacity to form hydrogen bonds than the carbonyl group,[10] the biological activities of **A** and **B** might be quite different.



A **B** **C**

1, X = O
2, X = S

Y = OR, NHR

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However, our synthetic expectations were not fulfilled; thus, when we attempted to prepare thiolactams **B**, through the acid chloride derived from **2**, we obtained 1-chloroisoquinoline derivatives **C**. This interesting reactivity of thioamides has not been previously reported and it is quite different from that of the corresponding amides.[8] Additionally, the obtained 1-chloroisoquinolines are not readily available by other means and the method can be employed for the synthesis of other chlorinated heterocycles. In this Letter, we report the transformations of some thiolactams to 1-chloroisoquinolines as well as a mechanistic insight from computational modelling, and the biological activity of thiolactams and 1-chloroisoquinoline as inhibitors of μ-calpain.

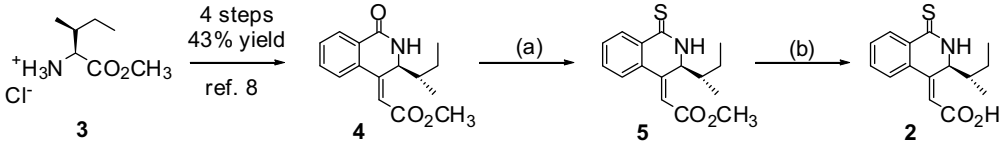
The methyl ester **4** was prepared in four steps from L-isoleucine methyl ester hydrochloride (**3**), as previously reported.[8] Compound **4** was reacted with Lawesson's reagent (LR)[11] to give the methyl ester **5**, which, in turn, was hydrolyzed to acid **2** in high overall yield (Scheme 1).[12]

In order to compare with the preceding biologically active compounds, our target molecule was diester **6**, which was planned to be prepared by the sequential treatment of acid **2** with thionyl chloride (to the acid chloride, not isolated) and 0.4 M equiv of 4-(hydroxymethyl)benzyl alcohol, as previously reported with the lactam analogues.[8] However, compound **6** was not formed, but 1-chloroisoquinoline **7** was obtained as the only non-polar compound. Although the isolated yield (18% after chromatography) was modest, it must be taken into account that the transformation of **2** into **7** involves six steps (formation of acyl chloride, addition of chloride, elimination of a sulfur-containing group, isomerization–aromatization and bisacylation).

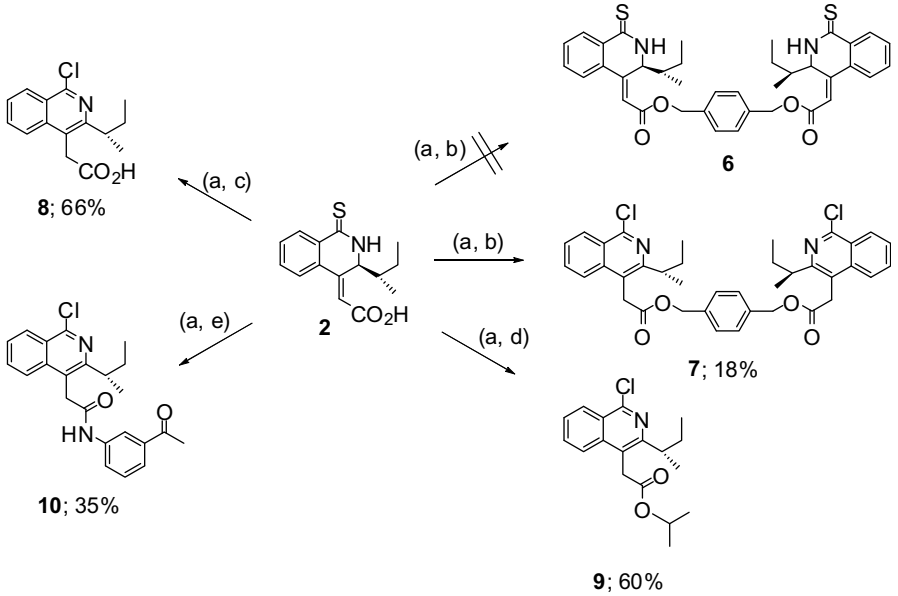
Other nucleophiles (water, alcohol and amine) were also used, obtaining higher isolated yields. Thus, acid **8** was prepared in 67% yield by quenching the reaction with water, the isopropyl ester **9** was synthesized in 60% yield by reaction with 2-propanol, and amide **10** was obtained in 35% yield by the reaction with the weakly nucleophilic 3-aminoacetophenone (Scheme 2).

Analogous to the transformations from **2**, we prepared 2-benzyl-1-chloroisoquinoline **13** (Scheme 3). The known[13] racemic lactam **11** was transformed into thiolactam **12** by Lawesson reaction (68% yield), which in turn was hydrolyzed (70% yield) to acid **13** (70% yield), that was submitted to the sequential treatment with thionyl chloride and 2-propanol to give **14** in 52% yield.

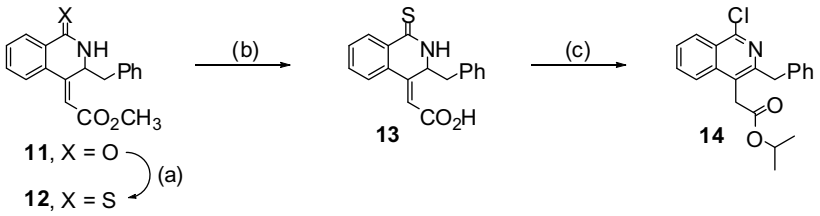
The different behaviour of lactams and thiolactams in the reaction with thionyl chloride can be rationalized by the higher nucleophilicity of sulfur in the thiolactam versus



Scheme 1. Reagents and conditions: (a) LR [2,4-bis(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiadiphosphetane], toluene, reflux, 1 h (91% yield); (b) 1 M aq LiOH/THF, rt, overnight (96% yield).

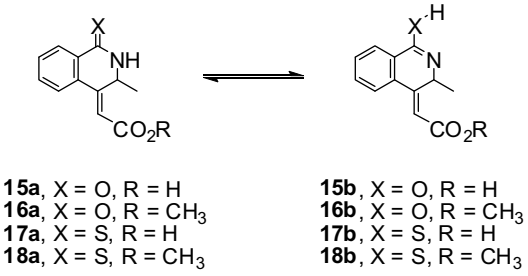


Scheme 2. Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, 0 °C to rt, 0.5 h; (b) $HOCH_2$-*p*-(C_6H_4)–CH_2OH, CH_2Cl_2, rt, 1 h; (c) NaOH, 0.5 h; (d) Me_2CHOH, CH_2Cl_2, rt, 1 h; (e) NH_2-*m*-(C_6H_4)–COMe, Et_3N, CH_2Cl_2, rt, overnight.



Scheme 3. Reagents and conditions: (a) LR, toluene, reflux, 1 h (68% yield); (b) 1 M aq LiOH/THF, rt, overnight (70% yield); (c) (i) $SOCl_2$, CH_2Cl_2, 0 °C to rt, 0.5 h; (ii) Me_2CHOH, CH_2Cl_2, rt, 1 h (52% yield).

the oxygen of the lactam. To test this hypothesis, a computational study was carried out on model compounds **15**–**18** (Scheme 4), which included both esters and acids of both oxygen- and sulfur-substituted heterocycles in the two tautomeric forms. For the sake of shortening calculations, the conformationally mobile *sec*-butyl group was changed by methyl. We studied equilibria between tautomers, calculation of orbitals and reactivity descriptors. Since the results of the corresponding methyl ester and acid are essentially identical, we present only the results on the acids.[14]



Scheme 4. Equilibria between tautomers.

The starting geometries used in the calculations were crystallographic structures of related oxo- and thioxo-dihydroisoquinolines.[15] All the calculations were carried out using GAUSSIAN 03 set of programs[16] at the B3LYP/6-31+G(d,p) theory level, and the geometries were optimized at high convergence. The relative energies of the two stable species and the transition states in the prototropies causing equilibria between tautomers are indicated in Figure 1. Each transition state has a single imaginary frequency corresponding to the vibration of the H–X bond (X = either O or S). It is observed that the keto-like form is the most stable for both compounds, but the enol-like form of the thio-analogue is kinetically and thermodynamically more stable than the oxygenated compound.

Since thionyl chloride is an electrophile, the substituent in position 1- of the starting isoquinoline has to act as nucleophile. Therefore, some molecular characteristics which gauge these electron-donor characteristics were computed. The shapes of the HOMOs of **15a**, **15b**, **17a** and **17b** (Fig. 2) show that the highest coefficient is placed on the

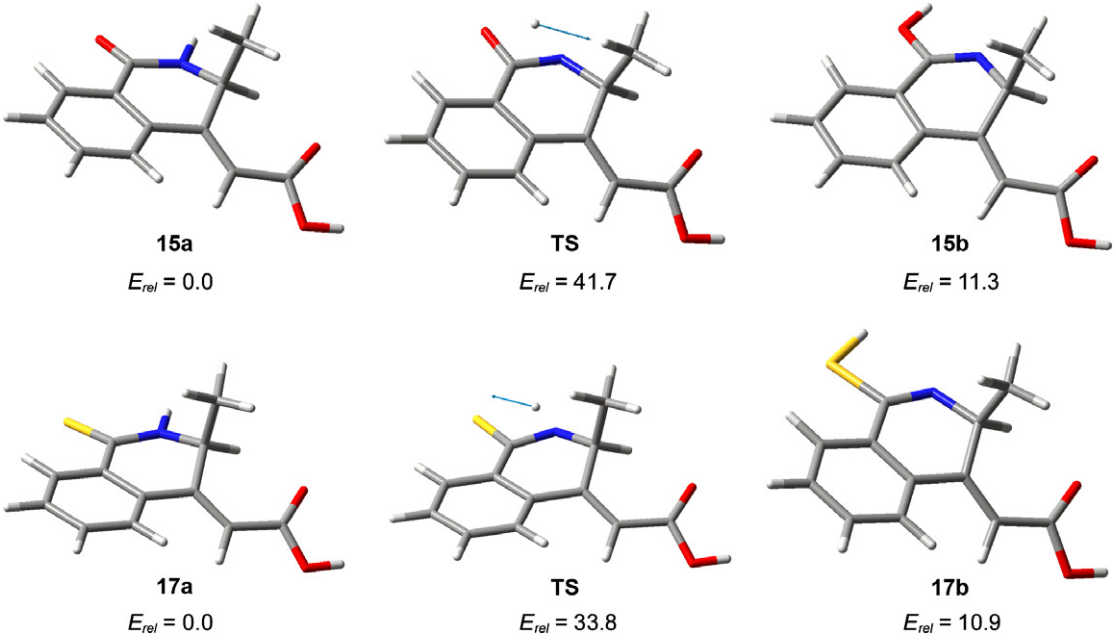


Fig. 1. B3LYP/6-31+G(d,p) optimized geometries and relative energies (kcal mol^{-1}) of the two tautomers of **15** and **17** and the corresponding transition states (**TS**).

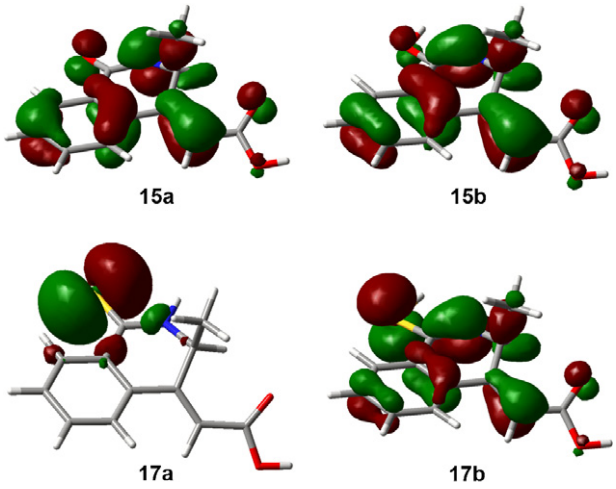


Fig. 2. HOMOs of the two tautomers of acids **15** and **17**. The orbitals are plotted on an isoelectronic density surface of 0.04 e bohr^{-3}.

sulfur atom of **17a**. Additional clues are obtained from the reactivity descriptors of acids **15a**, **15b**, **17a** and **17b** (Table 1).[17] It is observed that thiolactam **17a** has the highest energy HOMO and the smallest HOMO–LUMO gap as well as the highest polarizability α and the lowest ionization potential *I* of the four compounds. All these results explain the different reactivity of thionyl chloride with either the thiolactam or the lactam as well as that the reaction likely goes through the thiocarbonyl functionality. A plausible reaction course is indicated in Scheme 5, where the well-known transformation of COOH to COCl is not detailed.

Some of the synthesized thiolactams (**2**, **5** and **13**) and chloroisoquinolines (**7**–**10**) were tested as inhibitors of μ-calpain using labelled casein as substrate,[18] following the experimental procedure previously reported.[7a] The biological activity has been gauged by the IC_{50} values. The results shown in Table 2 indicate that all the tested compounds are either inactive or poor inhibitors of the enzyme.[19]

Table 2
Results of the inhibition of μ-calpain

Compound	IC_{50} (μM)
2	100
5	38
7	97
8	23
9	100
10	42
13	Inactive

In conclusion, starting from 3-alkyl-4-(methoxycarbonyl)methylene-3,4-dihydro-2*H*-isoquinoline-1-thione, we have prepared the derivatives of 4-(3-alkyl-1-chloroisoquinolinyl)acetic acid; which can be useful synthetic intermediates via nucleophilic substitution, metallation or transition-metal catalyzed transformations on the halogenated position;[20] as well as through the chemoselective functionalization of the substituent at position 4. Additionally, since the derivatives of 3-*sec*-butyl-1-chloroisoquinoline bear a stereogenic center and they can be prepared in both enantiomeric forms,[21] these compounds can be useful as catalysts or additives for asymmetric synthesis.

Acknowledgement

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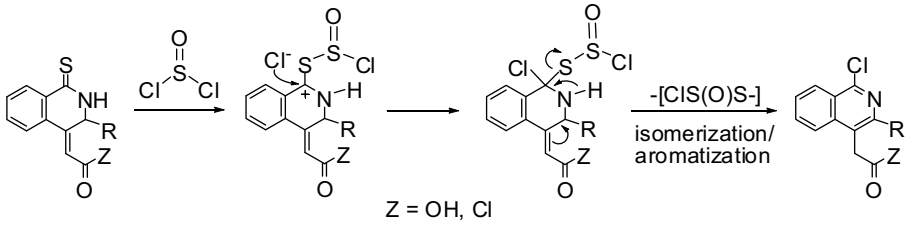
Supplementary data

Experimental details of synthetic procedures, spectroscopic and analytical data for new compounds and full

Table 1
Dipole moment (μ in Debyes), HOMO energy (ε_{HOMO} in eV), LUMO energy (ε_{LUMO} in eV), HOMO–LUMO energy difference ($\Delta E_{HOMO-LUMO}$), ionization potential (*I* in eV), electron affinity (*A* in eV), absolute hardness (η in eV), electrophilic global index (ω in eV) and polarizability (α in au) of compounds **15a**, **15b**, **17a** and **17b**

Compound	μ	ε_{HOMO}	ε_{LUMO}	$\Delta E_{HOMO-LUMO}$	*I*	*A*	η	ω	α
15a	3.73	−6.99	−2.58	4.41	8.59	0.99	3.80	3.02	165
15b	2.91	−6.69	−2.40	4.29	8.28	0.83	3.73	2.79	167
17a	4.47	−5.95	−2.76	3.19	7.93	1.26	3.33	3.17	192
17b	3.23	−6.65	−2.49	4.17	8.20	0.96	3.62	2.90	185



Scheme 5. Reaction course for the transformation of thiolactam into 1-chloroisoquinoline.

computational results. Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.tetlet.2008.02.023.

References and notes

1. (a) Goll, D. E.; Thompson, V. F.; Li, H.; Wei, W.; Cong, J. *Physiol. Rev.* **2003**, *83*, 731–801; (b) Tompa, P.; Buzder-Lantos, P.; Tantos, A.; Farkas, A.; Szilagyi, A.; Banoczi, Z.; Hudecz, F.; Friedrich, P. *J. Biol. Chem.* **2004**, *279*, 20775–20785; (c) *MEROPS the peptidase database* (release 7.70); http://merops.sanger.ac.uk/ (last accessed: 28th January, 2008).
2. (a) Hood, J.; Brooks, W.; Roszman, T. *Bioessays* **2006**, *28*, 850–859; (b) Croall, D. E.; Ersfeld, K. *Genome Biol.* **2007**, *8*, article 218; (c) Hara, M. R.; Snyder, S. H. *Ann. Rev. Pharmacol. Toxicol.* **2007**, *47*, 117–141.
3. (a) Harris, F.; Chatfield, L.; Singh, J.; Phoenix, D. A. *Mol. Cell. Biochem.* **2004**, *261*, 161–167; (b) Zatz, M.; Starling, A. *New Engl. J. Med.* **2005**, *352*, 2413–2423.
4. (a) Neffe, A. T.; Abell, A. D. *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 684–700; (b) Carragher, N. *Curr. Pharm. Des.* **2006**, *12*, 615–638.
5. Montero, A.; Mann, E.; Herradon, B. *Tetrahedron Lett.* **2005**, *46*, 401–405.
6. (a) Montero, A.; Mann, E.; Chana, A.; Herradon, B. *Chem. Biodiv.* **2004**, *1*, 442–457; (b) Montero, A.; Albericio, F.; Royo, M.; Herradon, B. *Org. Lett.* **2004**, *6*, 4089–4092; (c) Montero, A.; Alonso, M.; Benito, E.; Chana, A.; Mann, E.; Navas, J. M.; Herradon, B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2753–2757; (d) Montero, A.; Albericio, F.; Royo, M.; Herradon, B. *Eur. J. Org. Chem.* **2007**, 1301–1308.
7. (a) Mann, E.; Chana, A.; Sanchez-Sancho, F.; Puerta, C.; Garcia-Merino, A.; Herradon, B. *Adv. Synth. Catal.* **2002**, *344*, 855–867; (b) Salgado, A.; Mann, E.; Sanchez-Sancho, F.; Herradon, B. *Heterocycles* **2003**, *60*, 57–71.
8. Chicharro, R.; Alonso, M.; Mazo, M. T.; Aran, V. J.; Herradon, B. *ChemMedChem* **2006**, *1*, 710–714.
9. Herradón, B.; Chicharro, R.; Arán, V. J.; Alonso, M. PCT Application ES2005/070171.
10. Lee, H. J.; Choi, Y. S.; Lee, K. B.; Park, J.; Yoon, C. J. *J. Phys. Chem. A* **2002**, *106*, 7010–7017.
11. Pedersen, B. S.; Scheibye, S.; Nilsson, N. H.; Lawesson, S. O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 223–228.
12. The new compounds gave satisfactory analytical (C, H, N, ±0.3%) and spectroscopic (^{1}H and ^{13}C NMR, IR, MS) data. Detailed experimental procedures and spectroscopic data are included as Supplementary data.
13. Sanchez-Sancho, F.; Mann, E.; Herradon, B. *Adv. Synth. Catal.* **2001**, *343*, 360–368.
14. Complete computational data, including Cartesian coordinates of minima and transition states, are collected as Supplementary data.
15. Chicharro, R. Ph.D. Thesis, Universidad Autónoma de Madrid, 2007.
16. Frisch, M. J., et al. GAUSSIAN 03 *Revision B 04*, Gaussian: Wallingford, CT, 2004. The full reference is included as Supplementary data.
17. Parr, R. G.; Yang, W. *Density Functional Theory of Atoms and Molecules*; Oxford University Press: New York, 1989; pp 70–101.
18. (a) Jiang, S.-T.; Wang, J.-W.; Chang, T.; Chen, C.-S. *Anal. Biochem.* **1997**, *244*, 233–238; (b) Thomson, V. F.; Saldaña, S.; Cong, J.; Goll, D. E. *Anal. Biochem.* **2000**, *279*, 170–178.
19. The unique feature of calpain, that is activation by Ca^{2+}, allows to design inhibitors based on two distinct concepts: either electrophilic inactivation of the cysteine at the active site or hampering activation by Ca^{2+}. As previously discussed (Refs. 6 and 8), it is likely that the Michael acceptors **2** and **5** act by the former mechanism and the non-electrophilic isoquinolines **7**–**10** by the latter one.
20. Nair, M. D.; Premila, M. S. In *Isoquinolines*; Kathawala, F. G., Coppola, G. M., Schuster, H. F., Eds.; The Chemistry of Heterocyclic Compounds; Taylor, E. C., Ed.; John Wiley & Sons: New York, 1990; Vol. 38, Part 2, pp 1–133.
21. From D-isoleucine or from L-*allo*-isoleucine. The enantiomeric purity of **7**–**10** was assessed by HPLC (Nucleosil C_{18} as stationary phase and 100/0 to 10/90 CH_3CN/H_2O as mobile phase) with a UV/CD detector.