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INHIBITION OF HUMAN ERYTHROCYTE CALPAIN I BY NOVEL QUINOLINECARBOXAMIDES

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Abstract. 1,4-Dihydro-4-oxo-3-quinolinecarboxamides are a class of non-peptide reversible inhibitor of human erythrocyte Calpain I. The preparation and *in vitro* evaluation of these compounds are discussed.

Calcium-activated neutral proteases, or calpains, are ubiquitous, non-lysosomal cysteine endopeptidases that exist in two subclasses: calpain I (micromolar Ca^{2+} requiring form) and calpain II (millimolar Ca^{2+} requiring form). The biochemical properties and distribution of these isoforms have been the subject of recent reviews.[2-5] The putative role of calpains in a variety of physiological and pathological events,[2-11] such as signal transduction, homeostasis, memory function, hypertension, and degradation of myofibrillar or cytoskeletal proteins in degenerative diseases of muscle and nerve, have stimulated interest in the identification of potent, selective, membrane-permeable inhibitors of these enzymes. Such an inhibitor would be a welcomed biochemical tool for those unraveling calpain's physiological roles and might offer therapeutic utility as well.

The vast majority of the calpain inhibitors thus far reported contain an appropriate peptide-based scaffold[12] bearing functional groups that are known to inactivate cysteine proteases.[13,14] Examples include peptidyl aldehydes (e.g. leupeptin),[13,14] epoxides (e.g. E-64),[13,14] diazomethylketones,[15] halomethylketones,[13,14,16] acyloxymethylketones,[17] and sulfonium methylketones.[17] While reasonable levels of enzyme inhibition are often achieved with these inhibitor classes, selective inhibition of calpain versus other proteases,[13,14] especially the cysteine proteases cathepsins L and B,[16-19] has been difficult to achieve. In addition, one would anticipate that these inhibitors may exhibit poor pharmacokinetic profiles owing to the peptide-like nature of these agents. In light of these deficiencies, we sought to identify a novel class of selective non-peptide inhibitor of human erythrocyte calpain I.

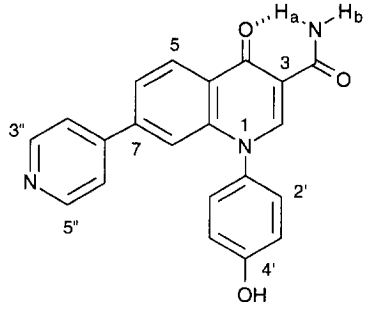

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Figure 1

3-Quinolinecarboxamide **1** (Figure 1) emerged from in-house screening efforts as a non-peptide lead of moderate potency (2 μM) and good selectivity versus a panel of in-house receptors and enzymes. Interestingly, compound **1** was devoid of antiherpetic properties[20] often associated with 1,4-dihydro-1,7-substituted 4-oxo-3-quinolinecarboxamides.[21] High throughput screening (>500 quinoline analogs) established that the 1-(4'-hydroxyphenyl) moiety (4'-OHPh) was essential for calpain I inhibition. Analogs of **1** with phenyl substituents other than 4'-hydroxyl (R^1 = 4'-XPh , X = H, Me, halogen, OMe, NHR, COR) showed little or no inhibition of calpain I (IC_{50} >50 μM).[22] We sought to increase the potency and selectivity of this class of non-peptide inhibitor by optimization of three substitutions, R^1 - R^3, present on the quinoline nucleus (Table 1). This paper describes the synthesis and structure activity relationships of 3-quinolinecarboxamides as human calpain I inhibitors.

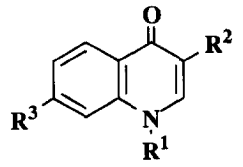
The 3-quinolinecarboxamide analogs **1-24** (Table 1) were prepared by expanding the methodology recently reported by Wentland and coworkers.[21] The preparations of analog **7**, a R^1-variant of **1**, and analog **15**, a R^3-variant, highlight the versatility of this methodology (Scheme 1 and 2). While in principle all of the analogs described here could have been prepared using Scheme 2, we chose to prepare the R^1- and R^2-variants via Scheme 1 since enamine **27** was readily available. The R^2-variants **3-6** were prepared by saponification of ethyl ester **29** (Scheme 1) followed by carboxyl activation and coupling with the requisite amines. The 7-bromo-3-quinolinecarboxamide **35** (highlighted in scheme 2) served as a common intermediate for the rapid synthesis of the R^3-variants **15-22**. The key palladium-catalyzed coupling of quinolinyl bromide **35** with aryl- and heteroarylstannanes proceeded in an efficient manner (yields >70%). Finally, the functionalized anilines required for this study (e.g. **26** and **31**) were synthesized from readily available starting materials according to the methods outlined in Schemes 3 and 4.

Quinolinecarboxamides **1-24** were evaluated *in vitro* for Calpain I inhibitory activity.[23] The microbial peptide aldehyde leupeptin **25** [13,14] (Ac-Leu-Leu-Arg-H, IC_{50} 0.3 μM) was employed as a reference inhibitor for this study. The results of this SAR study are shown in Table 1. Our first step was to determine whether the carboxamide functionality (R^2 = $CONH_2$) of **1** was important for calpain I inhibition. Our results suggest that the carboxamide functionality is in fact essential for retaining potency against calpain I. The planar, intramolecular hydrogen-bonded conformation of the ß-keto amide functionality (as indicated in Figure 1) is proposed as the bioactive conformation.[24] R^2 substituents that are unable to form this intramolecular, hydrogen-bonded conformation *and* form a second hydrogen bond (presumably to some H-bond acceptor on enzyme) were 10-fold less active (**2**) or completely inactive (**3-6**). Based on these results, the carboxamide functionality ($CONH_2$) at R^2 was retained.

While no potency increase was achieved by R^2 variation, a 4-fold increase in potency vs. **1** was observed with analogs (**7, 8**) which contained either a 2'-CH_3 or 2'-Cl substituent on the 4'-hydroxyphenyl ring of R^1. In contrast, analogs (**12** and **13**) bearing the strongly electron-withdrawing substituents 2'-F and 2'-CF_3 were 3- and 10-fold less potent than **1** respectively. Substitutions at the 3'- or 3',5'-positions were poorly tolerated or offered no advantages (**9-11, 14**). Since hydrophobic 2'-substituents provided an increase in potency and aniline **31** was easily prepared on a large scale, the 2'-Cl-4'-OHPh moiety of **8** was chosen as the preferred R^1 substitution for additional optimization studies.

Efforts to optimize the R^3 substituent were then begun. It was quickly determined that analogs which lack a heterocyclic R^3 substituent (e.g. **23**, R^3 = Ph; **24**, R^3 = H) did not inhibit calpain I at the highest concentration

Table 1. Structural and Inhibition Data for 1,4-Dihydro-4-oxo-3-quinolinecarboxamide Derivatives (**1-24**)

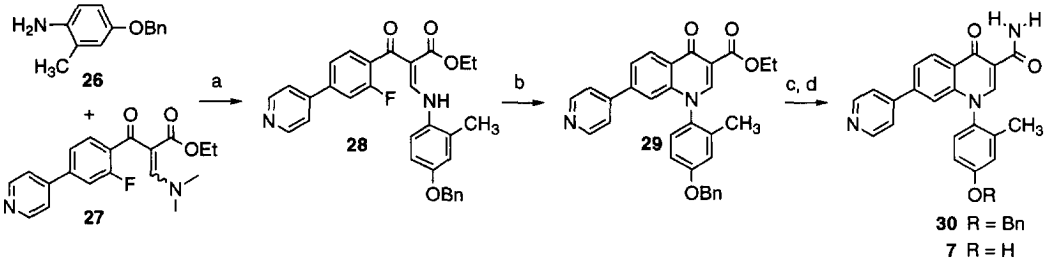


Compound[a]	R^1	R^2	R^3	Synthetic Scheme	IC_{50} (μM)[b]
R^2 SAR					
1	4'-HOC_6H_4	$CONH_2$	4"-pyridinyl	1	2
2	4'-HOC_6H_4	COOH	4"-pyridinyl	1	20
3	4'-HOC_6H_4	$CONHCH_3$	4"-pyridinyl	1	---[c]
4	4'-HOC_6H_4	CONHOH	4"-pyridinyl	1	---
5	4'-HOC_6H_4	$CONH(CH_2)_2OH$	4"-pyridinyl	1	---
6	4'-HOC_6H_4	$CON(CH_3)_2$	4"-pyridinyl	1	---
R^1 SAR					
1	4'-HOC_6H_4	$CONH_2$	4"-pyridinyl	1	2
7	2'-CH_3-4'-HOC_6H_3	$CONH_2$	4"-pyridinyl	1	0.5
8	2'-Cl-4'-HOC_6H_3	$CONH_2$	4"-pyridinyl	2	0.6
9	3'-Cl-4'-HOC_6H_3	$CONH_2$	4"-pyridinyl	1	2
10	3'-F-4'-HOC_6H_3	$CONH_2$	4"-pyridinyl	1	5
11	3',4'-OH-C_6H_3	$CONH_2$	4"-pyridinyl	1	7
12	2'-F-4'-HOC_6H_3	$CONH_2$	4"-pyridinyl	1	7
13	2'-CF_3-4'-HOC_6H_3	$CONH_2$	4"-pyridinyl	1	20
14	3',5'-CH_3-4'-HOC_6H_2	$CONH_2$	4"-pyridinyl	1	---
R^3 SAR					
8	2'-Cl-4'-HOC_6H_3	$CONH_2$	4"-pyridinyl	2	0.6
15	2'-Cl-4'-HOC_6H_3	$CONH_2$	2"-pyrazolyl	2	0.6
16	2'-Cl-4'-HOC_6H_3	$CONH_2$	3"-furanyl	2	2
17	2'-Cl-4'-HOC_6H_3	$CONH_2$	2"-Cl-4"-pyridinyl	2	2
18	2'-Cl-4'-HOC_6H_3	$CONH_2$	3"-pyridazinyl	2	2
19	2'-Cl-4'-HOC_6H_3	$CONH_2$	3"-pyridinyl	2	10
20	2'-Cl-4'-HOC_6H_3	$CONH_2$	2"-pyridinyl	2	30
21	2'-Cl-4'-HOC_6H_3	$CONH_2$	2"-F-4"-pyridinyl	2	30
22	2'-Cl-4'-HOC_6H_3	$CONH_2$	3",5"-CH_3-4"-pyridinyl	2	---
23	4'-HOC_6H_4	$CONH_2$	Ph	2	---
24	4'-HOC_6H_4	$CONH_2$	H	2	---
25	leupeptin (Ac-Leu-Leu-Arg-H)				0.3

[a] All new compounds gave satisfactory microanalytical and spectral data. [b]For assay description, see ref. 23.

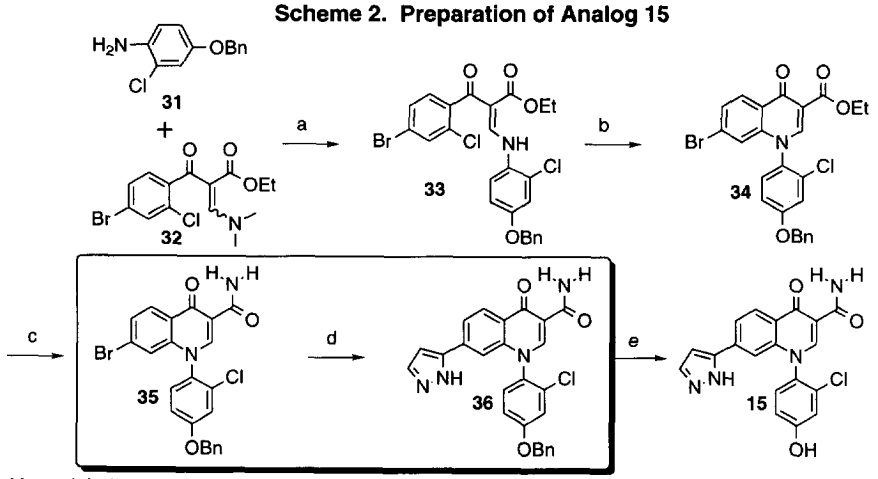
[c] The designation "---" indicates that the compound displayed <10% inhibition at 50 μM concentration.

Scheme 1. Preparation of Analog 7



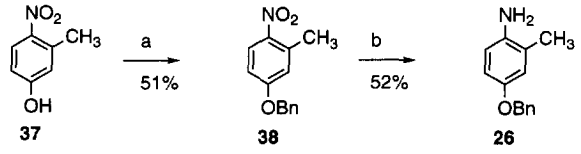
Key: (a) dioxane, reflux, 6 h, 82%; (b) NaH, dioxane, 70 °C, 6 h, 92%; (c) NH_3, EtOH, 120 °C, 15 h, 60%; (d) Pd black, cyclohexene, MeOH, 63%.

Scheme 2. Preparation of Analog 15

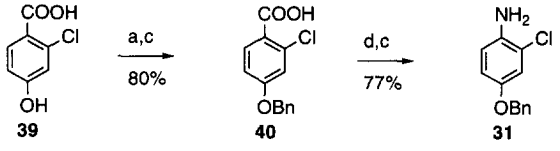


Key: (a) dioxane,100 °C, 7 h, then t-BuOH, 100 °C, 48 h, 90%; (b) NaH, dioxane, rt, 48 h, 79%; (c) NH_3, EtOH, 120 °C, 15 h, 86%; (d) 3-pyrazoletributylstannane, $PdCl_2(PPh_3)_2$, dioxane, 100 °C, 30 min, 90%; (e) Pd black, cyclohexene, MeOH, 91%.

Scheme 3



Scheme 4



Key: (a) BnBr, Cs_2CO_3, KI(cat), DMF, rt, 10 h; (b) $Na_2S_2O_4$, MeOH/H_2O, reflux, 2 h; (c) KOH, H_2O/EtOH, 90 °C, 2 -5 h; (d) i. TEA, diphenylphosphoryl azide, toluene; ii. EtOH, 105 °C, 2 h

tested (up to 50 μM). In contrast, analogs **8** and **15**, which bear the heterocyclic 4"-pyridinyl and 2"-pyrazolyl substituents at R^3, provided the maximum inhibition of human calpain I. Interestingly, the 3"- and 2"-pyridinyl R^3-substituted derivatives were about 20-fold and 50-fold less active than analog **8** (R^3 = 4"-pyridinyl). Additional attempts to increase potency (survey of heterocycles and substituent effects) were unsuccessful (**16-18**, **21**, **22**). Thus, a heterocyclic substituent, such as 4"-pyridinyl, is preferred at R^3 and plays a key, yet undefined, role in inhibitor binding. As a result of this SAR study, three analogs (**7**, **8**, and **15**) with moderate potency improvement vs. **1** were identified and selected for additional kinetic and selectivity studies.

Experiments were designed to investigate the mechanism of inhibition of these 3-quinolinecarboxamide derivatives. Using the calpain activity assay described by Gopalakrishna and Barsky (^{3}H-casein as substrate),[23] it was shown that these compounds are reversible inhibitors of calpain I. Interestingly, additional experiments using multiple concentrations of both ^{3}H-casein and inhibitor demonstrated that these compounds did not inhibit by a competitive mechanism of action. Since these compounds apparently inhibit calpain by binding to the enzyme at a site distinct from substrate, it is not surprising that these compounds show good selectivity versus proteases which share substrate specificity with calpain (Table 2).

Table 2. Inhibition (I) of Calpain I vs. Cathepsins B and L.

Compound	Calpain I IC_{50} (μM)[a]	Cathepsin B IC_{50} (μM)[ab]	Cathepsin L IC_{50} (μM)[ac]
1	2	32	37
8	0.5	25	22

[a] Replicate determinations gave standard deviations <20%. [b] Assay conditions as described in ref. 25. [c] Assay conditions as described in ref. 26.

The putative physiological and pathological roles of calpains have stimulated interest in the identification of potent, selective inhibitors of these enzymes. In-house screening efforts identified 3-quinolinecarboxamide **1** as a selective, non-peptide, reversible inhibitor of human erythrocyte calpain I. A versatile synthetic approach provided rapid preparation of some forty R^1-, R^2-, and R^3-analogs of screening lead **1**. Structure activity relationships have been identified for this novel class of calpain inhibitor, where a R^1 2'-chloro-4'hydroxyphenyl, a R^2 carboxamide, and a R^3 heterocycle are the preferred substituents. Compounds **7**, **8** and **15** embody these structural features. To date, these compounds have not been tested in therapeutically-relevant cellular or *in vivo* models.

References and Notes.

1. Present address: 3-Dimensional Pharmaceuticals Inc., 665 Stockton Drive, Exton, PA 19341.
2. Croall, D. E.; Demartino, G. N. *Physiol. Rev.* **1991**, *71*, 813-847.
3. Johnson, P. *Int. J. Biochem.* **1990**, *22*, 811-822.
4. *Intracellular Calcium-Dependent Proteolysis*; Mellgren, R. L.; Murachi, T., Eds.; CRC Press: Boca Raton, 1990.
5. Pontremoli, S.; Melloni, E. *Annu. Rev. Biochem.* **1986**, *55*, 455-481.

6. Suzuki, K.; Saido, T. C.; Hiraik, S. *Ann. N.Y. Acad. Sci.* **1992**, *674*, 218-227.
7. Yamamoto, S.; Shimizu, K.; Shimizu, K.; Suzuki, K.; Nakagawa, T. *Arthritis Rheum.* **1992**, *35*, 1309-1317.
8. Siman, R.; Card, J. P.; Davis, L. G. *J. Neurosci.* **1990**, *10*, 2400-2411.
9. Saito, K.; Elce, J. S.; Hamos, J. E.; Nixon, R. A. *Proc. Nat. Acad. Sci. USA* **1993**, *90*, 2628-2632.
10. Caner, H.; Collins, J. L.; Harris, S. M.; Kassell, N. F.; Lee, K. S. *Brain Res.* **1993**, *607*, 354-356.
11. Rami, A.; Krieglstein, J. *Brain Res.* **1993**, *609*, 67-70.
12. Takahashi, K. In *Intracellular Calcium-Dependent Proteolysis*; Mellgren, R. L.; Murachi, T., Eds.; CRC Press: Boca Raton, 1990; pp 55-74.
13. Shaw, E. In *Advances in Enzymology*; Meister, A., Ed.; Wiley: New York, 1990; pp 271-347.
14. Crawford, C. In *Intracellular Calcium-Dependent Proteolysis*; Mellgren, R. L.; Murachi, T., Eds.; CRC Press: Boca Raton, 1990; pp 75-89.
15. Wikstrom, P.; Anagli, J.; Angliker, H.; Shaw, E. *J. Enz. Inhib.* **1993**, *6*, 259-269.
16. Angliker, H.; Anagli, J.; Shaw, E. *J. Med. Chem.* **1992**, *35*, 216-20.
17. Pliura, D. H.; Bonaventura, B. J.; Smith, R. A.; Coles, P. J.; Krantz, A. *Biochem. J.* **1992**, *288*, 759-762.
18. Sasaki, T.; Kishi, M.; Saito, M.; Tanaka, T.; Higuchi, N.; Kominami, E.; Katunuma, N.; Murachi, T. *J. Enz. Inhib.* **1990**, *3*, 175-201.
19. Crawford, C.; Mason, R. W.; Wikstrom, P.; Shaw, E. *Biochem. J.* **1988**, *253*, 751-758.
20. Wentland, M. P., unpublished results (see reference 21).
21. Wentland, M. P.; Perni, R. B.; Dorff, P. H.; Brundaga, R. P.; Castaldi, J.; Bailey, T. R.; Carabateas, P. M.; Bacon, E. R.; Young, D. C.; Woods, M. G.; Rosi, D.; Drozd, M. L.; Kullnig, R. K.; Dutko, F. J. *J. Med. Chem.* **1993**, *36*, 1580-1596.
22. Unpublished results
23. Calpain I was partially purified from human erythrocytes using DEAE-sephacel and phenyl-sepharose CL-4B chromatography. The tritiated assay is a modification of that described in the literature (Gopalakrishna, R.; Barsky, S. H. *J. Biol. Chem.* **1986**, *261*, 13936-13942). The assay mixture contains 0.1 mg casein (0.004 mg, approx. 150,000 dpm, 3H-acetyl casein) in 50 mM HEPES (pH 7.5) containing 2 mM DTT. All reagents, with the exception of substrate (casein), were combined in 1 mL polystyrene titer plates. The plates were preincubated at 25 °C for 5 minutes with gentle shaking prior to the addition of substrate. The incubation was continued for an additional 2 hours and was terminated by the addition of 0.5 mL ice-cold 5% TCA. Unlabeled casein was added (2 mg/0.025 mL), the samples were centrifuged at 3,600 x g for 5 minutes and 0.5 mL of the supernatant was counted in 5 mL of Ready Protein liquid scintillation cocktail for 2 minutes in a Beckman LS5000 Scintillation Counter. Replicate determinations gave standard deviations <20%.
24. The large downfield shift of one of the two exchangeable N-H protons (^{1}H NMR of **1**, DMSO-d_6, δ 9.13 (H_a) and δ 7.65 (H_b)) and X-ray crystallography data for a related derivative[21] support this conformational assignment.
25. Knight, C. G.; *Biochem. J.* **1980**, *189*, 447-453.
26. Mason, R. W.; Green, G. K. J.; Barrett, A. J. *Biochem. J.* **1985**, *226*, 233-239.

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