of the reasons is the rather tedious procedure of establishing the effect on the induced histamine release from cortical slices. Therefore more simple test systems are highly wanted.

For therapeutical applications several possibilities seem to exist.

In the central nervous system the profile of selective H_3 agonists and antagonists is still largely unknown. The paper of Bristow and Bennett prove, however, that the H_3 receptor may constitute a new way to influence the activity pattern. Detailed studies are highly wanted here, too; it also seems attractive to investigate whether the effects of betahistine could be explained by the antagonistic effects of this compound on H_3 receptors indeed.

In peripheral systems too, suggestions have to remain rather speculative. However, the relaxation effects of histamine on the gut by interfering with the release of e.g. acetylcholine indicate that selective, nonabsorbed H_3 agonists could probably constitute an alternative way (to e.g. morphine) in order to reduce GI activity and to treat diarrhea. The prospects of H_3 antagonists are less clear in this perspective, although selective, nonabsorbed compounds could become of interest as well by means of their stimulatory properties.

The effects of H_3 agonists and antagonists on circulatory events have to be established. So far, the results point to attractive possibilities. It has been known for a long time that histamine itself is very active, on many systems; the effects of histamine on H_1 and H_2 will be antagonized by the effects mediated by H_3. A selective H_3 agonist could therefore antagonize histamine induced effects, without inducing histaminergic effects itself directly (e.g. smooth muscle contractions of gastric acid production); in a reverse way the same is true for H_3 antagonists. Investigations into the effects of both selective agonists and antagonists are highly wanted; one might speculate that the compounds constitute alternatives for existing agents which are useful for controlling blood pressure and circulation.

Note Added in Proof. After the manuscript of this paper had been finished, several new studies have been published. A very interesting finding has come from the group of Barnes. In two papers (Ichinose, M.; Stretton, C. D.; Schwartz, J.-C.; Barnes, P. J. Histamine H_3-receptors inhibit cholinergic neurotransmission in guinea-pig airways. *Br. J. Pharmacol.* **1989**, *57*, 13-15. Ichinose, M.; Barnes, P. J. Inhibitory histamine H_3-receptors on cholinergic nerves in human airways. *Eur. J. Pharmacol.* **1989**, *163*, 383-386.), it is shown that H_3-receptor stimulation reduces the cholinergic contractile responses to electrical stimulation without affecting acetylcholine-induced effects. The facts seem to indicate that H_3 receptors present on the vagus nerves modulate cholinergic neurotransmission in the airways and would open other appealing therapeutical possibilities of selective H_3 agonists.

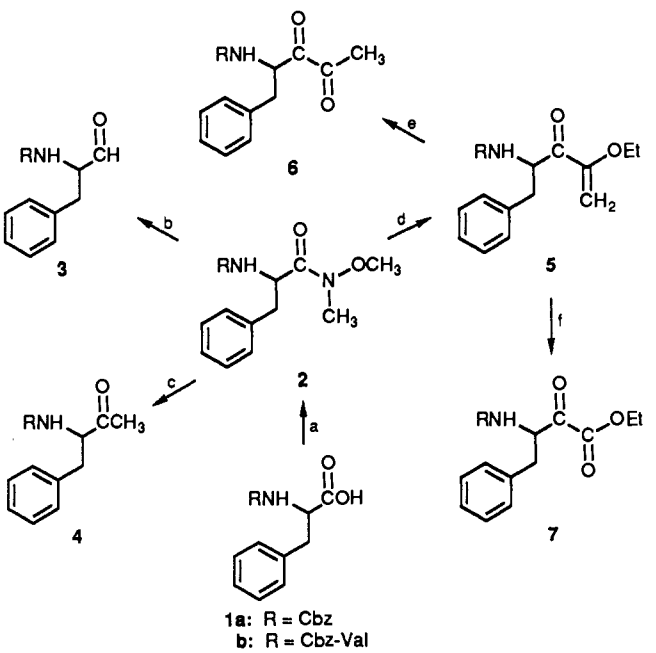
Communications to the Editor

α-Diketone and α-Keto Ester Derivatives of N-Protected Amino Acids and Peptides as Novel Inhibitors of Cysteine and Serine Proteinases

Sir:

A successful approach to specific inhibition of proteinases has been to design small peptide substrate analogues in which the scissile amide unit has been replaced by a functionality incorporating an electron-deficient carbonyl group, such as an α-fluorinated ketone[1-4] or an α-keto ester.[4,5] In this communication, we describe the synthesis and the evaluation of α-diketone derivatives of amino acids and dipeptides as a novel class of electron deficient carbonyl containing inhibitors for the serine and cysteine proteinases, α-chymotrypsin and calpain, respectively. We also demonstrate that peptidyl α-keto esters, which have been previously shown to inhibit serine proteinases,[4,5] are also potent inhibitors of calpain.

Scheme I[a]



[a] (a) *i*-BuOCOCl, *N*-methylmorpholine, *N,O*-dimethylhydroxylamine hydrochloride; (b) $LiAlH_4$, THF; (c) MeLi, THF; (d) *t*-BuLi, ethyl vinyl ether, $MgBr_2$, THF; (e) HCl, dioxane, H_2O; (f) O_3, CH_2Cl_2, pyridine.

Chemistry. The chemistry used to construct the α-diketone and α-keto ester inhibitors is shown in Scheme I. *N*-Carbobenzyloxy- and *N*-(*N*-carbobenzyloxyvalyl)-

(1) Stein, R. L.; Strimpler, A. M.; Edwards, P. D.; Lewis, J. J.; Mauger, R. C.; Schwartz, J. A.; Stein, M. M.; Trainor, D. A.; Wildonger, R. A.; Zottola, M. A. *Biochemistry* 1987, *26*, 2682.
(2) Imperiali, B.; Abeles, R. H. *Biochemistry* 1986, *25*, 3760.
(3) Dunlap, R. P.; Stone, P. J.; Abeles, R. H. *Biochem. Biophys. Res. Commun.* 1987, *145*, 509.
(4) Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L. Mehdi, S.; Kolb, M.; Neises, B.; Schirlin, D.; Bey, P. *J. Med. Chem.* In press.
(5) Hori, H.; Tasutake, A.; Minematsu, Y.; Powers, J. C. *Peptides, Structure and Function (Proceedings of the Ninth American Peptide Symposium)*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chem. Co.: Rockford, IL, 1985; p 819.

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phenylalanine (**1a** and **1b**) were converted to their respective *N*-methyl-*N*-methoxy carboxamides **2a** and **2b** by using a mixed anhydride coupling method. Compounds **2** were key intermediates for the preparation of α-diketone and α-keto ester derivatives. Treatment of **2a** and **2b** with lithiated ethyl vinyl ether (acyl anion equivalent;[6] Nahm and Weinreb procedure[7]) gave ethoxyvinyl ketones **5a** and **5b**, which were readily hydrolyzed with dilute hydrochloric acid (with dioxane as a cosolvent) to afford α-diketones **6a** and **6b**. Alternatively, the ethoxyvinyl ketones (in methylene chloride/pyridine) could be ozonolized to give α-keto esters **7a** and **7b**. Experimental details for the transformations shown in Scheme I are described in a recent report.[8] Conrow and Portoghese[9] have reported a lengthier route to polyfunctional α-diketones from carboxylic acids via α-enamino ketones.

The versatility of the *N*-methyl-*N*-methoxy carboxamides **2** was further exploited to prepare, for comparative studies, aldehydes **3a** and **3b**, via the procedure described by Fehrentz and Castro,[10] and methyl ketone **4a**. Thus, reduction of **2a** and **2b** with lithium aluminum hydride in tetrahydrofuran followed by hydrolysis with potassium hydrogen sulfate gave the respective aldehydes **3a** and **3b**. Likewise, addition of methyllithium to a tetrahydrofuran solution of **2a** gave methyl ketone **4a**, which has previously been prepared via the corresponding diazomethyl and chloromethyl derivatives.[11]

Proteinase Inhibitory Activity. Calpain, a ubiquitous cytosolic cysteine endopeptidase, preferentially cleaves peptide substrates with bulky residues, such as Phe, Arg, or Met, at the P_1 site and a Val or Leu residue at the P_2 site.[12] The serine proteinase α-chymotrypsin recognizes substrates with aromatic residues at the P_1 site and shows little specificity for the P_2 site. The α-diketones and α-keto esters synthesized for this study were found to be competitive inhibitors of calpain and α-chymotrypsin. Their apparent dissociation constants K_i, determined for purified calpain from chicken gizzard and commercially available α-chymotrypsin by using continuous enzyme assays, are presented in Table I. By analogy to mechanisms demonstrated for the inhibition of cysteine and serine proteinases by peptidyl aldehydes[13] and peptidyl fluoro ketones,[14] the α-diketone and α-keto ester inhibitors are presumed to inhibit calpain and α-chymotrypsin by the reversible formation of a covalent hemithioketal or hemiketal resulting from the attack of the sulfhydryl or hydroxyl group of the active site cysteine or serine residue at an electrophilic center of the α-dicarbonyl moieties. Thus, the inhibitory constants K_i of the aldehyde and fluoromethyl ketone analogues with the same amino acid side chain as the α-dicarbonyl inhibitors were also determined for comparative purposes.

Table I. Apparent Dissociation Constants for the Inhibition of Calpain and α-Chymotrypsin

compd[a]	K_i, μM: calpain[b]	K_i, μM: α-chymotrypsin[c]
Bz-Phe-H[d]	4	64
Cbz-Phe-H (**3a**)	3	360
Bz-Phe-CO_2CH_3[e]	150	0.15
Cbz-Phe-$CO_2CH_2CH_3$ (**7a**)	92	0.13
Cbz-Phe-$COCH_3$ (**6a**)	250	1.2
Bz-Phe-CHF_2[f]	>1000[g]	7.7
Bz-Phe-CF_3[f]	>1000[g]	2.3
Cbz-Phe-CH_3 (**4a**)	>2000[g]	
Cbz-Val-Phe-H (**3b**)	0.01[h]	54
Cbz-Val-Phe-CO_2CH_3[i]	0.4	0.06
Cbz-Val-Phe-$CO_2CH_2CH_3$ (**7b**)	0.6	0.06
Cbz-Val-Phe-$COCH_3$ (**6b**)	0.7	0.2
Cbz-Val-Phe-CF_3[j,k]	>180[g]	2.4[h]
Cbz-Val-D-Phe-CF_3[j,l]	>180[g]	45

[a] The structures of all compounds are consistent with the spectral data. [b] Assayed as described by Mehdi et al. (ref 24). Errors were smaller than 30%. [c] α-Chymotrypsin was assayed with *N*-Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide (Sigma) as the substrate as described by Geiger (ref 25), except that 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) rather than Tris was used as the buffer. K_i values were calculated as described by Mehdi et al. (ref 24) and were corrected [$K_i = K_{i\,app}/(1 + [S]/K_m)$] if the substrate concentration used was not negligible compared to the K_m (K_m = 0.12 mM). Errors were smaller than 30%. [d] Prepared by reduction of *N*-benzoylphenylalanine with borane–methyl sulfide complex followed by oxidation of the resulting alcohol with pyridinium chlorochromate. [e] Reference 26. [f] Reference 27. [g] Determination of definitive value precluded by limited solubility of inhibitor in the assay medium. [h] Slow-binding inhibitor. [i] Prepared similarly to Bz-Phe-CO_2CH_3 in ref 26. [j] Prepared according to the general procedure described in ref 4. [k] The L:D ratio at the C atom α to the trifluoromethyl ketone is 94:6. [l] The D:L ratio at the C atom α to the trifluoromethyl ketone is 89:11.

Although it is not obvious which carbonyl group of the α-diketone would be preferentially activated, ^{13}C NMR spectra of **6a** in DMSO-d_6 with added D_2O clearly show that only one of the two carbonyl groups is hydrated. Carbon–hydrogen coupling experiments show that the carbonyl group proximal to nitrogen is the one undergoing hydration. Although this interesting finding is not necessarily predictive of which carbonyl group is approached by enzyme active site nucleophiles, it is tempting to speculate that these nucleophiles are preferentially accessing the same carbonyl group.

From the K_i values listed in Table I, it appears that (1) the relative potency of the various classes of inhibitors as defined by the chemical nature of the electron-withdrawing group flanking the electron-deficient carbonyl differs for calpain and α-chymotrypsin and (2) within each class of inhibitors the potency increases when the P_2 site is occupied, as anticipated from the existence of extended substrate binding sites in both proteinases. In the *N*-benzoyl and *N*-carbobenzyloxy mono- and diamino acid series, the α-keto esters are the most potent inhibitors of α-chymotrypsin, followed by the α-diketones, the trifluoromethyl ketones, and the aldehydes in this order. This result is in accord with our recent study where tetrapeptide α-keto esters were found to be more potent than the corresponding tetrapeptide trifluoromethyl ketones as inhibitors of the serine proteinases, rat and human cathepsin G, porcine pancreatic elastase, and human neutrophil elastase.[4] In the present study, α-diketones and α-keto esters in each series appear to be equipotent inhibitors of calpain. However, these inhibitors are less potent than the corresponding aldehydes.

(6) Baldwin, J. E.; Hofle, G. A.; Lever, O. W. *J. Am. Chem. Soc.* **1974**, *96*, 7125.
(7) Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.
(8) Angelastro, M. R.; Peet, N. P.; Bey, P. *J. Org. Chem.* **1989**, *54*, 3913.
(9) Conrow, R.; Portoghese, P. S. *J. Org. Chem.* **1986**, *51*, 938.
(10) Fehrentz, J.-A.; Castro, B. *Synthesis* **1983**, 676.
(11) Fittkau, S.; Jahreis, G.; Peters, K.; Balaspiri, L. *J. Prakt. Chem.* **1986**, *328*, 529.
(12) Sasaki, T.; Kikuchi, T.; Yumoto, N.; Yoshimura, N.; Murachi, T. *J. Biol. Chem.* **1984**, *259*, 12489.
(13) (a) Gamcsik, M. P.; Malthouse, J. P. G.; Primrose, W.; Mackenzie, N. E.; Boyd, A. S. F.; Russell, R. A.; Scott, A. I. *J. Am. Chem. Soc.* **1983**, *105*, 6324. (b) Shah, D. O.; Lai, K.; Gorenstein, D. G. *J. Am. Chem. Soc.* **1984**, *106*, 4272.
(14) (a) Liang, T.; Abeles, R. H. *Biochemistry* **1987**, *26*, 7603. (b) Takahashi, L. H.; Radhakrishnan, R.; Rosenfield, R. E., Jr.; Meyer, E. F., Jr.; Trainor, D. A.; Stein, M. *J. Mol. Biol.* **1988**, *201*, 423.

Smith et al.[15] reported that for the cysteine proteinase cathepsin B the inhibitory constants of a series of peptidyl ketones (with the exception of the trifluoromethyl ketone) correlate with the equilibrium constants $K_{RSD,app}$ for the addition in aqueous medium of the thiol group of mercaptopropionic acid to the ketones CH_3COR, where R corresponds to the group flanking the electrophilic carbonyl in the inhibitor. A similar analysis of our inhibitors using the data of Burkey and Fahey[16] predicts that the α-diketone ($K_{RSD,app}$ = 33 M^{-1}) should be more potent than the α-keto ester ($K_{RSD,app}$ = 20 M^{-1}) and the aldehyde ($K_{RSD,app}$ = 29 M^{-1}). Thus, it would appear that the simple thermodynamically driven equilibrium system of Smith et al.[15] has poor predictive value for the inhibition of calpain by peptidyl α-diketones. It is also noteworthy that the K_i values for calpain of the fluoromethyl ketone derivatives in the mono- and diamino acid series are greater than 1000 and 180 μM, respectively. This finding corroborates the observation of Smith et al.[15] that Cbz-Phe-Ala-CF_3[17] was 4 orders of magnitude less potent than the corresponding aldehyde in inhibiting cathepsin B. Electronic factors not taken into account by the model of Smith et al.[15] could be important for inhibition.

In summary, peptides containing an α-dicarbonyl unit, such as an α-diketone or an α-keto ester, are potent inhibitors of the cysteine and serine proteinases calpain and α-chymotrypsin. These new calpain inhibitors may offer significant therapeutic utility.[18] Preliminary results indicate that peptidyl α-diketones also inhibit other cysteine and serine proteinases.

Registry No. **1a**, 1161-13-3; **1b**, 19542-51-9; **2a**, 114744-85-3; **2b**, 121253-52-9; **3a**, 59830-60-3; **3b**, 88191-84-8; **4a**, 111491-96-4; **5a**, 121253-53-0; **5b**, 121253-54-1; **6a**, 121253-55-2; **6b**, 121253-56-3; **7a**, 121253-57-4; **7b**, 121253-58-5; Bz-Phe-H, 35593-57-8; Bz-Phe-COOMe, 123540-99-8; Bz-Phe-CHF_2, 123541-00-4; Bz-Phe-CF_3, 123620-04-2; Cbz-Val-Phe-COOMe, 118943-12-7; Cbz-Val-Phe-CF_3, 123541-01-5; Cbz-Val-D-Phe-CF_3, 123541-02-6; $EtOCH{=}CH_2$, 109-92-2; Bz-Phe-OH, 2566-22-5; calpain, 78990-62-2; α-chymotrypsin, 9004-07-3; cysteine proteinase, 37353-41-6; serine proteinase, 37259-58-8.

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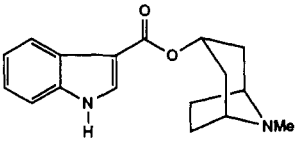
Aromatic Thiazole Derivatives: Structurally Novel and Selective Serotonin-3 Receptor Antagonists

Sir:

Antagonists of the serotonin-3 (5-HT_3) receptor subtype fall into two broad structural classes: the aromatic ester/amide series, represented by ICS-205-930 (**1**),[1] MDL-72,222,[2] BRL-43,674,[3] LY-278,584,[4] and zacopride,[5] and the indole-3-ketone series typified by the carbazole derivatives ondansetron (**2**)[6] and GR-65,630.[7] Because the 5-HT_3



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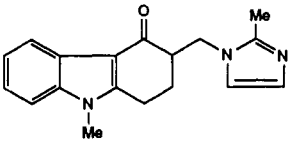

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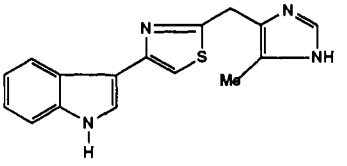

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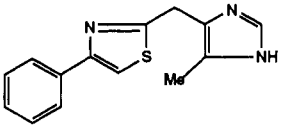

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receptor exists in both the peripheral[1] and central nervous systems,[7,8] 5-HT_3 antagonists exhibit a variety of pharmacological effects. Several 5-HT_3 receptor antagonists have demonstrated potent antagonism of chemotherapy- or radiation-induced emesis in man,[9] and various animal models also suggest that these drugs may have utility in the treatment of psychosis,[10] anxiety,[11] substance abuse

(15) Smith, R. A.; Copp, L. J.; Donnelly, S. L.; Spencer, R. W.; Krantz, A. *Biochemistry* **1988**, *27*, 6568.
(16) Burkey, T. J.; Fahey, R. C. *J. Am. Chem. Soc.* **1983**, *105*, 868.
(17) Cbz-Phe-Ala-CF_3 is a very slow-binding inhibitor of cathepsin B. The autolysis of calpain in the presence of activating calcium precluded the detection of a very slow onset of inhibition with our fluorinated ketones. However, the cysteine proteinase papain was not significantly inhibited when incubated for 6 h with 0.3 mM of Bz-Phe-CF_3 whereas the corresponding aldehyde **3a** inhibited papain with a K_i of 5 μM.
(18) Calpain has been implicated in important biological roles such as the modification of hormonal binding receptors,[19] the development of long-term memory,[20] the breakdown of neurofilaments at axon terminals,[21] muscle-protein turnover,[22] and metabolism of neuropeptides.[23]
(19) Vedeckis, W. V.; Freeman, M. R.; Schrader, W. T.; O'Malley, B. W. *Biochemistry* **1980**, *19*, 335.
(20) Lynch, G.; Baudry, M. *Science* **1984**, *224*, 1057.
(21) Zimmerman, U.-J. P.; Schlaepfer, W. W. *Biochemistry* **1982**, *21*, 3977.
(22) Kay, J. In *Proteases. Potential Role in Health and Disease*; Heidland, A., Horl, W. H., Eds.; Plenum Press: New York, 1983; p 519.
(23) Murachi, T.; Hatanaka, M.; Hamakubo, T. In *Neuropeptides and Their Peptidases*; Turner, A. J., Ed.; Ellis Horwood: Chichester, 1987; p 202.
(24) Mehdi, S.; Angelastro, M. R.; Wiseman, J. S.; Bey, P. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 1117.
(25) Geiger, R. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Ed.; Verlag Chemie: Weinheim, 1984; Vol V, p 99.
(26) Burkhart, J. P.; Peet, N. P.; Bey, P. *Tetrahedron Lett.* **1988**, *29*, 3433.
(27) Kolb, M.; Barth, J.; Neises, B. *Tetrahedron Lett.* **1986**, *27*, 1579.

(1) Richardson, B. P.; Engel, G.; Donatsch, P.; Stadler, P. A. *Nature* **1985**, *316*, 126. 2-Methylserotonin (**8**) was purchased from Research Biochemicals Incorporated.
(2) Fozard, J. R. *Arch. Pharmacol.* **1984**, *326*, 36.
(3) Fake, C. S.; King, F. D.; Sanger, G. J. *Br. J. Pharmacol.* **1987**, *91*, 335P.
(4) (a) Fludzinski, P.; Evrard, D. A.; Bloomquist, W. E.; Lacefield, W. B.; Pfeifer, W.; Jones, N. D.; Deeter, J. B.; Cohen, M. L. *J. Med. Chem.* **1987**, *30*, 1535. (b) Wong, D. T.; Robertson, D. W.; Reid, L. R. *Eur. J. Pharmacol.* **1989**, *166*, 107.
(5) Smith, W. W.; Sancilio, L. F.; Owera-Atepo, J. B.; Naylor, R. J.; Lambert, L. *J. Pharm. Pharmacol.* **1988**, *40*, 301.
(6) Butler, A.; Hill, J. M.; Ireland, S. J.; Jordan, C. C.; Tyers, M. B. *Br. J. Pharmacol.* **1988**, *94*, 397.
(7) Kilpatrick, G. J.; Jones, B. J.; Tyers, M. B. *Nature* **1987**, *330*, 746.
(8) Dumuis, A.; Bouhelal, R.; Sebben, M.; Bockaert, J. *Eur. J. Pharmacol.* **1988**, *146*, 187.
(9) (a) Cunningham, D.; Pople, A.; Ford, H. T.; Hawthorn, J.; Gazet, J.-C.; Challoner, T.; Coombes, R. C. *Lancet* **1987**, *1*, 1461. (b) Leibundgut, U.; Lancranjan, I. *Lancet.* **1987**, *1*, 1198. (c) Carmichael, J.; Cantwell, B. M. J.; Edwards, C. M.; Rapeport, W. G.; Harris, A. L. *Br. Med. J.* **1988**, *297*, 110.
(10) Costall, B.; Domeney, A. M.; Kelly, M. E.; Naylor, R. J.; Tyers, M. B. *Br. J. Pharmacol.* **1987**, *90*, 89P.

0022-2623/90/1833-0013$02.50/0