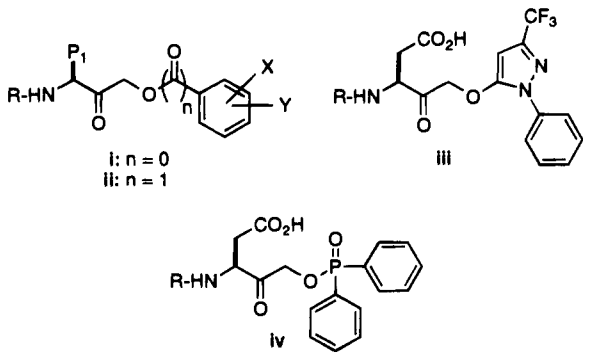
Aspartyl α-((Diphenylphosphinyl)oxy)methyl Ketones as Novel Inhibitors of Interleukin-1β Converting Enzyme. Utility of the Diphenylphosphinic Acid Leaving Group for the Inhibition of Cysteine Proteases

Roland E. Dolle,*,† Jasbir Singh,† David Whipple,† I. Kelly Osifo,† Gary Speier,† Todd L. Graybill,† Jill S. Gregory,‡ Alex L. Harris,‡ Carla T. Helaszek,‡ Robert E. Miller,‡ and Mark A. Ator‡

Departments of Medicinal Chemistry and Biochemistry, Sterling Winthrop Pharmaceuticals Research Division, 1250 South Collegeville Road, P.O. Box 5000, Collegeville, Pennsylvania 19426

Received October 19, 1994

One of the most significant advances in the design of cysteine protease inhibitors was the discovery of the peptidyl α-(aryloxy)- (**i**) and α-((arylacyl)oxy)methyl ketone (**ii**) classes of inhibitor.[1] In their seminal publication, Krantz and Smith demonstrated that these agents are potent and selective irreversible inactivators of this protease family.[1b] They showed that unlike the corresponding peptide chloromethyl ketones, **i** and **ii** possess low chemical reactivity and are in essence mechanism-based in their covalent labeling of the active site cysteine.[1] Dipeptides bearing an (arylacyl)oxy (benzoate) leaving group are orally bioavailable and reportedly lack gross toxicological liabilities.[2] In conjunction with our research interest regarding the discovery of novel cysteine protease inhibitors, particularly for interleukin-1β converting enzyme (ICE),[3,4,5] we have sought to identify new classes of quiescent affinity labels.[6] Our recent report concerning the inhibition of ICE by aspartylmethyl ketones **iii** containing the ((1-phenyl-3-(trifluoromethyl)pyrazol-5-yl)oxy) (or PTP) moiety is an example of such a new class of agent.[4] In this communication, we report on aspartyl α-((diphenylphosphinyl)oxy)methyl ketones **iv** as time-dependent inhibitors of ICE. We also demonstrate the utility of the diphenylphosphinate leaving group in inhibiting the cysteine proteases, cathepsin B and calpain I.



Several years ago we initiated a search for functionality other than phenols and benzoates to serve as leaving groups in the peptidyl-$COCH_2X$ type of inhibitor. This search for novel leaving groups was prompted by the report of Krantz and Smith who observed the rate of cathepsin B inactivation by **i** and **ii** is strongly dependent on the benzoate or phenol leaving group pK_a.[1b] However, the potency–pK_a relationship is not absolute.[7] This suggested to us that perhaps the *inherent structure* of the leaving group may be important for enzyme affinity. Tetrazoles, tetronic acids, heterocyclic acids, phosphorous-based acids, and many other structural classes were examined as potential leaving groups. An important selection criteria for leaving group screening was that the functionality react cleanly in an S_N2 fashion with peptide bromomethyl ketones in DMF using NaH or KF as the base. Three peptide scaffolds, Z-Asp, Z-Phe-Ala, and Z-Leu-Phe, were routinely employed in our screening strategy. The selection of the scaffolds was based on their known specificity for ICE,[5] cathepsin B,[1a] and calpain I.[8] This exercise led to the identification of peptidyl α-((diphenylphosphinyl)oxy)-methyl ketones as a new, generic class of cysteine protease inhibitor.

Scheme 1 highlights the synthesis of the novel diphenylphosphinate inhibitors **1**–**5** and related analogs **6**–**9**.[9] The bromomethyl ketones **10**–**12** were prepared from the corresponding peptide acids via decomposition of the intermediate diazo ketones with HBr–HOAc.[1b,5] The reaction of **10**–**12** with diphenylphosphinic acid (Aldrich) proceeded smoothly (1.1 equiv of diphenylphosphinic acid, 1.0 equiv of bromomethyl ketone, 1.5 equiv of KF, DMF, 25 °C, 2 h) to give the ((diphenylphosphinyl)oxy)methyl ketones **4**, **5**, and **13**. Treatment of *tert*-butyl ester **13** with trifluoroacetic acid (TFA) in CH_2Cl_2 (0.5 M solution of **13** in 25% v/v TFA–CH_2Cl_2, 25 °C, 3 h) furnished the aspartyl methyl ketone **1**. As demonstrated previously for the $AspCH_2PTP$ series,[4] the benzyloxycarbonyl protecting group in ester **13** was readily removed by catalytic hydrogenation (ambient H_2 pressure and temperature) in absolute ethanol (0.01 M) containing 4 equiv of 6 M aqueous HCl. Under these reaction conditions, amine hydrochloride **14** was obtained in virtually quantitative yield. Amine **14** was coupled (using BOP–HOBT or TPTU–HOBT as the coupling reagents)[10] to commercially available Z-Val-OH and Z-Val-Ala-OH, affording the desired di- and tripeptide methyl ketones **2** and **3** following TFA deprotection. Inhibitors **6**–**9** were prepared from Z-Val-Asp-CH_2Br and Z-Val-Ala-Asp-CH_2Br and phenylphosphinic acid (Aldrich), dimethylphosphinic acid (Aldrich), and bis(4-chlorophenyl)phosphinic acid,[11] using reaction conditions analogous to those used in the preparation of **1**.

The mono-, di-, and tripeptide aspartyl-based (phosphinyloxy)methyl ketones ($AspCH_2DPP$) **1**–**3** display potent time-dependent inhibition of ICE with second order rate constants of **11** 800 M^{-1} s^{-1}, 50 200 M^{-1} s^{-1} and **117** 000 M^{-1} s^{-1} (Table 1). The trend toward faster rates of inactivation upon increasing the peptide inhibitor length is consistent with that observed for the Asp-((2,6-dichlorobenzoyl)oxy)methyl ketone ($AspCH_2DCB$)[4] and Asp-((1-phenyl-3-(trifluoromethyl)pyrazol-5-yl)oxy)-methyl ketone ($AspCH_2PTP$)[5] classes of ICE inhibitor. The phenyl rings of the (diphenylphosphinyl)oxy departing group are important for potency since inhibitors **6** and **7** show attenuated activity relative to **2**. In inhibitor **6** a phenyl moiety is replaced with a hydrogen atom, while in inhibitor **7** both the aryl rings are

† Department of Medicinal Chemistry.
‡ Department of Biochemistry.

0022-2623/95/1838-0220$09.00/0

Scheme 1. Synthesis of the (Phosphinyloxy)methyl Ketones **1**–**9** (Absolute Stereochemistry is as Shown)

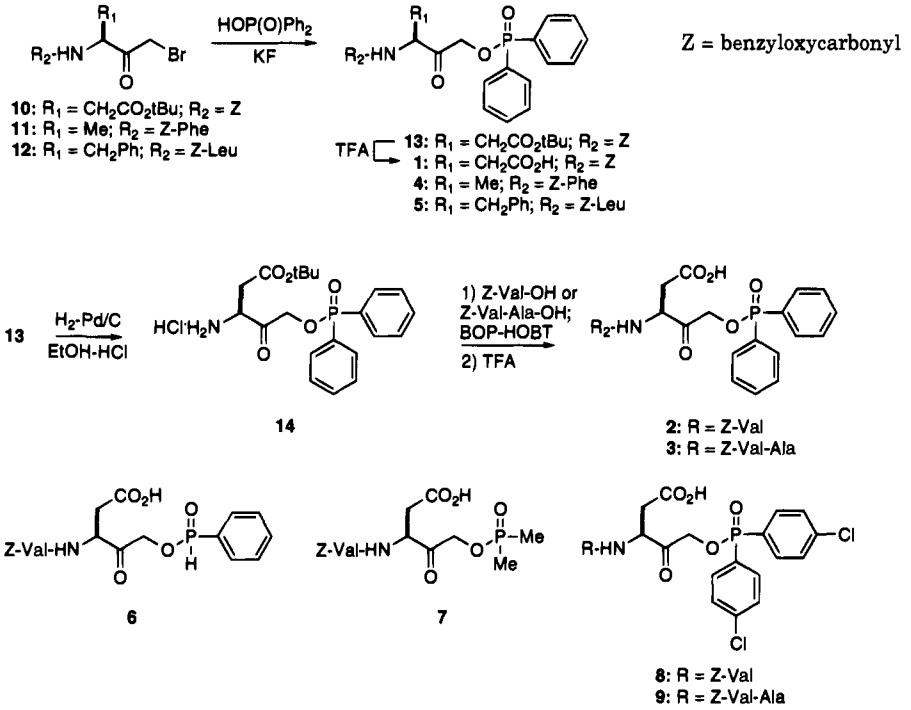


Table 1. Evaluation of Inhibitors **1**–**9** and Related Analogs **15**–**20** against ICE, Cathepsin B, and Calpain I

inhibitor	k_{obs}/[I] (M^{-1} s^{-1})		
	ICE[a,j]	cathepsin B[b,j]	calpain I[c,j]
Z-Asp-CH_2DPP[d] (**1**)	11800	g	g
Z-Val-Asp-CH_2DPP (**2**)	50200	<500	g
Z-Val-Ala-Asp-CH_2DPP (**3**)	117000	1700	g
Z-Val-Asp-CH_2HPP[d] (**6**)	<500	g	g
Z-Val-Asp-CH_2DMP[d] (**7**)	12300	g	g
Z-Val-Asp-CH_2DCPP[d] (**8**)	77300	g	g
Z-Val-Ala-Asp-CH_2DCPP (**9**)	230000	g	g
Z-Phe-Ala-CH_2DPP (**4**)	<500	400000	g
Z-Leu-Phe-CH_2DPP (**5**)	<500	<500	10000
Z-Phe-Ala-CH_2DCB[d] (**15**)	<500	1500000[h]	300
Z-Leu-Phe-CH_2DCB (**16**)	g	<1000[i]	11400
Z-Val-Asp-CH_2DCB[e] (**19**)	30000	g	g
Z-Phe-Ala-CH_2PTP[d] (**17**)	<500	2300	<500
Z-Leu-Phe-CH_2PTP (**18**)	g	<500	<500
Z-Val-Asp-CH_2PTP[f] (**20**)	20000	<500	g

[a] For a description of the ICE assay (pH 7.4, 37 °C), see ref 5. [b] Cathepsin B assay (pH 6.5, 25 °C, see ref 5. [c] Human erythrocyte calpain I assay was carried out using a continuous assay (pH 7.5, 8 °C) according to ref 18. [d] DPP = (diphenylphosphinyl)oxy; HPP = (phenylphosphinyl)oxy; DMP = (dimethylphosphinyl)oxy; DCPP = (bis(4-chlorophenyl)phosphinyl)oxy; DCB = 2,6-dichlorobenzoyl)-oxy; PTP = ((1-phenyl-3-(trifluoromethyl)pyrazol-5-yl)oxy. [e] Data taken from ref 4. [f] Data taken from ref 4. [g] Not determined. [h] 360 000 M^{-1} s^{-1} at 8 °C. [i] 250 M^{-1} s^{-1} at 8 °C. [j] Eleven different inhibitor concentrations were used for each k_{obs}/[I] determination. Standard error <10%.

exchanged for methyl groups. The phenyl replacements result in a *ca.* 4- to >100-fold loss in potency. Modulation of the inactivation rate is achieved by introduction of an electron-withdrawing group into the aryl rings as exemplified by inhibitors **8** and **9**. *p*-Chloro substitution of the phenyl rings leads to a doubling of the rate of inactivation for the Z-Val-Ala-Asp tripeptide from 117 000 M^{-1} s^{-1} for **3** to 230 000 M^{-1} s^{-1} for **9**. A similar rate increase is observed for the dipeptide pair **2** and **8**. The effect of the *p*-chloro substitution may be to decrease the pK_a of the leaving group, thereby giving rise to rate enhancement.[12,13] Finally, the lack of activity of Z-Phe-Ala-CH_2DPP (**4**) and Z-Leu-Phe-CH_2DPP (**5**) against ICE is predicted based on the strict P_1 Asp specificity requirement for the enzyme.[4]

The utility of the DPP departing group for inhibiting other cysteine proteases was investigated. Evaluation of Z-Phe-Ala-CH_2DPP (**4**) and Z-Leu-Phe-CH_2DPP (**5**) against cathepsin B and calpain I revealed these agents to be potent time-dependent inhibitors of the enzymes. The k_{obs}/[I] for inhibitor **4** is 400 000 M^{-1} s^{-1} (cathepsin B) while the k_{obs}/[I] for inhibitor **5** is 10 000 M^{-1} s^{-1} (calpain I). These second-order rate constants for **4** and **5** are comparable to those obtained for the corresponding DCB analogs **15** and **16**.[14] A similar study was conducted to ascertain the general utility of the PTP group to inhibit cathepsin B and calpain I. The PTP group had been previously shown by us to be a viable leaving group in the aspartic acid-based ICE inhibitors.[4] In striking contrast to **4** and **5**, the Z-Phe-Ala-CH_2PTP and Z-Leu-Phe-CH_2PTP analogs **17** and **18**[14] show greatly diminished activity against cathepsin B and calpain I. Collectively, the results suggest that the DCB and the newly discovered DPP departing groups may have general application in inhibiting cysteine proteases, while the PTP departing group is an enzyme (ICE) specific departing group (compare **2**, **19**, **20** to **4**, **15**, **17** and **5**, **16**, **18**).[15]

In summary, we have discovered the peptidyl α-((diphenylphosphinyl)oxy)methyl ketones as a novel class of cysteine protease inhibitor. The generic utility of the DPP leaving group has been demonstrated through the inhibition of ICE, cathepsin B, and calpain I by the Asp-CH_2DPP series **1**–**3** and inhibitors Z-Phe-Ala-CH_2DPP (**4**) and Z-Leu-Phe-CH_2DPP (**5**). We have also provided evidence that in contrast to the DCB and DPP leaving groups, the earlier reported PTP leaving group[4] appears specific for ICE. These results support our contention that the structure of the leaving group plays an important and as yet undefined role in enzyme recognition.[15,16] The disclosure of other departing groups,

structurally distinct from the DCB, DPP, and PTP functionality, will be forthcoming.

References

(1) (a) Smith, R. A.; Copp, L. J.; Coles, P. J.; Pauls, H. W.; Robinson, V. J.; Spencer, R. W.; Heard, S. B.; Krantz, A. New Inhibitors of Cysteine Proteinases. Peptidyl Acyloxymethyl Ketones and the Quiescent Nucleofuge Strategy. *J. Am. Chem. Soc.* **1988**, *110*, 4429–4431. (b) Krantz, A.; Copp, L. J.; Coles, P. J.; Smith, R. A.; Heard, S. B. Peptidyl (Acyloxy)methyl Ketones and the Quiescent Affinity Label Concept: The Departing Group as a Variable Structural Element in the Design of Inactivators of Cysteine Proteases. *Biochemistry* **1991**, *30*, 4678–4687. (c) Robinson, V. J.; Pauls, H. W.; Coles, P. J.; Smith, R. A.; Krantz, A. Carbon-13 NMR Characterization of the Papain Adduct Formed by Peptidyl Acyloxy-, Aryloxy-, and Chloromethyl Ketone Irreversible Inhibitors. *Bioorg. Chem.* **1992**, *20*, 42–54. (d) For reviews regarding cysteine protease inhibition, see: Shaw, E. Cysteinyl Proteinases and Their Selective Inactivation. *Adv. Enzymol.* **1990**, *63*, 271–347 and Demuth, H.-U. Recent Developments in Inhibiting Cysteine and Serine Proteases. *J. Enzyme Inhib.* **1990**, *3*, 249–278.

(2) Wagner, B. M.; Smith, R. A.; Coles, P. J.; Copp, L. J.; Ernest, M. J.; Krantz, A. *In vivo* Inhibition of Cathepsin B by Peptidyl (Acyloxy)methyl Ketones. *J. Med. Chem.* **1994**, *37*, 1833–1840.

(3) For leading references on ICE, see: Miller, D. K.; Calaycay, J. R.; Chapman, K. T.; Howard, A. D.; Kostura, M. J.; Molineaux, S. M.; Thornberry, N. A. The IL-1β Converting Enzyme as a Therapeutic Target. In *Immunosuppressive and Antiinflammatory Drugs*; Allison, A. C., Lafferty, K. J., Fliri, H., Eds.; Annals of the New York Academy of Sciences; New York Academy of Sciences: New York, 1993; Vol. 696, pp 133–148.

(4) Dolle, R. E.; Singh, J.; Rinker, J.; Hoyer, D.; Prasad, C. V. C.; Graybill, T. L.; Salvino, J. M.; Helaszek, C. T.; Miller, R. E.; Ator, M. A. Aspartyl α-(((1-Phenyl-3-trifluoromethyl)pyrazol)-5-oxy)-methyl Ketones as Interleukin-1β Converting Enzyme Inhibitors. Significance of the P_1 and P_3 Amido Nitrogens For Enzyme-Peptide Inhibitor Binding. *J. Med. Chem.* **1994**, *37*, 3863–3866.

(5) Dolle, R. E.; Hoyer, D.; Prasad, C. V. C.; Schmidt, S. J.; Helaszek, C. T.; Miller, R. E.; Ator, M. A. P1 Aspartate-Based Peptide α-((2,6-Dichlorobenzoyl)oxy)methyl Ketones as Potent Time-Dependent Inhibitors of Interleukin-1β Converting Enzyme. *J. Med. Chem.* **1994**, *37*, 563–564.

(6) Krantz, A. A Classification of Enzyme Inhibition. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1327–1334.

(7) Subtle or specific effects of the departing group do influence inhibitor potency. This is clearly evident by the data present in Figure 2 in ref 1b. At leaving group pK_a of 3.5, cathepsin B inactivation rates range some 200-fold. (b) Potency is dependent on the type and pattern of aryl substitution in the Asp-((aryl)-acyloxy)methyl ketone class of ICE inhibitor, unpublished observation.

(8) Crawford, D. In *Intracellular Calcium-Dependent Proteolysis*; Mellgren, R. L., Murachi, T., Eds.; CRC Press: Boca Raton, FL, 1990.

(9) All new compounds gave physical and spectroscopic data consistent with their structure.

(10) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. New Coupling Reagents in Peptide Chemistry. *Tetrahedron Lett.* **1989**, *30*, 1927–1930.

(11) Harger, M. J. P.; Westlake, S. Diarylphosphinic Azides. Photochemical Reactions Including Rearrangement in Methanol. *Tetrahedron* **1982**, *38*, 1511–1515.

(12) The pK_a for bis(4-chlorophenyl)phosphinic acid is 3.2 versus pK_a = 3.9 for diphenylphosphinic acid: Baldwin, R. A.; Cheng, M. T.; Homer, G. D. Application of the Hammett Equation to Organophosphorous-Substituted Phosphinic and Benzoic Acids. *J. Org. Chem.* **1967**, *32*, 2176–2180.

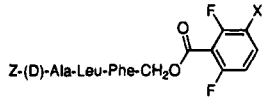
(13) The apparent potency–pK_a trend observed here for the DPP series is in contrast with (acyloxy)methyl ketones as reported by Thornberry: Thornberry, N. A.; Peterson, E. P.; Zhao, J. J.; Howard, A. D.; Griffin, P. R.; Chapman, K. T. *Biochemistry* **1994**, *33*, 3934–3940.

(14) Compounds **15**–**18** were prepared from **11** and **12** and 2,6-dichlorobenzoic acid and 1-phenyl-5-hydroxy-3-(trifluoromethyl)-pyrazole in DMF with KF as base.

(15) For other examples of the effect of leaving group on the inactivation of cysteine proteases, see: (a) Pliura, D. H.; Bonaventura, B. J.; Smith, R. A.; Coles, P. J.; Krantz, A. Comparative Behavior of Calpain and Cathepsin B Toward Peptidyl Acyloxymethyl Ketones, Sulphonium Methyl Ketones and Other Potential Inhibitors of Cysteine Proteases. *Biochem. J.* **1992**, *288*, 759–762. (b) Crawford, C.; Mason, R. W.; Wikstrom, P.; Shaw, E. The Design of Peptidyldiazomethane Inhibitors to Distinguish Between the Cysteine Proteases Calpain II. Cathepsin L and Cathepsin B. *Biochem. J.* **1988**, *253*, 751–758.

(16) It would be of interest to establish whether a potent, reversible inhibitor of ICE could be designed based on **1** or Z-AspCH_2PTP. Structurally simple phenylalkyl ketones derived from aspartic acid have been described as reversible inhibitors of ICE: Mjalli, A. M. M.; Chapman, K. T.; MacCross, M.; Thornberry, N. A. Phenyl Ketones as Potent Reversible Inhibitors of Interleukin-1β Converting Enzyme. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2689–2692.

(17) Another example of the profound influence leaving group structure can have on activity is seen with the inhibition of calpain I and cathepsins B and L by compounds **21** and **22**. Ketone **21** is a specific inhibitor of calpain I. Introduction of the morpholinoethoxy group in the meta position of the leaving groups leads to compound **22** which is no longer selective for calpain I. All data obtained at 8 °C. See ref 18.



	$k_{obs}/[I]$ ($M^{-1}s^{-1}$)		
	Calpain I	Cathepsin B	Cathepsin L
21: X = H	31,000	100	300
22: X = OCH_2CH_2N⟨O⟩	32,000	400	28,000

(18) Harris, A. L.; Gregory, J. S.; Maycock, A. L.; Graybill, T. L.; Osifo, I. K.; Schmidt, S. L.; Dolle, R. E. Characterization of a Continuous Fluorogenic Assay for Calpain I. Kinetic Evaluation of Peptidic Aldehydes and α-Substituted Methyl Ketones as Inhibitors of the Enzyme. *Bioorg. Med. Chem. Lett.*, in press.

JM9406920