3-Phosphonopropionic acids inhibit carboxypeptidase A as multisubstrate analogues or transition-state analogues

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A series of phosphonic acid analogues of 2-benzylsuccinate were tested as inhibitors of carboxypeptidase A. The most potent of these, (2*RS*)-2-benzyl-3-phosphonopropionic acid, had a K_i of $0.22 \pm 0.05 \mu\text{M}$, equipotent to (2*RS*)-2-benzylsuccinate and thus one of the most potent reversible inhibitors known for this enzyme. Lengthening by one methylene group to (2*RS*)-2-benzyl-4-phosphonobutyric acid increased the K_i to $370 \pm 60 \mu\text{M}$. The monoethyl ester (2*RS*)-2-benzyl-3-(*O*-ethylphosphono)propionic acid was nearly as potent as (2*RS*)-2-benzyl-3-phosphonopropionic acid, with a K_i of $0.72 \pm 0.3 \mu\text{M}$. The sulphur analogue of the monoethyl ester, 2-*ambo-P-ambo*-2-benzyl-3-(*O*-ethylthiophosphono)propionic acid, had a K_i of $2.1 \pm 0.6 \mu\text{M}$, nearly as active as (2*RS*)-2-benzyl-3-(*O*-ethylphosphono)propionic acid. These phosphonic acids and esters could be considered to be multisubstrate inhibitors of carboxypeptidase A by virtue of their structural analogy with 2-benzylsuccinate. Alternatively, the tetrahedral hybridization at the phosphorus atom suggests that they could be mimicking a tetrahedral transition state for the enzyme-catalysed hydrolysis of substrate.

INTRODUCTION

The zinc metalloproteinase carboxypeptidase A (EC 3.4.12.2) is strongly inhibited by the multisubstrate or bi-product analogue (2*RS*)-2-benzylsuccinate [K_i $0.2 \mu\text{M}$ (Galardy & Kortylewicz, 1984); K_i $1.1 \mu\text{M}$ (Byers & Wolfenden, 1973)]. In this type of inhibitor, functional groups of both hydrolysis products of a typical substrate (the carboxy group of the newly formed carboxylic acid product and the benzyl side chain and terminal carboxy group of the other product) are combined to form the inhibitor (Byers & Wolfenden, 1973). The discovery of this inhibitor ultimately led to the synthesis of another inhibitor with a better ligand for the zinc atom of the enzyme, namely (2*RS*)-2-benzyl-3-mercaptopropionic acid [K_i $0.011 \mu\text{M}$ (Ondetti *et al.*, 1979)]. This inhibitor could also be considered to be a multisubstrate analogue with an ionizable thiol group in place of the ionizable carboxylate group of 2-benzylsuccinate.

N^α-Phospho-L-phenylalanine [K_i $5 \mu\text{M}$ (Kam *et al.*, 1979); K_i $2.1 \mu\text{M}$ (Holmquist & Vallee, 1979)] could be considered to be a multisubstrate analogue related to 2-benzylsuccinate or to be a transition-state analogue with a tetrahedral phosphorus atom mimicking the tetrahedrally hybridized carbon atom that is formed from the carbonyl group during hydrolysis of amide substrates [see Wolfenden (1972) for a discussion of the concept of transition-state analogues, and Fig. 1]. For the zinc metalloproteinase thermolysin, 2-benzylsuccinate (Bolognesi & Matthews, 1979), a mercaptan-peptide (Monzingo & Matthews, 1982) and a phosphoramidate-peptide (Weaver *et al.*, 1977) have been shown, by X-ray crystallography, to bind with the appropriate functional group directly co-ordinated to the zinc atom. Thus these

functional groups are presumed to bind to carboxypeptidase A in the same manner.

The aldehyde analogue of 2-benzylsuccinate, (2*RS*)-2-benzyl-4-oxobutyric acid (K_i $0.048 \mu\text{M}$), has been proposed to bind to carboxypeptidase A as a tetrahedral transition-state analogue with a nucleophile of the enzyme or a water molecule taking the fourth position around the former aldehydic carbon atom (Galardy & Kortylewicz, 1984). This proposal has since been substantiated by X-ray crystallography, which shows that the fourth position is occupied by water, i.e. the aldehyde binds as its tetrahedral hydrate (D. W. Christianson & W. N. Lipscomb, Harvard University, personal communication). *N*^α-{[(Benzyloxycarbonyl)amino]methyl}-hydroxyphosphinoyl-L-phenylalanine (compound XIII, Cbz-NH-CH₂-P(O)(OH)-Phe) [K_i $0.09 \mu\text{M}$ (Jacobsen & Bartlett, 1981)] is an exact analogue of the carboxypeptidase A substrate *N*^α-benzyloxycarbonyl-glycyl-L-phenylalanine with the tetrahedral phosphonamide group replacing the trigonal planar carboxamide of the substrate. The phosphonamide compound (XIII) thus appears to be a candidate for a true transition-state analogue. The functional groups carboxylate, thiol, aldehyde, phosphoramidate and phosphonamide therefore all give powerful inhibitors of carboxypeptidase A when located in the correct position of carboxypeptidase A substrates or products (see Fig. 1).

In the present work we introduce two new functional groups, the phosphonic acid and thiophosphonic ester groups, which also produce powerful inhibitors of carboxypeptidase A when substituted in the correct position in a multisubstrate analogue. These phosphonic and thiophosphonic inhibitors are essentially equipotent at pH 7.5 to all of the other carboxypeptidase A

Abbreviation used: m.s.(c.i.), chemical-ionization mass spectrometry.

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Fig. 1. Model for the active site of carboxypeptidase A (Ondetti *et al.*, 1979), showing proposed modes of binding for (a) substrate, (b) a transition-state for substrate hydrolysis and (c) transition-state analogue inhibitors or multisubstrate analogue inhibitors of carboxypeptidase A

In (c): R' = OH, X = O, Y = NH, phospho-L-phenylalanine (compound IX); R' = OH, S = O, Y = CH₂, 2-benzyl-3-phosphonopropionic acid (compound V); R' = EtO, X = O, Y = CH₂, 2-benzyl-3-(O-ethylphosphono)propionic acid (compound VI); R' = EtO, X = S, Y = CH₂, 2-benzyl-3-(O-ethylthiophosphono)propionic acid (compound VIII). S₁, S₂', P, and P₁' respectively designate binding subsites and the amino acid residues of substrates that occupy those sites (Schechter & Berger, 1968).

inhibitors with the exception of (2*RS*)-2-benzyl-3-mercaptopropionic acid whose *K_i* is about 20-fold lower (Ondetti *et al.*, 1979). The phosphonic and thiophosphonic inhibitors can be considered as multisubstrate analogues based on benzy succinate, or as transition-state analogues due to the presence of a tetrahedrally hybridized phosphorus atom at the position occupied by a trigonal planar carbon atom in the ground state of the substrate and a tetrahedrally hybridized carbon atom in a transition state. Fig. 1 shows the proposed binding to carboxypeptidase A of substrate in the ground state and in a transition state, and of the phosphonic acid inhibitors.

EXPERIMENTAL

Chemicals and general methods

All the starting materials and solvents used in this work were reagent grade in generally 98% or higher purity, and were used without further purification or prepared by

standard literature procedures. T.l.c. was on silica gel 60F-254 (EM Reagents, Elmsford, NY, U.S.A.) in system A (chloroform/n-hexane, 3:1, v/v) or system B (propan-1-ol/conc. NH₃). Compounds were located by using the phosphomolybdate spray (Bandurski & Axelrod, 1951), u.v. light and I₂ vapour. Purification by column chromatography was performed on silica gel (100–200 mesh; EM Reagents). Proton-n.m.r. spectra were recorded on a Varian EM-390 spectrometer for dilute solutions (10%), with tetramethylsilane as internal standard. Chemical-ionization mass spectra [m.s.(c.i.)] were measured on a Finnigan 3300 GC/MS instrument at 130 eV with a solid probe. Melting points were taken on a hot-stage and are corrected. Boiling points, which are given for short-path evaporative distillation, are uncorrected. The yields given were based on isolated products after purification.

Syntheses

Methyl 2-methenyl-3-phenylpropionate (compound I). This was prepared in 76% yield, by alkylation of dimethyl benzylmalonate with formaldehyde by the general method of Stetter & Kuhlmann (1979): *R_F* (A) 0.75; n.m.r. δ (p.p.m.) (C²HCl₃) 3.60 (6H, s, 2OCH₃), 5.35 (1H, s, HCH=C), 6.15 (1H, s, HCH=C), 7.15 (5H, s, C₆H₅).

Phosphonothionic acid diethyl ester (compound II). This was prepared by the method of Wagner *et al.* (1971) by adding ethanol to P₄S₁₀ in 51% yield: b.p. (approx. 15 mmHg) 95–98 °C; n.m.r. δ (p.p.m.) (neat) 1.15 (6H, t, 2OCH₂CH₃), 2.80 (0.5H, s, PH), 3.90 (4H, m, 2OCH₂CH₃), 10.4 (0.5H, s, PH).

2-Benzyl-3-phosphonopropionic acid tripotassium salt (compound V). The addition of the sodium salt of diethyl phosphite to compound (I), by the method of Pudovik *et al.* (1950), gave 2-benzyl-3-(*OO*-diethylphosphono)propionic acid methyl ester (compound III) in 89% yield, as a colourless oil: *R_F* (A) = 0.52; n.m.r. δ (p.p.m.) (C²HCl₃) 1.25 (6H, m, 2OCH₂CH₃), 1.60–2.60 (2H, m, PCH₂), 2.90 (3H, m, PhCH₂CHCO), 3.55 (3H, s, OCH₃), 4.05 (4H, m, 2OCH₂CH₃), 7.20 (5H, m, C₆H₅); m.s.(c.i.) 315 (*M*+1), 333 (*M*+29), 355 (*M*+41). Refluxing of compound (III) (1.5 g; 4.8 mmol) with 15 ml of conc. HCl followed by evaporation of the solvent under reduced pressure and drying of the residue under high vacuum overnight gave 1.17 g (55% yield) of 2-benzyl-3-phosphonopropionic acid (compound IV), as a glassy hygroscopic solid: n.m.r. δ (p.p.m.) (C²HCl₃/[²H₆]dimethyl sulfoxide) 1.95 (2H, m, PCH₂), 2.95 (3H, m, PhCH₂CHCO₂H), 7.20 (5H, s, CH₅), 10.5 (3H, s, P(OH)₂, CO₂H); m.s.(c.i.) 227 [(*M*+1)–H₂O], 255 [(*M*+29)–H₂O], 267 [(*M*+41)–H₂O]. The pH of the solution of compound (IV) (0.72 g; 2.9 mmol) in 15 ml of water was adjusted to 8 with 1 M-KOH. Freeze-drying of the frozen mixture followed by washing of the residue with ethanol and acetone gave 1.035 g (98% yield) of compound (V): m.p. > 350 °C; *R_F* (B), 0.05; n.m.r. (p.p.m.) (²H₂O) 1.65–2.00 (2H, m, PCH₂), 2.55–3.25 (3H, m, PhCH₂CHCO₂K), 4.70 (¹H²O), 7.30 (5H, s, C₆H₅).

2-Benzyl-3-(*O*-ethylphosphono)propionic acid dipotassium salt (compound VI). A mixture of compound (III) (2 g, 6.4 mmol), 3.18 ml (12.8 mmol) of 4 M-KOH

and 5 ml of water in 5 ml of tetrahydrofuran was refluxed for 4 h. After evaporation of the organic solvent, the residue was diluted to 15 ml with water, and washed with diethyl ether (15 ml), frozen and freeze-dried. The solid residue was dissolved in 15 ml of ethanol, and product was precipitated by addition of anhydrous diethyl ether (50 ml), to give 1.9 g (86% yield) of compound (VI): m.p. > 350 °C; R_F (B) 0.35; n.m.r. δ (p.p.m.) ($^2\text{H}_2\text{O}$) 1.25 (3H, t, OCH_2CH_3), 1.60–2.05 (2H, m, $\text{P}-\text{CH}_2$), 2.65–3.15 (3H, m, $\text{C}_6\text{H}_5\text{CH}_2\text{CHCO}$), 3.85 (2H, m, OCH_2CH_3), 4.72 ($^1\text{H}^2\text{HO}$), 7.30 (5H, s, C_6H_5).

2-Benzyl-3-(*O*-ethylthiophosphonyl)propionic acid dipotassium salt (compound VIII). The addition of compound (I) (2 g; 11.35 mmol) to compound (II) (1.75 g; 11.35 mmol), performed as described for compound (III), gave 3.64 g (97% yield) of 2-benzyl-3-(*OO*-diethylthiophosphono)propionic acid methyl ester (compound VII) as an oil: R_F (A) 0.85; n.m.r. δ (p.p.m.) (C^2HCl_3) 1.25 (6H, m, $2\text{OCH}_2\text{CH}_3$), 1.65–2.60 (2H, m, PCH_2), 2.80–3.40 (3H, m, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}$), 3.60 (3H, s, OCH_3), 4.1 (4H, m, $2\text{OCH}_2\text{CH}_3$), 7.20 (5H, m, C_6H_5); m.s.(c.i.): 331 ($M+1$), 359 ($M+29$), 371 ($M+41$). The mixture of compound (VII) (0.91 g, 2.75 mmol) and 5.5 ml of 1 M-KOH in 10 ml of ethanol was refluxed for 20 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in 10 ml of ethanol and 100 ml of anhydrous diethyl ether was added. After the mixture had been kept for 12 h in the freezer (-10°C), the precipitate of compound (VIII) was filtered off and dried under reduced pressure: yield 0.3 g (30%); R_F (B) 0.37; n.m.r. δ (p.p.m.) ($^2\text{H}_2\text{O}$) 1.10 (3H, t, OCH_2CH_3), 1.50–2.20 (2H, m, PCH_2), 2.75 (3H, m, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}$), 3.87 (2H, m, OCH_2CH_3), 4.52 ($^1\text{H}^2\text{HO}$), 7.21 (5 H, s, C_6H_5).

2-Benzyl-4-phosphonobutyric acid (compound X). This was prepared as previously described (Galardy & Kortylewicz, 1985).

***N* $^{\alpha}$ -Phospho-L-phenylalanine (compound IX).** This was prepared as previously described for *N* $^{\alpha}$ -phospho-L-alanine (Galardy & Grobelny, 1983).

Kinetic studies

The hydrolysis of *O*-(trans-*p*-chlorocinnamoyl)-L- β -phenyl-lactate by carboxypeptidase A (Sigma Chemical Co., St. Louis, MO, U.S.A.) was measured spectrophotometrically at 25 °C as described by Suh & Kaiser (1976) in 50 mM-Tris base containing 0.5 M-NaCl adjusted to pH 7.5 with HCl. The suspension of enzyme as received from Sigma was diluted to give a stock solution with a concentration of 7 nM in the assay buffer. The enzyme concentration in the kinetic assay was 0.36 nM. Substrate concentrations were varied from 80 to 400 μM . K_m was found to be $170 \pm 30 \mu\text{M}$. Initial velocities were calculated from the linear initial slopes of the change in absorbance against reaction time where the amount of substrate consumed was always less than 10%. For each substrate and inhibitor at least four K_i values were determined, one from a Lineweaver-Burk plot and one from a Dixon plot from each of two independent experiments done on different days. The four K_i values were averaged, and a standard deviation was calculated. A stock solution of *N* $^{\alpha}$ -phospho-L-phenylalanine (compound IX) was prepared in water adjusted to pH 10 with NaOH in order to prevent cleavage of the P-N bond, which occurs at pH 7.5 with a half-time of about 1 h in similar phosphoramidates (Poncz *et al.*, 1984).

RESULTS

The K_i values for all of the inhibitors prepared in this study and for three reference compounds are given in Table 1. Fig. 2 shows a Lineweaver-Burk and a Dixon plot for the inhibition of carboxypeptidase A by (2*RS*)-2-benzyl-3-phosphonopropionic acid (compound V) (K_i 0.22 μM), the most powerful inhibitor prepared. This compound is equipotent to 2-benzylsuccinate (see Table 1). The phosphonic acid compound (V) is also nearly equipotent to Cbz-NH-CH₂-P(O)(OH)-Phe (compound XIII) [K_i 0.09 μM (Jacobsen & Bartlett, 1981)] and would be exactly equipotent if only one enantiomer of compound (V) is active. The K_i that we find for *N* $^{\alpha}$ -phospho-L-phenylalanine (compound IX), $0.24 \pm 0.05 \mu\text{M}$, is nearly 10-fold lower than the lowest

Table 1. Phosphonic acid and ester inhibitors of carboxypeptidase A

Values are given as means $\pm$ S.D. Abbreviation: Cbz, benzyloxycarbonyl.

Inhibitor	K_i (μM)
$\text{H}_2\text{PO}_3\text{-NH-CH}(\text{CH}_2\text{C}_6\text{H}_5)\text{-CO}_2\text{H}$ (IX)	$0.24 \pm 0.05^*$
$\text{H}_2\text{PO}_3\text{-CH}_2\text{-CH}(\text{CH}_2\text{-C}_6\text{H}_5)\text{-CO}_2\text{H}$ (V)	0.22 ± 0.05
$\text{EtO-P}(\text{O})(\text{OH})\text{-CH}_2\text{-CH}(\text{CH}_2\text{-C}_6\text{H}_5)\text{-CO}_2\text{H}$ (VI)	0.72 ± 0.3
$\text{EtO-P}(\text{O})(\text{SH})\text{-CH}_2\text{-CH}(\text{CH}_2\text{-C}_6\text{H}_5)\text{-CO}_2\text{H}$ (VIII)	2.1 ± 0.06
$\text{H}_2\text{PO}_3\text{-CH}_2\text{-CH}_2\text{-CH}(\text{CH}_2\text{-C}_6\text{H}_5)\text{-CO}_2\text{H}$ (X)	370 ± 60
$\text{HO}_2\text{C-CH}_2\text{-CH}(\text{CH}_2\text{-C}_6\text{H}_5)\text{-CO}_2\text{H}$	$0.2 \pm 0.1^\dagger$
$\text{HO}_2\text{C-CH}_2\text{-CH}_2\text{-CH}(\text{CH}_2\text{-C}_6\text{H}_5)\text{-CO}_2\text{H}$	$5^\ddagger$
$\text{Cbz-NH-CH}_2\text{-P}(\text{O})(\text{OH})\text{-NH-CH}(\text{CH}_2\text{-C}_6\text{H}_5)\text{-CO}_2\text{H}$ (XIII)	$0.09^\S$

* Literature values: 5 μM (Kam *et al.*, 1979); 2.1 μM (Holmquist & Vallee, 1979).

† Galardy & Kortylewicz (1984); literature value: 1.1 M (Byers & Wolfenden, 1973).

‡ Byers & Wolfenden (1973).

§ Jacobsen & Bartlett (1981); at pH 7.5.

Fig. 2. Lineweaver-Burk (a) and Dixon (b) plots for the inhibition of carboxypeptidase A by 2-benzyl-3-phosphonopropionic acid (compound V)

reported K_i of 2.1 μM (Holmquist & Vallee, 1979). This could be due to the difficulty of preparing phosphoramidates in pure form and to their decomposition at neutral pH with a half-time of about 1 h (Poncz *et al.*, 1984). In the present study N^α -phospho-L-phenylalanine (compound IX) was made up in a stock solution at pH 10 in order to prevent decomposition. Phosphate itself is a weak inhibitor of carboxypeptidase A, but it is too weak to exhibit any inhibition here (Lumry *et al.*, 1951).

The monoethyl ester of compound (V), (2*RS*)-2-benzyl-3-(*O*-ethylphosphono)propionic acid (compound VI) (K_i 0.72 μM), is nearly equipotent to compound (V). The invariance of K_i with monoesterification of the phosphonic acid of compound (V) is similar to the invariance of K_i with monoesterification by aliphatic alcohols on the phospho group in the angiotensin-converting-enzyme inhibitor N^α -phospho-L-alanyl-L-proline. The methyl, ethyl, benzyl (Galardy, 1982) and isopropyl (Galardy & Grobelny, 1985) esters of the phospho group of N^α -phospho-L-alanyl-L-proline are equipotent.

Substitution of one oxygen atom of compound (VI) with a sulphur atom gives (2-*ambo-P-ambo*)-2-benzyl-3-(*O*-ethylthiophosphono)propionic acid (compound VIII). Compound (VIII) is equipotent to compound (VI), assuming that one of the isomers at the chiral phosphorus atom of compound (VIII) is much more active than the other. Nishino & Powers (1979) found N^α -thiophospho-L-phenylalanyl-L-alanylglycine amide (K_i 7.0 μM) to be somewhat less potent than N^α -phospho-L-phenylalanyl-L-alanylglycine amide (K_i 2.6 μM) in inhibiting the zinc metalloproteinase thermolysin.

Extension of the phosphonic acid compound (V) by one methylene group to give (2*RS*)-2-benzyl-4-phosphonobutyric acid (compound X) increases the K_i by over three orders of magnitude. This result is surprising, since extending (2*RS*)-2-benzylsuccinate (compound XI) to (2*RS*)-2-benzylglutarate (compound XII) increased the K_i by only 25-fold (Byers & Wolfenden, 1973).

DISCUSSION

The simple phosphono-carboxylic acid (2*RS*)-2-benzyl-3-phosphonopropionic acid (compound V) has a K_i equal to that of (2*RS*)-2-benzylsuccinate (compound XI) (see

Table 1) and is thus one of the most potent reversible inhibitors known for carboxypeptidase A. If only one of the enantiomers of compound (V) is active then its K_i must be about 0.11 μM , equipotent to the exact substrate analogue Cbz-NH-CH₂-P(O)(OH)-Phe (compound XIII) [K_i 0.09 μM (Jacobsen & Bartlett, 1981)]. N^α -Phospho-L-phenylalanine (compound IX) also has a K_i nearly equipotent to both these inhibitors (see Table 1). Clearly the Cbz-NH-CH₂- moiety of compound (XIII) does not contribute to the low K_i of that compound.

If the above three inhibitors are all transition-state analogues their respective K_i values should be inversely proportional to the respective specificity-constant ($k_{\text{cat.}}/K_m$) values for their analogous substrates (see discussion in Bartlett & Marlowe, 1983). Taking acetyl-L-phenylalanine to be the nearest substrate analogue of compounds (V) and (IX), its $k_{\text{cat.}}/K_m$ can be compared with the corresponding value for N^α -benzyloxycarbonylglycyl-L-phenylalanine, the substrate analogue of compound (XIII). Since acetyl-L-phenylalanine is not a substrate for carboxypeptidase A (Quioco & Lipscomb, 1971), its $k_{\text{cat.}}/K_m$ must be very small compared with $k_{\text{cat.}}/K_m$ for N^α -benzyloxycarbonylglycyl-L-phenylalanine [$k_{\text{cat.}}/K_m$ 0.5 $\mu\text{M}^{-1}\cdot\text{min}^{-1}$ (Quioco & Lipscomb, 1971)]. If these inhibitors are transition-state analogues, then the inhibitor analogous to the better substrate (higher $k_{\text{cat.}}/K_m$) should be much more powerful than the analogues (compounds V and IX) of the very poor substrate. Bartlett & Marlowe (1983) have shown this relationship to hold for a series of thermolysin substrates and inhibitors that vary by over two orders of magnitude in $k_{\text{cat.}}/K_m$ and K_i respectively. The lack of even qualitative inverse proportionality between K_i for compounds (V) and (IX) versus compound (XIII) compared with the values of $k_{\text{cat.}}/K_m$ for their respective substrate analogues is thus surprising. Perhaps the structures of compounds (V) and (IX) are not analogous enough to acetyl-L-phenylalanine for a legitimate comparison with compounds (XIII) and its substrate analogue. Alternatively, compounds (V) and (IX) may be more like multisubstrate analogues than transition-state analogues and thus do not follow the expected relationship for transition-state analogues.

In summary, (2*RS*)-2-benzyl-3-phosphonopropionic

acid (compound V) (K_i 0.22 μM) is one of the most powerful reversible inhibitors known for carboxypeptidase A at pH 7.5 and is exceeded in potency only by (2*RS*)-2-benzyl-3-mercaptopropionic acid [K_i 0.011 μM (Ondetti *et al.*, 1979)]. Compound (VI), the monoethyl ester (on the phosphono group) of compound (V), and compound (VIII), the thiophosphono analogue of compound (VI), are approximately equipotent to compound (V). The low K_i of compound (V) is surprising considering that it is lacking both the NH group and the Cbz-NH-CH₂- group of the proposed transition-state analogue for carboxypeptidase A, Cbz-NH-CH₂-P(O)(OH)-Phe (compound XIII) [K_i 0.09 μM (Jacobsen & Bartlett, 1981)]. (2*RS*)-2-Benzyl-3-phosphonopropionic acid (compound V) could be considered to be a transition-state analogue for carboxypeptidase A by virtue of its tetrahedrally hybridized phosphorus atom or could be a multisubstrate analogue by virtue of its similarity to 2-benzylsuccinate.

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