# Molecular and Crystal Structures of MDL27,467A Hydrochloride and Quinapril Hydrochloride, Two Ester Derivatives of Potent Angiotensin Converting Enzyme Inhibitors

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*Received April 30, 1990*

The molecular structures of MDL27,467A hydrochloride, [4α,7α(R*),12bβ]-7-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1,2,3,4,6,7,12a,12b-octahydro-6-oxopyrido[2,1-a][2]benzazepine-4-carboxylic acid diphenylmethyl ester hydrochloride, and quinapril hydrochloride, [3S-[2[R*(R*)],3R]]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]-amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid hydrochloride, were determined by X-ray diffraction methods. The modified, C-terminal dipeptide portions and the phenylpropyl fragments in both crystal structures adopt similar conformations. The binding positions for several pharmacophores are defined by the constraint of the tricyclic system in the crystallographic structure of MDL27,467A hydrochloride. Conformational energy calculations show that the phenyl ring of the tetrahydro-3-isoquinoline system of quinapril does not fit into the $S_2'$ hydrophobic pocket of angiotensin converting enzyme.

## Introduction

Angiotensin converting enzyme (ACE, EC 3.4.15.1, dipeptidyl carboxypeptidase, kininase II), the regulatory zinc protease in the renin–angiotensin system, converts the inactive decapeptide, angiotensin I, to a potent vasoconstrictive octapeptide, angiotensin II, by cleaving the C-terminal dipeptide His-Phe.[1,2] The same enzyme inactivates the powerful vasodilator bradykinin, again by hydrolytic release of one or more C-terminal dipeptidyl residues.[2-5] Thus, both enzymatic reactions catalyzed by the converting enzyme may produce an elevation of blood pressure, either by increased vasoconstriction due to excessive synthesis of the peptide hormone angiotensin II or by decreased vasodilation due to bradykinin hydrolysis. Snake venom peptides, which contain proline as the C-terminal amino acid,[6] were shown to lower blood pressure in humans with various forms of hypertension by inhibition of the converting enzyme;[7-10] the clinical potential of the peptide inhibitors was limited, however, due to a lack of oral activity. Identification of the first potent and orally active ACE inhibitor, captopril[11,12] (1), was the result of extensive modifications of the hypotensive peptides. The mercaptoalkanoyl amino acid captopril was designed to establish the critical interaction with the active site zinc ion of ACE through a sulfhydryl group. Although the hypertension drug is well-tolerated in general, the agent eventually produces side effects;[13] some of the adverse reactions of captopril have been attributed to the sulfhydryl moiety.[14] Enalaprilat[15] (3), the second ACE inhibitor to reach clinical application in the treatment of essential hypertension and congestive heart failure, coordinates the active-site Zn with a carboxylate group. The carboxylalkanoyl amino acid is not only more potent than the merkaptoalkanoyl amino acid captopril but also has a prolonged duration of action. To increase bioavailability, the active diacid enalaprilat (3) is administered as a monoethyl ester, enalapril (2).

Ondetti et al.[11] and Cushman et al.[12] proposed a model for the ACE active site which involved three vital binding interactions between the enzyme and inhibitors of the sulfhydryl and carboxyl ligand classes: (1) coordination of the zinc cation through the thiol group in mercaptoalkanoyl amino acids and through the carboxylate moiety in carboxylalkanoyl amino acids, (2) hydrogen bonding of the carbonyl oxygen atom of the C-terminal amide group through a hydrogen donor function in the binding site, and (3) electrostatic interaction between the C-terminal carboxylate ion and a positively charged residue in the active site. The superior potency of inhibitors with proline at the C-terminal position and the beneficial effects of an α-methyl group of the acyl portion of prolyl inhibitors indicated two additional binding sites: a hydrophobic interaction between the proline ring and the $S_2'$ hydrophobic pocket,[16] and a hydrophobic contact between the α-methyl group and the $S_1'$ subsite of ACE. Entropic contributions to the binding energy, arising from the constraints of both the pyrrolidine ring and the hypothetical $S_1'$ ligand, are also likely and cannot be excluded a priori.

The existence of a $S_2'$ hydrophobic pocket was supported by the findings of Kim et al.[17] wherein the replacement

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of the pyrrolidine ring in captopril by an indoline moiety, WY44221 (4), improves ACE inhibition both in vivo and in vitro; in addition, the superior in vitro activity of ACE inhibitors with a large lipophilic group at the C-terminal position was confirmed by others.[18-20] Besides the five drug–receptor interactions of captopril, Patchett et al.[15] proposed two additional pharmacophores for enalaprilat (3): the phenylalkyl side chain which binds to the $S_1$ subsite and the amino group, –NH–, which interacts with the binding site through hydrogen bonding or through electrostatic forces after protonation of the amino group.

We report the crystal structures of two ester derivatives of new ACE inhibitors that are modeled after enalapril but have, in addition, additional hydrophobic groups or constraints in the $S_1'$-$S_2'$ region: MDL27,467A[21] (5), [4α,7α-(*R**),12bβ]-7-[[1-(ethoxycarbonyl)-3-phenylpropyl]-amino]-1,2,3,4,6,7,12a,12b-octahydro-6-oxopyrido[2,1-*a*]-[2]benzazepine-4-carboxylic acid diphenylmethyl ester hydrochloride, and quinapril (7),[22] [3*S*-[2[*R**-(*R**)],3*R**]]-1,2,3,4-tetrahydro-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-3-isoquinolinecarboxylic acid hydrochloride. Both crystal structures, together with conformational energy calculations, assist in the prediction of the binding conformation for ACE inhibitors, the pharmacophore separation in the binding conformers, and the definition of the requirements for the proposed $S_1'$ and $S_2'$ subsites.

MDL27,467A[21] (5) is the diester analogue of the most potent ACE inhibitor, MDL27,088 (6). The tricyclic compounds have been synthesized as lipophilic, conformationally restricted mimics of the carboxyl-terminal tripeptide sequence, ···Phe-His-Leu-OH, of the natural ACE substrate, angiotensin I. In MDL27,088 and its diester analogue, the histidine imidazole ring of the tripeptide is replaced by a phenyl group and the terminal leucine residue by piperidine-2-carboxylic acid. The bond between the phenyl ring and the piperidine ring is part of a seven-membered dehydro lactam ring which constrains the positions of the ACE binding groups along the C-terminal dipeptide backbone and the positions of the groups designed to occupy the $S_1'$ and $S_2'$ hydrophobic subsites of the enzyme. Thus, the relative positions of all but two of the pharmacophores are highly constrained in these tricyclic compounds. X-ray analysis of MDL27,467A hydrochloride was done to gain insight into the binding requirements of the converting enzyme; especially for the fused phenyl ring, which significantly contributes to the high potency of MDL27,088 ($K_i$ = 1.2 × $10^{-11}$ M).[21]

Quinapril (7) is the orally active ester analogue of the potent ACE inhibitor CI-928 (8). Klutchko et al.[22] modeled quinapril after the hypertension drug enalapril (2) with the proline residue replaced by tetrahydro-3-isoquinolinecarboxylic acid. The novel bicyclic moiety at the C-terminal position of quinapril, which is expected to bind the $S_2'$ hydrophobic pocket, was designed as a conformationally restricted derivative of phenylalanine. Differences in the pharmacokinetics and biodistribution for the two equipotent inhibitors, quinapril (7) and enalapril (2), were expected because quinapril has increased lipophilicity. This new nonsulfhydryl-type ACE inhibitor, which is undergoing clinical development, has shown significant efficacy in essential hypertension with a more rapid onset and a prolonged duration of action as compared to captopril; whereas the response profiles for quinapril and enalapril are comparable.[23] Surprisingly, the prodrug quinapril ($IC_{50}$ = 8.3 nM) is considerably more potent in vitro than enalapril ($IC_{50}$ = 140 nM), although CI-928 ($IC_{50}$ = 2.9 nM) and enalaprilat ($IC_{50}$ = 3.1 nM) are about equipotent. The reason for this potency difference is unclear. Although apparently well accepted by the $S_2'$ subsite of the converting enzyme, the larger hydrophobic ligand of quinapril does not improve the inhibition potency as compared to enalapril. This finding is in contrast to earlier reports of enhanced activities for both the indoline analogue of captopril, WY44221,[24] and the indoline derivative of enalapril, CGS13928C.[25] Thus, the crystal structure determination for quinapril was undertaken with special attention to the conformation of the novel bicyclic moiety.

**Table I.** Crystallographic Data for MDL27,467A Hydrochloride and Quinapril Hydrochloride

| | MDL27,467A·HCl | quinapril·HCl |
|---|---|---|
| mol formula | $C_{40}H_{42}N_2O_5$·HCl | $C_{25}H_{30}N_2O_5$·HCl |
| mol wt | 667.252 | 516.042 |
| crystal size (mm) | 0.1 × 0.1 × 0.5 | 0.4 (spherical) |
| space group, *Z* | $P2_1$, 2 | $P2_12_12_1$, 4 |
| *a*, Å | 19.704 (2) | 10.8568 (7) |
| *b*, Å | 7.169 (1) | 24.1146 (21) |
| *c*, Å | 13.270 (2) | 10.7823 (13) |
| β, deg | 105.902 (8) | |
| *V* (Å$^3$) | 1802.7 (4) | 2822.9 (5) |
| calcd density, g cm$^{-3}$ | 1.229 | 1.214 |
| obsd density, g cm$^{-3}$ | 1.230 | 1.207 |
| $\theta_{max}$, deg | 75 | 75 |
| unique reflections | 4008 | 6616 |
| iso extinction coeff | 27 (9) × $10^{-5}$ | 117 (3) × $10^{-4}$ |
| max shift/error | 0.0019 | 0.0018 |
| *R* | 0.056 | 0.051 |
| $R_w$ | 0.061 | 0.061 |

## Experimental Section

A crystalline sample of MDL27,467A hydrochloride was provided by Drs. G. A. Flynn and E. H. W. Bohme of the Merrell Dow Research Institute, Cincinnati, OH. The sample of quinapril hydrochloride was a gift from Dr. C. J. Blankley of the Warner-Lambert Company, Parke-Davis Pharmaceutical Research Division, Ann Arbor, MI. Treatment of the quinapril sample with acetonitrile permitted growth of a large crystal of the hydrochloride salt as an acetonitrile solvate which was subsequently shaped into a sphere. The crystal was covered with a thin layer of glue to protect the solid against moisture and oxygen. In addition, the sensitive quinapril crystal was kept shielded from light during the diffraction experiment. The crystal densities for both compounds were measured by suspending single crystals in a mixture of toluene and $CCl_4$.

The crystallographic data for both compounds are summarized in Table I. The diffraction data were collected at room temperature on an Enraf-Nonius CAD-4F automated diffractometer,

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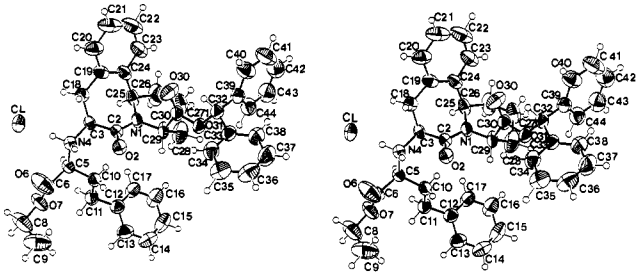

**Figure 1.** Molecular structure and atomic labeling scheme for MDL27,467A hydrochloride. This drawing was prepared by using the computer program ORTEP.[36]

using $\omega/2\Theta$ scans and CuK$\alpha$ radiation ($\lambda$ = 1.5418 Å, Ni filter, 32 mA, 44 kV). The $\Theta$ range for the 22 reflections which were used to define the orientation matrix and cell constants for MDL27,467A hydrochloride was 25.4°–30.7°, and that for quinapril hydrochloride 25.5°–39.4° based on 25 reflections. A total of 4008 unique reflections were collected for MDL27,467A hydrochloride ($+h,+k,\pm l$), of which 2922 had $I > 2.5\sigma(I)$ and were taken as observed. For quinapril hydrochloride, 6616 reflections were recorded ($+h,+k+l$ and $+h,-k,-l$) of which 3279 were unique ($R_{int}$ = 0.11) and 2946 had $I > 2.5\sigma(I)$. Correction for Lorentz, polarization, and secondary extinction effects were applied to the intensities of both data sets, but absorption corrections were not necessary. Unless otherwise stated, the programs used were those of the XRAY76 system.[26] The scattering factors used were those of Cromer and Mann,[27] except for the hydrogen atom scattering factors, which were from Stewart et al.[28] Both structures were solved with the direct method program SHELXS-86.[29]

**MDL27,467A Hydrochloride.** The hydrogen atoms were located in a difference Fourier synthesis, except those attached to C27. Large anisotropic temperature factors were found for C27; subsequently, the difference electron density map was calculated with C27 excluded. Two high electron density peaks of similar magnitude that were separated by 1.1 Å and in suitable bonding distances to C26 and C28 were located. Accordingly, the piperidine ring was treated as a combination of two alternative conformations with 50% occupancy for the two C27 positions, labeled C271 and C272. Twenty-five of the 43 hydrogen atoms adopted unsatisfactory bonding geometries during refinement and were placed in calculated positions with idealized geometry and isotropic thermal parameters set to 120% of the equivalent isotropic temperature factor of the attached non-hydrogen atom and were not refined further.

**Quinapril Hydrochloride.** Initial refinement revealed significant problems with the atomic positions for the ester group as they showed unrealistically high temperature factors and a poor bonding geometry. The parameters for the carbonyl oxygen atom, O6, and for the ethoxy group, O7–C8–C9, of the carboxylic acid ester were removed from the calculation and the difference electron density map recalculated. The geometry of the five highest electron density peaks suggested disorder in the ester group which was subsequently modeled as a combination of two conformations as shown in Figure 2. Refinement of the population parameters for the two C8 positions (C81 and C82) indicated about equivalent occupancy ($population_{C81}$ = 0.54; $population_{C82}$ = 0.57) which agreed with the corresponding electron densities in the difference Fourier map. The hydrogen atoms of the two alternative ester groups and those of the acetonitrile molecule were restrained to idealized positions with assigned thermal parameters as described for MDL27,467A hydrochloride.

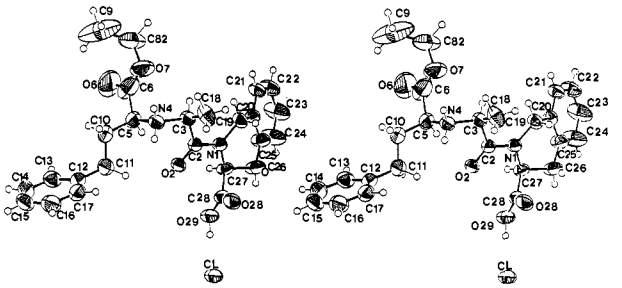

**Figure 2.** Molecular structure and atomic labeling scheme for quinapril hydrochloride. This drawing was prepared by using the computer program ORTEP.[36]

The positional and anisotropic thermal parameters for the non-hydrogen atoms, as well as the positional and isotropic thermal parameters for the unconstrained hydrogen atoms, were refined by weighted nonlinear least squares. The function minimized was $\sum w(|F_o| - |F_c|)^2$, where $w^{-1} = (\sigma|F_o|)^2 + k(|F_o|)^2$ with $k$ = 0.0003 for MDL27,467A hydrochloride and $k$ = 0.0001 for quinapril hydrochloride.

The positional parameters for the hydrogen atoms, the thermal parameters for all atoms, and lists of structure factors for both structures are available (see paragraph at the end of the paper regarding supplementary material).

**Calculation Methodology.** The conformations of analogues of the title molecules were obtained by MNDO[30] calculations. The MNDO program was from the MOPAC package (versions 2.08 and 4.0). MNDO calculations were performed on a CDC CYBER 205 supercomputer with a virtual storage operating system (VSOS). When rotamers were needed, the calculations were accomplished by a driver program written by R.J.H.;[31] the program generated the internal coordinates for a rotation about a specified torsion angle. Appropriate modifications to read in the coordinates were required for the MNDO routine.

## Results

The positional parameters and equivalent isotropic thermal parameters for MDL27,467A hydrochloride and for quinapril hydrochloride are given in Tables II and III, respectively. The molecular conformations and atomic labeling schemes for both compounds are shown in Figures 1 and 2.

The phenylpropyl fragments in both crystal structures, which were designed to occupy the $S_1$ hydrophobic pocket of the enzyme, adopt extended conformations with C5–C10–C11–C12 = 170.2 (4)° for MDL27,467A hydrochloride and with C5–C10–C11–C12 = 177.8 (3)° for quinapril hydrochloride. The planes defined by the propyl fragments are approximately perpendicular to the planes of the phenyl rings with torsion angles C10–C11–C12–C17 = −72.9 (6)° and C10–C11–C12–C17 = −99.0 (4)° for MDL27,467A hydrochloride and quinapril hydrochloride, respectively. This conformation for the phenylpropyl fragment was also observed in the crystal structures of the two potent ACE inhibitors, enalapril[32] and ramiprilat (**9**).[33] The conformational preference for phenylpropyl fragments was further investigated by a search of the Cambridge Structural Database.[34] The conformation of the 20


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**Table II.** Fractional Coordinates (×10⁴) and Equivalent Isotropic Thermal Parameters (×10) for the Non-Hydrogen Atoms of MDL27,467A Hydrochloride

| atom | x/a | y/b | z/c | $B_{eq}$[a] |
|---|---|---|---|---|
| N1 | 2593 (2) | 9716 (6) | 8742 (3) | 39 (2) |
| C2 | 3029 (2) | 8460 (7) | 9329 (3) | 33 (2) |
| O2 | 2832 (1) | 7218 (5) | 9832 (2) | 44 (1) |
| C3 | 3792 (2) | 8599 (6) | 9338 (3) | 32 (2) |
| N4 | 4230 (2) | 7674 (5) | 10293 (2) | 34 (1) |
| C5 | 4407 (2) | 8823 (6) | 11288 (3) | 37 (2) |
| C6 | 4800 (3) | 7478 (9) | 12151 (4) | 48 (2) |
| O6 | 4783 (3) | 5834 (7) | 12047 (3) | 81 (3) |
| O7 | 5156 (2) | 8373 (7) | 12983 (2) | 63 (2) |
| C8 | 5477 (4) | 7126 (12) | 13869 (5) | 84 (4) |
| C9 | 4952 (5) | 6208 (14) | 14357 (5) | 117 (6) |
| C10 | 3777 (2) | 9780 (7) | 11532 (3) | 41 (2) |
| C11 | 4007 (2) | 11293 (8) | 12363 (4) | 51 (2) |
| C12 | 3398 (2) | 12474 (8) | 12484 (4) | 47 (2) |
| C13 | 3159 (3) | 12359 (9) | 13366 (4) | 61 (3) |
| C14 | 2623 (3) | 13530 (12) | 13481 (4) | 77 (4) |
| C15 | 2327 (3) | 14808 (10) | 12722 (6) | 78 (4) |
| C16 | 2559 (3) | 14920 (9) | 11827 (5) | 77 (4) |
| C17 | 3090 (3) | 13750 (9) | 11724 (4) | 59 (3) |
| C18 | 3918 (2) | 7682 (7) | 8354 (3) | 43 (2) |
| C19 | 3455 (2) | 8268 (8) | 7294 (3) | 43 (2) |
| C20 | 3557 (3) | 7229 (10) | 6472 (4) | 66 (3) |
| C21 | 3169 (4) | 7622 (11) | 5447 (4) | 88 (4) |
| C22 | 2694 (3) | 9066 (13) | 5251 (4) | 87 (4) |
| C23 | 2595 (3) | 10078 (10) | 6064 (4) | 62 (3) |
| C24 | 2960 (2) | 9720 (8) | 7098 (3) | 45 (2) |
| C25 | 2833 (2) | 10895 (7) | 7984 (4) | 45 (2) |
| C26 | 2343 (4) | 12566 (12) | 7667 (6) | 94 (5) |
| C271 | 1655 (6) | 12684 (17) | 7720 (10) | 63 (6) |
| C272 | 2076 (6) | 13067 (16) | 8458 (11) | 61 (6) |
| C28 | 1579 (3) | 11763 (9) | 8789 (5) | 73 (4) |
| C29 | 1862 (2) | 9692 (7) | 8801 (4) | 43 (2) |
| C30 | 1396 (2) | 8529 (8) | 7956 (3) | 41 (2) |
| O30 | 1543 (2) | 7804 (6) | 7232 (3) | 75 (2) |
| O31 | 770 (1) | 8284 (6) | 8159 (2) | 50 (1) |
| C32 | 216 (2) | 7323 (8) | 7389 (3) | 46 (2) |
| C33 | −186 (2) | 6168 (8) | 8008 (4) | 47 (2) |
| C34 | 181 (3) | 5004 (8) | 8797 (4) | 58 (3) |
| C35 | −151 (4) | 3869 (8) | 9361 (4) | 69 (3) |
| C36 | −874 (4) | 3915 (10) | 9115 (5) | 83 (4) |
| C37 | −1252 (3) | 5082 (12) | 8356 (6) | 82 (4) |
| C38 | −906 (3) | 6203 (10) | 7801 (4) | 64 (3) |
| C39 | −222 (2) | 8696 (8) | 6632 (3) | 48 (2) |
| C40 | −473 (3) | 8232 (11) | 5587 (4) | 64 (3) |
| C41 | −910 (4) | 9502 (15) | 4887 (5) | 84 (4) |
| C42 | −1057 (4) | 11182 (14) | 5235 (6) | 82 (4) |
| C43 | −796 (3) | 11669 (11) | 6258 (6) | 72 (4) |
| C44 | −389 (3) | 10430 (9) | 6960 (4) | 57 (3) |
| Cl[c] | 57445 (5) | 82700[b] | 101971 (1) | 457 (4) |

[a] $B_{eq}$ is defined as $^1/_3(B_{11} + B_{22} + B_{33})$. [b] The origin was defined by fixing the y coordinate of atom Cl. [c] Fractional coordinates are ×10⁵; thermal parameter is ×10².

**Table III.** Fractional Coordinates (×10⁴) and Equivalent Isotropic Thermal Parameters (×10) for the Non-Hydrogen Atoms of Quinapril Hydrochloride

| atom | x/a | y/b | z/c | $B_{eq}$[a] |
|---|---|---|---|---|
| N1 | 9556 (2) | 9139 (1) | 10709 (2) | 37 (1) |
| C2 | 9176 (3) | 9667 (1) | 10727 (3) | 35 (1) |
| O2 | 9307 (2) | 9974 (1) | 9830 (2) | 40 (1) |
| C3 | 8536 (3) | 9878 (1) | 11897 (3) | 40 (1) |
| N4 | 8369 (3) | 10498 (1) | 11737 (2) | 44 (1) |
| C5 | 9485 (4) | 10858 (1) | 11759 (3) | 52 (2) |
| C6 | 9881 (10) | 10932 (3) | 13101 (7) | 123 (5) |
| O6 | 10826 (6) | 11234 (2) | 13230 (5) | 153 (4) |
| O7 | 9335 (5) | 10708 (2) | 13930 (3) | 118 (3) |
| C81 | 10871 (14) | 11286 (7) | 14773 (15) | 119 (10) |
| C82 | 10126 (11) | 10853 (6) | 15097 (9) | 99 (7) |
| C9 | 9769 (14) | 11507 (7) | 15488 (9) | 209 (11) |
| C10 | 9162 (4) | 11418 (2) | 11166 (4) | 55 (2) |
| C11 | 9180 (4) | 11403 (2) | 9753 (4) | 54 (2) |
| C12 | 8807 (3) | 11956 (1) | 9224 (3) | 44 (1) |
| C13 | 9622 (4) | 12399 (2) | 9189 (4) | 54 (2) |
| C14 | 9245 (5) | 12915 (2) | 8773 (4) | 61 (2) |
| C15 | 8067 (5) | 12998 (2) | 8368 (4) | 65 (2) |
| C16 | 7260 (4) | 12556 (2) | 8381 (4) | 62 (2) |
| C17 | 7606 (4) | 12045 (2) | 8807 (4) | 52 (2) |
| C18 | 7273 (3) | 9633 (2) | 12100 (4) | 59 (2) |
| C19 | 9454 (3) | 8751 (1) | 11761 (3) | 44 (1) |
| C20 | 10557 (3) | 8380 (1) | 11866 (3) | 42 (1) |
| C21 | 11072 (3) | 8242 (1) | 13005 (3) | 46 (2) |
| C22 | 12030 (4) | 7876 (2) | 13067 (4) | 64 (2) |
| C23 | 12519 (5) | 7654 (2) | 12009 (5) | 88 (3) |
| C24 | 12016 (6) | 7794 (2) | 10872 (4) | 96 (3) |
| C25 | 11020 (4) | 8155 (2) | 10801 (3) | 62 (2) |
| C26 | 10409 (5) | 8325 (2) | 9618 (4) | 73 (2) |
| C27 | 10194 (3) | 8960 (1) | 9583 (3) | 43 (1) |
| C28 | 9464 (4) | 9083 (1) | 8421 (3) | 45 (2) |
| O28 | 8372 (3) | 8993 (1) | 8332 (2) | 59 (1) |
| O29 | 10167 (2) | 9252 (1) | 7509 (2) | 53 (1) |
| Cl[b] | 89805 (9) | 92700 (4) | 50164 (7) | 525 (4) |
| N31 | 11727 (6) | 9882 (3) | 12035 (9) | 152 (5) |
| C32 | 12181 (6) | 9717 (3) | 12881 (10) | 119 (5) |
| C33 | 12677 (9) | 9530 (4) | 14041 (10) | 177 (8) |

[a] $B_{eq}$ is defined as $^1/_3(B_{11} + B_{22} + B_{33})$. [b] Fractional coordinates are ×10⁴; thermal parameter is ×10².

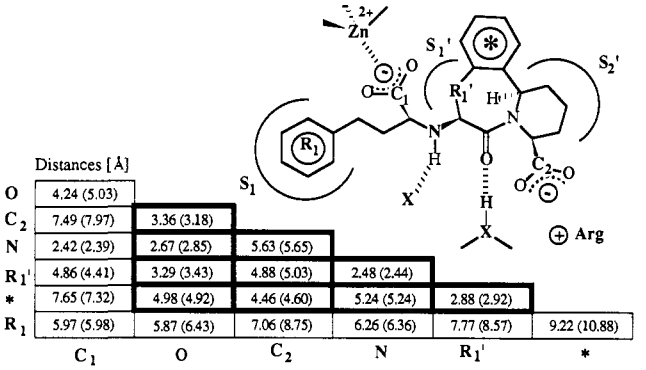


**Figure 3.** Pharmacophore separations in the crystallographic structure of MDL27,467A hydrochloride and in the corresponding MNDO optimized molecular structure of the diacid MDL27,088 (in parentheses). The five key separations are outlined in bold.

fragments observed by X-ray diffraction indicates that the extended geometry, like that observed in MDL27,467A hydrochloride and quinapril hydrochloride, is the favored arrangement ($N_{extended}$:$N_{folded}$ = 10:1) with the two planes, defined by the propyl portion and by the phenyl ring, either perpendicular, as found in the two compounds, or coplanar to each other ($N_{perpendicular}$:$N_{coplanar}$ = 2:1).

The validity of MDL27,467A hydrochloride and quinapril hydrochloride as models for the active diacids MDL27,088 and CI-928 was tested by MNDO optimization of molecular structures of the ester analogues found in the crystal, but with the ester groups replaced by hydrogen atoms and the protonation hydrogen atoms (H042 of MDL27,467A hydrochloride and H041 of quinapril hydrochloride) removed. The conformation of the tricyclic system observed in the crystal of MDL27,467A hydrochloride is preserved in the MNDO structure of MDL27,088 and the basic arrangement of the phenylpropyl fragment remains essentially unaltered. The amide bond that is essential for binding to ACE is constrained in the MDL molecular framework and adopts an almost ideal trans arrangement with a torsion angle C29–N1–C2–C3 of −174.9° in the crystal and remains unchanged in the MNDO structure of MDL27,088. Thus, considering the exceptionally high potency of the diacid, a trans arrangement for the amide function appears ideal for binding to the active site of the enzyme. The largest conformational changes in the calculated structures are observed for the C2–C3–N4–C5–C6–O6 fragment, which is due to the deprotonation of N4. The pharmacophore separations in the observed structure of MDL27,467A hydrochloride and

**Table IV.** Geometry of the Inter- and Intramolecular Hydrogen Bonds in MDL27,467A Hydrochloride and Quinapril Hydrochloride

| X–H···Y | X–H, Å | X···Y, Å | H···Y, Å | X–H···Y, deg | equivalent position[a] |
|---|---|---|---|---|---|
| | | MDL27,467A·HCl | | | |
| N4–H041···Cl | 0.85 (4) | 3.227 (3) | 2.70 (3) | 122 (3) | $1 - x,y - 0.5,2 - z$ |
| N4–H042···Cl | 0.95 (4) | 3.053 (4) | 2.19 (4) | 151 (3) | $x,y,z$ |
| | | Quinapril·HCl | | | |
| N4–H041···Cl | 0.86 (3) | 3.203 (3) | 2.45 (3) | 147 (3) | $1.5 - x,2 - y,z + 0.5$ |
| N4–H042···O28 | 0.91 (4) | 2.834 (4) | 1.95 (4) | 165 (3) | $1.5 - x,2 - y,z + 0.5$ |
| O29–H291···Cl | 0.90 (6) | 2.981 (3) | 2.09 (6) | 167 (5) | $x,y,z$ |

[a] The equivalent position refers to the hydrogen-atom acceptor.

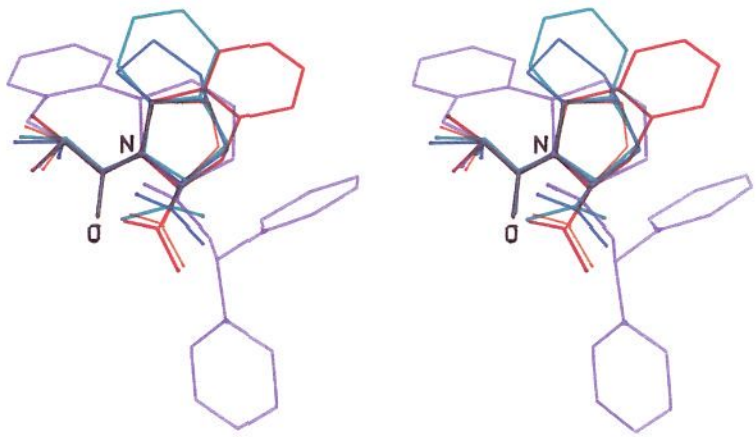


**Figure 4.** Superposition of the crystallographic conformations of the C-terminal dipeptide of five ACE inhibitors. The molecules are as follows: black, enalapril;[32] red, quinapril (this work); green, WY44221[35] (with the amide bond modeled as trans); blue, ramprilat;[32] and purple, MDL27,464A (this work). The phenylpropyl ester portion of enalapril, quinapril, ramprilat, and MDL27,464A, as well as the mercaptomethylene portion of WY44221, is omitted for clarity.

those in the MNDO structure of MDL27,088 are listed in Figure 3.

The conformation of the C-terminal dipeptide backbone and of the phenylpropyl fragment in quinapril hydrochloride is well-preserved in the calculated structure of CI-928, but the deprotonation of N4 shifts the torsion angles along the N1–C2–C3–N4–C5–C10 bond sequence by an average value of 17.5°. The nonaromatic portion of the tetrahydro-3-isoquinoline ring in the MNDO structure of CI-928 adopts an almost perfect chair conformation as was observed in the crystal structure of quinapril hydrochloride.

MNDO[30] optimizations of the two ring conformers, I and II, observed in the crystal structure of MDL27,467A hydrochloride reveal that both ring arrangements are stable conformations of the piperidine ring. The difference in the heat of formation is 0.48 kcal/mol, favoring the chair conformation. The chair arrangement for the 1,2,6-substituted piperidine ring was also observed for a precursor of MDL27,467A.[21]

The chlorine ions in each crystal lattice interact with the protonated nitrogen atoms in MDL27,467A and in quinapril through both hydrogen bonding and salt linkage. In the MDL27,467A hydrochloride crystal, the nitrogen cation N4 interacts with two Cl ions in the lattice and every chlorine anion interacts with two protonated N4 ions. The lattice is, therefore, stabilized by a chain of attractive charge–charge interactions. They are, however, somewhat diminished by the repulsion between the chlorine ions which are separated by 4.570 (1) Å. Each secondary amine group in the MDL27,467A hydrochloride crystal structure interacts with $Cl^-$ ions through a strong and a weak hydrogen bond, while the powerful hydrogen-acceptor atom of the amide group, O2, is lacking an appropriate donor and does not participate in hydrogen bonding. The hydrogen bond geometries are tabulated in Table IV. The short $N^{4+}\cdots Cl^-$ interaction is shorter than any $N^+\cdots Cl^-$ distance found in a search of the Cambridge Structural Database[34] (CSD) for the geometry of $[Cl^-\cdots NH_2^+(CHX_2)_2]$ fragments. The highest population for the 21 $Cl^-\cdots N^+$ distances observed by X-ray diffraction is near 3.15 Å, which indicates that the $N4^+\cdots Cl^-$ distance in the crystal of MDL27,467A hydrochloride is shortened by the strong hydrogen bond formed between Cl and H042.

In the quinapril hydrochloride crystal structure, both the hydroxyl group and the carbonyl oxygen atom of the terminal carboxyl function form strong hydrogen bonds: O29–H291 binds to the chlorine anion and O28 binds to the secondary amine, N4–H042, of another molecule. In addition, the secondary amine also forms a weak hydrogen bond between its second hydrogen atom, H041, and the chlorine anion. Thus, the two molecules of quinapril are linked by a strong hydrogen bond between the amine and the carbonyl group and by a chlorine anion bridge. The chlorine anion links molecules through a strong hydrogen bond to the hydroxyl group and a weak hydrogen bond to the secondary amine of the second molecule; see Table IV. The two remaining hydrogen acceptor functions, O2 of the amide group and the carbonyl oxygen atom of the ester group, O6, are not hydrogen bonded due to a lack of appropriate donor functions and the formation of only two-center hydrogen bonds. The high anisotropic thermal parameters for the atoms of the solvent molecules suggest some degree of freedom for the position of acetonitrile. In support of this possibility, neither polar nor hydrophobic contacts between acetonitrile and adjacent units in the crystal lattice are observed.

## Discussion

A major finding of this study is the determination of the

positions of the pharmacophores in the C-terminal dipeptide portion of inhibitors. This determination is based on two assumptions: first, that the high potency of MDL27,088 arises from a complete interaction of the inhibitor with the C-terminal binding portion of the ACE active site, and second, that the conformation of the constrained tricyclic dipeptide fragment of MDL27,088 is the same as that observed in the crystal structure of MDL27,467A hydrochloride. The first assumption is supported by the extremely high potency of MDL27,088,[21] which suggests that the inhibitor achieves maximal interaction with the enzyme. The second assumption is supported by comparison of the optimized structure of MDL27,088, obtained from MNDO calculations, with the experimental structure of MDL27,467A reported herein. For the five recognition points of the tricyclic system, the average difference for the pharmacophore separations between the calculated and the experimental structures is 0.1 Å; the proposed binding mode of MDL27,088 and the distance matrix for the pharmacophore separations are shown in Figure 3. Thus, except for the zinc ligand and the $S_1$ ligand, the binding positions of all expected recognition points for the inhibitor MDL27,088 can be postulated with some confidence based on the crystallographic results. The fused phenyl ring in MDL27,088 presumably contributes to the high potency of this compound by hydrophobic interaction with the binding site of ACE.

Comparison of the crystallographic structures of MDL27,467A hydrochloride, quinapril hydrochloride, enalapril,[32] and ramiprilat (9)[33] reveals that the basic conformations for the phenylpropyl fragments are consistent and that the arrangement of the C-terminal dipeptide portions places corresponding pharmacophores in similar positions. A superposition of the inhibitors, showing the C-terminal dipeptide portions and omitting the phenylpropyl ester of MDL27,467A, ramiprilat, enalapril, and quinapril and the mercaptomethylene of WY44221, is shown in Figure 4. In contrast to the agreement at the two terminii of the inhibitors, a superposition of these four structures, achieved by aligning the four amide groups, fails to find overall agreement because both the $S_1$ ligands and the crystallographic positions of the zinc-binding carboxyl groups of the structures are found in four completely different positions. This failure to superimpose is due to the apparent lack of conformational restriction for the three torsion angles along the bond sequence between the amide group and the phenylpropyl fragment. Thus, for both the $S_1$ ligand and the carboxyl groups, the binding position relative to the other pharmacophores of the inhibitor is not defined in this comparison. Determination of an accurate binding position for the $S_1$ ligand and the zinc ligand must await crystallographic analysis of potent inhibitors with a more rigid skeleton or a search of the conformational possibilities of this fragment using computational methods.

Compared to the pyrrolidine ring of enalapril, the larger $S_2'$ ligand contained in CI-928 (the diacid form of quinapril) does not increase the binding energy; the inhibitors are nearly equipotent. This lack of advantage can be explained by two possibilities: either a $S_2'$ ligand provides no gain in the overall binding energy, perhaps because the hydrophobic binding energy is compensated by an energetically unfavored ring conformation, or the phenyl ring in the tetrahydro-3-isoquinoline of CI-928 does not effectively occupy the $S_2'$ hydrophobic pocket. The first explanation can be disproven by the example of the replacement of the pyrrolidine ring in captopril by an indoline ring (inhibitor WY44221), which improved inhibition. The disadvantage of CI-928 is not due to an unfavored ring conformation because the indoline analogue and CI-928 both favor an approximately coplanar arrangement between the phenyl ring and the amide group. Thus, the second explanation may be valid; a superposition of WY44221, modeled with a trans amide bond,[35] and CI-928 shows that the centers of the two coplanar phenyl rings are separated by 2.28 Å (Figure 5). This difference suggests that the location of the phenyl portion of the 3-isoquinoline ring is not ideal for binding to the $S_2'$ subsite and would be better replaced by a quinoline ring which provides a closer overlap with the phenyl ring of WY44221.

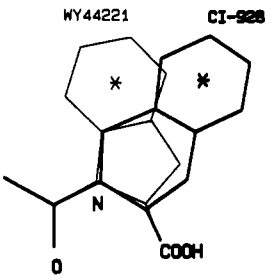


**Figure 5.** Comparison of the tetrahydro-3-isoquinoline ring of CI-928 and the indoline ring of WY44221. The C-terminal positions of both inhibitors are shown with their amide groups superimposed. For clarity, only the terminal dipeptide portion of the molecule is shown.

On the basis of crystallographic results, the aromatic portions of the phenylpropyl fragment in MDL27,088 and CI-928 are assumed to occupy the $S_1$ subsite of the enzyme with an extended conformation for the phenylpropyl portion and with the planes defined by the propyl fragment and by the phenyl ring either perpendicular or coplanar to each other.

In summary, this study has defined the positions of the C-terminal dipeptide portion of ACE inhibitors, has defined the requirements of the $S_2'$ subsite, and has identified a common conformation for the phenylpropyl portion of inhibitors which possess ligands for the $S_1$ subsite. ACE inhibitors are expected to bind to the active site with a trans amide bond and with the C$\alpha$–R1′ bond approximately perpendicular to the amide plane. Thus, the ligand for the $S_1'$ hydrophobic pocket is oriented above the plane of the amide group such that both the $S_1'$ ligand and the C-terminal carboxyl group are located on the same side of the amide plane. For ACE inhibitors like enalapril, the phenylpropyl fragment is likely to bind in the extended conformation with the planes defined by the propyl portion and by the phenyl ring either perpendicular or coplanar to each other. The aromatic portion of the indoline system in WY44221[24] and CGS13928C[25] is expected to bind the $S_2'$ hydrophobic pocket of the enzyme but no significant contribution to the overall binding energy is expected from the fused phenyl ring of the tetrahydro-3-isoquinoline moiety in quinapril and CI-928.

**Acknowledgment.** We thank Drs. G. A. Flynn and E. H. W. Bohme of the Merrell Dow Research Institute, Cincinnati, OH for supplying a sample of MDL27,467A hydrochloride. We are grateful to Dr. C. J. Blankley of the Warner-Lambert Company, Parke-Davis Pharmaceutical Research Division, Ann Arbor, MI for providing the quinapril hydrochloride sample. This work was supported

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by the Alberta Heritage Foundation for Medical Research and the National Science and Engineering Research Council of Canada.

**Supplementary Material Available**: Anisotropic thermal parameters for the non-hydrogen atoms, positional and isotropic parameters for the hydrogen atoms, and lists of bond distances, bond angles, and torsion angles (17 pages); the observed structure factors are available for both compounds (32 pages). Ordering information is given on any current masthead page.