Novel Inhibitors of Prolyl 4-Hydroxylase. 3.[1] Inhibition by the Substrate Analogue *N*-Oxaloglycine and Its Derivatives

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N-Oxaloglycine (3) is an α-ketoglutarate (1) analogue that is a competitive inhibitor of prolyl 4-hydroxylase (EC 1.14.11.2). A study of the structure–activity relationships of some other oxalo derivatives shows that substitution on the glycine moiety modulates activity stereoselectively and that if the ω-carboxylate is homologated or replaced by either acylsulfonamides or anilide, then activity is sharply reduced. This sensitivity to these changes is contrasted with the relative insensitivity of another putative α-ketoglutarate analogue, pyridine-2,5-dicarboxylic acid (2), and the implication is discussed that compounds of both series are unlikely to bind to prolyl hydroxylase in the same way even though both inhibit the enzyme competitively.

Introduction

Background. Inhibition of prolyl hydroxylase (EC 1.14.11.2) [procollagen-L-proline, 2-oxoglutarate:oxygen oxidoreductase (4-hydroxylating)][2] is of therapeutic interest because the enzyme is necessarily involved[3] in the biosynthesis of the collagen deposited during life-threatening fibroses. During these fibrotic states the involved organ or tissue becomes unbearably congested with inappropriately large amounts of collagen.

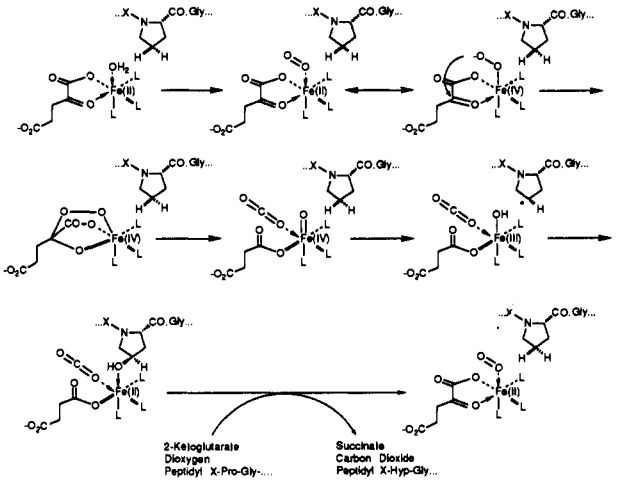
Chronic liver disease with its severe fibrotic component leads each year to the premature death of over 26 000 people in the United States alone,[4] where even in 1988[5] it was the eighth leading cause of death. Other fibrotic diseases[6] such as idiopathic and other pulmonary fibroses, renal fibrosis, and the diffuse cardiac fibrosis of progressive heart failure also lead to premature death, while the fibrotic component of less severe conditions such as scleroderma and rheumatoid arthritis disable and disfigure many more.

Collagenous proteins are characterized by the noncovalent association of three chains into a relatively inert rodlike triple helix.[3] It is the resistance of these triple-helical rods to proteolysis[7] that is responsible for the inability of the body to degrade the excessive accumulation of collagen.

The stability of the triple-helical domains is known[3] to be dependent on the extent of the conversion carried out by prolyl hydroxylase: the conversion of many of the X-Pro-Gly sequences into X-Hyp-Gly sequences (H-Hyp-OH = (2*S*,4*R*)-4-hydroxyproline) (Scheme I). Insufficiently hydroxylated chains do not form triple-helical domains that are stable at body temperature: they remain gelatinous rather than collagenous proteins and are susceptible to normal catabolism.

Inhibition of prolyl hydroxylase should therefore hinder the undesirable accumulation of newly synthesized collagen in fibrotic tissue by diverting collagen biosynthesis into a degradative pathway.

Scheme I. Catalytic Cycle of Oxidative Decarboxylation of 2-Ketoglutarate during the 4-Hydroxylation of Peptidylproline



There are, however, a dozen or more collagens,[8-10] which are each discrete and defined structural proteins of the extracellular matrix, as well as a number of other biologically important macromolecules that contain significant collagenous regions,[11] such as the C1q component of the classical pathway of complement,[12] the acetylcholine esterase of neuromuscular junction endplate,[13] conglutinin,[14]

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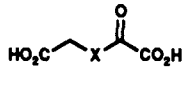
hepatic mannose binding proteins,[15] pulmonary surfactant apoproteins,[16] and the macrophage receptor for acetyl low density lipoprotein (Ac-LDL).[17]

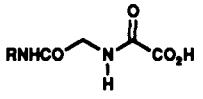
There are therefore serious concerns about the possible toxic consequences of interfering both with normal collagen turnover and with the biosynthesis of other vital collagenous molecules.

Clinical evaluation of the net benefit of a prolyl hydroxylase inhibitor in the treatment of life-threatening fibrotic conditions would be very desirable.[18,19] However, no agents are available[20] that would be suitable for such an investigation.

Enzyme Mechanism. It seems probable that the hydroxylation is carried out by an iron(IV)oxo species and that this species is generated by oxidative decarboxylation of 2-oxoglutarate (1) in the coordination shell of enzyme-bound iron(II) (Scheme I). The exact molecular details of the enzyme reaction are not known with certainty, but the active-site chemistry proposed by Hanauske-Abel and Guenzler[21] and summarized in Scheme I provides a sound basis for work in this area.



1 X = CH_2
3 X = NH

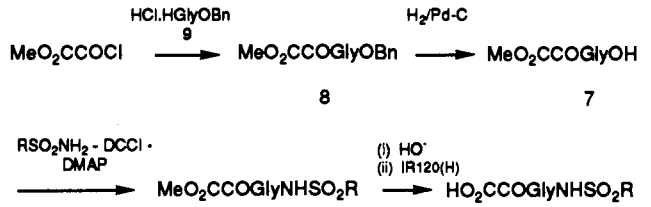


4 R = $MeSO_2$-
5 R = $PhSO_2$-
6 R = 4-EtO-C_6H_4-

This, the best mechanistic hypothesis available, emphasizes the availability of binding sites for 2-oxoglutarate (1) in the region of the Fe(II) held at the catalytic site of the enzyme. The iron(II) is assumed to form a 5-membered chelate with the planar, anionic, bidentate oxo carboxylate ligand while a lipophilic spacer presents the distal carboxylate to an unspecified anion binding-site on the enzyme.

Scheme II

Scheme III



Enzyme Inhibition. It was considered[22] that bidentate ligands that could not undergo the catalytically important oxidative decarboxylation would be inhibitors of the enzyme. The qualitatively successful prediction of the inhibitory properties of pyridine-2,5-dicarboxylic acid (2) (K_i = 0.8 μM) and its close analogues[22-24] lends support to the hypothesis on which the prediction was based and emphasizes the value of the pyrid-2-yl group as one of the ligands for active-site iron.

One of our analyses of the problem suggested that there should be a simpler modification of 2-oxoglutarate (1) that could fulfill all of the reasonable binding requirements while remaining resistant to oxidative decarboxylation: oxaloglycine (3)[25,26] is a similarly sized bidentate dicarboxylic acid in which an imino group has replaced one of the methylene groups of 2-oxoglutarate (1).

Oxaloglycine (3) is an interesting analogue of 2-oxoglutarate (1) in this context because it forms all of the polar interactions of 2-oxoglutarate (1), it contains an oxamic acid moiety as a potentially better bidentate ligand for the enzyme-bound iron(II), and its amide moiety should be relatively resistant to the nucleophilic attack postulated to occur at the 2-oxo group during the oxidative decarboxylation. The imino group also introduces additional polar interactions in a region thought to be predominantly nonpolar, the overall effect of which would be difficult to assess.

Oxaloglycine (3) is known to inhibit both glutamate dehydrogenase[27] [EC 1.4.1.4, L-glutamate:$NADP^+$ oxidoreductase (deaminating)][28] and isocitrate dehydrogenase[29] [EC 1.1.1.42, isocitrate:$NADP^+$ oxidoreductase (decarboxylating)],[30] to which it binds weakly (K_i = 4.5 and 29 μM,

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(20) HOE 077, the bis-2-methoxyethylamide and intended prodrug form of the known prolyl hydroxylase inhibitor pyridine-2,4-dicarboxylic acid,[21,22,49] is under investigation as a treatment for liver fibrosis by Hoechst A. G. However it is now known[50] that metabolism produces neither pyridine-2,4-dicarboxylic acid nor any other prolyl hydroxylase inhibitor that seems likely to account for the prophylactic antifibrotic effects of HOE 077. The therapeutic efficacy of HOE 077 is unknown but it seems unlikely that this compound can help assess the clinical value of a prolyl hydroxylase inhibitor.

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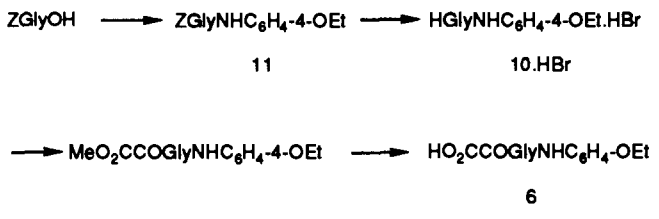
Scheme IV



respectively).[27,29] These two enzymes are mechanistically completely unrelated to prolyl hydroxylase, but it is probable that oxaloglycine (3) acts as an analogue of their product, 2-oxoglutarate (1), in each case.

The mechanism-based arguments in favor of oxaloglycine (3) coupled with the clear evidence that it can form interactions with some naturally occurring 2-oxoglutarate (1) binding sites led us to prepare and evaluate oxaloglycine (3) and some of its close analogues as inhibitors of prolyl hydroxylase.

This paper expands on our preliminary report[31] of *N*-oxalo amino acid analogues as novel inhibitors of prolyl hydroxylase that were discovered at ICI Pharmaceuticals during our search for clinically effective inhibitors of excessive collagen deposition. Baader, Berghard, and Guenzler-Pukall[32] have also recently disclosed their interest in oxaloglycine and some of its derivatives as inhibitors of prolyl hydroxylase. We now describe full details of our synthesis of this series of prolyl hydroxylase inhibitors, some of their structural limitations, and the startling dichotomy between the structure–activity relationships seen in this series and those seen in the apparently analogous pyridine-2,5-dicarboxylic acid series.[33]

Synthesis

Reports of oxaloglycine (3)[25-27,29,34-37] and its simple homologues[25,34,38-40] are surprisingly scarce in the literature: these are hygroscopic and exceptionally water-soluble compounds that are usually prepared as salts or used as material of undefined composition.

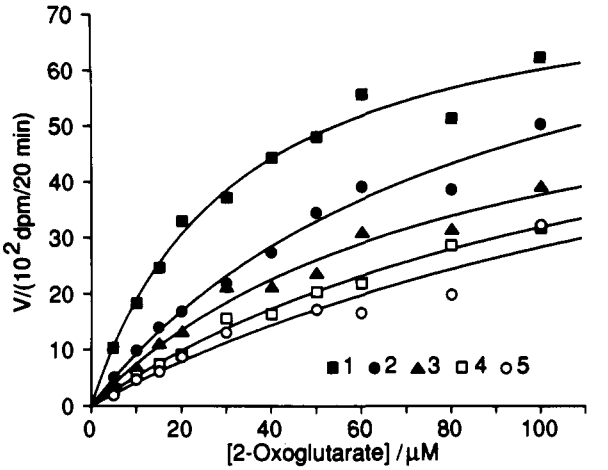


Figure 1. The prolyl hydroxylase velocity (V) vs substrate concentration (S) curves (1–5) at various oxaloglycine concentrations (0, 0.6, 1.2, 1.8, and 2.4 μM respectively).

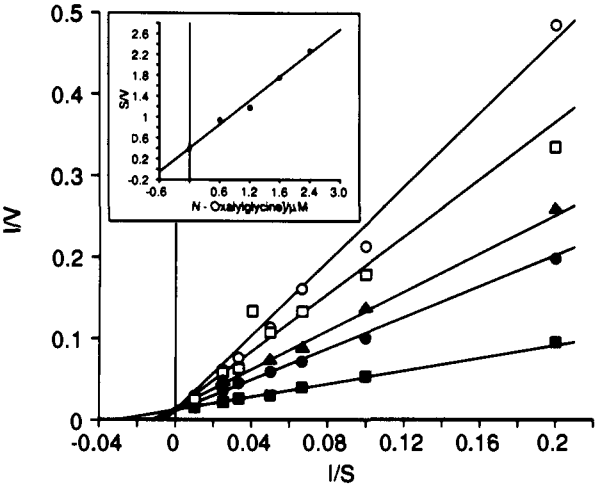


Figure 2. (a) Lineweaver–Burk replots of the data from Figure 1 show that oxaloglycine is a competitive inhibitor of prolyl hydroxylase (symbols as above); (b) (inset) the intercept at $S/V = 0$ shows that $K_i = 0.54$ μM.

We obtained oxaloglycine and a number of novel analogues by a modification of the method of Yanagawa and co-workers[25] (Scheme II) (Table I). This appears to be the first time that anyone has needed to isolate *N*-oxaloglycine, obtained as a white crystalline hemihydrate, and its simple analogues in analytically pure form, and so we report full details of our synthesis and of the characterization of these compounds. The key step in this work is the neutralization of the disodium salt using ion-exchange chromatography[25] on IR120(H) (see Experimental Section), and all of the other steps are unexceptional.

We also required the oxalo derivatives of *N*-(methylsulfonyl)-, *N*-(phenylsulfonyl)-, and *N*-(4-ethoxyphenyl)-glycinamide, 4, 5, and 6, respectively.

The acylsulfonamides were prepared from methoxalylglycine (7) and the appropriate sulfonamide using dicyclohexylcarbodiimide and 4-(dimethylamino)pyridine to activate the acyl group. The key steps in this sequence were firstly the formation of the mixed diester benzyl methoxalylglycinate (8) from benzyl glycinate (9) and secondly the selective hydrogenolytic mono-de-esterification (Scheme III).

N-(4-Ethoxyphenyl)glycinamide (Phenocoll) (10)[41] has

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Table I. Synthetic, Analytical, and Biochemical Data for Oxalo Amino Acids and Their Precursors

no.	compound (adduct)	% yield	method	analysis	mp/°C	solvent[a]	mean $IC_{50}/\mu M$ (n)[b] or [% inhibn]
2	pyridine-2,5-dicarboxylic acid (Aldrich)						5.18[c] (4)
3	$HO_2CCOGlyOH$ ($0.5H_2O$)	57	A (ii)	C,H,N	112.5–114.5	A	2.89[d] (7)
4	$HO_2CCOGlyNHSO_2Me$	46	B (iii)	C,H,N,S[e]	200.5–201.5 dec	D	414
5	$HO_2CCOGlyNHSO_2Ph$	35	B (iii)	C,H,N,S	203 dec	D	[34%][f]
6	$HO_2CCOGlyNHC_6H_4$-4-OEt	85	C (iv)	C,H,N	207 dec	C	[16%][f]
12	(*R,S*)-$HO_2CCOAlaOH$	31	A (ii)	C,H,N	109–111 dec	B	90.7 (2)
13	$HO_2CCOSarOH$	44	A (ii)	C,H,N	111–112.5 dec	A	[10%][f]
14	(*R,S*)-$HO_2CCOProOH$ ($0.25H_2O$)	49	A (ii)	C,H,N	94–120 dec	B	[10%][f]
15	$HO_2CCOAibOH$	55	A (ii)	C,H,N[g]	153–154 dec[h]	B	[26%][f]
16	(*S*)-$HO_2CCOAlaOH$ ($0.25H_2O$)	9	A (ii)	C,H,N	101–104	B	38.2 (2)
17	(*R*)-$HO_2CCOAlaOH$[i]	18	A (ii)	C,H,N	100–102	B	621 (2)
19	HO_2CCO-β-AlaOH	69	A (ii)	C,H,N	125.5–130.5 dec	B	[24%][f]
21	$HO_2CCOPabaOH$ ($0.5H_2O$)[j]	23	A (ii)	C,H,N	227–229	C	[09%][k]
	(*R,S*)-$HO_2CCOPheOH$	69	A (ii)	C,H,N	141–143.5	B	[18%][f]
	(*R,S*)-$HO_2CCOLeuOH$	36	A (ii)	C,H,N	99.5–102	B	[16%][f]
	(*R,S*)-$HO_2CCOMetOH$	64	A (ii)	C,H,N,S	118–120	B	[09%][f]
7	$MeO_2CCOGlyOH$	42	B (i)	C,H,N	123–126	D	
8	$MeO_2CCOGlyOBn$	76	A (i) (b)	C,H,N	104–105	E	
	(*R,S*)-$MeO_2CCOAlaOMe$ ($0.5H_2O$)	68	A (i) (b)	C,H,N[l]	(155/0.2)[m]		
	(*R*)-$MeO_2CCOAlaOMe$ ($0.25H_2O$)	48	A (i) (b)	C,H,N	(160/0.03)[m]		
	(*S*)-$MeO_2CCOAlaOMe$ ($0.25H_2O$)	70	A (i) (b)	C,H,N	(160/0.03)[m]		
	MeO_2CCO-β-AlaOEt	61	A (i) (b)	C,H,N	(150/0.03)[m]		
	$MeO_2CCOPabaOEt$	74	A (i) (b)	C,H,N	146–147	F	
	$MeO_2CCOAibOMe$ ($0.5H_2O$)	82	A (i) (b)	C,H,N	(157/0.2)[m]		
	(*R,S*)-$MeO_2CCOPheOMe$ ($0.25H_2O$)	80	A (i) (b)	C,H,N	(215/0.2)[m]		
	(*R,S*)-$MeO_2CCOLeuOMe$ ($0.5H_2O$)	82	A (i) (b)	C,H,N	(175/0.2)[m]		
	(*R,S*)-$MeO_2CCOMetOMe$ ($0.5H_2O$)	75	A (i) (b)	C,H,N,S[n]	(210/0.2)[m]		
	(*R,S*)-$MeO_2CCOProOMe$	67	A (i) (b)	C,H,N	(185/0.03)[m]		
	$EtO_2CCOSarOEt$ ($0.5H_2O$)	83	A (i) (b)	C,H,N	oil		
25	$MeO_2CCOGlyOMe$	72	A (i) (a)	C,H,N	52.5–54	none	
26	$MeO_2CCOGlyNHSO_2Me$	21	B (ii)	C,H,N,S	176–179 dec	none	
	$MeO_2CCOGlyNHSO_2Ph$	81	B (ii)	C,H,N,S	189–191 dec	D	
27	$HGlyNHC_6H_4$-4-OEt (HBr)[o]		C (ii)	C,H,N	195–210	none	
28	$MeO_2CCOGlyNHC_6H_4$-4-OEt	62	C (iii)	C,H,N[p]	182–183	D	

[a] A = ethyl acetate–dichloromethane (1:1); B = ethyl acetate–cyclohexane (1:1); C = water–2M HCl; D = acetone–petroleum ether; E = ethyl acetate–cyclohexane (1:3); F = dichloromethane–ether. [b] Geometric mean of n independent determinations. The activities determined for less active compounds are given in brackets as percent inhibition at 50 or 100 μg/mL as indicated in footnotes *f* and *k*. [c] K_i = 0.8 μM (ref 22). [d] K_i = 0.54 μM (this work). [e] C, 26.8; H, 3.4; N, 12.0; S, 14.5. $C_5H_8N_2O_6S$ requires C, 26.79; H, 3.57; N, 12.50; S, 14.28. [f] Percent inhibition at 50 μg/mL (ca. 200 μM). [g] C, 41.6; H, 5.2; N, 7.9. $C_6H_9NO_5$ requires C, 41.14; H, 5.14; N, 8.00. [h] Literature mp 114–115 °C on analytically unsatisfactory material (ref 40). [i] $[\alpha]^{20}_{589}$ = +37.2 (c = 1.00, DMSO). [j] Found 4.2%; $0.5H_2O$ requires 4.1% water. [k] Percent inhibition at 100 μg/mL (ca. 400 μM). [l] C, 42.8; H, 5.6; N, 6.9. $C_7H_{11}NO_5 \cdot 0.5H_2O$ requires C, 42.42; H, 6.06; N, 7.07. [m] Bp, °C/mmHg (Kuegelrohr). [n] C, 42.2; H, 6.3; N, 5.3; S, 12.9. $C_9H_{15}NO_5 \cdot 0.5H_2O$ requires C, 41.86; H, 6.20; N, 5.43; S, 12.40. [o] "Phenocoll" (**10**) (ref 41) hydrobromide. [p] 0.4-mg sample. C, 55.3; H, 5.0; N, 9.1. $C_{13}H_{16}N_2O_5$ requires C, 55.71; H, 5.71; N, 10.00.

long been known[42] to be available by reduction of the isonitrosoacetanilide produced by reaction of chloral, hydroxylamine, and *p*-phenetidine. For convenience we chose the less remarkable route from (benzyloxycarbonyl)glycine (Z-Gly-OH) shown in Scheme IV, in which it was important to decarbamylate intermediate **11** by using HBr in glacial acetic acid rather than by catalytic hydrogenation.

Results and Discussion

Under the assay conditions used in this work oxaloglycine (**3**) is an inhibitor of prolyl hydroxylase with an IC_{50} = 2.89 μM. Data on the enzyme kinetics as a function of inhibitor concentration were fitted[43] to a hyperbolic function (Figure 1). A Lineweaver–Burk[44] plot of the fitted data shows the inhibition to be competitive (Figure 2a) and a secondary replot of the data (Figure 2b) indicates that K_i = 0.54 μM.[31] The inhibitory potencies of this compound and of oxalo derivatives of other amino acids are listed in Table I.

Of the racemic C_α-substituted oxalo amino acids tested, only the alanine derivative (**12**) showed convincing inhibitory activity. Substitution on N_α as in oxalosarcosine (**13**) and oxaloproline (**14**) was also highly deleterious and the C_α-disubstituted and conformationally limited (oxaloamino)isobutyrate (**15**) was essentially inactive. These results suggested to us that the inhibition produced by oxaloglycine (**3**) was very sensitive to the overall shape of the molecule and possibly sensitive to steric effects near the main chain.

The oxalo derivatives of the individual (*R*)- and (*S*)-alanines exhibited quite different activities: while oxalo-(*S*)-alanine (**16**) was less potent than oxaloglycine by about 1 order of magnitude, it was at least 1 order of magnitude more potent than the *R*-enantiomer **17**, which was not convincingly active at all under our test conditions. We concluded that the inhibition of prolyl hydroxylase by oxaloglycine (**3**) is sensitive to enantiospecific steric interactions between the inhibitory ligand and its binding site(s).

Increasing the distance between the two carboxylate groups of both 2-oxoglutarate and oxaloglycine sharply reduces their interaction with the enzyme. Thus, 2-

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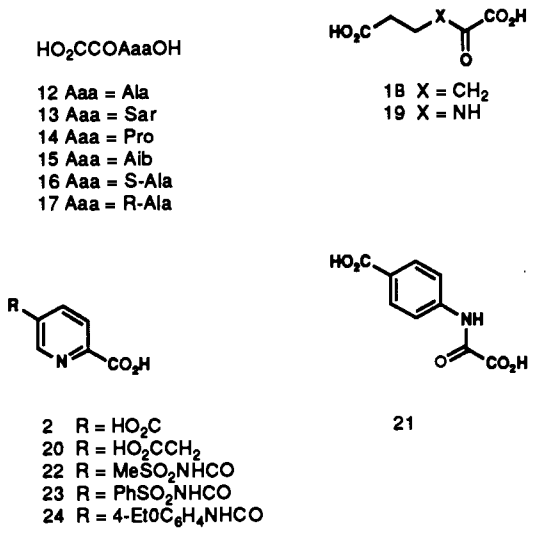
(43) Ratcliffe, J. HYPECALC.EXE, ICI Pharmaceuticals, 1985. A restrained least-squares fit of enzyme velocity (v) vs substrate concentration (S) data to the equation $v = (V_{max}S)/(K_m + S)$.
(44) Lineweaver, H.; Burk, D. The Determination of Enzyme Dissociation Constants. *J. Am. Chem. Soc.* **1934**, *56*, 658–666.



oxoadipate (18) will sustain the enzyme when used in place of its lower homologue 2-oxoglutarate (1), but it binds less well (K_m = 140 vs 3 μM).[22] In an analogous way, oxalo-β-alanine (19) is a much worse inhibitor of prolyl hydroxylase than is oxaloglycine (3) (IC_{50} > 620 μM vs 2.89 μM).

These data support the intuitively attractive idea that oxaloglycine (3) acts as an inhibitor of prolyl hydroxylase by binding to the enzyme in place of 2-oxoglutarate (1).

This unfavorable effect of homologation contrasts sharply with results seen[33] in the pyridine-2,5-dicarboxylic acid series, where homologation to the 5-acetic acid 20 is not attended by any significant change in inhibitory potency (5-carboxylic acid 2 IC_{50} = 5.18 μM vs 5-acetic acid 20 IC_{50}[33] = 9.6 μM) even though the changes appear to be strictly analogous.[22]

The lattitude allowed in the distance between the two carboxylate groups when it is spanned by an aromatic ring in the pyridine-2,5-dicarboxylic acid series also suggested that *N*-oxalo-4-aminobenzoic acid (21) might exhibit worthwhile inhibition of prolyl hydroxylase. However, it was inactive.

One potentially valuable feature of the binding site for the 5-carboxylic acid of pyridine 2,5-dicarboxylic acid analogues is its ability to accept groups other than carboxylate: both conventional acid mimics, such as the acylsulfonamides, e.g. 22 and 23 (IC_{50} = 1.8 and 1.1 μM, respectively),[33] and neutral H-bond acceptors, such as the anilides, e.g. 24 (IC_{50} = 5.0 μM),[1] are well tolerated by the enzyme. Again in sharp contrast to the methane- and the benzenesulfonamides 4 and 5 and the 4-ethoxyanilide 6 are all unacceptable as replacements for the carboxylate of the glycine moiety (IC_{50}s = 414, >350, and ≫370 μM vs 2.89 μM).

The structure–activity relationships reported here for oxaloglycine (3) are in startling contrast to those reported previously for derivatives of pyridine-2,5-dicarboxylic acid (2). These results suggest that it is unlikely that both oxaloglycine and pyridine-2,5-dicarboxylic acid bind in the same way to the same site on prolyl hydroxylase even though both are competitive inhibitors of the enzyme with respect to 2-oxoglutarate (1). If the ferrous iron of prolyl hydroxylase is hexacoordinate and assumed to be held in the enzyme by three mutually *cis*-ligands, there will be three other mutually *cis*-ligand-sites available for the co-substrates 2-ketoglutarate and dioxygen. There are three permutations of two from these three coordination sites, in one of which both of the 2-ketoglutarate coordination sites at the active-site iron are occupied whereas in the other two permutations only one of the 2-ketoglutarate coordination sites is occupied. In any event, a bidentate ligand will block the *bidentate* binding of 2-ketoglutarate, and it is unlikely that there would be any significant residual affinity of 2-ketoglutarate for the enzyme. Because the active site is unsymmetrical, there are two possible orientations that an unsymmetrical bidentate ligand could adopt in each of the three possible binding permutations. There are thus six possible arrangements of unsymmetrical bidentate ligands such as oxaloglycine or pyridine-2-carboxylic acid at the active site. Because each of these would severely interfer with the binding of 2-ketoglutarate, it is probable that inhibitors that adopt any—or all—of these arrangements would display competitive kinetics even though there were substantial differences at the molecular level.

In conclusion, we have shown that the rank order of potency of the inhibitors reported here is **3 > 2 > 16 ≫ 17** and that the other related compounds that were tested were essentially inactive. The inhibitory binding site for oxaloglycine derivatives on prolyl hydroxylase is thus highly intolerant of substitution on, or variation of, the glycyl moiety. In this respect as well as in many aspects of its molecular topography oxaloglycine (3) resembles the natural cofactor 2-oxoglutarate (1). It seems very likely that oxaloglycine (3) and 2-oxoglutarate (1) bind to prolyl hydroxylase in a closely analogous way. Thus, the discovery that the structure–activity relationships in the oxaloglycine series differ so completely from those in the pyridine-2,5-dicarboxylic acid series calls into question the published assumptions[22] about the nature of the pyridine-2,5-dicarboxylic acid (2) binding site on prolyl hydroxylase and suggests caution in interpreting the physical reality underlying the observation of strictly competetive inhibition.

Experimental Section

The following general procedures were followed unless otherwise stated: melting points were determined on a Büchi melting point apparatus and are uncorrected. Infrared spectra were recorded for Nujol mulls or thin films on a Philips PU9716 infrared spectrophotometer. ^{1}H-NMR spectra were obtained on a Bruker AC250, Bruker AM200, Varian EM390, or JEOL FX90Q instrument using dilute solutions in [$^2H_6$]DMSO (DMSO-d_6) or [^{2}H]$CHCl_3$ ($CDCl_3$) with tetramethylsilane as an internal standard. The ^{13}C-NMR spectrum of oxaloglycine (3) was obtained on a JEOL FX90Q using a dilute solution in [$^2H_2$]H_2O with the 41.5 ppm signal[25] as an internal standard. ^{1}H-NMR spectra were obtained for all isolated intermediates as well as for final products and were always consistent with the structural assignments. Optical rotations were measured on a Perkin-Elmer 241 polarimeter (1.0-dm path length) at room temperature. TLC was run on Merck Art. 5715 Kieselgel 60 F_{254} plates. Column chromatography was carried out by gravity filtration using Merck Art. 7734 Kieselgel 60. Extracts in water-immiscible solvents were dried using anhydrous magnesium sulfate and organic solvents were evaporated under reduced pressure using a Büchi R110 rotary evaporator. Elemental analyses were carried out under the direction of Mr. B. Crooks in the analytical section of ICI Pharmaceuticals at Alderley Park. Petroleum ether refers to the fraction boiling between 60–80 °C; water refers to distilled water. Amino acid ester hydrochlorides were usually freshly prepared from the amino acid, the corresponding alcohol, and hydrogen chloride gas,[45] methyl (*R*)- and (*S*)-alaninate hydrochlorides were prepared from (*R*)- and (*S*)-alanine, respectively, using thionyl chloride and methanol,[46] and benzyl glycinate hydrochloride (9)

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was prepared by the method of Erlanger and Brand.[47]

Biological Testing. The inhibitory potency of the compounds against prolyl 4-hydroxylase was determined using the indirect assay described by Cunliffe, Franklin, and Gaskell,[48] in which the conversion of the labeled cofactor 2-ketoglutarate into labeled succinate is measured. All points were determined in duplicate and IC_{50} values were obtained from six-point dose–response curves by interpolation. The logarithmic standard deviation estimated from the four sets of replicate data ($n = 15$, $\phi = 11$) was $\sigma = 0.14$; a compound is significantly different ($p < 0.05$) in potency to pyridine-2,5-dicarboxylic acid (**2**) if their IC_{50} values differ by a factor of more than 1.85 ($t_{95\%,\phi=11} = 2.20$).

A. Oxalo Amino Acids: General Procedure. Oxaloglycine (3). (i) Methyl Methoxalylglycinate (25). A stirred solution of methyl glycinate hydrochloride (dried at room temperature at 2 mmHg over P_4O_{10} overnight; 25.1 g, 200 mmol) in toluene (AR; 200 mL) was treated at room temperature with methoxalyl chloride (24.5 g, 200 mmol). The mixture was then heated under reflux for 4 h while a slow stream of dry argon was passed just over the surface. At this point the evolution of HCl gas was complete. The mixture was concentrated by evaporation under reduced pressure. (a) The oily residue was purified by column chromatography (300 g). Elution with ethyl acetate gave methyl methoxalylglycinate (**25**) as an oil that solidified on trituration [ether–petroleum ether (1:1)]: 25.24 g, 72%; mp 52.5–54 °C; ^{1}H NMR ($CDCl_3$) δ 7.60 (br s, 1 H), 4.15 (d, J = 6 Hz, 1 H), 3.93 (s, 3 H), 3.80 (s, 3 H). (b) In an alternative isolation procedure, which is especially suitable for more lipophilic esters, the oily residue may be partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The aqueous phase is then extracted twice with ethyl acetate. The combined organic phases are dried, evaporated to dryness, and purified either by chromatography as under method a or by distillation (Kuegelrohr).

(ii) Oxaloglycine (3). A stirred solution of **25** (24.5 g, 140 mmol) in water (467 mL) was treated under argon at room temperature with aqueous sodium hydroxide (1 M; 308 mL, 308 mmol). The reaction was maintained at room temperature for 2 h and then percolated during 20 min through a column of Amberlite IR-120(H) [600 mL, 1140 mequiv; previously washed with water (2 L)]. The column was eluted with water until the pH of the eluent had risen to 4. The eluent was evaporated to give an oil (17.3 g) that solidified on standing. The solid was dissolved in the minimum volume of boiling ethyl acetate, diluted with an equal volume of dichloromethane, cooled rapidly, and then filtered through cotton wool to remove some greasy residues. The crystals that formed from the filtrate were washed with dichloromethane and dried at room temperature over P_4O_{10} under vacuum to give oxaloglycine (**3**): 11.65 g, 57%; mp 112.5–114.5 °C; ^{1}H NMR (DMSO-d_6) δ 8.97 (t, J = 6 Hz, 1 H), 3.80 (d, J = 6 Hz, 2 H); ^{13}C NMR (D_2O) δ 172.5, 162.1, 160.5, 41.5. Anal. ($C_4H_5NO_5 \cdot 0.5H_2O$) C, H, N. This compound is hygroscopic, as are other members of this series, and should be stored under anhydrous conditions.

B. *N*-(Methylsulfonyl)oxaloglycinamide (4). (i) [(Methoxycarbonyl)carbonyl]glycine (7). A mixture of benzyl [(methoxycarbonyl)carbonyl]glycinate (**8**) (3.72 g, 0.15 mmol) and palladium on charcoal (10%; 0.37 g) was stirred under hydrogen (1 atm) at room temperature for 2 h. The mixture was filtered through Celite and evaporated to dryness to give an oil that slowly solidified. The solid was crushed and washed on a sinter with dichloromethane to give [(methoxycarbonyl)carbonyl]glycine (**7**): 1.01 g, 42%, mp 123–126 °C (acetone–petroleum ether); ^{1}H NMR (DMSO-d_6) δ 9.10 (br t, J = 6 Hz, 1 H), 4.80 (m, 4 H). Anal. ($C_5H_7NO_5$) C, H, N.

(ii) *N*-(Methylsulfonyl)[(methoxycarbonyl)carbonyl]glycinamide (26). A stirred solution of [(methoxycarbonyl)carbonyl]glycine (**7**) (0.403 g, 2.5 mmol), methanesulfonamide (0.238 g, 2.5 mmol), and 4-(dimethylamino)pyridine (0.305 g, 2.5 mmol) in dichloromethane (20 mL) maintained at 0 °C was treated rapidly with a solution of dicyclohexylcarbodiimide (0.529 g, 2.57 mmol) in dichloromethane (5 mL). The mixture was stirred overnight at room temperature under argon. The mixture was filtered and evaporated to dryness, and the residue was dissolved in methanol. The solution was percolated through a column of Amberlite IR-120(H) (20 mL; previously stirred with methanol overnight) and then eluted with methanol. The eluent was diluted with 2 volumes of ethyl acetate, filtered through silica gel in ethyl acetate–methanol (2:1), and evaporated. The residue crystallized on standing to give *N*-(methylsulfonyl)[(methoxycarbonyl)carbonyl]glycinamide (**26**): 0.124 g, 21%; mp 176–179 °C dec; ^{1}H NMR (DMSO-d_6) δ 11.0 (br s, 1 H), 9.10 (br t, J = 6 Hz, 1 H), 3.87 (d, J = 6 Hz, 2 H), 3.80 (s, 3 H), 3.20 (s, 3 H). Anal. ($C_6H_{10}N_2O_6S$) C, H, N, S.

(iii) *N*-(Methylsulfonyl)oxaloglycinamide (4). A stirred solution of *N*-(methylsulfonyl)[(methoxycarbonyl)carbonyl]glycinamide (**26**) (0.093 g, 0.39 mmol) in methanol (3 mL) was diluted with water (3 mL) and treated with aqueous sodium hydroxide (1 M; 0.6 mL, 0.6 mmol) under argon for 2 h. The reaction mixture was percolated through Amberlite IR 120(H) (previously washed with water) in water. The eluate was evaporated to an oil that was crystallized from acetone–petroleum ether to give *N*-(methylsulfonyl)oxaloglycinamide (**4**): 0.040 g, 46%; mp 200.5–201.5 °C dec; ^{1}H NMR (DMSO-d_6) δ ca. 11.0 (v br s, ca. 2 H), 8.95 (br t, J = 6 Hz, 1 H), 3.87 (d, J = 6 Hz, 2 H), 3.20 (s, 3 H). Anal. ($C_5H_8N_2O_6S$) C, H, N, S.

C. *N*-(4-Ethoxyphenyl)oxaloglycinamide (6). (i) *N*-(4-Ethoxyphenyl)(benzyloxycarbonyl)glycinamide (11). A stirred solution of (benzyloxycarbonyl)glycine (5.23 g, 25 mmol) and triethylamine (10.43 mL; 7.56 g, 75 mmol) in dichloromethane (150 mL) was treated at −30 °C with isobutyl chloroformate (3.63 mL; 3.82 g, 28 mmol). The mixture was allowed to warm to 0 °C under argon and then treated with 4-ethoxyaniline (3.22 mL; 3.43 g, 25 mmol). The mixture was stirred at room temperature for 2 h and then washed with hydrochloric acid (2 M; twice) and saturated aqueous sodium bicarbonate. The solution was dried and evaporated to give a solid that was dissolved in dichloromethane–methanol (10:1) and eluted through a small column of silica gel. The eluate was concentrated and diluted with petroleum ether to give a precipitate of *N*-(4-ethoxyphenyl)(benzyloxycarbonyl)glycinamide (**11**) [4.22 g, 51%; ^{1}H NMR ($CDCl_3$) δ 7.90 (br s, 1 H), 7.35 (m, 7 H), 6.83 (d, J = 9 Hz, 2 H), 5.60 (br s, 1 H), 5.15 (s, 2 H), 4.10 (m, 4 H), 1.40 (t, J = 6.5 Hz, 3 H)], which was used without further purification.

(ii) *N*-(4-Ethoxyphenyl)glycinamide Hydrobromide (27). A solution of *N*-(4-ethoxyphenyl)(benzyloxycarbonyl)glycinamide (**11**) (0.95 g, 37.1 mmol) in a solution of hydrogen bromide in acetic acid (30%, 20 mL) was stirred at room temperature for 45 min and then diluted with ether (100 mL). The resultant precipitate was isolated by filtration and then washed with ether to give *N*-(4-ethoxyphenyl)glycinamide hydrobromide (**27**): 0.58 g, 73%; mp 195–210 °C; ^{1}H NMR (DMSO-d_6) δ 10.20 (br s, 1 H), 8.05 (br s, 3 H), 7.50 (d, J = 9 Hz, 2 H), 6.91 (d, J = 9 Hz, 2 H), 4.00 (q, J = 7 Hz, 2 H), 3.75 (s, 2 H), 1.31 (t, J = 7 Hz, 3 H). Anal. ($C_{10}H_{15}N_2O_2 \cdot HBr$) C, H, N.

(iii) *N*-(4-Ethoxyphenyl)[(methoxycarbonyl)carbonyl]glycinamide (28). A stirred mixture of *N*-(4-ethoxyphenyl)glycinamide hydrobromide (**27**) (0.57 g, 21 mmol) in toluene (AR; 30 mL) was treated at room temperature with methyloxalyl chloride (0.245 g, 191 mmol). While a slow stream of dry argon was passed just over the surface, the mixture was then heated under reflux for 6 h. The inhomogeneous mixture was cooled to

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room temperature and filtered to give a product that was washed with toluene, dissolved in hot acetone, refiltered, isolated by evaporation, and then purified by chromatography on silica gel. A fraction eluting in dichloromethane–methanol (10:1) was further purified by precipitation from acetone with petroleum ether to give *N*-(4-ethoxyphenyl)[(methoxycarbonyl)carbonyl]glycinamide (**28**): 0.29 g, 49%; mp 182–183 °C; ^{1}H NMR (DMSO-d_6) δ 9.8 (br s, 1 H), 9.12 (br s, 1 H), 7.43 (d, J = 9 Hz, 2 H), 6.86 (d, J = 9 Hz, 2 H), 3.95 (m, 4 H), 3.80 (s, 3 H), 1.30 (q, J = 7 Hz, 3 H).

(iv) ***N*-(4-Ethoxyphenyl)oxaloglycinamide (6).** A solution of *N*-(4-ethoxyphenyl)[(methoxycarbonyl)carbonyl]glycinamide (**28**) (0.28 g, 1 mmol) in THF (20 mL) was diluted with water (10 mL) and then treated with aqueous sodium hydroxide (1 M; 1.5 mL, 1.5 mmol). The solution was stirred at room temperature for 2 h, filtered, and then acidified to pH 2 with hydrochloric acid (2 M). The solution was refrigerated for 1 h and the product that crystallized was isolated by filtration, washed with water, and dried to give *N*-(4-ethoxyphenyl)oxaloglycinamide (**6**): 0.23 g, 85%; mp 207 °C dec; ^{1}H NMR (DMSO-d_6) δ 13.80 (br s, 1 H), 9.80 (br s, 1 H), 8.85 (br m, 1 H), 7.45 (d, J = 9 Hz, 2 H), 6.85 (d, J = 9 Hz, 2 H), 3.95 (m, 4 H), 1.31 (t, J = 7 Hz, 3 H). Anal. ($C_{12}H_{14}N_2O_5$) C, H, N.

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Nonpeptide Angiotensin II Receptor Antagonists: Synthetic and Computational Chemistry of *N*-[[4-[2-(2*H*-Tetrazol-5-yl)-1-cycloalken-1-yl]phenyl]methyl]imidazole Derivatives and Their in Vitro Activity

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A series of nonpeptide angiotensin II receptor antagonists was synthesized and tested in vitro to investigate requirements for recognition by and binding to AT_1 receptors. Compared to a known series of *N*-(biphenylylmethyl)imidazoles, including losartan (DuP 753), which has a more rigid conformation in the 2′-tetrazolylbiphenyl moiety, the new series replaces the terminal phenyl with cycloalkenyls. Compounds were made with five- to seven-membered rings and with either a hydroxymethyl (**3**) or carboxyl (**4**) group at the 5 position on the imidazole ring. The effects of the lipophilicity and steric bulk of the terminal ring system, the amount of π-electron density in the terminal ring, and the relative spatial proximity of the tetrazolyl and the middle phenyl are explored in terms of binding affinity to AT_1 receptors in rat adrenal glomerulosa and rabbit aorta. The physicochemical variables of the new compounds were quantitated by computational chemistry and compared to those of losartan and its carboxyl metabolite. Potency at the AT_1 receptors is maximized when the terminal ring is six-membered; an aromatic ring binds better than a cycloalkenyl ring. The 5-carboxyimidazole compounds show higher affinity than the 5-hydroxymethyl series.

The renin–angiotensin system (RAS) is known to play an important role in the regulation of blood pressure and electrolyte and fluid balance under normal and a variety of pathophysiological conditions.[1,2] Angiotensin II (AII), the end product of the RAS cascade, has a powerful constricting action on arterioles and immediately elevates blood pressure. AII is one of the most studied hormones since it was isolated and crystallized 50 years ago by Lilly clinicians in Indianapolis.[3] The potent vasopressive action of AII is mediated through membrane-bound receptors coupled to G proteins in smooth muscle and other cells.[4] Although the receptors have been cloned, there is as yet no detailed three-dimensional structural information on them. In fact, there is not even a consensus on the most probable conformations adopted by the AII ligand itself. Being a linear octapeptide, AII is highly flexible and exists as populations of many conformations in polar and nonpolar environments. Hypotheses about its conformation at the receptor sites have been promoted.[5] However, the hypotheses have not been proven.

Over the last several of decades the RAS has been the target of therapeutic intervention in control of hypertension and related complications. Angiotensin-converting enzyme (ACE) inhibitors, such as captopril and enalapril, are useful in the treatment of hypertension, but they suffer from adverse side effects, such as hypotension, angioedema, and dry cough. Also, it is difficult to differentiate the ACE inhibitors clinically.[6] Antagonism of the AII receptors offers the prospect of a better intervention point in the RAS. There has been much work on saralasin, [Sar^1,Ala^8]AII, and other peptide AII receptor antagonists, but they have shortcomings of no oral bioavailability, poor in vivo stability, and partial agonist activity at high concentrations.[7]

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