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PREPARATION OF A NOVEL SERIES OF PHOSPHONATE NORSTATINE RENIN INHIBITORS

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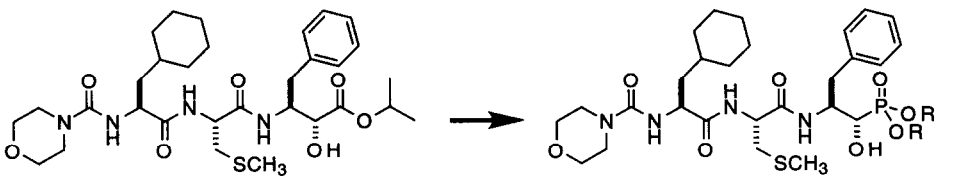
Abstract: Replacement of the C-terminal isopropyl ester in the orally active norstatine renin inhibitor terlakiren (**1**) with dialkyl phosphonate groups provided a novel series of phosphorus norstatine inhibitors **2**. Cyclic phosphonate **2f** (IC_{50} = 0.6 nM) was prepared in an attempt to overcome apparent steric limitations of the phosphonate binding pocket and was found to be equipotent to ester **1**. Like the ester norstatines, the phosphonate inhibitors preferred nonpolar P2 residues.

A major effort in the search for new agents to treat hypertension and congestive heart failure has focused on pharmacological interruption of the renin-angiotensin system (RAS)[1] as a way of preventing the actions of the highly vasoconstrictive octapeptide angiotensin II (AII). Early success with angiotensin converting enzyme (ACE) inhibitors[2] established the RAS as an important therapeutic target. Concerns over the unselective nature of ACE led to an extensive effort aimed at inhibitors of the aspartic protease renin,[3] while the recent discovery of potent nonpeptidic AII antagonists has produced an explosion of research directed toward compounds of that class.[3a,4]

Progress in the renin area has been hampered by poor oral bioavailability due to the size and peptidic nature of the inhibitors. However, in spite of these challenges, new renin inhibitors with improved oral activity have recently been reported.[5] Our interest in the renin inhibition area focused mainly on norstatine-containing compounds and led to the discovery of orally active terlakiren (**1**, CP-80,794; human plasma renin IC_{50} = 0.7 nM).[6]

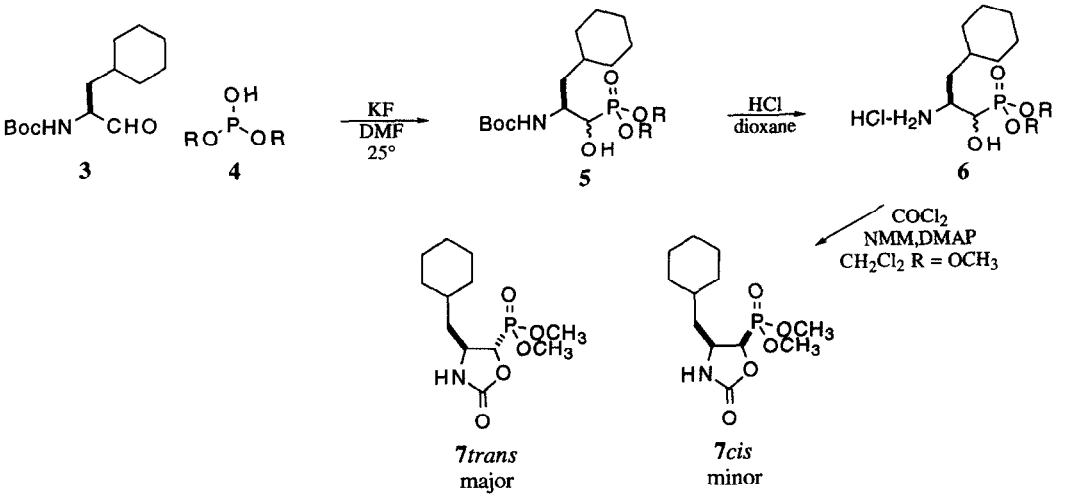
In an attempt to further improve activity in the norstatine series, we sought to identify replacements for the potentially metabolically labile isopropyl ester in terlakiren. One approach focused on the replacement of the ester with a dialkyl phosphonate[7] (Figure 1), a group which could maintain potential ester-like interactions (e.g., a hydrogen bond to the carbonyl/phosphonyl oxygen) while probing the ester binding region of renin with a tetrahedral moiety. This letter describes studies in the novel dialkyl phosphonate norstatine series.



terlakiren (**1**) **2**

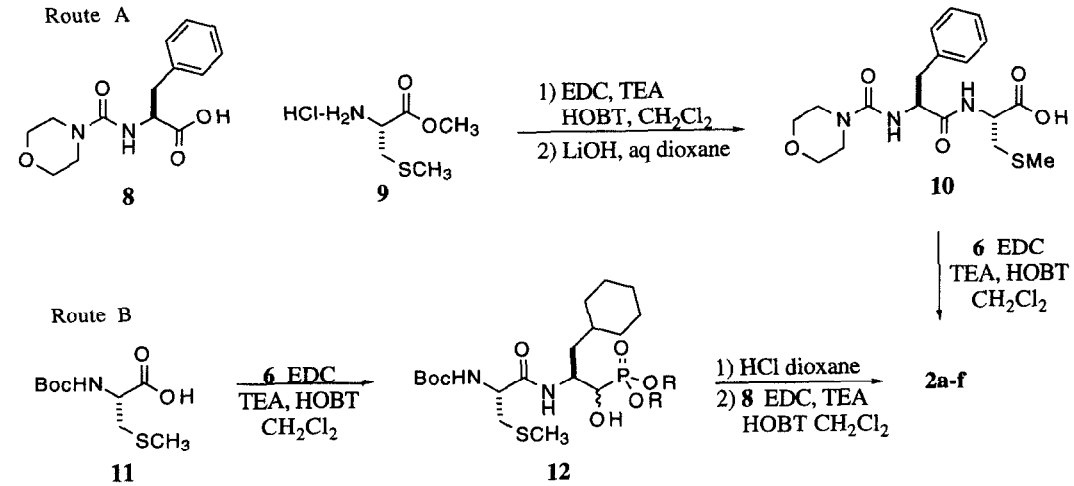
Figure 1

Scheme 1



Phosphonates **2a-f** (Tables 1 and 3) were prepared as outlined in Scheme 1.[8] Potassium fluoride-promoted addition[9] of dialkyl phosphites **4** to Boc-L-cyclohexylalanal **3**[10] (DMF, 25°) afforded 3-4:1 mixtures of inseparable epimeric phosphonates **5**. The stereochemistry of the major product in **5a** (R = CH_3) was determined by treating deprotected **6a** (HCl, dioxane) with phosgene (NMM, DMAP, CH_2Cl_2) and analyzing the crude oxazolidinones **7** by 500 MHz ^{1}H-NMR. Ring proton coupling constants[11] and NOE experiments[12] supported a *trans* relationship for the phosphonyl and cyclohexylmethyl groups in the major component in **7**, thereby establishing the S-configuration (desired) for the major isomer in **5a**.[13]

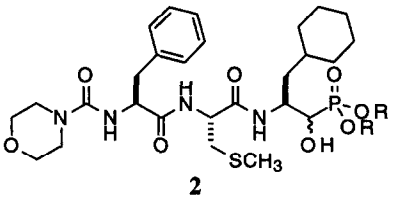
Scheme 2



The final compounds **2** were prepared as mixtures of diastereomers from **6** (Scheme 2), either by coupling to dipeptide **10** (Route A) or via intermediates **12** (Route B). Amines **6** were used directly in couplings without purification, and all couplings proceeded in high yield thus ensuring crude products which reflected the ratio of epimers in **5**. Product ratios were determined by ^{1}H-NMR and HPLC. Separation of epimers in the final products **2a-f** was difficult and the ratios of epimers in compounds prepared via Route A were not significantly improved over the corresponding ratios in **5**. Route B ultimately proved advantageous in obtaining products of higher diastereomeric purity by providing an additional opportunity for epimer separation with intermediates **12**.

The human plasma renin IC_{50}'s for ester **1** and phosphonates **2a-e** are presented in Table 1.[14] The first compound prepared, dimethyl phosphonate **2a**, was found to be a very good inhibitor with an IC_{50} of 5.8 nM. Although 10-fold less active than ester **1**, phosphonate **2a** clearly indicated that renin will tolerate a tetrahedral phosphonyl moiety in place of the planar norstatine carbonyl system. Compounds **2b-e** probed the steric requirements for this region of the enzyme. The best activity was restricted to the smallest groups thereby indicating a divergence of SAR between the ester and phosphonate series. A 4-fold drop in activity was observed for diethyl phosphonate **2b**, and decreases of 10- and 200-fold relative to the dimethyl phosphonate were seen for the di-*n*-propyl and di-isopropyl analogs, respectively. Dibenzyl compound **2e** was of intermediate potency with an IC_{50} of 190 nM.

Table 1 - Dialkyl Phosphonate Renin Inhibitors[14]



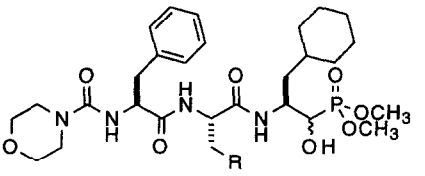
2

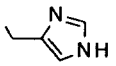
Cpd	R =	Synthetic Route	Epimer ratio (S:R)	Hu Renin IC_{50} (nM)
1	-	-	-	0.7
2a	$-CH_3$	A	3:1	5.8
2b	$-CH_2CH_3$	A	4:1	25
2c	$-CH_2CH_2CH_3$	B	16:1	60
2d	$-CH(CH_3)_2$	A	4:1	1400
2e	$-CH_2Ph$	A	3.5:1	190

Compounds **13** and **14** (Table 2) were prepared[15] as part of an effort to investigate the P_2 SAR of the new phosphonate norstatine series. Like the norstatine esters, the phosphonates generally showed better inhibitory

activity with non-branched, non-polar P2 residues (e.g., norleucine **13**, IC_{50} = 32 nM) than with the naturally-occurring P2 residue histidine (**14**, IC_{50} = 190 nM).

Table 2 - P2 Analogs[14]



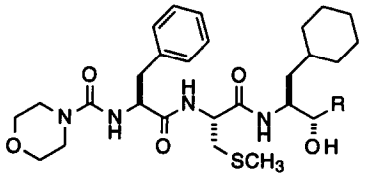
Cpd	R =	Synthetic Route	Epimer ratio (S:R)	Hu Renin IC_{50} (nM)
2a	$-SCH_3$	A	3:1	5.8
13	$-CH_2CH_2CH_3$	A	3:1	32
14		B	>10:1	190

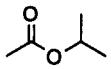
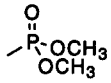
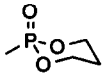
Our results indicated that good in vitro potency against plasma renin could be obtained with phosphonate norstatine compounds, and analog **2a** represented a promising lead toward identifying an equipotent replacement for the norstatine ester in terlakiren. However, in spite of encouraging in vitro potency, compound **2a** was found to be significantly weaker in vivo than the ester. Similar results were found with related phosphonates with IC_{50}'s in the 5 to 10 nM range. Additional studies were therefore undertaken to identify a compound in the phosphonate series with in vitro activity equal to ester **1** in order to directly test the in vivo effects of the phosphonate-ester substitution.

In contrast to the ester series, the SAR on the phosphonate group clearly indicated a preference for the smallest possible alkyl substituents (dimethyl), possibly as a result of the greater steric demands of the tetrahedral phosphonate relative to the planar carbonyl. Faced with the task of modifying the phosphonate group of inhibitor **2a** without greatly increasing steric bulk, we chose to investigate the activity of cyclic phosphonate **2f** (Table 3).[16]

Cyclic phosphonate **2f** (>10:1 S:R) was compared to ester **1** and dimethyl phosphonate **2a** for its ability to inhibit human plasma renin (Table 3). Cyclic phosphonate **2f** was found to have an IC_{50} of 0.6 nM, 10-fold better than dimethyl phosphonate **2a** and equipotent to terlakiren (**1**). Compound **2f** therefore represented the realization of our initial goal of identifying a novel potent replacement for the norstatine ester in **1**. The epimeric cyclic phosphonate (>10:1 R:S, structure not shown; IC_{50} = 14 nM) was significantly less active than isomer **2f** which had the statine-like hydroxyl configuration.[17]

Table 3 - Plasma Renin Inhibitory Activities[14]



Cpd	R =	Hu Plasma IC_{50} (nM)
1		0.7
2a[18]		5.8
2f		0.6

As a result of its subnanomolar potency, phosphonate **2f** was examined for in vivo activity. An intravenous dose of 3 mg/kg lowered blood pressure in the sodium-depleted guinea pig[6b] by 30 mm Hg before returning to baseline 3 hours later. A 30 mg/kg oral dose of **2f** produced a 10 mm Hg reduction which lasted for 2 hours. The results from these experiments were comparable to those obtained for ester **1**[6b] in spite of significantly weaker potency for the phosphonate against the guinea pig plasma enzyme (**2f**, GP IC_{50} = 8.4 nM; **1**, 0.3 nM). Based upon the comparison of guinea pig in vivo data, and the fact that similar IC_{50}'s were observed against monkey plasma renin (**2f**, MK IC_{50} = 0.2 nM; **1**, 0.5 nM), phosphonate **2f** was expected to have the same good oral activity in the sodium-depleted marmoset monkey that was observed for **1** (5 hours duration of action after a dose of 10 mg/kg, p.o.).[6b] It was therefore surprising to observe only weak blood pressure lowering activity for phosphonate **2f** in the monkey model after an oral dose of 10 mg/kg. Similar results with other in vitro potent compounds in this series led us to conclude that, as a class, the phosphonate norstatine renin inhibitors are less active in vivo than terlakiren (**1**) in the sodium-depleted marmoset monkey.

Although we did not reach our ultimate goal of identifying a compound with improved in vivo activity by replacing the ester in terlakiren (**1**) with the cyclic phosphonate in **2f**, we have demonstrated that excellent in vitro activity can be obtained for a renin inhibitor containing a phosphonate norstatine.

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8. All final products and intermediates provided satisfactory spectroscopic and high resolution MS data. Representative analytical data are given for **2f**: ^{1}H NMR (DMSO-d_6) δ 2.05 (s, 3 H), 2.63 (dd, J = 8, 14 Hz, 1 H), 2.81 (m, 2 H), 2.98 (dd, J = 4, 14 Hz, 1 H), 3.16 (m, 4 H), 3.43 (m, 4 H), 3.88 (m, 1 H), 4.10 (m, 1 H), 4.18-4.60 (m, 6 H), 6.61 (d, J = 9 Hz, 1 H), 7.23 (m, 5 H), 7.54 (d, J = 10 Hz, 1 H), 8.15 (d, J = 8 Hz, 1 H); FAB MS ($C_{30}H_{47}N_4O_8PS$+H) calcd 655.2933, found 655.2974.
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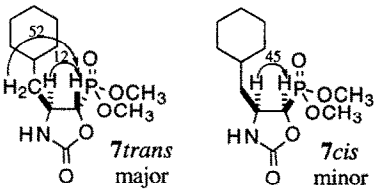
11. The coupling constants for the ring protons in the components in **7** supported the assigned stereochemistry: **7***trans* (major), J = 6.1 Hz; **7***cis* (minor), 8.7 Hz. See: Kempf, D. J.; de Lara, E.; Stein, H. H.; Cohen, J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1978. and Sham, H. L.; Rempel, C. A.; Stein, H.; Cohen, J. *J. Chem. Soc., Chem. Commun.* **1987**, 683; and reference 7a.
12. The indicated NOE's were observed for **7** *trans* and **7** *cis*. The arrow tails indicate irradiated protons and the arrow heads point to enhanced protons. The numbers over the arrows are magnitudes in percent.



13. The stereochemistries of the major isomers in **5b-f** were assigned by analogy to **5a**.
14. Reported IC_{50}'s values are the means of at least three determinations. Calculated SEM's range from 12% to 30% of the mean IC_{50} value.
15. Compounds **13** and **14** were prepared by substituting the appropriately protected amino acid in Scheme 2.
16. The corresponding 5-membered ring phosphonate was our original target. However, the required 5-membered cyclic phosphite **4** was very unstable and addition to aldehyde **5** could not be achieved. See, Nifant'ev, E. E.; Nasonovskii, I. S.; Miklashevskii, A. V.; Zavalishina, A. I.; Smirova, E. I. *J. Org. Chem., USSR (Eng.)* **1975**, *11*, 2235.
17. The presence of a few percent of **2f** having been responsible for the majority of the epimer's 14 nM activity could not be ruled out due to estimated HPLC and NMR detection limits of 10%.
18. Compound **2a** was isolated as a 3:1 mixture of S- to R-hydroxyl group epimers. See Table 1.

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