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NON-PEPTIDIC INHIBITORS OF NEUTRAL ENDOPEPTIDASE 24.11
2. DESIGN AND PHARMACOLOGY OF ORALLY ACTIVE PHOSPHONATE PRODRUGS

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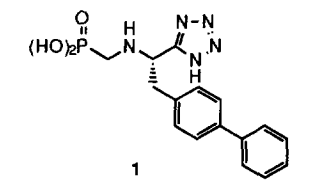
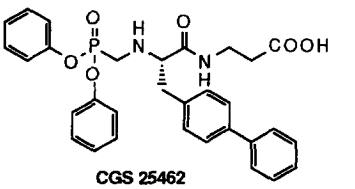
Abstract: Prodrugs of **1** (CGS 26303), a novel non-peptidic neutral endopeptidase (NEP 24.11) inhibitor, have been designed. In particular, the diphenylphosphonate **6** (CGS 26393) was found to be a potent, orally bioavailable and long-acting NEP inhibitor. Accordingly, **6** protected atrial natriuretic peptide from enzymatic degradation and displayed a significant antihypertensive activity in the DOCA-salt rat.

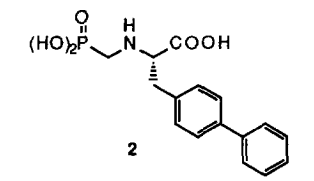
Introduction. In the preceding paper, we have described the design of **1** (CGS 26303), a structurally novel and highly potent inhibitor of neutral endopeptidase (NEP 24.11; IC_{50} = 0.93 nM) and a modest inhibitor of endothelin-converting enzyme (ECE; IC_{50} = 1.1 μM).[1] The pharmacological evaluation of **1** in rats has demonstrated its ability to protect exogenously administered atrial natriuretic peptide (ANP) from enzymatic degradation, and has been consistent with a functional inhibition of ECE.[2] Unfortunately, **1** lacked biological activity after oral administration. Recently, we have shown that some diphenyl phosphonates, such as CGS 25462, behave as orally active prodrugs of α-amino phosphonic acids.[3] A combination of features has been identified to contribute to the success of this approach. First, since our data support an enzymatic catalysis for the 2-step hydrolysis of the phosphonate to the phosphonic acid in plasma,[4] substrate recognition by this putative enzyme is critical.[5] Second, the nature of the leaving group (e.g. aryl alcohol) affects the conversion *in vivo* and, possibly, the oral absorption of the phosphonate. Among several analogues, diphenylphosphonates maintain the optimum balance between a suitable chemical stability and a steady processing *in vivo*.[3] Third, the presence of an α-amino group, most likely protonated at physiological pH, enhances the hydrolytic susceptibility of the phosphonate.[4] Finally, participation of the amide group should also facilitate the hydrolysis.[6] In that case, other groups, such as carboxylates or tetrazolides would provide a more effective anchimeric assistance than the amide group during the phosphonate hydrolysis, perhaps allowing the use of other leaving groups than aryl alcohols. Therefore, a series of phosphonates derived from **1** and from its carboxylic acid analogue **2**, a weaker NEP inhibitor (IC_{50} = 15 nM), were synthesized and evaluated for oral activity in rats.



1

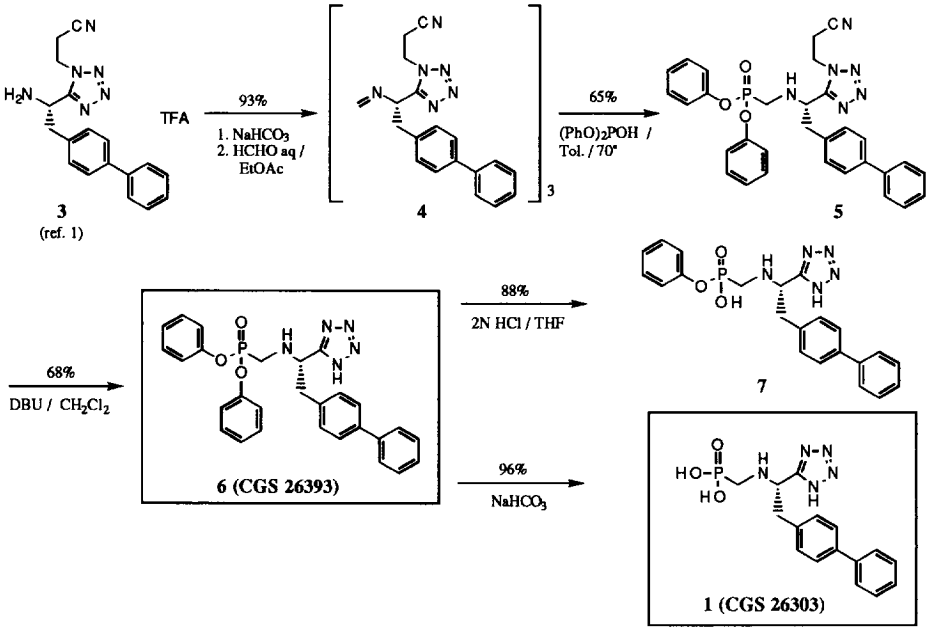


CGS 25462



2

Chemistry. Diaryl phosphonate derivatives of **1** were prepared according to the methods previously used in the synthesis of CGS 25462,[3] i.e. by addition of a diarylphosphite to the [6H]-triazine **4**, followed by deprotection of the tetrazole group in **5** under anhydrous basic conditions.[7] A similar protocol was applied to obtain the phosphonates derived from **2**, using the [6H]-triazine generated by the cyclocondensation of (S)-(4-phenyl)phenylalanine benzyl ester with formaldehyde. Palladium-catalyzed hydrogenolysis of the benzyl ester completed the synthesis of the C-terminal carboxylic acids. The preparation of **6** is representative of the synthetic procedures used in this work (Scheme). Acidic treatment of **6** produced selectively the monophosphonate **7**, while its sodium bicarbonate-induced hydrolysis[4] gave directly and conveniently the phosphonic acid **1** in excellent yield. The di(carboethoxymethyl)phosphonates **9** and **11** were also obtained by the [6H]-triazine method, using the crude diglycolyl phosphite intermediate ($HP(O)(CH_2COOEt)_2$) produced by dialkylation of phosphorous acid (H_3PO_3 / $BrCH_2COOEt$ /DIPEA / CH_3CN).

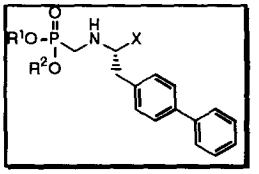


Scheme

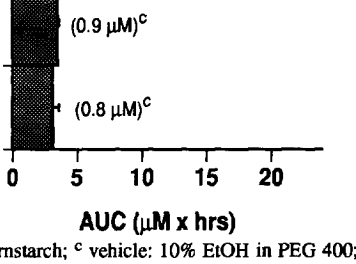
Results and Discussion. The ability of the phosphonates to act as prodrugs was evaluated by an *ex vivo* assay which allows the determination of NEP inhibitory activity generated in plasma after oral administration of the compounds.[8] The NEP inhibition was assumed to result from the production *in vivo* of the corresponding unbound phosphonic acids **1** or **2**.[9] Data were collected at 1 hr intervals during 6 hrs (n= 3-9). Determination of the area under the curve (AUC) and the maximum concentration of inhibitor produced are presented in the Table. In the tetrazole series, results indicate that the simple dimethylphosphonate **8** was not a prodrug, probably due to its resistance towards hydrolysis *in vivo*. However, the more activated diglycolyl derivative **9** displayed substantial oral activity, comparable to that of the substituted diarylphosphonate **10**. By far the best pharmacokinetic profile was achieved with the diphenyl phosphonate **6**, an observation which parallels our

previous finding with phosphonomethyl dipeptides.[4] Weak activity was observed with the monophosphonate **7**, most likely because of poor absorption properties. In the carboxylic acid series, a change of vehicle was usually required for solubility reasons, making a direct comparison with the tetrazole analogues difficult. However, within this series, a similar trend emerged in favor of the diphenyl phosphonate **12**.

Table. Structures of Phosphonate Prodrugs and Plasma Levels of Free NEP Inhibitor[a]



Cpd	R^1	R^2	X	
8	MeO	MeO	tetrazole	(0.01 μM)[b]
9	EtO	EtO	tetrazole	(0.45 μM)[b]
6	Ph	Ph	tetrazole	(5.2 μM)[b]
10	Ph	Ph	tetrazole	(0.36 μM)[b]
7	Ph	H	tetrazole	(0.19 μM)[b]
11	EtO	EtO	COOH	(1.1 μM)[c]
12	Ph	Ph	COOH	(1.6 μM)[b]
13	Ph	Ph	COOH	(0.9 μM)[c]
14	Ph	Ph	COOH	(0.8 μM)[c]



[a] all doses : 30 mg/kg (n = 3-8); [b] vehicle: 3% cornstarch; [c] vehicle: 10% EtOH in PEG 400; maximum concentration levels in parentheses; values are means ± SEM.

Since the diphenylphosphonate **6** displayed an attractive pharmacokinetic profile, its protective effect on exogenously infused ANP was investigated[10] (Fig. 1). Results show that a single low oral dose (1 mg/kg) of **6** resulted in a large potentiation of immunoreactive ANP (irANP) levels in rats for the entire duration of the

experiment (4 hrs). The antihypertensive activity of **6** was also verified in the DOCA-salt rat, a low renin model of hypertension responsive to NEP inhibitors. As expected from its pharmacokinetic profile, **6** produced a significant and sustained lowering of mean arterial pressure (-27 mmHg at 5 hrs) after oral administration (Fig. 2).

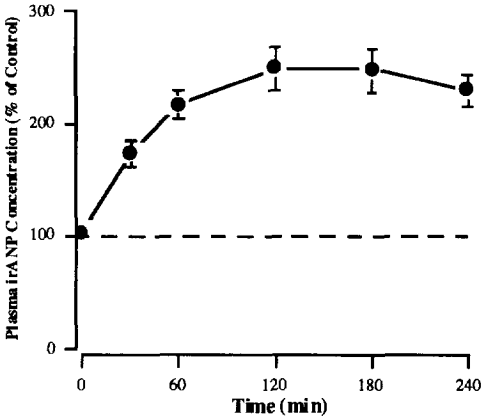


Fig. 1. Effect of **6** (●, 1 mg/kg, p.o.) on plasma irANP concentration in conscious rats (n=3) infused with ANP (450 ng/kg/min). Values are mean ± SEM expressed as a percent of those measured in vehicle-treated rats.

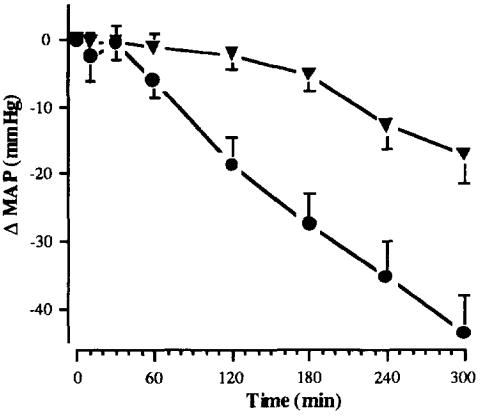


Fig. 2. Effect of **6** (●, 30 mg/kg, p.o.) in conscious DOCA-salt hypertensive rats (n=12). Values are mean ± SEM changes in mean arterial pressure (MAP). A significant antihypertensive effect ($p<0.05$) was measured with **6** relative to vehicle (▼, PEG 400).

Conclusion. The diphenylphosphonate tetrazole **6** constitutes an efficient, orally active prodrug of **1**, capable of slowing down ANP metabolism, and exerting a long-lasting antihypertensive effect in the DOCA-salt rat. It fulfills our goal to discover a novel, structurally unique, highly potent, and orally bioavailable inhibitor of NEP, and provides further support for the usefulness of diphenylphosphonates as phosphonic acid prodrugs. Based on the pharmacological profile of the parent compound **1** (CGS 26303), which extends beyond the effects of NEP inhibition,[2] **6** (CGS 26393) holds significant promise as a therapeutic agent. It has been selected for toxicological evaluation.

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References and Notes

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(9) This assumption has only been formally verified with **6** using HPLC. Like **CGS 25462**, **6** is intrinsically inactive (IC_{50} > 1 μM) but rapidly metabolized *in vivo* in rat plasma to the corresponding phosphonic acid inhibitor (S. Kuzmich, personal communication). A similar behavior is expected for the other phosphonates **8-14**.
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