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THE IDENTIFICATION OF A NOVEL RENIN INHIBITOR OF EQUIVALENT EFFICACY FOLLOWING ORAL OR INTRAVENOUS ADMINISTRATION.

S. A. Smith*, K. A. Al-Barazanji, R. E. Buckingham, F. Cassidy, M. C. Coldwell, F. J. L. Finney, M. S. Hadley, P. Ham*, S. A. Lawrence, D. J. Nash, E. J. R. Tingley, J. M. Watson.

SmithKline Beecham Pharmaceuticals, Discovery Research, Coldharbour Road, The Pinnacles, Harlow, Essex, CM19 5AD, U.K.

Abstract: The identification and *in vitro* potency of a novel series of renin inhibitors, based on pyrido-fused [6,6] ring systems is described. The syntheses of representative members of the series are reported. The most interesting compound, the pyrido[4,3-b]thiazine **10**, shows equivalent efficacy in the conscious, sodium-depleted, marmoset following oral or intravenous administration.

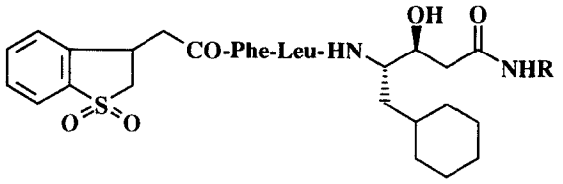
The renin-angiotensin system (RAS) is known to play a crucial role in the regulation of blood pressure and in the maintenance of electrolyte balance[1]. This system can be blocked by the inhibition of angiotensin converting enzyme (ACE), and such an approach has led to useful antihypertensive drugs. ACE inhibitors may also be of value in other areas, such as congestive heart failure[2]. Inhibition of renin rather than ACE may, however, have potential therapeutic advantages, since the action of renin, unlike ACE, is directed toward a single, RAS-specific substrate, angiotensinogen. Moreover, renin is the rate limiting enzyme in the RAS.

A large number of potent inhibitors of renin have been identified[3]. However, as a result of problems due, in the main, to low oral activity and bioavailability[4], few of the reported inhibitors appear likely to become widely useful drugs. We now report some of our work that has given, in the marmoset, an inhibitor of equivalent efficacy following oral or intravenous administration.

Our work in this area initially led to the identification of the dihydrobenzothiophene dioxide (DHBT) derivative **1** (Table 1), containing the 4(S)-amino-5-cyclohexyl-3(S)-hydroxypentanoic acid (ACHPA) moiety[5], as a novel inhibitor of good potency (IC_{50} 10nM)[6]. Attempting to modify the physicochemical properties of this compound (e.g., very high lipophilicity, pH 7.4 aqueous buffer solubility < 100ng/ml), thereby reducing potential problems with low and variable bioavailability[7], the C-terminal moiety was replaced with a variety of more polar groups. The requisite bifunctional amines, which were either commercially available or prepared by literature or analogous routes[8,9], were incorporated into inhibitors **2**-**8**[10] (Table 1) using carbodiimide methodology[9].

The optimal C-terminal group identified from this approach is a 3-carbon linked imidazolyl moiety, as in **3**. Alteration of chain length (as in **2** or **4**), or of the basic moiety (as in **5**, an alternative aromatic heterocycle, **6**, a saturated heterocycle used effectively in other series[11], or **7**, an acyclic amine), gave compounds of lower potency. The acid **8**, although not devoid of activity, was of less interest than inhibitors containing basic heteroaromatic moieties.

Table 1: The Effect of C-Terminal Amide Variation



Compound No.	R	IC_{50} (nM)[a]
1	$(CH_2)_2CH(CH_3)_2$	10
2	$(CH_2)_2$(1-Imidazolyl)	41
3	$(CH_2)_3$(1-Imidazolyl)	12
4	$(CH_2)_4$(1-Imidazolyl)	37
5	$(CH_2)_3$(3-Pyridyl)	34
6	$(CH_2)_2$(4-Morpholinyl)	170
7	$(CH_2)_3N(CH_3)_2$	95
8	$(CH_2)_3CO_2H$	55

[a] Compounds were tested against human plasma renin at pH 6[9]. The quoted figures are the result of at least two determinations performed in duplicate.

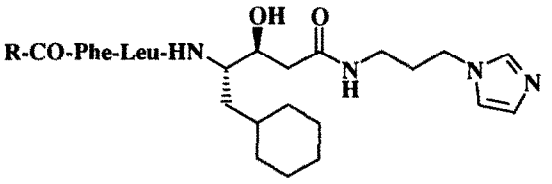
As predicted, the physicochemical properties of **3** were much improved over those of **1**. Indeed, in pH 7.4 aqueous buffer, **3** exhibits solubility of 11μg/ml, which is 100 times that of **1**; its solubility in water, as the hydrochloride, is 15mg/ml.

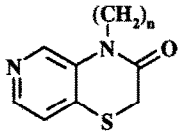
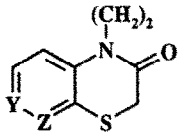
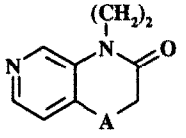
With the identification of **3** in hand, we next turned our attention to the N-terminus with the aims of removing the stereocentre inherent in the DHBT ring system and further increasing the polarity of the inhibitors, whilst retaining or increasing potency. We reasoned that the stereocentre could be removed if the rest of the inhibitor molecule was linked to the bicyclic system at a nitrogen atom, whilst increased polarity could be achieved by replacement of the benzo ring of the DHBT moiety by a heterocyclic ring. With these aims in mind, we identified the 3-oxopyrido-1,4-thiazine and related ring systems as likely replacements for our original N-terminus[12]. Compounds prepared to test this hypothesis, all of which contain the favoured 3-(1-imidazolyl)propyl amide (IPA) C-terminus, appear in Table 2.

The syntheses of these inhibitors were achieved as follows. Compound **10** was prepared, as shown in Scheme 1, *via* alkylation of the parent 3-oxopyrido[4,3-b]thiazine ring system **16**, followed by hydrolysis and elaboration using carbodiimide methodology. The 3-oxopyrido[3,4-b] analogue **12** was prepared *via* lithiation[13] of the pivaloyl derivative **18** as shown in Scheme 2. The sulphone **14** was prepared *via* oxidation of **17** with magnesium monoperoxyphthalate[12]; this reagent gave a much cleaner product than MCPBA[14]. Other members of Table 2 were prepared in a similar manner, or by literature or analogous routes[14,15].

In the 3-oxopyrido[4,3-b]thiazine series, the propanoyl derivative appears optimal (**10** vs **9** and **11**), and, perhaps curiously, the position of the pyridine nitrogen atom does not appear crucial (**10** vs **12** and **13**).

Table 2: The Effect of N-terminal Bicyclic Variation



Compound No.	R		IC_{50} (nM)[a]
9		n = 1	38
10		n = 2	4
11		n = 3	14
12		Y = N, Z = CH	4
13		Y = CH, Z = N	6
14		A = SO_2	8
15		A = O	20

[a] Compounds were tested against human plasma renin at pH 6[9]. The quoted figures are the result of at least two determinations performed in duplicate.

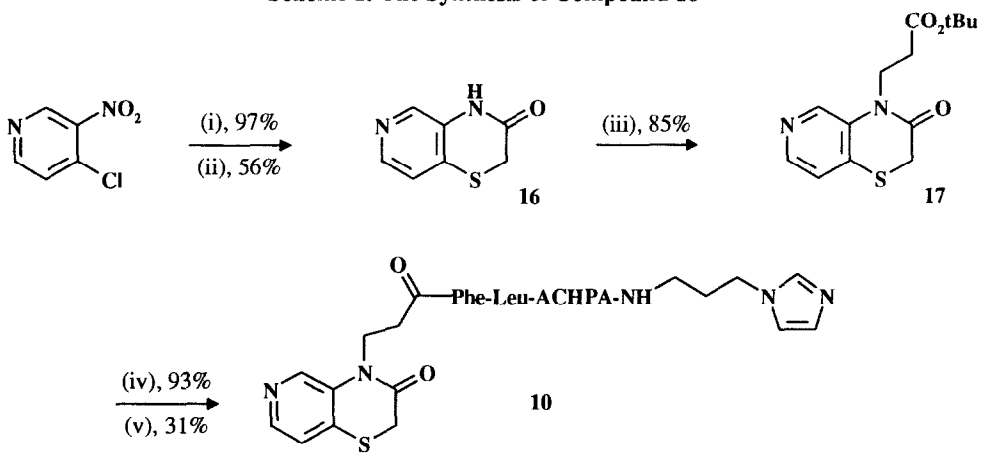
Oxidation to the sulphone causes some loss in potency (**14** vs **10**), whereas replacement of sulphur with oxygen is more detrimental (**15** vs **10**). Gratifyingly, the most potent members of this series, **10** and **12**, are 3 times more potent than **3**. Furthermore, **10** exhibits a solubility in pH 7.4 aqueous buffer of 100 µg/ml, which is 9 times that of **3**.

Compounds **10-15** were tested for their blood pressure lowering effects in the sodium depleted marmoset[12,16]. In this model, the most attractive compound was **10**, as shown in Figure 1.

Calculation of the areas under the blood pressure/time curves for the two different routes of administration show compound **10** to be similarly efficacious whether given by either the oral or intravenous route. Whilst this comparison cannot be interpreted as a direct measure of bioavailability[17], it remains remarkable for an inhibitor to show equivalent efficacy when given by either route of administration. Some

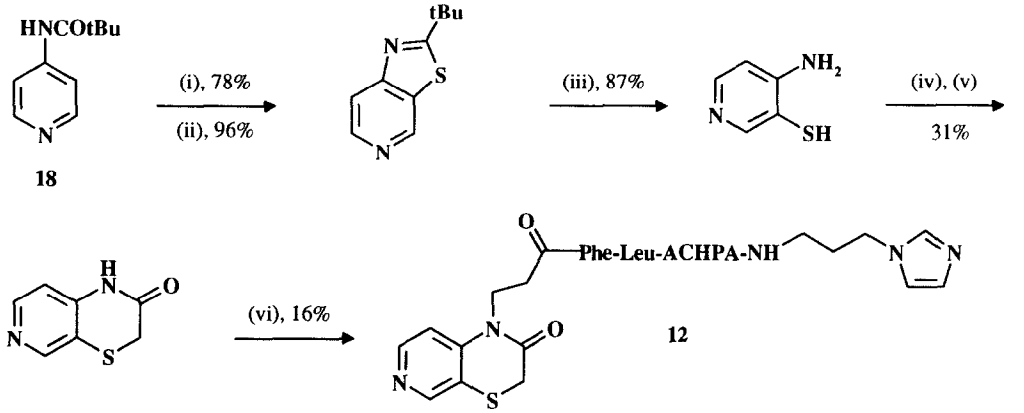
rather general criteria for obtaining respectable inhibitor bioavailability (in particular, the presence of a lipophilic P_2-site residue, and a (basic) C- or N-terminal residue with a pK_a not significantly greater than physiological pH) have recently been set by other workers in the field[4]. **10** does accord reasonably well with these criteria. However, if the characteristics could be clearly delineated, and introduced into inhibitors of even greater potency (perhaps incorporating more active scissile bond replacements[1,2]), this would greatly advance the prospects of identifying the ultimate target of this area: a potent, long acting, orally effective renin inhibitor, which would provide a realistic challenge to the ACE inhibitors available today.

Scheme 1: The Synthesis of Compound 10[18]



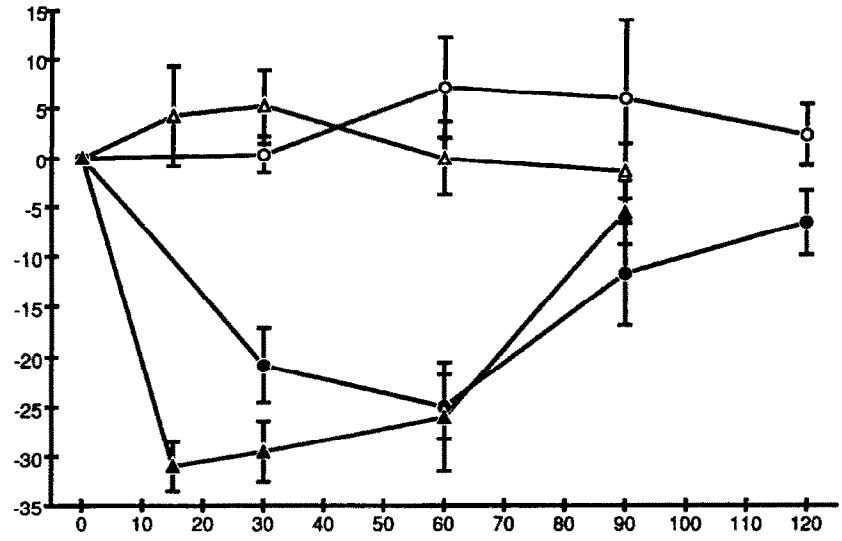
(i) $HSCH_2CO_2Et$ / K_2CO_3 / DMF (ii) Fe / AcOH (iii) t-Butyl acrylate / K_2CO_3 / DMF (iv) TFA / CH_2Cl_2
(v) H-Phe-Leu-ACHPA-IPA / DEC / HOBT / DMF

Scheme 2: The Synthesis of Compound 12[18]



(i) n-BuLi then $(iPr_2NCSS)_2$ (ii) HCl (iii) NaOH (iv) $BrCH_2CO_2Et$ / K_2CO_3 / DMF (v) Xylene, reflux
(vi) (a) t-Butyl acrylate / K_2CO_3 / DMF (b) TFA / CH_2Cl_2 (c) H-Phe-Leu-ACHPA-IPA / DEC / HOBT / DMF

Figure 1: The Effect of 10 on Mean Blood Pressure in Sodium-depleted Conscious Marmosets[16].



▲ Compound **10** 30μmol/kg **i.v.** (n=4)

Δ Vehicle 1ml/kg **i.v.** (n=4)

• Compound **10** 30μmol/kg **p.o.** (n=7)

o Vehicle 1ml/kg **p.o.** (n=5)

Values are mean and SEM, n = number of animals.

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16. Marmosets (Callithrix jacchus) of both sexes, weighing 300-400g, trained to tolerate custom-built restrainers permitting non-invasive blood pressure measurement (tailcuff, Contron W + W 8005), were fed a low-sodium diet supplemented with fruit. To stimulate renin release, the marmoset received a dose of the diuretic furosemide (5 mg/kg i.m.), given 30-45 min before the start of the experiment. This was performed following the procedure of Wood, J. M.; Criscione, L.; de Gasparo, M.; Buhlmayer, P.; Rueger, H.; Stanton, J. L.; Jupp, R. A.; Kay, J. *J. Cardiovasc. Pharmacol.* **1989**, *14*, 221-226. Compounds were administered as their hydrochloride salts in water.
17. See Rush, B. D.; Wilkinson, K. F.; Zhong, W. Z.; Closson, S. K.; Lakings, D. B.; Ruwart, M. J. *Int. J. Pharmaceut.* **1991**, *73*, 231-237, and cf. Pals, D. T.; Thaisrivongs, S.; Lawson, J. A.; Kati, W. M.; Turner, S. R.; DeGraaf, G. L.; Harris, D. W.; Johnson, G. A. *Hypertension* **1986**, *8*, 1105-1112.
18. Data for compound **10**:

NMR (d_6-DMSO) (400MHz) δ 0.70-0.85 (1H, m), 0.85 (3H, d, J=7Hz), 0.90 (3H, d, J=7Hz), 1.05-1.65 (14H, m), 1.70-1.85 (3H, m), 2.05-2.15 (2H, m), 2.35-2.45 (2H, m), 2.65-2.75 (1H, dd, J=12, 9Hz), 2.90-3.10 (3H, m), 3.55 (2H, s), 3.75-3.90 (2H, m), 3.95 (2H, t, J=7Hz), 3.90-4.10 (2H, m), 4.30 (1H, q, J=7Hz), 4.55 (1H, m), 4.85 (1H, d, J=5Hz), 6.85 (1H, s), 7.15 (1H, s), 7.10-7.25 (5H, m), 7.30 (1H, d, J=9Hz), 7.45 (1H, d, J=6Hz), 7.60 (1H, s), 7.75 (1H, t, J=9Hz), 8.10-8.20 (2H, m), 8.30 (1H, d, J=9Hz), 8.50 (1H, s). M.S. (FAB) (m/z) $[M+H]^+$ = 803 (45%).

Data for compound **12** (.2HCl):

NMR (d_6-DMSO) (400MHz) δ 0.70-1.00 (1H, m), 0.85 (3H, d, J=7Hz), 0.90 (3H, d, J=7Hz), 1.00-1.85 (15H, m), 1.90 (2H, t, J=9Hz), 2.10 (2H, m), 2.35-2.50 (2H, m), 2,70 (1H, t, J=9Hz), 2.85-3.15 (3H, m), 3.55-4.40 (10H, m), 4.20 (2H, t, J=7Hz), 4.50 (1H, m), 7.10-7.35 (5H, m), 7.45 (1H, d, J=9Hz), 7.50 (1H, d, J=7Hz), 7.70 (1H, s), 7,80 (1H, s), 7.90 (1H, m), 8.30 (1H, d, J=8Hz), 8.40 (1H, d, J=9Hz), 8.50 (1H, d, J=7Hz), 8.75 (1H, s), 9.15 (1H, s). M.S. (FAB) (m/z) $[M+H]^+$ = 803 (25%).

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