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A new class of prolylcarboxypeptidase inhibitors, Part 2: The aminocyclopentanes

Thomas H. Graham [a,*], Wensheng Liu [a], Andreas Verras [a], Mikhail Reibarkh [a], Kelly Bleasby [b], Urmi R. Bhatt [c], Qing Chen [b], Margarita Garcia-Calvo [c], Wayne M. Geissler [c], Judith N. Gorski [d], Huaibing He [b], Michael E. Lassman [c], JeanMarie Lisnock [c], Xiaohua Li [b], Zhu Shen [c], Xinchun Tong [b], Elaine C. Tung [b], Judyann Wiltsie [c], Dan Xie [c], Suoyu Xu [b], Jianying Xiao [d], Jeffrey J. Hale [a], Shirly Pinto [c], Dong-Ming Shen [a]

[a] Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA
[b] Department of Drug Metabolism, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA
[c] Department of Metabolic Disorders, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA
[d] Department of In Vivo Pharmacology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065-0900, USA

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ABSTRACT

A series of potent inhibitors of prolylcarboxypeptidase (PrCP) was developed by modifying a lead structure that was discovered by high-throughput screening. The *tert*-butyl pyrrolidine was replaced by an aminocyclopentane to reduce the metabolic liabilities of the original lead. The compounds demonstrated sub-nanomolar in vitro IC_{50} values, minimal activity shifts in pure plasma and improved pharmacokinetics. Complete ex vivo plasma target engagement was achieved with low brain exposure at the 20 h time point following p.o. dosing in a mouse. The results indicate that the aminocyclopentanes are useful tools for studying the therapeutic potential of peripheral (non-CNS) PrCP inhibition.

Prolylcarboxypeptidase (PrCP) is a widely distributed serine protease that cleaves the amide bond between a C-terminal amino acid and a proline residue (i.e., peptide-Pro-Xxx-OH).[1] Substrates of PrCP include angiotensins II and III,[2] plasma prekallikrein[3] and α-melanocyte stimulating hormone (α-MSH).[4] Many hypotheses have been proposed for the biological role of PrCP including cardiovascular effects,[5] inflammation induction,[6] and metabolism regulation.[7] The presence of PrCP in both the CNS and peripheral (non-CNS) system complicates the evaluation of small molecule inhibitors. As a consequence, the identification of structurally diverse inhibitors of PrCP that act in the peripheral system would further define the biological role and therapeutic potential of PrCP.

The search for novel small molecule inhibitors of PrCP has resulted in the discovery of several classes of potent PrCP inhibitors.[8] The preceding communication described a new class of potent PrCP inhibitors that was discovered by high-throughput screening of the Merck sample collection. Modifications of the initial lead structure **1** focused on improving the pharmacokinetics (PK) and understanding the pharmacodynamics (PD) (Fig. 1). Specifically, the aryl triazole portion was modified to address the suspected metabolism of the tolyl methyl group in **1** and a pyrazole was found to be an adequate replacement for the triazole. The modifications resulted in structures, such as **2**, that demonstrated enhanced in vitro inhibition of PrCP, improved pharmacokinetics and confirmed ex vivo target engagement.

Enhancement in the stability of **2**, as compared to **1**, toward liver microsomes was correlated with an improved mouse pharmacokinetic profile. An ex vivo target engagement assay indicated 98% inhibition of PrCP in mouse plasma at 20 h post-dose when **2** was dosed orally at 30 mpk but the brain to plasma ratio was 1.35 ± 0.33. However, an additional compound indicated that structural variations in the series can influence the brain and plasma drug levels and would allow for targeting of the peripheral system.

Additional studies to improve the pharmacokinetic and pharmacodynamic profiles focused on modifying the *tert*-butyl pyrrolidine. The preceding communication revealed that compounds lacking the *tert*-butyl group generally showed improved stability in the presence of liver microsomes. In addition, metabolite identification using LC/MS/MS techniques revealed the potential for metabolism

* Corresponding author. Tel.: +1 732 594 2918; fax: +1 732 594 3007.
E-mail address: thomas.graham@merck.com (T.H. Graham).

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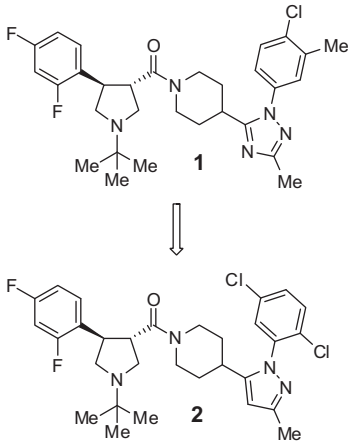


Figure 1. The lead discovered with high-throughput screening (**1**) and a modified structure (**2**); h = human, m = mouse.

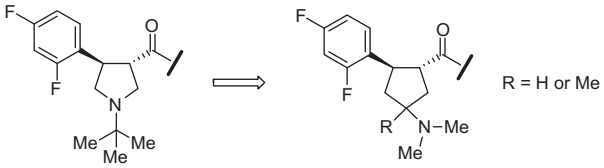
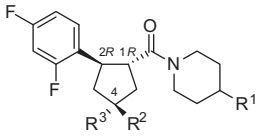


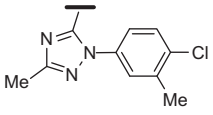
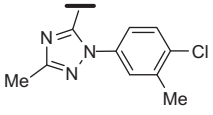
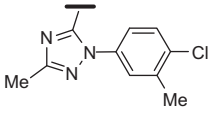
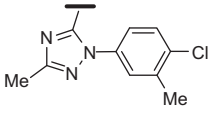
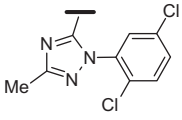
Figure 2. Structural modification of the *tert*-butyl pyrrolidine lead structure affords the aminocyclopentanes.

at the *tert*-butyl as well as the pyrrolidine ring.[9] Therefore, efforts were focused on reducing the potential metabolic instabilities of the *tert*-butyl pyrrolidine core by structural modifications.

A possible modification to the *tert*-butyl pyrrolidine involved moving the nitrogen to an exocyclic position, affording an aminocyclopentane (Fig. 2). A series of aminocyclopentanes were prepared and profiled in a manner similar to previously described methods (Table 1).[10,11] Compound **3**, a close analog of **1**, demonstrated a 3- to 5-fold decrease in the in vitro inhibition for mouse and human PrCP while the epimer *epi*-**3** was less potent. In addition, the large shift in potency with the mouse enzyme was an undesirable property for testing in the in vivo mouse models. However, the improved pharmacokinetic profile for **3** relative to **1** suggested that replacing the pyrrolidine ring with the aminocyclopentane was a promising strategy. Compound **4a**, a structural isomer of **1**, incorporated a methyl group on the cyclopentane to block a potential site of metabolism and was comparable in inhibitory activity to **1**. A decreased shift for the inhibition of human versus mouse PrCP was observed with **4a**. The pharmacokinetic profile of **4a**, while improved relative to **1**, was less than satisfactory. Replacement of the potentially metabolically labile dimethylamine with a simple amino group afforded **4b**, which displayed substantially decreased potency relative to **4a**. The increase in stability of **4b** toward liver microsomes suggested the dimethylaminocyclopentane in **4a** was detrimental to the metabolic stability of the molecule. A similar trend was observed with **5a**–**b** and **6a**–**b** where the aminocyclopentane was less potent but had improved iv pharmacokinetic parameters relative to the dimethylaminocyclopentane. Notably, there was only a 2- to 4-fold decrease in in vitro potency for **5b** relative to **5a**. Similar to *epi*-**3**

Table 1
Data for the aminocyclopentanes[a]



Cmpd	R^1	R^2	R^3	PrCP IC_{50}[b] (nM)		PPB[c]		Microsomal stability[d]		Mouse pharmacokinetics[e]				
				h	m	h	m	h	m	Cl (mL/min/kg)	V_d (L/kg)	$t_{½}$ (h)	$AUCN_{po}$ (M·h·kg/mg)	%F
3		$-NMe_2$	–H	6.4	41	22	32	–	–	37	9.2	2.9	0.55	63
epi-**3**		–H	$-NMe_2$	12.5	65	–	–	–	–	–	–	–	–	–
4a		$-NMe_2$	–Me	1.1	2.5	5	22	27	47	62	8	1.9	0.12	24
4b		$-NH_2$	–Me	49.9	85.1	7.6	10.2	78	72	–	–	–	–	–
5a		$-NMe_2$	–Me	1.6	2.0	33	14	18	6	135	14.7	1.3	0.03	12

(*continued on next page*)