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Design and Synthesis of Prolylcarboxypeptidase (PrCP) Inhibitors To Validate PrCP As A Potential Target for Obesity

Changyou Zhou,[†,∥] Margareta Garcia-Calvo,[‡] Shirly Pinto,[‡] Matthew Lombardo,[†] Zhe Feng,[†] Kate Bender,[†] KellyAnn D. Pryor,[‡] Urmi R. Bhatt,[‡] Renee M. Chabin,[‡] Wayne M. Geissler,[‡] Zhu Shen,[‡] Xinchun Tong,[§] Zhoupeng Zhang,[§] Kenny K. Wong,[‡] Ranabir Sinha Roy,[‡] Kevin T. Chapman,[†] Lihu Yang,[†] and Yusheng Xiong*[,†]

[†]*Department of Medicinal Chemistry,* [‡]*Department of Metabolic Disease, and* [§]*Department of Drug Metabolism, Merck Research Laboratories, Rahway, New Jersey 07065.* [∥] *Current address: BioDuro, A PPD Company, Building E, no. 29, Life Science Park Road, Changping District, Beijing 102206, P. R. China*

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Prolylcarboxypeptidase (PrCP) is a serine protease that may have a role in metabolism regulation. A class of reversible, potent, and selective PrCP inhibitors was developed starting from a mechanism based design for inhibiting this serine protease. Compound **8o** inhibits human and mouse PrCP at IC_{50} values of 1 and 2 nM and is not active ($IC_{50} > 25\ \mu M$) against a panel of closely related proteases. It has lower serum binding than its close analogues and is bioavailable in mouse. Subchronic dosing of **8o** in $PrCP^{-/-}$ and WT mice at 100 mg/kg for 5 days resulted in a 5% reduction in body weight in WT mice and a 1% reduction in PrCP KO mice.

Introduction

Prolycarboxypeptidase (PrCP,[a] EC 3.4.16.2) was identified 40 years ago[1] from kidney extract and cloned[2] in 1993 from a human kidney library. It is a 58 kDa serine protease which specifically cleaves C-terminal amino acid residue connected to proline. Substrate specificity studies[1b,1e] indicated that the enzyme has preferences for Xxx-Pro-Phe-OH and Xxx-Pro-Val-OH. The enzyme is found in lysosomes[1e] and extracellularly as membrane-bound or soluble form in plasma and urine.[3] The PRCP gene is widely expressed in many tissues such as lung, liver, pancreas, kidney, adrenal gland, and white fat as well as in brain tissues.

In vitro, PrCP was found to cleave angiotensin II and III,[1b,c] and plasma prekallikrein[4] which in turn activates bradykinin. More recently, PrCP was also found to metabolize α-melanocyte-stimulating hormone (α-MSH).[5] Because PrCP can degrade many bioactive peptides, it is postulated to have important biological functions including a cardiovascular effect through its possible role in the RAS pathway and body weight regulation[5−7] via its metabolism of α-MSH or other unknown mechanisms.[8] PrCP is also implicated in inflammation because of its upregulation in LPS stimulated endothelium cells and its role in bradykinin activation.[9] However, there are no in vivo studies to confirm PrCP's biological functions, partly due to the lack of suitable small molecule tools.

In the study of PrCP's function in regulating α-MSH, the authors reported[5] that the PrCP gene trap mice $PrCP^{gt/gt}$ (PrCP gene expression was suppressed) demonstrated reduced body weight and food intake as well as fat mass reduction vs the control WT mice. Our in-house effort in phenotyping of PrCP gene knock out mice confirmed these findings, and additionally we did not observe any blood pressure change between the KO and WT groups. The encouraging PrCP KO phenotyping results prompted us to look for a small molecule PrCP inhibitor to validate PrCP as a potential target for obesity treatment.

Synthesis

The synthesis of compounds in this study is illustrated in Scheme 1. The substituted pyrrolidinylimidazoles were made from condensation of *N*-Boc protected proline with the corresponding α-halo ketones in a two-step reaction. The final compounds were then obtained from coupling of the deprotected pyrrolidinylimidazole with appropriate carboxylic acids, with or without subsequent transformations pertinent to each individual compounds, see reaction 1. For the benzimidazole type of compounds in this study, *N*-Boc-proline was first coupled with the corresponding diamino arenes. The resulting amide is then cyclized in acetic acid to the corresponding pyrrolidinylbenzimidazole. Again, most of the compounds were obtained from coupling of the deprotected pyrrolidinylbenzimidazoles, with or without subsequent transformations.

The asymmetric synthesis of (*S*,*S*)1-amino-2-methyl-3-arylpropionic acid (**12**), an important fragment used as acid in reactions 1 and 2, is an improvement over Burks' Rh catalyzed hydrogenation.[10] In that study, the enantiomer of compound **12** (compound **5b** in ref 10) was obtained with 80% ee under 90 psi of hydrogen in methanol with (*R*,*R*)-Me-BPE as the ligand. We sought to improve the enantiomeric excess by a chiral ligand screening with compound **11**, and found that

*To whom correspondence should be addressed. Phone: 1-732-594-7507. E-mail: yusheng_xiong@merck.com. Address: Merck Research Laboratories, Mail Code RY50G-147, P.O. Box 2000, Rahway, New Jersey 07065.

[a] Abbreviations: PrCP, prolylcarboxypeptidase; RAS, renin angiotensin aldosterone pathway; Mca, (7-methoxycoumarin-4-yl)acetyl, Dnp, 2,4-dinitrophenyl; MSA, mouse serum albumin; Cl, clearance; Vd, volume of distribution; AUC, area under the curve; *F*%, bioavailability; AIB, α-amino isobutyrate; QPP, quiescent cell proline dipeptidase; FAP, fibroblast activation protein; PEP, prolyl endopeptidase; ACE2, angiotensin converting enzyme 2; DPP4, dipeptidyl peptidase 4.

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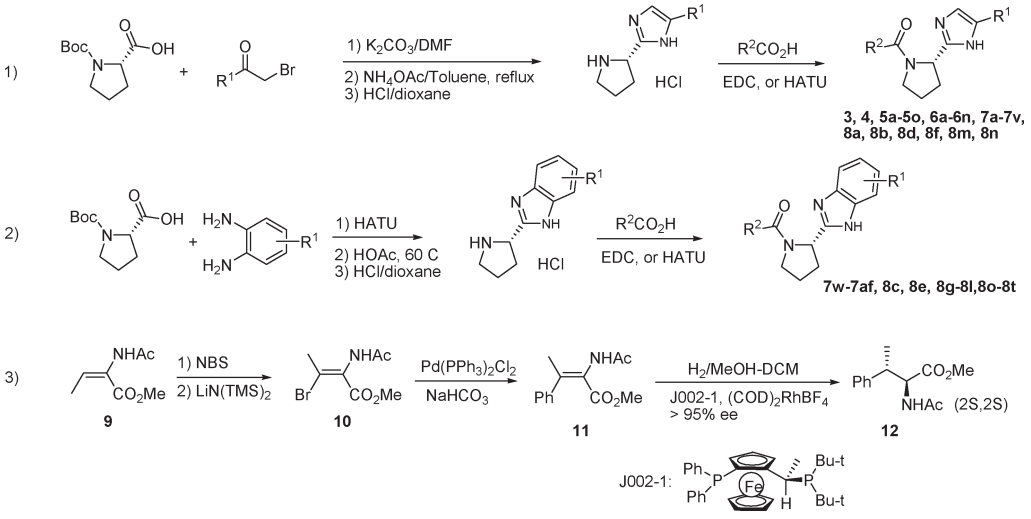
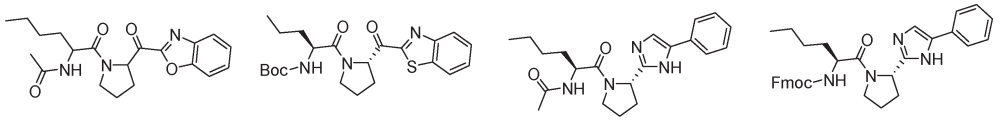
Scheme 1



Scheme 2



JP002-1 was the best ligand for the asymmetric hydrogenation of **11** to **12** (> 95% ee). The detailed procedure was illustrated in the synthesis of compound **8o**. The highly regioselective synthesis of bromoenamide **10** from **9**[11] was accomplished by the use of strong base $LiN(TMS)_2$ instead of triethylamine to afford the (*E*) isomer as the dominant product.[12] This important precursor also afforded easy access to many analogues of compound **8o**.

PrCP Inhibitor Design and Potency Optimization

An enzyme activity assay was developed using recombinant PrCP and a FRET peptide substrate Mca-Ala-Pro-Lys(Dnp)-OH to screen compounds for PrCP inhibition activity. The assay was run with substrate concentration of 25 μM (at its K_m value) and by continuously monitoring (up to 30 min) the appearance of fluorescence product upon PrCP cleavage in the absence and in the presence of different concentrations of inhibitors.

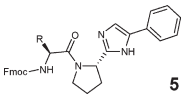
It is well-known that compounds with an electrophilic carbonyl attached to heterocycles[13] are good source of inhibitors for serine protease/hydrolases. For prolyl endopeptidase (PEP), there has been several inhibitor designs like *Z*-Pro-prolinal and other electrophilic carbonyl derivatives.[14] PrCP is a serine protease that has a strong preference for cleaving Peptide-Pro-Xxx-OH. Therefore, we searched the Merck sample collection for compounds with an *N*-acylpyrollidine attached to a ketone. Compound **1**,[14c,15] which was a member of a library designed to target serine proteases in general, showed some moderate potency against PrCP and provided us with a critical starting point for lead identification, Scheme 2. With a few modifications, we obtained compound **2**, which has (*S*,*S*) configuration and showed increased potency against PrCP (IC_{50} 0.3 μM.). In the absence of structural biology information, it is our working hypothesis that compounds **1** and **2** bind to PrCP similar to substrate where the pyrrolidine ring sits in the S1 pocket and carbonyl group interact with the active serine hydroxyl group of the catalytic triad. The following discussion uses the nomenclature system of Schechter[16] regarding the substrate-active site interaction.

The electrophilic ketone in compounds **1** and **2** are important for their activity because it forms a covalent bond with the catalytic serine hydroxyl group. For the same reason, it can sometimes be difficult to make these covalent inhibitors selective against other serine proteases. The electrophilic ketone may also become a liability when it comes to drug metabolism and bioavailability.[17] In addition, the complexity in the synthesis of a ketone group α to the amide is an obstacle to rapid and thorough optimization of the lead series. Therefore we decided to explore ketone-free noncovalent inhibitors early in the program. Instead of using an electrophilic ketone to replace the scissile amide group in the substrate mimics, we tested a variety of heterocycles, as an amide isostere, attached to the pyrrolidine in scaffold **2**. A variety of changes were made, but only compounds **3** and **4** showed moderate activity at 5 μM (Scheme 1). Thus compound **4** became the lead structure for our medicinal chemistry effort to deliver a compound suitable for pharmacological study of the biological functions of PrCP.

We took advantage of the highly modular feature of compound **4** and started optimization with a library of analogues to probe the purported P2 binding pocket (S2 site). A few representative compounds are listed in Table 1 to highlight the characteristic of the P2 pocket. This pocket clearly prefers neutral and nonpolar groups. Compounds with basic side chains, **5f** and **5g**, lost activity completely, and those with acidic groups such as **5b** and **5c** were very weak. The simple alkyls faired much better in comparison, and *para*-biphenyl methyl and 3-phenylpropyl groups stand out as the best fit for this pocket.

We then proceeded to replace the bulky Fmoc group present in compound **5** while we kept *para*-biphenylmethyl

Table 1. h-PrCP Inhibition Data for Compounds **5**



5

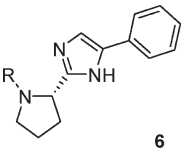
R	compd	activity[a]
$HOCH_2$	**5a**	24%
$HOOCCH_2$	**5b**	6%
$HOOCCH_2CH_2$	**5c**	6%
$CH_3SO_2CH_2CH_2$	**5d**	14%
$CH_3SCH_2CH_2$	**5e**	4.37
$H_2N(CH_2)_3$	**5f**	0%
$H_2N(CH_2)_4$	**5g**	2%
CH_3CH_2	**5h**	25%
$BnOCH_2$	**5i**	0.85
t-$BuOOC(CH_2)_5$	**5j**	6.20
Z-$NH(CH_2)_4$	**5k**	74%
Alloc-$NH(CH_2)_4$	**5l**	0.80
$Ph(CH_2)_3$	**5m**	0.24
$CH_3(CH_2)_5$	**5n**	0.57
p-biphenyl-CH_2	**5o**	0.09

[a] Activity is IC_{50}s in μM, or % inhibition at 5 μM.

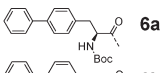
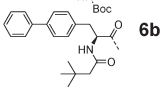
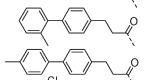
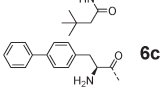
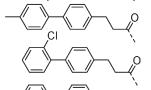
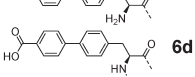
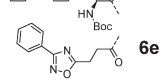
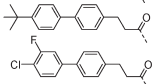
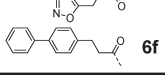
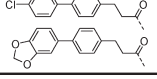
or a phenylpropyl group constant at the P2 position. The SAR showed the same general trends with the two groups, and only compounds with the *para*-biphenylmethyl group are presented in Table 2. The bulky Fmoc group can be replaced with Boc (**6a**) or completely removed (**6f**) with only 2-fold loss in potency. Many other polar or nonpolar groups in addition to the neopentyl group in **6b** were tolerated at the purported P3 position (S3 site). Attempts to install acidic or basic groups in the terminal phenyl ring, i.e., compounds **6d**, **6g**, led to loss of activity, similar to what was found in the compound **5** series. Replacement of the middle phenyl ring with oxadiazole, as in compound **6e**, was not tolerated well. Further refinement in the biphenyl area revealed some preference for meta and para substitution at the terminal phenyl ring. Compound **6k** featuring a *para*-Cl, and compound **6n** with a piperonyl group showed 5−7-fold increase in potency vs the unsubstituted compound **6f**.

Optimization around the imidazole was first carried out with the biphenylpropionic amide in the left side as shown in Table 3. The SAR around the imidazole is sensitive to the substitution pattern and nature of the substituents. The unsubstituted imidazole compound **7a** is not very active, but small substitution groups like trifluoroethyl (**7c**, IC_{50} 476 nM) and particularly *t*-butyl (**7d**, IC_{50} 21 nM) increase the activity significantly. It seems that an electron withdrawing group lowers the activity, compound **7e** is completely inactive, and compound **7j** is at least 10-fold less active than **7l**. With *t*-butyl as an exception, substitution with a flat sp^2 carbon is favored vs an sp^3 carbon, and compound **7f** with an isopropenyl group (IC_{50} 92 nM) is 8-fold more active than **7g** (IC_{50} 710 nM). We did not extensively explore aliphatic substituted imidazole analogues because of potential metabolic stability issues. Rather, we focused our efforts on aryl or heteroaryl substitutions. Substitution at the ortho position of the phenyl ring disrupts the binding to PrCP, as illustrated by compound **7n** (*ortho*-$OCHF_2$, 35%@5 μM). Meta substitution is generally favored on the phenyl ring, as shown in **7m** (*meta*-Me, IC_{50} 24 nM) and **7p** (*meta*-Cl, IC_{50}, 32 nM). The phenyl imidazole group in **6f** can also be replaced with benzimidazoles. Here again the substitution pattern

Table 2. h-PrCP Inhibition Data for Compounds **6**[a]



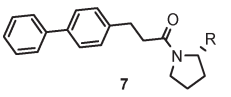
6

R	Compound	Activity	R	Compound	Activity
	**6a**	0.068		**6g**	4.09
	**6b**	0.399		**6h**	0.352
	**6c**	0.518		**6i**	0.056
	**6d**	0.849		**6j**	0.122
	**6e**	14%		**6k**	0.049
	**6f**	0.223		**6l**	0.072
				6m	0.073
				6n	0.030

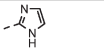
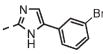
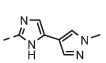
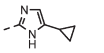
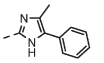
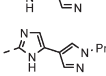
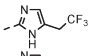
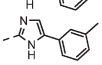
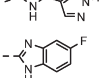
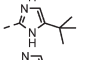
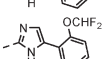
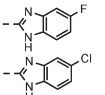
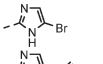
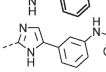
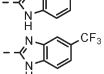
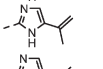
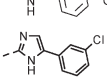
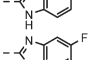
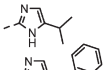
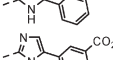
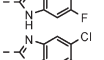
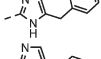
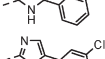
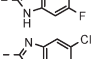
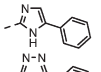
[a] Activity is IC_{50} in μM, or % inhibition at 5 μM.

around the benzimidazole is quite sensitive: small groups at 5- and 6-positions are favored over larger groups, and 4- and 7-substitutions are not tolerated. Benzimidazole derivative **7ab** showed a remarkable IC_{50} of 78 nM. Mindful of the high lipophilicity of our inhibitors series **6** and **7**, we made an effort to install polar groups wherever they were tolerated. Polar groups on the phenyl ring such as **7o** (3-methylsulfonamido) and **7q** (3-carboxylic acid) lowered activity, and substitution on the imidazole with heterocycles such **7u** and **7v** with pyrazole was not productive either. Even the sterically least perturbed changes by installing nitrogen into the benzimidazole (**7ad**) failed to yield any active PrCP inhibitors. The sensitivity to polarity can partly explain why compound **7i**, with a triazole replacing imidazole, is not active.

Table 3. h-PrCP Inhibition Data for Compounds **7**[a]



7

R	Compound	Activity	R	Compound	Activity	R	Compound	Activity
	**7a**	25%		**7k**	27		**7u**	**44%**
	**7b**	38%		**7l**	338		**7v**	**7%**
	**7c**	476		**7m**	32		**7w**	280
	**7d**	21		**7n**	35%		**7x**	221
	**7e**	0%		**7o**	16%		**7y**	38%
	**7f**	92		**7p**	23		**7z**	163
	**7g**	710		**7q**	827		**7aa**	184
	**7h**	13%		**7r**	320		**7ab**	78
	**6f**	223		**7s**	74		**7ac**	146
	7i	7%		**7t**	274		**7ad**	7%
	7j	20%						

[a] Activity is IC_{50} in nM, or % inhibition at 5 μM.

The improvement of potency of this inhibitor series was achieved fairly quickly through parallel synthesis, aided by high throughput purification, and we found that the SAR in both left and right side of the molecules are somewhat independent of each other: combining favorable groups from Tables 2 and 3 led to potent inhibitors.

Optimizing PK Properties and Reducing Protein Binding

While the in vitro potency of our PrCP inhibitors reached IC_{50} of low nM level, most of them do not have adequate metabolic stability and oral bioavailability required for an in vivo study.

Compounds **5o** with a bulky Fmoc, and **6c** with a bare amino group, do not have adequate oral drug levels in mice, as indicated by the low oral AUC values and low *F*%. Compound **8a**, with no α substitution, also suffered from high metabolism, as indicated by the high clearance. Amide hydrolysis does not seem to be the main clearance mechanism because compound **8b**, which is unlikely a substrate for amide hydrolase with *gem*-dimethyl groups at the α position, still showed high plasma clearance of 92 mL/min/kg. In an in vitro metabolite identification study with compound **7k**, no amide hydrolysis products were detected. A clear trend we observed is that benzimidazole tends to have better oral bioavailability vs the corresponding arylimidazole. Arylimidazoles, even the heavily fortified compound **8d**, have low bioavailability.

The conventional medicinal chemistry approaches such as replacing H with halogen, replacing CH_2 with oxygen, and preempting the oxidation with a hydroxyl substitution, showed variable improvement when used separately, and in combination. Fusing a cyclopropyl ring to the pyrrolidine ring reduced PrCP activity and did not improve the metabolic stability (Table 4). We were surprised to find that the hydroxyl group in the pyrrolidine ring (compound **8f**) enhanced PrCP inhibition, but **8f** does not show any improvement in metabolic stability.

A significant improvement in PK property was realized when we reintroduced the α amino group back as shown in compound **8g**, in this case, the amine was capped with an α amino isobutyrate (AIB). The selection of AIB draws from our experience[18] that this group can sometime confer unique improvement in oral bioavailability. Compound **8g** showed reasonable metabolic stability (low plasma clearance) and good oral drug level (AUC of 1 μM·h·kg/mg) and 31% bioavailability. Compounds **8h** and **8i**, which feature the benzimidazole at the right-hand side and arylpropionate at the left side, all showed significant improvements in bioavailability. The effect of AIB in this structural scaffold is quite remarkable because minor modifications of the AIB group resulted in loss of bioavailability and even metabolic stability in cases. The terminal amine is critical. Replacing the amine with a hydroxyl group, as in compounds **8j**, **8r**, and **8s**, invariably led to loss of bioavailability (low oral AUC and *F*%) and reduced metabolic stability (high clearance) to some extent. Insertion of a CH_2 group in the AIB part, as in compound **8k** and **8l**, resulted in loss of bioavailability even though the metabolic stability is not affected. We also noticed that the effect of AIB is limited; compounds with *t*-butylimidazole **8m** and with polar groups in the biaryl portion (**8t**) did not benefit from the presence of AIB group in terms of DMPK properties.

Another desirable property for molecules useful in animal studies is low plasma protein binding.[19] We relied on a serum shift assay,[20] which measures PrCP inhibition in the presence of mouse serum albumin (MSA). Because of the high protein binding of the assay substrate Mca-Ala-Pro-Lys(Dnp)-OH, the shift assay is limited to 1% MSA content (mouse blood contains about 4% MSA). This serum shift assay served well to rank our inhibitors in terms of protein binding. Table 4 also lists the serum shifted IC_{50} values for some selected compounds.

As the calculated LogD (at pH 7.4) indicated in Table 4, many of the potent inhibitors are highly lipophilic. One of the consequences of high LogD is that compounds tend to have high protein binding, resulting in low unbound drug level. The serum shift in IC_{50} is large for many of the high LogD compounds in Table 4. The relatively more polar compounds, such as **8m**, **8n**, and **8t**, have lower LogD values and small or no shift in the serum shift assay. Unfortunately, the bioavailability of these low LogD compounds still needs improvement.

Plasma protein binding to small molecules can also be structurally specific,[21] and compounds with similar LogD can have different levels of plasma protein binding. We have noticed a clear trend in compounds with the β-methyl group, such as **8o**, **8p**, and **8q**, **8r**, to have a relatively low serum shift in IC_{50} vs compounds without β-methyl. The presence of β-methyl group[22] not only reduced the serum shift but also increased potency against PrCP, particularly against mouse PrCP. Compound **8q** is the most potent among the four enantiomers of β-methyl phenylalanine derivatives, but all four enantiomers showed relatively low serum shift (data not shown). The β,β-dimethyl phenylalanine compound **8u** also showed a relatively low serum shift. This dramatic effect of a methyl group supports the hypothesis that the presence of a methyl group disrupts the specific binding to mouse serum albumin. Whether this effect holds true in human serum remains to be tested, but for use as a tool in animal model studies, this class of inhibitor is adequate.

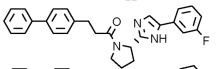
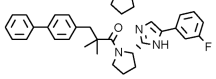
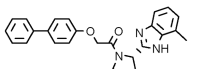
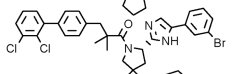
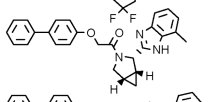
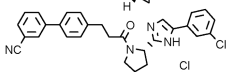
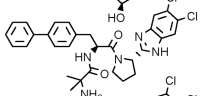
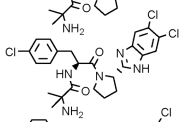
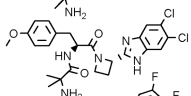
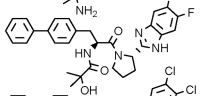
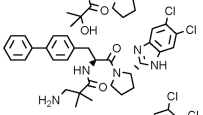
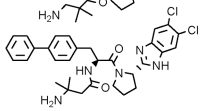
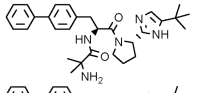
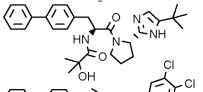
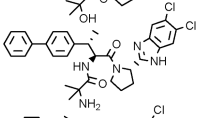
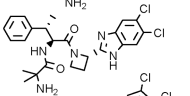
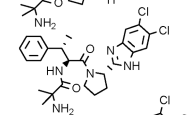
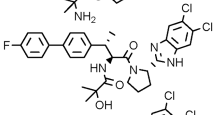
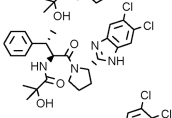
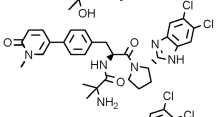
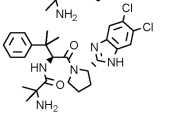
In Vivo Study in DIO Mice

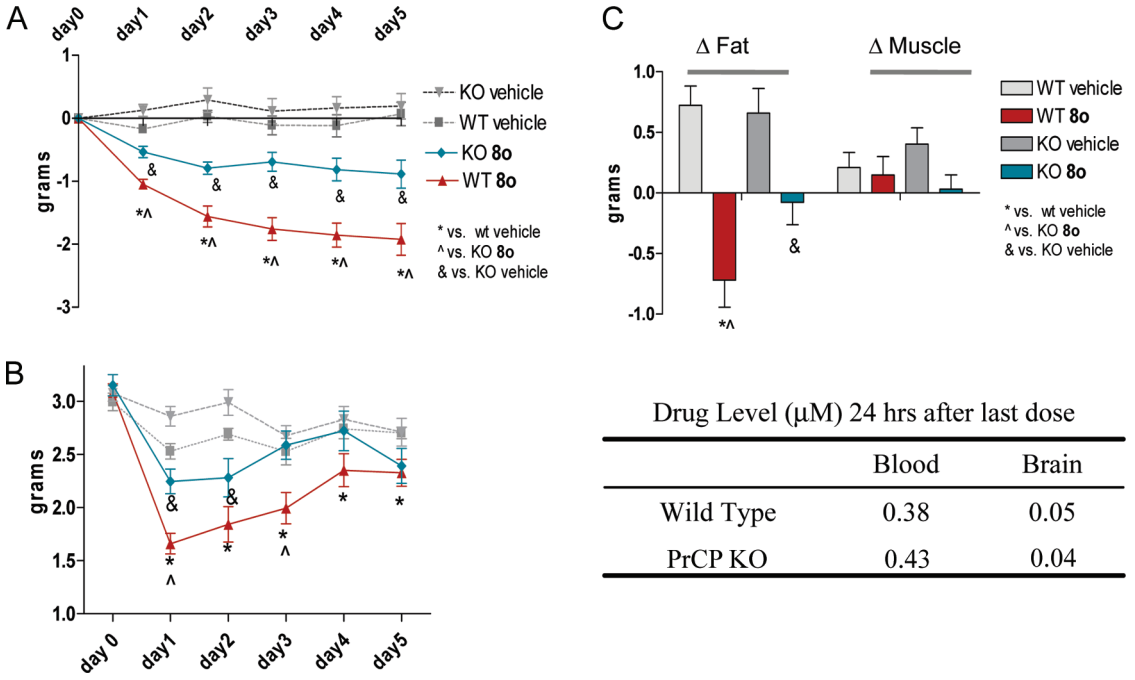
Among the closely homologous serine proteases[23] to PrCP are QPP[24], FAP,[25] PEP,[14] ACE2,[26] and DPP4,[27] DPP8,[28] DPP9.[29] Selected compounds were profiled in the counter screens[30] against this panel of proteases. Compound **8o** showed high selectivity (IC_{50} all >25 μM) against all of the counter screen proteases, confirming that our PrCP inhibitor series without a serine trap is highly specific for PrCP. Further counter screen in a large panel of enzymes, ion channels, and receptors did not uncover any alarming activities. Compound **8o** does not have big species difference between human and mouse, its IC_{50} for m-PrCP is 1.8 nM, slightly less potent compared to that of h-PrCP.

To investigate the effect of PrCP inhibition in vivo, we carried out a subchronic study with compound **8o** in two groups of mice, $PrCP^{-/-}$ (KO) and $PrCP^{+/+}$ (WT), on high fat diet. Figure 1 shows the result of this study. The mice were of similar body weights (40−45 g) at the beginning of the study. Upon daily oral dosing of compound **8o** at 100 mg/kg for 5 days, the WT mice in the treatment group showed a 4.9% body weight loss vs its vehicle control group, while the PrCP KO mice only showed a 1.1% body weight loss (Figure 1a). The 1.1% weight difference in KO groups (treatment vs vehicle) is difficult to explain. It could be due to some unknown off-target activity of compound **8o**, which should occur to a similar degree in the WT group. Other than body weight reduction, compound **8o** did not cause any apparent adverse effect in mice.

Figure 1b shows that food intake in the WT mice was also reduced (vs vehicle control) more than the KO group.

Table 4. PrCP Inhibition Activity and Pharmacokinetic Data

Compound	Structure	h-PrCP IC_{50} (nM)		m-PrCP IC_{50} (nM)		cLogD	Cl	Vd	$T_1/_2$	AUC	F%
		no MSA	1% MSA	no MSA	1% MSA		ml/min/kg	L/kg	hr	uM.hr.kg/mg	
5o	--	90				9.5	26	1.7	2.4	0.012	1%
6c	--	410				4.6	43	5.5	2.5	0.032	4%
8a		158	3314	871		5.8	75	4.6	0.8	0.025	5%
8b		79	1460			6.5	92	8.8	2.3	0.021	5%
7w	--	281				5.2	88	2.5	0.4	0.159	35%
8c		432				4.6	54	4.0	3.4	0.390	49%
8d		22	20000			8.2	15	4.0	9.6	0.082	5%
8e		308	1970			4.4	41	2.3	0.6	0.197	20%
8f		24				5.2	82	2.2	0.6	0.003	1%
8g		4	356	15	789	4.7	12	2.7	2.8	1.010	31%
8h		54	114			3.5	7	2.6	3.0	4.560	100%
8i		9	829	251	3320	2.6	25	2.2	1.4	0.451	33%
8j		21				5.4	39	1.5	0.5	0.131	9%
8k		3	42			3.9	23	7.9	4.3	0	0%
8l		5	71			4.0	14	6.8	5.2	0	0%
8m		7	2	41	17	3.2	11	11	15	0.170	6%
8n		4	4	16	15	4.5	117	2.9	0.4	0	0%
8o		1	1	2	8	5.1	23	4.4	2.3	0.180	13%
8p		37	67			3.0					
8q		5	10	32	60	3.3	17	1.5	1.5	0.790	41%
8r		2	2			6.3	23	1.2	0.8	0.150	12%
8s		21	59	29	98	4.6	72	2.4	0.5	0.020	4%
8t		7	5			3.1	70	14	2.6	0	0%
8u		12	39								



Drug Level (μM) 24 hrs after last dose

	Blood	Brain
Wild Type	0.38	0.05
PrCP KO	0.43	0.04

Figure 1. Four groups ($N = 8$ each) of mice were dosed with compound **8o** orally once a day for 5 days. The body weight changes (a) and food intake (b) were recorded. Parts (a) and (b) share the same set of annotation. At the end of the study (24 h after the last dose), the NMR imaging (c) of the animal were taken before drug level analysis. P values for data points marked with *,^, and & are all < 0.05.

Furthermore, NMR body imaging (Figure 1c) showed that in the WT group, the body weight loss is largely due to fat mass reduction, in contrast to the the PrCP KO group where body weight reduction has a significant portion of muscle weight loss. The drug levels 24 h post the last dose were about the same in both WT and KO groups in the range of 0.3−0.4 μM in blood. Given that compound **8o** inhibits mouse PrCP with IC_{50} of 8 nM (with 1% MSA), it is reasonable to assume that at 300−400 nM trough level there was significant inhibition of PrCP throughout the experiment. Therefore the bulk of the weight loss in WT group was likely a result of PrCP inhibition. Compound **8o** did not have brain exposure in mice. The efficacy observed is likely the result of inhibition of peripheral PrCP. The full potential of PrCP inhibition in CNS remains to be explored.

Conclusion

We have developed a class of potent PrCP inhibitors starting from rational design based on known serine protease scaffold. At the beginning, compounds **1** and **2** resemble PrCP substrates, and the electrophilic ketone group may function as the serine trap. In the end, the aryl imidazole and benzimidazoles diverged far away from the mechanism based covalent inhibitors. Consequently, this series of inhibitors is readily progressed to reversible and PrCP specific inhibitors. Pharmacokinetic property was also improved to the level suitable for animal studies. Serendipitously, we found that the β-methyl group in the biphenylpropionic acid increased potency against both h- and m-PrCP and significantly reduced mouse serum albumin binding. Compound **8o** possessed a relatively better profile overall in this class and showed statistically significant mechanism based body weight lowering in DIO mice, thus providing some indication that PrCP is a therapeutic target for obesity.

Further validation of PrCP as an obesity target would require the correlation of efficacy to the level of target engagement, which is lacking at the moment. Because the current inhibitors are not brain penetrating, the potential of inhibiting PrCP in CNS remains to be explored. But the results we have obtained are encouraging, and the tool compounds we discovered will help in elucidating biological functions of PrCP in the future.

Experimental Section

General Methods. All commercial chemicals and solvents are reagent grade and were used without further purification unless otherwise specified. ^{1}H NMR spectra were recorded on a Varian InNova 500 MHz spectrometer. Because many of the pyrrolidine amides are mixture of two rotamers with variable ratios, chemical shifts for the major rotamer were listed if any. Low-resolution mass spectra (MS) were determined on a Micromass platform liquid chromatography−mass spectrometer (LC-MS), using a Waters Xterrra MSC18 3.5 μM, 50 mm × 3.0 mm column with a binary solvent system where solvent A was water, 0.06% trifluoroacetic acid (by volume), and solvent B was acetonitrile, 0.05% trifluoroacetic acid (by volume). The LC method used a flow rate of 1.0 mL/mim with the following gradient: $t = 0$ min, 90% solvent A; $t = 3.75$ min, 2.0% solvent A; $t = 4.75$ min, 2% solvent A; $t = 4.76$ min, 90% solvent A; $t = 5.5$ min, 90% solvent A. LC-MS HPLC method served as analysis of purity over a broad range, and all final compounds showed a single peak (>95% purity) using this analytical method. Preparative TLC was done on Analtech Uniplate: cat. no. 02015, Silica Gel GF, 20 cm × 20 cm, 2000 μM.

Preparation of *tert*-Butyl {(2*S*)-1-[(2*S*)-2-(1,3-Benzothiazol-2-ylcarbonyl)pyrrolidin-1-yl]-1-oxopentan-2-yl}carbamate (2). Step 1. To a solution of benzothiazole (10.6 g, 78.3 mmol) in dried THF (300 mL), *n*-BuLi (80 mmol) was added dropwise at −78 °C and then the mixture was stirred at the temperature for 2 h. To the mixture was added a solution of *tert*-butyl (2*S*)-2-[methoxy-(methyl)carbamoyl]pyrrolidine-1-carboxylate (20.2 g, 78.3 mmol) in THF (120 mL) at −78 °C and also kept stirred at that temperature for 3 h. Then to the resulting mixture was added saturated NH_4Cl solution and stirred at room temperature for another 1 h. The solution was extracted with EtOAc (3 × 200 mL), and the organic layers were washed with brine, dried over Na_2SO_4,

concentrated, and purified by flash chromatography (PE:EtOAc = 15:1) to give *tert*-butyl (2*S*)-2-(1,3-benzothiazol-2-ylcarbonyl)-pyrrolidine-1-carboxylate (13.9 g, yield 53.5%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23−8.25 (m, 2H), 7.66−7.70 (m, 2H), 5.50 (dd, 1H, J = 7.16, J = 6.03), 3.47−3.49 (m, 2H), 2.83−2.87 (m, 1H), 2.17−2.19 (m, 3H). MS: m/z 333 $(M + 1)^+$.

Step 2. To the above product (2.0 g, 8.7 mmol) in dioxane (15 mL) was added 15 mL of HCl/Et_2O. The mixture was stirred at room temperature for about 1 h and then concentrated until solid precipitated. The mixture was filtered, and the solid was washed with EA and Et_2O and dried to give 1,3-benzothiazol-2-yl[(2*S*)-pyrrolidin-2-yl]methanone as solid (1.03 g, yield 62.5%). ^{1}H NMR (300 MHz, MeOD) δ 8.21−8.25 (m, 2H), 7.67−7.69 (m, 2H), 5.50−5.53 (m, 1H), 3.48−3.50 (m, 2H), 2.82−2.85 (m, 1H), 2.17−2.19 (m, 3H). MS: m/z 233 $(M + 1)^+$.

Step 3 (Method A). To a solution of 1,3-benzothiazol-2-yl-[(2*S*)-pyrrolidin-2-yl]methanone (268 mg, 1 mmol, 1.0 equiv), Boc-L-norvaline-OH (218 mg, 1 mmol,1.0 equiv), EDC (236 mg, 1.2 mmol, 1.2 equiv), and HOBT (162 mg, 1.2 mmol, 1.2 equiv) in dried DCM (10 mL) was added DIEA (575 mg, 5.0 mmol, 5.0 equiv) and DMAP (0.1 equiv), the resulting mixture was stirred at rt overnight. The mixture was then partitioned between water and DCM. The organic layer was washed with saturated citric acid, saturated $NaHCO_3$ solution, brine consequently, and then dried over Na_2SO_4, concentrated, and purified by TLC to give compound **2** (242 mg, 58.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16−8.18 (m, 1H), 7.96−7.98 (m, 1H), 7.50−7.58 (m, 2H), 5.80−5.84 (m, 1H), 5.25 (d, 1H, J = 8.59 Hz), 4.43−4.49 (m, 1H), 3.86−3.90 (m, 1H), 3.72−3.78 (m, 1H), 2.46−2.53 (m, 1H), 2.05−2.14 (m, 3H), 1.82−1.88 (m, 1H), 1.60−1.67 (m, 1H), 1.41 (s, 9H), 0.96−0.99 (m, 3H). MS: m/z 418 (M + 1).

Method B: Preparation of 5-Phenyl-2-[(2*S*)-pyrrolidin-2-yl]-1*H*-imidazole. Step 1. A mixture of chloroacetobenzene (7.73 g, 50 mmol), Boc-L-proline (10.76 g, 50 mmol), and potassium carbonate (20.73 g, 150 mmol) in DMF was stirred at rt overnight, dumped into water, extracted with ethyl acetate, washed with water and brine, dried over sodium sulfate, and evaporated. The residue was mixed with ammonium acetate (38.5 g, 500 mmol) and toluene, refluxed on a Dean−Stark to remove water for 2 h, cooled, dumped into water, and basified with satd sodium bicarbonate. Some material was lost due to bubble forming. Organic phase was washed with water, dried, and evaporated. The residue was purified on Combi-Flash (20%-50% EtOAc/hexane) to give 8.6 g of intermediate *tert*-butyl (2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate as a light-brown solid. LC-MS: 313.92.

Step 2. TFA (15 mL) was added dropwise to a solution of (2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate (6.0 g, 20 mmol) in DCM (15 mL) at room temperature. The solution was stirred overnight. The reaction mixture was concentrated and followed by triturating with ether (25 mL). The white precipitate was collected, washed with more ether and dried to give 2.15 g (83%) of 5-phenyl-2-[(2*S*)-pyrrolidin-2-yl]-1*H*-imidazole as TFA salt. ^{1}H NMR (500 MHz, MeOH-d_4), δ 8.04 (s, 1H), 7.84 (d, 2 H), 7.55 (t, 2H), 7.51 (t, 1H), 5.20 (t, 1H), 3.60 (m, 2H), 2.74 (m, 1H), 2.55 (m, 1H), 2.42 (m, 1H), 2.26 (m, 1H).

The following compounds (**3**, **4**, **5a**−**5o**, and **6a**−**6n**) were synthesized from coupling of protected amino acid and 5-phenyl-2-[(2*S*)-pyrrolidin-2-yl]-1*H*-imidazole with EDC using method A. Most of the final compounds were purified via reverse phase HPLC or Gilson system, or prep TLC.

***N*-{(2*S*)-1-Oxo-1-[(2*S*)-2-(5-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]hexan-2-yl}acetamide (3).** MS: calcd, 368.22; obsd, 369.03.

9*H*-Fluoren-9-ylmethyl {(2*S*)-1-Oxo-1-[(2*S*)-2-(5-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]hexan-2-yl}carbamate (4). MS: calcd, 548.28; obsd, 549.04.

9*H*-Fluoren-9-ylmethyl {(2*S*)-3-Hydroxy-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-2-yl}carbamate (5a). *O*-*t*-Butyl-*N*-Fmoc-L-serine (77 mg, 0.2 mmol) and 5-phenyl-2-[(2*S*)-pyrrolidin-2-yl]-1*H*-imidazole (49.9 mg, 0.2 mmol) were coupled using method A. The product (28 mg, 24%) was then treated with 4 M HCl in dioxane for 1 h to give titled product after evaporation and drying. MS: calcd, 522.23; obsd, 522.93.

(3*S*)-3-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-4-oxo-4-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]butanoic Acid (5b). MS: calcd, 550.22; obsd, 550.95.

(4*S*)-4-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-5-oxo-5-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]pentanoic Acid (5c). MS: calcd, 564.24; obsd, 565.33.

9*H*-Fluoren-9-ylmethyl {(2*S*)-4-(Methylsulfonyl)-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]butan-2-yl}carbamate (5d). MS: calcd, 598.22; obsd, 598.99.

9*H*-Fluoren-9-ylmethyl {(2*S*)-4-(Methylsulfanyl)-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]butan-2-yl}carbamate (5e). MS: calcd, 566.24; obsd, 566.94.

9*H*-Fluoren-9-ylmethyl {(2*S*)-5-Amino-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]pentan-2-yl}carbamate (5f). MS: calcd, 549.27; obsd, 549.94.

9*H*-Fluoren-9-ylmethyl {(2*S*)-6-Amino-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]hexan-2-yl}carbamate (5g). MS: calcd, 563.29; obsd, 564.01.

9*H*-Fluoren-9-ylmethyl {(2*S*)-1-Oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]butan-2-yl}carbamate (5h). MS: calcd, 520.25; obsd, 521.

9*H*-Fluoren-9-ylmethyl {(2*S*)-3-(Benzyloxy)-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-2-yl}carbamate (5i). MS: calcd, 612.27; obsd, 612.99.

***tert*-Butyl (7*S*)-7-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-8-oxo-8-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]octanoate (5j).** MS: calcd, 662.35; obsd, 663.04.

Benzyl {(5*S*)-5-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-6-oxo-6-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]hexyl}carbamate (5k). MS: calcd, 697.33; obsd, 697.98.

Prop-2-en-1-yl {(5*S*)-5-{[(9*H*-Fluoren-9-ylmethoxy)carbonyl]amino}-6-oxo-6-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]hexyl}carbamate (5l). MS: calcd, 647.31; obsd, 648.02.

9*H*-Fluoren-9-ylmethyl {(2*S*)-1-Oxo-5-phenyl-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]pentan-2-yl}carbamate (5m). MS: calcd, 610.29; obsd, 610.99.

9*H*-Fluoren-9-ylmethyl {(2*S*)-1-Oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]octan-2-yl}carbamate (5n). MS: calcd, 576.31; obsd, 577.01.

9*H*-Fluoren-9-ylmethyl {(2*S*)-3-(Biphenyl-4-yl)-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-2-yl}carbamate (5o). MS: calcd, 658.29; obsd, 658.99.

***tert*-Butyl {(2*S*)-3-(Biphenyl-4-yl)-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-2-yl}carbamate (6a).** MS: calcd, 536.28; obsd, 536.97.

***N*-{(2*S*)-3-(Biphenyl-4-yl)-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-2-yl}-3,3-dimethylbutanamide (6b).** Coupling of **6c** and 3,3-dimethylbutanoic acid using method A gave the titled compound. MS: calcd, 534.30; obsd, 534.96.

(2*S*)-2-Amino-3-(biphenyl-4-yl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6c). Compound **6a** was treated with 4 M HCl in dioxane to give the titled compound. MS: calcd, 436.23; obsd, 436.97.

4′-{(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propyl}biphenyl-4-carboxylic Acid (6d). Step 1 (Method C). *N*-Boc-4-iodophenylalanine (1.96 g, 5 mmol) was mixed with 5-phenyl-2-[(2*S*)-pyrrolidin-2-yl]-1*H*-imidazole (1.43 g, 5 mmol), DIEA (1.94 g, 15 mmol), and HATU (2.28 g, 6 mmol) in DMF (30 mL) at room temperature for 1 h. The mixture was diluted with water and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4, and the crude product was separated to two diastereomers after flash column chromatography with 10−80% EA/hexane gradient: *tert*-butyl {(2*R*)-3-(4-iodophenyl)-1-oxo-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-2-yl}carbamate (1.1 g) as the first fraction and the (S,S) isomer (1.6 g) as the second fraction. The more active isomer (*S*,*S*) was used in the next step.

Step 2. The (*S*,*S*) iodo compound from above (290 mg, 0.5 mmol), 4-(dihydroxyboranyl)benzoic acid (150 mg, 0.9 mmol), 1,1′-bis(di-t-butylphosphino)ferrocene palladium dichloride (65 mg), and sodium carbonate in DMF (5 mL, contained two drops of water) was degassed and then stirred at 110 °C under nitrogen for 4 h, cooled at RT, diluted with ethyl acetate, and acidified with 2N aq HCl and separated. The organic phase was washed with water, dried over sodium sulfate, and evaporated. The residue was purified on Comb-Flash (100% EtOAc and then 10% MeOH/EtOAc) to give 8 mg of pure product (and 25 mg impure fraction). MS: calcd: 580.27; obsd, 581.39.

1-[(2*S*)-2-(4-Phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]-3-(3-phenyl-1,2,4-oxadiazol-5-yl)propan-1-one (6e). MS: calcd, 413.19; obsd, 413.96.

3-(Biphenyl-4-yl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6f). MS: calcd, 421.22; obsd, 421.93.

Preparation of 3-(4-Iodophenyl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one. 3-(4-Iodophenyl)propanoic acid (1.518 g, 5.5 mmol) was coupled to 5-phenyl-2-[(2*S*)-pyrrolidin-2-yl]-1*H*-imidazole (5.5 mmol) with HATU using method C to give 2.17 g of the titled compound. MS: calcd, 471.08; obsd, 472.05.

3-(3′-Aminobiphenyl-4-yl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6g). 3-(4-Iodophenyl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (41 mg, 0.1 mmol), 3-aminophenyl boronic acid (26 mg, 0.15 mmol), Na_2CO_3 (32 mg, 0.3 mmol), and 1,1′-bis(di-*t*-butylphosphino)-ferrocene palladium dichloride (10 mg) were stirred in DMF (2 mL) at 100 °C for 5 h. The mixture was diluted with ethyl acetate (50 mL) and washed with water. The crude product was then purified through automated reverse phase HPLC to give the titled product: MS: calcd, 436.23; obsd, 437.57.

The following compounds (**6h**−**6n**) were prepared similarly.

3-(2′-Methylbiphenyl-4-yl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6h). MS: calcd, 435.23; obsd, 436.58.

3-(4′-Methylbiphenyl-4-yl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6i). MS: calcd, 435.23; obsd, 436.26.

3-(2′-Chlorobiphenyl-4-yl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6j). MS: calcd, 455.18; obsd, 456.21.

3-(4′-Chlorobiphenyl-4-yl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6k). MS: calcd, 455.18; obsd, 456.15.

3-(4′-*tert*-Butylbiphenyl-4-yl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6l). MS: calcd, 477.28; obsd, 478.38.

3-(4′-Chloro-3′-fluorobiphenyl-4-yl)-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6m). MS: calcd, 473.17; obsd, 474.15.

3-[4-(1,3-Benzodioxol-5-yl)phenyl]-1-[(2*S*)-2-(4-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (6n). MS: calcd, 465.21; obsd, 466.28.

Preparation of *tert*-Butyl (2*S*)-2-(1*H*-Imidazol-2-yl)pyrrolidine-1-carboxylate. *tert*-Butyl (2*S*)-2-formylpyrrolidine-1-carboxylate (1 g, 5 mmol) was stirred with 28% ammonia aq solution (5.6 mL) in EtOH (20 mL) for 30 min, followed a slow addition of oxaldehyde (0.88 g, 6 mmol) in 50% EtOH (20 mL) in 10 h. The resulting solution was stirred for 3 days. After aqueous workup, the crude product was purified by silica gel chromatography to give 1.2 g of the titled compound. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (s, 2H), 4.98 (m, 1H), 3.40 (m, 2H), 2.92 (m, 1H), 2.15 (m, 2H), 2.00 (m, 1H), 1.50 (s, 9H).

3-(Biphenyl-4-yl)-1-[(2*S*)-2-(1*H*-imidazol-2-yl)pyrrolidin-1-yl]-propan-1-one (7a). *tert*-Butyl (2*S*)-2-(1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate (65 mg, 0.27 mmol) was treated with 4N HCl in dioxane for 1 h. The residue from evaporation and drying was then coupled with 3-(biphenyl-4-yl)propanoic acid using method A to give 68 mg of the titled compound as TFA salt. MS: calcd, 345.18; obsd, 346.18. ^{1}H NMR (500 MHz, DMSO-d_3) δ 7.63 (d, 2H), 7.58 (s, 2H), 7.57 (d, 2H), 7.46 (t, 2H), 7.35 (t, 1H), 7.32 (d, 2H), 5.16 (m, 1H), 3.72 (m, 1H), 3.4 (m, 1H), 2.82 (t, 2H), 2.68 (t, 2H), 2.31 (m, 1H), 1.94 (m,3 H).

3-(Biphenyl-4-yl)-1-[(2*S*)-2-(4-cyclopropyl-1*H*-imidazol-2-yl)-pyrrolidin-1-yl]propan-1-one (7b) Method D. Compound **7e** (12 mg, 0.028 mmol), cyclopropyl boronic acid pinacolate (95 mg, 20 equiv), and 1,1′-bis (di-*t*-butylphosphino)ferrocene palladium dichloride (2 mg) was mixed in THF (1 mL) with $NaHCO_3$ (satd, 0.2 mL) and purged with N_2. The sealed vessel was heated in microwave for 10 min at 100 °C. The crude product after aqueous work up was purified through reverse phase HPLC to give the titled compound. MS: calcd, 385.22; obsd, 386.11. ^{1}H NMR (500 MHz, DMSO-d_3) δ 7.61 (d, 2H), 7.55 (d, 2H), 7.44 (t, 2H), 7.33 (t, 1H), 7.30 (d, 2H), 7.24 (s, 1H), 5.05 (m, 1H), 4.11 (m, 1H), 3.70 (m, 1H), 2.82 (t, 2H), 2.66. (t, 2H), 2.28 (m, 1H), 1.90 (m, 3H), 1.42 (m, 1H), 0.96 (m, 2H), 0.72 (m, 2H).

3-(Biphenyl-4-yl)-1-{(2*S*)-2-[5-(2,2,2-trifluoroethyl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7c). Step 1. *N*-Boc-*R*-proline (0.93 g, 4.29 mmol) and 1-amino-4,4,4-trifluorobutan-2-ol (4.29 mmol) were coupled with EDC in the same condition as step 3 for compound **2** to give 0.8 g of *tert*-butyl 2-[(4,4,4-trifluoro-2-hydroxybutyl)carbamoyl]pyrrolidine-1-carboxylate after prep TLC (SiO_2, 1:1 hexane:EA). MS: calcd, 340.16; obsd, 241.08, 362.95.

Step 2. The intermediate amide alcohol was oxidized to amide ketone with Dess−Martin reagent (1.25 equiv) in DCM overnight to give 0.72 g of *tert*-butyl 2-[(4,4,4-trifluoro-2-oxobutyl)-carbamoyl]pyrrolidine-1-carboxylate after removal of solid and evaporation of solvent. This crude product was then refluxed with ammonium acetate (3.2 g) in toluene for 3 h with a Dean−Stark trap to remove water. The resulting residue was washed with sodium bicarbonate solution. The crude product was purified on PreTLC (EA) to give 0.11 g of *tert*-butyl 2-[5-(2,2,2-trifluoroethyl)-1*H*-imidazol-2-yl]pyrrolidine-1-carboxylate. MS: calcd, 319.15; obsd, 320.06.

Step 3. The above intermediate was treated with 4 N HCl in dioxane for 1 h, and the residue was coupled with 3-(biphenyl-4-yl)propanoic acid with EDC in the same condition as step 3 from compound **2**. The final product was purified by reverse phase prep HPLC to give 7 mg of the titled compound (**7c**) as white powder after freeze-drying. MS: calcd, 427.19; obsd, 427.96.

3-(Biphenyl-4-yl)-1-[(2*S*)-2-(5-*tert*-butyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (7d). Step 1. *N*-Boc-*S*-proline (100 mg, 0.465 mmol) was stirred with 1-bromo-3,3-dimethylbutan-2-one (0.465 mmol) and K_2CO_3 (96 mg) in DMF overnight. The crude product after aqueous workup was refluxed with ammonium acetate (700 mg) in toluene for 3 h using a Dean−Stark trap. Aqueous work up afforded 20 mg of the intermediate *tert*-butyl (2*S*)-2-(5-*tert*-butyl-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate. MS: calcd, 293.21; obsd, 294.23.

Step 2. This was treated with 4N HCl in dioxane to remove the Boc group. The crude product (9 mg) was coupled with 3-(biphenyl-4-yl)propanoic acid using method A to give 10 mg of the titled compound (**7d**). MS: calcd, 401.25; obsd, 402.05. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.58 (d, 2H, J = 8 Hz), 7.52 (d, 2H, J = 7 Hz), 7.40 (t, 2H, J = 8 Hz), 7.29 (t, 1H, J = 8 Hz), 7.27 (d, 2H, J = 7 Hz), 7.24 (s, 1H), 5.06 (m, 1H), 3.70 (m, 1H), 3.49 (m, 1H), 2.78 (t, 2H), 2.63 (t, 2H), 2.18 (m, 1H), 1.90 (m, 3H), 1.25 (s, 9H).

3-(Biphenyl-4-yl)-1-[(2*S*)-2-(4-bromo-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (7e). Step 1. To a solution of *tert*-butyl (2*S*)-2-(1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate (100 mg, 0.42 mmol) and TEA (0.1 mL, 0.84 mmol) in DMF (3 mL) was added trityl chloride (235 mg, 0.84 mmol). The mixture was stirred overnight, followed by aqueous work up and flash chromatography to give 88 mg of *tert*-butyl (2*S*)-2-(1-trityl-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate. ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.38 (br, 15H), 6.84 (AB, 2H), 4.40 (m, 1H), 3.50 (m, 2H), 1.60 (m, 2H), 1.28 (m, 2H).

Step 2. *tert*-Butyl (2*S*)-2-(1-trityl-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate (1.35 g, 2.8 mmol) was stirred with NBS (0.5 g, 2.8 mmol) in THF (100 mL) for 30 min after aqueous work up and flash chromatograph eluting with EA:hexane (0−40% gradient) to give 0.6 g of *tert*-butyl (2*S*)-2-(4-bromo-1-trityl-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate. ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.40 (br, 15H), 6.80 (s, 1H), 4.40 (m, 1H), 3.55 (m, 2H), 1.60 (m, 2H), 1.28 (m, 2H). MS: calcd, 557.17; obsd, 580.04.

Step 3. A solution of *tert*-butyl (2*S*)-2-(4-bromo-1-trityl-1*H*-imidazol-2-yl)pyrrolidine-1-carboxylate (400 mg, 0.72 mmol) in DCM (5 mL) was mixed with TFA (5 mL). The solution was stirred for 2 h at room temperature. This was evaporated to dryness and taken up in DCM (10 mL). 3-(*p*-Biphenyl)propionic acid (182 mg, 0.8 mmol), HOBt (165 mg, 1 mmol), and TEA (0.14 mL, 1 mmol) and EDC (275 mg, 1.4 mmol) were added. The mixture was stirred for 1 h and was diluted with water and extracted with EA (3 × 20 mL). The combined EA solution was washed with 0.1 N HCl, $NaHCO_3$, and brine. The crude product was flash chromatographed through silica gel column (40% EA/hexane) to give 257 mg of compound **7e**. ^{1}H NMR (500 MHz, DMSO-d_3) δ 7.61 (d, 2H), 7.58 (s, 1H), 7.54 (d, 2H), 7.43 (t, 2H), 7.32 (t, 1H), 7.30 (d, 2H), 4.95 (m, 1H), 3.6 (m, 2H), 2.82 (t, 2H), 2.61. (t, 2H), 2.18 (m, 1H), 1.90 (m, 3H). MS: calcd, 423.09; obsd, 424.05, 426.05.

3-(Biphenyl-4-yl)-1-{(2*S*)-2-[5-(prop-1-en-2-yl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7f). This compound was prepared from **7e** and 2-propenylboronic acid using method D. MS: calcd, 385.22; obsd, 386.20.

3-(Biphenyl-4-yl)-1-{(2*S*)-2-[5-(propan-2-yl)-1*H*-imidazol-2-yl]-pyrrolidin-1-yl}propan-1-one (7g). This compound was prepared from **7f** by Pd/C catalyzed hydrogenation in MeOH. MS: calcd, 387.23; obsd, 388.23.

1-[(2*S*)-2-(5-Benzyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]-3-(biphenyl-4-yl)propan-1-one (7h). This compound was prepared from compound **7e** and benzylboronic acid pinacolate using method D. MS: calcd, 435.23; obsd, 436.15.

3-(Biphenyl-4-yl)-1-[(2*S*)-2-(5-phenyl-4*H*-1,2,4-triazol-3-yl)-pyrrolidin-1-yl]propan-1-one (7i). Step 1. *N*-Boc-*S*-proline (300 mg, 1.56 mmol) was coupled to hydrazine with EDC in DCM. The crude product was purified through reverse phase prep HPLC to give 190 mg of the hydrazide. The hydrazide, methyl benzenecarboximidoate (1 equiv), and excess DIEA (3 equiv) were heated in 1:1 mix of toluene and DMF to 150 °C for 20 min in a microwave reactor. The crude product was purified through reverse phase prep HPLC to give the triazole *tert*-butyl (2*S*)-2-(5-phenyl-4*H*-1,2,4-triazol-3-yl)pyrrolidine-1-carboxylate in 20% yield. MS: calcd, 314.17; obsd, 337.23 (M + Na^+).

Step 2. The Boc-protected triazole (6 mg) was treated with 1 mL of 4N HCl (dioxane) for 30 min to give 5 mg of 3-phenyl-5-[(2*S*)-pyrrolidin-2-yl]-4*H*-1,2,4-triazole as HCl salt. MS: calcd, 214.12; obsd, 215.28.

Setp 3. The triazole HCl salt (2 mg, 7.2 μmol) was coupled with 3-(biphenyl-4-yl)propanoic acid in the same condition as step 3 for compound **2** to give 0.7 mg of the titled compound **7i**. MS: calcd, 422.21; obsd, 455.21 (M + Na).

3-(Biphenyl-4-yl)-1-{(2*S*)-2-[4-chloro-5-(3-methylphenyl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7j). Compound **7m** (5 mg) was heated with 10 equiv of NCS in THF to 60 °C for 1 h. PrepTLC purification afforded 0.8 mg of the titled compound. MS: calcd, 469.19, 471.19; obsd, 470.15, 472.12.

3-(Biphenyl-4-yl)-1-{(2*S*)-2-[5-(3-bromophenyl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7k). The intermediate 5-(3-bromophenyl)-2-[(2*S*)-pyrrolidin-2-yl]-1*H*-imidazole, prepared using method B, was coupled to 3-(biphenyl-4yl)propionic acid using method A to give the titled compound. MS: calcd, 499.13, 501.12; obsd, 500.02, 502.02. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.06 (br, 1H), 8.04 (s, 1H), 7.80 (d, 1H), 7.61 (d, 2H), 7.60 (d, 1H), 7.55 (d, 2H), 7.46 (t, 1H), 7.45 (t, 2H), 7.35 (t, 1H), 7.33 (s, 1H), 7.32 (d, 2H), 5.17 (m, 1H), 3.75 (m, 1H), 3.60 (m, 1H), 2.86 (t, 2H), 2.70 (t, 2H), 2.30 (m, 1H), 2.02 (m, 3H).

3-(Biphenyl-4-yl)-1-[2-(4-methyl-5-phenyl-1*H*-imidazol-2-yl)-pyrrolidin-1-yl]propan-1-one (7l). To a mixture methylboronic acid (6 mg, 0.1 mmol), bis(triphenylphohpine)palladium(II) chloride (1.4 mg), and 3-(biphenyl-4-yl)-1-[(2*S*)-2-(4-bromo-5-phenyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]propan-1-one (prepared from NBS bromination of compound **6f** in the same condition as for **7j**) (10 mg, 0.02 mmol) in THF (1 mL) and K_2CO_3−LiCl (0.1 mL, 5 mM) was heated in a microwave to 140 °C for 15 min. The crude product after aqueous work up was purified through Gilson HPLC to give 4 mg of the titled compound. MS: calcd, 435.23; obsd, 436.15.

3-(Biphenyl-4-yl)-1-{(2*S*)-2-[5-(3-methylphenyl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7m). The compound was prepared from the corresponding bromo ketone following method B and then method A. MS: calcd, 435.23; obsd, 436.10. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.93 (br, 1H), 7.60 (d, 1H), 7.56 (d, 2H), 7.52 (s, 1H), 7.50 (d, 2H), 7.40 (t, 2H), 7.35 (t, 1H), 7.29 (t, 1H), 7.27 (s, 1H), 7.26 (d,2H), 7.20 (d, 1H), 5.10 (m, 1H), 3.60 (m, 1H), 3.52 (m, 1H), 2.80 (t, 2H), 2.64 (t, 2H), 2.32 (s, 3H), 2.02 (m, 1H), 1.98 (m, 3H).

3-(Biphenyl-4-yl)-1-(2-{5-[2-(difluoromethoxy)phenyl]-1*H*-imidazol-2-yl}pyrrolidin-1-yl)propan-1-one (7n). This compound was prepared from **7e** and corresponding boronic acid using method D. MS: calcd, 487.21; obsd, 488.18.

The following compounds (**7o**−**7t**) were prepared from the corresponding bromo ketone following method B to form the imidazole intermediate which were then coupled to biphenylpropionic acid using method A.

***N*-[3-(2-{(2*S*)-1-[3-(Biphenyl-4-yl)propanoyl]pyrrolidin-2-yl}-1*H*-imidazol-5-yl)phenyl]-methanesulfonamide (7o).** MS: calcd, 514.20; obsd, 515.13. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.0 (s, 1H), 8.01 (s, 1H), 7.62 (d, 2H), 7.58 (s, 1H), 7.57 (d, 2H), 7.52 (s, 1H), 7.51 (d, 1H), 7.46 (t, 2H), 7.46 (t, 1H), 7.35 (t, 1H), 7.33 (d, 2H), 7.25 (d, 1H), 5.20 (m, 1H), 3.76 (m, 1H), 3.50 (m, 1H), 3.08 (s, 3H), 2.85 (t, 2H), 2.70 (t, 2H), 2.35 (m, 1H), 2.02 (m, 3H).

3-(Biphenyl-4-yl)-1-{2-[5-(3-chlorophenyl)-1*H*-imidazol-2-yl]-pyrrolidin-1-yl}propan-1-one (7p). MS: calcd, 455.18; obsd, 456.10. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.01 (br, 1H), 7.85 (s, 1H), 7.70 (d, 1H), 7.56 (d, 2H), 7.50 (d, 2H), 7.49 (d, 1H), 7.40−7.35 (m, 4H), 7.29 (t, 1H), 7.26 (d, 2H), 5.20 (m,1H), 3.70 (m, 1H), 3.55 (m, 1H), 2.80 (t, 2H), 2.64 (t, 2H), 2.25 (m 1H), 1.96 (m, 3H).

3-(2-{1-[3-(Biphenyl-4-yl)propanoyl]pyrrolidin-2-yl}-1*H*-imidazol-5-yl)benzoic acid (7q). This compound was obtained from hydrolysis of the corresponding cyano compound. MS: calcd, 465.21; obsd, 466.19. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.20 (s, 1H), 11.80 (s, 1H), 8.37 (d, 1H), 7.98 (s, 1H), 7.75 (s, 1H), 7.60 (d, 1H), 7.55 (d, 2H), 7.49 (m, 4H), 7.46 (d, 2H), 7.25 (d, 1H), 5.20 (m,1H), 3.59 (m, 1H), 3.50 (m, 1H), 2.85 (t, 2H), 2.65 (t, 2H), 2.30 (m 1H), 2.10 (m, 3H).

3-(Biphenyl-4-yl)-1-{2-[5-(3-chloro-4-fluorophenyl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7r). MS: calcd, 473.17; obsd, 474.02.

3-(Biphenyl-4-yl)-1-{2-[5-(furan-2-yl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7s). MS: calcd, 411.19; obsd, 412.20.

3-(Biphenyl-4-yl)-1-{2-[5-(4-methylthiophen-2-yl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7t). MS: calcd, 441.19; obsd, 442.09. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.76 (s, 1H), 7.62 (d, 1H), 7.56 (d, 2H), 7.46 (d, 2H), 7.42 (s, 1H), 7.35 (t, 1H), 7.32 (d, 2H), 7.29 (s, 1H), 7.21 (s, 1H), 5.15 (m,1H), 3.75 (m, 1H), 3.55 (m, 1H), 2.85 (t, 2H), 2.70 (t, 2H), 2.28 (m 1H), 2.00 (m, 3H).

Compounds **7u** and **7v** were prepared from Suzuki coupling of **7e** with the corresponding boronic acids using method D.

3-(Biphenyl-4-yl)-1-{2-[5-(1-methyl-1*H*-pyrazol-4-yl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7u). MS: calcd, 425.22; obsd, 426.19.

3-(Biphenyl-4-yl)-1-{2-[5-(1-propyl-1*H*-pyrazol-4-yl)-1*H*-imidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7v). MS: calcd, 453.25; obsd, 454.21.

Compounds **7w**−**7ae** were prepared from coupling of biphenylproprionic acid and the corresponding pyrrolidinylbenzimidazole

using method C. The pyrrolidinylbenzimidazoles were prepared using method E (see compound **7ab**).

3-(Biphenyl-4-yl)-1-[2-(6-fluoro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]propan-1-one (7w). MS: calcd, 413.19; obsd, 414.31.

3-(Biphenyl-4-yl)-1-[2-(6-chloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]propan-1-one (7x). MS: calcd, 429.16; obsd, 430.26.

3-(Biphenyl-4-yl)-1-{2-[6-(trifluoromethyl)-1*H*-benzimidazol-2-yl]pyrrolidin-1-yl}propan-1-one (7y). MS: calcd, 463.19; obsd, 464.30.

3-(Biphenyl-4-yl)-1-[2-(5,6-difluoro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]propan-1-one (7z). MS: calcd, 431.18; obsd, 432.30.

3-(Biphenyl-4-yl)-1-[2-(6-chloro-5-fluoro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]propan-1-one (7aa). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.75 (d, 1H, J_{F-H}), 7.60 (d, 2H), 7.53 (d, 2H), 7.43 (t, 2H), 7.42 (d, 1H, J_{F-H}), 7.33 (t, 1H), 7.31 (d, 2H), 5.16 (d, 1H), 3.72 (m, 1H), 3.55 (m, 1H), 2.84 (t, 2H), 2.68 (t, 2H), 2.23 (m, 1H), 2.08 (m, 2H), 1.96 (m, 1H). MS: calcd, 447.15; obsd, 448.46.

3-(Biphenyl-4-yl)-1-[2-(5,6-dichloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]propan-1-one (7ab). Step 1. Preparation of 5,6-Dichloro-2-(pyrrolidin-2-yl)-1*H*-benzimidazole (Method E). To a DMF (50 mL) of 4,5-dichlorobenzene-1,2-diamine (6.29 g, 35.5 mmol), *N*-Boc-*S*-proline (7.64 g, 35.5 mmol), and DIEA (7.56 mL, 43.4 mmol) was added HATU (15 g, 39.4 mmol). The solution was stirred for 5 h, poured into water, and extracted with ethyl acetate. The organic layer was dried over sodium sulfate and evaporated. The crude residue was mixed with 30 mL of acetic acid and heated at 60 °C overnight. Acetic acid was removed under reduced pressure, and the residue was treated with 50 mL of 5N NaOH. The resulting solid was filtered and washed with water and dried in air. Purification on comb-flash gave a brown solid, which was further crystallized in ethyl acetate/hexane to give a light-brown solid. Weight: 8.5 g. LC-MS: calcd, 355.09; obsd, 356.21.

The Boc-protected product was treated with 4N HCl in THF for 2 h. Evaporation and vacuum drying left 7.85 g solid of 5,6-dichloro-2-(pyrrolidin-2-yl)-1*H*-benzimidazole as the HCl salt. MS: calcd, 255.03; obsd, 256.45.

Step 2. The pyrrolidinylbenzimidazole from above was coupled with 3-(biphenyl-4-yl)proprionic acid using method C to give compound **7ab**. ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.86 (s, 2H), 7.55 (d, 2H), 7.48 (d, 2H), 7.40 (t, 2H), 7.30 (t, 1H), 7.27 (d, 2H), 5.31 (m, 1H), 3.81 (m, 1H), 3.62 (m, 1H), 2.94 (t, 2H), 2.80 (t, 2H), 2.48 (m, 1H), 2.16 (m, 1H), 2.10 (m, 2H). MS: calcd, 463.12; obsd, 463.93.

3-(Biphenyl-4-yl)-1-[2-(4,5-dimethyl-1*H*-benzimidazol-2-yl)-pyrrolidin-1-yl]propan-1-one (7ac). MS: calcd, 423.23; obsd, 424.35.

3-(Biphenyl-4-yl)-1-[2-(3*H*-imidazo[4,5-*c*]pyridin-2-yl)pyrrolidin-1-yl]propan-1-one (7ad). ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.36 (br, 1H), 8.58 (br, 1H), 8.10 (br, 1H), 7.61 (d, 2H), 7.54 (d, 2H), 7.44 (t, 2H), 7.33 (t, 1H), 7.32 (d, 2H), 5.25 (d, 1H), 3.76 (m, 1H), 3.62 (m, 1H), 2.78 (t, 2H), 2.61 (t, 2H), 2.34 (m, 1H), 2.08 (m, 2H), 2.01 (m, 1H). MS: calcd, 396.20; obsd, 397.22.

3-(Biphenyl-4-yl)-1-{2-[5-(3-fluorophenyl)-1*H*-imidazol-2-yl]-pyrrolidin-1-yl}propan-1-one (8a). The intermediate pyrrolidinylbenzimidazole, prepared from the corresponding bromo ketone using method B, was coupled with 3-(biphen-4-yl)propionic acid using method A. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.02 (br, 1H), 7.7 (br, 2H), 7.62 (d, 2H), 7.55 (d, 2H), 7.45 (t, 2H), 7.35 (t, 1H), 7.33 (d, 2H), 7.22 (t, br, 1H), 5.18 (m,1H), 3.80 (m, 1H), 3.51 (m, 1H), 2.85 (t, 2H), 2.70 (t, 2H), 2.30 (m 1H), 2.05 (m, 3H). MS: calcd, 439.21; obsd, 440.13.

3-(Biphenyl-4-yl)-1-{2-[5-(3-fluorophenyl)-1*H*-imidazol-2-yl]-pyrrolidin-1-yl}-2,2-dimethylpropan-1-one (8b). MS: calcd, 467.24; obsd, 468.39.

2-(Biphenyl-4-yloxy)-1-[(2*S*)-2-(4-methyl-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]ethanone (8c). MS: calcd, 411.19; obsd, 412.11.

1-{2-[5-(3-Bromophenyl)-1*H*-imidazol-2-yl]-4,4-difluoropyrrolidin-1-yl}-3-(2′,3′-dichlorobiphenyl-4-yl)propan-1-one (8d). The intermediate 5-(3-bromophenyl)-2-[(2*S*)-4,4-difluoropyrrolidin-2-yl]-1*H*-imidazole was prepared from 1-(*tert*-butoxycarbonyl)-4,4-difluoro-L-proline and 2-bromo-1-(3-bromophenyl)ethanone using method B followed by HCl de-Boc. This was then coupled with 3-(2′,3′-dichlorobiphenyl-4-yl)propanoic acid using method A to give the titled compound. ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.92 (br, 1H), 7.68 (br, 1H), 7.53 (m, 1H), 7.40 (m, 1H), 7.32 (m, 2H), 7.29 (m, 4H), 7.22 (m, 1H), 7.12 (d, 1H), 5.40 (m, 1H), 4.10 (m, 2H), 3.00 (t, 2H), 2.58 (m, 1H), 2.55 (t, 2H), 2.53 (m, 1H). MS: calcd, 603.03; obsd, 604.01.

2-(Biphenyl-4-yloxy)-1-[(1*R*,2*S*,5*S*)-2-(4-methyl-1*H*-benzimidazol-2-yl)-3-azabicyclo[3.1.0]hex-3-yl]ethanone (8e). MS: calcd, 423.19; obsd, 424.15. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.58 (d, 2H), 7.53 (d, 3H), 7.43 (t, 2H), 7.32 (t, 1H), 7.34 (br, 1H), 7.31 (t, 1H), 7.25 (br, 1H), 7.03 (d, 2H), 5.45 (d, 1H), 4.92 (d, 1H), 4.82 (d, 1H), 3.99 (m, 1H), 3.85 (d, 1H), 2.52 (s, 3H), 2.10 (m, 1H), 2.04 (m, 1H), 0.80 (m, 2H).

4′-(3-{(2*S*,4*R*)-2-[5-(3-Chlorophenyl)-1*H*-imidazol-2-yl]-4-hydroxypyrrolidin-1-yl}-3-oxopropyl)biphenyl-3-carbonitrile (8f). Step 1. *N*-Boc-4(*R*)-hydroxy-L-proline was reacted with 2-bromo-1-(3-chlorophenyl)ethanone using method B to give *tert*-butyl (2*S*,4*R*)-2-[5-(3-chlorophenyl)-1*H*-imidazol-2-yl]-4-hydroxypyrrolidine-1-carboxylate in 70% yield. MS: calcd, 363.13; obsd, 364.17. The Boc group was removed with 4N HCl in dioxane to give (3*R*,5*S*)-5-[5-(3-chlorophenyl)-1*H*-imidazol-2-yl]pyrrolidin-3-ol as a solid. MS: calcd, 263.08; obsd, 264.28. ^{1}H NMR (500 MHz, MeOH-d_4) δ 7.96 (s, 1H), 7.89 (t, 1H), 7.76 (d, 1H), 7.50 (t, 1H), 7.45 (d, 1H), 5.29 (t, 1H), 4.56 (d, 1H), 3.70 (dd, 1H), 3.48 (d, 1H), 2.64 (d, 2H).

Step 2. The imidazole from above was coupled with cyanobiphenylpropionic acid using method A to give **8f**. MS: calcd, 496.17; obsd, 497.23. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.08 (br, 2H), 7.95 (d, 1H), 7.88 (s, 1H), 7.82 (d, 1H), 7.74 (d, 1H), 7.65 (t, 1H), 7.62 (d, 2H), 7.54 (t, 1H), 7.47 (d, 1H), 7.34 (d, 2H), 5.15 (m, 1H), 4.50 (m, 1H), 3.75 (m, 1H), 3.60 (m, 1H), 2.85 (m, 2H), 2.72 (m, 1H), 2.64 (m, 1H), 2.31 (m, 1H), 2.19 (m, 1H).

2-Amino-*N*-{(2*S*)-3-(biphenyl-4-yl)-1-[(2*S*)-2-(5,6-dichloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]-1-oxopropan-2-yl}-2-methylpropanamide (8g). MS: calcd, 563.19; obsd, 564.29.

5,6-Dichloro-2-[(2*S*)-1-(2-methylalanyl-4-chloro-L-phenylalanyl)pyrrolidin-2-yl]-1*H*-benzimidazole (8h). MS: calcd, 521.12; obsd, 522.13.

5,6-Dichloro-2-[(2*S*)-1-(2-methylalanyl-*O*-methyl-L-tyrosyl)-azetidin-2-yl]-1*H*-benzimidazole (8i). MS: calcd, 503.15; obsd, 504.20.

***N*-{(2*S*)-3-(Biphenyl-4-yl)-1-[(2*S*)-2-(5,6-difluoro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]-1-oxopropan-2-yl}-2-hydroxy-2-methylpropanamide (8j).** MS: calcd, 532.23; obsd, 533.20.

3-Amino-*N*-{(2*S*)-3-(biphenyl-4-yl)-1-[(2*S*)-2-(5,6-dichloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]-1-oxopropan-2-yl}-2,2-dimethylpropanamide (8k). MS: calcd, 577.20; obsd, 578.20

3-Amino-*N*-{(2*S*)-3-(biphenyl-4-yl)-1-[(2*S*)-2-(5,6-dichloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]-1-oxopropan-2-yl}-3-methylbutanamide (8l). MS: calcd, 577.20; obsd, 578.20.

2-Amino-*N*-{(2*S*)-3-(biphenyl-4-yl)-1-[(2*S*)-2-(5-*tert*-butyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]-1-oxopropan-2-yl}-2-methylpropanamide (8m). MS: calcd, 501.31; obsd, 502.24.

***N*-{(2*S*)-3-(Biphenyl-4-yl)-1-[(2*S*)-2-(5-*tert*-butyl-1*H*-imidazol-2-yl)pyrrolidin-1-yl]-1-oxopropan-2-yl}-2-hydroxy-2-methylpropanamide (8n).** MS: calcd, 502.29; obsd, 503.23.

2-Amino-*N*-{(2*S*,3*S*)-3-(biphenyl-4-yl)-1-[(2*S*)-2-(5,6-dichloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]-1-oxobutan-2-yl}-2-methylpropanamide (Nonpreferred Name) (8o). Step 1: Preparation of (*E*)-Methyl 2-Acetamido-3-bromobut-2-enoate. Into a 1000 mL four-necked round-bottom flask was placed a solution of (*Z*)-methyl 2-acetamidobut-2-enoate[16] (36 g, 229.30 mmol, 1.00 equiv) in DCM (400 mL). To the above was added NBS (48.75 g, 273.91 mmol, 1.20 equiv) in several batches. The reaction mixture was stirred overnight at room temperature. The resulting solution was diluted with 500 mL of H_2O qand extracted with 3 × 200 mL of DCM. The combined organic layer was washed with 3 × 200 mL

of brine, dried, and concentrated under vacuum. The residue was dissolved in THF (400 mL), followed by addition of LiHDMS (45.74 g, 273.89 mmol, 1.20 equiv) dropwise with stirring at −78 °C. The resulting solution was stirred for an additional 30 min at −78 °C and quenched by the addition of 1000 mL of water. The resulting solution was extracted with 6 × 500 mL of ethyl acetate. The organic layers were combined, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was washed with 600 mL of PE and dried. This resulted in 39.5 g (78%) of (*E*)-methyl 2-acetamido-3-bromobut-2-enoate as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.68 (s, 1H), 3.62 (s, 3H), 2.32 (s, 3H), 1.92 (s, 3H).

Step 2. Preparation of Methyl (2*E*)-2-(Acetylamino)-3-(biphenyl-4-yl)but-2-enoate. Into a 5 L three-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen was placed a solution of (4-phenylphenyl)boranediol (150 g, 757.58 mmol, 1.00 equiv) in benzene (2250 mL), then added (*E*)-methyl 2-acetamido-3-bromobut-2-enoate (178.8 g, 757.63 mmol, 1.00 equiv), a solution of Cs_2CO_3 (247 g, 758.13 mmol, 1.00 equiv) in water (758 mL), and bis(triphenylphosphine)-palladium(II) chloride (15.75 g, 22.44 mmol, 0.03 equiv). The resulting solution was stirred for 3 h at 80 °C, then cooled and diluted with 2000 mL of EtOAc. The isolated solid was collected by filtration, washed with 1 × 500 mL of EtOH, and dried. This resulted in 215 g (92%) of the titled compound as a white solid.

Step 3: Asymmetric Hydrogenation of Methyl (2*E*)-2-(Acetylamino)-3-(biphenyl-4-yl)but-2-enoate.[15] Into a 1000 mL pressure tank reactor were added a solution of methyl (2*E*)-2-(acetylamino)-3-(biphenyl-4-yl)but-2-enoate (30 g, 96.97 mmol, 1.00 equiv) in MeOH (300 mL), a solution of J002-1 (2.6 g, 4.79 mmol, 0.05 equiv), and $(COD)_2RhBF_4$ (1.86 g, 4.58 mmol, 0.05 equiv) in MeOH (30 mL) DCM (30 mL), and an additional amount of DCM (200 mL). The resulting solution was pressurized with hydrogen to 35 atm and stirred for 18 h at 30 °C. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column and eluted with ethyl acetate/hexane (3:2). This resulted in 23 g (76%) of methyl (2*S*,3*S*)-2-(acetylamino)-3-(biphenyl-4-yl)butanoate as a white solid.

Step 4. Into a 20 L four-necked round-bottom flask were placed methyl (2*S*,3*S*)-2-(acetylamino)-3-(biphenyl-4-yl)butanoate (38 g, 122.19 mmol, 1.00 equiv), hydrochloric acid (3805 mL), and water (2650 mL). The resulting mixture was stirred for 10 h at 100 °C, then cooled and filtered. To the solid was added tetrahydrofuran (500 mL), sodium bicarbonate (30.57 g, 366.55 mmol, 3.00 equiv), and $(Boc)O_2$ (53.37 g, 244.37 mmol, 2.00 equiv). The resulting solution was stirred for 3 h at room temperature, then adjusted to pH 5−6 with hydrochloric acid (1 mol/L). The resulting solution was extracted with 3 × 300 mL of ethyl acetate. The organic layers were combined, washed with 3 × 300 mL of brine, dried, and concentrated under vacuum. The residue was applied onto a silica gel column and eluted with ethyl acetate/petroleum ether (1:5−1:1). This resulted in 21.6 g (50%) of (2*S*,3*S*)-3-(biphenyl-4-yl)-2-[(*tert*-butoxycarbonyl)amino]-butanoic acid as a light-yellow solid.

Step 5. Into a 500 mL four-necked round-bottom flask purged and maintained with an inert atmosphere of nitrogen were placed a solution of (2*S*,3*S*)-3-(biphenyl-4-yl)-2-[(*tert*-butoxycarbonyl)amino]butanoic acid (21.6 g, 60.85 mmol, 1.00 equiv) in DMF (100 mL), 5,6-dichloro-2-(pyrrolidin-2-yl)-1*H*-benzimidazole (30.5 g, 92.71 mmol, 1.50 equiv), and HOBT (15.78 g, 103.14 mmol, 1.70 equiv), followed by the addition of *N*-ethyl-*N*-isopropylpropan-2-amine (31.43 g, 243.64 mmol, 4.00 equiv) and EDC (17.45 g, 91.36 mmol, 1.50 equiv). The resulting solution was stirred overnight at room temperature, then quenched by the addition of 500 mL of H_2O. The resulting solution was extracted with 3 × 300 mL of ethyl acetate. The organic layers were combined, washed with 3 × 300 mL of $NaHCO_3$ solution, 3 × 300 mL of NH_4Cl solution, and 3 × 300 mL of brine, dried over anhydrous sodium sulfate, and concentrated under vacuum. The residue was applied onto a silica gel column and eluted with ethyl acetate/petroleum ether (85:15−1:1). The collected fractions were combined and concentrated under vacuum to give 18 g (50%) of *tert*-butyl {(2*S*,3*S*)-3-(biphenyl-4-yl)-1-[(2*S*)-2-(5,6-dichloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]-1-oxobutan-2-yl}carbamate as a light-red solid.

Step 6. Acetyl chloride (1.9 g, 24.20 mmol, 1.20 equiv) was added dropwise to a stirred mixture of methanol (50 mL) and 1,4-dioxane (20 mL). This was followed by the addition of *tert*-butyl {(2*S*,3*S*)-3-(biphenyl-4-yl)-1-[(2*S*)-2-(5,6-dichloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]-1-oxobutan-2-yl}carbamate (5 g, 8.43 mmol, 1.00 equiv) in several batches. The resulting solution was stirred for 2 h at room temperature. The resulting mixture was concentrated under vacuum. The residue was coevaporated with 3 × 100 mL of EtOAc to afford 2 g (42%) of 2-{(2*S*)-1-[(2*S*,3*S*)-2-ammonio-3-(biphenyl-4-yl)butanoyl]pyrrolidin-2-yl}-5,6-dichloro-1*H*-benzimidazol-1-ium dichloride as a white solid.

Step 7. Into a 25 mL three-necked round-bottom flask were placed a solution of 2-{(2*S*)-1-[(2*S*,3*S*)-2-ammonio-3-(biphenyl-4-yl)butanoyl]pyrrolidin-2-yl}-5,6-dichloro-1*H*-benzimidazol-1-ium dichloride (700 mg, 1.24 mmol, 1.00 equiv) in dichloromethane (5 mL), *tert*-butyl 2-methyl-3-oxobutan-2-ylcarbamate (378 mg, 1.86 mmol, 1.51 equiv), HATU (703.5 mg, 1.85 mmol, 1.50 equiv), and *N*-ethyl-*N*-isopropylpropan-2-amine (560 mg, 4.34 mmol, 3.51 equiv). The resulting solution was stirred overnight at room temperature and quenched by the addition of 20 mL of water. The resulting solution was extracted with 3 × 20 mL of ethyl acetate. The organic layers were combined, washed with 3 × 20 mL of sodium bicarbonate solution, and 3 × 20 mL of NH_4Cl solution, then dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by eluting through a silica gel column with a (1:4−4:1) EtOAc:PE solvent system. This resulted in 0.5 g (58%) of *tert*-butyl [1-({(2*S*,3*S*)-3-(biphenyl-4-yl)-1-[(2*S*)-2-(5,6-dichloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]-1-oxobutan-2-yl}amino)-2-methyl-1-oxopropan-2-yl]carbamate as a yellow solid.

Step 8. Into a 250 mL 3-necked round-bottom flask were placed methanol (200 mL) and 1,4-dioxane (100 mL), then added acetyl chloride (1.9 g, 24.36 mmol, 1.20 equiv) dropwise with stirring, followed by addition of *tert*-butyl [1-({(2*S*,3*S*)-3-(biphenyl-4-yl)-1-[(2*S*)-2-(5,6-dichloro-1*H*-benzimidazol-2-yl)pyrrolidin-1-yl]-1-oxobutan-2-yl}amino)-2-methyl-1-oxopropan-2-yl]carbamate (14 g, 20.65 mmol, 1.00 equiv) in several batches. The resulting solution was stirred for 2 h at room temperature and concentrated under vacuum. The residue was coevaporated with 3 × 200 mL of EtOAc to give 12 g (89%) of compound **8o** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.927 (3H, s), 1.18−1.19 (3H, d, J = 6.8 Hz), 1.35 (3H, s), 2.04−2.07 (1H, t), 2.18−2.40 (2H, m), 3.22−3.26 (1H, m), 3.86−3.90 (1H, m), 4.14−4.16 (1H, m), 4.90−4.95 (1H, m), 5.26−5.29 (1H, m), 7.33−7.37 (3H, t), 7.44−7.50 (4H, m), 8.07−7.59 (2H, d), 8.09−8.30 (2H, d), 8.42(1H, d). MS: calcd, 577.20; obsd, 578.12.

5,6-Dichloro-2-{(2*S*)-1-[2-methylalanyl-(*βS*)-*β*-methyl-L-phenylalanyl]azetidin-2-yl}-1*H*-benzimidazole (8p). MS: calcd, 487.15; obsd, 488.18.

5,6-Dichloro-2-{(2*S*)-1-[2-methylalanyl-(*βS*)-*β*-methyl-L-phenylalanyl]pyrrolidin-2-yl}-1*H*-benzimidazole (8q). MS: calcd, 501.17; obsd, 502.25.

***N*-[(2*S*,3*S*)-1-[(2*S*)-2-(5,6-Dichloro-1*H*-benzimidazol-2-yl)-pyrrolidin-1-yl]-3-(4′-fluorobiphenyl-4-yl)-1-oxobutan-2-yl]-2-hydroxy-2-methylpropanamide (8r).** MS: calcd, 596.18; obsd, 597.11.

***N*-{(2*S*,3*S*)-1-[(2*S*)-2-(5,6-Dichloro-1*H*-benzimidazol-2-yl)-pyrrolidin-1-yl]-1-oxo-3-phenylbutan-2-yl}-2-hydroxy-2-methylpropanamide (8s).** MS: calcd, 502.15; obsd, 503.22.

5,6-Dichloro-2-{(2*S*)-1-[2-methylalanyl-4-(1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-L-phenylalanyl]pyrrolidin-2-yl}-1*H*-benzimidazole (8t). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.55 (d, 1H, α-NH), 8.06 (br, 3H, NH_3), 8.03 (d, 1H), 7.79 (s, 2H), 7.70

(dd, 1H), 7.34 (d, 2H), 7.27 (d, 2H), 6.45 (d, 1H), 5.16 (dd, 1H), 4.78 (m, 1H), 3.84 (m, 1H), 3.77 (m, 1H), 3.50 (s, 3H), 3.12 (dd, 1H), 2.82 (dd, 1H), 2.0−2.22 (m, 4H), 1.42 (s, 3H), 1.30 (s, 3H). MS: calcd, 594.19; obsd, 595.09, 597.05.

5,6-Dichloro-2-[(2*S*)-1-(2-methylalanyl-*β*,*β*-dimethyl-L-phenylalanyl)pyrrolidin-2-yl]-1*H*-benzimidazole (8u). MS: calcd, 515.19; obsd, 516.71.

Enzyme Assay. The activity of recombinant human or mouse PrCP was measured using continuous fluorometric assays in 96-well plate format (Costar Black polystyrene plates (cat. no. 3792)). Briefly, appropriate dilutions of enzyme (1nM final concentration) were added to reaction mixtures containing the substrate Mca-Ala-Pro-Lys(Dnp)-OH (Anaspec, Inc., San Jose, CA. (cat. no. 60757)) at a concentration of 25 μM (K_m = 50 μM) in the presence or absence of the inhibitor of interest. The final reaction volume was 100 μL, and different dilutions of inhibitors were added as DMSO solutions to give a total 1% organic solvent content. Experiments were carried out at 37 °C under standard reaction conditions defined as 10 mM sodium acetate, 100 mM NaCL, 25 μg/mL BSA, pH 5.5. Serum shift assays were carried out in the same conditions described above, including 1% mouse serum albumin (SIGMA, cat. no. A3139, containing free fatty acid) and increasing the concentration of substrate to 100 μM in order to detect some measurable PrCP activity. Fluorescence was monitored continuously for 30 min in a Molecular Devices Spectramax GeminiXS fluorescent plate reader, using an excitation wavelength of 320 nm and an emission wavelength of 405 nm.

Single-point inhibition assay was run in duplicate or triplicate, and standard deviation was generally within < 50%. IC_{50} titration was run with 11 points starting from 10 μM to 0.0655 μM once. For potent compounds, the titration was repeated starting at 1 μM.

In Vivo Study. Male C57BL/6NT wild-type and PrCP −/− mice (N6 C57BL/6NT background) mice from Taconic Farms, Inc. at 6 weeks of age, were placed on an irradiated High Fat Diet (HFD: RD12942i Research Diets) at Taconic Farms, Inc. Animals were subsequently transferred to the animal vivarium at Merck Research Laboratories in Rahway, NJ. Obese mice, approximately 18−20 weeks old and weighing approximately 40−45 g, were individually housed and conditioned to PO QD dosing until body weights were stable.

Mice were grouped according to their body weights (N = 8 per group) and orally dosed by gavage with either vehicle (10% Tween−water) alone or with 100 mpk compound **8o** prior to the onset of the dark cycle for 5 consecutive days. Daily food intake and body weight were measured and analyzed. Four days prior to the study and at the fifth day of the study, whole-body NMR was used to measure body composition. All data are presented as mean ± SEM. Statistical significance was calculated using Student's t test with differences considered significant when 2-tailed $p < 0.05$.

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