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Benzo[*d*]imidazole inhibitors of Coactivator Associated Arginine Methyltransferase 1 (CARM1)—Hit to Lead studies

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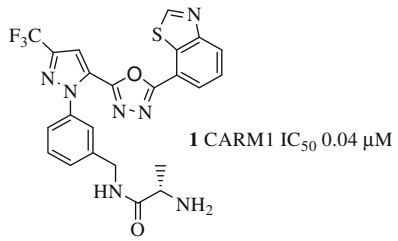
ABSTRACT

Hit to Lead optimization and SAR development led to the identification of the potent and selective benzo[*d*]imidazole inhibitor (**17b**) of Co-activator Associated Arginine Methyltransferase (CARM1).

Post-translational modifications of proteins are increasingly being recognized as essential to their function in cells. In particular, methylation of histones which are an essential component of chromatin, has been shown to play an important role in the regulation of gene transcription.[1] Protein arginine methyl transferases (PRMTs), which transfer a single methyl group from the methy donor S-Adenosyl-L-Methionine (SAM) to an individual arginine residue in the target substrate, have recently emerged as crucial players in this process.[2] The PRMT family, for which 11 different transcripts have been identified to date, has been implicated in processes as varied as chromatin remodeling, signal transduction, proliferation, mRNA splicing, RNA processing, nucleo-cytoplasmic shuttling and DNA repair.[3–5] PRMT4 or CARM1 (Co-activator associated arginine methyltransferase-1) specifically drew our interest given its contributions to nuclear hormone receptor (NHR) signaling, NF-kB signaling and cancer. It was recently shown that CARM1 was up-regulated during the progression of prostate cancer.[6] Over-expression of CARM1 was seen in both androgen-stimulated and castration resistant prostate cancer tumors. We therefore rationalized that targeting CARM1 would be a viable approach for anti-cancer therapy. Hence, the identification of selective CARM1 inhibitors as tools to understand its function in cells is of significant interest.

To date, there have been only a few publications describing small molecule chemical modulators of CARM1.[7] Earlier, we described a pyrazole inhibitor **1** of CARM1 that showed selectivity versus related methyltranseferases (PRMT1 and PRMT3) (Fig. 1).[8] The identification of selective CARM1 inhibitors from different chemotypes as tools to investigate CARM1 cellular function and its relevance in disease state would be of significant interest. Herein, we report the Hits to Lead optimization studies that led to the identification of a selective and potent benzo[*d*]imidazole inhibitor of CARM1.

During the screening campaign of the corporate compound collection, benzo[*d*]imidazole related analogs represented by compound **2** (Fig. 2) were identified as hits with modest activity (IC_{50} 0.84 μM) in the CARM1 mediated methylation assay.[9] Based on this hit, we initiated Hits to Lead optimization efforts to further improve the in vitro potency of this series.



1 CARM1 IC_{50} 0.04 μM

Figure 1.

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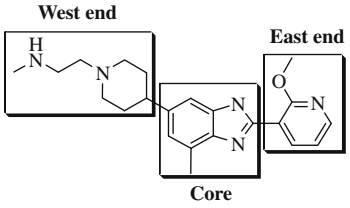


2 CARM-1 IC_{50} = 0.84 μM

Figure 2.

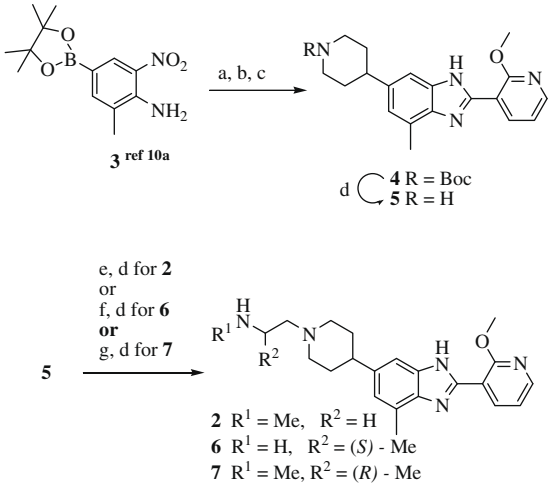
We first embarked on evaluating the West end of the molecule. Towards this objective, West end bearing terminal amines with a variety of dispositions and substitutions were synthesized using the approaches depicted in Scheme 1.

Very close modifications in the West end such as introduction of α-methyl group (as in **6** and **7**) as well as removal of internal nitrogen atom or abolishing basicity of either of nitrogen atoms (data not shown) led to a significant loss of potency (Table 1). This observation suggests that the binding pocket near the West end is tight, and there is very little tolerance for any changes.

Next, we turned our attention to the center core. Towards this end, we synthesized analogs (**8** and **9**) to understand the effect of a methyl group at the 4- and 1-position of the benzo[*d*]imidazole. The 4-des-methyl analog **8** and the 1-methyl analog **9** were prepared from 4-bromo-6-nitroaniline and 4-bromo-*N*-methyl-2-nitro aniline, respectively, using a similar approach as shown in Scheme 1. Also, to evaluate the effect of introduction of a nitrogen atom at the 4-position, imidazo-pyridine analog **14** was synthesized as shown in Scheme 2.

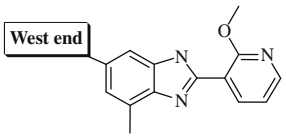
The SAR of the core region is summarized in Table 2. The 4-des-methyl analog (**8**) was slightly less potent while 1-methyl substitution at the nitrogen of the benzo[*d*]imidazole (as in **9**) resulted in further loss of activity. Introduction of a nitrogen atom (**14**) however was tolerated but did not offer any additional advantage.

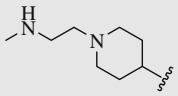
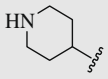
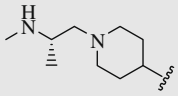
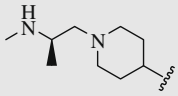
We then explored the SAR of the East end (Table 3). A modified synthetic route as depicted in Scheme 3 was used to assemble these analogs.

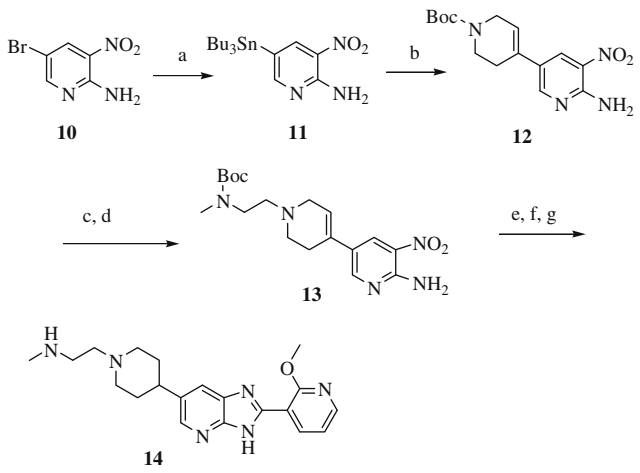


Scheme 1. Reagents and conditions: (a) *tert*-butyl 4-(trifluoro-methylsulfonyloxy)-5,6-dihydropyridine-1(2*H*)-carboxylate,[10d] $PdCl_2dppf$, Cs_2CO_3, DMF, 90 °C, 86%; (b) H_2 (50 psi), Pd/C (10%), MeOH, 70–80%; (c) 2-methoxynicotinaldehyde, MeOH, rt; (d) 25% TFA/DCE; (e) *tert*-butyl methyl(2-oxoethyl)carbamate, sodium triacetoxyborohydride, DCE, 70%; (f) *tert*-butyl (*S*)-methyl(1-oxopropan-2-yl)carbamate, sodium triacetoxyborohydride, DCE, 65%; (g) *tert*-butyl (*R*)-methyl(1-oxopropan-2-yl)carbamate, sodium triacetoxyborohydride, DCE, 70%.

Table 1
SAR for the West end



Compd[11]	West end	CARM 1 IC_{50} (μM)[9]
2		0.84
5		17
6		>30
7		>30

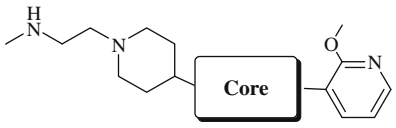


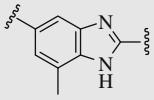
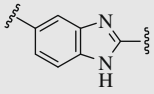
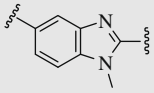
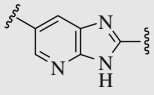
Scheme 2. Reagents and conditions: (a) $(Bu_3Sn)_2$, $Pd(PPh_3)_4$, dioxane, microwave at 200 °C, 1 h, 54%; (b) *tert*-butyl 4-(trifluoromethylsulfonyloxy)-5,6-dihydropyridine-1(2*H*)-carboxylate, Pd_2dba_3, $AsPPh_3$, CuI, NMP, 50 °C, 55%; (c) 25% TFA/DCE; (d) *tert*-butyl methyl(2-oxoethyl)carbamate, sodium triacetoxyborohydride, DCE; (e) H_2 (50 psi), Pd/C (10%), MeOH; (f) 2-methoxynicotinaldehyde; (g) 25% TFA/DCE, 28% from **12**.

SAR from this study showed that almost all of the substituted phenyl and pyridyl groups were tolerated (only partial data displayed). Interestingly, an unsubstituted phenyl group was not tolerated. Within the set of substituted phenyls, compounds with 2,6-disubstituted phenyl group were preferred (as in **17b** and **17d**), indicating conformational preferences for the activity. Generally, phenyl ring bearing at least one hydrogen bond accepting *ortho*-substituents were favored.

Compound **17b** was further evaluated in biochemical assays using other CARM1 substrates (HuR and PABP). The compound showed a similar level of activity using these substrates.[8a] In order to assess the selectivity of this series versus various closely related PRMT's, compound **17b** was evaluated for its ability to inhibit

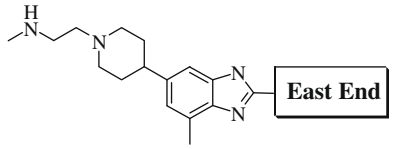
Table 2
SAR for the Core

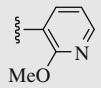
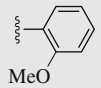
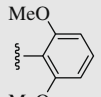
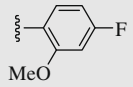
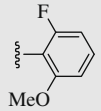


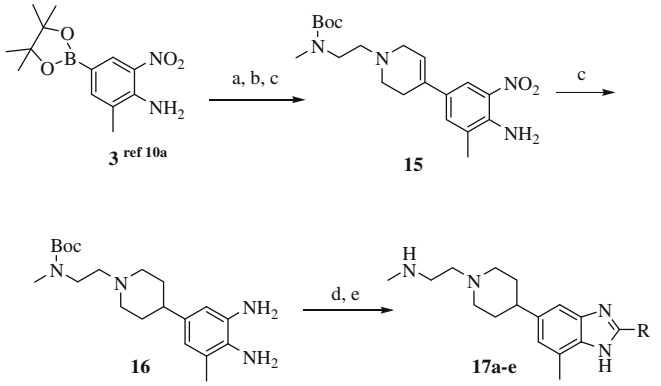
Compd[11]	Core	CARM 1 IC_{50}[9] (μM)
2		0.84
8		1.2
9		2.9
14		0.7

PRMT1 and PRMT3.[12] Compound **17b** was found to be significantly less active against these enzymes (IC_{50} >25 μM) suggesting selectivity in binding and inhibition.

Table 3
SAR for the East end



Compd[11]	East end	CARM 1 IC_{50}[9] (μM)
2		0.84
17a		0.41
17b		0.07
17c		0.26
17d		0.12
17e		4.6



Scheme 3. Reagents and conditions: (a) *tert*-butyl 4-(trifluoro-methylsulfonyloxy)-5,6-dihydropyridine-1(2*H*)-carboxylate,[10d] $PdCl_2dppf$, Cs_2CO_3, DMF, 90 °C, 89%; (b) 25% TFA/DCE; (c) *tert*-butyl methyl(2-oxoethyl)carbamate, sodium triacetoxyborohydride, DCE, 96% over two steps; (d) H_2(50 psi), Pd/C(10%), MeOH, 100%; (e) aromatic aldehydes; (e) 25% TFA/DCE.

In summary, through Hits to Lead optimization and SAR exploration, we have identified benzo[*b*]imidazole derivative **17b** as a potent and selective inhibitor of CARM1. Further work leading to optimization of this series will be reported in the future.

References and notes

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9. A filter based assay as described in Ref. 8a was used to test compounds that specifically inhibited CARM1 dependent methylation. Human full length GST-tagged CARM1 purified from baculovirus infected insect cells was used as the source for enzyme. Histone H3 (Roche Applied Science) and tritiated Sadenosyl-L-methionine (SAM) (Amersham Pharmacia Biotech) were used as the substrate and cofactor, respectively, for the assay. The values reported are the average of three with <10% variability.
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11. (a) All compounds were characterized by LC–MS and NMR analysis. In addition, the yields were based on the weight of pure product unless mentioned otherwise. (b) Analytical data for compound **17b**: ^{1}H NMR (500 MHz, methanol-d_3, 55 °C) δ ppm 7.68 (t, J = 8.52 Hz, 1H) 7.52 (s, 1H) 7.35 (s, 1H) 6.95 (s, 1H), 6.93 (s, 1H), 3.96 (s, 6H) 3.50–3.57 (m, 2H) 3.40–3.45 (m, 2H) 3.20–3.28 (m, 2H), 2.96–3.04 (m, 1H) 2.84–2.95 (m, 2H) 2.80 (s, 3H), 2.65 (s, 3H) 2.07–2.17 (m, 4H) MS (ESI) *m/z* 514.12 (M+H).
12. PRMT1 and PRMT3 specific methylation assays were performed using assay conditions as reported in Ref. 8.