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Pyrazole inhibitors of coactivator associated arginine methyltransferase 1 (CARM1)

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ABSTRACT

This study reports the identification and Hits to Leads optimization of inhibitors of coactivator associated arginine methyltransferase (CARM1). Compound **7b** is a potent, selective inhibitor of CARM1.

Members of the protein arginine methyl transferase (PRMT) family have been implicated in a wide variety of cellular processes including nuclear hormone receptor (NHR) mediated signaling, protein–protein interactions, protein trafficking, mRNA splicing and processing, and transcriptional regulation.[1] The family, which consists of at least 9 members (PRMTs 1–9), catalyzes the generation of asymmetric (type I) or symmetric (type II) di-methyl arginine residues on substrate proteins using *S*-adenosyl methionine (SAM) as the methyl donor.[2] CARM1 (or PRMT4), a type I enzyme, has been shown to methylate a wide variety of substrate proteins that can be roughly classified into three categories. In the first category, CARM1 methylates histone H3 and p300/CBP, thereby altering chromatin architecture and impacting transcriptional initiation.[3] Thus, the recruitment of CARM1 to the promoter regions of genes regulated by transcription factors such as nuclear hormone receptors, NF-κB, p53, etc. and the subsequent methylation of histone H3 result in increased gene expression by making the chromatin more accessible to the transcriptional machinery. In the second category, methylation by CARM1 of proteins involved in splicing such as SmB, U1C, SAP49, and CA150 implicates CARM1 in regulating alternative splicing.[4] Finally, methylation of RNA-binding proteins such as HuR, HuD, and PABP indicates a role for CARM1 in mRNA processing and stabilization.[5] Taken together, these observations point to a crucial role for CARM1 in modulating gene expression at multiple critical levels. More recently, CARM1 has been shown to be up-regulated during the progression of prostate cancer.[6] Over-expression of CARM1 is seen in both androgen-stimulated and castration-resistant prostate cancer tumors. A convincing argument can therefore be made to support the hypothesis that targeting CARM1 would be a viable approach for anti-cancer therapy.

To date, there have been only a few publications describing small molecule chemical modulators of the PRMTs,[7] and none of these report selectivity toward inhibition of CARM1. For instance, Bedford and colleagues have identified compounds that inhibit arginine, but not lysine methylation by multiple PRMTs in vitro, and do not interfere with Ado-Met binding. The exact mechanism of inhibition by these compounds is unclear especially since some of the compounds can inhibit the methylation of some substrates, but not others. Therefore, the identification of selective CARM1 inhibitors as tools to interrogate its function in cells would be of significant interest. Herein, we report Hits to Lead SAR studies that led to the identification of a selective potent pyrazole inhibitor (**7b**) of CARM1.

From a high throughput screening effort, a pyrazole amide derivative (**1**) and closely related analogs were identified as 'hits' with modest activity in the CARM1 mediated methylation assay (Fig. 1).[8] Based on these hits, we initiated Hits to Lead efforts to further improve the in vitro potency of this series (see Table 1).

First, we embarked on exploration of the pyrazole amide (East end of core). The required scaffold was synthesized using the reported intermediate **2**.[9,10] SAR from this exercise indicated that truncation of the biphenyl moiety was permitted (**5d**) whereas small alkyl amides were not tolerated (**5a**–**b**) (see Scheme 1).

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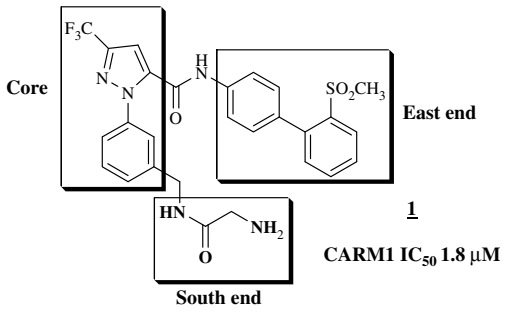
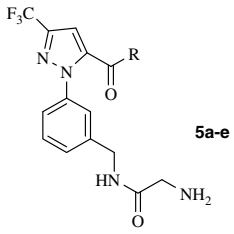


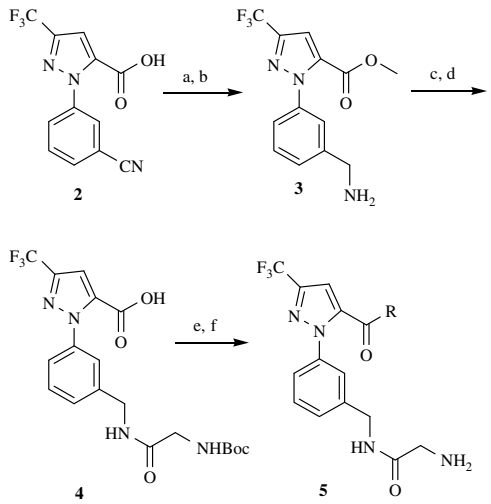
Figure 1. Initial Hit.

Table 1
Preliminary SAR for East end

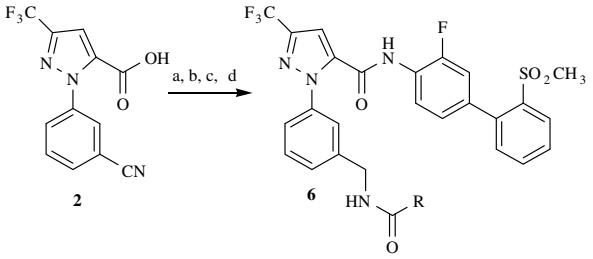


Compound #[10]	R	CARM1 IC_{50} (μM)[8]
5a	–$NHCH_3$	10.6
5b	–$N(CH_3)_2$	29.8
5c	–NH-Ph	7.7
5d	–NH-(3-SO_2Me)-Ph	0.23
5e	–NH-(4-biphenyl)	1.42

We then examined the glycinamide portion (South end of core) of **1**. The synthesis of these analogs was readily achieved using the approach outlined in Scheme 2. As shown in Table 2, α-amino acids at the South end were found to be preferred over either β or α, α′ di-substituted amino acid amides. Within the subset of analogs prepared from α-amino acids, the (*S*)-enantiomer (**6b**) was about 150-fold more active than the corresponding (*R*)-enantiomer (**6c**) (only data for alanine are shown). Additionally, neither bulky substituents on the α-carbon nor methylation of the terminal amino group were tolerated (see Table 2).

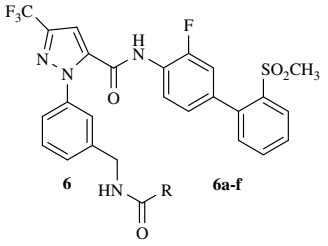


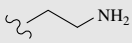
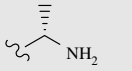
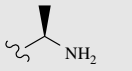
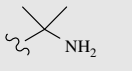
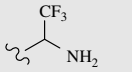
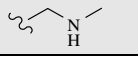
Scheme 1. Reagents: (a) MeOH, $SOCl_2$, 92%; (b) H_2, 10% Pd/C, MeOH, 78%; (c) N-Boc-Gly-OSu, Et_3N, CH_2Cl_2, 95%; (d) 1 N aq KOH, MeOH, 90%; (e) R^1R^2NH, DIC, HOAt, DMF, 60–80%; (f) TFA–CH_2Cl_2 (1:1), 90–100%.



Scheme 2. Reagents: (a) 3-fluoro-2′-(methylsulfonyl)biphenyl-4-amine, EDCI, HOAt, Hunig's base, CH_2Cl_2, 90%; (b) H_2, Pd/C (10%), TFA (cat), MeOH, 80%; (c) $HCO_2(CR^1R^2)_n$ NHBoc.

Table 2
SAR for South end



Compound #[10]	R	CARM1 IC_{50} (μM)[8]
6a		16.9
6b		0.16
6c		20.1
6d		>30
6e		>30
6f		>30

Next we evaluated the amide portion (East end of core) of the lead while retaining the (*S*)-alanine benzylamide at the South end. New analogs were assembled using the approach outlined in Scheme 3. Among the various amines tried (only partial data shown), benzyl amines were found to be superior to aniline and aliphatic amines. Within the set of α-methyl benzyl amines, the (*R*)-enantiomer was ~60-fold more active than the (*S*)-enantiomer (Table 3).

Next, we evaluated alternative linkages for the alanine amide side chain, including positional isomers. These analogs were prepared as per Schemes 4 and 5, respectively. An approach identical to Scheme 5 was also used to prepare the *ortho*-benzylamide regioisomer (**16**).

The data from this exercise indicated that none of these modifications were tolerated. We also examined other pyrazole amide alternatives (East end of core), such as the benzyl carbamate (**17**) (Scheme 6). Among the various substitutes (data not shown for others), the carbamate was tolerated the best, and displayed only an 8-fold loss of potency compared to **7b**.

In order to assess the selectivity of this series versus various closely related PRMT's, we evaluated the most active compound (**7b**) for its ability to inhibit PRMT1 and PRMT3.[11] Compound **7b** was found to be significantly less active against these enzymes (IC_{50} > 25 μM) suggesting selectivity in binding and inhibition.