



Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Pyrazole inhibitors of coactivator associated arginine methyltransferase 1 (CARM1)

Ashok V. Purandare *, Zhong Chen, Tram Huynh, Suhong Pang, Jieping Geng, Wayne Vaccaro, Michael A. Poss, Jonathan Oconnell, Kimberly Nowak, Lata Jayaraman

Bristol-Myers Squibb Pharmaceutical Research and Development, Princeton, NJ 08543, USA

ARTICLE INFO

Article history:

Received 23 April 2008

Revised 6 June 2008

Accepted 9 June 2008

Available online 12 June 2008

Keywords:

Coactivator associated arginine

methyltransferase 1

CARM1

PRMT4

Protein arginine methyltransferase

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.

© 2008 Elsevier Ltd. All rights reserved.

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- κ B, 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).

* Corresponding author. Tel.: +1 609 252 4320; fax: +1 609 252 7446.

E-mail address: ashok.purandare@bms.com (A.V. Purandare).

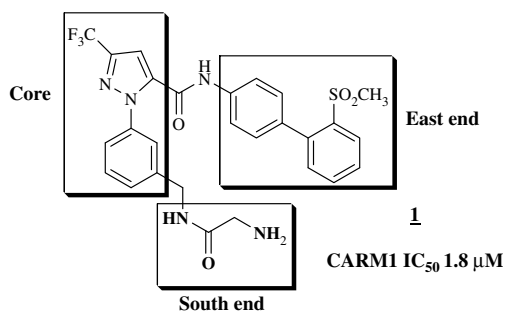
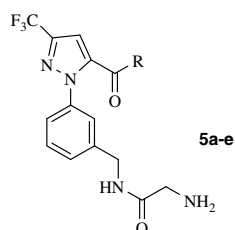
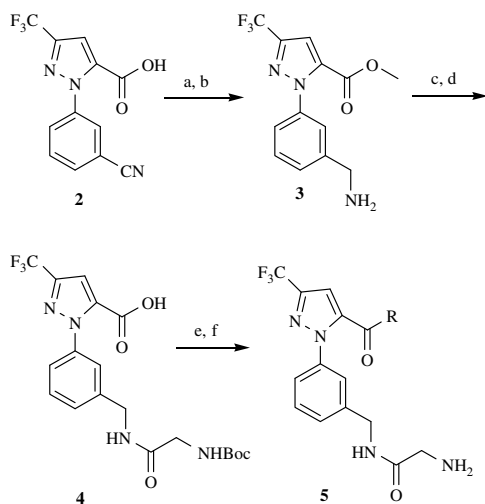
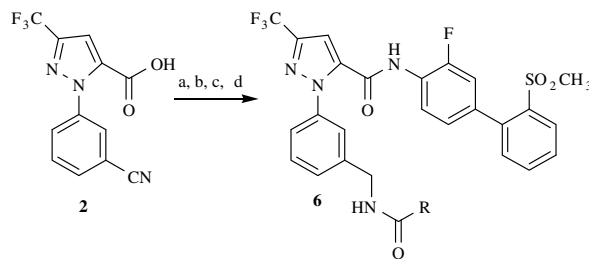
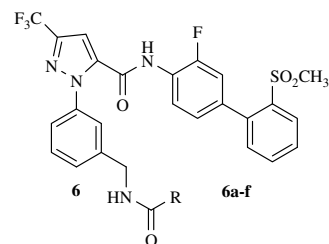


Figure 1. Initial Hit.

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 ₂ Me)–Ph	0.23
5e	–NH–(4–biphenyl)	1.42

We then examined the glycine amide 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, SOCl₂, 92%; (b) H₂, 10% Pd/C, MeOH, 78%; (c) N-Boc-Gly-OSu, Et₃N, CH₂Cl₂, 95%; (d) 1 N aq KOH, MeOH, 90%; (e) R¹R²NH, DIC, HOAt, DMF, 60–80%; (f) TFA–CH₂Cl₂ (1:1), 90–100%.Scheme 2. Reagents: (a) 3-fluoro-2'-(methylsulfonyl)biphenyl-4-amine, EDCl, HOAt, Hunig's base, CH₂Cl₂, 90%; (b) H₂, Pd/C (10%), TFA (cat), MeOH, 80%; (c) HCO₂(CR¹R²)_n NHBoc.Table 2
SAR for South end

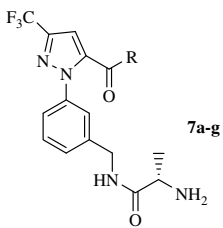
Compound # ¹⁰	R	CARM1 IC ₅₀ (μM) ⁸
6a		16.9
6b		0.16
6c		20.1
6d		>30
6e		>30
6f		>30

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).

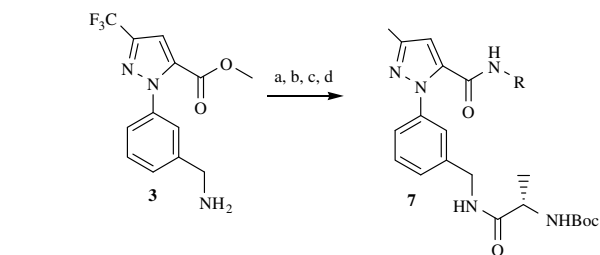
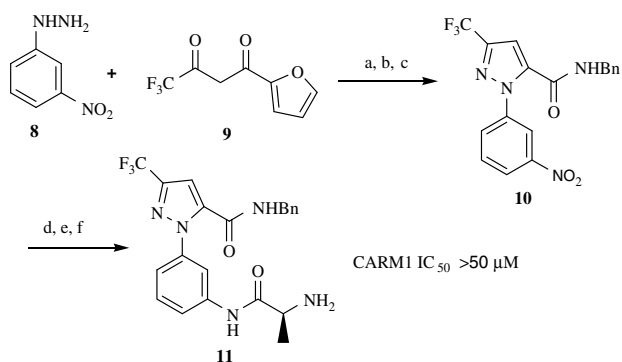
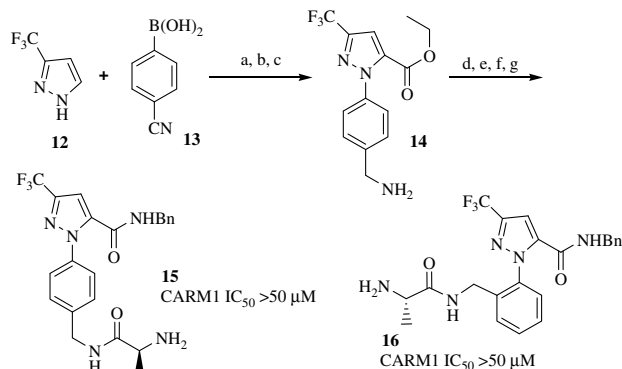
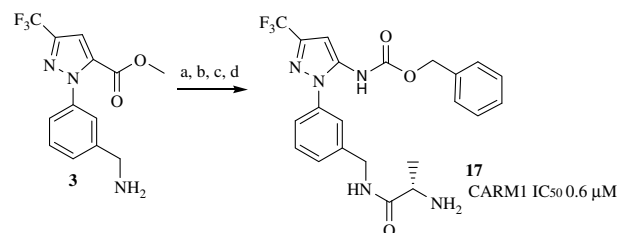
Next, we evaluated alternative linkages for the alanine amide side chain, including positional isomers. These analogs were prepared as per Schemes 4 and 5, respectively. An approach identical to Scheme 5 was also used to prepare the *ortho*-benzylamide regioisomer (**16**).

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 (IC₅₀ > 25 μM) suggesting selectivity in binding and inhibition.

Table 3
SAR for East end


Compound # ¹⁰	R	CARM1 IC ₅₀ (μM) ⁸
7a		1.32
7b		0.08
7c		3.4
7d		7.1
7e		0.18
7f		1.1
7g		4.3

**Scheme 3.** Reagents and conditions: (a) Boc-L-Ala-OSu, CH₂Cl₂, Et₃N, 92%; (b) 1 N aq KOH, MeOH, 90%; (c) RNH₂, DIC, HOAt, DMF, 60–75%; (d) TFA–CH₂Cl₂ (1:1), RT, 80–95%.**Scheme 4.** Reagents and conditions: (a) AcOH, 100 °C, 6 h, 60%; (b) KMnO₄, acetone–water, 60 °C, 3 h, 30%; (c) BnNH₂, EDCI, HOAt, Et₃N, DCE, 40%; (d) Zn, NH₄Cl, MeOH, 1 h, 86%; (e) Boc-L-Ala-OSu, CH₂Cl₂, Et₃N, 92%; (f) TFA–CH₂Cl₂ (4:1), 70%.**Scheme 5.** Reagents and conditions: (a) Cu(OAc)₂, pyridine, CH₂Cl₂, air, 2 days, 50%; (b) *n*-BuLi, NCCO₂Et, THF, 74%; (c) H₂, Pd/C, PtO₂ (1:1), EtOH, 2 h, 69%; (d) Boc-L-Ala-OSu, CH₂Cl₂, Et₃N, 82%; (e) aq NaOH, 60 °C, 1 h, 78%; (f) BnNH₂, EDCI, HOAt, Et₃N, 40%; (g) TFA–CH₂Cl₂ (1:4); 80%.**Scheme 6.** Reagents: (a) Boc-L-Ala-OSu, CH₂Cl₂, Et₃N, 92%; (b) 1 N aq KOH, MeOH, 90%; (c) DPPA, BnOH, 80–85%; (d) TFA–CH₂Cl₂ (1:1), 90–95% s.

In summary, through a Hits to Lead approach, we have identified pyrazole amide **7b** as a potent and selective inhibitor of CARM1. Further work related to the optimization of this class of inhibitors will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found in the online version, at [doi:10.1016/j.bmcl.2008.06.026](https://doi.org/10.1016/j.bmcl.2008.06.026).

References and notes

- Krause, C.; Yang, Z.; Kim, Y.; Lee, J.; Cook, J.; Pestka, S. *Pharm. Ther.* **2007**, *113*, 50.
- Zhang, Y.; Reinberg, D. *Genes Dev.* **2001**, *15*, 2343.
- Chen, D.; Ma, H.; Hong, H.; Koh, S.; Huang, S.; Schurter, B.; Aswad, D.; Stallcup, M. *Science* **1999**, *284*, 2174; Xu, W.; Chen, H.; Du, K.; Asahara, H.; Tini, M.; Emerson, B.; Montimony, M.; Evans, R. *Science* **2001**, *294*, 2507; Chevillard-Briet, M.; Trouche, D.; Vandel, L. *EMBO J.* **2002**, *21*, 5457.
- Cheng, D.; Cote, J.; Shaaban, S.; Bedford, M. *Mol. Cell* **2007**, *25*, 71.
- Li, H.; Park, S.; Kilburn, B.; Jelinek, M.; Henschen-Edman, A.; Aswas, D.; Stallcup, M.; Laird-Offringa, I. *J. Biol. Chem.* **2002**, *277*, 44623; Lee, J.; Bedford, M. *EMBO Rep.* **2002**, *3*, 268; Fujiwara, T.; Mori, Y.; Chu, D.; Koyama, Y.; Miyata, Y.; Tanaka, H.; Yachi, K.; Kubo, T.; Yoshikawa, H.; Tohyama, M. *Mol. Cell Biol.* **2006**, *26*, 2273.
- (a) Hong, H.; Kao, C.; Jeng, M.; Eble, J.; Koch, M.; Gardner, T.; Zhang, S.; Li, L.; Pan, C.; Hu, Z.; M. G.; Cheng, L. *Cancer* **2004**, *101*, 83; (b) Majumdar, S.; Liu, Y.; Ford, O.; Mohler, J.; Whang, Y. *Prostate* **2006**, *66*, 1292.
- (a) Cheng, D.; Yadav, N.; King, R. W.; Swanson, M. S.; Weinstein, E. J.; Bedford, M. T. *J. Biol. Chem.* **2004**, *279*, 23892; (b) Ragno, R.; Simeoni, S.; Castellano, S.; Vicidomini, C.; Mai, A.; Caroli, A.; Tramontano, A.; Bonaccini, C.; Trojer, P.; Bauer, I.; Brosch, G.; Sbardella, G. *J. Med. Chem.* **2007**, *50*, 1241; (c) Spannhoff, A.; Heinke, R.; Bauer, I.; Trojer, P.; Metzger, E.; Gust, R.; Schuele, R.; Brosch, G.; Sippl, W.; Jung, M. *J. Med. Chem.* **2007**, *50*, 2319; (d) Spannhoff, A.; Machmur, R.; Heinke, R.; Trojer, P.; Bauer, I.; Brosch, G.; Schuele, R.; Hanefeld, W.; Sippl, W.; Jung, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4150; (e) Mai, A.; Cheng, D.; Bedford, M.; Valente, S.; Nabbioso, A.; Perrone, A.; Brosch, G.; Sbardella, G.; Bellis, F. D.; Miceli, M.; Altucci, L. *J. Med. Chem.* **2008**.

8. **CARM1 assay.** A methylation based filter assay was devised 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 S-adenosyl-L-methionine (SAM) (Amersham Pharmacia Biotech) were used as the substrate and cofactor, respectively, for the assay. Methylation reactions were performed for 75–90 min at room temperature using enzyme (CARM1), substrate (histone H3) and cofactor (SAM) in the presence of methylation buffer (20 mM Tris-HCl, pH 8.0, 200 mM NaCl, 0.4 mM EDTA) and in the presence or absence of compound. Reactions were terminated using TCA and the reaction mixture was precipitated with BSA overnight. Reaction mixtures were then harvested the next morning by filtering the samples, and the filters washed. The signal on the filters was read in a Top Count after addition of MicroScint-20. Three independent IC₅₀ determinations were done. Values are reported as the average of three IC₅₀ values. The difference between the three IC₅₀ determinations was less than 25% in all cases.
9. Pinto, D. J. P.; Orwat, M. J.; Wang, S.; Fevig, J. M.; Quan, M. L.; Amparo, E.; Cacciola, J.; Rossi, K. A.; Alexander, R. S.; Smallwood, A. M.; Luetttgen, J. M.; Liang, L.; Aungst, B. J.; Wright, M. R.; Knabb, R. M.; Wong, P. C.; Wexler, R. R.; Lam, P. Y. S. *J. Med. Chem.* **2001**, 444, 566.
10. 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 **7b**: NMR (CD₃OD, δ): 8.2 (s, 1H), 7.96 (s, 1H), 7.71 (d, 1H), 7.33 (m, 5H), 7.2 (m, 1H), 7.1 (d, 1H), 4.6 (s, 1H), 4.4 (s, 1H), 3.9 (m, 1H), 1.3 (d, 3H); MS (ESI) m/z 446.18 (M+H).
11. PRMT1 and PRMT3 specific methylation assays were performed using assay conditions identical to the CARM1 assay⁸ with the following exceptions: GST-PRMT1 or GST-PRMT3, expressed and purified from bacteria, were used as the enzymes instead of CARM1. Histone H4 (Roche Applied Science) was used as the substrate in the PRMT1 assay. GST-GAR, expressed and purified from bacteria, was used as the substrate in the PRMT3 assay.