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RESEARCH ARTICLE

# Design and synthesis of selective, small molecule inhibitors of coactivator-associated arginine methyltransferase 1 (CARM1)†‡

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H. Ü. Kaniskan,[§,a] M. S. Eram, [§,b] J. Liu,[a] D. Smil,[b] M. L. Martini,[a] Y. Shen,[a] V. Santhakumar,[b] P. J. Brown,[b] C. Arrowsmith,[b,c] M. Vedadi,*[,b,d] and J. Jin*[,a]

Coactivator-associated arginine methyltransferase 1 (CARM1) is a type I protein arginine methyltransferase (PRMT) that catalyzes the conversion of arginine into monomethylarginine (MMA) and further into asymmetric dimethylarginine (ADMA). CARM1 methylates histone 3 arginines 17 and 26, as well as numerous non-histone proteins including CBP/p300, SRC-3, NCOA2, PABP1, and SAP49, while also functioning as a coactivator for various proteins that have been linked to cancer such as p53, NF-κβ, β-catenin, E2F1 and steroid hormone receptor ERα. As a result, CARM1 is involved in transcriptional activation, cellular differentiation, cell cycle progression, RNA splicing and DNA damage response. It has been associated with several human cancers including breast, colon, prostate and lung cancers and thus, is a potential oncological target. Herein, we present the design and synthesis of a series of CARM1 inhibitors. Based on a fragment hit, we discovered compound **9** as a potent inhibitor that displayed selectivity for CARM1 over other PRMTs.

## Introduction

Coactivator-associated arginine methyltransferase 1 (CARM1, also known as PRMT4) is a type I protein arginine methyltransferase (PRMT) along with PRMT1, PRMT3, PRMT6 and PRMT8, which are responsible for converting arginine into monomethylarginine (MMA) and further into asymmetric dimethylarginine (ADMA).[1] CARM1 methylates histone 3 arginines 17 and 26,[2, 3] as well as numerous non-histone proteins including CBP/p300, SRC-3, NCOA2, PABP1, SmB, HuR, HuD, CA150, SAP49, and U1C.[1] Therefore, it plays a role in cellular processes such as transcriptional activation,[4] RNA splicing,[4, 5] cellular differentiation,[6] cell cycle progression[7] and DNA repair.[8] It has been shown that the enzymatic activity of CARM1 is essential for the most of its *in vivo* functions.[9] CARM1 functions as a coactivator for various proteins that have been linked to cancer including p53, NF-κβ, β-catenin, E2F1 and steroid hormone receptor ERα.[1, 10-12] Overexpression of CARM1 in several human cancers including breast,[7] colon,[13] prostate[14] and lung[15] cancers has been reported. Thus, CARM1 is a potentially attractive therapeutical target for cancer and as a result there have been numerous studies directed toward the discovery of small molecule CARM1 inhibitors.[16, 17]

Several high throughput screening (HTS) campaigns resulted in the identification of pyrrazole-amide as well as benzo[d]imidazole CARM1 inhibitors.[18, 19] These initial reports were followed by structure-activity relationship (SAR) studies, which led to the discovery of small molecule inhibitors **1** and **2** with $IC_{50}$ values around 30 nM. These inhibitors displayed selectivity for CARM1 over PRMT1 and PRMT3 while the selectivity over other PRMTs was not reported (Figure 1).[20-23] We have recently reported a type I PRMT chemical probe, MS023 (**3**), which is potent against all type I PRMTs, but inactive against type II and type III PRMTs as well as other methyltransferases.[24] A series of adenosine-based CARM1 inhibitors with high potency and selectivity over PRMT1 and PRMT6 was also published.[25] Herein we report the discovery of selective CARM1 inhibitors and describe their design, synthesis and biological evaluation.

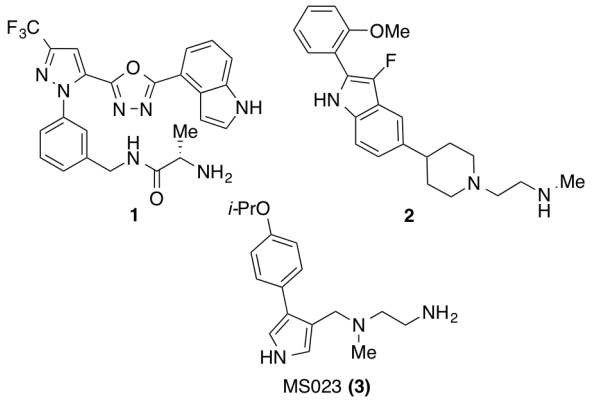


**Figure 1.** Structures of known CARM1 inhibitors **1**, **2** and the type I PRMT chemical probe **3**.

[a] *Departments of Pharmacological Sciences and Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, New York 10029, United States. E-mail: jian.jin@mssm.edu*
[b] *Structural Genomics Consortium, University of Toronto, Toronto, Ontario M5G 1L7, Canada. E-mail: m.vedadi@utoronto.ca*
[c] *Princess Margaret Cancer Centre and Department of Medical Biophysics, University of Toronto, Toronto, Ontario M5G 2M9, Canada.*
[d] *Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON M5S 1A8, Canada.*
† The authors declare no competing interests.
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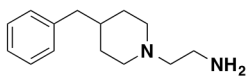
§ These authors contributed equally to this work.

## Results and Discussion

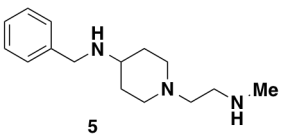
The alkyl-diamino tail, shown to be bound in the substrate-binding site of CARM1,[21] is the shared feature of inhibitors **1**-**3** (Figure 1) as well as of a recently published PRMT6 inhibitor.[26] In a recent study, a commercially available, diverse fragment library of compounds mimicking this alkyl-diamino tail were tested against PRMT6 and compound **4** was reported as a PRMT6 inhibitor with $IC_{50}$ of 300 ± 40 nM (Figure 2).[27] Compound **4** was 3- and 7-fold less potent for CARM1 ($IC_{50}$ = 1,000 ± 40 nM) and PRMT8 ($IC_{50}$ = 2100 ± 200 nM), respectively. It was not very potent against PRMT1 and PRMT3 (>40-fold less potent) and did not inhibit other methyltransferases. We sought to discover potent CARM1 selective inhibitors by using the fragment hit **4** as a starting point. Our initial SAR studies resulted in compounds **5** and **6** as promising CARM1 inhibitors (Figure 2). Compound **5** shares the (piperidinyl)ethan-1-amine core of **4**, but it is connected to the phenyl group via a methylene-amine (-$CH_2$NH-) linker instead of methylene (-$CH_2$-) linker. It was found to be 10-fold more potent for CARM1 ($IC_{50}$ = 471 ± 36 nM) over PRMT6 ($IC_{50}$ = 4,564 ± 1210 nM), exhibiting some selectivity (Figure 2). In addition, it had no appreciable inhibitory activity against PRMT1, PRMT3 and PRMT8 as well as PRMT5 and PRMT7 ($IC_{50}$ > 50,000 nM for all). On the other hand, compound **6** contains an (azetidinyl)ethan-1-amine core and –$CH_2$O- connection to the aryl ring (Figure 2). This inhibitor displayed good potency for CARM1 ($IC_{50}$ = 144 ± 37 nM) and was less than 10-fold selective over PRMT6 and PRMT8 ($IC_{50}$ = 1,079 ± 75 and 1,200 ± 70 nM, respectively) while it was inactive against other PRMTs.

Since inserting a nitrogen between the piperidine and benzyl groups of compound **4** (compound **5**, Figure 2) resulted in some selectivity for CARM1 over PRMT6, we further explored this scaffold and synthesized derivatives of **5** by adding substituents on the phenyl ring (Table 1). We mainly investigated meta-substitution based on the encouraging potency of compound **6** for CARM1. Among the meta-fluoro (**7**), meta-chloro (**8**) and meta-bromo (**9**) derivatives, **9** displayed the best potency for CARM1 with $IC_{50}$ of 94 ± 23 nM as well as selectivity over PRMT6 (around 20–fold, $IC_{50}$ = 2,160 ± 68 nM). The electron-withdrawing trifluoromethyl (**10**), or electron-donating triflourometoxy (**11**) substitution did not result in a more potent or selective inhibitor than **9.** The phenyl-substituted derivative (**12**) reversed the selectivity in favour of PRMT6 ($IC_{50}$ = 120 ± 13 nM) over CARM1 ($IC_{50}$ = 330 ± 43 nM).



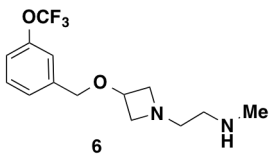

4

CARM1 ($IC_{50}$) = 1000 ± 40 nM
PRMT6 ($IC_{50}$) = 300 ± 40 nM




5

CARM1 ($IC_{50}$) = 471 ± 36 nM
PRMT6 ($IC_{50}$) = 4564 ± 1210 nM

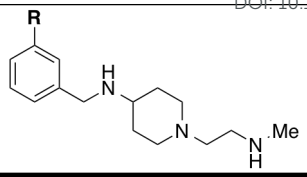



6

CARM1 ($IC_{50}$) = 144± 37 nM
PRMT6 ($IC_{50}$) = 1079 ± 75 nM

**Figure 2.** Structures of the fragment hit **4**, and initial hits **5** and **6**.

**Table 1.** $IC_{50}$ values of compounds **5**, **7-12**.

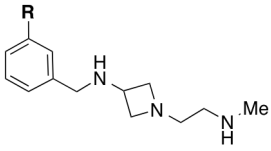



| Cmpd | R | CARM1 $IC_{50}$ (nM) | PRMT6 $IC_{50}$ (nM) |
|---|---|---|---|
| **5** | H | 471 ± 36 | 4,560 ± 1210 |
| **7** | F | 223 ± 57 | 1,890 ± 456 |
| **8** | Cl | 157 ± 54 | 2,920 ± 303 |
| **9** | Br | 94 ± 23 | 2,160 ± 68 |
| **10** | $CF_3$ | 302 ± 41 | 2,980 ± 298 |
| **11** | $OCF_3$ | 342 ± 48 | 4,730 ± 174 |
| **12** | Ph | 330 ± 43 | 120 ± 13 |

We then focused our attention on the (azetidinyl)ethan-1-amine scaffold represented by compound **6** (Figure 2). Since this scaffold with an oxygen linker did not show good selectivity over either PRMT6 or PRMT8, we utilized the methylene-amine (-CH2NH-) linker as in the piperidinyl series and synthesized substituted phenyl derivatives **13-18** (Table 2). While there was an overall increase in potency of these inhibitors as compared to compounds **7-12** for CARM1, the potency for PRMT6 was even greater in comparison and thus, the selectivity over PRMT6 suffered significantly. For example the meta-chloro (**14**) and meta-bromo (**15**) derivatives were potent for CARM1 with $IC_{50}$ of 50 ± 14 and 67 ± 9 nM, respectively. However, **14** and **15** displayed high potency for PRMT6 with $IC_{50}$ of 133 ± 8 and 249 ± 22 nM respectively, resulting in only 3 to 4-fold selectivity for CARM1 over PRMT6.

The synthetic route for the preparation of compounds **7-12** is shown in Scheme 1. The synthesis started with a nucleophilic displacement reaction between 4-amino-1-Boc-piperidine (**19**) and

**Table 2.** $IC_{50}$ values of compounds **13-18**.




| Cmpd | R | CARM1 $IC_{50}$ (nM) | PRMT6 $IC_{50}$ (nM) |
|---|---|---|---|
| **13** | F | 130 ± 29 | 101 ± 7 |
| **14** | Cl | 50 ± 14 | 133 ± 8 |
| **15** | Br | 67 ± 9 | 249 ± 22 |
| **16** | $CF_3$ | 94 ± 17 | 469 ± 64 |
| **17** | $OCF_3$ | 90 ± 25 | 434 ±27 |
| **18** | Ph | 69 ± 10 | 67 ± 13 |



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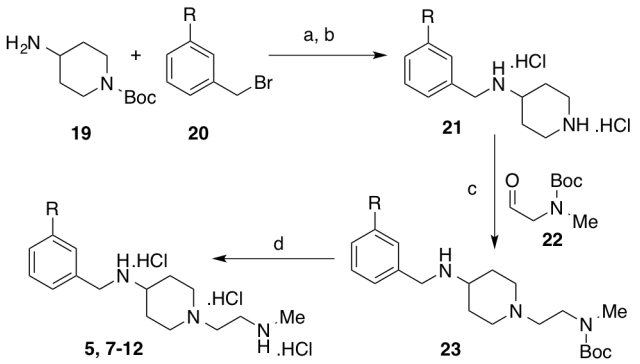


**Scheme 1.** General scheme for the synthesis of compounds **5**, **7-12**. Conditions: a. $Et_3N$, DCM b. Methanolic HCl (3N) c. $Et_3N$, $NaBH(OAC)_3$ d. Methanolic HCl (3N).

meta-substituted benzyl bromides (**20**). The resulting diamines were then treated with methanolic HCl to remove the *tert*-butoxy carbamate group. The reductive amination of amines **21** with N-Boc-(methylamino)acetaldehyde (**22**), followed by a deprotection reaction, afforded the desired compounds (**5** and **7-12**). A similar synthetic route was employed for the synthesis of compounds **13-18** starting with 3-amino-1-Boc-azetidine (see supporting information for detailed procedures).

Among all the derivatives synthesized, compound **9** emerged as the best inhibitor with good potency and around 20-fold selectivity toward CARM1 over PRMT6. In addition, we have tested Inhibitor **9** against other PRMTs in biochemical assays and found that it showed excellent selectivity for CARM1 over other type I PRMTs, PRMT1, PRMT3 ($IC_{50}$ > 50,000 nM) and PRMT8 ($IC_{50}$ = 9,200 ± 500 nM) as well as the type II and type III PRMTs, PRMT 5 and 7 ($IC_{50}$ > 50,000 nM).

To assess the mechanism of action (MOA) of inhibitor **9**, we evaluated the effect of the peptide substrate and cofactor S-adenosyl-*L*-methionine (SAM) concentrations on $IC_{50}$ values of **9** against CARM1. As shown in Figure **3**, increasing the peptide substrate or SAM concentrations had no effect on the $IC_{50}$ values of **9** against CARM1, suggesting that this inhibitor is noncompetitive with both the cofactor SAM and peptide substrate. It has been previously reported that active site binding inhibitors can display noncompetitive behavior in MOA studies.[28, 29] We and others have observed this phenomenon with PRMT inhibitors.[24, 26] For example, MOA studies of MS023 (**3**) suggested that the inhibitor was noncompetitive with both SAM and peptide substrate while the X-ray crystal structure of MS023 in complex with PRMT6 clearly showed that it occupies the substrate binding pocket. Therefore, based on the literature precedents, it is very likely that inhibitor **9** interacts with the substrate binding site of CARM1 to assert its inhibitory effect.

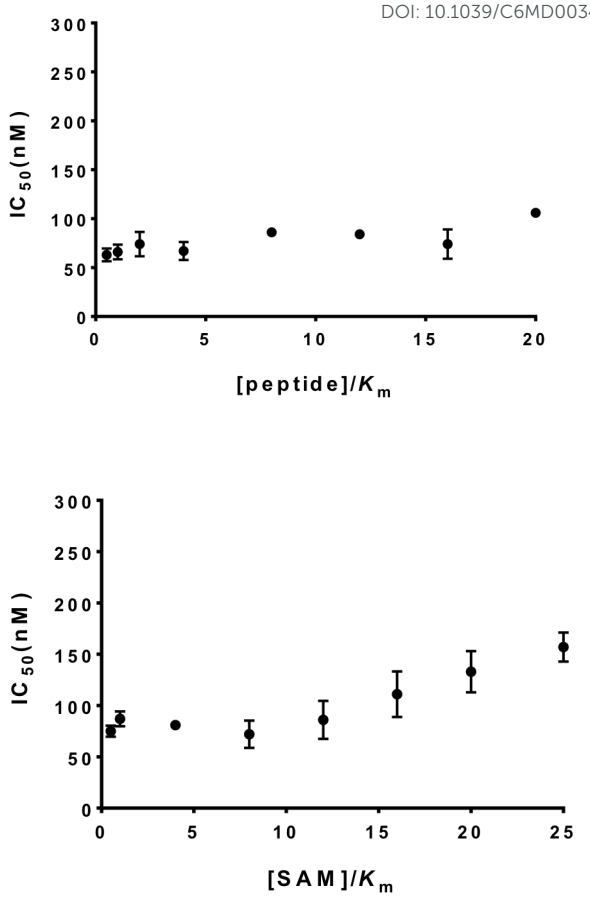


**Figure 3.** MOA study of compound **9** against CARM1 with changing concentrations of the peptide substrate (top) and the cofactor SAM (bottom).

## Conclusions

Starting from a reported fragment hit (**4**), a PRMT6 inhibitor, we discovered compound **9**, a potent and selective inhibitor of CARM1, through a SAR study. In biochemical assays, **9** displayed high potency ($IC_{50}$ = 94 ± 23 nM) for CARM1 and was around 20-fold selective for CARM1 over PRMT6 and highly selective over PRMT1, PRMT3, PRMT8 as well as PRMT5 and PRMT7. We believe that inhibitor **9** could be a useful tool for studying the role of CARM1 in health and disease. We anticipate that the work described here will facilitate further development of CARM1 selective inhibitors and chemical tools.

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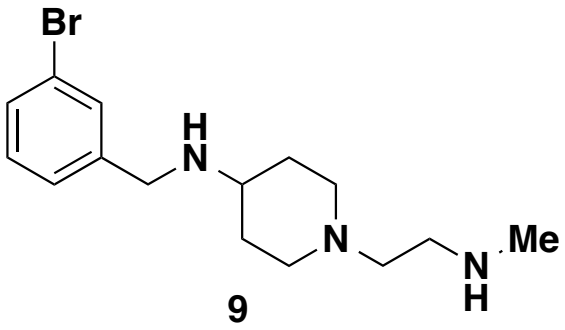
Structure-activity relationship studies, starting from a fragment hit, resulted in discovery of the compound **9**, an inhibitor of CARM1 with high potency and selectivity.




**9**

CARM1 ($IC_{50}$) = 94 ± 23 nM