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1,2-Diamines as inhibitors of co-activator associated arginine methyltransferase 1 (CARM1)

Eric Therrien [a], Guillaume Larouche [a], Sukhdev Manku [a], Martin Allan [a], Natalie Nguyen [a], Sylvia Styhler [b], Marie-France Robert [b], Anne-Christine Goulet [b], Jeffrey M. Besterman [b], Hannah Nguyen [b], Amal Wahhab [a,*]

[a] MethylGene Inc., Department of Medicinal Chemistry, 7220 rue Frederick-Banting, Montreal, Quebec, Canada H4S 2A1
[b] MethylGene Inc., Department of Pharmacology and Cell Biology, 7220 rue Frederick-Banting, Montreal, Quebec, Canada H4S 2A1

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ABSTRACT

We have identified the N^1-benzyl-N^2-methylethane-1,2-diamine unit as a substitute for the (*S*)-alanine benzylamide moiety for the design of co-activator associated arginine methyltransferase 1 (CARM1) inhibitors. The potency of these inhibitors is in the same order of magnitude as their predecessors and their clearance, volume of distribution, and half lives were greatly improved.

There is an emerging interest in the enzymes that modulate the methylation of protein lysine and arginine residues of histones. One such set of enzymes is the mammalian protein arginine methyltransferases (PRMTs), a family of at least nine members with diverse biological functions.[1] These enzymes catalyze the transfer of methyl groups from *S*-adenosyl methionine to Arg17 and Arg26 residues in histone H3, resulting in the formation of either asymmetric (Type I; PRMTs 1–4, 6 and 8) or symmetric (Type II; PRMTs 5, 7, and 9) ω-N_G,N_G-dimethylarginine tails on a wide variety of protein substrates.[2]

The co-activator associated arginine methyltransferase 1 (CARM1), also known as PRMT4, methylates proteins with roles in gene regulation at the level of chromatin remodeling (histone H3 and CBP/p300),[3a–d] RNA processing and stability (PABP, HuR and HuD)[3d–g] and RNA splicing (CA150, SAP49, SmB and U1C).[3h,i] CARM1 was initially identified as a secondary co-activator for the p160 steroid co-activator protein in transcription mediated by nuclear hormone receptors,[4] and was later found to be a positive co-regulator for a number of other transcriptional modulators such as SRC-3,[5a] CBP/p300,[3a,5b] β-catenin,[5c] p53,[5d,e] NF-kappa-B,[5f,g] CIITA,[5h] HTLV-1 Tax,[5i] PPARγ5j and c-fos.[5k] As CARM1 is recruited by a diverse panel of transcription factors, it is not surprising that its target genes encompass a variety of roles including cell growth regulation (estrogen receptor,[4] pS2,[5l] complement C3,[5j] glutathione peroxidase 3,[5j] UCP-1[5j]), inflammation (MIP-2,[5f] IP-10,[5f] Class II MHC) and adipogenesis (THRSP-14, FABP4/aP2; ACS5; adipsin, APOA-1 and -IV).[5j] The co-activator activity of CARM1 coincides with the arginine methylation of histone H3 and CBP/p300 on several target promoters. Mutations of critical residues in the catalytic domain of CARM1 has been shown to compromise transcriptional activation, suggesting that the integrity of the methyltransferase domain of CARM1 is important for its co-activator function.[4,5c,h]

Several studies demonstrated a role of CARM1 in cancer. Knockout or silencing of CARM1 impedes estrogen-stimulated gene expression, cell cycle progression and growth of breast cancer cells,[3d,5l] and evidence also links CARM1, cyclin E and steroid co-activator overexpression to high-grade breast cancer tumors.[6a] Genome-wide positional analyses show that overexpression gene signatures from ER-alpha positive primary breast tumors, derived from over 20 studies, are significantly related to enhancer-rich clusters associated with the induction of CARM1 activity in response to estrogen.[6b] Furthermore, modulation of CARM1 levels affect androgen-dependent transcription and prostate cancer cell growth,[6c] and elevated CARM1 levels correlate with the development of prostate carcinoma as well as the progression of androgen-independent prostate cancer.[6d] These studies support CARM1 as a plausible target for anti-cancer drug development.

Early reports describing novel inhibitors of PRMTs originated either from molecular modeling studies or high-throughput screening.[7a–e] The majority of these were micromolar inhibitors that lacked selectivity and, in some cases, did not possess drug-like

* Corresponding author.
E-mail address: wahhaba@methylgene.com (A. Wahhab).

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properties. In addition, the bisubstrate inhibitor reported by Osborne et al.[8] was over fourfold more active against PRMT1 compared to CARM1. Just recently, we disclosed a new series of thiophene-based nanomolar inhibitors of CARM1 (Fig. 1, compound **1**).[9a] The activity of these inhibitors were comparable to those reported by Purandare et al.[9b,c] (Fig. 1, compound **2a–b**).

Preliminary investigation of the pharmacokinetic properties of **1**, and **2a** proved that both the phenyl and thiophene-based inhibitors had poor bioavailability in rats and low exposure when dosed ip in mouse. The recent report of Huynh et al.[9d] confirmed our observations that **2a** had poor permeability (PAMPA assay). However, the authors were able to improve the permeability of these inhibitors by exchanging the amide unit on the right-hand side with an 1,3,4-oxadiazole moiety. In addition, they reported that the (*S*)-alanine benzylamide, on the left of **2** is optimal for CARM1 enzymatic activity and simple modifications such as in **3**, **4**, or **5** were not tolerated[9b,d] (Table 1). In this Letter, we disclose our efforts to replace the (*S*)-alanine benzyl amide moiety and to identify N^1-benzyl-N^2-methylethane-1,2-diamine unit for the design of CARM1 inhibitors, as we suspected that the former unit might be responsible for the observed poor PK of these inhibitors. Unlike their predecessors, these diamines have improved clearance, volume of distribution, and half lives.

Compound **6**, a homolog of **2b**, was prepared starting from 2-(3-aminophenyl)acetonitrile utilizing known procedures as depicted in Scheme 1. Diazotization of 2-(3-aminophenyl)acetonitrile followed by a tin chloride reduction of the intermediate diazonium salt, and cyclocondensation of the resultant hydrazide with 4,4,4-trifluoro-1-(furan-2-yl)butane-1,3-dione gave trisubstituted pyrazole **7**. Borane reduction of **7** and Boc-protection of the resulting amine, followed by the oxidative cleavage of the furan ring affor-

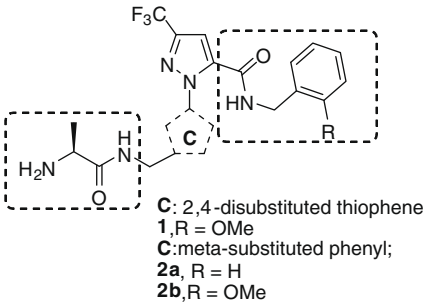
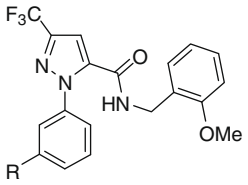
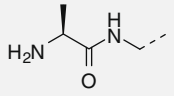
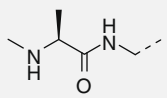
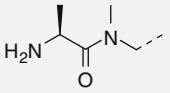
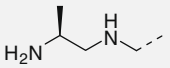
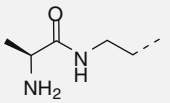
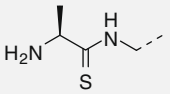
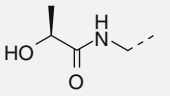
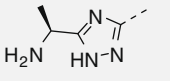
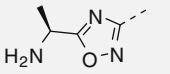
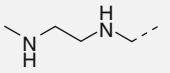
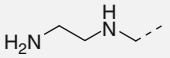
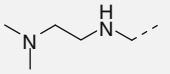
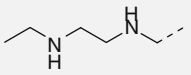
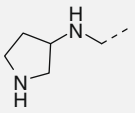
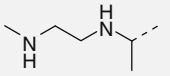
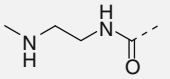


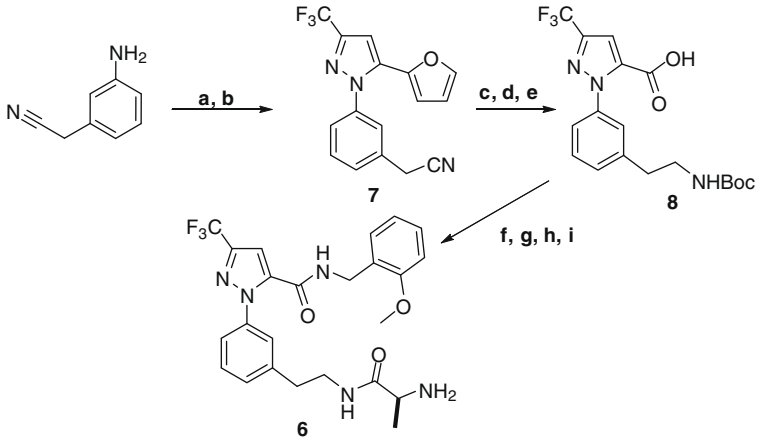
Figure 1. (*S*)-Alanine amides as CARM1 inhibitors.

Table 1
Effect of Ala-benzylamide replacement on CARM1 inhibitory activity[a]

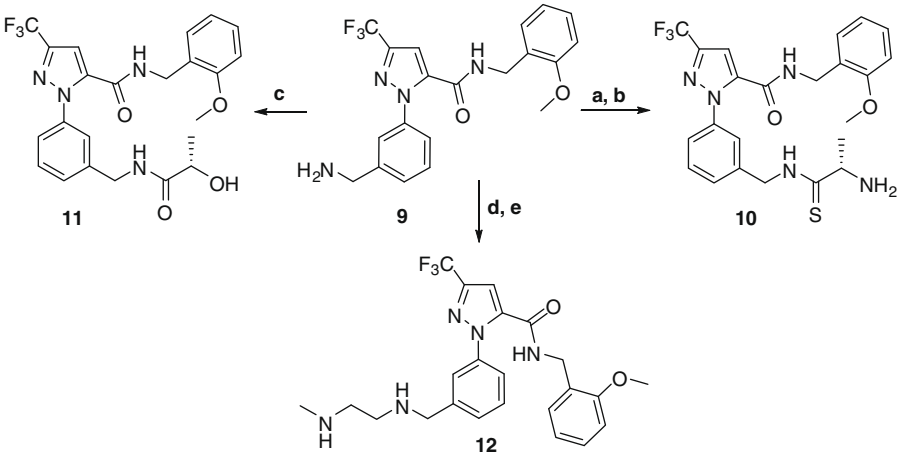


Compd	R	CARM-1 IC_{50} (μM)
2b[b]		0.06
3		>20[c]
4		>20[d]
5		>20[d]
6		>20
10		4.5
11		>20
19		>20
20		>20
12		0.20
15a		1.0
15b		7.9
15c		7.7
15d		>20
16		21
17		>20

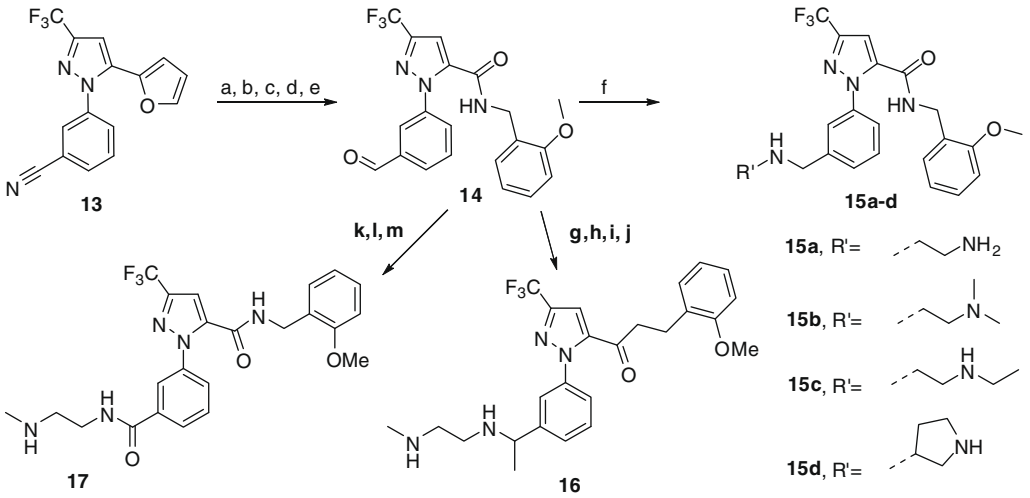
[a] Values are means of at least two experiments.
[b] Compound **2b** (Fig. 1) CARM1 IC_{50} value determined in-house.
[c] Ref. 9c reported methylation of the terminal amino acid (glycine) analog bearing a different amide on the right-hand side.
[d] Ref. 9d reported analogues with the same modification of the amino acid terminus but bearing an oxadiazole unit on the right-hand side of the molecule.



Scheme 1. Reagents and conditions: (a) $NaNO_2$, HCl, $SnCl_2{\cdot}H_2O$, 0 °C, 65%; (b) 4,4,4-trifluoro-1-(furan-2-yl)butane-1,3-dione, AcOH, 120 °C, 46%; (c) $BH_3{\cdot}THF$, THF, crude; (d) Boc_2O, Et_3N, DCM, 53% for two steps; (e) $NaClO_2$, NaH_2PO_4, MeCN, H_2O, crude; (f) (2-methoxyphenyl)methanamine, BOP, Et_3N, DCM, 46% for two steps; (g) 1:1 TFA/DCM, 1.5 h, rt, crude; (h) Boc-Ala-OSu, Et_3N, DCM, 58%; (i) 1:1 TFA/DCM, 4 h, rt, quantitative.



Scheme 2. Reagents and conditions: (a) (*S*)-*tert*-butyl 1-(6-fluoro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-1-thioxopropan-2-ylcarbamate, DMF, 0 °C, 2 h, then rt, 18 h, 39%; (b) 1:4 TFA/DCM, 3 h, rt, 96%; (c) lactic acid, BOP, Et_3N, DCM, 14%; (d) *tert*-butyl methyl(2-oxoethyl)carbamate, AcOH, MeOH, rt, 30 min then $NaCNBH_3$, 16 h, 37%; (e) 1:4 TFA: DCM, rt, 1.5 h, 22%.



Scheme 3. Reagents and conditions: (a) DIBAL-H, DCM, 0 °C to rt over 2 h, crude; (b) 1,3-propanediol, *p*-toluene sulfonic acid, toluene, Dean-Stark, 16 h, 79%; (c) $NaClO_2$, NaH_2PO_4, MeCN, H_2O, crude; (d) (2-methoxyphenyl)methanamine, BOP, Et_3N, DCM, 48% for two steps; (e) 4:1 AcOH/H_2O, 90 °C, 2 h, crude; (f) for **15a**, *tert*-butyl *N*-(2-aminoethyl)carbamate, DCM, AcOH, followed by $NaBH(OAc)_3$, rt, 16 h, 62%; then 1:6.5 TFA/DCM, rt, 2.5 h, 32%; (g) for **16**, MeMgBr, THF, −78 to 0 °C, 81%; (h) Dess–Martin periodinane, DCM, rt, 87%; (i) *tert*-butyl 2-aminoethyl(methyl)carbamate, AcOH, DCM, 15 min, then $NaBH(OAc)_3$, rt, 18 h, 30%; (j) 1:4 TFA/DCM, 2 h, rt, 80%; (k) for **17**, 2-methyl-2-butene, 10:1 *t*-BuOH/H_2O, KH_2PO_4, $NaClO_2$, rt, 1 h, crude, (l) *tert*-butyl 2-aminoethyl(methyl)carbamate, BOP, Et_3N, DCM, rt, 36 h, 74%; (m) 1:4 TFA/DCM, rt, 2 h, 9%.

ded acid **8**. BOP coupling of **8** with (2-methoxyphenyl)methanamine, followed by removal of the Boc group with TFA, reaction of the produced amine with Boc-Ala-OSu, and further amine deprotection gave the desired analog **6**.

Intermediate **9**, prepared according to published procedures,[9] was used for the synthesis of analogues **10**, **11**, and **12** (Scheme 2). The thioamide **10** was accessible by the reaction of **9** with (*S*)-*tert*-butyl 1-(6-fluoro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-1-thioxopropan-2-ylcarbamate, prepared according to the procedure of Zacharie et al.[10] followed by removal of the Boc group.

Alcohol **11** was obtained in one step by coupling of **9** with lactic acid, while diamine **12** was prepared by reductive amination of *tert*-butyl methyl(2-oxoethyl)carbamate, followed by the Boc-deprotection.

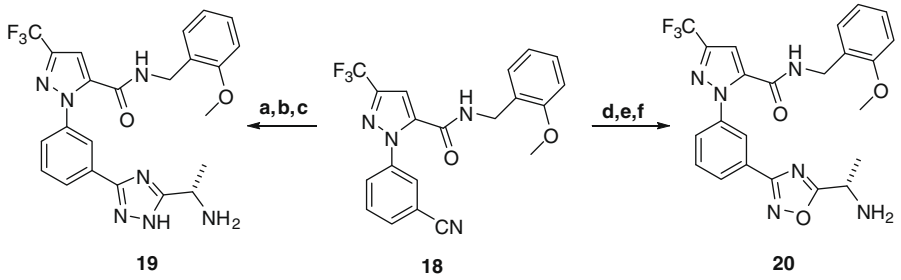
The conversion of the nitrile **13**[9] gave access to the aldehyde **14**, which was a key building block for the synthesis of analogues **15**, **16** and **17** (Scheme 3). Reduction of **13** with DIBAL-H followed by an acetal protection of the resulting aldehyde (not shown in the scheme), oxidation of the furan ring to the acid, coupling of the resulting acid with (2-methoxyphenyl)methanamine and finally hydrolysis of the acetal group with acetic acid gave the aldehyde **14**. Reductive amination of the aldehyde **14** with the appropriate amine utilizing $NaBH(OAc)_3$ as the reducing agent, followed by the removal of the Boc-protecting groups gave diamines **15a–d** (Table 1). Reaction of the aldehyde **14** with the methyl magnesium bromide and oxidation of the resulting alcohol with the Dess–Martin periodinane to the ketone, followed by reductive amination with *tert*-butyl 2-aminoethyl(methyl)carbamate in the presence of $NaBH(OAc)_3$, and further Boc-deprotection gave diamine **16**. Sodium chlorite oxidation of **14** to the corresponding acid, followed by BOP coupling with *tert*-butyl 2-aminoethyl(methyl)carbamate and subsequent Boc-deprotection provided amide **17**.

Nitrile intermediate **18**[9] was used to access triazole **19** and oxadiazole **20** (Scheme 4). Reaction of **18** with $HCl_{(gas)}$ in MeOH, followed by the condensation of the resulting amidate with (*S*)-*tert*-butyl 1-hydrazinyl-1-oxopropan-2-ylcarbamate, dehydration, and subsequent Boc-deprotection gave triazole **19**. The reaction of **18** with hydroxylamine, followed by condensation of the resulting *N*-hydroxyamidine with Boc-Ala-OH utilizing TBTU, dehydration, and deprotection gave oxadiazole **20**.

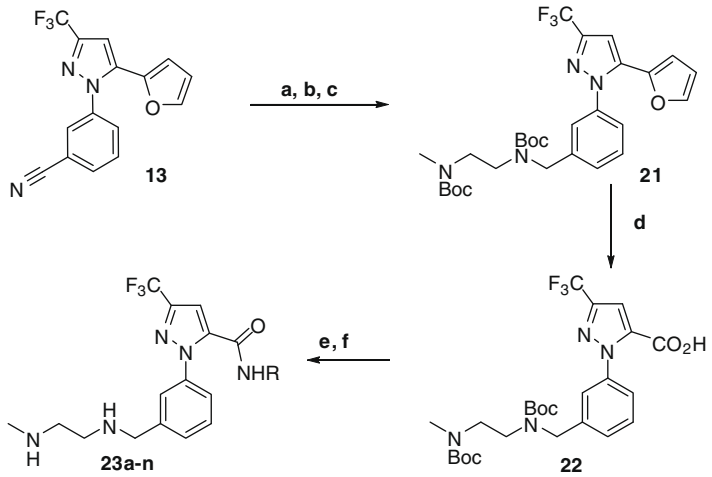
The synthesis of amides **23a–n** is depicted in Scheme 5 below. The nitrile **13** was converted to the bis-Boc-protected intermediate **21** following procedures described above. Oxidation of **21** to the carboxilic acid **22**, followed by a coupling of the carboxylic acid with different amines, and subsequent Boc-deprotection gave the desired amides in good yields.

Compound **24** was prepared according to Scheme 6 starting from the acid **22** by cyclocondensation with 2-methoxybenzhydrazide utilizing 2-chloro-1,3-dimethylimidazolinium chloride as the dehydrating agent, followed by the Boc-deprotection with TFA.

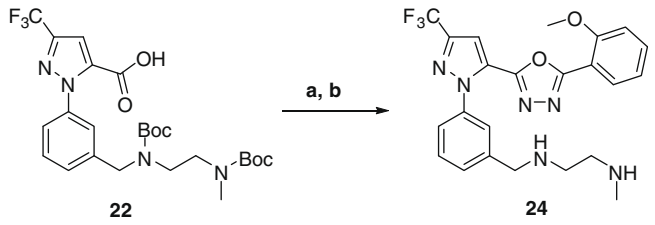
Both compounds **25** and **26** (Table 3) were prepared following the same procedure described for **23** starting from 5-amino-2-fluorobenzonitrile and 5-amino-2-methylbenzonitrile, respectively. The trifluoromethyl substituted analog **27**, was prepared starting from methyl 3-amino-5-(trifluoromethyl)benzoate as illustrated in Scheme 7 below. The ester **28**, prepared following published



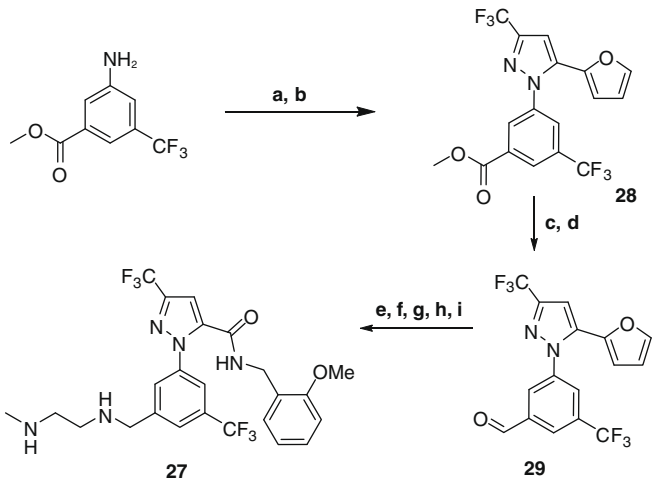
Scheme 4. Reagents and conditions: (a) 1:1 MeOH/Et_2O, −5 °C, $HCl_{(gas)}$, 30 min, then rt, 2 h, 81%; (b) (*S*)-*tert*-butyl 1-hydrazinyl-1-oxopropan-2-ylcarbamate, Et_3N, reflux, 6 h, then xylenes, 140 °C, 20 min, 18%; (c) 1:4 TFA/DCM, 1 h, rt, 54%; (d) $NH_2OH{\cdot}HCl$, $NaHCO_3$, MeOH, reflux 4 h, 60%; (e) Boc-Ala, TBTU, HOBt, DIPEA, DMF, rt 1 h, then 110 °C, 2 h, 55%; (f) 1:1 TFA/DCM, rt, 1 h, quantitative.



Scheme 5. Reagents and conditions: (a) DIBAL-H, DCM, 0 °C to rt over 2 h, crude; (b) *tert*-butyl 2-aminoethyl(methyl)carbamate AcOH, MeOH, 1 h, rt, then $NaBCNH_3$, rt, 18 h, crude; (c) Boc_2O, DCM, 0 °C to rt, 5 h, 78% over three steps; (d) $NaClO_2$, NaH_2PO_4, MeCN, H_2O, crude; (e) RNH_2, BOP, Et_3N, DCM, 18 h; (f) 1:4 TFA/DCM, rt, 1–3 h.



Scheme 6. Reagents and conditions: (a) 2-methoxybenzhydrazide, 2-chloro-1,3-dimethylimidazolinium chloride, Et_3N, DMF, rt, 24 h, 15%; (b) 1:4 TFA/DCM, rt, 1 h, quantitative.



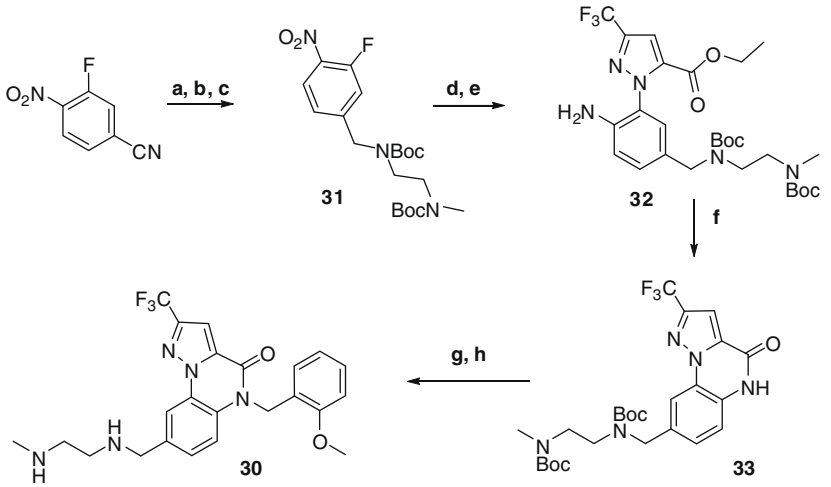
Scheme 7. Reagents and conditions: (a) $NaNO_2$, HCl, $SnCl_2{\cdot}H_2O$, 0 °C, crude; (b) 4,4,4-trifluoro-1-(furan-2-yl)butane-1,3-dione, AcOH, 120 °C, 67%; (c) DIBAL-H, toluene, −78 °C, 1 h, then warm to rt over 1.5 h, 94%; (d) Dess–Martin periodinane, DCM, rt, 2 h, crude; (e) *tert*-butyl 2-aminoethyl(methyl)carbamate AcOH, MeOH, rt, 1 h, then $NaBH_3CN$, rt, 16 h, crude; (f) Boc_2O, DCM, 0 °C to rt, 16 h, 81% over three steps; (g) $NaClO_2$, NaH_2PO_4, MeCN, H_2O, crude; (d) (2-methoxyphenyl)methanamine, EDC, DMAP, DCM, 48 h, 10% for two steps; (n) 1:4 TFA/DCM, rt, 2 h, 84%.

procedures,[9] was converted to the aldehyde **29** in two steps by reduction with DIBAL-H followed by oxidation with Dess–Martin periodinane. Reductive amination of **29**, Boc-protection of the resulting amine, oxidative cleavage of the furan to the carboxylic acid, and the EDC assisted coupling with (2-methoxyphenyl)methanamine, followed by the TFA removal of the Boc-protecting groups gave analog **27**.

The tricyclic compound **30** was prepared starting from 3-fluoro-4-nitrobenzonitrile, as shown in the Scheme 8. Borane reduction of the nitrile group to the amine, followed by reductive amination of *tert*-butyl methyl(2-oxoethyl)carbamate and titanium(IV) isopropoxide and $NaBH_4$, followed by a subsequent Boc-protection of the resulting amine gave the bis-Boc diamine **31**. This material reacting with ethyl 3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate in the presence of NaH, followed by catalytic reduction of the nitro group gave the aniline **32**. Cyclization of the aniline **32** to the lactam **33** was achieved with Me_3Al. Abstraction of the acidic proton of lactam **33** and alkylation with 2-methoxybenzyl chloride, followed by removal of the Boc-protecting groups gave the analog **30**.

The effects of compounds **3–6**, **10–13**, **19**, **20**, **15a–d**, **16**, **17**, **23a–n**, **24–27**, and **30** on the inhibitory activity of CARM1 was measured by means of a histone methyltransferase assay using recombinant CARM1 enzyme, and histone H3 as the substrate.[11a,d] Table 1 shows the CARM1 inhibitory activity of some of the compounds we prepared to investigate replacements of the Ala-benzylamide end of the molecule. The lack of enzymatic inhibitory activity of compounds **3**, **4**, and **5** is consistent with the recent reports of Purandare et al.[9b,c] and Huynh et al.[9d] In addition, the inactivity of **6**, and **11** impresses the requirement for an unsubstituted terminal amine being a certain distance away from the core. The amide functionality of **2b** seems to be essential for activity while the amide isosteres such as the thiourea (**10**), triazole (**19**), and oxadiazole (**20**) were not active, despite the presence of the unsubstituted amine functionality (Table 1). The inactivity of the desamide analog **5** and the observed activity of analogues **12** and **15a** was intriguing and prompted us to investigate the possible binding site for this unit in order to rationalize the observed trend in the activities of these analogues.

A close inspection of the published crystal structure of the catalytically active site of CARM1 in complex with the co-factor product SAH, illustrated the importance of key interactions of SAH with two active site residues, ARG169 and ASP191.[12] The carboxylate group of SAH makes a salt bridge with the arginine residues



Scheme 8. Reagents and conditions: (a) $BH_3{\cdot}THF$, THF, 0 °C, then rt for 16 h, crude; (b) *tert*-butyl methyl(2-oxoethyl)carbamate, titanium(IV) isopropoxide, THF, rt, 5 h, then $NaBH_4$, EtOH, rt, 16 h, 73%; (c) Boc_2O, DCM, Et_3N, 0 °C to rt, 2.5 h, 22%; (d) ethyl 3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylate, NaH, DMF, 25 min at rt, then cool and add **31** dropwise in DMF, warm to rt, 1 h, 30%; (e) 10% Pd/C, $H_{2\ (gas)}$ 1 atm, EtOH, 1 h, rt, 94%; (f) DCM, 0 °C, Me_3Al/toluene, warm up to rt for 1 h, then 2 h at 40 °C, 27%; (g) NaH, DMF, rt, 10 min, then add 2-methoxybenzyl chloride, 0 °C, then at rt, 16 h, 70%; (h) 4:1 TFA/DCM, rt, 2 h, 39%.

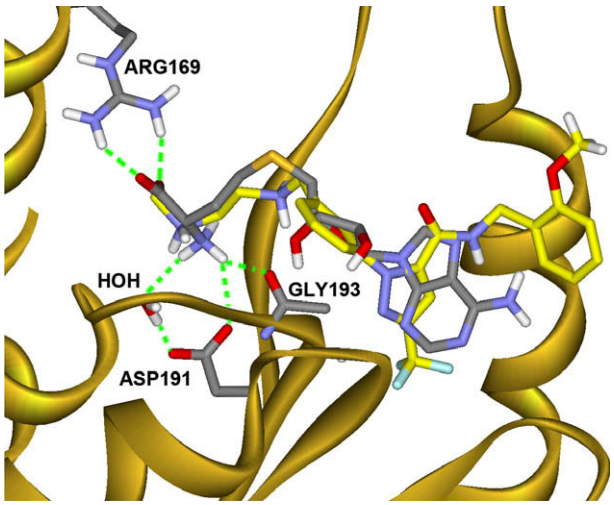


Figure 2. Overlay of the conformations of **12** (yellow) and SAH (gray) docked in the active site of CARM1. Dashed green lines indicate hydrogen bonds.

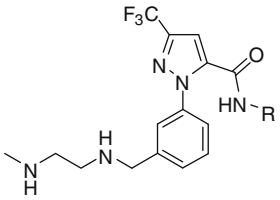
whereas the ammonium moiety is involved in a bifurcated H-bonded interaction with ASP191 and a water molecule in the active site. Docking experiments provided a plausible explanation for the observed behavior of the synthesized analogues.

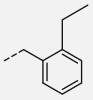
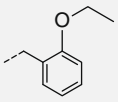
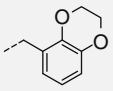
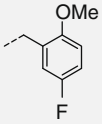
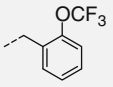
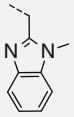
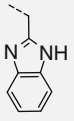
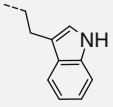
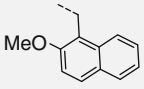
Figure 2 shows an overlay of diamine **12** with SAH obtained from the docking experiments using the automated docking software FITTED 2.6[13] with the CARM1 crystal structure (PDB code 2v74). Analysis of the docking results highlighted the importance of the flexible ethylene fragment of **12** for productive binding. Substituents or group modifications on this ethylene unit that constrain the flexibility or the size are detrimental to the activity (see Table 1). We hypothesize that such modifications prohibit the possibility for the ligand to adopt the required conformation to interact with ASP191 and the water molecule in the active site, while avoiding the positive surroundings of ARG169.[13d]

Having established that the unsubstituted ethylene unit of **12** is most favorable, we turned our attention to the other parts of our lead. Table 2, shows the CARM1 inhibitory activity of some of the diverse amides we prepared to optimize the right-hand moiety. We first investigated the tolerance of substitution on the benzyl moiety of **12**. However, none of the analogues prepared, **23a–23h**, were more active than **12**; indicating that an *o*-methoxy substituent is optimal for activity. Replacement of the benzyl moiety of the amide with an alkyl (**23i**) or a heterocycle (**23j–l**) led to more than one log loss of inhibitory activity against the CARM1 enzyme. A bicyclic naphthalen-1-ylmethyl group (**23m)** was tolerated and resulted in a slight loss of inhibitory activity.

However, unlike the benzyl analog, an *o*-methoxy substituent off the naphthyl (**23n**) led to a marked decrease of activity (see Table 2). In contrast to the observations of Huynh et al.[9d] replacement of the amide group of **12** with the 1,3,4-oxadiazole moiety, compound **24**, resulted in a threefold loss of activity against the CARM1 enzyme (IC_{50} of 0.59 μM). Since there was no gain in potency by replacement of right-hand side amide group, we next turned our attention to the core of **12** and studied the effect of substitution off the phenyl ring. Simple substituents such as fluoro (**25**), methyl (**26**), or trifluoromethyl (**27**) groups were not tolerated leading to substantial or total loss of activity (Table 3). Cyclization of the side chain amide unto the core of the compound, producing the constrained analog **30**, abolished the ability of the compound to inhibit CARM1 activity. These results confirm that diamine **12** is the leading inhibitor in this series.[14] However, despite the observed CARM1 enzymatic activity, compound **12** failed to show any cellular activities when tested in MTT assays.

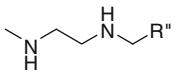
Table 2
SAR of diamine analogues **23a–n**[a]

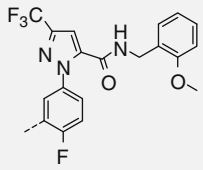
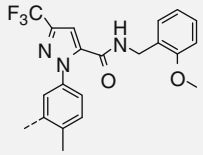
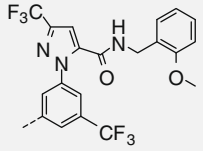
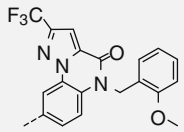


Compd	R	CARM-1 IC_{50} (μM)
23a		>10
23b		13.1
23c		1.61
23d		0.9
23e		0.71
23f		0.71
23g		6.5
23h		>10
23i		3.6
23j		4.7
23k		4.3
23l		1.8
23m		0.32
23n		3.35

[a] Values are means of at least two experiments.

Table 3
Substitutions on the core phenyl ring[a]



Compd	R″	CARM-1 IC_{50} (μM)
25		5.6
26		7.94
27		>20
30		>10

[a] Values are means of at least two experiments.

To test whether our inhibitors had better PK properties than the previously reported compounds, we evaluated the PK profiles of **12**, **23d**, **23m**, and **24** in rats (Table 4). The results revealed that these diamine inhibitors have lower clearance, longer half lives and smaller volumes of distribution as compared to **2b** (Table 4). However, they still suffered from poor oral absorption as indicated by the low AUC values and require further optimization.

In conclusion, we identified the N^1-benzyl-N^2-methylethane-1,2-diamine unit as a substitute for the (*S*)-alanine benzylamide moiety for the design of CARM1 small molecule inhibitors. The inhibitory activities of the diamines were of the same order of magnitude as their predecessors, and they exhibited improved clearance, volume of distribution and longer half lives.

Table 4
PK profile in rat of selected compounds

Compd	N. AUC (PO)[b] μM h/(mg/Kg)	N. AUC (IV)[b] μM h/(mg/Kg)	Cl (IV) (L/h)/Kg	V_{ss}(IV) L/Kg	$t_{1/2}$(IV) h	%F
2b[a]	0.01	0.04	86	33.6	0.07	—[c]
12	0.07	2.1	1.2	1.4	2.1	4
23d	0.03	1.8	1.2	0.1	0.5	2
23m	0.03	1.3	2.3	0.2	1.4	3
24	0.03	1.0	2.2	0.6	1.8	3

[a] Values obtained in our laboratories.
[b] N. AUC is the normalized AUC values.
[c] The compound is cleared extremely rapidly. An accurate value could not be calculated due to the very low N. AUC (IV).

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11. (a) All experimental details can be found in MethylGene patent application, Wahhab, A.; Therrien, E.; Allan, M.; Manku, S. International Patent WO 08/104077 A1, 2008; (b) The CARM1 enzyme (N-terminal His-tagged, recombinant mouse CARM1, expressed in Sf9 cells) was purchased from Millipore (cat# is 14-575, Lot # is DAM1473541). Histone H3 (Sigma–Aldrich)

was used as the substrate, and the methylation was monitored using tritiated S-Adenosyl-Methionine (SAM) (Amersham Pharmacia Biotech) as a methyl donor. The reactions were performed at 30 °C for a total of 15 min, using enzyme (CARM1), substrate (histone H3), and co-factor (SAM) in the absence and presence of compound. Assay protocol: 2 μl of the diluted compound was added to a U-Bottom of a PP 96-well plate. CARM1 enzyme was diluted in a Tris–HCl (pH 9) buffer (to a final enzyme concentration of 0.005 μg/μl) and 8 μl of the cold enzyme solution was immediately added to the compound and allowed to pre-incubate for 10 min at room temperature. A mixture of SAM (20% [3*H*] SAM) and histone H3 (10 μl) was then added and incubated for 15 min at 30 °C for a final concentration of 0.0125 μg/μl histone and 2 μM SAM (0.033 mCi/ml). The reaction was stopped by adding 20 μl of SAH for a final concentration of 60 μM and, 10 μl of the quenched reaction is spotted onto P30 Filtermat paper. The Filtermat is washed twice for 15 min with 10% TCA solution and then once for 5 min with 95% ethanol. A wax scintillant is used with the filtermat and the radioactivity was read using a Wallac Microbeta counter (CCPM).

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14. To test the specificity of our inhibitors for CARM1 versus other arginine or lysine methyltransferases, compound **12** was tested against the PRMT1 arginine and the Set7/9 lysine methyltransferases and was found to be significantly less active against both enzymes (IC_{50} >100 μM). The PRMT1 enzyme (N-terminal GST-tagged, human PRMT1 recombinant protein, expressed in *E. coli*) was purchased from upstate (Cat # 14-474, Lot # 25336). Histone H4 (recombinant protein expressed in *E. coli*, Cat # 14-412, Lot # 23215, purchased from upstate) was used as the substrate, and the methylation was monitored using tritiated S-Adenosyl-Methionine (SAM) (Amersham Pharmacia Biotech) as a methyl donor. The reactions were performed at 30 °C for a total of 15 min, using enzyme (PRMT1), substrate (histone H4), and co-factor (SAM) in the absence and presence of compound. Assay protocol: 2 μl of the diluted compound was added to a U-Bottom of a PP 96-well plate. PRMT1 enzyme was diluted in a Tris–HCl (pH 9) buffer (to a final enzyme concentration of 0.2 μM) and 10 μl of the cold enzyme solution was immediately added to the compound and allowed to pre-incubate for 10 min at room temperature. A mixture of SAM (20% [3*H*] SAM) and histone H4 (10 μl) was then added and incubated for 15 min at 30 °C for a final concentration of 3.5 μM histone and 0.2 μM SAM (0.033 mCi/ml). The reaction was stopped by adding 20 μl of SAH for a final concentration of 60 μM and, 10 μl of the quenched reaction is spotted onto P30 Filtermat paper. The Filtermat is washed twice for 15 min with 10% TCA solution and then once for 5 min with 95% ethanol. A wax scintillant is used with the filtermat (Wallac, filtermat A 1450-421) and the radioactivity was read using a Wallac Microbeta counter (CCPM). The SET9 enzyme (N-terminal His-tagged, human SET9 recombinant protein, expressed in *E. coli*) was purchased from upstate (Cat # 14-469, Lot # 32194). Histone H3 (purchased from upstate) was used as the substrate, and the methylation was monitored using tritiated S-Adenosyl-Methionine (SAM) (Amersham Pharmacia Biotech) as a methyl donor. The reactions were performed at 37 °C for a total of 45 min, using enzyme (SET9), substrate (histone H3), and co-factor (SAM) in the absence and presence of compound. Assay protocol: 2 μl of the diluted compound was added to a U-Bottom of a PP 96-well plate. SET9 enzyme was diluted in a Tris–HCl (pH 9) buffer (to a final enzyme concentration of 0.0005 μg/μl) and 8 μl of the cold enzyme solution was immediately added to the compound and allowed to pre-incubate for 10 min at room temperature. A mixture of SAM (20% [3*H*] SAM) and histone H3 (10 μl) was then added and incubated for 45 min at 37 °C for a final concentration of 0.06 μg/μl histone and 0.2 μM SAM (0.011 mCi/ml). The reaction was stopped by adding 100 μl of water and transferred to a White Flashplate (Perkin Elmer basic flashplate). The flashplate was washed twice with 10% TCA. The dry plate was read using a Wallac Microbeta counter (CCPM).