A Potent, Selective and Cell-active Inhibitor of Human Type I Protein Arginine Methyltransferases

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Abstract

Protein arginine methyltransferases (PRMTs) play a crucial role in a variety of biological processes. Overexpression of PRMTs has been implicated in various human diseases including cancer. Consequently, selective small-molecule inhibitors of PRMTs have been pursued by both academia and pharmaceutical industry as chemical tools for testing biological and therapeutic hypotheses. PRMTs are divided into three categories: type I PRMTs which catalyze mono- and asymmetric dimethylation of arginine residues, type II PRMTs which catalyze mono- and symmetric dimethylation of arginine residues, and type III PRMT which catalyzes only monomethylation of arginine residues. Here, we report the discovery of a potent, selective and cell-active inhibitor of human type I PRMTs, MS023, and characterization of this inhibitor in a battery of biochemical, biophysical and cellular assays. MS023 displayed high potency for type I PRMTs including PRMT1, 3, 4, 6 and 8, but was completely inactive against type II and type III PRMTs, protein lysine methyltransferases and DNA methyltransferases. A crystal structure of PRMT6 in complex with MS023 revealed that MS023 binds the substrate binding site. MS023 potently decreased cellular levels of histone arginine asymmetric dimethylation. It also reduced

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ASSOCIATED CONTENT
Supporting information
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global levels of arginine asymmetric dimethylation and concurrently increased levels of arginine monomethylation and symmetric dimethylation in cells. We also developed MS094, a close analog of MS023, which was inactive in biochemical and cellular assays, as a negative control for chemical biology studies. MS023 and MS094 are useful chemical tools for investigating the role of type I PRMTs in health and disease.

Arginine methylation is a common post-translational modification in eukaryotic cells.[1-3] Protein arginine methyltransferases (PRMTs) catalyze the transfer of the methyl group from the cofactor S-5′-adenosyl-*L*-methionine (SAM) to arginine residues of a variety of histone and non-histone proteins.[4] The arginine guanidinium group can be mono- and/or dimethylated.[5] After one of the two guanidine terminal nitrogens is monomethylated (Rme1), the same nitrogen can be further methylated to provide arginine asymmetrical dimethylation (Rme2a) or the other nitrogen can be methylated to give arginine symmetrical dimethylation (Rme2s). To date, nine PRMTs have been identified and they are grouped into three categories: type I, type II and type III.[2, 5, 6] Type I PRMTs catalyze mono- and asymmetric dimethylation of arginine residues. Most of the known PRMTs, including PRMT1, 3, 4 (also known as CARM1 (Co-activator-associated arginine methyltransferase 1)), 6 and 8, belong to this type. Type II PRMTs catalyze mono- and symmetric dimethylation of arginine residues and they include PRMT5 and PRMT9.[7] PRMT7 is the only known type III PRMT, which catalyzes monomethylation of arginine residues.[5]

Arginine methylation does not change the cationic charge of arginine residues.[8] Instead, it increases the size and hydrophobicity of the protein, thus affecting its interactions with other proteins and regulating its physiological function,[9] including RNA processing, DNA repair, transcriptional activation/repression, signal transduction, cell differentiation, and embryonic development.[4] Dysregulation of PRMTs has been implicated in a variety of human diseases.[4-6] For example, overexpression of PRMT1 was reported in leukemia[10] and breast,[11] prostate,[12] lung,[13] bladder[13] and colon[14] cancers. PRMT6 upregulation was observed in melanoma[15] and bladder, lung[13] and prostate[16] cancers. It has been shown that abrogation of *PRMT1* or *PRMT6* genes significantly reduces the growth of bladder and lung cancer cells.[13] In addition, PRMT4 levels are elevated in breast,[17] prostate[18] and colorectal[19] cancers. Moreover, increased levels of arginine asymmetrical dimethylation (Rme2a), which is the main product of Type I PRMTs, are associated with cardiovascular disease and pulmonary hypertension.[20-22] A growing body of evidence suggests that these PRMTs are potential therapeutic targets.[5, 6]

Small-molecule chemical probes[23, 24] that selectively inhibit the catalytic activity of PRMTs are valuable tools for deciphering the complex regulatory mechanisms enabled by protein arginine methylation. Although the selective PRMT inhibitor discovery field is gaining momentum, only a limited number of selective inhibitors have been reported.[25-37] An inhibitor that is selective for type I PRMTs over other PRMTs, PKMTs (protein lysine methyltransferases) and DNMTs (DNA methyltransferases) has not yet been reported.

Inspired by the recent discoveries of EPZ020411 (Figure 1),[36] a potent and selective PRMT6 inhibitor, and CMPD-1 (Figure 1),[29] a PRMT4 (CARM1) inhibitor, we designed and synthesized MS023 (Figure 1) and its close analogs, and discovered MS023 as a

selective inhibitor of type I PRMTs. We also discovered a close analog of MS023 as a negative control for cell-based studies. We characterized these compounds in a battery of biochemical, biophysical, and cellular assays. Here, we report the design, synthesis, and biological characterization of this chemical probe and its negative control.

RESULTS AND DISCUSSIONS

Design and Synthesis

The ethylenediamine side chain was seen in both the PRMT6 inhibitor EPZ020411 and PRMT4 (CARM1) inhibitor CMPD-1.[29, 36] We rationalized that the ethylenediamino group is an arginine mimetic and a major contributor to PRMT6 and PRMT4 (both of which are type I PRMTs) inhibitory activities of these compounds. Based on this analysis, we hypothesized that an ethylenediamino group would be an excellent moiety for targeting type I PRMTs. We therefore designed compounds **1** – **3** (Table 1), all of which contain an ethylenediamino group. We also explored two additional regions of the EPZ020411 scaffold.[36] Because the substituted cyclobutoxy group on the phenyl ring likely contributed to the selectivity for PRMT6, we replaced this group with a smaller functional group such as trifluoromethyl or isopropoxy group (compounds **1** – **3** in Table 1) to gain inhibitory activities for other type I PRMTs. In addition, we probed the electronic nature of the main heteroaromatic core by replacing the pyrazole ring with a 1,2,3-triazole or pyrrole ring (compounds **1** – **3** in Table 1). To generate a negative control for chemical biology studies, we designed compounds **4** – **6** (Table 1) by replacing the crucial ethylenediamino group with a hydroxyethylamino (compound **4**) or aminoamide (compounds **5** and **6**) group. It is worth noting that compounds **4** – **6** are excellent tools for testing our hypothesis that the ethylenediamino group is critical for maintaining type I PRMT inhibitory activities. The compounds in Table 1 were synthesized according to the synthetic routes outlined in Scheme 1 and Supporting Schemes S1 – S5.

SAR Results

Compounds **1** – **6** were evaluated against all known human PRMTs (except PRMT2, which is not catalytically active in our assay) in biochemical assays using tritiated SAM (^{3}H-SAM) as the methyl donor. Results of our structure-activity relationship (SAR) studies are summarized in Table 1 (K_i values were calculated as previously described[38]). Compound **1** which contains a triazole core displayed weak potency for most of type I PRMTs. It was most potent against PRMT6 ($IC_{50} = 230 \pm 12$ nM), had a modest potency against PRMT8 ($IC_{50} = 3,000 \pm 500$ nM) and was inactive against PRMT1, 3 and 4 ($IC_{50} > 20,000$ nM). The pyrrole core is better than the triazole core at inhibiting type I PRMTs. For example, compound **2** was greater than 70-fold more potent for PRMT1, 3, 4 and 8 than compound **1**. It was also about 10-fold more potent for PRMT6 than compound **1**. Compound **2** displayed high potency for PRMT6 ($IC_{50} = 9 \pm 0.9$ nM) and PRMT8 ($IC_{50} = 42 \pm 3$ nM), but was less potent for PRMT1 ($IC_{50} = 250 \pm 15$ nM), PRMT4 ($IC_{50} = 260 \pm 10$ nM) and PRMT3 ($IC_{50} = 1,100 \pm 180$ nM). Most interestingly, we found that changing the *meta*-trifluoromethyl group (compound **2**) to the *para*-isopropoxy group (compound **3**) improved potency against all type I PRMTs. MS023 (compound **3**) potently inhibited PRMT1 ($IC_{50} = 30 \pm 9$ nM), PRMT3 ($IC_{50} = 119 \pm 14$ nM), PRMT4 ($IC_{50} = 83 \pm 10$ nM), PRMT6 ($IC_{50} = 4 \pm 0.5$ nM)

and PRMT8 ($IC_{50} = 5 \pm 0.1$ nM) (Figure 2A). Preincubation of MS023 with PRMT6 for 30 minutes had no effect on IC_{50} values (Supporting Figure S1). Experiments were performed under linear initial velocities, and the presence of the cofactor product SAH (S-5′-adenosyl-*L*-homocysteine) did not affect IC_{50} values for MS023 (Supporting Figure S2) or its binding to PRMT6 (Supporting Figure S3). Importantly, MS023 did not inhibit any of type II PRMTs (PRMT5 and 9) and type III PRMT (PRMT7) at concentrations up to 10 μM (Figure 2A). Similarly, compounds **1** and **2**, two earlier analogs of MS023, did not inhibit type II and type III PRMTs. We also found that switching the terminal primary amino group (MS023) to a hydroxyl group (MS094, compound **4**) completely abolished the inhibitory activity for type I PRMTs. In addition, replacing either of the two basic amino groups of MS023 with an amide group (compounds **5** and **6**) resulted in a total loss of potency. Compounds **4** – **6** were also inactive against type II and type III PRMTs. These results provide strong support to our hypothesis that the ethylenediamino group is an excellent arginine mimetic and a critical moiety for targeting type I PRMTs. Furthermore, the high structural similarity and drastic potency difference between MS023 and MS094 suggest they will be excellent positive and negative control tool compounds for chemical biology studies.

Characterization in Biophysical Assays and Further Assessment of Selectivity

We next evaluated MS023 in biophysical assays. Using isothermal titration calorimetry (ITC), we confirmed that MS023 binds PRMT6 with high affinity ($K_d = 6$ nM) (Figure 2B). A molar ratio of 0.5 was observed suggesting that only half of the expected binding sites were available to bind MS023 under the conditions that ITC was performed. The high binding affinity of MS023 to PRMT6 was also confirmed by differential scanning fluorimetry (DSF). A melting temperature increase (T_m) of 20 °C was observed upon binding of MS023 to PRMT6 (Figure 2C).

To further assess selectivity of MS023, we tested it against 25 PKMTs and DNMTs, and 3 histone lysine demethylases. We were pleased to find that MS023 did not inhibit these methyltransferases and demethylases at up to 10 μM (Figure 2D). In addition, MS023 did not show binding to any of the 9 methyllysine or methylarginine reader proteins when tested using DSF or DSLS (differential static light scattering) assays (Supporting Table 1).

Mechanism of Action (MOA) Studies and the Cocrystal Structure of MS023

To assess MOA of MS023, we evaluated the effect of SAM and peptide concentrations on IC_{50} values of MS023 against PRMT6. As illustrated in Figures 2E and F, increasing the cofactor SAM or peptide substrate concentrations had no effect on IC_{50} values of MS023 against PRMT6, suggesting that this inhibitor is noncompetitive with both the cofactor SAM and peptide substrate. The peptide competition experiment was also repeated using different SAM concentrations (i.e., IC_{50} values were determined at different peptide and SAM concentrations) to determine whether MS023 is a cofactor-dependent peptide competitive inhibitor, which has been reported previously.[39] However, we did not observe any change in the potency (IC_{50}) of MS023 against PRMT6 when the SAM concentration was modified either below or above the saturation concentration. Similar noncompetitive pattern of inhibition has been observed for all other human type I PRMTs, except PRMT3, for which

MS023 appears to be noncompetitive with the peptide substrate but uncompetitive with the cofactor SAM (Supporting Figure S4).

We obtained an X-ray cocrystal structure of PRMT6 in complex with MS023 and the cofactor product SAH (PDB code: 5E8R, Supporting Table 2). We were pleased to find that the ethylenediamino group indeed occupies the substrate arginine-binding site (Figure 3), thus validating our inhibitor design hypothesis. This moiety forms multiple hydrogen bonds with PRMT6. The terminal primary amino group forms two direct hydrogen bonds: one with Glu155 side-chain and the other one with the backbone carbonyl group of Met157. This amino group also forms water-mediated hydrogen bonds with the backbone carbonyl group of Trp156 and the side-chain of Glu164. The tertiary amino group forms a direct hydrogen bond with the His317 side-chain. These multiple hydrogen bond interactions further confirmed the importance of the ethylenediamine moiety. Our SAR results have shown that disturbing one or more of these interactions (e.g., the replacement of the terminal primary amino group with a hydroxyl group (compound **4**), the replacement of either of the two basic amino groups with an amide group (compounds **5** and **6**)) resulted in the total loss of potency for PRMT6. Furthermore, other moieties of MS023 make a number of additional interactions with PRMT6. For example, the pyrrole nitrogen interacts with Glu59 through a hydrogen bond. The phenyl group of MS023 makes π-π interactions with Tyr159. The isopropoxy oxygen forms a hydrogen bond with His163 while the isopropyl group is solvent exposed.

By comparing of cocrystal structures of MS023, CMPD-1 and EPZ020411 with SAH and PRMTs, we found that the ethylenediamino moieties of these three inhibitors make conserved interactions with PRMT6 (His317 and Glu155) and PRMT4 (His415 and Glu258) (Supporting Figure S5A). Surprisingly, a number of residues contributing to the binding pocket of MS023 in our crystal structure are not conserved in PRMT4 (e.g., PRMT6 Cys50 versus PRMT4 Phe153, PRMT6 Val56 versus PRMT4 Gln159) (Supporting Figure S5B). We hypothesize that aligned but non-conserved side-chains in PRMT6 and PRMT4 can accommodate the inhibitor in different ways. EPZ020411 is larger than MS023 and is extending in an area of the binding site unoccupied by MS023, where differences between PRMT6 and PRMT4 (e.g., PRMT6 Leu46 versus PRMT4 Gln149) may prohibit binding to the latter.

On the basis of that the crystal structure clearly shows the ethylenediamine moiety of MS023 occupies the substrate binding site of PRMT6, one expects that MS023 would be competitive with the peptide substrate in the MOA studies. However, it has been well documented that active site-binding small-molecule inhibitors can appear to be noncompetitive in MOA studies.[40, 41] One possibility is that PRMT6 derives most of its binding affinity to the peptide substrate from regions outside of the arginine-binding pocket. MS023 can effectively occupy the arginine side chain binding pocket of the substrate and likely form the substrate-inhibitor-PRMT6 complex without significant penalty of free energy. Another plausible explanation is that the binding of MS023 to PRMT6 induces a major protein confirmation change, thus traditional enzyme kinetics may not apply. An α-helix at the N-terminus of type I PRMT structures adopts distinct conformations in apo, cofactor-bound, substrate-bound and inhibitor-bound states (Supporting Figure S6).[42]

Probable conformational differences of this helix between histone-bound and inhibitor-bound PRMT6 may contribute to the discrepancy observed between our crystal structure and the MOA derived from kinetic experiments.

Cell-based Studies

We first assessed the effect of pharmacological inhibition of endogenous PRMT1 by MS023 in cells. It has been reported that PRMT1 is the main contributor to H4R3 (histone H4 arginine 3) asymmetric dimethylation (H4R3me2a) in cells.[43, 44] We found that knocking down PRMT1, but not PRMT3, 4, or 6, resulted in decreased basal H4R3me2a level in MCF7 cells (Supporting Figure S7). Similar to the PRMT1 knockdown, MS023 treatment (48 h exposure) potently and concentration-dependently reduced cellular levels of H4R3me2a ($IC_{50} = 9 \pm 0.2$ nM (n = 3)) (Figure 4A). We also tested MS094, compounds **5** and **6** in the same cellular assay. As expected, these compounds did not decrease cellular levels of H4R3me2a (Figure 4B), consistent with their lack of inhibitory activity in biochemical assays. To determine the cellular activity of MS023 against PRMT6, we used HEK293 cells with ectopic expression of PRMT6. Overexpression of the wild type (WT) but not catalytically inactive mutant (V86K/D88A) PRMT6 robustly increased endogenous asymmetric dimethylation of H3R2 (histone H3 arginine 2) (Figure 4C, first two columns)[45]. MS023 (20 h exposure) concentration-dependently reduced the H3R2me2a mark in HEK293 cells ($IC_{50} = 56 \pm 7$ nM (n = 3)) (Figure 4C). The effect of the 333nM or 1,000 nM MS023 treatment matched with that of the catalytically inactive mutant (Figure 4C).

We next assessed the effect of MS023, as a pan inhibitor of type I PRMTs, on arginine methylation patterns in cells. As introduced earlier, type I PRMTs catalyze the mono- and asymmetric dimethylation of arginine residues. After treating MCF7 and HEK293 cells with MS023 for two days, we observed a significant decrease in global levels of arginine asymmetric dimethylation (Rme2a) and a concurrent increase in global levels of arginine monomethylation (Rme1) and arginine symmetric dimethylation (Rme2s) (Figure 5 and Supporting Figure S8). The effect is consistent with that of PRMT1 knockout,[46] which accounts for about 90% of global arginine asymmetric dimethylation. We also tested MS094, compounds **5** and **6** in the cellular assays. As expected, these compounds did not change global levels of arginine monomethylation, asymmetric dimethylation and symmetric dimethylation in MCF7 cells (Supporting Figures S9-S10), confirming that these compounds are excellent negative controls for cell-based studies.

Lastly, we evaluated the effect of MS023 on cell growth using eight different cell lines (Supporting Figure S11). With the exception of HEK293 cells, 96 h treatment with MS023 did not significantly impact cell growth at concentrations up to 1 μM. At higher concentrations (e.g., 10 μM, 50 μM), MS023 decreased cell growth in most of tested cell lines. PRMTs were shown to play important roles in cell growth and proliferation.[10, 13, 46-52] However, the effects on cell proliferation maybe time and cell type dependent as PRMT1 conditional knock out in MEF cells led to cell death 10-12 days after the knockout induction[46]. Since MCF7 cells seemed to be the least sensitive to the inhibitor treatment, we evaluated the effect of MS023 on MCF7 cell growth over a period of 10 days together with

the most sensitive cells – HEK293, as a control. Long-term treatment of HEK293 cells with MS023 showed the same results as the 4-day treatment, with 1 μM concentration greatly affecting cell growth (Supporting Figure S11 and S12A). However, longer treatment of MCF7 cells revealed a significant decrease of cell growth at 10 μM concentration (Supporting Figure S12A). Since these results are based on cell confluency and it was shown previously that PRMT1 and PRMT6 inhibition decreases cell proliferation and leads to cell senescence and increased cell size/flattening morphology,[48, 50] we determined the cell number at the end of experiment using two fluorescent nuclear dyes Vybrant Dye Cycle Green and Enzo Nuclear ID Red. As shown in Supporting Figure S12B, MS023 even at 0.1 μM affected cell growth in MCF7 cells (the cell number after 10 days of treatment decreased by 30%). Thus, MS023 at low concentrations inhibits cell growth indicated by decreased cell number and also potentially induces growth arrest and flattening morphology (Supporting Figure S12C).

Conclusions

We discovered MS023, a potent, selective and cell-active inhibitor of type I PRMTs. In biochemical assays, MS023 was highly potent and selective for type I PRMTs over a broad range of epigenetic modifiers including type II and type III PRMTs, PKMTs, DNMTs, histone lysine demethylases, and methyl-lysine and methylarginine reader proteins. The X-ray cocrystal structure of MS023 in complex with PRMT6 confirmed that MS023 occupies the peptide substrate binding site. Our SAR results together with the insights revealed by the cocrystal structure support our inhibitor design hypothesis that the ethylenediamino group of MS023 is an excellent arginine mimetic and a critical moiety for targeting type I PRMTs. Importantly, MS023 potently inhibited the catalytic activity of PRMT1 and reduced levels of H4R3me2a in cells. It also inhibited PRMT6-dependent increase in H3R2me2a levels in cells. Furthermore, MS023 reduced global levels of arginine asymmetric dimethylation and concurrently increased arginine monomethylation and symmetric dimethylation in cells. We also discovered MS094, a close analog of MS023, which was inactive in biochemical and cellular assays, as a negative control for chemical biology studies. Taken together, MS023 and MS094 are valuable tools for the biomedical community to investigate the role of type I PRMTs in health and disease.

METHODS

Synthesis of MS023 and its analogs

Synthetic procedures and full characterization data of all new compounds are described in Supporting Information.

PRMT biochemical assays

A scintillation proximity assay (SPA) was used for assessing the effect of test compounds on inhibiting the methyl transfer reaction catalyzed by PRMTs as described previously.[27] In brief, the tritiated S-adenosyl-*L*-methionine (^{3}H-SAM, PerkinElmer Life Sciences) was used as the donor of methyl group. The (^{3}H) methylated biotin labelled peptide was captured in streptavidin/scintillant-coated microplate (FlashPlate® PLUS; PerkinElmer Life Sciences) which brings the incorporated ^{3}H-methyl and the scintillant to close proximity resulting in

light emission that is quantified by tracing the radioactivity signal (counts per minute) as measured by a TopCount NXT™ Microplate Scintillation and Luminescence Counter (PerkinElmer Life Sciences). When necessary, non-tritiated SAM was used to supplement the reactions. The IC$_{50}$ values were determined under balanced conditions at K_m concentrations of both substrate and cofactor by titration of test compounds in the reaction mixture.

Selectivity assays; MOA studies; biophysical assays; cloning, expression and purification of PRMT6 for crystallography; crystallization, data collection and structure determination; nuclear staining with fluorescent membrane permeable dyes; Cell growth assay

Protocols are described in Supporting Information.

Cellular PRMT1 assay

MCF7 cells were grown in 12-well plates in DMEM supplemented with 10% FBS, penicillin (100 units mL^{-1}) and streptomycin (100 μg mL^{-1}). 40% confluent cells were treated with different concentrations of MS023 and compounds **4** – **6** at indicated concentrations or DMSO control for 48 h. Cells were lysed in 100 μL of total lysis buffer (20 mM Tris-HCl pH 8, 150 mM NaCl, 1 mM EDTA, 10 mM MgCl$_2$, 0.5% TritonX-100, 12.5 U mL^{-1} benzonase (Sigma), complete EDTA-free protease inhibitor cocktail (Roche)). After 3 min incubation at RT, SDS was added to final 1% concentration. Lysates were run on SDS-PAGE and immunoblotting was done as outlined below to determine H4R3me2a, arginine asymmetric dimethylation, arginine symmetric dimethylation and arginine monomethylation in western blot.

Cellular PRMT6 assay

HEK293 cells were grown in 12-well plates in DMEM supplemented with 10% FBS, penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}). 50 % confluent cells were transfected with FLAG-tagged PRMT6 or mutant V86K/D88A PRMT6 (1 μg of DNA per well) using jetPRIME® transfection reagent (Polyplus-Transfection), following manufacturer instructions. After 4 h media were removed and cells were treated with MS023 at indicated concentrations or DMSO control. After 20 h, media was removed and cells were lysed in 100 μL of total lysis buffer.

Western blot

Total cell lysates were resolved in 4-12% Bis-Tris Protein Gels (Invitrogen) with MOPS buffer (Invitrogen) and transferred in for 1.5h (80 V) onto PVDF membrane (Millipore) in Tris-Glycine transfer buffer containing 20% MeOH and 0.05% SDS. Blots were blocked for 1 h in blocking buffer (5% milk in 0.1% Tween 20 PBS) and incubated with primary antibodies: mouse anti-H4 (1:1000, Abcam #174628), rabbit anti-H4R3me2a (1:1000, Active Motif #39705), mouse anti-FLAG (1:5000, Sigma #F1804), mouse anti-β actin (1:3000, Abcam #3280), mouse anti-H3 (1:1000, Abcam #174628), rabbit anti-H3R2me2a (1:1000, Millipore #04-808), rabbit anti-Rme2a (1:1000, Cell Signaling Technology #13522), rabbit anti-Rme2a Asym24 (1:1000, Millipore#07-414), rabbit anti-Rme2S (1:1000, Cell Signaling Technology #13222), rabbit anti-Rme1 (1:1000, Cell Signaling

Technology #8015) in blocking buffer overnight at 4 °C. After five washes with 0.1% Tween 20 PBS the blots were incubated with goat-anti rabbit (IR800 conjugated, LiCor #926-32211) and donkey anti-mouse (IR 680, LiCor #926-68072) antibodies (1:5000) in Odyssey Blocking Buffer (LiCor) for 1 h at RT and washed five times with 0.1% Tween 20 PBS. The signal was read on an Odyssey scanner (LiCor) at 800 nm and 700 nm.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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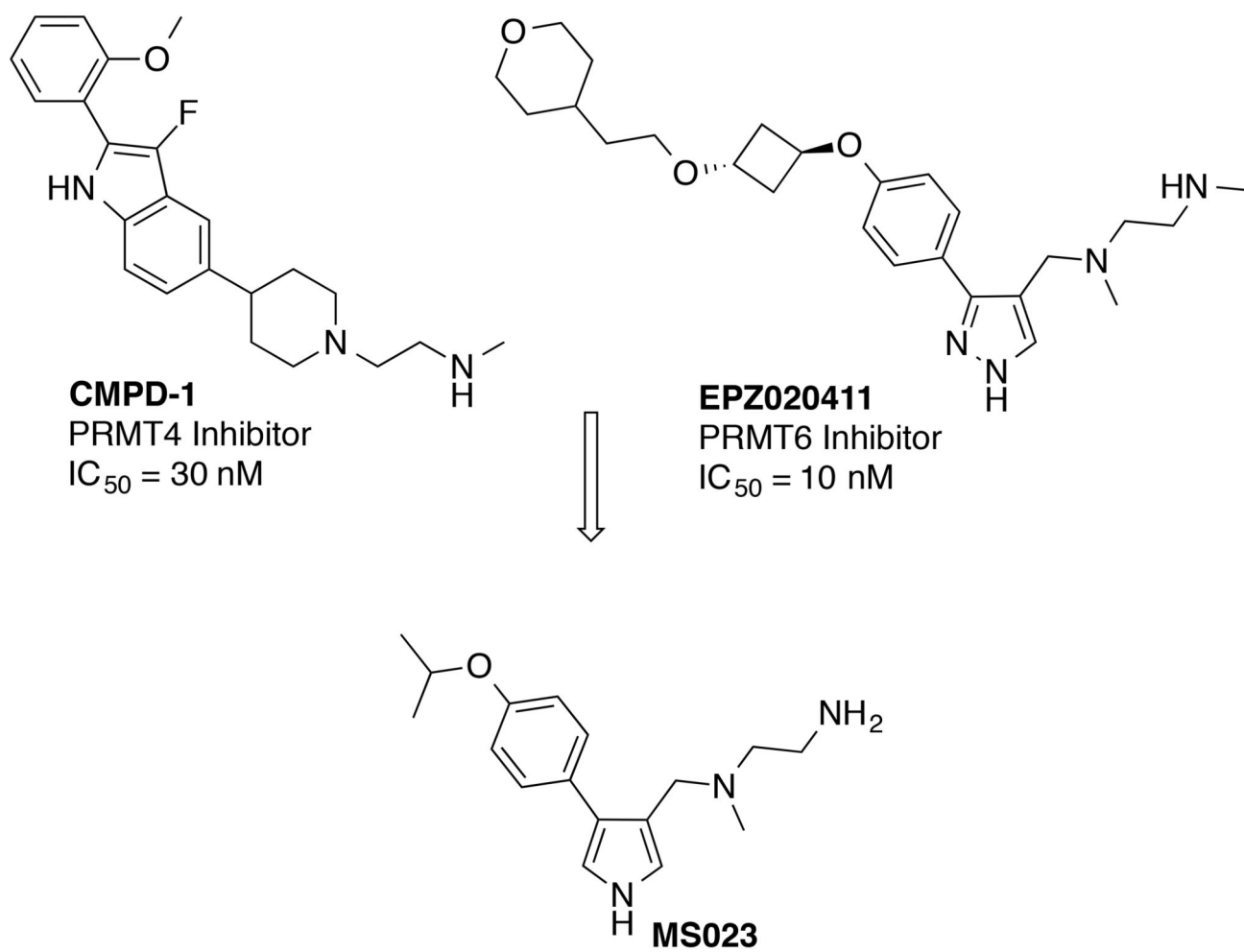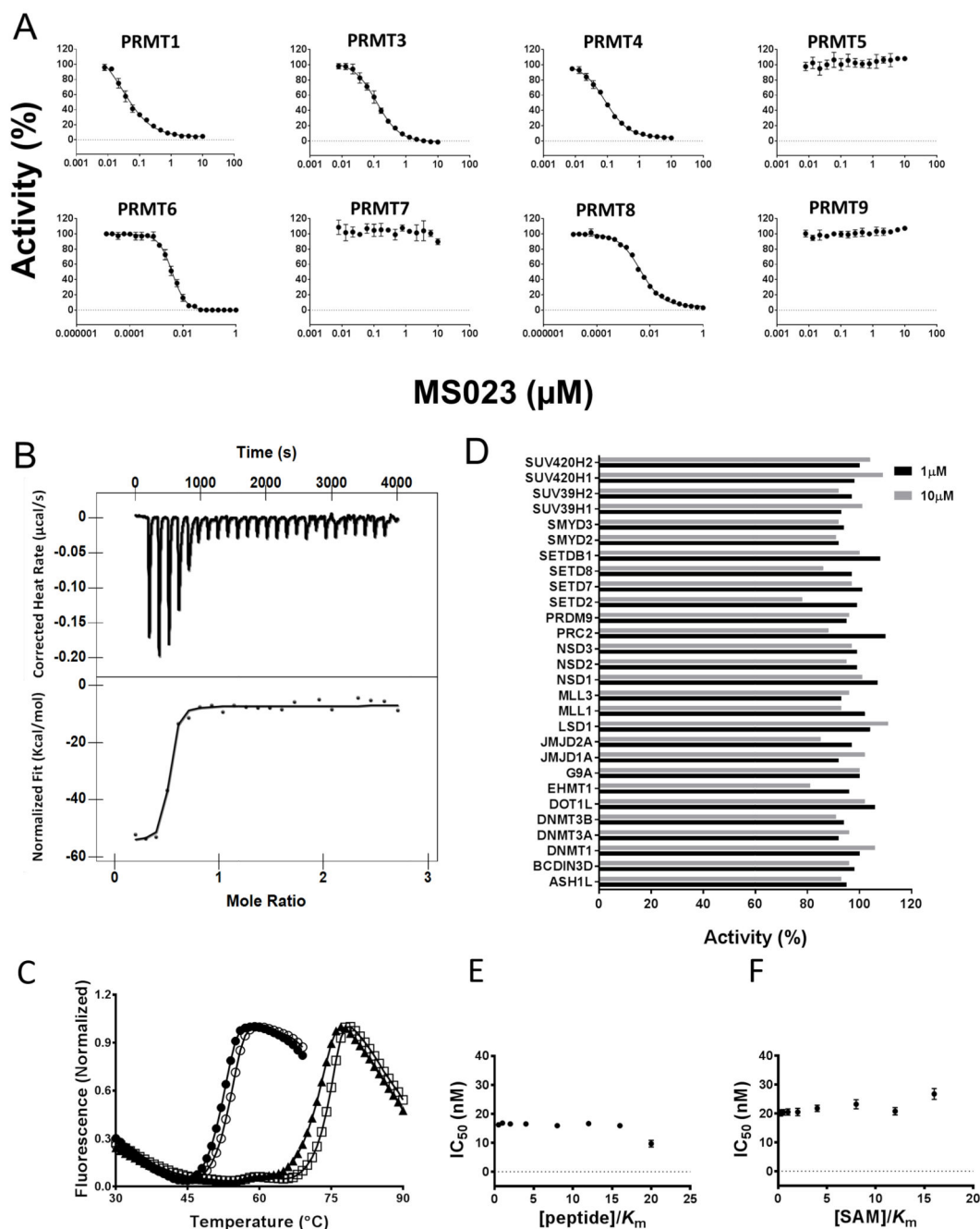
Figure 1. Design of the type I PRMT inhibitor MS023

Figure 2. Characterization of MS023 in biochemical and biophysical assays

(A) IC_{50} determination for MS023 against PRMTs. (B) Isothermal titration calorimetry (ITC) was used to assess binding of PRMT6 to MS023 (K_d = 6 nM). (C) Differential scanning fluorimetry (DSF) was used to confirm the binding of PRMT6 to MS023. Experiments were performed for PRMT6 (●) in the absence of any compound as a control (T_m = 52 °C), and in the presence of (○) 100 μM SAM (T_m = 1.6 °C), (▲) 200 μM MS023 (T_m = 20 °C) and (□) 100 μM SAM plus 200 μM MS023 (T_m = 22 °C). The inflection point of each transition curve is considered melting temperature (T_m) and the increase in T_m

is an indication of binding. (D) The selectivity of MS023 was determined against a panel of 25 PKMTs and DNMTs and 3 histone lysine demethylases at two compound concentrations of 1 μM and 10 μM. No change was observed in the IC_{50} values when determined under different peptide (E) and SAM (F) concentrations for PRMT6 indicating a noncompetitive pattern of inhibition.
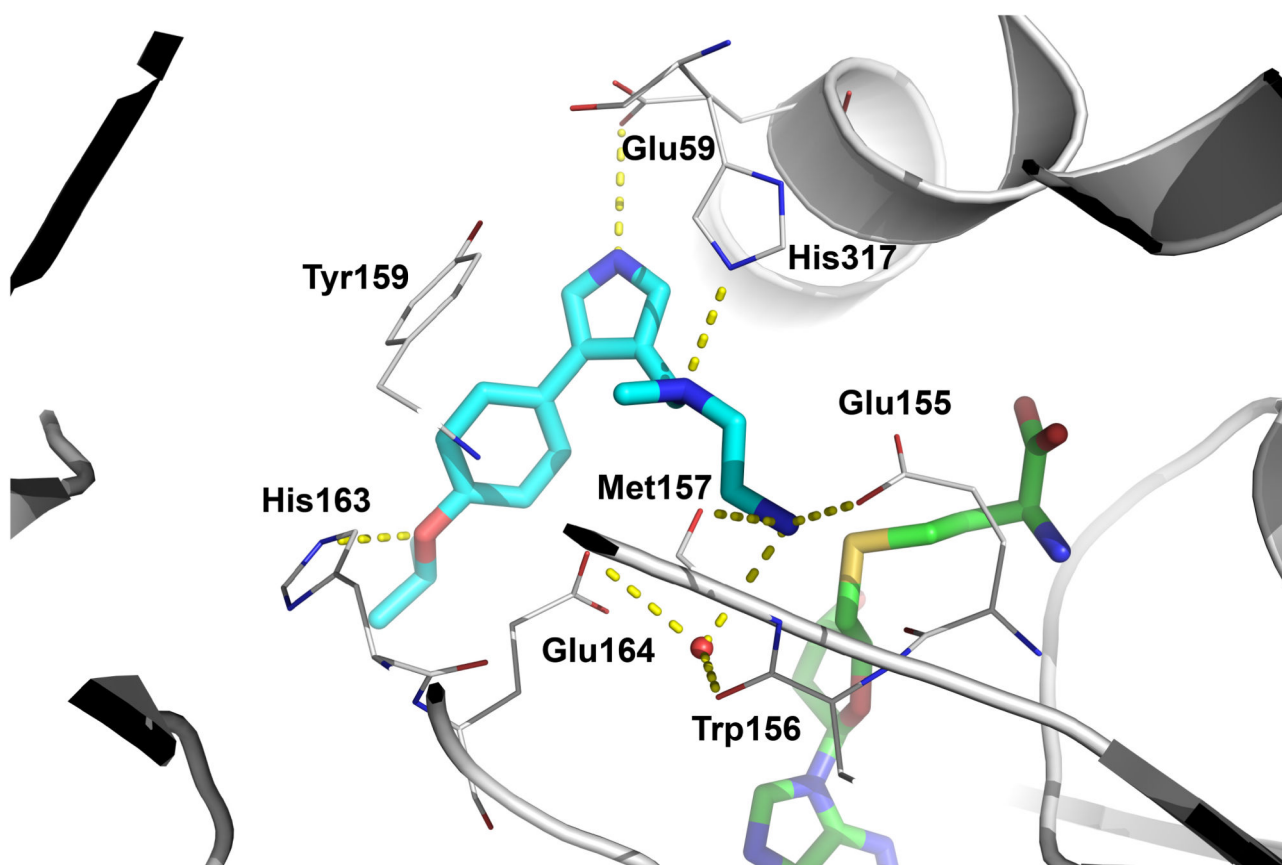
Figure 3.
The X-ray crystal structure of a ternary complex of MS023 (cyan), SAH (green) and
PRMT6 (gray). Key interactions shown in yellow dotted lines.
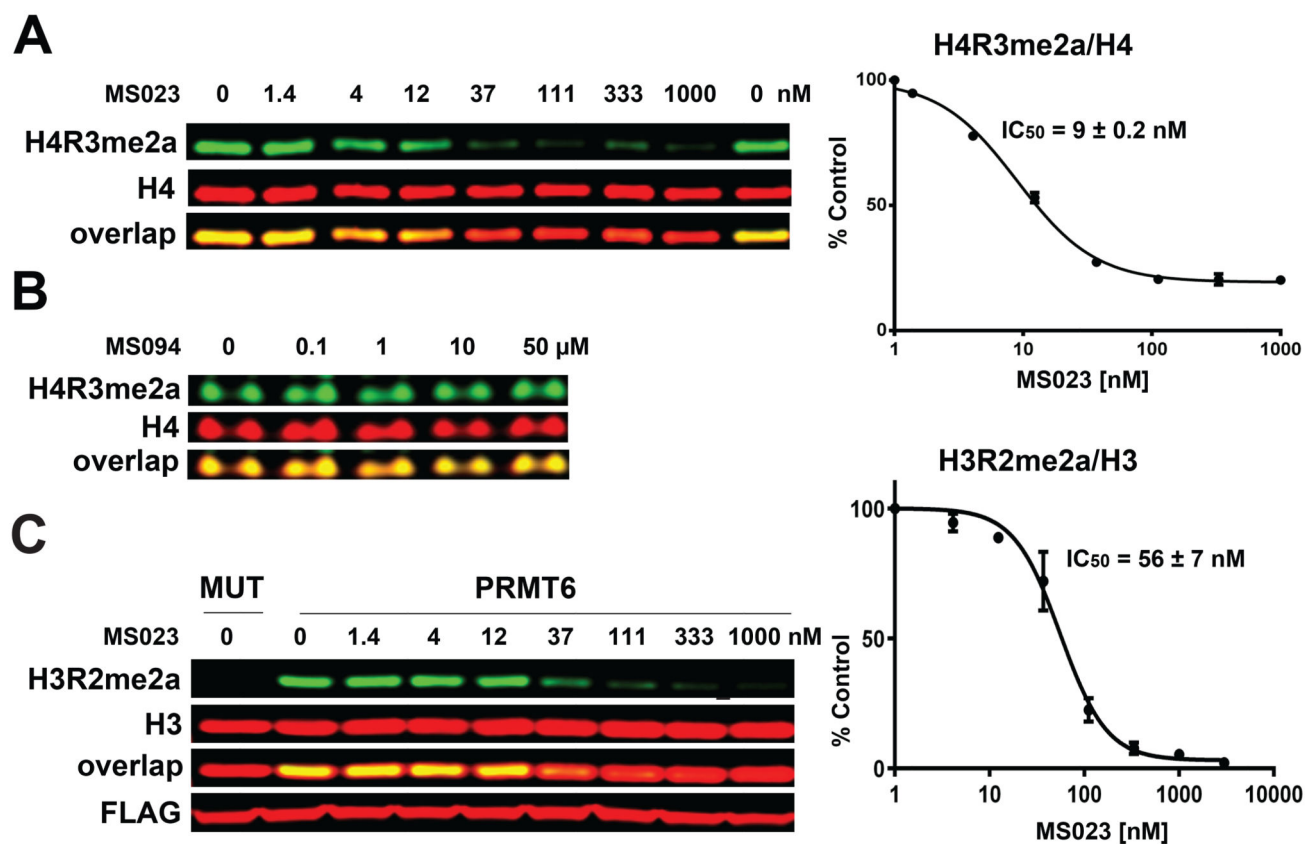
Figure 4. The effect of MS023 and MS094 on inhibiting PRMT1 and PRMT6 in cells
(A) MS023 inhibits PRMT1 methyltransferase activity in MCF7 cells. (B) MS094 does not inhibit PRMT1 methyltransferase activity in MCF7 cells. MCF7 cells were treated with MS023 (A) or MS094 (B) at indicated concentrations for 48 h and H4R3me2a levels were determined by Western blot. The graphs represent nonlinear fits of H4R3me2a signal intensities normalized to total histone H4. The results are MEAN ± SEM of two experiments done in triplicate. (C) MS023 inhibits PRMT6 methyltransferase activity in HEK293 cells. HEK293 cells were transfected with FLAG-tagged PRMT6 or its catalytically inactive mutant V86K/D88A (MUT) and treated with MS023 at indicated concentrations for 20 h. H3R2me2a levels were determined by Western blot. The graphs represent nonlinear fits of H3R2me2a signal intensities normalized to total histone H3. The results are MEAN ± SEM of 3 replicates.
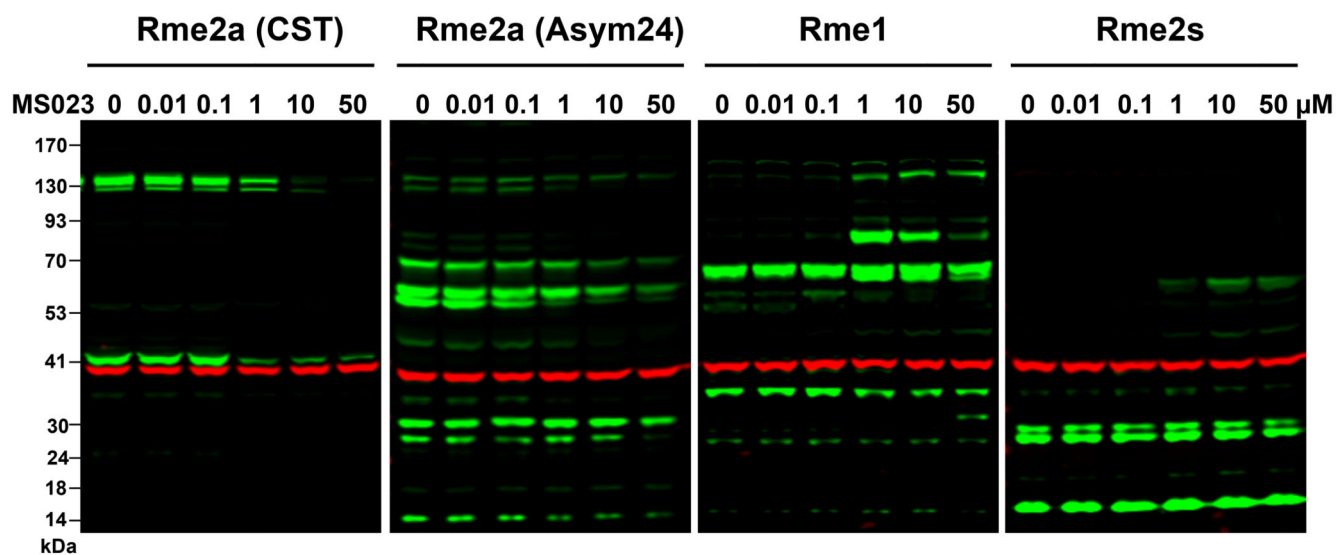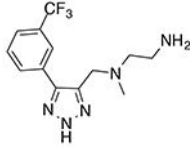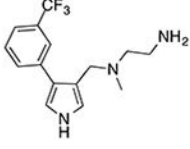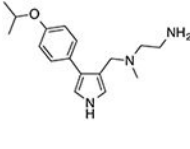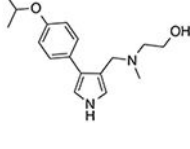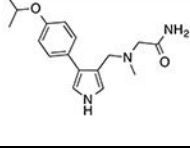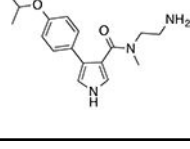
Figure 5. The effect of MS023 on arginine asymmetric dimethylation (Rme2a), symmetric dimethylation (Rme2s) and monomethylation (Rme1) in cells

MS023 reduces cellular levels of Rme2a and concurrently increases cellular levels of Rme1 and Rme2s. MCF7 cells were treated with MS023 at indicated compound concentrations for 48 h. Arginine asymmetric dimethylation, arginine symmetric dimethylation and arginine monomethylation were detected by western blot using indicated pan-arginine antibodies (green). β-actin was used as a loading control (red).
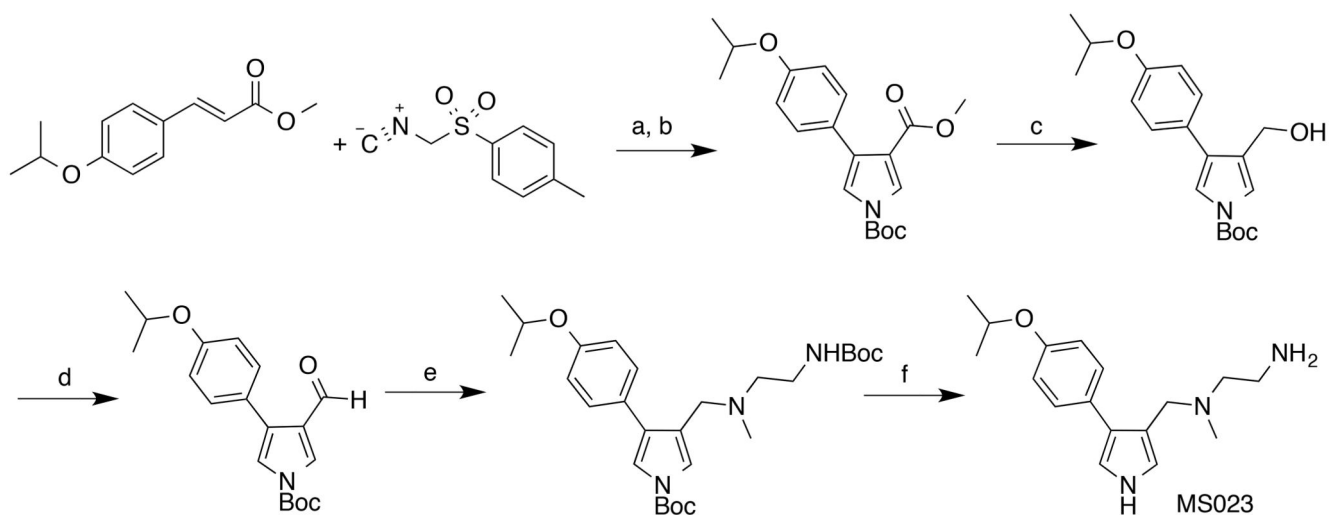
Scheme 1. Synthetic route for MS023

Reagents and conditions: (a) NaH, DMF, THF; (b) Boc$_2$O, Et$_3$N, DMAP, DCM, 82% over two steps; (c) DIBALH, DCM, −78 °C, 71%; (d) DMP, DCM, 75%; (e) *tert*-butyl (2-(methylamino)ethyl)carbamate, NaBH(OAc)$_3$, DCM, 94%; (f) HCl, MeOH, 45 °C, 87%.

Table 1

SAR of synthesized compounds

Compound	Structure	IC$_{50}$ (nM) / K_i (nM)				
		PRMT1	PRMT3	PRMT4	PRMT6	PRMT8
1		>20,000/ >10,000	>100,000/ >50,000	>75,000/ >37,500	230 ± 12/ 110 ± 6	3,000 ± 500/ 1,500 ± 250
2		250 ± 15/ 120 ± 4	1,100 ± 180/ 550 ± 85	260 ± 10/ 120 ± 5	9 ± 0.9[*]/ 3 ± 0.3	42 ± 3/ 17 ± 0.9
MS023 (3)		30 ± 9/ 11 ± 3	119 ± 14/ 55 ± 7	83 ± 10/ 23 ± 3	4 ± 0.5[*]/ 0.8 ± 0.1	5 ± 0.1[*]/ 1.3 ± 0.03
MS094 (4)		NI	NI	NI	NI	NI
5		NI	NI	NI	NI	NI
6		NI	NI	NI	NI	NI

IC$_{50}$ determination experiments were performed at substrate concentrations equal to the respective K_m values for each enzyme. K_i values were calculated as previously described. IC$_{50}$ determination experiments were performed in triplicate and the values are presented as Mean ± SD. NI: no inhibition.

[*] Due to the high potency of the compounds, lower enzyme concentrations (5 nM) were used for testing these compounds to avoid enzyme concentrations higher than IC$_{50}$ values as much as possible.