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A novel arginine methyltransferase inhibitor with cellular activity

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Abstract—Via virtual screening we identified a thioglycolic amide as an arginine methyltransferase (PRMT) inhibitor and tested it and related compounds against the fungal PRMT RmtA and human PRMT1. Compound RM65 was the most potent druglike inhibitor (IC_{50}-PRMT1: 55.4 μM) and showed histone hypomethylation in HepG2 cells. Docking studies proposed binding at the substrate and SAM cofactor binding pocket. It may serve as a lead for further PRMT inhibitors useful for the treatment for hormone dependent cancers.

Histones are subject to an intricate pattern of posttranslational modifications such as acetylation and methylation which govern the access of transcription factors to DNA. The same modifications also dictate the activity of certain non-histone proteins.[1] There is little precedence for inhibitors of arginine methyltransferases (PRMTs). Beside cofactor analogues like Sinefungin,[2] there are only three reports on inhibitors of these enzymes. In one study certain dyes and related molecules were discovered to inhibit PRMT by high throughput screening[3] and subsequently structure-activity relationships[4] were obtained. But some of these compounds were also identified as histone acetyltransferase inhibitors.[5]

The proteotypical inhibitor AMI-1 (**1**)[3] (see Fig. 1) shows the ability to block transcriptional activation of hormone receptors by agonists and therefore this class of inhibitors has potential as anticancer drugs. We have reported on the first rational approach to PRMT inhibitors using a protein-based virtual screening (see Fig. 1).[6]

These new inhibitors stilbamidine (**2**) and allantodapsone (**3**) lead to cellular hypomethylation and a block of estrogen receptor activation in a reporter gene model.[6]

Here we report on further lead structures that were identified by virtual screening, respectively, were generated by testing of analogues of the initial lead in this study.

First, we have screened the Hans-Knöll-Database (HKI, including about 9000 compounds) virtually for novel fragment-like leads (molecular weight <200) representing as starting points for the development of novel PRMT inhibitors.[7] The pre-filtering of the HKI resulted in about 900 compounds that were docked into a homology model of a fungal PRMT (RmtA).[8] RmtA is a homologue of human PRMT1 which we used for the primary biological screening. The docking was carried out using the GOLD[9] program (CCDC, Cambridge, UK) as previously published.[6] The top-ranked fragments were visually inspected in MOE (Chemical Computing Group, Montreal, Canada) whether their binding mode is in agreement with GRID (Molecular Discovery,

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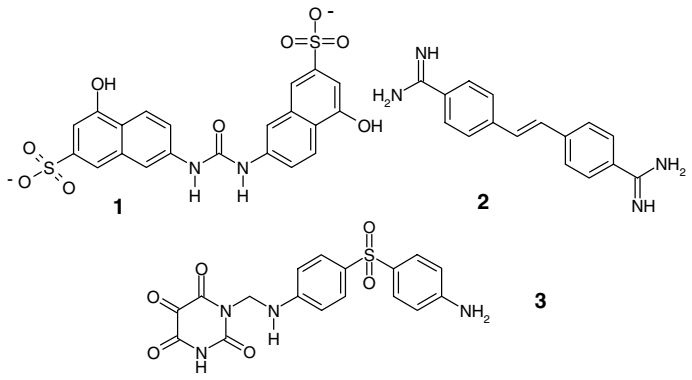

Figure 1. PRMT inhibitors.

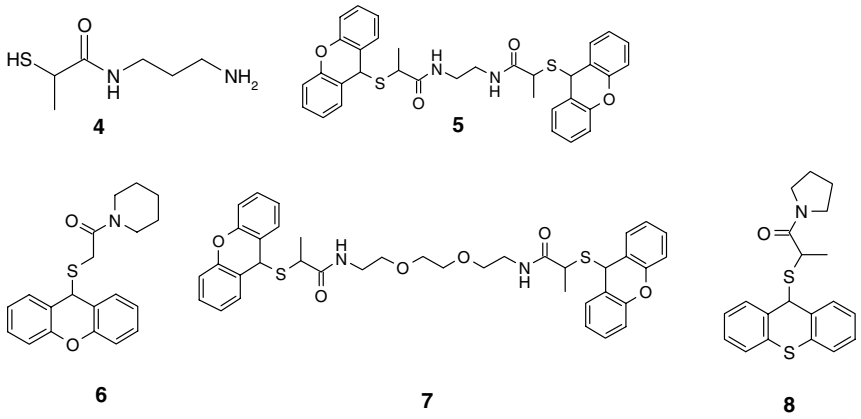

Figure 2. Thioglycolic amides tested for PRMT inhibition.[12]

Oxford, UK) interaction fields calculated for the binding pocket.

Compound **4** (see Fig. 2) was identified by the virtual screening of the HKI as a candidate for biological testing and found to be a micromolar inhibitor of PRMT activity. As it is chemically not very stable and is not a druglike molecule, we subsequently searched our in house database for structurally related compounds that also contain an α-methylthioglycolic amide substructure.[10]

Thus, we identified compounds **5**–**8** as potential inhibitors (see Fig. 2) and tested them in an enzyme assay that is based on antibody mediated recognition of arginine methylation, a secondary europium labelled antibody, and finally measurement of time-resolved fluorescence (see Table 1).[6] Initial biological screening was performed using a fungal PRMT from Aspergillus nidulans (RmtA) which is homologous to hPRMT1[11] and all compounds[12] were subsequently tested on hPRMT1[6] (see Table 1).

Using our in vitro assay, we identified compound **5** as an inhibitor of both fungal and human PRMTs. It showed no inhibition of the lysine methyltransferase SET7/9 at 50 μM (data not shown).[13] Structural differences between **4** and **5** may serve as a starting point towards species selective inhibitors as potential antifungal agents.

Table 1. In vitro enzyme inhibition using the TRF assay

Compounds	RmtA IC_{50}[a] (μM)	hPRMT1 IC_{50} [a] (μM)
1[b]	33.8 (±7.8)	1.2 (±0.5)
4	46.8 (±2.9)	na
5	46.2 (±1.4)	55.4 (±3.4)
6	na	na
7	na	na
8	na	na

[a] Values are means of duplicates, standard deviation is given in parentheses (na = not active, <20% at 40 μM).
[b] Data from the literature.[5]

Hit validation was performed using antibody mediated detection of histone hypomethylation in HepG2 cells as described before.[6,14] As mentioned in previous reports on PRMT inhibitors rather high concentrations are necessary to reverse hypermethylation on arginine 3 on histone H4. Compound **5** shows a robust effect at concentrations of 150 μM and higher (Fig. 3). An antiproliferative effect as judged by the protein content as a measure of cell mass and hence, proliferation, could not be observed. Therefore, we report a novel cell permeable PRMT inhibitor. Compound **5** may serve as a new lead structure for further synthetic optimization.

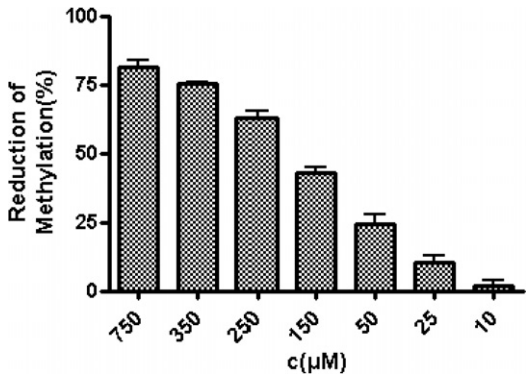

Figure 3. Cellular hypomethylation by compound **5** in HepG2 cells (normalized to protein content).

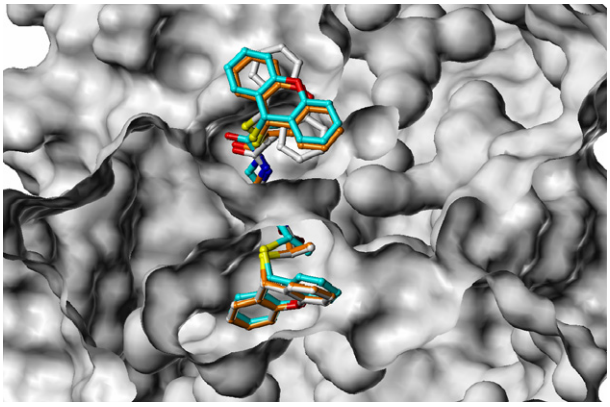

Figure 4. Top ranked docking solution for the bisubstrate inhibitor 5 (all three stereoisomers shown) at hPRMT1. The Connolly molecular surface of the enzyme is displayed (white) showing the substrate and SAM binding pocket.

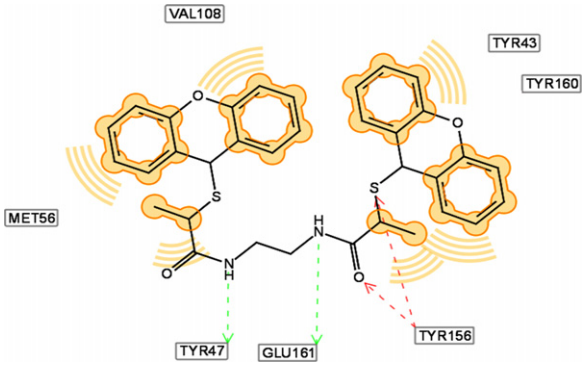

Figure 5. Schematic representation of the interaction between 5 (*R,R* isomer) and hPRMT1. Hydrogen bonds between 5 and enzyme are displayed dashed line, whereas hydrophobic interactions are indicated by the orange lines (Generated with LigandScout[16]).

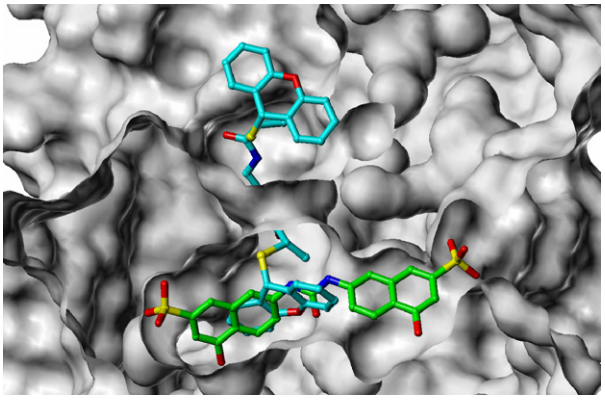

Figure 6. Comparison of the top ranked docking solution of 5 (*R,R* isomer, cyan) and 1 (AMI-1, green) at hPRMT1.

To analyze the binding mode of the developed inhibitors, compounds **4**–**8** were docked into a hPRMT1 protein model. The docking of **4** showed a similar interaction type as the recently developed inhibitors **2** and **3**, (i.e., interaction of the basic amine with the active site Glu152) which were shown to be competitive in regard to the peptide substrate.[6] In contrast, favourable docking solutions for **1** and **5** could only be obtained when the cofactor *S*-adenosyl methionine (SAM) was omitted from the protein model. All docked stereoisomers of compound **5** show favourable van der Waals interaction with several aromatic residues of the SAM and substrate binding pocket (Tyr43, Tyr47, Met56, Val108, Tyr156, Tyr160, and Glu161) as well as hydrogen bonds with Tyr47, Tyr156, and Glu161 (Figs. 4 and 5).[15] Thus, **5** represents, in contrast to the substrate-competitive inhibitors **2**–**4**, a bisubstrate type PRMT1 inhibitor.

For **1** our modelling raises doubt on the reported mode[3] of peptide substrate competition. Figure 6 shows the interaction mode of **1** in comparison with the bisubstrate-type inhibitor **5** in the human PRMT1 model. Both compounds interact with residues of the SAM binding pocket. The sulfonyl groups of **1** make electrostatic and hydrogen bonding interaction with several amino acid residues also involved in binding of SAM (not shown). To give experimental proof for the competition of **5** to SAM, we tested the inhibition of hPRMT1 by **5** using varying cofactor and inhibitor concentrations. Indeed, competition with SAM could be shown (see Fig. 1 of Supporting Information).

Since we started with the fragment **4**, which contains a basic amino group that is not observed in the bisubstrate-type inhibitor, it was interesting to compare the type of interaction of both inhibitors. The docking of **4** showed that the compound makes a salt-bridge between protonated nitrogen and Glu152. Interestingly, the α-methylthioglycolic amide substructure of **5** shows the same orientation and interaction as observed for **4**. The interaction mode of **4** is displayed together with the docking result of **5** in Fig. 7.

Due to the limited space between SAM and substrate pocket no favourable docking solutions could be derived for **6** and **8**. The more bulky pyrrolidine or piperidine ring does not fit into the narrow part of the binding pocket, as observed for **5**. Due to the increased spacer length between the two amide moieties, compound **7** is too large to fit completely into the binding pocket.

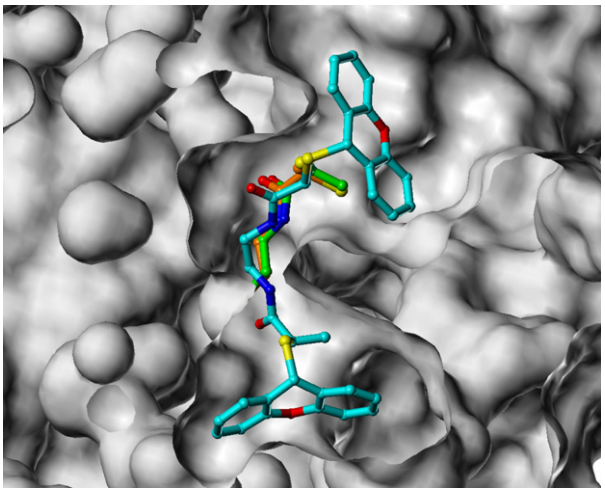

Figure 7. Comparison of the top ranked docking solution of 5 (*R*,*R* isomer, cyan) and 4 (both isomers R and S, green and orange) in the substrate and SAM binding pockets of hPRMT1.

The docking results are in qualitative agreement with the biological data, showing that among the novel compounds only **5** is a hPRMT1 inhibitor.

In summary, for the first time a fragment-focussed virtual screening approach has successfully been applied in the search for new PRMT inhibitors. Compound **5** has been identified as a cell permeable inhibitor that leads to histone hypomethylation in cancer cells. Due to the link between arginine methylation and transcription, especially via androgen and estrogen dependent receptors, it may serve as new lead for potential drugs for the treatment of hormone dependent cancers. The reference inhibitor **1** is about fifty-fold more potent in vitro but also only shows significant histone hypomethylation above 100 μM.[3,6] Docking studies suggested a mode of bisubstrate inhibition of the new inhibitor **5** which opens up interesting possibilities for future structural variations. The modelled protein-inhibitor complexes will be valuable tools towards new potent and selective PRMT inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.05.088.

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7. The program OMEGA, Openeye was used to generate the 3D structures of the HKI compounds considering all possible stereoisomers and the MOE 2006.8 program was used to store all structures and docking results. The Hans-Knöll-Institute Database was obtained from the collection of natural products, derivatives and synthetic analogues and comprises about 9000 compounds. Those compounds have been mainly provided by more than 80 research groups worldwide.
8. The RmtA homology model based on rat PRMT1 and PRMT3 X-ray structures including the cofactor SAM was used for the virtual screening as previously published.[6]
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10. Using MACCS fingerprints, a Tanimoto coefficient >0.5 and the similarity search module within MOE we searched for analogues of **4**.
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12. Purity and identity of the compounds was assessed by NMR, IR, mass spectrometry and elemental analyses (not shown). The synthesis of (*R*,*S*)-*N*-(3-aminopropyl)-2-sulfanylpropionic acid amide **4** and corresponding analytical data have been described by R. Ortmann, Ph.D. Thesis, University of Marburg, Germany 2001, page 311. The syntheses of (*N*,*N*′-ethane-1,2-diyl)di[(*R*,*S*)-2-(9-xanthenyl-sulfanyl)propionic acid amide **5**, *N*,*N*′-(3,6-dioxaoctane-1,8-diyl)-di[(*R*,*S*)-2-(9-xanthenylsulfanyl)propionic acid amide **6** and 2-(9-xanthenylsulfanyl)-[*N*,*N*-butane-1,4-diyl]propionic acid amide **7** have been described by R. Machmur, Ph.D. Thesis, University of Marburg, Germany 2006, **5** page 182, **6** page 186, **7** page 177, **8** page 242.
13. The PRMT assays were performed as in the literature.[6] The SET7/9 assays are based on the same system. However, hSET7/9 from Biotrend was used as the source of enzyme activity. Biotinylated Histone H3 peptide, aa 1–21 (Upstate), served as a substrate. The incubation with the enyzme was performed at 37 °C for 1 h. The amount of the substrate's turnover was detected by the usage of anti-monomethyl-Histone H3 (Lys4) rabbit IgG (Upstate).
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15. Compounds **4**–**8** are chiral compounds and were tested as racemic mixtures. The docking was carried out with all possible stereoisomers, and comparable docking poses were obtained.
16. LigandScout, Inteligand, www.inteligand.com, Vienna, Austria.