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Acyl derivatives of *p*-aminosulfonamides and dapsone as new inhibitors of the arginine methyltransferase hPRMT1

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ABSTRACT

Arginine methylation is an epigenetic modification that receives increasing interest as it plays an important role in several diseases. This is especially true for hormone-dependent cancer, seeing that histone methylation by arginine methyltransferase I (PRMT1) is involved in the activation of sexual hormone receptors. Therefore, PRMT inhibitors are potential drugs and interesting tools for cell biology. A dapsone derivative called allantodapsone previously identified by our group served as a lead structure for inhibitor synthesis. Acylated derivatives of *p*-aminobenzenesulfonamides and the antilepra drug dapsone were identified as new inhibitors of PRMT1 by in vitro testing. The bis-chloroacetyl amide of dapsone selectively inhibited human PRMT1 in the low micromolar region and was selective for PRMT1 as compared to the arginine methyltransferase CARM1 and the lysine methyltransferase Set7/9. It showed anticancer activity on MCF7a and LNCaP cells and blocked androgen dependent transcription specifically in a reporter gene system. Likewise, a transcriptional block was also demonstrated in LNCaP cells using quantitative RT-PCR on the mRNA of androgen dependent genes.

1. Introduction

Epigenetics is defined as inheritable changes in the phenotype without changes in the genome.[1] The major biochemical mechanisms behind epigenetics are DNA methylation and posttranslational histone modifications such as acetylation or methylation.[2] Histone methylation is mediated by histone methyltransferases, which can be divided into lysine and arginine methyltransferases. These enzymes have repeatedly been linked to cancer formation and progression and have therefore emerged as interesting targets for drug discovery.[3] Especially arginine methyltransferases also target non-histone protein substrates and are therefore often called protein methyltransferases (PRMTs).[3] The subtype PRMT1 is particularly interesting as it has been linked to the activation of estrogen and androgen[4–6] receptors and therefore may represent a new treatment option for hormone-dependent cancer.[5] So far, only a limited number of inhibitors are available for PRMT1.[5,6] Examples are AMI-1,[4,5,7–9] inhibitor **6e**,[7] stilbamidine,[5] allantodapsone[5] and C-7280948[8,9] (see Fig. 1).

Stilbamidine and allantodapsone showed indirect antiestrogen properties in a reporter gene based assay,[5] thus in this study we set out to synthesize analogs of allantodapsone. Due to the potential lability of the aminal structure we wanted to prepare more stable simple acyl derivatives of allantodapsone as well as similar derivatives of C-7280948.[8] The synthesis of the modified analogs of these lead inhibitors was based on docking studies carried out using structural information of the PRMT1 protein. Inspection of potential interaction points at the substrate binding pocket of PRMT1 was used to guide the synthesis of the new analogs (details in the Modelling section). These new compounds were tested for PRMT1 inhibition in vitro and inhibitors in the low micromolar region were obtained. A chloroacetyl derivative showed potent antiproliferative activity and blocked androgen-dependent protein expression in a reporter gene model.

2. Chemistry

2.1. Analogs of allantodapsone

Acyl analogs of allantodapsone were synthesized by adding acyl chlorides at room temperature to commercially available dapsone

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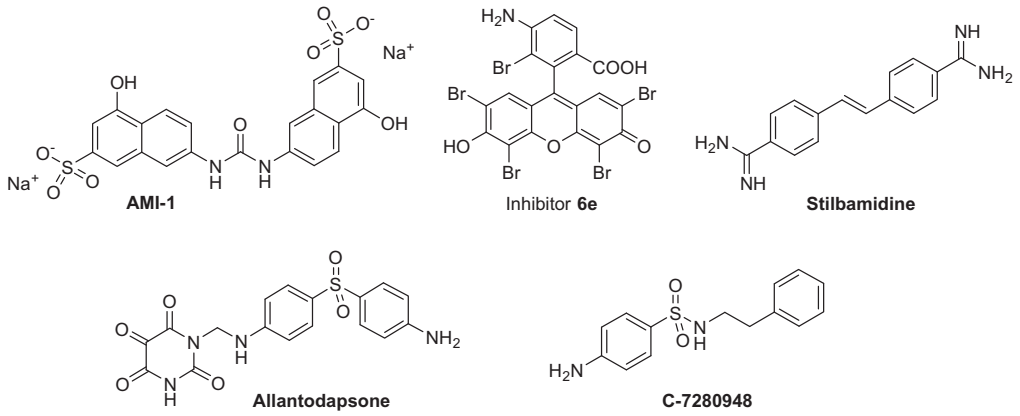


Figure 1. Examples of known PRMT1 inhibitors.

in pyridine (see Fig. 2a). In most cases diacetylation products were formed as well. Due to their very similar physicochemical behaviour, mono- and diacetylated compounds were difficult to separate from each other by column chromatography and only a limited amount of the respective compounds could be obtained in a pure form. Although typical yields were below 10%, the easy accessibility of dapsone allowed collection of sufficient amounts of testing material and did not require optimization of the synthesis.

To analyze the role of the amide function, we also synthesized a sulfonamide and an urea analogue. Using 4-chlorobenzenesulfonyl chloride, which was conjugated with dapsone (see Fig. 2b), we obtained compound **3** as a sulfonyl analogue of **1b.** To obtain the urea derivative **4**, benzyl isocyanate and dapsone were used as the starting materials (see Fig. 2b).

2.2. Acylated derivatives of C-7280948

A similar approach was used with the lead inhibitor C-7280948, which was also treated with various acyl chlorides (see Fig. 3a) to give a set of acylated derivatives. Since the first inhibition studies with the acylated dapsones (see below) revealed a higher potency for derivatives containing an aromatic residue, only aromatic acyl chlorides were used as reagents. To obtain an acylated derivative that would again bear a *p*-amino function for a possible hydrogen bond interaction with the active site, derivative **7h** (see Fig. 3a) was reduced with stannous chloride dihydrate to the aniline **7i**. Again, 4-chlorobenzenesulfonyl chloride was used to synthesize a representative sulfonamide analogue **7j** (see Fig. 3a).

For further structural variation of the lead compound **6a**, we reduced the spacer length between the sulfonamide nitrogen and the aromatic substituent, also introducing branched chain substituents. 4-Nitrobenzenesulfonyl chloride was treated with benzyl amine or racemic α-methylbenzyl amine, respectively, using the same conditions as for the synthesis of **6a** (see Fig. 3a). For further structural variation, substituted benzyl and phenethyl amines were also used as starting materials. The nitro group was again reduced to the corresponding amine by the use of stannous chloride dihydrate. Additionally, the benzene in the amine part

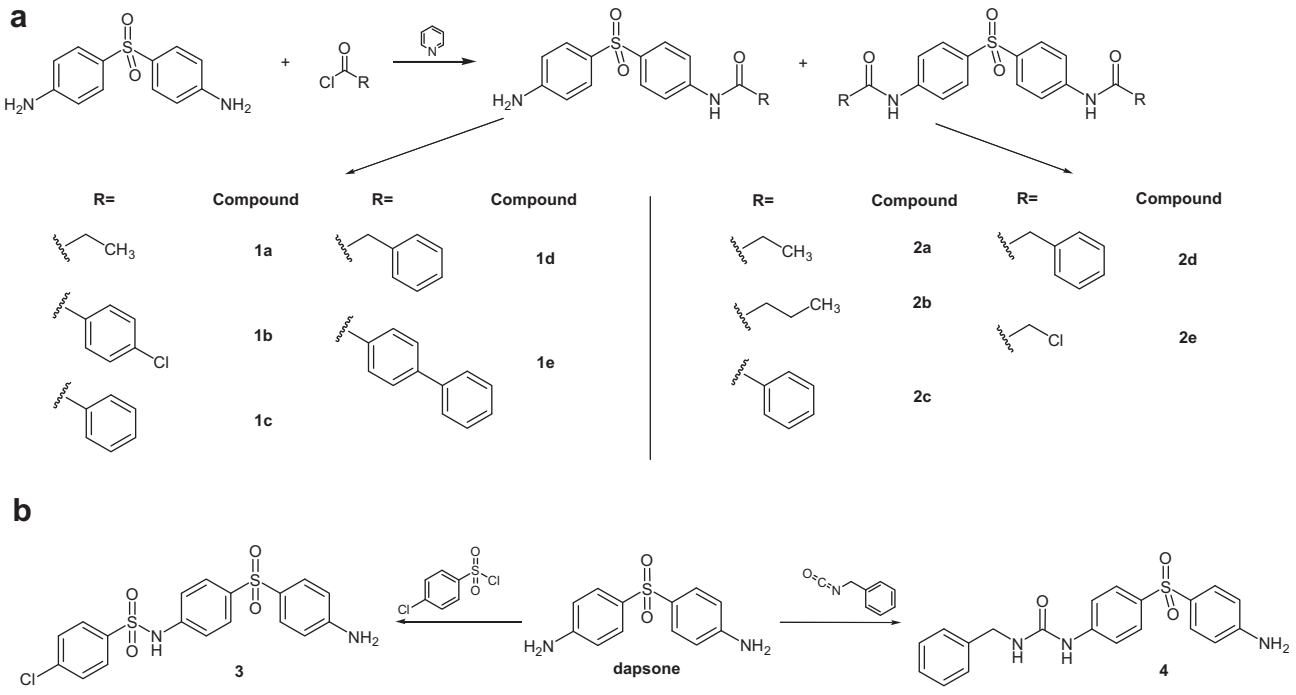


Figure 2. (a) Route for the synthesis of acylated dapsone derivatives with monoacylated and diacylated dapsone derivatives, (b) synthesis of a sulfonyl and an urea derivative of dapsone.

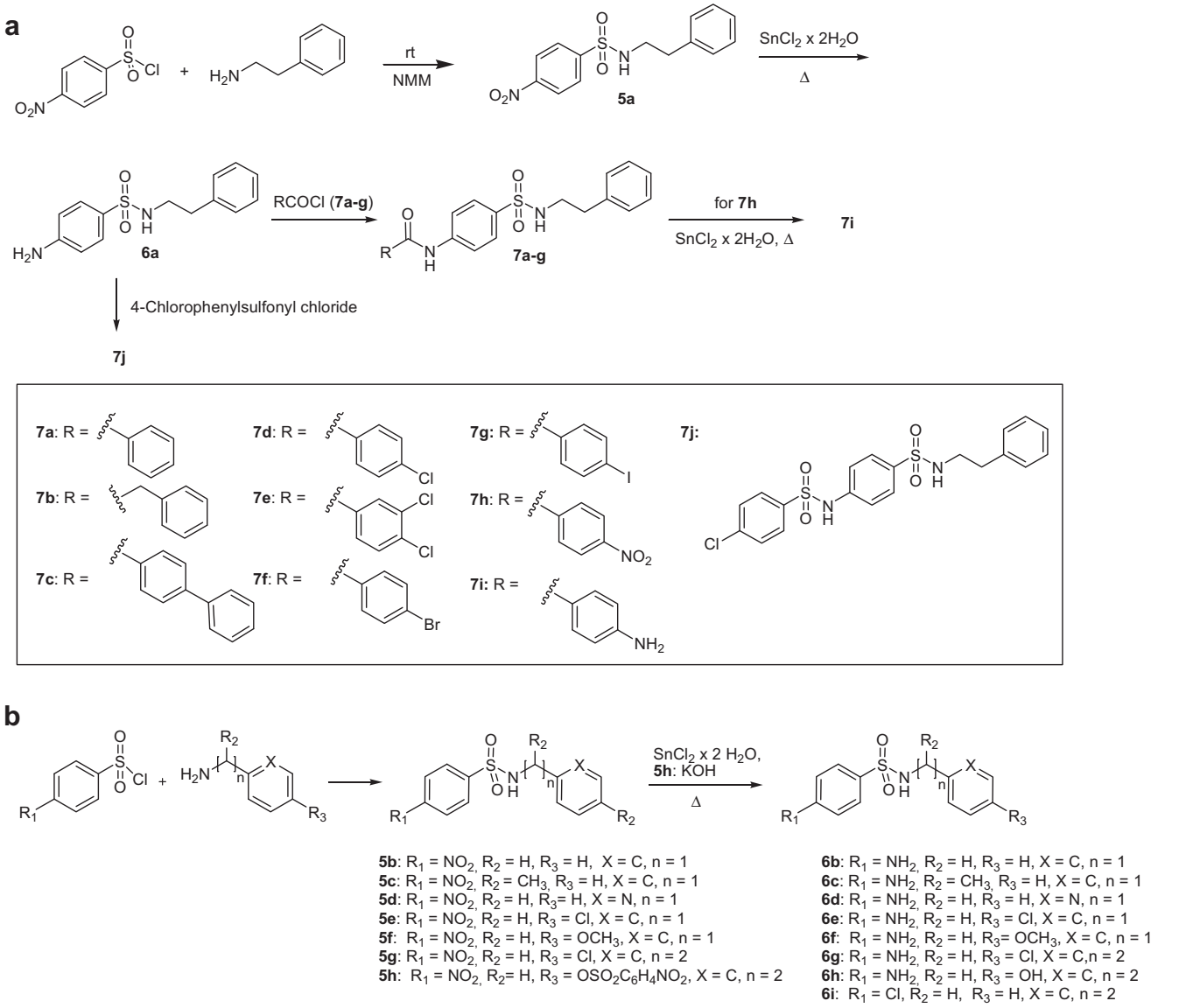


Figure 3. (a) Synthesis of acylated derivatives of C-7280948 (**6a**) and (b) derivatives with varied aliphatic side chain length.

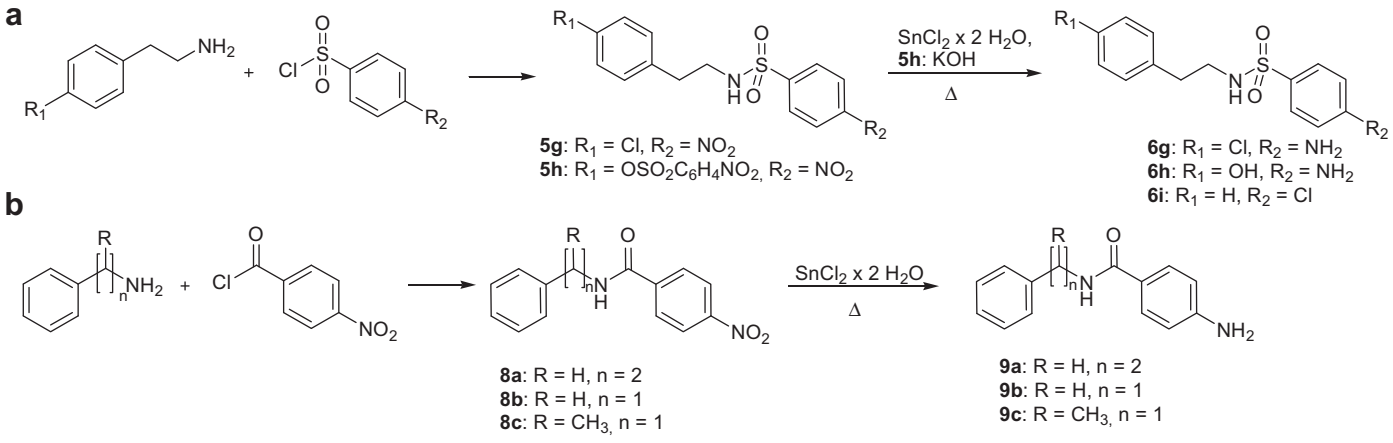


Figure 4. (a) Synthesis of derivatives of **6a** with *p*-substituted phenethylamides, (b) synthesis of carbamide analogues of **6a** with varied aliphatic side chain.

was replaced by a pyridine in a representative compound (**6d**, see Fig. 3b).

Furthermore, polar substituents were introduced to give rise to the substituted phenethyl amides **6g–i** (see Fig. 4a). For the synthesis of carbamide derivates of **6a**, phenethyl and benzyl amines were treated with acid chlorides. Again, the aniline was synthesized from the nitro precursor by reduction with stannous chloride (see Fig. 4b).

3. Molecular modelling

Since there is no crystal structure of human PRMT1 (hPRMT1) available yet, a recently generated homology model[5,8] was used for the docking studies. The hPRMT1 model has been successfully used before to identify the inhibitors stilbamidine, allantodapsone and C-7280948.[5,8] The substrate binding pocket was analyzed by calculating the molecular interaction fields using the GRID software (Molecular Discovery Inc.). The GRID molecular interaction fields[10] show favourable areas of interaction calculated with the hydrophobic probe, the aromatic C2 probe (see Fig. 5), an amine probe and a chlorine probe (see Fig. S1). Starting from the docking solutions of allantodapsone and C-7280948 (see Fig. 5, S2), together with the GRID, fields of the binding ideas for the derivatization of both compounds were obtained.

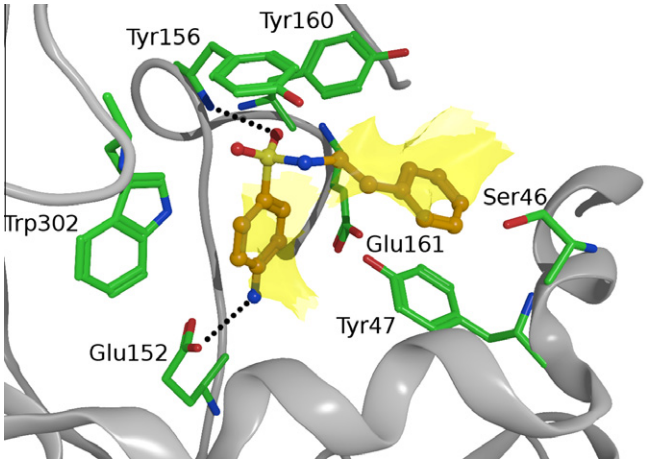


Figure 5. Docking result for C-7280948 (colored orange) at the PRMT1 substrate binding pocket. Only relevant amino acids are displayed in green, whereas the PRMT1 backbone is shown as grey ribbon. The favourable interaction field derived with the aromatic C2 GRID probe is colored orange (contour level −2.0 kcal/mol). Hydrogen bonds are shown as dashed lines.

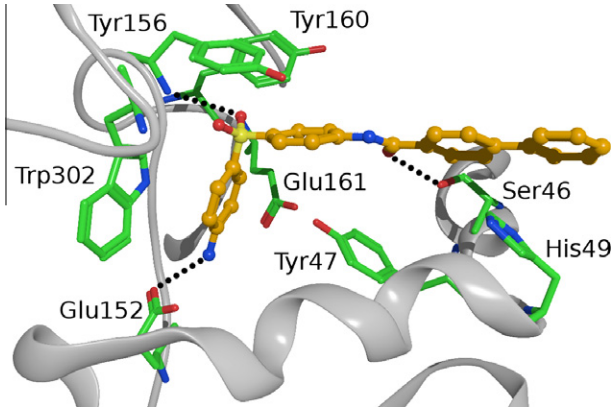


Figure 6. Docking solution obtained for **1e**. Only relevant amino acids are displayed in green, whereas the PRMT1 backbone is shown as grey ribbon. Hydrogen bonds are shown as dashed lines.

3.1. Ligand docking

All compounds were docked in the hPRMT1 substrate binding pocket using the GOLD 4.0 docking software (Cambridge Crystallographic Centre).[11] The top-ranked docking solution obtained for compounds **1a–e**, **2a**, **2b**, **2e**, **3**, and **4** was similar to the one obtained for the original compound allantodapsone, with the aromatic amino group interacting with Glu152 of the catalytic site (**1e**, see Fig. 6). The docking of compound **2a** and **2b** showed that the modification of the amino group with an alkyl group (ethyl or *n*-propyl) is sterically unfavourable. Compounds **2c** and **2d** as well as the anilide derivatives of C-7280948 (**7a–j**) could not be docked into the substrate binding pocket due to steric clashes of the aromatic ring attached to the anilide group and the SAM cofactor. However, when docking the inhibitors into the PRMT1 structure without the bound cofactor SAM, a favourable interaction with cofactor and substrate binding pocket could be observed. The orientation of the most active compounds **2c** and **7e** is shown exemplarily in Figures S3 and S4.

Interestingly, the active inhibitor **6h**, in which the aromatic amino group has been replaced by a chlorine substituent, showed a similar binding mode as observed for C-7280948 (**6a**). This is in agreement with the GRID interaction fields obtained with the chlorine probe, where a favourable interaction site for chloro-substituents was detected near the catalytic site (see Fig. S1). A *para* substitution of the phenyl ring of C-7280948 (**6a**) significantly lowers the activity (**6i**, **g**). The same effect is observed even with a shorter linker (**6e**, **f**). The docking results showed that this *para* substituent hinders the favourable π/π-stacking with Tyr47 as observed for C-7280948. As expected, derivatives in which the sulfoneamide moiety is replaced by a heteroaromatic ring (**6d**) or the sulfoneamide is replaced by an amide (**11a–c**) showed a binding mode similar to the lead structure.

4. Biological testing

All compounds were tested for inhibition of recombinant human PRMT1 using the non-histone protein Npl3 as a substrate in an ELISA in vitro assay. IC_{50} values were usually only determined if more than 50% inhibition was observed at 50 μM.

4.1. Analogues of allantodapsone

Among the monoacyl derivatives of dapsone, only the biphenyl compound **1e** showed good enzyme inhibition in vitro with an IC_{50}-value of 11.8 μM. Among the bisubstituted dapsones, aromatic rings in **2c** and **2d** led to good inhibition and the bis-chloroacetyl compound **2e** was the strongest inhibitor with an IC_{50} around 1 μM, allantodapsone has been published with an IC_{50} of 1.7 μM (see Table 1).[5]

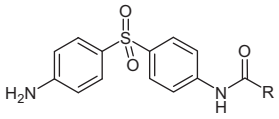
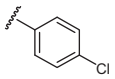
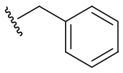
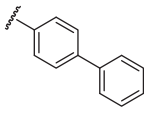
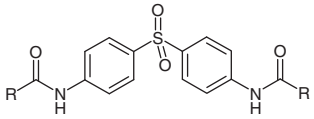
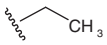
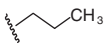
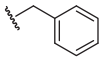
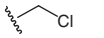
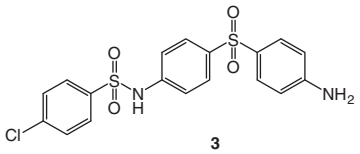
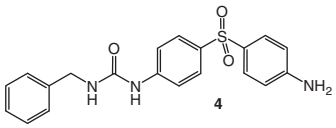
4.2. Derivatives of C-7280948

Among the derivatives of **6a**, the best inhibition was seen with the 4-chlorobenzoyl (**7d**) and the 3,4-dichlorobenzoyl amide **7e**. The sulfonyl analogue **7j** showed a somewhat improved activity as compared to its carbonyl congener **7d** (see Table 2).

Reduction of the spacer length in the phenethylamide part of **6a** did not lead to improved derivatives (see Table 3).

To check the binding mode of the two most potent inhibitors **2e** and **7e**, a jump dilution test of reversibility was performed by incubation of the compounds at the IC_{50} with 10× enzyme, followed by

Table 1
Inhibitory properties of the acylated dapsone derivatives against hPRMT1

Core	R	Compound	IC_{50} ± SEM (μM) or inhibition (%) (concentration)
		**1a**	70.6 (50 μM) 23.5 (10 μM)
		**1b**	45.9 ± 2.7
		**1c**	65.6 ± 7.9
		**1d**	41.2 (50 μM) 35.3 (10 μM)
		**1e**	11.8 ± 3.9
		**2a**	ni[a] (50 μM)
		**2b**	44.1 (50 μM) 14.7 (10 μM)
		**2c**	14.1 ± 1.7
		**2d**	30.4 ± 3.8
		**2e**	1.5 ± 0.2
 **3**		**3**	35.1 ± 2.9
 **4**		**4**	13.6 (50 μM) ni[a] (10 μM)

[a] ni—no inhibition, inhibition <5%.

dilution of enzyme to 1× in the reaction volume. The compounds went to about 0.1× IC_{50}. The loss of inhibition in this procedure indicates a reversible mode of inhibition (see Fig. S5). Furthermore, a mechanism of action study was conducted by varying the substrate concentration over a large range within the in vitro assay with fixed inhibitor concentrations for inhibitors **2e** and **7e**. Both inhibitors showed greater inhibitory potential compared to normal assay conditions at low substrate concentration whereas higher substrate concentrations led to lower inhibitory potential (see Figs. S6 and S7). Thus, the analogues seem to retain the substrate competitive mode of inhibition of the lead compound allantodapsone.

To analyze for selectivity among arginine methyltransferases, we tested the inhibitors **2e** and **7j** for inhibition of the isoform CARM1 (=PRMT4), which has also been linked to hormone dependent gene transcription.[12,13] Up to very high micromolar concentrations, no inhibition of CARM1 could be shown using a radioactive in vitro assay (see Fig. S8).

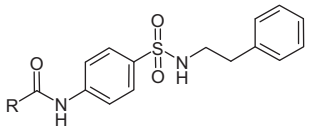
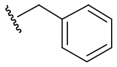
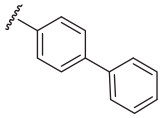
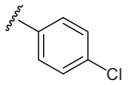
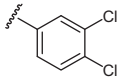
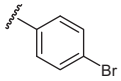
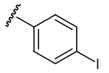
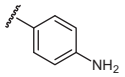
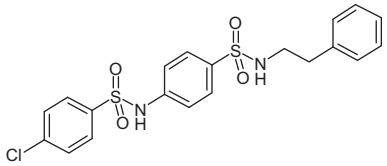
In order to test the selectivity towards lysine methyltransferases, the best inhibitors were assayed for the inhibition of human recombinant Set7/9 as a representative example. Among others, Set7/9 has recently been shown to play a role in stabilizing estrogen receptor α by methylation which is necessary for efficient recruitment of the receptor to its target genes.[14] Again, we used an antibody based detection of methylation on a peptide substrate.[14] All compounds exhibited little inhibition below 50 μM. The phenylacetic acid derivative of dapsone **1d** and the bis-chloromethyl compound **2e** were very selective for PRMT1 (see Table 4).

4.3. Cellular characterization of the most potent inhibitors

Selected inhibitors were then characterized for their cellular activity. The bis-chloroacetyl compound **2e** was analyzed for growth inhibition of MCF7a breast cancer and LNCaP prostate cancer cells using an MTS assay. The GI_{50} values for **2e** were 1.97 ± 0.14 μM on MCF7a cells and 4.49 ± 0.14 μM on LNCaP cells (growth curves not shown). Inhibitor **7e** showed very moderate growth inhibition on MCF7a cells (52.24 ± 5.4 μM) and was not analyzed on LNCaP cells.

To analyze for potential indirect antihormone properties, we used a reporter gene assay that analyzes luciferase activity in an androgen dependent expression system. Among the most potent inhibitors, only compound **2e** showed the desired effect. In order

Table 2
Inhibitory properties of amide derivatives of **6a** against hPRMT1

Core	R	Compound	IC_{50} ± SEM (μM) or inhibition (%) (concentration)
		**6a**	26.7 ± 3.5
		**7a**	20 (50 μM) 15 (10 μM)
		**7b**	10 (50 μM) ni[a] (10 μM)
		**7c**	42 (50 μM) 39 (10 μM)
		**7d**	48.9 ± 2.4
		**7e**	10.2 ± 0.5
		**7f**	41 (50 μM) 23 (10 μM)
		**7g**	68.1 ± 1.2
		**7i**	40 (50 μM) 40 (10 μM)
		**7j**	5.0 ± 0.2

[a] ni—no inhibition, inhibition <5%.

to rule out unspecific effects on transcription or translation, we also used a control reporter gene system. Here, the luciferase gene is under control of a thymidine kinase promotor which is not responsive to androgen stimulation. At 10 and 1 μM no effect was observed, indicating a specific effect on androgen dependent transcription. The reduction seen at 25 μM is due to cytotoxicity, as activity also decreased in non-stimulated cells (see Fig. 7).

We then analyzed the effects of the inhibitor **2e** on androgen dependent gene transcription in LNCap cells that are not transfected. To this end, we quantitated the mRNA of androgen dependent target genes after androgen stimulation with and without treatment with **2e**. For all genes investigated, a statistically significant reduction in the mRNA levels could be detected. The expression of GAPDH, which is not androgen dependent, was not altered (see Fig. 8).

5. Conclusion

Synthetic variation of sulfonamide and sulfone inhibitors of PRMT1 led to the establishment of structure–activity relationships. Among the monoacyl analogues of allantodapsone, the most potent inhibitor was the biphenylylamide **1e**. Among the diacyl derivatives, especially the bis-chloroacetylated derivative **2e** showed potent enzyme inhibition. It has also significant cytotoxic activity. While this might at least in part be due to unspecific cytotoxicity caused by its potentially alkylating properties, it specifically blocks androgen dependent gene transcription in a reporter gene model and in untransfected prostate cancer cells. Chloroacetamidines possess a similar alkylating group and were shown to be inhibitors of histone deamidation in vitro[15,16] but at least in vitro our data suggests a reversible mode of action. In the case of the sulfonamide lead inhibitor **6a**, structural variation of the phenethyl group did not lead to optimized derivatives. Acylated derivatives **7a** were also less active, with the exception of the 3,4-dichloro derivative **7e**.

Thus, structure–activity studies led to an improved inhibitor of PRMT1 with cellular activity. This makes **2e** an interesting starting point for the further optimization of inhibitors as useful tools in epigenetic research, and may also serve as a basis for the development of therapeutic agents against prostate cancer.

6. Experimental section

6.1. Materials and methods

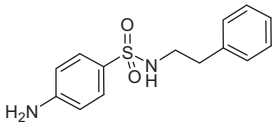
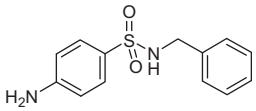
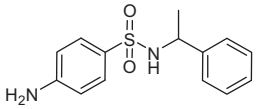
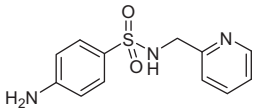
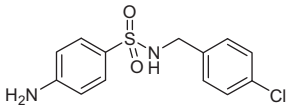
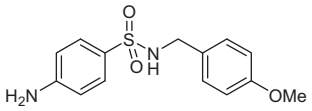
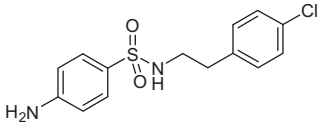
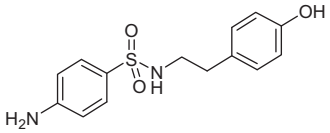
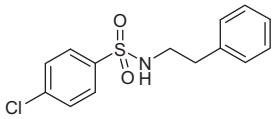
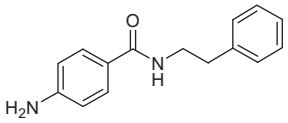
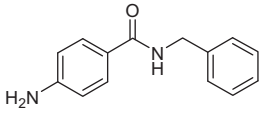
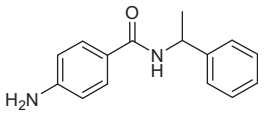
6.1.1. In vitro methylation assay (hPRMT1)

The assay was performed as published.[4]

6.1.2. In vitro methylation assay (CARM1)

A solution containing 20 μM of recombinant histone H3, 10 μg/mL of bovine serum albumin and 250 nM of flag-CARM1 (CARM1 was expressed from the original mouse FlagCARM1 construct given by Evans[17] using the baculorvirus system) in 50 mM Tris–HCl, 0.2 M NaCl pH 8.0, 0.5 mM DTT was incubated with increasing

Table 3
Inhibitory properties of further analogues of **6a** against hPRMT1

Core	Compound	IC_{50} ± SEM (μM) or inhibition (%) (concentration)
	**6a**	26.7 ± 3.5
	**6b**	28 (50 μM) 22 (10 μM)
	**6c**	40 (50 μM) 28 (10 μM)
	**6d**	70.7 ± 4.4
	**6e**	ni[a] (50 μM)
	**6f**	19 (50 μM) 12 (10 μM)
	**6g**	ni[a] (50 μM)
	**6h**	37 (50 μM) 30 (10 μM)
	**6i**	ni[a] (50 μM)
	**9a**	49.7 ± 1.7
	**9b**	47 (50 μM)[b]
	**9c**	34 (50 μM)[b]

[a] ni—no inhibition, inhibition <5%.
[b] Data obtained as published.[5]

concentrations of inhibitor (0.01–2 mM) for 30 min at room temperature. A 5 min centrifugation step at 15,000*g* was performed to eliminate any precipitated protein. The reaction was initiated in a volume of 10 μL by addition of 10 μM ^{14}C-labeled SAM at 50 mCi mmol^{-1} (GE Healthcare), performed for 15 min at 25 °C and stopped by mixing to 4 μL of SDS PAGE loading buffer. The reaction product was analyzed on a 12% polyacrylamide denaturing gel. After electrophoresis, the gel was stained with Coomassie Brilliant Blue, dried on a Whatman 3MM paper sheet and exposed for 24 h to 5 days against a GP2025 imaging plate (Kodak). The amount of radioactivity incorporated specifically by histone H3 was quantified by phosphorimager analysis (Typhoon, Molecular Dynamic).

Table 4
Selectivity of the best inhibitors tested against Set7/9

Compound	IC_{50} ± SEM (μM) or inhibition (%)	Compound	IC_{50} ± SEM (μM) or inhibition (%) (concentration)
1d	ni[a] (50 μM)	**7e**	37 (50 μM)
1e	66.4 ± 0.7	**7j**	58 (50 μM)
2d	31 (50 μM)	**6d**	14 (50 μM)
2e	ni[a] (50 μM)	**11a**	20 (50 μM)

[a] ni—no inhibition, inhibition <5%.

6.1.3. In vitro time-resolved fluorescence immunosorbent methyltransferase assay (Set7/9)

The heterogeneous assay is performed in streptavidine-coated 96-well plates (Nunc). After each incubation step, six washing steps are necessary to remove the non bound fraction (Tecan Columbus Plate washer; 300 μL/ step in an overflow modus, using 100 mM Tris, containing 0.1% Tween 20; pH 7.5). In the first incubation step, the biotinylated histone peptide (aa 1–21 of human histone H3, Upstate, 20 pmol/ well in the same buffer as mentioned above) is bound to the cavities. In the second step, the bound substrate is turned over in an enzymatic reaction. Therefore, preincubation of enzyme (Set7/9, BPS biosciences, human, recombinant, N-terminal GST tag, 5 μL/well, diluted in 50 mM Tris, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 8.5), 5 μL of inhibitor solution (DMSO) and 10 μL of 15 mM Tris-buffer takes place for 10 min at 30 °C. Controls are treated in the same manner, using buffer instead of the inhibitor solution. Then, each mixture is transferred to the plate. Each well is filled with 15 mM Tris-buffer to obtain an incubation volume of 100 μL in the end. To start the enzymatic reaction, 5 μL of an 800 μM SAM solution is added to each well. The amount of the turnover is detected by a primary rabbit antibody (anti-methyl H3K4, Upstate, 100 μL of a 1:1000 dilution in 100 mM Tris containing 0.1% Tween 20 and 0.5 % BSA, protease-free; pH 7.5) followed by an europium-labeled secondary antibody directed against rabbit IgG (Perkin–Elmer, 5 pmol/well, using the same buffer as for the dilution of the first antibody). The europium label is cleaved off by adding 100 μL of enhancement solution (Perkin–Elmer) to all wells. After shaking the plate for 10 min at room temperature, the TRF measurement (340/615 nm, BMG Polarstar) is performed for the quantitation.

6.2. Luciferase assay

The day before transfection, 2×10^5 LNCaP cells were cultured in RPMI1640 supplemented with 10% Fetal Calf Serum (FCS). LNCaP cells were washed one time with PBS and cultured in phenol-red-free RPMI1640 supplemented with 10% double-stripped FCS (dsFCS) just before transfection with Effecten (Qiagen). 500 ng of MMTV-Luc or TK-Luc reporter plasmid[18] were used for each well. Six hours later, cells were treated with indicated inhibitors at indicated concentrations. When cells were not treated by inhibitor, DMSO was added as vehicle. Just after adding inhibitors or vehicle, cells were treated over night with or without 10^{-10} M R1881 as indicated. Luciferase activity was assayed as described.[19] All experiments were repeated three times in duplicate. Bars represent mean + SD (n = 6).

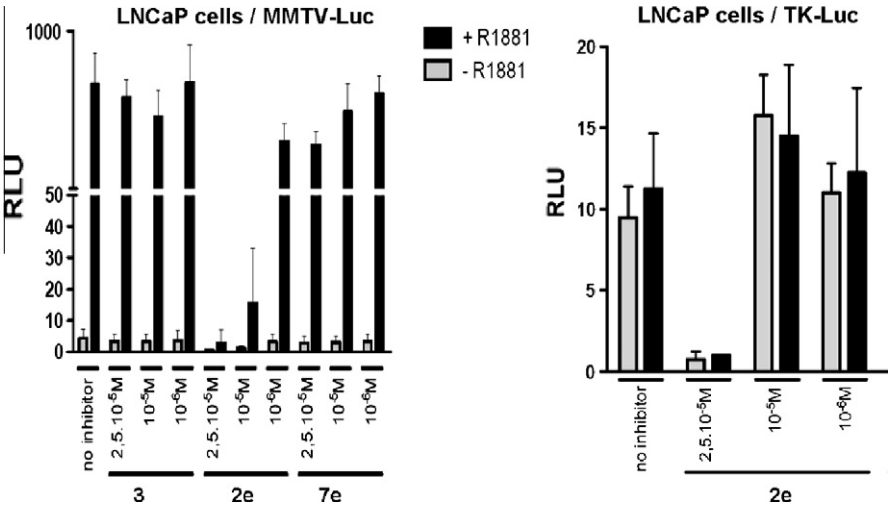


Figure 7. Transcriptional regulation by PRMT1 inhibitors, investigated in a reporter gene system. The MMTV-Luc reporter is androgen driven, the TK-Luc promotor not. R1881 is a synthetic androgen that is used for receptor activation. Differences for androgen dependent cells are only significant for **2e** (all concentrations) and **7e** (25 μM, p <0.01).

6.3. Effects on androgen receptor target genes

LNCaP cells were washed one time with PBS and have been starved during 24 h in phenol-red-free RPMI1640 supplemented with 0.5% double stripped FCS (dsFCS). Cells were then treated or not with inhibitor as indicated. When cells were not treated by inhibitor, DMSO was added as vehicle. After (10 min) adding inhibitors or vehicle, cells were treated over night with or without 10^{-9} M R1881 as indicated.[20] DNaseI-treated RNA isolated using Trizol (Invitrogen) was used for reverse transcription. Quantitative PCR was performed in a LightCycler 480 (Roche). Product formation was detected by incorporation of SYBR green I using ROX as a passive reference (ABgene). For qRT-PCR, the following primers were used: TMPRSS2[21] 5′-TCACACCAGCCATGATCTGT-3′ and 5′-CTGTCACCCTGGCAAGAATC-3′; ELK4 5′-CTGTTGCTCCCCTAAGTC CA-3′ and 5′-CCAGCCCAGACAGAGTGAAT-3′; IGF1-R[21] 5′-CTGTAT GCCTCTGTGA ACC-3′ and 5′-TAGACCATCCCAAACGAC-3′; NKX3.1[22] 5′-AGAACGACCAGCTGA GCAC-3’ and 5′-AAGACCC-CAAGTGCCTTTCT-3′; CXCR4[23] 5′-CTGTGAGCAGAGGGTCCAG-3′ and 5′-ATGAATGTCCACCTCGCTTT-3′; MAK[24] 5′-TGGACTTGCAAGA GAATTAAGGT-3′ and 5′-CTTCAGGGGCACGATACC-3′; MAF[25] 5′-AGCGGCTTCCGAGAAAAC-3′ and 5′-GCGAGTGGGCTCAGTTATG-3′; N4A1 5′-ACAGCTTGCTTGTCGATGTC-3′ and 5′-GGTTCTGC AGCTCCTCCAC-3′; PER1[26] 5′-AGGTACCTGGAGAGCTGCAA-3’ and 5′-GATCTTTCTTGGTCCCCACA-3′; GREB1[25] 5′-AAGCTGAGCAGCA CAGACAA-3′ and 5′-GGCTTCTCTTCTCCGAGGTAG-3′; FKBP5 5′-TTT TTGAGATTGAGCTCCTTGA-3′ and 5′-TTGTGTTCACCTTTGCCAAC-3′; GAPDH[27] 5′-GAGTCCACTGGCGTCTTCAC-3′ and 5′-GTTCACAC CCATGACGAACA-3′. Bars represent mean + SD (n = 6). P-value: ns = non significant; * = <0.05; *** <0.001 (Supplementary data).

6.4. Inhibitors

Standard chemicals were purchased from Acros, Aldrich, Sigma or Fluka. NMR spectra were obtained on a Bruker Avance DRX 400 MHz spectrometer or a Varian 100 MHz, respectively. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane. EI- and CI-mass spectra were measured with a TSQ700 mass spectrometer (Thermoelectron). ESI- and PCI-mass spectra were recorded with a LCQ-Advantage mass spectrometer. Merck Silica Gel 60 was used for flash chromatography with cyclohexane/ethyl acetate mixtures as eluent so that the R_f value of the desired product was about 0.3.

6.5. Purity

If purity of the final compounds was determined by HPLC analyses, the following protocols were used:

System 1:

Column: Phenomenex Luna® 5 μ Phenyl–Hexyl 250 × 4.6 mm; mobile phase: acetonitrile/water (70/30 v/v); flow: 0.5 mL/min; detection UV 254 nm; samples: 0.1 mg/mL; injection volume: 20 μl; manual integration after blind subtraction from same series.

System 2:

Column: Phenomenex Synergi® 4 μm Max-RP 80 Å, 150 × 4.60 mm; mobile phase: A: water + 0.05% TFA, B: acetonitrile + 0.05% TFA; flow: 1 mL/min; detection UV 210 nm; samples: 1 mg/mL; injection volume: 5 μl; gradient elution:

Time (min)	Eluent A (%)	Eluent B (%)
0.0	90.0	10.0
4.0	90.0	10.0
29.0	0.0	100.0
31.0	0.0	100.0
31.5	90.0	10.0
40.0	90.0	10.0

Manual integration after blind subtraction from same series.

Compounds were synthesized according to general procedures as described in the following.

6.6. General procedure 1: synthesis of amides with acyl chlorides and dapsone[28]

Dapsone (3 mmol) is dissolved in pyridine (5 mL/mmol). The acyl chloride (1 mmol) is dissolved in dioxane (5 mL/mmol) and added dropwise to the reaction mixture which is then stirred over night at room temperature.

To purify the crude product, approximately 50 mL of diethylether are added to the solution. The formed precipitate is separated and dissolved in DMF. The solution is poured into 200 mL of 2 M hydrochloric acid and the resulting precipitate is filtered off and washed thoroughly with water. The product is dried in vacuo and can be further purified by flash column chromatography.

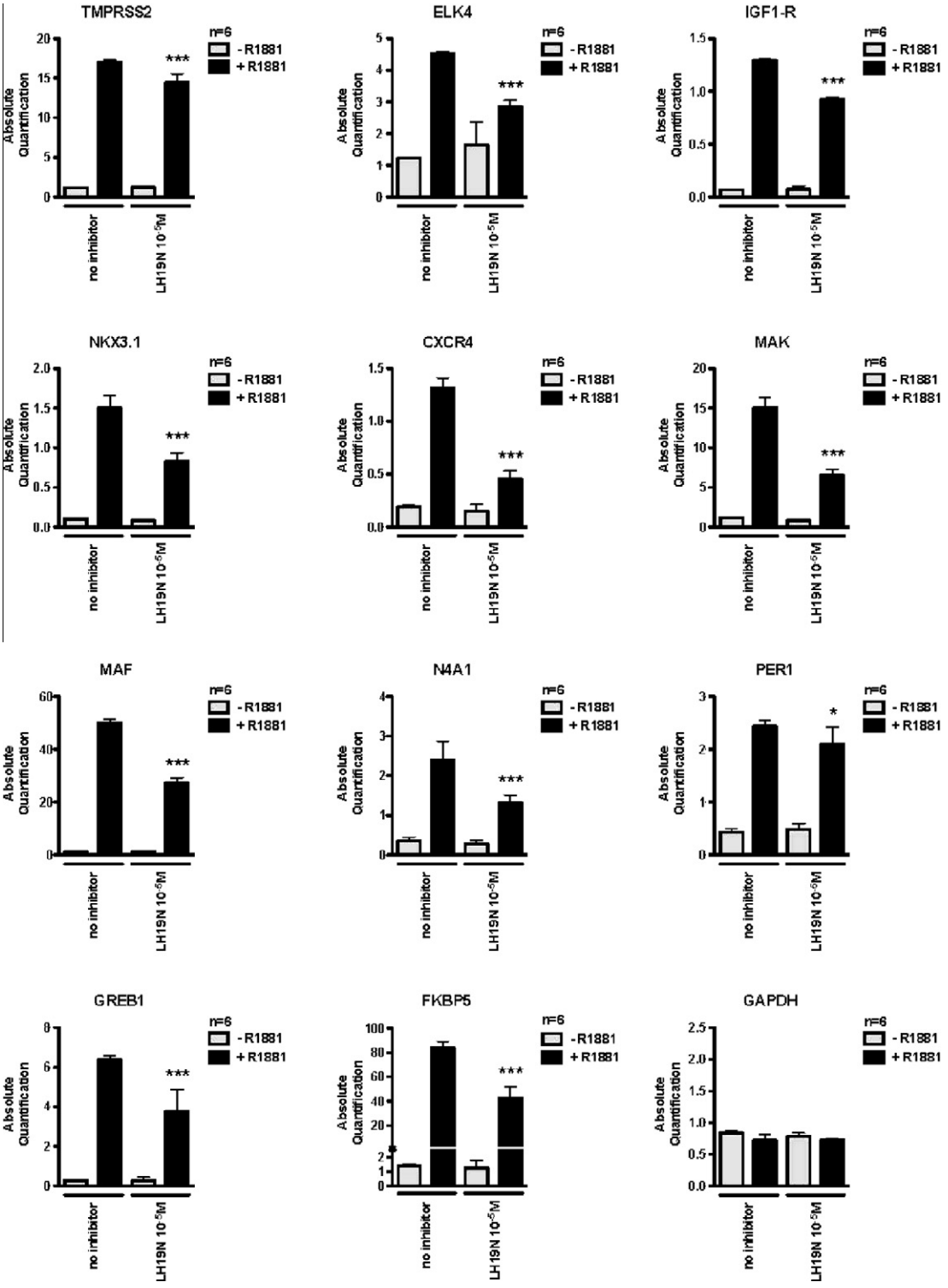


Figure 8. Block of androgen dependent gene transcription by PRMT1 inhibitor **2e**. mRNA levels of androgen dependent target genes were measured by qRT-PCR. R1881 is a synthetic androgen that is used for receptor activation.

6.7. General procedure 2: synthesis of urea derivatives with isocyanates[29]

The amine (1 mmol) is dissolved in dioxane (20 mL/mmol) and the isocyanate (1 mmol), also dissolved in dioxane, is added dropwise. The reaction mixture is heated under reflux for 3 h, controlling the end of the reaction by thin layer chromatography. Water is added to the cooled reaction mixture and the resulting precipitate is filtered off. The crude product is then purified by reversed phase column chromatography.

6.8. General procedure 3: reaction of amines with 4-nitrobenzenesulfonylchloride[30]

The amine is dissolved in dichloromethane, cooled to 0 °C and *N*-methylmorpholine (0.24 mL/mmol amine) is added. An

equimolar amount of sulfonylchloride, also dissolved in dichloromethane, is added dropwise to the solution. After stirring over night at room temperature, the solution is washed three times with 2 M hydrochloric acid, once with a saturated solution of $NaHCO_3$ and once with brine. The organic layer is dried over $MgSO_4$. To obtain the crude product, the solvent is removed in vacuo. The crude product may be purified by flash column chromatography.

6.9. General procedure 4: reduction of aromatic nitro compounds[30]

The aromatic nitro compounds are dissolved in ethyl acetate and $SnCl_2 \times 2H_2O$ (1.125 g/mmol) is added. The reaction mixture is heated under reflux for at least 2 h. The reaction is monitored by thin layer chromatography. When the reaction is completed, the batch is cooled. A solution of $NaHCO_3$ is then added until pH 7–8 is reached. The organic layer is separated and the aqueous residue is washed three times with ethyl acetate. The combined organic layers are washed with brine and dried over $MgSO_4$. The solvent is removed in vacuo to obtain the crude product.

6.10. General procedure 5: synthesis of amides with acyl chlorides and aromatic amines

The corresponding acyl chlorides is dissolved in dioxane and added to a solution of the appropriate aromatic amine in hot toluene. The reaction mixture is heated under reflux for 2 h. When the reaction is over, the solvent is removed in vacuo and the crude product is purified by recrystallisation or flash column chromatography.

6.11. General procedure 6: hydrolysis of esters under basic conditions

The ester is dissolved in ethanol and refluxed with a 10% solution of KOH (40 mL/mmol) for 30 min. After the end of the reaction, monitored by thin layer chromatography, the reaction mixture is cooled to room temperature. The excess solvent is evaporated. The crude product is dissolved in ethyl acetate and is washed three times with water, once with a saturated solution of $NaHCO_3$ and once with brine. The organic layer is dried over Na_2SO_4 and the solvent is evaporated. The obtained crude product can be further purified by recrystallisation or flash column chromatography.

6.12. *N*-[4-(4-Aminophenyl)sulfonylphenyl]propanamide, 1a

General procedure: 1, yellowish crystals; Yield: 2%; ^{1}H NMR (DMSO-d_6): δ = 10.2 (s, 1H, NH), 7.74 (s, 4H, H_{ar} 2/3/5/6), 7.53–7.47 (m, 2H, H_{ar} 2′/6′), 6.64–6.58 (m, 2H, H_{ar} 3′/5′), 6.09 (s, 2H, NH_2), 2.35 (q, 2H, ^3J = 7.52 Hz, CH_2), 1.11 (t, 3H, ^3J = 7.49 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ = 173.07 (C=O), 153.83 (C–NH_2), 143.45 (C–NH), 132.26 (C–SO_2), 129.56 (CH_{ar}, C2′/6′), 128.13 (CH_{ar}, C2/6), 126.63 (C′–SO_2), 119.26 (CH_{ar}, C3/5), 113.41 (CH_{ar}, C3′/5′), 30.02 (CH_2), 9.81 (CH_3); CIMS (direct mode): calcd/found (*m/z*): 322.1/322.1 $[M+NH_4]^+$; Purity (HPLC): 95.6% (System 1).

6.13. *N*-[4-(4-Aminophenyl)sulfonylphenyl]-4-chlorobenzamide, 1b

General procedure: 1, yellow crystals; Yield: 4%; ^{1}H NMR (DMSO-d_6): δ = 10.62 (s, 1H, NH), 8.01–7.92 (m, 4H, H_{ar}), 7.85–7.80 (m, 2H, H_{ar}), 7.65–7.59 (m, 2H, H_{ar}), 7.56–7.50 (m, 2H, H_{ar}), 6.65–6.58 (m, 2H, H_{ar}), 6.12 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6): δ = 165.32 (C=O), 153.87 (C–NH_2), 143.13 (C–NH), 138.11 (C–Cl), 137.24 (C_{ar}), 133.51 (C_{ar}), 130.19 (CH_{ar}), 129.64 (CH_{ar}), 128.96 (CH_{ar}), 127.99 (CH_{ar}), 126.41 (C_{ar}), 120.62 (CH_{ar}), 113.40 (CH_{ar}); CIMS (direct mode): calcd/found (*m/z*): 386.0/386.0 $[M]^+$, 404.0/404.1 $[M+NH_4]^+$; Purity (HPLC): 99.3% (System 2).

6.14. *N*-[4-(4-Aminophenyl)sulfonylphenyl]benzamide, 1c

General procedure: 1, white crystals; Yield: 4%; ^{1}H NMR (DMSO-d_6): δ = 10.59 (s, 1H, NH), 8.00–7.92 (m, 4H, H_{ar}), 7.85–7.79 (m, 2H, H_{ar}), 7.65–7.58 (m, 1H, H_{ar}), 7.57–7.50 (m, 4H, H_{ar}), 6.65–6.58 (m, 2H, H_{ar}), 6.14 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6): δ = 166.44 (C=O), 153.86 (C–NH_2), 143.33 (C–NH), 137.89 (C–SO_2), 134.80 (C_{ar}), 132.39 (C_{ar}), 129.64 (CH_{ar}), 128.88 (CH_{ar}), 128.22 (CH_{ar}), 127.99 (CH_{ar}), 126.41 (CH_{ar}), 120.52 (CH_{ar}), 113.37 (CH_{ar}); EIMS (direct mode): calcd/found (*m/z*): 352.1/352.1 $[M]^+$, 353.1/353.2 $[M+H]^+$; Purity (HPLC): 95.4% (System 2).

6.15. *N*-[4-(4-Aminophenyl)sulfonylphenyl]-2-phenylacetamide, 1d

General procedure: 1, beige crystals; Yield: 1%; ^{1}H NMR (DMSO-d_6): δ = 10.54 (s, 1H, NH), 7.80–7.71 (m, 4H, H_{ar}), 7.53–7.47 (m, 2H, H_{ar}), 7.34–7.29 (m, 4H, H_{ar}), 7.28–7.21 (m, 1H, H_{ar}), 6.63–6.56 (m, 2H, H_{ar}), 6.13 (s, 2H, NH_2), 3.67 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ = 170.20 (C=O), 153.82 (C–NH_2), 143.23 (C–NH), 137.50 (C–SO_2), 135.93 (C_{ar}), 129.58 (CH_{ar}), 129.54 (CH_{ar}), 128.75 (CH_{ar}), 128.17 (CH_{ar}), 127.05 (CH_{ar}), 126.42 (C_{ar}), 119.41 (CH_{ar}), 113.35 (CH_{ar}), 43.69 (CH_2); ESIMS (positive mode): calcd/found (*m/z*): 367.1/367.3 $[M+H]^+$; Purity (HPLC): 95.5% (System 2).

6.16. *N*-[4-(4-Aminophenyl)sulfonylphenyl]-4-phenylbenzamide, 1e

General procedure: 1, white crystals; Yield: 6%; ^{1}H NMR (DMSO-d_6): δ = 10.63 (s, 1H, NH), 8.11–7.94 (m, 4H, H_{ar}), 7.89–7.78 (m, 4H, H_{ar}), 7.79–7.72 (m, 2H, H_{ar}), 7.58–7.47 (m, 4H, H_{ar}), 7.46–7.40 (m, 1H, H_{ar}), 6.66–6.59 (m, 2H, H_{ar}), 6.15 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6): δ = 166.04 (C=O), 153.86 (C–NH_2), 143.91 (C–NH), 143.36 (C_{ar}), 139.41 (C_{ar}), 137.88 (C_{ar}), 133.51 (C_{ar}), 129.64 (CH_{ar}), 129.51 (C_{ar}), 128.96 (CH_{ar}), 128.67 (CH_{ar}), 128.01 (CH_{ar}), 127.63 (CH_{ar}), 127.07 (CH_{ar}), 126.42 (CH_{ar}), 120.55 (CH_{ar}), 113.39 (CH_{ar}); EIMS (direct mode): calcd/found (*m/z*): 429.1/429.1 $[M]^+$; Purity (HPLC): 99.2% (System 2).

6.17. *N*-[4-[4-(Propanoylamino)phenyl]sulfonylphenyl]-propanamide, 2a

General procedure: 1, yellowish crystals; Yield: 5%; ^{1}H NMR (DMSO-d_6): δ = 10.26 (s, 2H, NH), 7.86–7.74 (m, 8H, H_{ar}), 2.35 (q, 4H, ^3J = 7.57 Hz, CH_2), 1.07 (t, 6H, ^3J = 7.57 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ = 173.17 (C=O), 144.10 (C–NH), 135.42 (C–SO_2), 128.77 (C_{ar}, C2/6), 119.35 (C_{ar}, C3/5), 30.01 (CH_2), 9.76 (CH_3); CIMS (direct mode): calcd/found (*m/z*): 360.1/360.1 $[M]^+$, 378.1/378.1 $[M+NH_4]^+$; Purity (HPLC): >99.5% (System 1).

6.18. *N*-[4-[4-(Butanoylamino)phenyl]sulfonylphenyl]-butanamide, 2b

General procedure: 1, white crystals; Yield: 34%;^{1}H NMR (DMSO-d_6): δ = 10.30 (s, 2H, NH), 7.86–7.76 (m, 8H, H_{ar}), 2.31 (t, 4H, ^3J = 7.38 Hz, α-CH_2), 1.65–1.54 (m, 4H, β-CH_2), 0.90 (t, 6H, ^3J = 7.38 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ = 173.35 (C=O), 144.03 (C–NH), 135.44 (C–SO_2), 128.79 (C_{ar}, C2/6), 119.36 (C_{ar}, C3/5), 38.76 (α-CH_2), 18.77 (β-CH_2), 13.95 (CH_3); CIMS (direct mode): calcd/found (*m/z*): 388.2/388.2 $[M]^+$, 406.2/406.2 $[M+NH_4]^+$; Purity (HPLC): 97.8% (System 1).

6.19. *N*-[4-(4-Benzamidophenyl)sulfonylphenyl]benzamide, 2c

General procedure: 1, white crystals; Yield: 8%; ^{1}H NMR (DMSO-d_6): δ = 10.65 (s, 2H, NH), 8.06–8.00 (m, 4H, H_{ar}), 7.98–7.90 (m, 8H, H_{ar}), 7.65–7.59 (m, 2H, H_{ar}), 7.58–7.51 (m, 4H, H_{ar}); ^{13}C NMR (DMSO-d_6): δ = 166.54 (C=O), 144.11 (C–NH), 136.06 (C–SO_2), 134.74 (C_{ar}), 132.46 (C_{ar}), 128.90 (CH_{ar}), 128.71 (CH_{ar}), 128.26 (CH_{ar}), 120.64 (CH_{ar}); CIMS (direct mode): calcd/found (*m/z*): 474.1/474.1 [M+NH4]$^+$, 456.1/456.1 [M]$^+$; Purity (HPLC): 95.9% (System 2).

6.20. 2-Phenyl-*N*-[4-[4-[(2-phenylacetyl)amino]phenyl]-sulfonylphenyl]acetamide, 2d

General procedure: 1, white crystals; Yield: 1%; ^{1}H NMR (DMSO-d_6): δ = 10.60 (s, 2H, NH), 7.87–7.75 (m, 8H, H_{ar}), 7.33–7.21 (m, 10H, H_{ar}), 3.67 (s, 4H, CH_2); ^{13}C NMR (DMSO-d_6): δ = 170.29 (C=O), 143.94 (C–NH), 135.86 (C–SO_2), 135.70 (CH_{ar}), 129.53 (CH_{ar}), 128.83 (CH_{ar}), 128.74 (CH_{ar}), 127.05 (CH_{ar}), 119.55 (CH_{ar}), 43.71 (CH_2); EIMS (direct mode): calcd/found (*m/z*): 484.1 /484.1 [M]$^+$; Purity (HPLC): 95.4% (System 2).

6.21. 2-Chloro-*N*-[4-[4-[(2-chloroacetyl)amino]phenyl]-sulfonylphenyl]acetamide, 2e

General procedure: 1, yellowish crystals; Yield: 6%; ^{1}H NMR (DMSO-d_6): δ = 10.73 (s, 2H, O=C–NH), 7.93–7.87 (m, 4H, H_{ar}), 7.82–7.77 (m, 4H, H_{ar}), 4.30 (s, 4H, CH_2); ^{13}C NMR (DMSO-d_6): δ = 165.82 (C=O), 143.30 (C_{ar}–NH), 136.17 (C–SO_2), 129.00 (CH_{ar}), 119.88 (CH_{ar}), 43.95 (CH_2); EIMS (direct mode): calcd/found (*m/z*): 400.0/400.1 [^{35}Cl^{35}ClM]$^+$, 401.0/401.1 [^{35}Cl^{35}ClM+H]$^+$, 402.0/402.1 [^{37}Cl^{35}ClM]$^+$ 404.0/404.1 [^{37}Cl^{37}ClM]$^+$; Purity (HPLC): 97.9% (System 2).

6.22. *N*-[4-(4-Aminophenyl)sulfonylphenyl]-4-chloro-benzenesulfonamide, 3

General procedure: 1, yellowish crystals; Yield: 24%; ^{1}H NMR (DMSO-d_6): δ = 11.01 (s, 1H, NH), 7.84–7.78 (m, 2H, H_{ar}), 7.73–7.68 (m, 2H, H_{ar}), 7.37–7.62 (m, 2H, H_{ar}), 7.49–7.43 (m, 2H, H_{ar}), 7.26–7.19 (m, 2H, H_{ar}), 6.61–6.55 (m, 2H, H_{ar}), 6.15 (s, 2H, NH_2); ^{13}C NMR (DMSO-d_6): δ = 153.93 (C–NH_2), 141.91 (C–NH), 138.64 (C–SO_2–NH), 138.54 (C–SO_2), 138.23 (C–SO_2), 130.12 (C_{ar}), 129.66 (C_{ar}), 128.96 (C_{ar}), 128.62 (C_{ar}), 125.97 (C_{ar}–Cl), 119.28 (C_{ar}, C3′/5′), 113.38 (C_{ar}, C3/5); EIMS (direct mode): calcd/found (*m/z*): 422.0/422.0 [M]$^+$; Purity (HPLC): 96.5% (System 2).

6.23. 1-[4-(4-Aminophenyl)sulfonylphenyl]-3-benzyl-urea, 4

General procedure: 1, white crystals; Yield: 79%; ^{1}H NMR (DMSO-d_6): δ = 9.06 (s, 1H, Ar-NH), 7.71–7.65 (m, 2H, H_{ar}), 7.5–7.53 (m, 2H, H_{ar}), 7.52–7.46 (m, 2H, H_{ar}), 7.37–7.19 (m, 5H, H_{ar}), 6.79 (t, 1H, ^3J = 5.94 Hz, CH_2–NH), 6.63–6.56 (m, 2H, H_{ar}), 6.09 (s, 2H, NH_2), 4.30 (d, 2H, ^3J = 2.93 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 155.15 (C=O), 153.65 (C–NH_2), 144.80 (C_{ar}) 140.39 (C_{ar}–NH), 135.13 (C–SO_2), 129.43 (CH_{ar}), 128.74 (CH_{ar}), 128.21 (CH_{ar}), 127.53 (CH_{ar}), 127.21 (CH_{ar}), 126.98 (C_{ar}), 117.69 (CH_{ar}), 113.33 (CH_{ar}), 43.15 (CH_2); ESIMS (direct positive mode): calcd/found (*m/z*): 382.1/382.2 [M+H]$^+$; Purity (HPLC): 97.7% (System 2).

6.24. 4-Nitro-*N*-phenethyl-benzenesulfonamide, 5a

General procedure: 3, yellowish crystals; Yield: 81%; ^{1}H NMR ($CDCl_3$): δ = 8.35–8.29 (m, 2H, H_{ar}), 7.99–7.93 (m, 2H, H_{ar}), 7.32–7.22 (m, 3H, H_{ar}), 7.12–7.06 (m, 2H, H_{ar}), 4.66 (t, 1H, ^3J = 5.97 Hz, NH), 3.33 (dt, 2H, ^3J = 6.68, 6.31 Hz, NH-CH_2), 2.82 (t, 2H, ^3J = 6.84 Hz, CH_2); ^{13}C NMR ($CDCl_3$): δ = 149.96 (C–NO_2), 145.79 (C_{ar}), 137.09 (C–SO_2), 128.89 (CH_{ar}), 128.66 (CH_{ar}), 128.18 (CH_{ar}), 127.05 (CH_{ar}), 124.32 (CH_{ar}), 44.33 (CH_2), 35.81 (NH–CH_2); CIMS (direct mode): calcd/found (*m/z*): 324.1/324.2 [M+NH_4]$^+$.

6.25. *N*-Benzyl-4-nitro-benzenesulfonamide, 5b

General procedure: 3, shining yellow crystals; Yield: 89%; ^{1}H NMR (DMSO-d_6): δ = 8.56 (s, 1H, NH), 8.39–8.33 (m, 2H, H_{ar}, H2/6), 8.04–7.97 (m, 2H, Har, H3/5), 7.29–7.15 (m, 5H, H_{ar}), 4.07 (s, 1H, CH_2); ^{13}C NMR (DMSO-d_6): δ = 149.82 (C–NO_2), 146.84 (C_{ar}), 137.55 (C–SO_2), 128.68 (CH_{ar}), 128.84 (CH_{ar}), 128.08 (CH_{ar}), 127.68 (CH_{ar}), 124.90 (CH_{ar}), 46.58 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 310.1/310.1 [M+NH_4]$^+$.

6.26. 4-Nitro-*N*-(1-phenylethyl)benzenesulfonamide, 5c

General procedure: 3, yellow crystals; Yield: 65%; ^{1}H NMR (DMSO-d_6): δ = 8.63 (d, 1H, ^3J = 8.08 Hz, NH), 8.24–8.17 (m, 2H, H_{ar}, H3/5), 7.86–7.79 (m, 2H, H_{ar}, H2/6), 7.16–7.05 (m, 5H, H_{ar}), 4.50–4.38 (*pq*, 1H, CH), 1.27 (d, 3H, ^3J = 6.98 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ = 149.46 (C–NO_2), 147.45 (C_{ar}), 143.02 (C–SO_2), 128.50 (CH_{ar}), 128.32 (CH_{ar}), 127.27 (CH_{ar}), 126.51 (CH_{ar}), 124.53 (CH_{ar}), 53.67 (CH), 23.94 (CH_3); APCIMS (direct negative mode): calcd/found (*m/z*): 305.1/305.1 [M−H]$^-$, 306.1/306.1 [M]$^-$.

6.27. 4-Nitro-*N*-(2-pyridylmethyl)benzenesulfonamide, 5d

General procedure: 3, yellow crystals; Yield: 45%; ^{1}H NMR (DMSO-d_6): δ = 8.70 (s, 1H, NH), 8.42–8.19 (m, 3H, Pyr-H6, H_{ar}, H3/5), 8.04–7.97 (m, 2H, H_{ar}, H2/6), 7.71 (dt, 1H, ^3J = 7.74 Hz, ^4J = 1.89 Hz, Pyr-H5), 7.33 (d, 1H, ^3J = 7.74 Hz, Pyr-H4), 7.25–7.18 (m, 1H, Pyr-H3), 4.18 (s, 1H, CH_2); ^{13}C NMR (DMSO-d_6): δ = 156.98 (Pyr-C2), 149.82 (C–SO_2), 149.22 (C–NO_2), 146.76 (Pyr-C6), 137.16 (Pyr-C4), 128.52 (CH_{ar}), 124.82 (CH_{ar}), 122.90 (Pyr-C3), 122.20 (Pyr-C5), 48.33 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 294.0/294.0 [M+H]$^+$.

6.28. *N*-[(4-Chlorophenyl)methyl]-4-nitro-benzenesulfonamide, 5e

General procedure: 3, yellow crystals; Yield: 71%; ^{1}H NMR (DMSO-d_6): δ = 8.62 (br s, 1H, SO_2–NH), 8.40–8.32 (m, 2H, H_{ar}3/5), 8.05–7.96 (m, 2H, H_{ar}, H2/6), 7.37–7.27 (m, 2H, H_{ar}), 7.26–7.20 (m, 2H, CH_{ar}), 4.08 (s, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ = 149.81 (C–NO_2), 146.76 (C–Cl), 136.65 (C–SO_2), 132.34 (C_{ar}), 129.93 (CH_{ar}), 128.61 (CH_{ar}), 128.50 (CH_{ar}), 124.87 (CH_{ar}), 45.83 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 140.0/140.1 [Cl–C_6H_4–CH_2–NH]$^+$, 344.0/344.1 [M+NH_4]$^+$, 346.0/346.1 [^{37}ClM+NH_4]$^+$.

6.29. *N*-[(4-Methoxyphenyl)methyl]-4-nitro-benzene-sulfonamide, 5f

General procedure: 3, yellow crystals; Yield: 95%; ^{1}H NMR (DMSO-d_6): δ = 8.48 (t, 1H, ^3J = 6.13 Hz, SO_2–NH), 8.38–8.31 (m, 2H, H_{ar}, H3/5), 8.00–7.94 (m, 2H, H_{ar}, H2/6), 7.13–7.06 (m, 2H, H_{ar}), 6.82–6.75 (m, 2H, H_{ar}), 4.00 (t, 1H, ^3J = 5.00 Hz, CH_2), 3.69 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ = 158.94 (C–NO_2), 149.74 (C_{ar}), 146.95 (C–SO_2), 129.52 (CH_{ar}), 129.34 (C_{ar}), 128.47 (CH_{ar}), 124.82 (CH_{ar}), 114.03 (CH_{ar}), 55.46 (CH_3), 46.15 (CH_2); ESIMS (direct negative mode): calcd/found (*m/z*): 321.1/321.4 [M−H]$^-$.

6.30. *N*-[2-(4-Chlorophenyl)ethyl]-4-nitro-benzenesulfonamide, 5g

General procedure: 3, white crystals; Yield: 76%; ^{1}H NMR (DMSO-d_6): δ = 8.40–8.31 (m, 2H, H_{ar}, H3/5), 8.09 (br s, 1H, NH),

8.01–7.91 (m, 2H, H_{ar}, H2/6), 7.30–7.22 (m, 2H, H_{ar}), 7.20–7.13 (m, 2H, H_{ar}), 3.09 (t, 2H, ^3J = 7.14 Hz, NH–CH_2), 2.69 (t, 2H, ^3J = 7.14 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 149.82 (C–NO_2), 146.48 (C_{ar}), 137.96 (C–SO_2), 131.45 (C_{ar}), 131.09 (CH_{ar}), 128.59 (CH_{ar}), 128.40 (CH_{ar}), 124.96 (CH_{ar}), 44.28 (NH–CH_2), 34.82 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 358.0/358.1 $[M+NH_4]^+$, 360.0/360.1 $[^{37}ClM+NH_4]^+$.

6.31. 4-Amino-*N*-phenethyl-benzenesulfonamide, 6a

General procedure: 4, yellow crystals; Yield: 89%; ^{1}H NMR (CDCl$_3$): δ = 7.63–7.56 (m, 2H, H_{ar}, H2/6), 7.34–7.19 (m, 4H, H_{ar}, H2′/3′/5′/6′), 7.14–7.05 (m, 1H, H_{ar}, H4′), 6.72–6.63 (m, 2H, H_{ar}, H3/5), 4.26 (t, 1H, ^3J = 6.03 Hz, SO_2–NH), 4.13 (s, 2H, NH_2), 3.25–3.17 (m, 2H, NH–CH_2), 2.78 (t, 2H, ^3J = 6.89 Hz, CH_2); ^{13}C NMR (CDCl$_3$): δ = 150.42 (C–NH_2), 137.75 (C_{ar}), 129.20 (CH_{ar}), 128.72 (CH_{ar}), 127.98 (C–SO_2), 126.73 (C_{ar}), 114.05 (CH_{ar}), 44.06 (CH_2), 35.69 (NH–CH_2); CIMS (direct mode): calcd/found (*m/z*): 277.1/277.1 $[M+H]^+$. Purity (HPLC): 97.3% (System 2).

6.32. 4-Amino-*N*-benzyl-benzenesulfonamide, 6b

General procedure: 4, yellow crystals; Yield: 98%; ^{1}H NMR (DMSO-d_6): δ = 7.63 (t, 1H, ^3J = 6.37 Hz, NH), 7.48–7.42 (m, 2H, H_{ar}, H2/6), 7.33–7.19 (m, 5H, H_{ar}), 6.64–6.58 (m, 2H, H_{ar}, H3/5), 5.93 (s, 2H, NH_2), 3.87 (d, 2H, ^3J = 6.23 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 152.89 (C–NH_2), 138.49 (C_{ar}), 128.88 (CH_{ar}), 128.59 (CH_{ar}), 127.97 (CH_{ar}), 127.43 (CH_{ar}), 125.98 (C–SO_2), 113.08 (CH_{ar}), 46.51 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 263.1/263.1 $[M+H]^+$, 280.1/280.1 $[M+NH_4]^+$; Purity Elemental Anal. Calcd C, 59.52; H, 5.38; N, 10.68; S, 12.22. Found: C, 59.53; H, 5.48; N, 10.44; S, 11.92.

6.33. 4-Amino-*N*-(1-phenylethyl)benzenesulfonamide, 6c

General procedure: 4, colorless crystals; Yield: 1%; ^{1}H NMR (DMSO-d_6): δ = 7.68 (d, 1H, 8.03 Hz, NH), 7.39–7.32 (m, 2H, H_{ar}, H 2/6), 7.27–7.13 (m, 5H, H_{ar}), 6.56–6.48 (m, 2H, H_{ar}, H3/5), 5.86 (s, 2H, NH_2), 4.26–4.14 (m, 1H, CH), 2.09 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ = 152.63 (C–NH_2), 144.58 (C_{ar}), 128.74 (CH_{ar}), 128.46 (CH_{ar}), 127.01 (CH_{ar}), 126.47 (CH_{ar}), 112.93 (CH_{ar}), 52.86 (CH), 23.83 (CH_3); CIMS (direct mode): calcd/found (*m/z*): 277.1/277.2 $[M+H]^+$, 294.1/294.2 $[M+NH_4]^+$; Purity (HPLC): 99.8% (System 2).

6.34. 4-Amino-*N*-[(4-chlorophenyl)methyl]benzenesulfonamide, 6e

General procedure: 4, yellow crystals; Yield: 70%; ^{1}H NMR (DMSO-d_6): δ = 7.69 (t, 1H, ^3J = 4.42 Hz, SO_2–NH), 7.46–7.70 (m, 2H, H_{ar}), 7.37–7.32 (m, 2H, H_{ar}), 7.30–7.23 (m, 2H, H_{ar}, H2/6), 6.63–6.57 (m, 2H, H_{ar}, H3/5), 5.94 (s, 2H, NH_2), 3.87 (d, 2H, ^3J = 6.42 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 152.95 (C–NH_2), 137.67 (C–Cl), 131.96 (C_{ar}), 129.79 (CH_{ar}), 128.88 (CH_{ar}), 128.52 (CH_{ar}), 125.86 (C–SO_2), 113.09 (CH_{ar}), 45.73 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 297.0/297.1 $[^{35}ClM+H]^+$, 299.0/299.1 $[^{37}ClM+H]^+$; Purity (HPLC): 98.6% (System 2).

6.35. 4-Amino-*N*-[(4-methoxyphenyl)methyl]benzenesulfonamide, 6f

General procedure: 4, light yellow crystals; Yield: 84%; ^{1}H NMR (DMSO-d_6): δ = 7.54 (s, 1H, ^3J = 6.45 Hz, SO_2-NH), 7.46–7.40 (m, 2H, H_{ar}), 7.17–7.11 (m, 2H, H_{ar}), 6.87–6.81 (m, 2H, H_{ar}), 6.63–6.57 (m, 2H, H_{ar}), 6.93 (s, 2H, NH_2), 3.79 (d, 2H, ^3J = 6.21 Hz, CH_2), 3.72 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ = 158.78 (C–NH_2), 152.87 (C_{ar}), 130.29 (C_{ar}), 129.32 (CH_{ar}), 128.90 (CH_{ar}), 126.06 (C–SO_2), 114.01 (CH_{ar}), 113.09 (CH_{ar}), 55.49 (CH_3), 46.05 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 121.1/121.1 $[H_3CO–C_6H_4–CH_2]^+$, 136.1/136.1 $[H_3CO–C_6H_4–CH_2–NH]^+$, 293.1/293.2 $[M+H]^+$; Purity (HPLC): 98.3% (System 2).

6.36. 4-Amino-*N*-[2-(4-chlorophenyl)ethyl]benzenesulfonamide, 6g

General procedure: 4, yellow crystals; Yield: 89%; ^{1}H NMR (DMSO-d_6): δ = 7.42–7.36 (m, 2H, H_{ar}, H2/6), 7.34–7.29 (m, 2H, H_{ar}), 7.21–7.14 (m, 3H, SO_2–NH, H_{ar}), 6.62–6.57 (m, 2H, H_{ar}), 5.93 (s, 2H, NH_2), 2.90–2.81 (m, 2H, NH–CH_2), 2.64 (t, 2H, ^3J = 7.17 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 152.86 (C–NH_2), 138.46 (C_{ar}), 131.25 (C_{ar}), 130.93 (CH_{ar}), 128.84 (CH_{ar}), 128.59 (CH_{ar}), 125.96 (C–SO_2), 113.15 (CH_{ar}), 44.18 (NH–CH_2), 34.87 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 311.1/311.1 $[^{35}ClM+H]^+$, 313.1/313.1 $[^{37}ClM+H]^+$; Purity (HPLC): 98.3% (System 2).

6.37. 4-Amino-*N*-[2-(4-hydroxyphenyl)ethyl]benzenesulfonamide, 6h

General procedure: 6, brown crystals; Yield: 84%; ^{1}H NMR (DMSO-d_6): δ = 9.18 (s, 1H, OH), 7.50–7.37 (m, 2H, H_{ar}, H2′/6′), 7.11 (t, 1H, ^3J = 5.85 Hz, SO_2–NH), 6.99–6.87 (m, 2H, H_{ar}, H3′/5′), 6.73–6.56 (m, 4H, H_{ar}), 5.87 (s, 2H, NH_2), 2.89–2.75 (m, 2H, NH–CH_2), 2.60–2.47 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6): δ = 156.11 (C–OH), 152.83 (C–SO_2), 129.87 (CH_{ar}), 129.43 (C_{ar}), 128.88 (CH_{ar}), 125.98 (C–NH_2), 115.56 (CH_{ar}), 113.16 (CH_{ar}), 44.95 (NH–CH_2), 35.86 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 293.1/293.1 $[M+H]^+$; Purity (HPLC): 96.8% (System 2).

6.38. 4-Chloro-*N*-phenethyl-benzenesulfonamide, 6i

General procedure: 3, white crystals; Yield: 60%; ^{1}H NMR (DMSO-d_6): δ = 7.84 (s, 1H, NH), 7.80–7.74 (m, 2H, H_{ar}), 7.68–7.62 (m, 2H, H_{ar}), 7.29–7.22 (m, 2H, H_{ar}), 7.21–7.12 (m, 3H, H_{ar}), 2.98 (t, 2H, ^3J = 7.29 Hz, NH–CH_2) ,2.68 (t, 2H, ^3J = 7.37 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 139.68 (C_{ar}), 138.99 (C_{ar}), 137.61 (C_{ar}), 129.76 (CH_{ar}), 129.08 (CH_{ar}), 128.85 (CH_{ar}), 128.74 (CH_{ar}), 126.67 (CH_{ar}), 44.42 (NH–CH_2), 35.63 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 296.0/296.1 $[^{35}ClM+H]^+$, 298.0/298.1 $[^{37}ClM+H]^+$; Purity (HPLC): 99.1% (System 2).

6.39. *N*-[4-(Phenethylsulfamoyl)phenyl]benzamide, 7a

General procedure: 5, white crystals; Yield: 92%; ^{1}H NMR (DMSO-d_6): δ = 10.58 (s, 1H, CONH), 8.02–7.92 (m, 4H, H_{ar}), 7.81–7.73 (m, 2H, H_{ar}), 7.65–7.50 (m, 4H, H_{ar}), 7.31–7-23 (m, 2H, H_{ar}), 7.22–7.12 (m, 3H, H_{ar}), 3.02–2.91 (m, 2H, CH_2–NH), 2.69 (t, 2H, ^3J = 7.62 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 166.47 (C=O), 143.12 (C_{ar}–NH), 139.15 (C_{ar}), 135.06 (C–SO_2), 134.91 (C_{ar}), 132.37 (C_{ar}), 129.08 (CH_{ar}), 128.89 (CH_{ar}), 128.75 (CH_{ar}), 128.22 (CH_{ar}), 127.95 (CH_{ar}), 126.65 (C_{ar}), 120.39 (CH_{ar}), 44.53 (NH–CH_2), 35.66 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 381.1/381.2 $[M+H]^+$, 389.1/398.3 $[M+NH_4]^+$; Purity (HPLC): 99.5% (System 2).

6.40. *N*-[4-(Phenethylsulfamoyl)phenyl]-2-phenyl-acetamide, 7b

General procedure: 5, yellow needles; Yield: 70%; ^{1}H NMR (DMSO-d_6): δ = 10.53 (s, 1H, Ar-NH), 7.82–7.75 (m, 2H, CH_{ar}), 7.74–7.68 (m, 2H, H_{ar}), 7.65 (t, 1H, ^3J = 5.76 Hz, SO_2–NH), 7.37–7.30 (m, 4H, H_{ar}), 7.29–7.22 (m, 3H, H_{ar}), 7.21–7.12 (m, 3H, H_{ar}), 3.69 (s, 2H, CH_2–CO), 2.98–2.89 (m, 2H, CH_2–NH), 2.67 (t, 2H, ^3J = 7,58 Hz, Phe-CH_2); ^{13}C NMR (DMSO-d_6): δ = 170.17 (C=O),

143.02 (C_{ar}–NH), 139.15 (C_{ar}), 136.01 (C_{ar}), 134.76 (C_{ar}), 129.56 (CH_{ar}), 129.05 (CH_{ar}), 128.75 (CH_{ar}), 128.72 (CH_{ar}), 128.09 (CH_{ar}), 127.05 (CH_{ar}), 126.62 (CH_{ar}), 119.28 (CH_{ar}), 44.47 (CH_2), 43.74 (CH_2–NH), 35.66 (Phe-CH_2); EIMS (direct positive mode): calcd/found (*m/z*): 395.1/395.2 $[M+H]^+$, 412.1/412.3 $[M+NH_4]^+$; Purity (HPLC): 98.6% (System 2).

6.41. *N*-[4-(Phenethylsulfamoyl)phenyl]-4-phenyl-benzamide, 7c

General procedure: 5, white crystals; Yield: 40%; ^{1}H NMR (DMSO-d_6): δ = 10.62 (s, 1H, CONH), 8.12–8.04 (m, 2H, H_{ar}), 8.03–7.95 (m, 2H, H_{ar}), 7.90–7.84 (m, 2H, H_{ar}), 7.82–7.73 (m, 4H, H_{ar}), 7.60 (t, 1H, SO_2–NH, ^3J = 5.82 Hz), 7.55–7.49 (m, 2H, H_{ar}), 7.47–7.41 (m, 1H, H_{ar}), 7.31–7.24 (m, 2H, H_{ar}), 7.23–7.13 (m, 3H, H_{ar}), 3.02–2.93 (m, 2H, CH_2–NH), 2.70 (t, 2H, ^3J = 7.65 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 166.11 (C=O), 143.92 (C_{ar}–NH), 143.18 (C_{ar}) 139.45 (C_{ar}), 139.17 (C–SO_2), 135.08 (C_{ar}), 133.63 (C_{ar}), 129.52 (CH_{ar}), 129.09 (CH_{ar}), 128.98 (CH_{ar}), 128.75 (CH_{ar}), 128.67 (C_{ar}), 127.98 (CH_{ar}), 127.37 (CH_{ar}), 127.08 (CH_{ar}), 126.66 (C_{ar}), 120.44 (CH_{ar}), 44.56 (CH_2–NH), 35.69 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 457.2/457.3 $[M+H]^+$, 474.2/474.3 $[M+NH_4]^+$; Purity (HPLC): >99.5% (System 2).

6.42. 4-Chloro-*N*-[4-(phenethylsulfamoyl)phenyl]benzamide, 7d

General procedure: 5, off-white crystals; Yield: 10%; ^{1}H NMR (DMSO-d_6): δ = 10.64 (s, 1H, CONH), 8.05–7.92 (m, 4H, H_{ar}), 7.83–7.74 (m, 2H, H_{ar}), 7.67–7.55 (m, 3H, H_{ar}, SO_2–NH), 7.31–7.23 (m, 2H, H_{ar}), 7.22–7.11 (m, 3H, H_{ar}), 3.03–2.92 (m, 2H, CH_2–NH), 2.70 (t, 2H, ^3J = 7.59 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 165.51 (C=O), 142.86 (C_{ar}–NH), 139.08 (C–SO_2), 137.27 (C_{ar}), 135.20 (C_{ar}), 133.50 (C_{ar}), 130.16 (CH_{ar}), 129.06 (CH_{ar}), 128.99 (CH_{ar}), 128.77 (CH_{ar}), 127.96 (CH_{ar}), 126.68 (C_{ar}), 120.57 (CH_{ar}), 44.51 (NH–CH_2), 35.596 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 415.0/415.0 $[^{35}ClM+H]^+$, 417.0/417.1 $[^{37}ClM+H]^+$; Purity (HPLC): 97.5% (System 2).

6.43. 3,4-Dichloro-*N*-[4-(phenethylsulfamoyl)phenyl]benzamide, 7e

General procedure: 5, yellowish crystals; Yield: 78%; ^{1}H NMR (DMSO-d_6): δ = 10.68 (s, 1H, CONH), 8.23 (d, 1H, ^4J = 2.03 Hz, H_{ar}, H2′), 8.00–7.91 (m, 3H, H_{ar}, A/A′, H5′), 7.84 (d, 2H, ^3J = 8.42 Hz, H_{ar}, H6′), 7.60 (1H, t, 5.88 Hz, SO_2–NH), 7.31–7.23 (m, 2H, H_{ar}, B/B′), 7.22–7.13 (m, 3H, H_{ar}), 3.03–2.95 (m, 2H, CH_2–NH), (t, 2H, ^3J = 7.40 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 164.11 (C=O), 142.66 (C_{ar}–NH), 139.14 (C–SO_2), 135.49 (C_{ar}), 135.17 (C_{ar}), 135.16 (C_{ar}), 131.79 (C_{ar}), 131.27 (C_{ar}), 130.16 (C_{ar}), 129.07 (CH_{ar}), 128.75 (CH_{ar}), 128.62 (C_{ar}), 127.99 (CH_{ar}), 126.67 (C_{ar}), 120.58 (CH_{ar}), 44.53 (NH–CH_2), 35.65 ($CHar_2$); CIMS (direct mode): calcd/found (*m/z*): 449.0/448.9 $[^{35}Cl^{35}ClM+H]^+$, 466.0/466.1 $[^{35}Cl^{35}ClM+NH_4]^+$ 468.0/468.1 $[^{35}Cl^{37}ClClM+H]^+$, 470.1 $[^{37}Cl^{37}ClM+NH_4]^+$; Purity (HPLC): 98.9% (System 2).

6.44. 4-Bromo-*N*-[4-(phenethylsulfamoyl)phenyl]benzamide, 7f

General procedure: 5, off-white crystals; Yield: 98%; ^{1}H NMR (DMSO-d_6): δ = 10.65 (s, 1H, CONH), 8.02–7.86 (m, 4H, H_{ar}), 7.81–7.72 (m, 4H, H_{ar}), 7.63 (t, 1H, SO_2–NH, ^3J = 5.96 Hz), 7.31–7-23 (m, 2H, H_{ar}), 7.22–7.10 (m, 3H, H_{ar}), 3.02–2.89 (m, 2H, CH_2-NH), 2.68 (t, 2H, ^3J = 7.37 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 165.49 (C=O), 142.89 (C_{ar}–NH), 139.15 (C–SO_2), 135.26 (C_{ar}), 133.94 (C_{ar}), 131.93 (CH_{ar}), 130.37 (CH_{ar}), 129.08 (CH_{ar}), 128.75 (CH_{ar}), 127.97 (CH_{ar}), 126.66 (CH_{ar}), 126.19 (C_{ar}), 120.48 (CH_{ar}), 44.53 (NH–CH_2), 35.66 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 459.0/458.9 $[^{79}BrM+H]^+$, 461.0/461.0 $[^{81}BrM]^+$; Purity (HPLC): 98.4% (System 2).

6.45. 4-Iodo-*N*-[4-(phenethylsulfamoyl)phenyl]benzamide, 7g

General procedure: 5, white crystals; Yield: 32%; ^{1}H NMR (DMSO-d_6): δ = 10.66 (s, 1H, CONH), 8.04–7.88 (m, 4H, H_{ar}, A/A′, C/C′), 7.81–7.73 (m, 4H, H_{ar}, B/B′), 7.64 (t, 1H, ^3J = 5.91 Hz, SO_2–NH), 7.32–7-23 (m, 2H, H_{ar}), 7.22–7.10 (m, 3H, H_{ar}), 3.02–2.89 (m, 2H, CH_2–NH), 2.68 (t, 2H, ^3J = 7.52 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 165.75 (C=O), 142.93 (C_{ar}–NH), 139.15 (C–SO_2), 137.76 (CH_{ar}), 135.20 (C_{ar}), 134.22 (C_{ar}), 130.17 (CH_{ar}), 129.08 (CH_{ar}), 128.75 (CH_{ar}), 127.95 (CH_{ar}), 126.65 (CH_{ar}), 120.47 (CH_{ar}), 100.27 (C–I), 44.53 (NH–CH_2), 35.65 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 507.0/506.9 $[M+H]^+$, 524.0/524.0 $[M+NH_4]^+$; Purity (HPLC): 99.0% (System 2).

6.46. 4-Nitro-*N*-[4-(phenethylsulfamoyl)phenyl]benzamide, 7h

General procedure: 5, yellowish crystals; Yield: 5%; ^{1}H NMR (DMSO-d_6): δ = 10.90 (s, 1H, CONH), 8.42–8.34 (m, 2H, H_{ar}), 8.23–8.15 (m, 2H, H_{ar}), 8.01–7.93 (m, 2H, H_{ar}), 7.83–7.75 (m, 2H, H_{ar}), 7.66 (t, 1H, ^3J = 5.80 Hz, SO_2–NH), 7.30–7.23 (m, 2H, H_{ar}), 7.22–7.10 (m, 3H, H_{ar}), 3.01–2.90 (m, 2H, CH_2–NH), 2.68 (t, 2H, ^3J = 7.49 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 164.85 (C=O), 149.77 (C_{ar}–NO_2), 142.60 (C_{ar}), 140.54 (C_{ar}), 139.14 (C–SO_2), 135.63 (C_{ar}), 129.81 (CH_{ar}), 129.08 (CH_{ar}), 128.75 (CH_{ar}), 128.03 (CH_{ar}), 126.66 (CH_{ar}), 124.04 (CH_{ar}), 120.62 (CH_{ar}), 44.51 (CH_2–NH), 35.66 (CH_2); APCIMS (direct positive mode): calcd/found (*m/z*): 426.1/ 425.9 $[M+H]^+$.

6.47. 4-Amino-*N*-[4-(phenethylsulfamoyl)phenyl]benzamide, 7i

General procedure: 4, yellowish crystals; Yield: 97%; ^{1}H NMR (DMSO-d_6): δ = 10.09 (s, 1H, CONH), 8.00–7.89 (m, 2H, H_{ar}), 7.76–7.68 (m, 4H, H_{ar}), 7.57 (t, 1H, ^3J = 5.75 Hz, SO_2-NH), 7.30–7.23 (m, 2H, H_{ar}), 7.22–7.12 (m, 3H, H_{ar}), 6.64–6.57 (m, 2H, H_{ar}), 5.85 (s, 2H, NH_2), 3.00–2.90 (m, 2H, CH_2-NH), 2.68 (t, 2H, ^3J = 7.67 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 152.95 (C=O), 143.72 (C_{ar}), 139.12 (C–SO_2), 134.11 (C_{ar}), 130.04 (CH_{ar}), 129.06 (CH_{ar}), 128.78 (CH_{ar}), 127.84 (CH_{ar}), 126.74 (C_{ar}), 126.69 (C_{ar}), 120.75 (CH_{ar}), 120.13 (CH_{ar}), 113.06 (CH_{ar}), 44.52 (CH_2–NH), 35.56 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 396.1/396.1 $[M+H]^+$; Purity (HPLC): 99.2% (System 2).

6.48. 4-Chloro-*N*-[4-(phenethylsulfamoyl)phenyl]benzenesulfonamide, 7j

General procedure: 5, white crystals; Yield 82%; ^{1}H NMR (DMSO-d_6): δ = 10.93 (s, 1H, SO_2-NH-AR), 7.88–7.79 (m, 2H, H_{ar}), 7.71–7.64 (m, 2H, H_{ar}), 7.67–7.79 (m, 2H, H_{ar}), 7.56 (t, 1H, ^3J = 5.70 Hz, SO_2-NH), 7.33–7.14 (m, 5H, H_{ar}), 7.12–7.04 (m, 2H, H_{ar}), 3.00–2.84 (m, 2H, CH_2-NH), 2.62 (t, 2H, ^3J = 7.60 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 141.49 (C–NH_2), 139.05 (C–SO_2), 138.66 (C_{ar}), 138.39 (C_{ar}), 135.73 (C_{ar}), 130.04 (CH_{ar}), 129.01 (CH_{ar}), 128.71 (CH_{ar}), 128.57 (CH_{ar}), 126.63 (CH_{ar}), 119.38 (CH_{ar}), 44.44 (CH_2–NH), 35.58 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 541.0/541.0 $[^{35}ClM+H]^+$, 453.0/453.0 $[^{37}ClM+H]^+$, 468.0/468.0 $[^{35}ClM+NH_4]^+$, 470.0/470.0 $[^{37}ClM+NH_4]^+$; Purity (HPLC): 97.8% (System 2).

6.49. [4-[2-[(4-Aminophenyl)sulfonylamino]ethyl]phenyl] 4-aminobenzenesulfonate, 8

General procedure: 4, yellowish crystals; Yield: 45%; ^{1}H NMR (DMSO-d_6): δ = 7.48–7.35 (m, 4H, H_{ar}), 7.18–7.07 (m, 3H, H_{ar},

NH), 6.93–6.84 (m, 2H, H_{ar}), 6.69–6.56 (m, 4, H_{ar}), 6.28 (s, 2H, NH_2), 5.84 (s, 2H, NH_2), 2.92–2.81 (m, 2H, NH-CH_2), 2.65 (t, 2H, ^3J = 7.19 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 154.91 (C_{ar}), 152.88 (C–SO_2–O), 148.17 (C_{ar}), 138.25 (C–SO_2), 130.75 (CH_{ar}), 130.31 (CH_{ar}), 128.90 (CH_{ar}), 125.65 (C–NH_2), 122.43 (CH_{ar}), 118.37 (C–NH_2), 113.18 (CH_{ar}), 113.12 (CH_{ar}), 44.16 (NH–CH_2), 34.79 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 465.1/465.1 $[M+NH_4]^+$.

6.50. 4-Nitro-*N*-phenethyl-benzamide, 8a

General procedure: 3, yellow crystals; Yield: 43%; ^{1}H NMR (DMSO-d_6): δ = 8.91 (t, 1H, ^3J = 5.41 Hz, NH), 8.34–8.28 (m, 2H, H_{ar}, A/A′), 8.07–8.01 (m, 2H, H_{ar}, B/B′), 7.34–7.16 (m. 5H, H_{ar}), 3.57–3.47 (m, 2H, CH_2-NH), 2.87 (t, 2H, ^3J = 7.35 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 164.93 (C=O), 149.37 (C–NO_2), 140.61 (C–CO), 139.76 (CH_{ar}), 129.08 (CH_{ar}), 129.02 (CH_{ar}), 128.78 (CH_{ar}), 126.57 (C_{ar}), 123.95 (CH_{ar}), 41.47 (NH–CH_2), 35.30 (Phe-CH_2); CIMS (direct mode): calcd/found (*m/z*): 270.1/270.1 $[M+H]^+$.

6.51. *N*-Benzyl-4-nitro-benzamide, 8b

General procedure: 3, yellow crystals; Yield: 79%; ^{1}H NMR (DMSO-d_6): δ = 9.39 (t, 1H, ^3J = 5.69 Hz, NH), 8.36–8.30 (m, 2H, H_{ar}, A/A′), 8.15–8.10 (m, 2H, H_{ar}, B/B′), 7.36–7.31 (m, 4H, H_{ar}), 7.29–7.23 (m, 1H, H_{ar}), 4.52 (d, 2H, ^3J = 5.92 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 165.03 (C=O), 149.47 (C–NO_2), 140.37 (C–CO), 139.55 (CH_{ar}), 129.20 (CH_{ar}), 128.77 (CH_{ar}), 127.72 (CH_{ar}), 127.32 (C_{ar}), 124.00 (CH_{ar}), 43.27 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 274.1/274.1 $[M+NH_4]^+$.

6.52. 4-Nitro-*N*-(1-phenylethyl)benzamide, 8c

General procedure: 3, light yellow needles; Yield: 59%; ^{1}H NMR (DMSO-d_6): δ = 9.18 (d, 1H, ^3J = 7.56 Hz, NH), 8.35–8.29 (m, 2H, H_{ar}, A/A′), 8.15–8.08 (m, 2H, H_{ar}, B/B′), 7.43–7.38 (m, 2H, H_{ar}), 7.37–7.30 (m, 2H, H_{ar}), 7.27–7.20 (m, 1H, H_{ar}), 5.18 (m, 1H, CH), 1.5 (d, 3H, ^3J = 7.09 Hz, CH_3); ^{13}C NMR (DMSO-d_6): δ = 164.35 (C=O), 149.40 (C–NO_2), 144.87 (CH_{ar}), 140.58 (C–CO), 129.33 (CH_{ar}), 128.72 (CH_{ar}), 127.16 (C_{ar}), 126.47 (CH_{ar}), 123.88 (CH_{ar}), 49.25 (CH), 22.56 (CH_3); EIMS (direct mode): calcd/found (*m/z*): 270.1/270.2 $[M]^+$, 271.1/271.2 $[M+H]^+$.

6.53. 4-Amino-*N*-phenethyl-benzamide, 9a

General procedure: 4, light yellow crystals; Yield: 84%; ^{1}H NMR (DMSO-d_6): δ = 8.09 (t, 1H, ^3J = 5.51 Hz, NH), 7.58–7.52 (m, 2H, H_{ar}, A/A′), 7.33–7.26 (m, 2H, H_{ar}), 7.25–7.16 (m, 3H, H_{ar}), 6.56–6.50 (m, 2H, H_{ar}, B/B′), 5.58 (s, 2H, NH_2), 3.46–3.38 (m, 2H, CH_2-NH), 2.81 (t, 2H, ^3J = 7.56 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 166.59 (C=O), 151.93 (C_{ar}-NH), 140.18 (CH_{ar}), 129.04 (CH_{ar}), 128.72 (CH_{ar}), 126.42 (C_{ar}), 121.75 (C_{ar}-CO), 112.93 (CH_{ar}), 41.14 (NH–CH_2), 35.84 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 241.1/241.2 $[M+H]^+$; Purity (HPLC): 98.7% (System 2).

6.54. 4-Amino-*N*-benzyl-benzamide, 9b

General procedure: 4, yellow crystals; Yield: 91%; ^{1}H NMR (DMSO-d_6): δ = 8.57 (t, 1H, ^3J = 5.59 Hz, NH), 7.65–7.59 (m, 2H, H_{ar}, A/A′), 7.34–7.26 (m, 4H, H_{ar}), 7.25–7.19 (m, 1H, H_{ar}), 6.58–6.51 (m, 2H, H_{ar}, B/B′), 5.62 (s, 2H, NH_2), 4.43 (d, 2H, ^3J = 6.03 Hz, CH_2); ^{13}C NMR (DMSO-d_6): δ = 166.62 (C=O), 152.08 (C–NH_2), 140.76 (CH_{ar}), 129.19 (CH_{ar}), 128.59 (CH_{ar}), 127.55 (C_{ar}), 126.95 (CH_{ar}), 121.41 (*C*–CO), 112.95 (CH_{ar}), 42.76 (CH_2); CIMS (direct mode): calcd/found (*m/z*): 226.1/226.2 $[M]^+$, 227.1/227.2 $[M+H]^+$; Purity (HPLC): 96.5% (System 2).

6.55. 4-Amino-*N*-(1-phenylethyl)benzamide, 9c

General procedure: 4, yellow crystals; Yield: 98%; ^{1}H NMR (DMSO-d_6): δ = 8.31 (d, 1H, ^3J = 8.12 Hz, NH), 7.66–7.59 (m, 2H, H_{ar}, A/A′), 7.39–7.34 (m, 2H, H_{ar}), 7.33–7.26 (m, 2H, H_{ar}), 7.23–7.17 (m, 1H, H_{ar}), 6.57–6.51 (m, 2H, H_{ar}, B/B′), 5.61 (s, 2H, NH_2), 5.13 (m, 1H, CH), 2.52–2.49 (m, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ = 165.90 (C=O), 152.01 (C–NH_2), 145.89 (CH_{ar}), 129.31 (CH_{ar}), 128,53 (CH_{ar}), 126.80 (C_{ar}), 126.47 (CH_{ar}), 121.59 (C_{ar}-CO), 112.87 (CH_{ar}), 48.43 (CH), 22.80 (CH_3); EIMS (direct mode): calcd/found (*m/z*): 120.0/120.0 [Phe–CH–(CH3)–NH]$^+$, $[H_2N$–Phe–CO$]^+$; Purity (HPLC): 97.5% (System 2).

6.56. Molecular modeling

All calculations were performed on a Pentium IV 2.6 GHz based Linux cluster (16 CPUs). The compounds were transformed into three-dimensional molecular structures using the MOE modeling package (Chemical Computing Group). All compounds were generated in the protonation state that can be assumed under physiological condition.

6.57. Protein modeling

A homology model for human PRMT1 was generated using ClustalW sequence alignment of mouse PRMT1 and PRMT3 and the COMPOSER module within Sybyl 7.1. The model generation and validation is described in detail in the previous publications.[5,8]

6.58. GRID calculations

Interaction possibilities were analyzed using the GRID program (Molecular Discovery Inc.). GRID is an approach to predict noncovalent interactions between a molecule of known three-dimensional structure (i.e., PRMT1) and a small group as a probe (representing chemical features of a ligand).[10] The calculations were performed using version 22 of the GRID program and the hPRMT1 homology model.

6.59. Ligand docking

The docking studies were performed using GOLD (version 4.0, Cambridge Crystallographic Data Centre) with default parameters together with the GoldScore fitness function.[11] GoldScore was chosen because it has outperformed other scoring functions in our previous studies. Docking was carried out to obtain a population of possible conformations and orientations for the inhibitors at the putative active site. All torsion angles in each compound were allowed to rotate freely. For each ligand, docking runs were performed with a maximum allowed number of 10 poses.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.02.032.

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