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Steric structure–activity relationship of cyproheptadine derivatives as inhibitors of histone methyltransferase Set7/9

Takashi Fujiwara [a], Kasumi Ohira [a], Ko Urushibara [b], Akihiro Ito [c,d], Minoru Yoshida [c,d,e], Misae Kanai [b], Aya Tanatani [b], Hiroyuki Kagechika [a], Tomoya Hirano [a,*]

[a] Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU), 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan
[b] Department of Chemistry, Faculty of Science, Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112-8610, Japan
[c] Chemical Genetics Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[d] Chemical Genomics Research Group, RIKEN Center for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[e] Drug Discovery Platforms Cooperation Division, RIKEN Center for Sustainable Resource Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

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ABSTRACT

Set7/9 is a histone lysine methyltransferase, but it is also thought to be involved in a wide variety of pathophysiological functions. We previously identified cyproheptadine, which has a characteristic butterfly-like molecular conformation with bent tricyclic dibenzosuberene and chair-form *N*-methylpiperidine moieties, as a Set7/9 inhibitor. In this work, we synthesized several derivatives in order to examine the steric structure–inhibitory activity relationship. We found that even a small change of molecular shape due to reduction or replacement of the 10,11-olefinic bond of the tricyclic ring generally resulted in a drastic decrease of the inhibitory activity. Our results should be useful not only for development of more potent and selective inhibitors, but also for the construction of novel inhibitor scaffolds.

1. Introduction

Histone lysine methyltransferases (HKMTs) are 'writers' of histone protein methylation, transferring the methyl group of *S*-adenosylmethionine (SAM) to specific lysine residues.[1–4] The HKMTs include SET domain-containing lysine methyltransferase 7/9 (Set7/9, also known as SETD7 or KMT7), which was reported to methylate lysine 4 on histone H3 (H3K4), leading to activation of gene transcription.[5] Since then, it has also been found to regulate other biomolecules,[6] including TAF10,[7] p53,[8] estrogen receptor α (ERα),[9] androgen receptor (AR),[10] retinoic acid receptor (RAR),[11] STAT3,[12] FoxO3,[13] Tat,[14] HIF-1α[15] and DNA methyltransferase (DNMT1).[16] Due to this diversity of substrates, Set7/9 is considered to influence a wide range of physiological functions, including differentiation, cell cycle, metabolism and inflammation, and altered function of Set7/9 is associated with various diseases.[17–20] However, its exact function is still unclear, as Set7/9 knockdown or depletion appears not to induce expected function.[21–25] Inhibitors of Set7/9 are needed for studies of its physiological function and for potential therapeutic use, but only a few have been reported, in contrast to the situation for other KHMTs, such as G9a and Dot1L.[26–33] We have reported a bisubstrate-type inhibitor, DAAM-3, based on an aza-derivative of coenzyme adenosylhomocysteine (AzaAdoMet) bearing an alkylamino group (Fig. 1a).[34] Recently, the first practically selective and potent inhibitor, (*R*)-PFI-2, was reported,[35] and subsequently DC-S239 was also developed.[36] In addition, we recently identified cyproheptadine (**1a**) based on fluorescence-based assay system,[37] which has been used clinically as a serotonin receptor antagonist or histamine receptor (H1) antagonist, as a Set7/9 inhibitor.[38] Cyproheptadine is reported to inhibit methylation-mediated stabilization of estrogen receptor α (ERα). It also inhibits estrogen-dependent growth of breast cancer MCF-7 cells, and has been proposed as a potential therapeutic agent for breast cancer.

DAAM-3 was shown to bind to the coenzyme-binding site by analysis of the crystal structure of the complex of DAAM-3 with Set7/9,[39] and DC-S239 is expected to bind to the same site.[36] On the other hand, (*R*)-PFI-2 binds to the substrate-binding site of Set7/9.[35] Cyproheptadine (**1a**) may also bind to the substrate-binding site, as indicated by its co-crystal structure and kinetic studies. Crystal structure and NMR analyses showed that cyproheptadine (**1a**) exists in a butterfly-like conformation with a bent tricyclic dibenzosuberene moiety and chair-form piperidine ring.[40,41] This characteristic structure is retained in the co-crystal of cyproheptadine (**1a**) with Set7/9, but interestingly, the only interaction

* Corresponding author. Tel.: +81 3 5280 8128; fax: +81 3 5280 8127.
E-mail address: hira.chem@tmd.ac.jp (T. Hirano).

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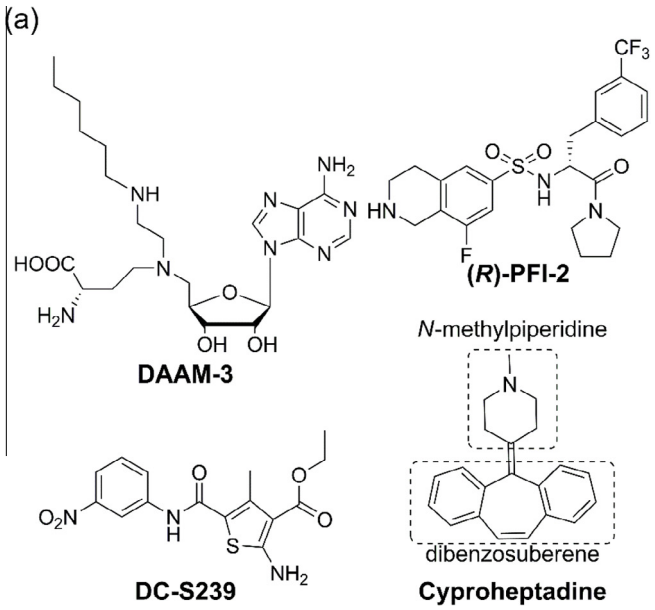


Figure 1. (a) Structures of reported Set7/9 inhibitors. (b) Schematic representation of the three-dimensional structure of cyproheptadine (**1a**).

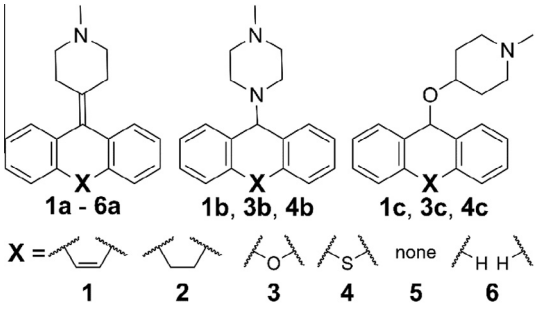


Figure 2. Derivatives of cyproheptadine (**1a**).

between the inhibitor and the receptor appeared to occur at the *N*-methylpiperidine ring, especially the nitrogen atom, of cyproheptadine (**1a**). Thus, the importance of the overall molecular shape remains unclear. So in this study, we synthesized several derivatives of cyproheptadine (**1a**) with different tricyclic ring systems or different linking groups between the tricyclic ring and monocyclic amine, in order to investigate the structural requirements for inhibition of Set7/9.

2. Results and discussion

2.1. Design and synthesis of derivatives of cyproheptadine

We designed three types of cyproheptadine derivatives. The 10,11-olefinic bond in the tricyclic ring (X in Fig. 2) was replaced with a saturated bond (**2a**), an oxygen atom (**3a**), a sulfur atom (**4a**), a direct linkage between the rings (**5a**) or no linkage (**6a**). Ab initio calculations indicated that these conversions would change the ring geometry.[42] In addition, the olefinic bond linking the tricyclic ring to piperidine was changed to a carbon–nitrogen bond (compounds **1b**, **3b** and **4b**) or an ether bond (compounds **1c**, **3c** and **4c**) (Fig. 2).

Compounds **1a** and **4a** are commercially available. Compounds **2a**, **3a**, **5a** and **6a** were prepared via Grignard reaction of 4-chloro-1-methylpiperidine with the corresponding arylketones (**2d**, **3d**, **5d** and **6d**), followed by dehydration, according to the reported method (Scheme 1).[42,43]

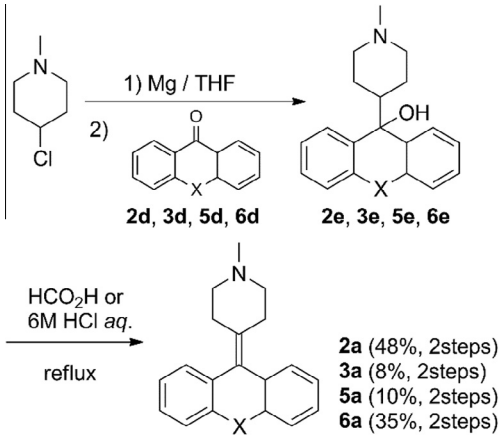
Compounds **1f** and **3f** were converted to the corresponding chlorinated compounds (**1g** and **3g**), and then treated with 1-methylpiperazine to yield **1b** and **3b** (Scheme 2). Compound **4b** was similarly prepared from **4f**, which was derived from thioxanthone by reduction. Compounds **1c** and **3c** were synthesized by condensation of 4-hydroxy-1-methylpiperidine with the corresponding alcohols (**1f** and **3f**) under acidic conditions. Compound **4c** was prepared from thioxanthone via **4f**.

2.2. Inhibitory activities towards Set7/9

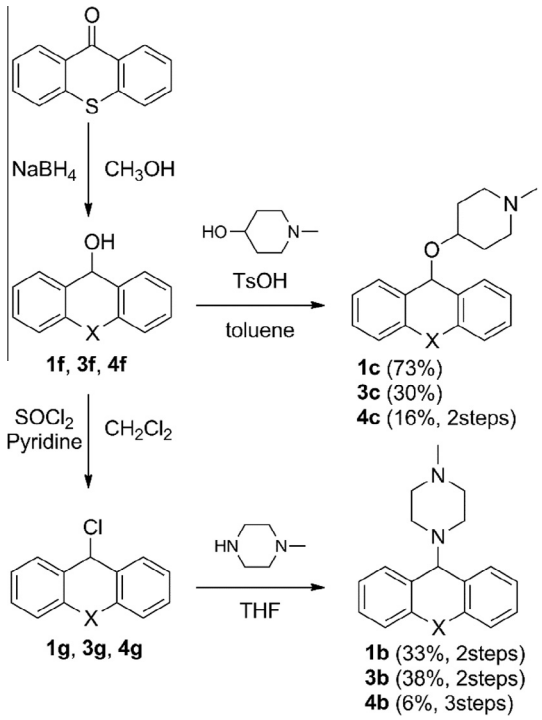
The Set7/9-inhibitory activities of the synthesized compounds were evaluated by means of the AlphaLISA method, which is one of reported methods for evaluating the activity of Set7/9,[44] and consists of biotylated substrate peptide, two beads conjugated with streptavidin and antibody against methylated substrate, respectively (Table 1). In methylated state of substrate peptide by Set7/9, both beads could be attached, resulting in emission of luminescence. In this assay, cyproheptadine (**1a**) inhibited Set7/9 with an IC_{50} value of 3.4 μM. Among test compounds with a modified tricyclic ring, compound **4a** (X = S) showed similar inhibitory activity (IC_{50}: 4.3 μM for **4a**) to cyproheptadine. The compound with the oxygen atom (**3a**) showed a small decrease of inhibitory activity, and those with the saturated bond (**2a**), direct linkage (**5a**) or no linkage (**6a**) showed greatly decreased activities. Compounds in which the double bond linking the tricyclic ring and piperidine was converted to a carbon–nitrogen bond (**1b**, **3b**, **4b**) or an ether bond (**1c**, **3c**, **4c**) showed a dramatic loss of inhibitory activity, which indicated that this double bond is important for the inhibitory activity.

2.3. Structural study for the inhibition of Set7/9

Although calculated optimized structures of **1a**–**6a** have been reported,[42] we performed X-ray crystallographical analysis of



Scheme 1. Synthesis of **2a**, **3a**, **5a** and **6a**.



Scheme 2. Synthesis of **1b**, **3b**, **4b**, **1c**, **3c** and **4c**.

Table 1
Inhibitory activity towards Set7/9

	IC_{50} (μM)
1a	3.4
2a	59.1
3a	16.3
4a	4.3
5a	41.5
6a	94.6
1b	>100
3b	>100
4b	>100
1c	>100
3c	>100
4c	>100

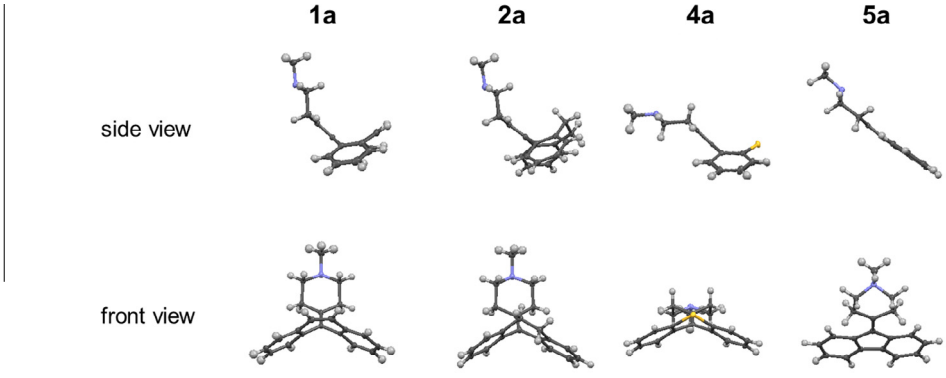
selected compounds, cyproheptadine (**1a**), **2a**, **4a** and **5a** (Table 2), for precise evaluation of the steric structure–activity relationship.

As shown in Figure 3, the crystal structure of cyproheptadine (**1a**) was similar to that bound to Set7/9.[38] Since there are no obvious direct interactions such as hydrogen bonds between its tricyclic dibenzosuberene moiety and Set7/9 in the crystal structure, we assumed that the molecular shape would be important for the activity. Compound **2a** (X = $-CH_2CH_2-$) showed rather similar molecular shapes with cyproheptadine (**1a**), though significant distortion of the benzene rings was observed in **2a**. Compound **4a** also showed a similar structure of the tricyclic part to cyproheptadine (**1a**), while the conformation of the piperidine ring was different. In the front view, it can be seen that the dihedral angles of the two benzene rings of **1a**, **2a** and **4a** were similar, about 120–130°. Compound **5a** formed a flatter structure (see side view). Previously reported structural study by ab initio calculations indicated that **4a** would have similar butterfly-like conformation to cyproheptadine (**1a**), **2a** would be also similar, whereas **5a** would have similar flat structure.[42] Our crystallographic study showed similar insights of **4a** and **5a**, however, structural characteristic only for **2a**, which would be distortion of benzene ring due to flexible saturated bond, was also observed. Cyproheptadine (**1a**) and its derivatives are reported to be serotonin 5-HT_{2A} receptor ligands, and the binding affinities of **1a**, **2a**, **4a** and **5a** have also been reported. Compound **5a** lacked affinity for both Set7/9 and 5-HT_{2A}, probably due to its flatter structure, whereas **4a** showed similar activity to cyproheptadine (**1a**) for both targets, and **2a** showed 5-fold less activity for 5-HT_{2A} and 18-fold less activity for Set7/9. Thus, rigidity of the tricyclic structure due to the presence of the unsaturated central double bond may be important for Set7/9-inhibitory activity.

In our previous report, the *N*-methyl group of cyproheptadine (**1a**) was shown to be important for Set7/9 inhibition: even the change to an ethyl group decreased the activity. In accordance with this, the crystal structure of cyproheptadine (**1a**) bound to Set7/9 indicates that the *N*-methylpiperidine moiety is held within the lysine access channel by hydrogen bond formation and hydrophobic van der Waals interactions, while the importance of other structural components is harder to predict. In this work, we have shown that the folded molecular shape of cyproheptadine (**1a**) is important, because loss of the rigid olefinic bond linking the dibenzosuberene and piperidine rings drastically decreased the inhibitory activity. Interestingly, reduction of the 10,11-olefinic bond of the tricyclic ring had a greater effect on the inhibitory activity towards Set7/9 than on that towards 5-HT_{2A}, the original target molecule of cyproheptadine. The present findings may provide

Table 2
X-ray structural analysis of cyproheptadine (**1a**), **2a**, **4a** and **5a**

	Cyproheptadine (**1a**)	**2a**	**4a**	**5a**
Recryn. solvent	EtOH, H_2O	CH_2Cl_2, hexane	Hexane	Hexane
Formula	$C_{21}H_{21}N$	$C_{21}H_{23}N$	$C_{19}H_{19}NS$	$C_{19}H_{19}N$
Crystal system	Monoclinic	Monoclinic	Triclinic	Orthorhombic
Space group	$P2_1/c$	$P2_1/n$	$P\bar{1}$	*P*nma
a (Å)	5.4909(8)	10.0058(12)	10.5730(9)	20.141(3)
b (Å)	10.5745(16)	5.6890(7)	17.3009(15)	12.653(2)
c (Å)	27.313(3)	27.915(3)	17.9732(15)	5.3569(9)
α (°)	90	90	105.9721(14)	90
β (°)	92.410(2)	93.533(2)	101.3888(14)	90
γ (°)	90	90	96.0237(14)	90
Z	4	4	8	4
T (K)	93	93	93	93
GOF	0.987	1.004	1.043	1.151
R_1 [$I > 2\sigma(I)$]	0.0494	0.0358	0.0369	0.0420
wR_2 [$I > 2\sigma(I)$]	0.1173	0.0876	0.0995	0.0963



	1a	2a	4a	5a
Dihedral angle between two phenyl rings	127.4°	119.5°	124.6°, 129.8° 133.0°, 133.7°	175.1°
IC_{50} (μM) for Set7/9	3.4	59.1	4.3	41.5
K_i (nM) for 5-HT_{2A}[a]	1.6	9.0	2.5	199

Figure 3. Steric structure and biological activities of cyproheptadine (**1a**), and its derivatives **2a**, **4a** and **5a**. In the crystal of **4a**, four molecules exist independently in the asymmetric unit. All of them have similar structures, and only one of them is shown. [a]Data from Ref. 42.

clues for the development of more selective and potent inhibitors of Set7/9, to avoid any supposed adverse effects in its clinical application due to the activity against those receptors.

3. Conclusion

In this work, we synthesized several derivatives of cyproheptadine in order to examine the steric structure–activity relationship for Set7/9-inhibitory activity. We found that the overall folded molecular shape of cyproheptadine is important. Our results should be helpful for the development of more potent and selective Set7/9 inhibitors, and for finding novel inhibitor scaffolds.

4. Experimental

4.1. General

All reagents were purchased from Sigma–Aldrich Chemical, Tokyo Kasei Kogyo, Wako Pure Chemical Industries, and Kanto Kagaku. Silica gel for column chromatography was purchased from Kanto Kagaku. NMR spectra were recorded on a Bruker Avance 400 or Bruker Avance 500 spectrometer. Mass spectral data was obtained on a Bruker Daltonics microTOF-2focus in the positive and negative ion detection modes. Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected.

4.2. Preparation of compounds

4.2.1. Preparation of 1a–6a

Cyproheptadine (**1a**) and **4a** were obtained commercially. Compounds **2a**, **3a**, **5a** and **6a** were prepared according to reported methods from 4-chloro-1-methylpiperidine and corresponding arylketones (**2d**, **3d**, **5d** and **6d**) via Grignard reaction and acidic dehydration.[42,43] Those analytical data were shown below.

2a: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.14–7.07 (m, 8H), 3.46–3.37 (m, 2H), 2.86–2.78 (m, 2H), 2.65–2.60 (m, 2H), 2.47–2.38 (m, 4H), 2.28 (s, 3H), 2.17–2.11 (m, 2H); HRMS (ESI+) Calcd for $C_{21}H_{24}N$ $[M+H]^+$: 290.1903. Found 290.1906.

3a: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (dd, J = 1.6, 7.6 Hz, 2H), 7.26–7.18 (m, 4H), 7.13–7.09 (m, 2H), 2.85 (t, J = 6.0 Hz, 4H), 2.37 (t, J = 6.0 Hz, 4H), 2.26 (s, 3H); HRMS (ESI+) Calcd for $C_{19}H_{20}NO$ $[M+H]^+$: 278.1539. Found 278.1538.

5a: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.89 (br d, J = 7.6 Hz, 2H), 7.77 (m, 2H), 7.34–7.25 (m, 4H), 3.28 (t, J = 6.0 Hz, 4H), 2.65 (t, J = 6.0 Hz, 4H), 2.36 (s, 3H); HRMS (ESI+) Calcd for $C_{19}H_{20}N$ $[M+H]^+$: 262.1590. Found 262.1586.

6a: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.26 (m, 4H), 7.22–7.18 (m, 2H), 7.14–7.12 (m, 4H), 2.46–2.39 (m, 8H), 2.30 (s, 3H); HRMS (ESI+) Calcd for $C_{19}H_{22}N$ $[M+H]^+$: 264.1747. Found 264.1748.

4.2.2. Preparation of 1b and 3b

Under an argon atmosphere, thionyl chloride (0.30 ml, 4.1 mmol) was added to a solution of **1f** (0.30 g, 1.4 mmol) and pyridine (0.20 ml, 2.5 ml) in dichloromethane (3 ml) at 0 °C. The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure to yield **1g**, which was used without further purification. Compound **1g** was dissolved in THF (8 ml), and *N*-methylpiperadine (0.32 ml, 2.9 mmol) was added slowly to the resulting solution at −70 °C. The reaction mixture was stirred for 3 h at room temperature, then diluted with diethylether, and the organic layer was washed with brine. The extract was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate–methanol = 10:1) to afford **1b** (0.14 g, 0.48 mmol, y. 33%) as a white powder. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40–7.34 (m, 4H), 7.33–7.27 (m, 4H), 6.58 (s, 2H), 4.28 (s, 1H), 2.17 (br, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 138.2, 134.4, 130.4, 130.1, 129.6, 128.0, 127.0, 78.0, 54.9, 51.4, 45.7; Anal. Calcd for $C_{20}H_{22}N_2$: C, 82.72; H, 7.64, N, 9.65. Found C, 82.46; H, 7.59; N, 9.57.; mp (hexane): 144.5–146.0 °C.

3b was similarly prepared from **3f** and *N*-methylpiperadine. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34 (q, J = 1.5, 7.8 Hz, 2H), 7.31–7.28 (m, 2H), 7.15–7.10 (m, 4H), 4.81 (s, 1H), 2.39 (br, 8H), 1.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 153.4, 130.4, 128.6, 122.9, 120.1, 116.1, 60.5, 55.2, 47.7, 45.7; Anal. Calcd for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19, N, 9.99; O, 5.71. Found C, 77.29; H, 7.18; N, 9.85.; mp (hexane): 115.0–117.0 °C.

4.2.3. Preparation of 4b

Sodium borohydride (0.70 g, 19 mmol) was added to a solution of thioxanthone (1.0 g, 4.7 mmol) in methanol (30 ml) at 0 °C. The mixture was heated at reflux for 20 min, then cooled to room temperature, and quenched with water. The aqueous layer was extracted with ethyl acetate. The organic layer was dried over

anhydrous sodium sulfate. The solvent was removed under reduced pressure to yield **4f**. This product was dissolved in dichloromethane under an argon atmosphere, and thionyl chloride (1.0 ml, 14 mmol) and pyridine (0.60 ml, 7.4 mmol) were added to the solution at 0 °C. The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure to yield **4g**, which was dissolved in THF (25 ml). *N*-Methylpiperadine (1.0 ml, 9.0 mmol) was added slowly to the resulting solution at −70 °C. The reaction mixture was stirred for 5 h at room temperature, and then diluted with diethylether. The organic layer was washed with brine and dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate–methanol = 10:1) to afford **4b** (89 mg, 0.30 mmol, y. 6.0%) as a brown powder. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.45–7.43 (m, 2H), 7.36–7.34 (m, 2H), 7.25–7.23 (m, 4H), 4.52 (s, 1H), 2.33 (br, 8H) 2.18 (s, 3H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 134.1, 132.9 131.4, 127.2, 126.8, 125.7, 70.3, 55.1, 50.0, 45.7; Anal. Calcd for $C_{18}H_{20}N_2S$: C, 72.93; H, 6.80, N, 9.45; S, 10.82. Found C, 73.02; H, 6.68; N, 9.24.; mp (hexane): 126.0–147.5 °C.

4.2.4. Preparation of 1c and 3c

4-Hydroxy-1-methylpiperidine (0.31 g, 2.7 mmol) was added to a solution of **1f** (0.50 g, 2.4 mmol) and *p*-toluenesulfonic acid (0.20 g, 1.2 mmol) in toluene (40 ml). The mixture was heated at reflux overnight, then cooled to room temperature, and 2 M aqueous sodium hydroxide was added to it. The aqueous layer was extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (ethyl acetate–methanol = 10:1) to afford **1c** (0.53 g, 1.7 mmol, y. 73%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ7.74 (d, *J* = 7.6 Hz, 2H), 7.39 (dt, *J* = 1.2, 7.6 Hz, 2H), 7.29 (dd, *J* = 1.2, 7.6 Hz, 2H), 7.21 (dt, *J* = 1.2, 7.4 Hz, 2H), 4.86 (s, 1H), 3.55 (m, 1H), 2.73 (m, 2H), 2.29 (s, 3H), 2.19 (br, 2H), 1.93 (br, 2H), 1.85 (m, 2H); HRMS (ESI+) Calcd for $C_{21}H_{23}NO$ $[M + H]^+$: 306.1852. Found 306.1848.

3c was similarly prepared from **3f** and 4-hydroxy-1-methylpiperidine. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.52 (dd, *J* = 1.8, 7.8 Hz, 2H), 7.35 (dt, *J* = 1.6, 7.6 Hz, 2H), 7.16 (m, 4H), 5.81 (s, 1H), 3.39 (m, 1H), 2.58 (m, 2H), 2.12 (s, 3H), 1.98 (br, 2H), 1.67 (br, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.3, 130.2, 129.6, 123.2, 121.1, 116.8, 68.7, 53.5, 46.1, 32.5; HRMS (ESI+) Calcd for $C_{19}H_{21}NO_2$ $[M+H]^+$: 296.1645. Found 296.1640.

4.2.5. Preparation of 4c

Sodium borohydride (0.56 mg, 15 mmol) was added to a solution of thioxanthone (0.80 g, 3.8 mmol) in methanol (25 ml) at 0 °C. The mixture was heated at reflux for 1 h, then cooled to room temperature, and quenched with water. The aqueous layer was extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to yield **4f**, which was dissolved in toluene (55 ml). 4-Hydroxy-1-methylpiperidine (0.31 g, 2.7 mmol) and *p*-toluenesulfonic acid (0.27 mg, 1.5 mmol) were added to the resulting solution. The mixture was heated at reflux overnight, then cooled to room temperature, and 2 M aqueous sodium hydroxide was added to it. The aqueous layer was extracted with dichloromethane. The organic layer was dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (ethyl acetate–methanol = 10:1) to afford **4c** (0.19 mg, 0.59 mmol, y. 16%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.60 (dd, *J* = 0.8, 7.2 Hz, 2H), 7.49 (dd, *J* = 1.2, 7.6 Hz, 2H), 7.31 (dt, *J* = 1.6, 7.4 Hz, 2H), 7.24 (dt, *J* = 1.6, 7.8 Hz, 2H), 5.15 (s, 1H), 3.62 (m, 1H), 2.79 (m, 2H), 2.32 (s, 3H), 2.24 (br, 1H), 1.98 (m, 2H), 1.85 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 136.8, 132.7, 127.2, 127.2, 126.6, 126.2, 45.9, 31.3; HRMS (ESI+) Calcd for $C_{19}H_{21}NOS$ $[M+H]^+$: 312.1417. Found 312.1408.

4.3. AlphaLISA assay

An AlphaLISA enzymatic assay was performed as described elsewhere.[44] Briefly, recombinant Set7/9 protein was incubated with a biotinylated histone H3-derived peptide (final concentration 50 nM) and SAM (final concentration 400 nM) in 10 μL of assay buffer (50 mM Tris–HCl [pH 8.8], 0.01% Tween-20, 5 mM $MgCl_2$, 1 mM DTT). After 60 min at room temperature, anti-H3K4me1-2 acceptor beads (final concentration 20 μg/mL) and streptavidin donor beads (final concentration 20 μg/mL) were added and incubated for an additional 30 min at room temperature. Then, the α signal was detected with an EnSpire Alpha plate reader (PerkinElmer, Waltham, MA, USA).

4.4. X-ray crystallographic analysis

Crystallographic data were collected on a Bruker SMART APEX II ULTRA diffractometer attached with a CCD detector and graphite-monochromated MoKα (λ = 0.71073 Å) radiation. Data were corrected for absorption by the multiscan semiempirical method implemented in SADABS[45] and their crystal structures were solved by direct methods SHELXS-97[46] end refined by SHELXTL-2014.[47] Full-matrix least-squares refinement was performed on F^2 for all unique reflections with anisotropic displacement parameters for non-hydrogen atoms. All hydrogen atoms were included as their calculated positions. The crystallographic data have been deposited at the Cambridge Crystallographic Data Centre as CCDC 1484063 for **1a**, CCDC 1484064 for **2a**, CCDC 1484065 for **4a** and CCDC 1484066 for **5a**.

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