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Development of novel inhibitors for histone methyltransferase SET7/9 based on cyproheptadine

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Abstract: The histone methyltransferase SET7/9 methylates not only histone but also non-histone proteins as substrates, and therefore SET7/9 inhibitors are considered candidates for treatment of diseases. Previously, our group identified cyproheptadine, which is used clinically as a serotonin receptor antagonist or histamine receptor (H1) antagonist, as a novel scaffold of SET7/9 inhibitor. In this work, we focused on dibenzosuberene as a substructure of cyproheptadine, and synthesized derivatives with various functional groups. Among them, compound bearing a 2-hydroxy group showed the most potent activity. On the other hand, a 3-hydroxy group or other hydrophilic functional groups such as acetamide decreased the activity. Structural analysis clarified a rationale for the improved potency only by tightly restricted location and type of hydrophilic group. In addition, a SET7/9 loop, which was only partially visible in the complex with cyproheptadine, became more clearly visible in that with 2-hydroxycyproheptadine. These results are expected to be helpful for further structure-based development of SET7/9 inhibitors.

Introduction

Posttranscriptional modifications of histone proteins are well-known regulatory mechanisms of gene transcription. [1-2] Among the enzyme families involved, histone lysine methyltransferases (HKMTs) serve as “writers” of histone protein methylation.[3-6] SET domain-containing lysine methyltransferase 7/9 (SET7/9, also known as SETD7 or KMT7) is a member of the HKMT family, and methylates lysine 4 of histone H3 (H3K4). However, its substrate scope is not limited to histone protein, as it also methylates 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] Thus, SET7/9 plays important roles in various physiological functions and related diseases, and SET7/9 inhibitors are expected to be useful as therapeutic agents.[17-20] Recently, (*R*)-PFI-2 was reported as the first practical SET7/9 inhibitor,[21] followed by a few inhibitors such as DC-S239.[22] We have reported a bisubstrate-type inhibitor, DAAM-3, that consists of the coenzyme derivative AzaAdoMet, in which the bridging sulfur atom of the coenzyme *S*-adenosylmethionine (SAM) is replaced with nitrogen, mimicking the side chain of lysine (a SET7/9 substrate).[23,24] However, structure-activity studies of these inhibitors are quite limited, and no compound has yet been approved for clinical use. We also identified cyproheptadine (Figure 1, **1**), a clinically used serotonin receptor antagonist or histamine receptor (H1) antagonist, as a SET7/9 inhibitor.[25,26] Cyproheptadine (**1**) blocks the ubiquitination of ERα by the methylation of lysine (Lys302), and induces the digestion of ERα, so it could be a candidate therapeutic agent for diseases associated with upregulation of ERα, such as breast cancer. As cyproheptadine is already approved for clinical use, we considered that it would be a promising lead compound for optimization as a SET7/9 inhibitor. Cyproheptadine (**1**) consists of *N*-methylpiperidine and tricyclic dibenzosuberene moieties. Our previous study indicated the importance of the *N*-methyl group on the piperidine ring, and a key role of the characteristic butterfly-like conformation.[27] Replacement of the *N*-methyl group with any other substituent examined resulted in a reduction of the inhibitory activity. X-ray structural analysis confirmed that the space around the *N*-methyl group is quite restricted in the cyproheptadine-bound SET7/9 structure. Nevertheless, the structural analysis revealed that a flexible loop prior to the C-terminal helix interacted with the dibenzosuberene moiety, suggesting potential for optimization of the compound. In the present study, we thus focused on the dibenzosuberene moiety, and introduced various functional groups on the phenyl ring that might potentially interact with proximal amino-acid residues, including those in the associated loop. We present here cyproheptadine derivatives with improved potency, and also describe a structural analysis of one of the derivatives, which provided a rationale for the improved potency.

Results and Discussion

In the co-crystal structure of the SET7/9 complex with cyproheptadine (**1**), amino acid residues around the dibenzosuberene ring were Asn263, Gly264, Tyr335 and Gly336

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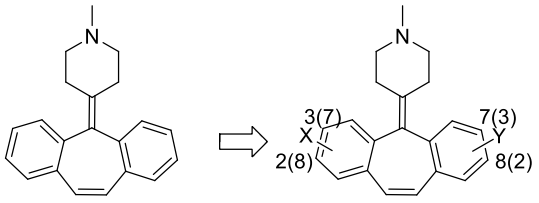
[26], so the introduction of suitable functional groups that could form favorable interactions with these amino acid residues might be good strategy. In addition, as described above, the loop (residues 337 to 349) located prior to the C-terminal helix seemed to be associated at least in part with the dibenzosuberene moiety. Hence, in order to develop more potent inhibitors, we introduced various types of functional groups including a hydrophilic hydroxy group, hydrophobic halogen atom and methoxy group into the dibenzosuberene moiety (Figure 1, **2a** – **2p**).



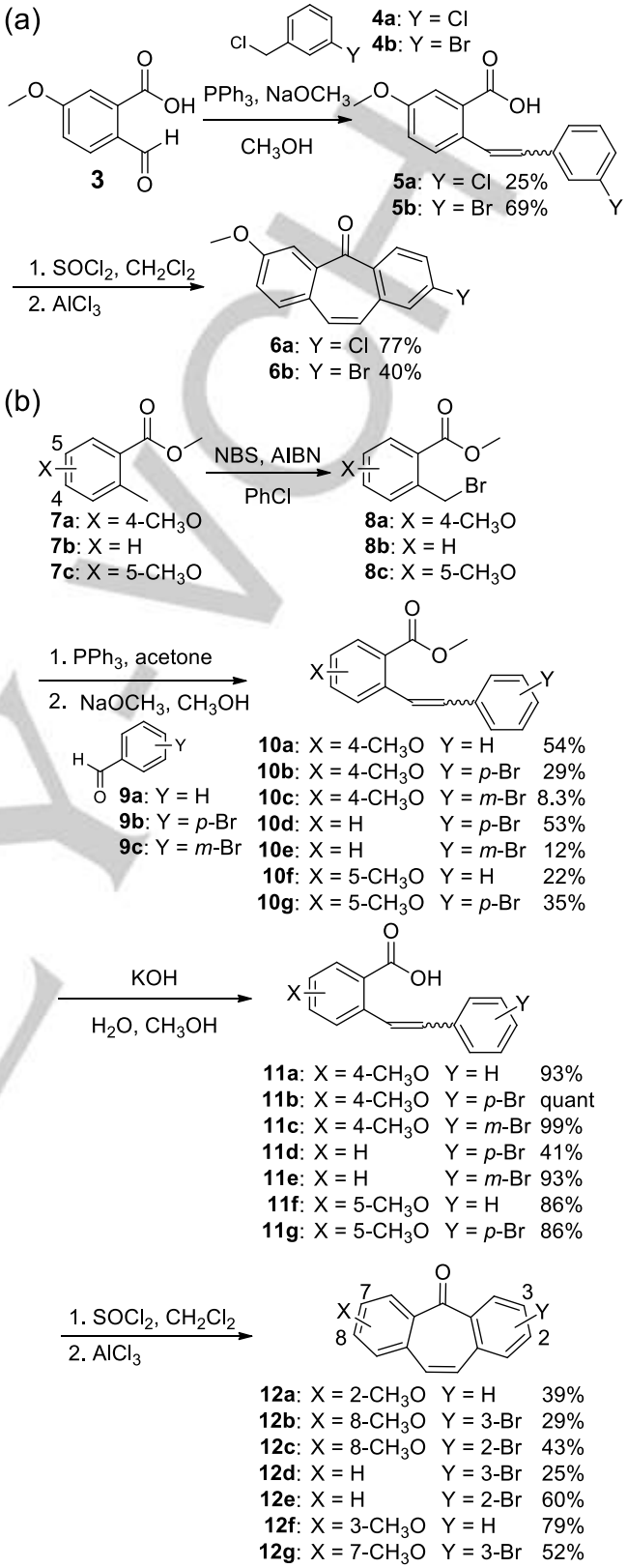
Cyproheptadine (**1**) **2a - 2p**

Comp.	X	Y	Comp.	X	Y
2a	3-CH_3O	H	**2j**	3-OH	H
2b	7-CH_3O	3-Br	**2k**	7-OH	3-Br
2c	7-CH_3O	2-Br	**2l**	7-OH	2-Br
2d	7-CH_3O	2-Cl	**2m**	7-OH	2-Cl
2e	2-CH_3O	H	**2n**	2-OH	H
2f	8-CH_3O	3-Br	**2o**	8-OH	3-Br
2g	8-CH_3O	2-Br	**2p**	8-OH	2-Br
2h	H	3-Br			
2i	H	2-Br			

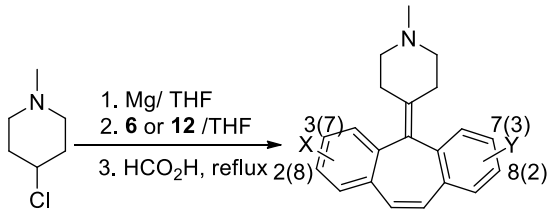
Figure 1. Design of cyproheptadine derivatives as candidate SET7/9 inhibitors.

Cyproheptadine derivatives were synthesized via Grignard reaction between 4-chloro-1-methylpiperidine and dibenzosuberenone derivatives according to the reported method.[28,29] 2-Chloro-7-methoxydibenzosuberenone (**6a**) was prepared by the reported procedure[30] as shown in Scheme 1a. Wittig reaction of 2-formyl-5-methoxybenzoic acid (**3**) with the phosphonium salt of *m*-chlorobenzyl chloride (**4a**) afforded a mixture of *cis*- and *trans* **5a**. Transformation of **5a** into the acid chloride, followed by ring-closing Friedel-Crafts acylation, gave compound **6a**. Compound **6b** bearing a bromo atom was similarly prepared from **3**, though the yield of compound **5** without a halogen atom was relatively low. So, another synthetic scheme was employed, as shown in Scheme 1b. Bromination of 2-methyl-4-methoxybenzoic acid ester (**7a**) with NBS afforded compound **8a**, which reacted with benzaldehyde (**9a**) to yield **10a**. After ester hydrolysis, **11a** was cyclized to **12a**. Other dibenzosuberenone moieties (**12b** – **12g**) were prepared similarly.

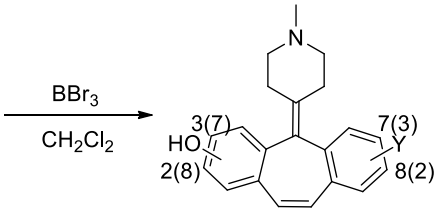
Reaction of the Grignard reagent prepared from 4-chloro-*N*-methylpiperidine with **6** or **12** afforded cyproheptadine derivatives (**2a** – **2i**). Demethylation of compounds **2a** – **2i** yielded the corresponding hydroxy compounds (**2j** – **2p**) (Scheme 2).



Scheme 1. Synthesis of dibenzosuberenones (**6** and **12**)



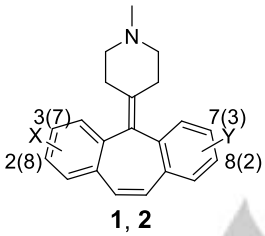
2a:	X = 3-CH_3O	Y = H	69%
2b:	X = 7-CH_3O	Y = 3-Br	31%
2c:	X = 7-CH_3O	Y = 2-Br	54%
2d:	X = 7-CH_3O	Y = 2-Cl	54%
2e:	X = 2-CH_3O	Y = H	60%
2f:	X = 8-CH_3O	Y = 3-Br	39%
2g:	X = 8-CH_3O	Y = 2-Br	54%
2h:	X = H	Y = 3-Br	36%
2i:	X = H	Y = 2-Br	63%



2j:	X = 3-OH	Y = H	92%
2k:	X = 7-OH	Y = 3-Br	51%
2l:	X = 7-OH	Y = 2-Br	47%
2m:	X = 7-OH	Y = 2-Cl	81%
2n:	X = 2-OH	Y = H	34%
2o:	X = 8-OH	Y = 3-Br	26%
2p:	X = 8-OH	Y = 2-Br	86%

Scheme 2. Synthesis of cyproheptadine derivatives **2**

Table 1. Inhibitory activity of **2** towards SET7/9.



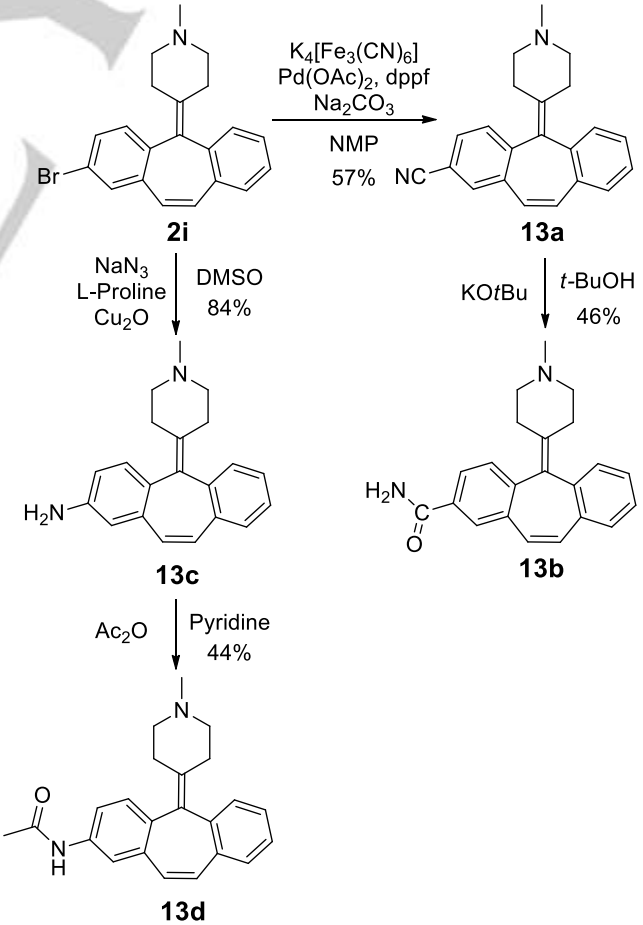
1, **2**

Comp.	X	Y	IC_{50} (μM)[a]
2a	3-CH_3O	H	6.6 ± 0.73
2b	7-CH_3O	3-Br	8.8 ± 0.50
2c	7-CH_3O	2-Br	7.1 ± 0.77
2d	7-CH_3O	2-Cl	7.2 ± 3.0
2e	2-CH_3O	H	2.0 ± 0.27
2f	8-CH_3O	3-Br	7.6 ± 0.33
2g	8-CH_3O	2-Br	4.4 ± 0.11
2h	H	3-Br	9.6 ± 0.68
2i	H	2-Br	6.7 ± 0.66
2j	3-OH	H	7.1 ± 0.58
2k	7-OH	3-Br	10.6 ± 0.51
2l	7-OH	2-Br	27.4 ± 0.97
2m	7-OH	2-Cl	11.0 ± 2.6
2n	2-OH	H	0.41 ± 0.14
2o	8-OH	3-Br	2.1 ± 0.61
2p	8-OH	2-Br	6.8 ± 1.7
1	H	H	3.4 ± 0.37

[a]IC_{50} determination experiments were performed in triplicate, and each standard deviation is also shown.

The SET7/9-inhibitory activities of the synthesized compounds were evaluated by the AlphaLISA method (Table 1).[31]Most compounds showed decreased inhibitory activities, including compounds with a hydrophobic bromo group (**2h** and **2i**). Only the compound with a hydrophilic hydroxy group at the 2-position (**2n**) showed more potent activity: its IC_{50} was 0.41 μM. The enhancement of the inhibitory activity of compound **2n** compared with cyproheptadine was also observed in the immunoblotting for the detection of methylation level of H3K4 (Figure S1). Interestingly, the isomeric compound **2j** with the hydroxy group at the 3-position (IC_{50}: 7.1 μM), and compounds **2o** and **2p** with an additional bromo group on the other phenyl ring showed decreased activities (IC_{50}: 2.1 and 6.8 μM). Most compounds showed only weak inhibitory activity against another HKMT, G9a (Table S1).

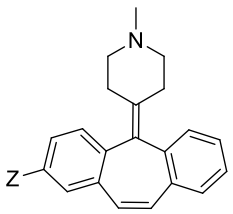
To further explore the effects of hydrophilic groups, we synthesized compounds **13a** - **d** with cyano, aminocarbonyl, amino, acetamido and carboxylate groups, respectively. These compounds were prepared from 2-bromocyproheptadine (**2i**), as shown in Scheme 3. The bromo group of **2i** could be transformed to a cyano group by reaction with potassium ferrocyanide and palladium catalyst[32] to yield **13a**, which was subsequently transformed to aminocarbonyl compound (**13b**). Compound **13c** bearing an amino group was prepared from **2i** by reaction with sodium azide and cuprous oxide,[33] and its acetylated derivative **13d** was also synthesized. In addition, **2i** was shown to be easily transformed to those with various functional groups, which would be useful for further work.



Scheme 3. Synthesis of cyproheptadine derivatives (**13**) with various hydrophilic functional groups.

The inhibitory activities of compounds **13** are summarized in Table 2. Compounds **13b** and **13c** with a 2-aminocarbonyl or 2-amino group showed similar enhancement of inhibitory activity to compound **2n** bearing a 2-hydroxy group, while **13d** with a 2-acetamido group showed weaker activity. So, for the improvement of SET7/9-inhibitory activity, introduction of a suitable hydrophilic group at an appropriate position is necessary

Table 2. Inhibitory activity towards SET7/9.



Comp.	Z	IC_{50} (μM)[a]
1	H	3.4 ± 0.37
2i	Br	6.7 ± 0.66
2n	OH	0.41 ± 0.14
13a	CN	1.6 ± 0.32
13b	$C(O)NH_2$	0.43 ± 0.025
13c	NH_2	0.75 ± 0.13
13d	NHAc	5.1 ± 0.60

[a]IC_{50} determination experiments were performed in triplicate, and each standard deviation is also shown.

In order to clarify the reason for the favorable effect of the 2-hydroxy group of **2n**, we conducted X-ray crystallographic analysis of SET7/9 complexed with a cyproheptadine derivative. We crystallized the ternary complex of SET7/9, a coenzyme analogue Sinefungin, and **2n** by the soaking method, and determined its structure at the resolution of 1.69 Å (Table 3 and Figure 2A). Compound **2n** was clearly observed in the electron density map, and showed a bulge corresponding to the 2-hydroxy group on the benzene ring near the substrate-binding site (Figure 2B). Residues from Tyr337 to Ser345 were visible in the electron density map, although residues 346–348 just before the C-terminal helix could not be refined because the electron density was too low.

Compound **2n** binds to SET7/9 at the same position and with the same orientation as cyproheptadine (Figure 3). As in the SET7/9–SAM–cyproheptadine complex,[26] the *N*-methylpiperidine nitrogen atom of **2n** forms a 2.6 Å hydrogen bond with the carbonyl oxygen atom of Thr266, and the methylpiperidine ring makes hydrophobic contacts with the side chain of Tyr335. Importantly, the hydroxy group of **2n** forms 2.6 Å and 3.0 Å hydrogen bonds with the side-chain oxygen atom and main-chain nitrogen atom of Asp338, respectively, which contributes to the increased affinity for SET7/9 (Figures 2B and 3). In the case of the 3-hydroxy compound **2j**, such an interaction presumably does not exist, because the hydroxy group lies further from the protein and is more exposed to the solvent. The dibenzosuberene moiety is surrounded by hydrophobic residues Pro341, Pro342, Pro350, Trp352 and Tyr353. Interestingly, the side chain of Tyr337, which occupies the cyproheptadine-binding site to form the lysine access channel when bound with a substrate peptide, is completely flipped outwards (Figures 2B and 3). In the SET7/9–SAM–cyproheptadine complex, the electron density of Tyr337 is absent, and only weak electron density of a short stretch in the loop region is visible (Figure 3).[26] On the other hand, the interactions between compound **2n** and Asp338 are likely to restrain the conformation of the flexible loop, thereby making Tyr337 and the subsequent residues clearly visible in the electron density map. Addition of bromine to the other side of the benzene ring of **2n** (i.e. **2o** and **2p**) decreased the inhibitory activity (Table 1). The bromine atom would be close to the carbonyl group of Trp260 in **2o** or to the side chain of Trp260 in **2p**. In either case, the interaction seems to be unfavorable for inhibitor binding.

Table 3. Crystallographic data collection and refinement statistics

	SET7/9–Sinefungin–**2n**
Data Collection	
Space group	$P2_12_12$
Unit-cell dimensions (Å)	a = 110.18, b = 34.96, c = 67.87
Resolution range (Å)	50 – 1.69 (1.72 – 1.69) [a]
R_{sym}	0.107 (1.284)
R_{meas}	0.117 (1.425)
R_{pim}	0.046 (0.604)
$CC_{1/2}$	0.997 (0.467)
$I/\sigma(I)$	22.6 (1.9)
Completeness (%)	99.9 (99.3)
Redundancy	6.3 (5.4)
Refinement	
Resolution range (Å)	42.77 – 1.69
No. of reflections (total)	30193
No. of reflections (test set)	1762
R_{work}/R_{free}	0.168/0.200
No. of atoms, Average *B*-factors (Å^2)	
Total	2217, 30.7
Protein	1952, 30.1
Coenzyme (Sinefungin)	27, 17.9
Inhibitor (**2n**)	23, 24.3
Glycerol	18, 55.8
Water	197, 37.3
R.m.s. deviations	
Bond lengths (Å)	0.006
Bond angles (°)	0.89
Ramachandran plot (%)	
Favored	95.5
Allowed	4.5
Outliers	0

[a] Values in parentheses are for the highest resolution shell.

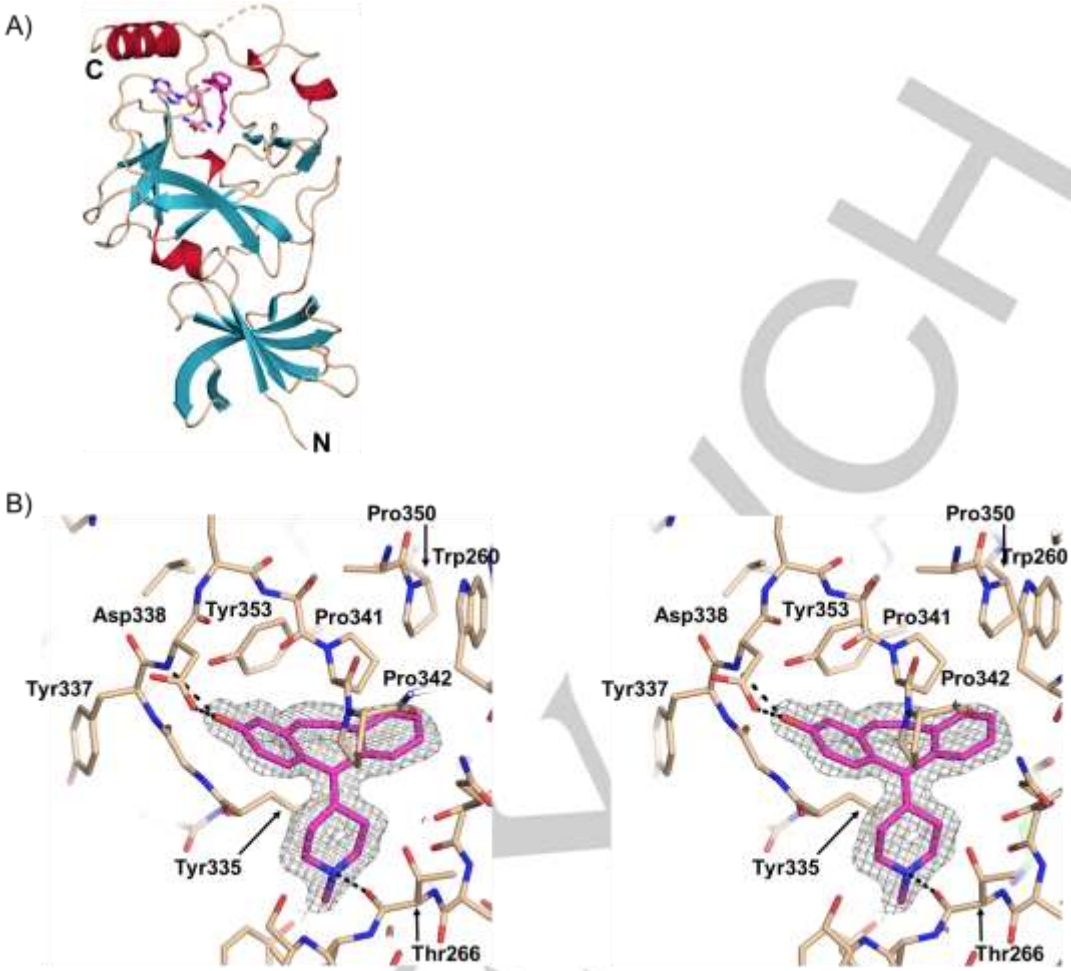


Figure 2. X-ray crystallographic analysis of the compound **2n**-bound SET7/9 SET domain. A) Ribbon representation of the overall structure. *α*-Helices, *β*-strands and the loop regions are shown in red, cyan and pale orange, respectively. Compound **2n** (magenta) and Sinfungin (pink) are shown in stick models. B) Compound **2n** (magenta) and surrounding residues represented by a stick model are shown in stereo. Hydrogen bonds are indicated by dashed lines. A simulated annealing 2*mF*o-*DF*c omit map for the compound contoured at 1.2 σ is shown in gray mesh.

We also tried co-crystallization of **13b** and **13c** with SET7/9–Sinefungin, as well as the soaking method, but without success. Simple substitution of the hydroxy group of **2n** with an aminocarbonyl group would cause a steric clash with the side chain of Asp338. Therefore, the flexible loop might form a different conformation to accommodate these compounds (i.e. **13b** and **13c**) for submicromolar inhibitory activity (IC_{50}) comparable to **2n**, which might alter the crystal packing.

Superimposition of the structures of SET7/9–Sinefungin bound with compound **2n** and SET7/9–SAM bound with the reported inhibitor (*R*)-PFI-2[21] is shown in Figure 4. The both inhibitors block the binding tunnel for the side chain of the methylation target lysine of a substrate protein between the peptide-binding site and the coenzyme. The piperidine ring of compound **2n** and the pyrrolidine ring of (*R*)-PFI-2 occupy a similar position, and the side chain of Tyr335 has hydrophobic contacts with both rings. However, their binding modes are different in several points. First, compound **2n** occupies only the binding site of the substrate lysine side chain, whereas (*R*)-PFI-2 additionally blocks the site of the residues at the (-1) and (-2) positions from the target lysine. Second, although both inhibitors interact with and stabilize the flexible C-terminal loop, its conformations in the two complexes largely differ. In particular, Tyr337 in compound **2n**-bound SET7/9 is flipped out as described above, whereas Tyr337 in (*R*)-PFI-2-bound SET7/9 does not change its conformation. It should be noted that although the compound **2n** itself does not occupy the main chain-binding site of the substrate protein, residues at Pro342, Gly343 and Lys344 in the C-terminal loop occupy the binding groove for the side chain of the residue at the (-2) position from the target lysine. Third, hydrophilic interactions formed by the two inhibitors are distinct from each other and there are no common hydrophilic interactions between the two complexes (Figure 4). Thus, the binding mode of compound **2n** toward SET7/9 is different from that of (*R*)-PFI-2, confirming its unique scaffold as a SET7/9 inhibitor.

Our results suggest that introduction of a hydrophilic functional group of an appropriate type at an appropriate position in cyproheptadine (**1**) is necessary to increase the inhibitory potency. Similar tight restriction was also observed in our previous report,[27] where a change of molecular shape due to change of the linking group between the tricyclic dibenzosuberene moiety and *N*-methylpiperidine or replacement of the 10,11-olefinic bond of dibenzosuberene, or even a small change due to reduction of 10,11-olefinic bond, caused a drastic decrease of the inhibitory activity.

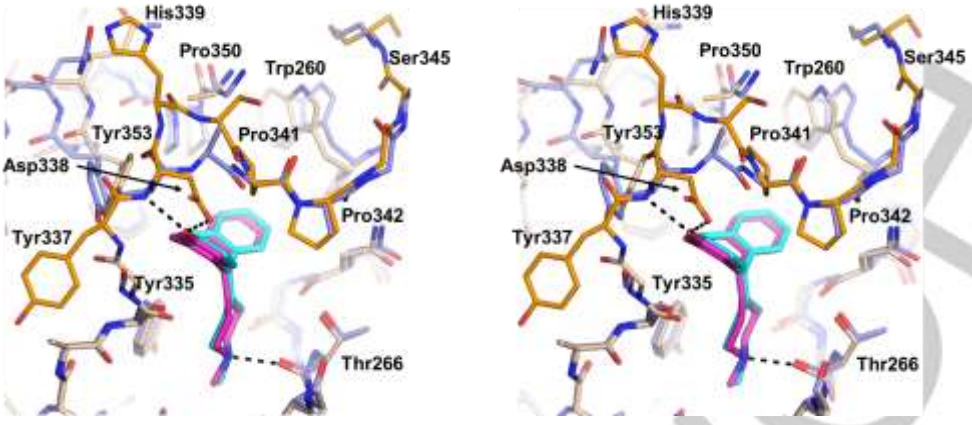


Figure 3. Stereoview of the superimposition of SET7/9–Sinefungin–compound **2n** and SET7/9–SAM–cyproheptadine. SET7/9 in the complex with compound **2n** is shown in pale orange, and the residues from Tyr337 to Ser345, which became clearly visible in this structure, are colored orange. Compound **2n** is shown in magenta. SET7/9 in the complex with cyproheptadine is shown in pale blue color, and cyproheptadine is shown in cyan.

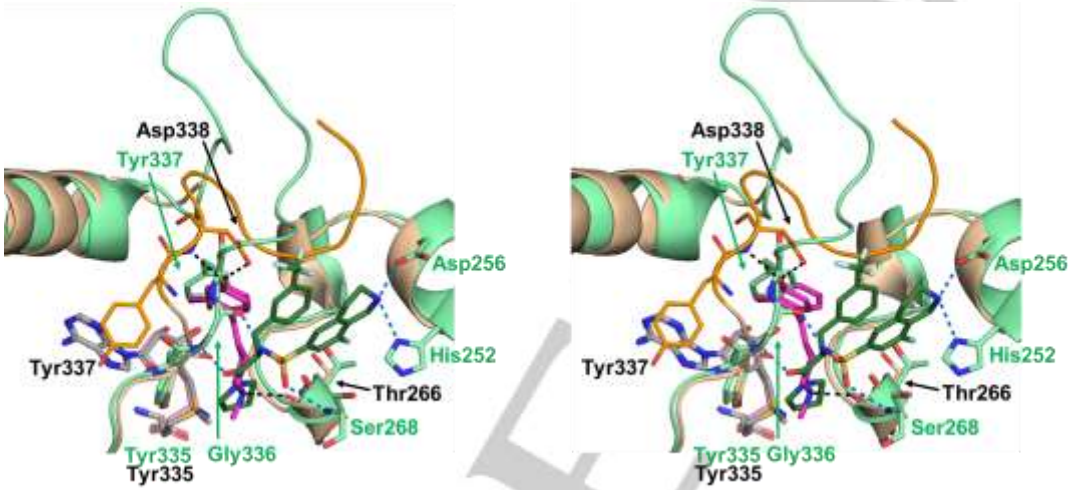


Figure 4. Stereoview of the superimposition of SET7/9–Sinefungin–compound **2n** and SET7/9-SAM-(*R*)-PFI-2. The former complex is depicted as follows: SET7/9 (orange), Sinefungin (light pink), compound **2n** (magenta), key residue labels (black), and hydrogen bonds (dashed black lines). The latter complex is depicted as follows: SET7/9 (light green), SAM (gray), (*R*)-PFI-2 (dark green), key residues labels (green), and hydrogen bonds (dashed green lines).

Conclusions

We have developed a potent SET7/9 inhibitor by structural development of our previously obtained inhibitor, cyproheptadine, with the aid of a fine co-crystal structure. The presence of a hydrophilic group on the dibenzosuberene moiety is important, but its permissible size and location are tightly restricted. To date, only a few inhibtors such as (*R*)-PFI-2 have been repored, and the study of structure and activity relationship seems still immature. Our newly developed inhibitor **2n** and the structural insights should be helpful for developing superior SET7/9 inhibitors, which might be candidate agents for treatment of diseases such as breast cancer.

Experimental Section

Chemistry

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 Brucker 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.

Preparation of 6: *m*-Chlorobenzyl chloride (**4a**, 1.3 g, 8.4 mmol) was added to a solution of triphenylphosphine (2.2 g, 8.4 mmol) in methanol (10 ml), and the reaction mixture was heated under reflux for 2 h under argon. The reaction mixture was cooled to 0°C, then **3** (1.0 g, 5.6 mmol) was added, followed by a solution of sodium methoxide (0.75 g, 14 mmol) in methanol (15 ml). The reaction mixture was stirred overnight, and poured into ice water. The suspension was washed with dichloromethane, acidified with aqueous hydrochloric acid, and then extracted with dichloromethane. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 5) to afford a mixture of *cis*- and *trans*-**5a** (4 : 1, 0.40 g, 1.4 mmol, y. 25%) as white powder, which was used in the next reaction without further purification. The obtained mixture was dissolved in dichloromethane (7 ml), and a solution of thionyl chloride (1.1 ml) in dichloromethane (3 ml) was slowly added to the solution. The reaction mixture was heated under reflux for 1.5 h. Then, a suspension of aluminium chloride (0.22 g) in dichloromethane (10 ml) was added, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was poured into ice water, and the aqueous phase was extracted with dichloromethane. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 20), and **6a** (0.29 g, 1.0 mmol, y. 77%) was obtained as a brown powder. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.21 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 3.2 Hz, 1H), 7.52-7.46 (m, 3H), 7.22 (dd, *J* = 2.8, 8.4 Hz, 1H), 7.06 (d, *J* = 12.0 Hz, 1H), 6.95 (d, *J* = 12.0 Hz, 1H), 3.94 (s, 3H).

6b was similarly prepared from *m*-bromobenzyl chloride (**4b**) and compound **3**. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.13 (d, *J* = 8.8 Hz, 1H), 7.77 (d, *J* = 2.8 Hz, 1H), 7.70 (d, *J* = 2.0 Hz, 1H), 7.63 (dd, *J* = 2.0, 8.8 Hz, 1H), 7.50 (d, *J* = 8.4 Hz,1H), 7.23 (dd, *J* = 2.8, 8.4 Hz, 1H), 7.07 (d, *J* = 12.0 Hz, 1H), 6.85 (d, *J* = 12.0 Hz, 1H), 3.95 (s, 3H).

Preparation of 10: The reaction mixture of **7a** (3.8 g, 21 mmol) and *N*-bromosuccinimide (4.1 g, 23 mmol) in chlorobenzene (30 ml) was heated to 70°C, and a solution of 2,2'-azobis(isobutyronitrile) (0.13 g, 0.80 mmol) in chlorobenzene (10 ml) was added. This mixture was stirred for 1 h at 70°C, then cooled to room temperature, and the solvent was removed in vacuo. The residue containing compound **8a** was used for the next step without purification. It was added to a solution of triphenylphosphine (5.5 g, 21 mmol) in acetone (25 ml), and the reaction mixture was refluxed for 1 h. It was cooled to room temperature, and the precipitate was collected. This precipitate and sodium methoxide (1.3 g, 25 mmol) were dissolved in methanol (30 ml), and the solution was stirred for 30 min at room temperature, and then heated under reflux. Benzaldehyde (**9a**, 1.1 g, 11 mmol) was added, and stirring was continued for 6 h under reflux. The reaction mixture was poured into ice water, and the suspension was washed with dichloromethane, acidified with aqueous hydrochloric acid, and extracted with dichloromethane. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 15), affording a mixture (2 : 1) of *cis*- and *trans*-**10a** (1.6 g, 5.8 mmol, 54%) as colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09 (d, *J* = 16.0 Hz, trans-1H), 7.98 (d, *J* = 9.0 Hz, cis-1H), 7.96 (d, *J* = 9.0 Hz, trans-1H), 7.56 (d, *J* = 7.5 Hz, trans-2H), 7.36 (t, *J* = 7.5 Hz, trans-2H), 7.27 (t, *J* = 7.5 Hz, trans-1H), 7.18-7.07 (m, cis-6H + trans-1H), 6.98 (d, *J* = 16.0 Hz, trans-1H), 6.82 (dd, *J* = 2.5, 8.5 Hz, trans-1H), 6.79 (dd, *J* = 2.5, 8.5 Hz, cis-1H), 6.68 (d, *J* = 2.5 Hz, cis-1H), 6.65 (d, *J* = 12 Hz, cis-1H), 3.91 (s, trans-3H), 3.89 (s, trans-3H), 3.57 (s, cis-3H).

10b – **10g** were similarly prepared from corresponding compounds **7**, **8** and **9**. Those analytical data were given below.

10b (obtained as a mixture (5 : 2) of *cis*- and *trans*-compounds): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.08 (d, *J* = 16.4 Hz, trans-1H), 8.00 (d, *J* = 8.8 Hz, cis-1H), 7.97 (d, *J* = 8.4 Hz, trans-1H), 7.48 (d, *J* = 8.8 Hz, trans-2H), 7.42 (d, *J* = 8.8 Hz, trans-2H), 7.27 (d, *J* = 8.4 Hz, cis-2H), 7.15 (d, *J* = 2.4 Hz, trans-1H), 7.10 (d, *J* = 12.0 Hz, cis-1H), 6.94 (d, *J* = 8.4 Hz, cis-2H), 6.85 (d, *J* = 16.4 Hz, trans-1H), 6.83 (dd, *J* = 2.4, 8.8 Hz, trans-1H), 6.81 (dd, *J* = 2.4, 8.8 Hz, cis-1H), 6.64 (d, *J* = 2.4 Hz, cis-1H), 6.56 (d, *J* = 12.0 Hz, cis-1H), 3.90 (s, trans-3H), 3.89 (s, trans-3H), 3.86 (s, cis-3H), 3.63 (s, cis-3H).

10c (obtained as a mixture (2 : 1) of *cis*- and *trans*-compounds): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.11 (d, *J* = 16.5 Hz, trans-1H), 8.03 (d, *J* = 8.5 Hz, cis-1H), 8.00 (d, *J* = 9.0 Hz, trans-1H), 7.71-7.70 (m, trans-1H), 7.50 (brd, *J* = 7.5 Hz, trans-1H), 7.43-7.40 (m, trans-1H), 7.27-7.23 (m, cis-2H + trans-1H), 7.18-7.16 (m, trans-1H), 7.15 (d, *J* = 12.5 Hz, cis-1H), 7.04-6.98 (m, cis-2H), 6.92-6.83 (m, trans-2H), 6.84 (dd, *J* = 2.5, 9.0 Hz, cis-1H), 6.68 (d, *J* = 2.5 Hz, cis-1H), 6.59 (d, *J* = 12.5 Hz, cis-1H), 3.93 (s, trans-3H), 3.92 (s, trans-3H), 3.89 (s, cis-3H), 3.65 (s, cis-3H).

10e (obtained as a *cis*-compound): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.01 (m, 1H), 7.34-7.32 (m, 2H), 7.25-7.13 (m, 3H), 7.11 (d, *J* = 12.0 Hz, 1H), 6.99-6.92 (m, 2H), 6.57 (d, *J* =12.0 Hz, 1H), 3.90 (s, 3H).

10f (obtained as a *cis*-compound): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.50 (d, *J* = 2.5 Hz, 1H), 7.17-7.07 (m, 6H), 6.98 (d, *J* = 12.0 Hz, 1H), 6.85 (dd, *J* = 2.5, 8.5 Hz, 1H), 6.59 (d, *J* = 12.0 Hz, 1H), 3.89 (s, 3H), 3.84 (s, 3H).

10g (obtained as a mixture (2 : 1) of *cis*- and *trans*-compounds): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.91 (d, *J* = 16.5 Hz, trans-1H), 7.64 (d, *J* = 8.5 Hz, trans-1H), 7.51 (d, *J* = 3.0 Hz, cis-1H), 7.46 (d, *J* = 8.5 Hz, trans-2H), 7.44 (d, *J* = 2.5 Hz, trans-1H), 7.38 (d, *J* = 8.5 Hz, trans-2H), 7.26 (d, *J* = 8.5 Hz, cis-2H), 7.08 (d, *J* = 8.5 Hz, cis-1H), 7.08-7.05 (m, trans-1H), 7.02 (d, *J* = 12.0 Hz, cis-1H), 6.94 (d, *J* = 8.5 Hz, cis-2H), 6.84 (dd, *J* = 3.0, 8.5 Hz, cis-1H), 6.84 (d, *J* = 16.5 Hz, trans-1H), 6.51 (d, *J* = 12.0 Hz, cis-1H), 3.93 (s, trans-3H), 3.89 (s, cis-3H), 3.86 (s, trans-3H), 3.84 (s, cis-3H).

Preparation of 11: A mixture of **10a** (1.26 g, 4.7 mmol) and potassium hydroxide (0.53 g, 9.4 mmol) in methanol (20 ml) and water (0.8 ml) was heated under reflux for 4h, and then cooled to room temperature. The reaction mixture was poured into ice water, acidified with aqueous hydrochloric acid, and extracted with ethyl acetate. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo to afford a mixture (2 : 1) of *cis*- and trans-**11a** (1.1 g, 4.4 mmol, 93%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.14-8.08 (m, cis-1H + trans-2H), 7.56 (d, *J* = 7.2 Hz, trans-2H), 7.38 (t, *J* = 7.2 Hz, trans-2H), 7.28 (t, *J* = 7.2 Hz, trans-1H), 7.19-7.09 (m, cis-6H + trans-1H), 7.00 (d, *J* = 16.0 Hz, trans-1H), 6.87 (dd, *J* = 2.4, 8.8 Hz, trans-1H), 6.82 (dd, *J* = 2.4, 8.4 Hz, cis-1H), 6.70-6.67 (m, cis-2H), 3.93 (s, trans-3H), 3.57 (s, cis-3H).

11b – **11g** were similarly prepared from corresponding compounds **10**. Those analytical data were given below.

11b (obtained as a mixture (5 : 2) of *cis*- and *trans*-compounds): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13-8.08 (m, cis-1H + trans-2H), 7.49 (d, *J* = 8.8 Hz, trans-2H), 7.42 (d, *J* = 8.8 Hz, trans-2H), 7.28 (d, *J* = 8.4 Hz, cis-2H), 7.16 (d, *J* = 2.4 Hz, trans-1H), 7.13 (d, *J* = 12.0 Hz, cis-1H), 6.95 (d, *J* = 8.4 Hz, cis-2H), 6.91 (d, *J* = 15.6 Hz, trans-1H), 6.88 (dd, *J* = 2.4, 8.8 Hz, trans-1H), 6.84 (dd, *J* = 2.8, 8.8 Hz, cis-1H), 6.66 (d, *J* = 2.8 Hz, cis-1H), 6.58 (d, *J* = 12.0 Hz, cis-1H), 3.91 (s, trans-3H), 3.64 (s, cis-3H).

11c (obtained as a mixture (2 : 1) of *cis*- and *trans*-compounds): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.13-8.08 (m, cis-1H + trans-2H), 7.71-7.70 (m, trans-1H), 7.57-7.52 (m, trans-1H), 7.42-7.39 (m, trans-1H), 7.27-7.22 (m, cis-2H + trans-1H), 7.16 (d, *J* = 12.0 Hz, cis-1H), 7.16-7.14 (m, trans-1H), 7.02-6.97 (m, cis-2H), 6.92-6.87 (m, trans-2H), 6.85 (dd, *J* = 3.0, 8.5 Hz, cis-1H), 6.67 (d, *J* = 3.0 Hz, cis-1H), 6.58 (d, *J* = 12.0 Hz, cis-1H), 3.92 (s, trans-3H), 3.63 (s, cis-3H).

11e (obtained as a *cis*-compound): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.13-8.11 (m, 1H), 7.38-7.34 (m, 2H), 7.24-7.18 (m, 3H), 7.14 (d, *J* = 12.0 Hz, 1H).7.01-6.94 (m, 2H), 6.59 (d, *J* = 12.0 Hz, 1H).

11f (obtained as a *cis*-compound): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.64 (d, *J* = 2.5 Hz, 1H), 7.19-7.08 (m, 6H), 7.04 (d, *J* = 12.0 Hz, 1H), 6.90 (dd, *J* = 2.5, 9.0 Hz, 1H), 6.62 (d, *J* = 12.0 Hz, 1H), 3.85 (s, 3H).

11g (obtained as a mixture (2 : 1) of *cis*- and *trans*-compounds): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.98 (d, *J* = 16.5 Hz, trans-1H), 7.66 (d, *J* = 8.5 Hz, trans-1H), 7.64 (d, *J* = 3.0 Hz, cis-1H), 7.59 (d, *J* = 2.5 Hz, trans-1H), 7.47 (d, *J* = 8.5 Hz, trans-2H), 7.39 (d, *J* = 8.5 Hz, trans-2H), 7.27 (d, *J* = 8.5 Hz, cis-2H), 7.14-7.11 (m, trans-1H), 7.12 (d, *J* = 8.5 Hz, cis-1H), 7.07 (d, *J* = 12.0 Hz, cis-1H), 6.95 (d, *J* = 8.5 Hz, cis-2H), 6.92 (dd, *J* = 3.0, 8.5 Hz, cis-1H), 6.86 (d, *J* = 16.5 Hz, trans-1H), 6.54 (d, *J* = 12.0 Hz, cis-1H), 3.88 (s, trans-3H), 3.86 (s, cis-3H).

Preparation of 12: **11a** (0.25 g, 0.98 mmol) was dissolved in dichloromethane (20 ml), and thionyl chloride (0.13 g, 1.1 mmol) was slowly added to the solution. The reaction mixture was heated under reflux for 1.5 h, then aluminium chloride (0.17 g) was added to it, and stirring was continued overnight at room temperature. The reaction mixture was poured into ice water, and the aqueous phase was extracted with dichloromethane. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate : *n*-hexane = 1 : 3) to afford **12a** (91 mg, 0.38 mmol, y. 39%) as a brown powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.32-8.28 (m, 2H), 7.67-7.63 (m, 1H), 7.59-7.55 (m, 2H), 7.12 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.07 (d, *J* = 12.5 Hz, 1H), 7.01 (d, *J* = 12.5 Hz, 1H), 6.99 (d, *J* = 2.0 Hz, 1H), 3.95 (s, 3H).

12b – **12g** were similarly prepared from corresponding compounds **11**. Those analytical data are given below.

12b: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.43 (d, *J* = 2.0 Hz, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 7.68 (dd, *J* = 2.0, 8.4 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.11 (dd, *J* = 2.8, 8.8 Hz, 1H), 7.02-6.95 (m, 3H), 3.93 (s, 3H).

12c: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.26 (d, *J* = 8.5 Hz, 1H), 8.16 (d, *J* = 8.5 Hz, 1H), 7.68 (d, *J* = 2.0 Hz, 1H), 7.64 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.11 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.02 (d, *J* = 12.0 Hz, 1H), 6.96 (d, *J* = 2.5 Hz, 1H), 6.92 (d, *J* = 12.0 Hz, 1H), 3.92 (s, 3H).

12d (obtained from : ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.37 (d, *J* = 2.4 Hz, 1H), 8.21 (d, *J* = 8.0 Hz, 1H), 7.72 (dd, *J* = 2.4, 8.4 Hz, 1H), 7.68-7.63 (m, 1H), 7.59-7.54 (m, 2H), 7.41 (d, *J* = 8.4 Hz, 1H), 7.09 (d, *J* = 12.0 Hz, 1H), 7.00 (d, *J* = 12.0 Hz, 1H).

12e: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.23-8.20 (m, 1H), 8.08 (d, *J* = 8.8 Hz, 1H), 7.70 (d, *J* = 2.0 Hz, 1H), 7.68-7.63 (m, 2H), 7.59-7.53 (m, 2H), 7.10 (d, *J* = 12.0 Hz, 1H), 6.95 (d, *J* = 12.0 Hz, 1H).

12f: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.27-8.25 (m, 1H), 7.77 (d, *J* = 2.8 Hz, 1H), 7.65-7.61 (m, 1H), 7.55-7.50 (m, 2H), 7.49 (d, *J* = 8.8 Hz, 1H), 7.21 (dd, *J* = 2.8, 8.8 Hz, 1H), 7.04 (d, *J* = 12.0 Hz, 1H), 6.97 (d, *J* = 12.0 Hz, 1H), 3.94 (s, 3H).

12g: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.40 (d, *J* = 2.0 Hz, 1H), 7.75 (d, *J* = 2.5 Hz, 1H), 7.72 (dd, *J* = 2.0, 8.5 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 7.23 (dd, *J* = 2.5, 8.5 Hz, 1H), 7.06 (d, *J* = 12.0 Hz, 1H), 6.91 (d, *J* = 12.0 Hz, 1H), 3.95 (s, 3H).

Preparation of 2: Magnesium (46 mg, 1.9 mmol) and iodine (2 pieces) were added to a two-necked flask. Under an argon atmosphere, dry tetrahydrofuran (8 ml), dibromoethane (2 drops) and 4-chloro-1-methylpiperidine (0.25 g, 1.9 mmol) were added to the flask. The mixture was heated under reflux with stirring for 1.5 h, then cooled to room temperature, and a solution of **12f** (0.15 g, 0.63 mmol) in dry tetrahydrofuran (8 ml) was added to it. The reaction mixture was stirred overnight at room temperature. After the addition of saturated aqueous ammonium chloride, the aqueous phase was extracted with ethyl acetate. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was dissolved in 6 M hydrochloric acid (20 ml), and the mixture was stirred under reflux for 3 h. After the addition of aqueous sodium hydroxide, the aqueous phase was extracted with ethyl acetate. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (dichloromethane : methanol = 8 : 1) to afford **2a** (0.14 mg, 0.44 mmol, y. 69%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33-7.29 (m, 2H), 7.25-7.23 (m, 2H), 7.18 (brd, *J* = 7.5 Hz, 1H), 6.86 (d, *J* = 12.0 Hz, 1H), 6.81 (d, *J* = 12.0 Hz, 1H), 6.80 (dd, *J* = 2.5, 8.5 Hz, 1H), 6.74 (d, *J* = 2.5 Hz, 1H), 3.81 (s, 3H), 2.60-2.49 (m, 2H), 2.41-2.31 (m, 2H), 2.24 (s, 3H), 2.25-2.05 (m, 4H); ^{13}CNMR ($CDCl_3$, 125 MHz) δ 159.7, 140.4, 138.4, 135.1, 135.0, 133.5, 130.5, 129.5, 129.1, 128.5, 128.1, 128.0, 127.5, 126.3, 113.2, 112.4, 57.2, 55.4, 46.0, 30.2, 30.0; HRMS (ESI+) Calcd. for $C_{22}H_{24}NO$ $[M+H]^+$: 318.1852. Found 318.1853.

2b – **2i** were similarly prepared from corresponding compounds **6** and **12**. Those analytical data are given below.

2b: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35-7.33 (m, 2H), 7.23 (d, *J* = 8.5 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H), 6.87 (d, *J* = 11.5 Hz, 1H), 6.81 (dd, *J* = 3.0, 8.5 Hz, 1H), 6.72 (d, *J* = 11.5 Hz, 1H), 6.71 (d, *J* = 3.0 Hz, 1H), 3.82 (s, 3H), 2.62-2.55 (m, 2H), 2.42-2.34 (m, 2H), 2.26 (s, 3H), 2.26-2.17 (m, 2H), 2.11-2.06 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.0, 140.04, 140.01, 136.0, 134.2, 132.5, 131.3, 131.1, 129.8, 129.7, 129.5, 128.0, 121.7, 113.3, 112.9, 57.1, 55.6, 46.0, 30.2, 30.1; HRMS (ESI+) Calcd. for $C_{22}H_{23}BrNO$ $[M + H]^+$: 396.0958. Found 396.0958.

2c: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.44-7.40 (m, 2H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 1H), 6.88 (d, *J* = 11.6 Hz, 1H), 6.81 (dd, *J* = 2.4, 8.4 Hz, 1H), 6.71 (d, *J* = 2.4 Hz, 1H), 6.70 (d, *J* = 11.6 Hz, 1H), 3.81 (s, 3H), 2.54-2.51 (m, 2H), 2.40-2.28 (m, 2H), 2.25 (s, 3H), 2.25-2.12 (m, 2H), 2.10-2.03 (m, 2H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ159.9, 140.2, 137.1, 137.0, 135.7, 132.5, 131.9, 130.6, 130.3, 130.1, 129.8, 127.7, 127.6, 120.0, 113.3, 112.5, 57.0, 55.4, 45.9, 30.1, 30.0; HRMS (ESI+) Calcd. for $C_{22}H_{23}BrNO$ $[M + H]^+$: 396.0958. Found 396.0956.

2d: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.25-7.21 (m, 3H), 7.08 (d, *J* = 8.8 Hz, 1H), 6.87 (d, *J* = 11.6 Hz, 1H), 6.80 (dd, *J* = 2.4, 8.8 Hz, 1H), 6.70 (d, *J* = 2.4 Hz, 1H), 6.68 (d, *J* = 11.6 Hz, 1H), 3.80 (s, 3H), 2.55-2.52 (m, 2H), 2.39-2.31 (m, 2H), 2.24-2.15 (m, 2H), 2.24 (s, 3H), 2.13-2.04 (m, 2H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ159.9, 140.3, 136.7, 136.6, 135.9, 132.4, 132.0, 131.8, 129.8, 129.8, 127.8, 127.7, 127.7, 127.4, 113.3, 112.5, 57.0, 55.4, 45.9, 30.2, 30.0; HRMS (ESI+) Calcd. for $C_{22}H_{23}ClNO$ $[M + H]^+$: 352.1463. Found 352.1454.

2e: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33-7.29 (m, 2H), 7.24-7.17 (m, 2H), 7.10 (d, *J* = 8.4 Hz, 1H), 6.92 (d, *J* = 12.0 Hz, 1H), 6.89 (dd, *J* = 2.4, 8.4 Hz, 1H), 6.85 (d, *J* = 12.0 Hz, 1H), 6.82 (d, *J* = 2.4 Hz, 1H), 2.53-2.49 (m, 2H), 2.38-2.32 (m, 2H), 2.23 (s, 3H), 2.22-2.15 (m, 2H), 2.11-2.05 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.1, 139.7, 136.0, 135.2, 134.8, 133.1, 132.5, 131.6, 131.0, 129.8, 128.6, 128.4, 127.9, 126.3, 114.4, 112.5, 57.4, 55.5, 46.2, 30.32, 30.28; HRMS (ESI+) Calcd for $C_{22}H_{24}NO$ $[M + H]^+$: 318.1852. Found 318.1849.

2f: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.35-7.32 (m, 2H), 7.16 (d, *J* =8.5 Hz, 1H), 7.09 (d, *J* = 8.0 Hz, 1H), 6.91 (dd, *J* = 2.5, 8.5 Hz, 1H), 6.87 (d, *J* = 11.5 Hz, 1H), 6.83 (d, *J* = 11.5 Hz, 1H), 6.81 (d, *J* = 2.5 Hz, 1H), 3.80 (s, 3H), 2.57-2.50 (m, 2H), 2.39-2.30 (m, 2H), 2.24 (s, 3H), 2.21-2.16 (m, 2H), 2.10-2.06 (m, 2H); ^{13} C NMR ($CDCl_3$, 125 MHz) δ 158.1, 141.0, 136.1, 135.7, 133.7, 131.7, 131.6, 131.5, 130.9, 130.2, 129.7, 129.1, 121.9, 114.4, 112.5, 57.11, 57.08, 55.3, 46.0, 30.2, 30.1; HRMS (ESI+) Calcd. for $C_{22}H_{23}BrNO$ $[M + H]^+$: 396.0958. Found 396.0957.

2g: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.44 (d, *J* = 2.0 Hz, 1H), 7.42 (dd, *J* = 2.0, 8.4 Hz, 1H), 7.08 (d, *J* = 8.4 Hz, 1H), 7.04 (d, *J* = 8.0 Hz, 1H), 6.91 (dd, *J* = 2.8, 8.4 Hz, 1H), 6.89 (d, *J* = 11.6 Hz, 1H), 6.82 (d, *J* = 2.8 Hz, 1H), 6.81 (d, *J* = 11.6 Hz, 1H), 3.80 (s, 3H), 2.61-2.56 (m, 2H), 2.41-2.34 (m, 2H), 2.26 (s, 3H), 2.28-2.08 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.1, 138.2, 136.6, 135.5, 132.4, 132.3, 131.9, 131.0, 130.8, 130.6, 130.0, 129.9, 129.6, 119.9, 114.6, 112.6, 56.9, 55.3, 45.8, 29.8, 29.7; HRMS (ESI+) Calcd. for $C_{22}H_{23}BrNO$ $[M + H]^+$: 396.0958. Found 396.0948

2h: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36-7.30 (m, 4H), 7.26 (d, *J* = 1.6, 7.2 Hz, 1H), 7.18 (dd, *J* = 1.6, 7.2 Hz, 1H), 7.17 (d, *J* = 8.8 Hz, 1H), 6.94 (d, J = 11.6 Hz, 1H), 6.84 (d, *J* = 11.6 Hz, 1H), 2.56-2.50 (m, 2H), 2.41-2.30 (m, 2H), 2.24 (s, 3H), 2.22-2.05 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 140.6, 138.6, 134.6, 133.7, 132.1, 131.6, 130.9, 129.8, 129.6, 129.2, 128.5, 128.2, 128.0, 126.5, 121.8, 57.00, 56.98, 45.9, 30.0; HRMS (ESI+) Calcd. for $C_{21}H_{21}BrN$ $[M + H]^+$: 366.0852. Found 366.0859.

2i: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45 (br, 1H), 7.42 (d, *J* = 2.0, 8.4 Hz, 1H), 7.35-7.31 (m, 2H), 7.26-7.24 (m, 1H), 7.18 (brd, *J* = 7.6 Hz, 1H), 7.06 (d, *J* = 8.4 Hz, 1H), 6.95 (d, *J* = 12.0 Hz, 1H), 6.81 (d, *J* = 12.0 Hz, 1H), 2.54-2.50 (m, 2H), 2.37-2.30 (m, 2H), 2.23 (s, 3H), 2.19-2.14 (m, 2H), 2.10-2.05 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 139.0, 137.9, 136.7, 136.0, 134.5, 132.4, 132.3, 130.7, 130.6, 130.1, 129.5, 128.5, 128.4, 128.1, 126.5, 119.9, 57.13, 57.11, 46.0, 30.2; HRMS (ESI+) calcd. for $C_{21}H_{21}BrN$ $[M + H]^+$: 366.0852. Found 366.0845.

Preparation of compound 2j: A solution of compound **2a** (50 mg, 0.16 mmol) in dichloromethane (2 ml) was cooled to -78°C under an argon atmosphere, and a 1.0 M solution of boron tribromide in dichloromethane (0.78 ml, 0.78 mmol) was added to it. The reaction mixture was stirred for 2 h at room temperature, then poured into ice-water, and extracted with dichloromethane. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (dichloromethane : methanol = 8 : 1) to afford **2j** (45 mg, 0.15 mmol, y. 92%) as a white solid. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.41-7.31 (m, 3H), 7.25 (d, *J* = 8.5 Hz, 1H), 7.24 (brd, *J* = 7.5 Hz, 1H), 6.89 (d, *J* = 11.5 Hz, 1H), 6.82 (d, *J* = 11.5 Hz, 1H), 6.79 (dd, *J* = 2.0, 8.5 Hz, 1H), 6.70 (d, *J* = 2.0 Hz, 1H), 3.31-3.25 (m, 2H), 2.88 (br, 2H), 2.80 (s, 3H), 2.70-2.56 (m, 2H), 2.51-2.37 (m, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 160.0, 141.3, 139.6, 139.2, 137.2, 132.6, 132.0, 130.3, 130.2, 130.1, 129.71, 129.68, 128.9, 128.94, 128.88, 116.2, 115.9, 57.31, 57.28, 44.7, 29.4, 29.3; HRMS (ESI+) Calcd. for $C_{21}H_{22}NO$ $[M + H]^+$: 304.1696. Found 304.1685.

2k – **2p** were similarly prepared from corresponding compounds **2**. Those analytical data were given below.

2k: ^{1}H NMR (CD_3OD, 400 MHz) δ 7.37 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.28 (d, *J* = 2.0 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 6.86 (d, *J* = 11.6 Hz, 1H), 6.80 (d, *J* = 2.8, 8.4 Hz 1H), 6.71 (d, *J* = 11.6 Hz, 1H), 6.68 (d, *J* = 2.8 Hz, 1H), 3.01 (br, 2H), 2.70 (br, 2H), 2.51 (s, 3H), 2.51-2.38 (m, 4H); ^{13}C NMR (CD_3OD, 125 MHz) δ 158.0, 138.9, 134.8, 134.2, 131.2, 130.1, 129.8, 129.5, 129.4, 126.61, 126.57, 121.0, 114.1, 113.9, 55.4, 55.3, 43.2, 27.9, 27.7; HRMS (ESI+) Calcd. for $C_{21}H_{21}BrNO$ $[M + H]^+$: 382.0801. Found 382.0806.

2l: ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.44 (d, *J* = 1.5 Hz, 1H), 7.38 (brd, *J* = 8.5 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 6.97 (brd, *J* = 8.5 Hz, 1H), 6.88 (d, *J* = 12.0 Hz, 1H), 6.75 (brd, *J* = 8.5 Hz, 1H), 6.67 (d, *J* = 12.0 Hz, 1H), 6.62 (d, *J* = 2.0 Hz, 1H), 2.79-2.71 (m, 2H), 2.57-2.25 (m, 4H), 2.24 (s, 3H), 2.01-1.93 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.8, 140.0, 137.3, 136.8, 133.9, 133.6, 132.2, 130.9, 130.4, 130.3, 130.1, 127.2, 126.9, 120.3, 115.1, 115.0, 56.4, 45.8, 30.1, 29.3; HRMS (ESI+) Calcd. for $C_{21}H_{21}BrNO$ $[M+H]^+$: 382.0801. Found 382.0790.

2m: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.21-7.17 (m, 2H), 7.14, (dd, *J* = 2.0, 8.0 Hz), 6.91-6.88 (m, 1H), 6.86 (d, *J* = 12.0 Hz, 1H), 6.82 (dd, *J* = 2.4, 8.4 Hz, 1H), 6.76 (br, 1H), 6.66 (d, *J* = 12.0 Hz, 1H), 3.08-2.99 (m, 2H), 2.77-2.47 (m, 4H), 2.50 (s, 3H), 2.44-2.31 (m, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 159.7, 140.4, 138.3, 137.8, 136.9, 133.9, 133.4, 131.6, 130.7, 130.1, 129.0, 128.8, 127.9, 115.8, 115.3, 56.36, 56.34, 43.9, 28.6, 28.4; HRMS (ESI+) Calcd. for $C_{21}H_{21}ClNO$ $[M + H]^+$: 338.1306. Found 338.1297.

2n: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.37-7.34 (m, 2H), 7.27 (td, *J* = 1.0, 7.5 Hz, 1H), 7.19 (dd, *J* = 1.5, 7.0 Hz, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 6.90 (d, *J* = 12.0 Hz, 1H), 6.82 (dd, *J* = 2.0, 8.5 Hz, 1H), 6.82 (d, *J* = 12.0 Hz, 1H), 6.77 (d, *J* = 2.0 Hz, 1H), 2.57-2.50 (m, 2H), 3.11-3.09 (m, 2H), 2.70-2.64 (m, 2H), 2.65 (s, 3H), 2.57-2.50 (m, 2H), 2.39-2.30 (m, 2H); ^{13}C NMR (CD_3OD, 125 MHz) δ 158.3, 140.6, 138.7, 138.1, 136.8, 132.9, 132.1, 131.2, 131.0, 130.4, 129.9, 129.7, 128.6, 117.4, 116.2, 57.62, 57.59, 45.2, 29.80, 29.76; HRMS (ESI+) Calcd. for $C_{21}H_{22}NO$ $[M + H]^+$: 304.1696. Found 304.1702.

2o: ^{1}H NMR (CD_3OD, 400 MHz) δ 7.40 (dd, *J* = 2.0, 8.4 Hz, 1H), 7.34 (d, *J* = 2.0 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 1H), 6.85 (s, 2H), 6.83 (dd, *J* = 2.4, 8.0 Hz, 1H) , 6.75 (d, *J* = 2.4 Hz, 1H), 2.81-2.77 (m, 2H), 2.45-2.19 (m, 6H), 2.38 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 156.1, 140.5, 135.7, 133.8, 133.6, 132.7, 131.4, 130.2, 129.9, 129.7, 129.3, 129.1, 129.0, 121.2, 115.3, 113.9, 55.84, 55.80, 43.9, 28.52, 28.47; HRMS (ESI+) calcd. for $C_{21}H_{21}BrNO$ $[M + H]^+$: 382.0801. Found 382.0803.

2p: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.52 (s, 1H), 7.63 (d, *J* = 2.0 Hz, 1H), 7.55 (d, *J* = 2.0, 8.0 Hz, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 6.93 (d, *J* = 12.0 Hz, 1H) , 6.91 (d, *J* = 12.0 Hz, 1H), 6.84 (dd, *J* = 2.5, 8.5 Hz, 1H), 6.78 (d, *J* = 2.5 Hz, 2H), 2.48-2.40 (m, 2H), 2.30-2.23 (m, 2H), 2.14 (s, 3H), 2.06-1.97 (m, 4H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 156.2, 137.3, 136.7, 135.5, 134.9, 132.1, 130.6, 130.4, 129.7, 129.4, 129.0, 128.9, 119.8, 115.5, 114.1, 55.4, 55.3, 43.1, 27.7; HRMS (ESI+) Calcd. for $C_{21}H_{21}BrNO$ $[M + H]^+$: 382.0801. Found 382.0791.

Preparation of 13a: Sodium carbonate (20 mg, 0.19 mmol) and potassium hexacyanoferrate(II) trihydrate (18 mg, 0.041 mmol) were ground to a fine powder, placed in pressure tube, and dried in vacuo at 80°C overnight. A solution of **2i** (70 mg, 0.19 mmol) in *N*-methylpyrrolidone (1.5 ml), a 3.8 mM solution of 1,1'-bis(diphenylphosphino)ferrocene in *N*-methylpyrrolidone (0.1 ml, 0.38 μmol) and a 1.9 mM solution of palladium acetate in *N*-methylpyrrolidone (0.1 ml, 0.19 μmol) were then added to it. The atmosphere in the pressure tube was replaced with argon, and the tube was sealed. The contents were heated with stirring at 120°C for 19 h, then cooled to room temperature, poured into water, and extracted with dichloromethane. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (dichloromethane : methanol = 10 : 1) to afford **13a** (34 mg, 0.11 mmol, y. 57%) as a brown solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.60 (d, *J* = 1.5 Hz, 1H), 7.57 (dd, *J* = 1.5, 8.0 Hz, 1H), 7.39-7.26 (m, 4H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 11.5 Hz, 1H), 6.86 (d, *J* = 11.5 Hz, 1H), 2.54-2.52 (m, 2H), 2.39-2.30 (m, 2H), 2.24 (s, 3H), 2.20-2.04 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 143.7, 138.7, 137.4, 136.1, 134.7, 133.6, 132.5, 132.3, 131.0, 129.8, 129.1, 129.0, 128.9, 127.2, 119.2, 110.5, 57.3, 46.3, 30.5, 30.4; HRMS (ESI+) Calcd. for $C_{22}H_{21}N_2$ $[M + H]^+$: 313.1699. Found 313.1708.

Preparation of compound 13b: A flame-dried flask fitted with a magnetic stirring bar was charged with **13a** (30 mg, 0.10 mmol), potassium *tert*-butoxide (32 mg, 0.30 mmol) and *tert*-butanol (3 ml). The reaction mixture was stirred at room temperature for 21 h under an argon atmosphere. After addition of water, the aqueous phase was extracted with dichloromethane. The organic phase was dried over sodium sulfate, and the solvent was removed in in vacuo. The residue was purified by silica gel column chromatography (dichloromethane : methanol = 5 : 1) to afford **13b** (15 mg, 0.045 mmol, y. 46%) as a white powder. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.91 (d, *J* = 2.0 Hz, 1H), 7.88 (dd, *J* = 2.0, 8.0 Hz,

1H), 7.42-7.24 (m, 5H), 7.04 (dd, *J* = 11.5 Hz, 1H), 7.01 (d, *J* = 11.5 Hz, 1H), 2.65-2.62 (m, 2H), 2.43-2.37 (m, 2H), 2.27 (s, 3H), 2.22-2.11 (m, 4H) ; ^{13}C NMR (CD_3OD, 125 MHz) δ 172.8, 144.3, 140.6, 137.2, 136.89, 136.88, 135.8, 134.1, 133.9, 132.1, 130.5, 130.4, 130.3, 130.2, 129.6, 128.8, 128.7, 58.6, 46.8, 31.44, 31.40; HRMS (ESI+) Calcd. for $C_{22}H_{23}N_2O$ $[M + H]^+$: 331.1805. Found 331.1801.

Preparation of compound 13c: **2i** (0.10 g, 0.27 mmol), proline (40 mg, 0.35 mmol), sodium azide (35 mg, 0.54 mmol), and copper(I) oxide (39 mg, 0.27 mmol) were added to a round-bottomed flask. Under an argon atmosphere, dimethyl sulfoxide (2.5 mL) was added to the flask, and the reaction mixture was stirred at 100°C for 7 h. Saturated aqueous ammonium chloride was added, and the aqueous phase was extracted with dichloromethane. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (dichloromethane : methanol = 10 : 1) to afford **13c** (69 mg, 0.23 mmol, y. 84%) as a brown solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31-7.15 (m, 4H), 6.98 (d, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 11.5 Hz, 1H), 6.78 (d, *J* = 11.5 Hz, 1H), 6.68 (dd, *J* = 2.5, 8.0 Hz, 1H), 6.20 (d, *J* = 2.5 Hz, 1H), 3.61 (br, 2H), 2.40-2.33 (m, 2H), 2.27 (s, 3H); ^{13}C NMR (CD_3OD, 125 MHz) δ 148.1, 141.5, 137.7, 137.0, 136.9, 134.7, 133.2, 132.6, 131.9, 130.9, 130.2, 130.0, 129.5, 128.1, 117.6, 116.5, 58.64, 58.61, 46.7, 31.32, 31.27; HRMS (ESI+) Calcd. for $C_{21}H_{23}N_2$ $[M + H]^+$: 303.1856. Found 303.1852.

Preparation of compound 13d: A solution of **13c** (20 mg, 0.066 mmol) and acetic anhydride (2 drops) in pyridine (1 ml) was stirred at room temperature for 5 h, then poured into ice-water, and extracted with ethyl acetate. The organic phase was dried over sodium sulfate, and the solvent was removed in vacuo. The residue was purified by silica gel column chromatography (dichloromethane : methanol = 10 : 1) to afford **13d** (11 mg, 0.029 mmol, y. 44%) as a white solid. ^{1}H NMR (CD_3OD, 400 MHz) δ 7.90 (s, 1H), 7.56 (d, *J* = 2.0 Hz, 1H), 7.51 (dd, *J* = 2.0, 8.0 Hz, 1H), 7.36-7.17 (m, 4H), 7.13 (d, *J* = 8.0 Hz, 1H), 6.94 (d, *J* = 12 Hz, 1H), 6.86 (d, *J* = 12 Hz, 1H), 2.62-2.56 (m, 2H), 2.40-2.32 (m, 2H), 2.23 (s, 3H), 2.12 (s, 3H), 2.21-2.06 (m, 4H); ^{13}C NMR (CD_3OD, 125 MHz) δ 170.2, 138.8, 137.0, 135.2, 134.8, 134.6, 134.0, 133.5, 131.0, 130.3, 128.3, 128.0, 127.9, 127.6, 126.1, 119.4, 119.1, 56.3, 44.4, 29.0, 22.4; HRMS (ESI+) Calcd. for $C_{23}H_{25}N_2O$ $[M + H]^+$: 345.1961. Found 345.1954.

AlphaLISA assay

AlphaLISA enzymatic assay was performed as described elsewhere.[31] 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 incubation was continued for an additional 30 min at room temperature. Then, the α signal was detected with an EnSpire Alpha plate reader (PerkinElmer, Waltham, MA, USA). The signal was fitted to the equation "% of control (without inhibitor) of α signal = m1 + (m2 – m1) / [1 + ([inhibitor] / m3)^m4]" using the Origin software (OriginLab, Northampton, MA, USA), and IC_{50} can be estimated as "m3".

X-ray crystallographic analysis

A SET7/9 methyltransferase domain (residues 111−366) containing an N-terminal GSSGSSG sequence was prepared as previously described.[26] Briefly, a solution of 8 mg/ml of SET7/9 protein in 20 mM Tris-HCl (pH 7.5) buffer solution containing 200 mM NaCl and 5 mM DTT was mixed with 1.5 mM coenzyme analogue, Sinefungin, which was used in place of SAM. Crystals of the SET7/9–Sinefungin complex were obtained using the hanging drop method at 293 K against a reservoir solution containing 100 mM Tris (pH 8.5) and 34% (w/w) PEG 6000. These crystals were soaked in reservoir solution containing 3 mM **2n** for 10 days, then cryoprotected with 15% glycerol, and flash-cooled in liquid nitrogen. Data were collected at the BL17A beamline at the Photon Factory (KEK, Tsukuba, Japan), and processed using *HKL-2000* and the CCP4 suite to 1.69 Å resolution. Molecular replacement was performed with *Phaser* using the protein portion of the SET7/9–SAM–cyproheptadine complex (PDB code 5AYF). The topology and parameter files for the inhibitor were generated with the *eLBOW* module of *PHENIX*. Model building and refinement were carried out with *COOT* and *PHENIX*, respectively. The crystallographic statistics are summarized in Table 3. The figures were prepared using *PyMOL* (Schrödinger, LLC). The coordinates and structure factors have been deposited in the Protein Data Bank (PDB) with the accession code 5YLT.

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Keywords: SET7/9 • inhibitor • histone methyltransferase • cyproheptadine • epigenetics

References:

[1] S. Khorasanizadeh, *Cell* **2004**, *116*, 259-272.
[2] M. Biel, V. Wascholowski, A. Giannis, *Angew. Chem. Int. Ed*. **2005**, *44*, 3186-3216.
[3] S. Rea, F. Eisenhaber, N. O'Carroll, B.D. Strahl, Z. W. Sun, M. Schmid, S. Opravil, K. Mechtler, C. P. Ponting, C. D. Allis, T. Jenuwein, *Nature* **2000**, *406*, 593-599.
[4] Y. Zhang, D. Reinberg, *Gene. Dev.* **2001**, *15*, 2343-2360.
[5] R. A. Varier, H. T. M. Timmers, *Biochim. Biophys. Acta* **2011**, *1815*, 75-89.
[6] X. Zhan, H. Wen, X. Shi, *Acta. Biochim. Biophys. Sin.* **2012**, *44*, 14-27.
[7] K. Nishioka, S. Chuikov, K. Sarma, H. Erdjument-Bromage, C. D. Allis, P. Tempst, D. Reinberg, *Genes Dev.* **2002**, *16*, 479-489.
[8] A. Dhayalan, S. Kudithipudi, P. Rathert, A. Jeltsch, *Chem. Biol.* **2011**, *18*, 111-120.
[9] A. Kouskouti, E. Scheer, A. Staub, L. Tora, I. Talianidis, *Mol. Cell* **2004**, *14*, 175-182.
[10] S. Chuikov, J. K. Kurash, J. R. Wilson, B. Xiao, N. Justin, G. S. Ivanov, K. McKinney, P. Tempst, C. Prives, S. J. Gamblin, N. A. Barlev, D. Reinberg, *Nature* **2004**, *432*, 353-360.
[11] K. Subramanian, D. Jia, K. -V. Priya, D. R. Powell, R. E. Collins, D. Sharma, J. Peng, X. Cheng, P. M. Vertino, *Mol. Cell* **2008**, *30*, 336-347.
[12] L. Gaughan, J. Stockley, N. Wang, S. R. C. McCracken, A. Treumann, K. Armstrong, F. Shaheen, K. Watt, I. J. McEwan, C. Wang, R. G. Pestell, C. N. Robson, *Nucl. Acids Res.* **2011**, *39*, 1266-1279.
[13] M. D. M. Huq, N. -P. Tsai, S. A. Khan, L. -N. Wei, *Mol. Cell. Proteomics* **2007**, *6*, 677-688.
[14] J. Yang, J. Huang, M. Dasgupta, N. Sears, M. Miyagi, B. Wang, M. R. Chance, X. Chen, Y. Du, Y. Wang, L. An, Q. Wang, T. Lu, X. Zhang, Z. Wang, G. R. Stark, *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 21499-21504.

[15] D. R. Calnan, A. E. Webb, J. L. White, T. R. Stowe, T. Goswami, X. Shi, A. Espejo, M. T. Bedford, O. Gozani, S. P. Gygi, A. Brunet, *Aging* **2012**, *4*, 462-479.
[16] S. Pagans, S. E. Kauder, K. Kaehlcke, N. Sakane, S. Schroeder, W. Dormeyer, R. C. Trievel, E. Verdin, M. Schnolzer, M. Ott, *Cell Host Microbe.* **2010**, *7*, 234-244.
[17] X. Liu, Z. Chen, C. Xu, X. Leng, H. Cao, G. Ouyang, W. Xiao, *Nucl. Acids Res.* **2015**, *43*, 5081-5098.
[18] P. O. Esteve, H. G. Chin, J. Benner, G. R. Feehery, M. Samaranayake, G. A. Horwitz, S. E. Jacobsen, S. Pradhan, *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 5076-5081.
[19] S. M. Carr, N. B. La Thangue, *Cell Cycle* **2011**, *10*, 733-734.
[20] S. M. Carr, S. Munro, B. Kessler, U. Oppermann, N. B. La Thangue, *EMBO J.* **2011**, *30*, 317-327.
[21] D. Barsyte-Lovejoy, F. Li, M. J. Oudhoff, J. H. Tatlock, A. Dong, H. Zeng, H. Wu, S. A. Freeman, M. Schapira, G. A. Senisterra, E. Kuznetsova, R. Marcellus, A. Allali-Hassani, S. Kennedy, J. P. Lambert, A. L. Couzens, A. Aman, A. C. Gingras, R. Al-Awar, P. V. Fish, B. S. Gerstenberger, L. Roberts, C. L. Benn, R. L. Grimley, M. J. Braam, F. M. Rossi, M. Sudol, P. J. Brown, M. E. Bunnage, D. R. Owen, C. Zaph, M. Vedadi, C. H. Arrowsmith, *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 12853-12858.
[22] F. Meng, S. Cheng, H. Ding, S. Liu, Y. Liu, K. Zhu, S. Chen, J. Lu, Y. Xie, L. Li, R. Liu, Z. Shi, Y. Zhou, Y.-C. Liu, M. Zheng, H. Jiang, W. Lu, H. Liu, C. Luo, *J. Med. Chem.* **2015**, *58*, 8166-8181.
[23] S. Mori, K. Iwase, N. Iwanami, Y. Tanaka, H. Kagechika, T. Hirano, *Bioorg. Med. Chem.* **2010**, *18*, 8158-8166.
[24] H. Niwa, N. Handa, Y. Tomabechi, K. Honda, M. Toyama, N. Ohsawa, M. Shirouzu, H. Kagechika, T. Hirano, T. Umehara, S. Yokoyama, *Acta Cryst, D* **2013**, *69*, 595-602.
[25] H. Chi, Y. Takemoto, K. T. Nsiama, T. Kato, N. Nishino, A. Ito, M. Yoshida, *Bioorg. Med. Chem.* **2014**, *22*, 1268-1275.
[26] Y. Takemoto, A. Ito, H. Niwa, M. Okamura, T. Fujiwara, T. Hirano, N. Handa, T. Umehara, T. Sonoda, K. Ogawa, M. Tariq, N. Nishino, S. Dan, H. Kagechika, T. Yamori, S. Yokoyama, M. Yoshida, *J. Med. Chem.* **2016**, *59*, 3650-3660.
[27] T. Fujiwara, K. Ohira, K. Urushibara, A. Ito, M. Yoshida, M. Kanai, A. Tanatani, H. Kagechika, T. Hirano, *Bioorg. Med. Chem.* **2016**, *24*, 4318-4323.
[28] E. L. Engelhardt, H. C. Zell, W. S. Saari, M. E. Christy, D. Colton, *J. Med. Chem.* **1965**, *8*, 829-835.
[29] A. M. Honrubia, J. Rodrigues, R. Dominguez, E. Lozoya, F. Manut, A. J. Seijas, C. M. Villaverde, M. J. Calleja, I. M. Cadavid, S. Maayani, F. Sanz, I. M. Losa, *Chem. Pharm. Bull.* **1997**, *45*, 842-848.
[30] S. C. Koeberle, S. Fisher, D. Schollmeyer, V. Schattel, C. Grütter, D. Rauh, S. A. Laufer, *J. Med. Chem.* **2012**, *55*, 5868-5877.
[31] N. Gauthier, M. Caron, L. Pedro, M. Arcand, J. Blouin, A. Labonte, C. Normand, V. Paquet, A. Rodenbrock, M. Roy, N. Rouleau, L. Beaudet, J. Padros, R. Rodriguez-Suarez, *J. Biomol. Screening* **2012**, *17*, 49-58.
[32] T. Schareina, A. Zapf, M. Beller, *Chem. Commun.* **2004**, *12*, 1388-1389.
[33] J. T. Markiewicz, O. Wiest, P. Helquist, *J. Org. Chem.* **2010**, *75*, 4887-4890.

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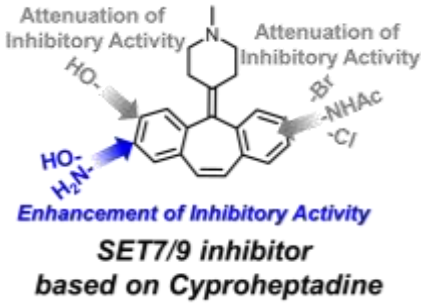

New series of SET7/9 inhibitors: SET7/9 methylates not only histone but also non-histone proteins as substrates, so its inhibitors are considered candidates for treatment of diseases. Here, we have developed a new family of inhibitors based on our previously identified scaffold, cyproheptadine.