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# Design, synthesis, and protein methyltransferase activity of a unique set of constrained amine containing compounds

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Abstract

Epigenetic alterations relate to various human diseases, and developing inhibitors of Kme regulatory proteins is considered to be a new frontier for drug discovery. We were inspired by the known multicyclic ligands, **UNC669** and **UNC926**, which are the first reported small molecule ligands for a methyl-lysine binding domain. We hypothesized that reducing the conformational flexibility of the key amine moiety of **UNC669** would result in a unique set of ligands. Twenty-five novel compounds containing a fused bi- or tricyclic amine or a spirocyclic amine were designed and synthesized. To gauge the potential of these amine-containing compounds to interact with Kme regulatory proteins, the compounds were screened against a panel of 24 protein methyltransferases. Compound **13** was discovered as a novel scaffold that interacts with SETD8 and could serve as a starting point for the future development of PKMT inhibitors.

Graphical abstract

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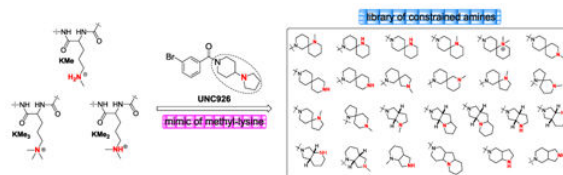
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Keywords

Cyclic fused and spirocyclic amines; Histone methyltransferases; Cross-screening; Structure based design

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Mounting evidence suggests that epigenetic alterations relate to various human diseases including inflammation, brain disorders, metabolic diseases, and cancer.[1-3] One such epigenetic modification is histone lysine methylation. Protein lysine methyltransferases (PKMTs) catalyze the transfer of a methyl group from the cofactor S-adenosyl-L-methionine (SAM) to lysine residues of histone and non-histone substrates, leading to lysine mono-, di-, and/or trimethylation. Lysine methylation is reversible in that the methyl groups can be removed by either the Jumonji family of 2-oxoglutarate-dependent demethylases[4] or the flavin dependent enzymes, lysine-specific histone demethylase 1 (LSD1) and LSD2.[5] Lysine methylation has been identified at various positions of the histone tails, mainly on histone 3 at lysine 4 (K4), K9, K27, K36, and K79, and on histone 4 at K20.[6] All methylated forms of lysine are cationic at physiological pH, while trimethyllysine contains a fixed positive charge irrespective of its environment.

In contrast to the enzymes that install and remove the methyl groups on lysine, methyl-lysine (Kme) readers recognize and bind the Kme marks non-covalently, most commonly via an interaction between the methyl-ammonium group and an aromatic cage in the protein. This binding interaction is largely the result of favorable cation-π and van der Waals interactions, while hydrophobic desolvation effects also contribute. Depending on the methylation state, nearby acidic residues in the binding pocket are also known to form salt bridges with the methylated lysine residue, offering an additional stabilizing effect.[7, 8] Kme readers are categorized into three main families by their respective binding domains: the plant homeodomain (PHD) zinc finger proteins, the WD40 repeat domain-containing proteins, and the so-called Royal family of reader proteins, including tudor, chromo, Pro-Trp-Trp-Pro (PWWP), and malignant brain tumor (MBT) domain-containing proteins.

Overall, histone lysine methylation can be associated with either transcriptional activation or repression. The mutation, overexpression, and aberrant regulation of Kme regulatory proteins have been linked to many diseases, especially cancer.[3] For example, overexpression of the key developmental histone lysine methyltransferase, EZH2, has been observed in several types of leukemias and in various solid tumors.[9] Thus, developing inhibitors of Kme regulatory proteins is considered to be a new frontier for drug discovery. Additionally, the exact biological mechanisms of many of these proteins still require elucidation, and therefore small molecule chemical probes would facilitate further study and understanding of their functions.[10-12]

We were initially inspired by the known multicyclic ligands, **UNC669** (**1a**, Fig. 1a)[13] and **UNC926** (**1b**, Fig. 1b),[14] which are the first reported small molecule ligands for a methyl-lysine binding domain, specifically the second MBT domain of both L3MBTL1 and L3MBTL3. As shown in the high-resolution co-crystal structure of **UNC669** and L3MBTL1 (pdb 3P8H), the pyrrolidine amine makes a key hydrogen bond with aspartic acid D355 and the ligand also engages the aromatic binding cage (F379, W382, Y386; Fig. 1c). We

hypothesized that preparing a variety of amines with reduced conformational flexibility relative to the piperidine-pyrrolidine amine (Fig. **1a** and **1b**, red) may generally result in ligands with enhanced affinity for proteins that interact with methylated lysine.

Beyond targeting methyl-lysine regulatory proteins, nitrogen-based heterocycles are of broad interest in medicinal chemistry. Furthermore, compounds with fused ring systems and spirocyclic heterocycles have found applications as antibacterial drugs,[15] antitumour drugs,[16] 5-HT$_{2C}$ receptor agonists,[17] ChK1 kinase inhibitors,[18] and CGRP receptor antagonists.[19] In this article we describe the design and synthesis of a set of compounds containing fused bi- or tricyclic amines and spirocyclic amines that are well poised to both interact with Kme regulatory proteins as well as provide value to a number of medicinal chemistry efforts.

We designed 25 fused and spirocyclic amines (Fig. 2) that: (1) contain a basic amine, (2) are fragment-like in size (less than 200 g/mol), (3) are non-aromatic, and (4) contain a synthetic handle for further chemistry to attach the amines to a variety of scaffolds. Upon preparation, each amine was reacted with 3-bromobenzoyl chloride by the general synthetic route shown in Scheme 1. When necessary, protected amines were coupled to 3-bromobenzoyl chloride and subsequent deprotection gave the desired products. At this point, secondary amines could also be alkylated to give the corresponding tertiary or quaternary amine-containing compounds. In the end, this resulted in a unique set of fused and spirocyclic compounds (**2–26**) well poised for screening efforts and future development, and their synthesis is discussed below.

While several of the proposed constrained amines or precursors of these amines were synthesized according to methods in the literature, a number of the amines including those of compounds **2**, **3**, **18**, and **19** have not been reported previously. In the preparation of compounds **2** and **3**, 1-benzyl-3-piperidone (**27**) was used as a starting material (Scheme 2). Treatment of **27** with allylamine gave the corresponding imine, and the crude product was then treated with allylmagnesium bromide to provide the aminodiene (**28**). Compound **29** was obtained by protecting the secondary amino group of **28** as the trifluoroacetamide. Treatment of **29** with Grubbs catalyst in DCM gave spirocyclic compound **30**. The trifluoroacyl group of compound **30** was converted to a Boc protecting group in two steps to generate **32**. The key intermediate (**33**) was obtained by reduction of **32** with palladium. We coupled amine **33** to 3-bromobenzoyl chloride and removed the Boc group to provide target compound **2**. Compound **3** was achieved via installation of a methyl group on the basic nitrogen of compound **2** via reductive amination. The Boc protected 1,9-diazaspiroundecane amine used to prepare compounds **4**-**6** was similarly synthesized from 1-benzyl-4-piperidone. [20] The reaction of **5** with methyl iodide gave the corresponding quaternary amine containing compound (**6**). It is possible that such quaternary amines may interact more favorably with slightly larger binding pockets such as those of Kme3 reader domains and methyltransferases that install the trimethyl mark.

The syntheses of the bi- or tricyclic amines of compounds **7**-**10** were initiated with the protection of aminoacetaldehyde dimethyl acetal as the ethyl carbamate, followed by alkylation with allyl bromide. After deprotection with formic acid, the desired aldehyde was

obtained. A 1,3-dipolar cycloaddition of the aldehyde and N-methylglycine, proline, piperidine-2-carboxylic acid, or N-benzylglycine provided the amines used to prepare compounds **7-10**, respectively.[17, 21] This racemic synthesis afforded the amine precursors of compounds **7**–**10** as *cis* racemates, as has been reported previously.[17] We additionally prepared the 4-nitrobenzoyl analogue of compound **8** and confirmed its *cis*-configuration by single-crystal X-ray diffraction (Supplementary Figure 1). The fused cyclic amines of compounds **11** and **12** were synthesized with the common starting material, 3,3-dimethoxypropan-1-amine, using a 1,3-dipolar cycloaddition as the key step, analogous to the preparation of compounds **7** and **10**, respectively.[22] Compounds **7** and **11** were obtained after deprotection of the benzyl group upon coupling to 3-bromobenzoyl chloride. Additionally, we prepared an analogue of compound **11** (containing two 4-nitrobenzoyl groups) and its *cis*-configuration was confirmed by single-crystal X-ray diffraction (Supplementary Figure 2).

The fused cyclic amine containing compounds **13-16** were synthesized from 2,3-pyridinedicarboxylic acid. Dehydration of the pyridinedicarboxylic acid with acetic anhydride and then ammonolysis with benzyl ammonium gave 6-benzyl-5H-pyrrolo[3,4-*b*]pyridine-5,7(6*H*)-dione. Hydrogenation with palladium gave key intermediate 6-benzyltetrahydro-5*H*-pyrrolo[3,4-*b*]pyridine-5,7(6*H*)-dione, and subsequent reduction by lithium aluminium hydride led to the protected bicyclic fused amine precursor used to prepare compounds **13**-**16**.[23] Key intermediate 6-benzyltetrahydro-5*H*-pyrrolo[3,4-*b*]pyridine-5,7(6*H*)-dione was determined to be the *cis* racemate by comparison of the NMR spectra with previous literature reports.[24] Compound **17** was prepared analogously to compound **16** from 3,4-pyridinedicarboxylic acid.[22]

Compounds **18** and **19** were prepared according to the sequence outlined in Scheme 3. First, compound **35** was converted to the corresponding oxime (**36**) which was subsequently oxidized with TFAA/H_2O_2 to give the Cbz protected 3-nitro piperidine (**37**). The nitro ester intermediate (**38**) was obtained by a Michael addition of **37** with methyl acrylate under mild conditions. Subsequent reduction of the nitro group by catalytic hydrogenation over Raney Nickel gave the corresponding amino intermediate, which spontaneously cyclized to the spirolactam (**39**). Reduction of **39** by lithium aluminium hydride lead to 7-methyl-1,7-diazaspiro [4. 5] decane, which was subsequently acylated to give compound **18.** Alternatively, methylation of the amide of **39** by iodomethane followed by Cbz deprotection with palladium provided intermediate **41**, which could be converted to compound **19** by a similar reduction and acylation sequence. The diamine spirocycle scaffolds used to prepare compounds **20** and **21** were synthesized from Cbz-protected 4-nitropiperidine via the same route.[25]

The boc protected spirocyclic amines used in the preparation of compounds **22** and **23** were obtained by cyclization of 1-benzyl-4-piperidinone, or 1-benzyl-3-piperidinone in the case of **24**, with ethyl cyanoacetate followed by hydrolysis and decarboxylation as reported previously.[26] Finally, the methylated spirocyclic amines of compounds **25** and **26** were prepared utilizing a key double alkylation of ethyl cyanoacetate. Subsequent nitrile reduction

and lactamization to establish the first heterocyclic ring followed by imide formation with methyl amine and global reduction furnished the target spirodiamines.[27]

With a unique set of compounds in hand containing bi- or tricyclic fused ring systems or spirocyclic amines, we sought to investigate the ability of these compounds to interact with a panel of 24 lysine and arginine methyltransferases (Supplementary Table 1). The activity of each enzyme was initially measured in the presence of 50 μM compound using a radioactivity-based Scintillation Proximity Assay.[28] Of the compounds screened, three reduced the enzymatic activity of the methyltransferase SETD8 to less than 50% that of the untreated enzyme (Table 1).

SETD8 is the sole PKMT known for the *in vivo* monomethylation of histone H4 lysine 20 (H4K20),[29, 30] and it has also been shown to monomethylate K382 of p53 and K248 of the proliferating cell nuclear antigen (PCNA).[31, 32] This enzyme has been implicated in DNA damage response, cell cycle progression, and the silencing of euchromatic genes.[33] Due to the growing interest in the perturbation of SETD8 activity with small molecules, a number of inhibitors of SETD8 have recently been discovered including the SAM-competitive inhibitor and marine natural product, nahuoic acid A,[34] and the substrate competitive inhibitor, UNC0379 (Supplementary Figure 3).[35] Most recently, Blum and coworkers reported three irreversible SETD8 inhibitors which exhibited modest selectivity and were active in cells.[36]

We next sought to confirm these results by determining the IC_{50} values of compounds **5**, **6**, and **13** for SETD8. While compounds **5** and **6** demonstrated no appreciable activity up to 200 μM (Supplementary Figure 4), compound **13** showed robust but weak activity against SETD8 ($IC_{50} = 39 \pm 11$ μM; Table 2 and Figure 3). Hoping to further characterize the activity of compound **13** for SETD8, we performed isothermal titration calorimetry (ITC) studies both in the presence and absence of *S*-Adenosyl-L-homocysteine (SAH). While significant binding was not observed, this is not overly surprising, as the ligand is inherently weak which prevents the ITC studies from being performed under optimal conditions to detect and quantify binding, particularly considering that prior reports suggest that we would expect a dissociation constant about 3-fold weaker than the observed IC_{50}.[35]

In conclusion, we were inspired by the known multicyclic ligands, **UNC669** and **UNC926**, and we hypothesized that reducing the conformational flexibility of the piperidine-pyrrolidine amine would generate a novel set of nitrogen-based heterocyclic ligands well poised to engage the proteins that interact with methylated lysine, as well as provide value in other areas of medicinal chemistry. In order to explore this hypothesis further, we designed and synthesized a unique set of 25 compounds with bi- or tricyclic fused amines or spirocyclic heterocycles and a simple bromobenzene core. We then investigated the ability of this set of amine-containing compounds to interact with a panel of protein lysine and arginine methyltransferases, which led to the identification of compound **13** as a novel scaffold that interacts with SETD8. Despite its weak affinity, compound **13** serves as an attractive scaffold for further development as a SETD8 inhibitor. In addition, we believe that the set of constrained amines developed will find wide utility when incorporated into a

variety of diverse scaffolds, and we are presently expanding our compound set to be broadly screened against different classes of Kme regulatory proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

References and notes

1. Jones PA, Baylin SB. Cell. 2007; 128:683. [PubMed: 17320506]

2. Copeland RA, Solomon ME, Richon VM. Nat. Rev. Drug. Disc. 2009; 8:724.

3. Arrowsmith CH, Bountra C, Fish PV, Lee K, Schapira M. Nat. Rev. Drug. Disc. 2012; 11:384.

4. Tsukada YI, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P, Zhang Y. Nature. 2005; 439:811. [PubMed: 16362057]

5. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, Casero RA, Shi Y. Cell. 2004; 119:941. [PubMed: 15620353]

6. Schotta G, Sengupta R, Kubicek S, Malin S, Kauer M, Callén E, Celeste A, Pagani M, Opravil S, Rosa-Velazquez IADL, Espejo A, Bedford MT, Nussenzweig A, Busslinger M, Jenuwein T. Genes. Dev. 2008; 22:2048. [PubMed: 18676810]

7. Daze KD, Hof F. Accounts. Chem. Res. 2012; 46:937.

8. Gao C, Herold JM, Kireev D, Wigle T, Norris JL, Frye SV. J. Am. Chem. Soc. 2011; 133:5357. [PubMed: 21428286]

9. Simon JA, Lange CA. Mutat. Res-Fund. Mol. O. M. 2008; 647:21.

10. Frye SV. Nat. Chem. Biol. 2010; 6:159. [PubMed: 20154659]

11. Bunnage ME, Chekler ELP, Jones LH. Nat. Chem. Biol. 2013; 9:195. [PubMed: 23508172]

12. Workman P, Collins I. Chem. Biol. 2010; 17:561. [PubMed: 20609406]

13. Herold JM, Wigle TJ, Norris JL, Lam R, Korboukh VK, Gao C, James LI, Kireev DB, Senisterra G, Vedadi M, Tripathy A, Brown PJ, Arrowsmith CH, Jin J, Janzen WP, Frye SV. J. Med. Chem. 2011; 54:2504. [PubMed: 21417280]

14. Herold JM, James LI, Korboukh VK, Gao C, Coil KE, Bua DJ, Norris JL, Kireev DB, Brown PJ, Jin J, Janzen WP, Gozani O, Frye SV. Med. Chem. Comm. 2012; 3:45.

15. Østergaard C, Sørensen TK, Knudsen JD, Frimodt-Møller N. Antimicrob. Agents. Chem. 1998; 42:1706.

16. Shao Y, Zhang HK, Ding H, Quan HT, Lou LG, Hu LH. J. Nat. Prod. 2009; 72:1170. [PubMed: 19499938]

17. Huck BR, Llamas L, Robarge MJ, Dent TC, Song J, Hodnick WF, Crumrine C, Stricker-Krongrad A, Harrington J, Brunden KR, Bennani YL. Bioorg. Med. Chem. Lett. 2006; 16:2891. [PubMed: 16546379]

18. Converso A, Hartingh T, Garbaccio RM, Tasber E, Rickert K, Fraley ME, Yan YW, Kreatsoulas C, Stirdivant S, Drakas B, Walsh ES, Hamilton K, Buser CA, Mao XZ, Abrams MT, Beck SC, Tao WK, Lobell R, Sepp-Lorenzino L, Zugay-Murphy J, Sardana V, Munshi SK, Jezequel-Sur SM, Zuck PD, Hartman GD. Bioorg. Med. Chem. Lett. 2009; 19:1240. [PubMed: 19155174]

19. Burgey CS, Potteiger CM, Deng JZ, Mosser SD, Salvatore CA, Yu S, Roller S, Kane SA, Vacca JP, Williams TM. Bioorg. Med. Chem. Lett. 2009; 19:6368. [PubMed: 19818613]

20. Jenkins ID, Lacrampe F, Ripper J, Alcaraz L, Van Le P, Nikolakopoulos G, Leone PD, White RH, Quinn RJ. J. Org. Chem. 2009; 74:1304. [PubMed: 19105637]

21. Schenke, T.; Petersen, U. 1990. EP393424A2

22. Wu, Y.; Qi, M. 2010. CN101619064A

23. Zhang, W.; Yang, D.; Huang, L.; Ma, J.; Guo, A.; Jiang, L.; Ma, R.; Chen, S. 2011. CN 102060855

24. Pallavicini M, Bolchi C, Fumagalli L, Piccolo O, Valoti E. Tetrahedron-Asymmetr. 2011; 22:379–380.

25. Mullen P, Miel H, McKervey MA. Tetrahedron Lett. 2010; 51:3216.

26. Chen, H.; Zhang, Z.; Ma, R.; Chen, S.; Li, G. 2008. CN101255160A

27. Weinberg K, Stoit A, Kruse CG, Haddow MF, Gallagher T. Tetrahedron. 2013; 69:4694.

28. Ibáñez G, Shum D, Blum G, Bhinder B, Radu C, Antczak C, Luo M, Djaballah H. Comb. Chem. High. T. Scr. 2012; 15:359.

29. Nishioka K, Rice JC, Sarma K, Erdjument-Bromage H, Werner J, Wang YM, Chuikov S, Valenzuela P, Tempst P, Steward R, Lis JT, Allis CD, Reinberg D. Mol. Cell. 2002; 9:1201. [PubMed: 12086618]

30. Wu S, Rice JC. Cell Cycle. 2011; 10:68. [PubMed: 21200139]

31. Shi XB, Kachirskaia L, Yamaguchi H, West LE, Wen H, Wang EW, Dutta S, Appella E, Gozani O. Mol. Cell. 2007; 27:636. [PubMed: 17707234]

32. Takawa M, Cho HS, Hayami S, Toyokawa G, Kogure M, Yamane Y, Iwai Y, Maejima K, Ueda K, Masuda A, Dohmae N, Field HI, Tsunoda T, Kobayashi T, Akasu T, Sugiyama M, Ohnuma S, Atomi Y, Ponder BA, Nakamura Y, Hamamoto R. Cancer Res. 2012; 72:3217. [PubMed: 22556262]

33. Beck DB, Oda H, Shen SS, Reinberg D. Genes Dev. 2012; 26:325. [PubMed: 22345514]

34. Williams DE, Dalisay DS, Li F, Amphlett J, Maneerat W, Chavez MAG, Wang YA, Matainaho T, Yu W, Brown PJ, Arrowsmith CH, Masoud V, Andersen RJ. Org. lett. 2012; 15:414. [PubMed: 23272941]

35. Ma A, Yu W, Li F, Bleich RM, Herold JM, Butler KV, Norris JL, Korboukh V, Tripathy A, Janzen WP, Arrowsmith CH, Frye SV, Vedadi M, Brown PJ, Jin J. J. Med. Chem. 2014; 57:6822. [PubMed: 25032507]

36. Blum G, Ibanez G, Rao X, Shum D, Radu C, Djaballah H, Rice JC, Luo M. ACS Chem. Biol. 2014; 9:2471. [PubMed: 25137013]
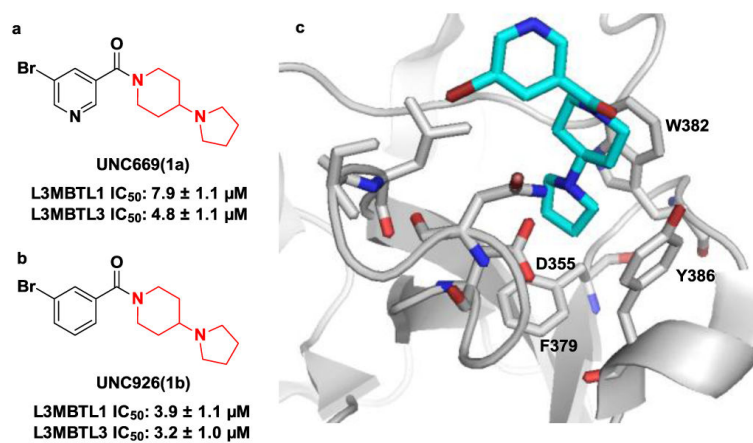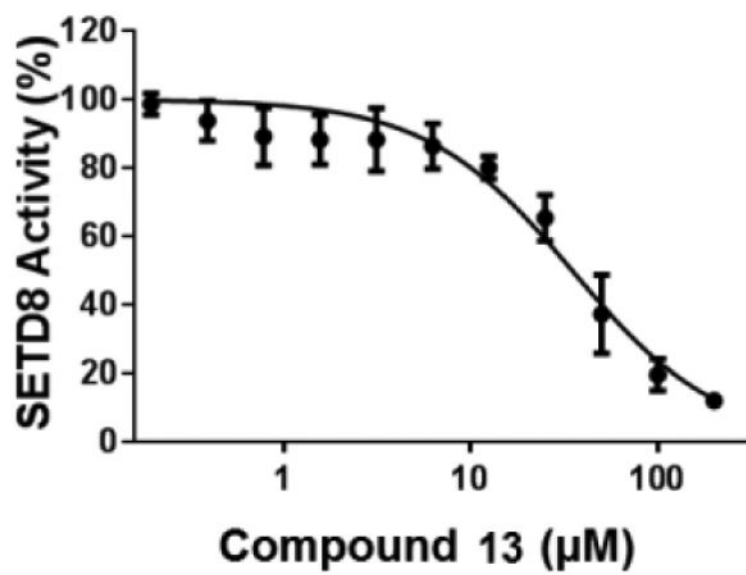
Figure 1.
Structures of L3MBTL1/L3MBTL3 inhibitors. (a) Structure of **UNC669**. (b) Structure of **UNC926**. (c) Co-crystal structure of **UNC669** with L3MBTL1 (pdb 3P8H).
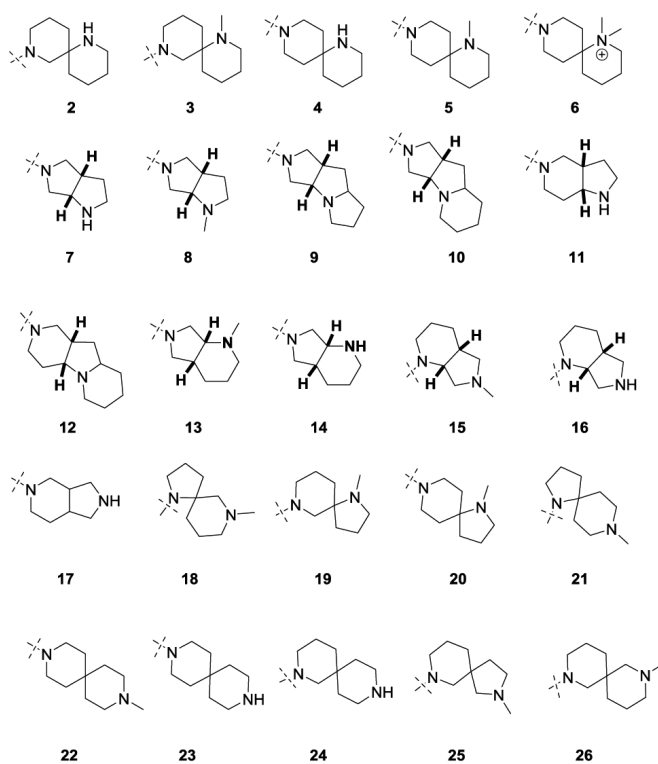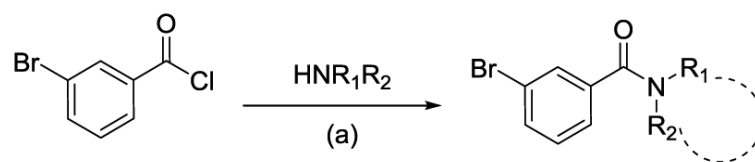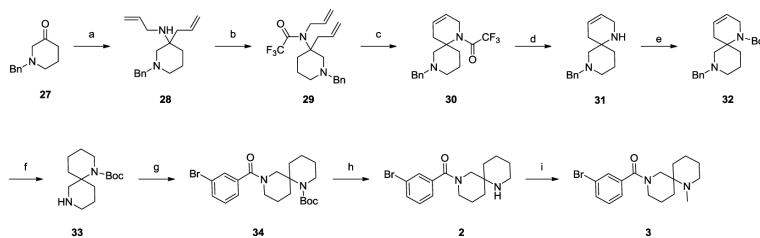
Figure 2.
Structures of compounds containing bi- or tricyclic fused amines or spirocyclic heterocycles.
The core structure of all final compounds is shown in Scheme 1.

Figure 3.
Compound **13** binds SETD8 with an IC_{50} of 39 ± 11 μM.

Scheme 1.
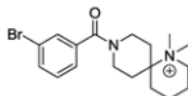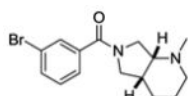General procedure for target compound synthesis. (a) Et$_3$N, DCM, 0 °C to rt.

Scheme 2.
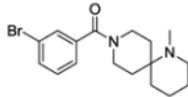
Route to compounds **2** and **3**. (a) i. 3-aminopropene, K_2CO_3, toluene, N_2, 12 h; ii. 10% allylmagnesium bromide/Et_2O, toluene, N_2, 0 °C to rt, 8 h (two steps, 37%-45%); (b) TFAA, DMAP, DCM, 0 °C to rt, 21 h (82%); (c) Grubbs II, DCM, Ar, 24 h, (73%); (d) K_2CO_3, MeOH, reflux, 5 h, (98%); (e) (Boc)$_2$O, MeCN, 3 d, (99%); (f) 10% Pd/C, HOAc, HCOONH4, MeOH, reflux, Ar, 5 h, (82%); (g) 3-bromobenzoyl chloride, Et_3N, DCM, 0 °C to rt, 2 h, (95%); (h) i. CF_3COOH, DCM, 2 h; ii. TEA, DCM, 4 h, (95%); (i) NaCNBH$_4$, HCHO/H_2O, HOAc, MeOH, 20 h, (85%).
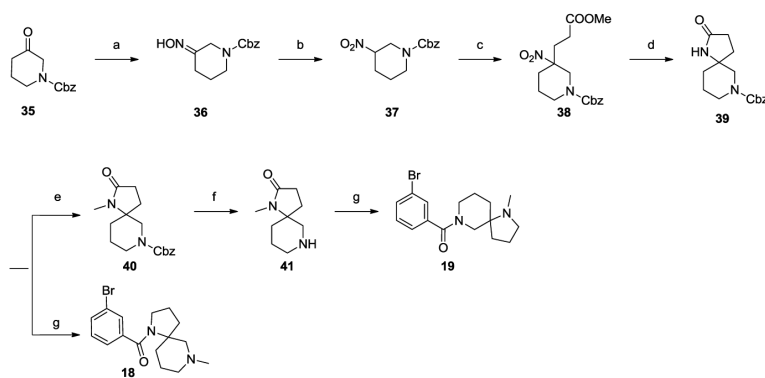
Scheme 3.

Route to compounds **18** and **19**. (a) NH$_2$OH·HCl, CH$_3$COONa, EtOH, 2 h (86%); (b) TFAA, 30% H$_2$O$_2$, urea, NaHCO$_3$, CH$_3$CN, reflux, 11 h (33%-39%); (c) methyl acrylate, K$_2$CO$_3$, MeOH, 12 h (98%); (d) Raney Ni, H$_2$, EtOH, 50 °C; (e) CH$_3$I, NaH, THF, 19 h (97%); (f) Pd/C, H$_2$, 24 h; (g) i. LiAlH$_4$, THF, reflux, 12 h; ii. 3-bromobenzoyl chloride, Et$_3$N, DCM, 0 °C to rt, 2 h, (two steps, 56%-97%).

Table 1

Structures of preliminary SETD8 hit compounds and percent SETD8 activity in the presence of 50 μM compound.

Compound	Structure	% SETD8 Activity
5		43
6		49
13		45

Table 2

IC_{50} determination of compounds 5, 6, and 13 for SETD8.

Compound	IC_{50} (μM)	Hill Slope
5	>200	NA
6	>200	NA
13	39 ± 11	2