Bioorganic & Medicinal Chemistry Letters 26 (2016) 4436–4440

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

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

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ARTICLE INFO

Article history:
Received 29 June 2016
Revised 1 August 2016
Available online 3 August 2016

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

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.

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

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http://dx.doi.org/10.1016/j.bmcl.2016.08.004
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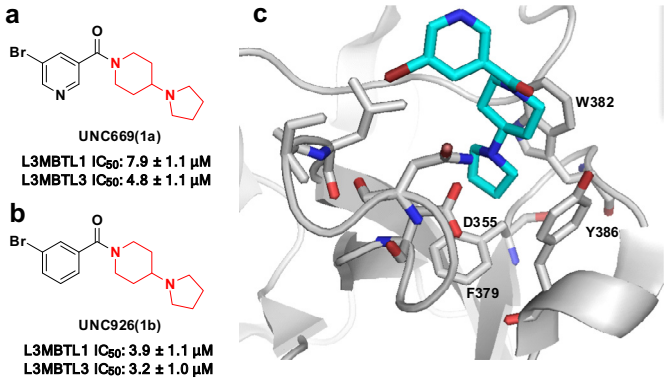


Figure 1. Structures of L3MBTL1/L3MBTL3 inhibitors. (a) Structure of **UNC669**. (b) Structure of **UNC926**. (c) Co-crystal structure of **UNC669** with L3MBTL1 (pdb 3P8H).

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 b, 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] anti-tumour 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.

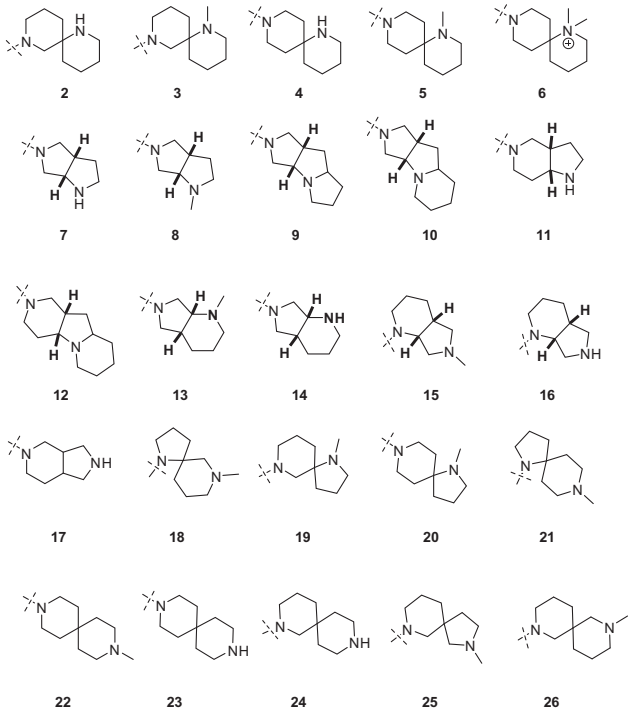
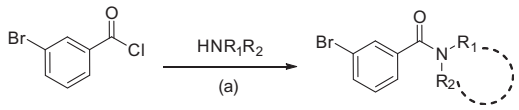


Figure 2. Structures of compounds containing bi- or tricyclic fused amines or spirocyclic heterocycles. The core structure of all final compounds is shown in Scheme 1



Scheme 1. General procedure for target compound synthesis. (a) Et_3N, DCM, 0 °C to rt.

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 Fig. 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,