Recent Progress in Histone Demethylase Inhibitors

Tom E. McAllister[†,#], Katherine S. England[‡,§], Richard J. Hopkinson[†], Paul E. Brennan[‡,§], Akane Kawamura[†,#,], Christopher J. Schofield[†,*]*

† Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA, UK; # Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Old Road Campus, Roosevelt Drive, Headington, OX3 7BN, UK; ‡ Structural Genomics Consortium, University of Oxford, Old Road Campus, Roosevelt Drive, Headington, OX3 7DQ, UK; § Target Discovery Institute, University of Oxford, NDM Research Building, Roosevelt Drive, Headington, OX3 7FZ, UK.

Abstract

There is increasing interest in targeting histone *N*-methyl-lysine demethylases (KDM) with small molecules both for the generation of probes for target exploration and for therapeutic purposes. Here we update on previous reviews on the inhibition of the lysine-specific demethylases (LSDs or KDM1s) and JmjC families of *N*-methyl-lysine demethylases (JmjC KDMs, KDM2-7) focusing on the academic and patent literature from 2014 to date. We also highlight recent biochemical, biological and structural studies which are relevant to KDM inhibitor development.

Introduction

The biological importance of the methylation and demethylation of lysine and arginine side-chains is of increasing interest from both basic science and pharmaceutical perspectives.[1] Along with other post-translational modifications, including acetylation, phosphorylation and ubiquitination, the dynamic methylation of the tails of the histone H3 and H4 proteins plays central roles in the regulation of transcription.[2] There are now multiple genetic links between the catalytic domains that catalyse histone tail modifications and diseases, both in development and in adults (principally cancer at present).[3] The clinical success of histone deacetylase inhibitors has stimulated work on the analogous demethylases (KDMs);[4] clinical trials have been recently initiated on KDM inhibitors. Two families of KDMs have been identified, i.e. the flavin-dependent lysine-specific demethylases (LSDs or KDM1s) and the larger family of 2-oxoglutarate (2OG)-, ferrous iron- and oxygen-dependent demethylases (JmjC KDMs).[5] The peptidyl arginine deiminases (PADIs) also catalyse loss of methyl groups from *N*-methylated arginine residues, but with concomitant hydrolysis, and therefore are not strictly demethylases.[6]

The purpose of this mini-perspective is to provide an update on previous reviews of histone demethylase inhibitors[7] focusing on the academic and patent literature from the beginning of 2014 to date, as well as highlighting related research that may be of use in inhibitor development. We begin by giving a brief overview of the field, identifying challenges and then summarising recent advances in structural / mechanistic studies relevant to inhibitor development, highlighting relevant contemporary controversies in functional assignment.

1. Overview of KDM catalysis

Although evidence for the reversible methylation of histones has long been available,[8] until the discovery of the first KDM (lysine specific demethylase 1, LSD1 or KDM1A) in 2004,

lysyl methylation on histone proteins (and indeed on other proteins) was considered by many to be an irreversible modification.[9] To date, two families of KDMs (KDM1s and JmjC KDMs) have been identified, which both use oxidative mechanisms to catalyse *N*-methyl-lysine demethylation.[10] The LSD enzymes (KDM1A/B in humans) are members of the amine oxidase superfamily that couple substrate oxidation to the reduction of flavin adenine dinucleotide (FAD). Mechanistic studies suggest that this process involves electron transfer / hydride transfer steps that requires a lone pair on the methyl lysyl amine; as a consequence, KDM1s do not accept N^{ε}-trimethyl-lysyl residues as substrates (Figure 1).[11] The JmjC KDMs, which are members of the 2OG- and ferrous iron-dependent oxygenase (2OG oxygenase) superfamily, catalyse the oxidative decarboxylation of 2OG to form a highly reactive iron(IV)-oxo species that acts to hydroxylate the *N*-methyl group, forming an unstable hemi-aminal which fragments to give the demethylated product and formaldehyde (Figure 1). The JmjC-KDM mechanism does not require a lone pair on the *N*-methylated substrate and therefore, in contrast to the KDM1 enzymes, the JmjC KDMs can additionally catalyse demethylation of N^{ε}-trimethyl-lysine (note: some JmjC KDMs do not accept N^{ε}-trimethyl-lysine substrates, possibly for steric reasons).[10a] Overall, in structural terms, both the KDM1 and JmjC KDMs appear to be reasonably representative of the enzyme families to which they belong, as reflected in their general active site architectures and overall folds (though the precise folds are subfamily-specific).[12] However, the oligomeric nature of the KDM substrates means that the KDM-histone / nucleosome interactions should be considered as much protein-protein interactions as 'normal' enzyme-substrate interactions. Here we do not discuss structures of the KDMs in detail, since these have been previously reviewed;[12] however, it should be noted that multiple new crystal structures of KDM1A (PDB IDs: 4KUM,[13] 4CZZ,[14] 4UVA,[15] 4UVB,[15] 4UVC,[15] 4UV8,[15] 4UV9[15] and 4UXN[16]) and of JmjC KDMs

(KDM2A structures, PDB IDs: 4QXB,[17] 4QXC,[17] 4QXH,[17] 4QX7,[17] 4QX8,[17] 4QWN[17] and 4TN7;[17] KDM4 structures, PDB IDs: 4XDO,[18] 4XDP,[18] and 4URA;[19] KDM6B structures, PDB IDs: 4V2V[20] and 4V2W[20]) have been reported. These structures along with those of related human enzymes should be of use in developing inhibitors selective for specific sets of KDMs.

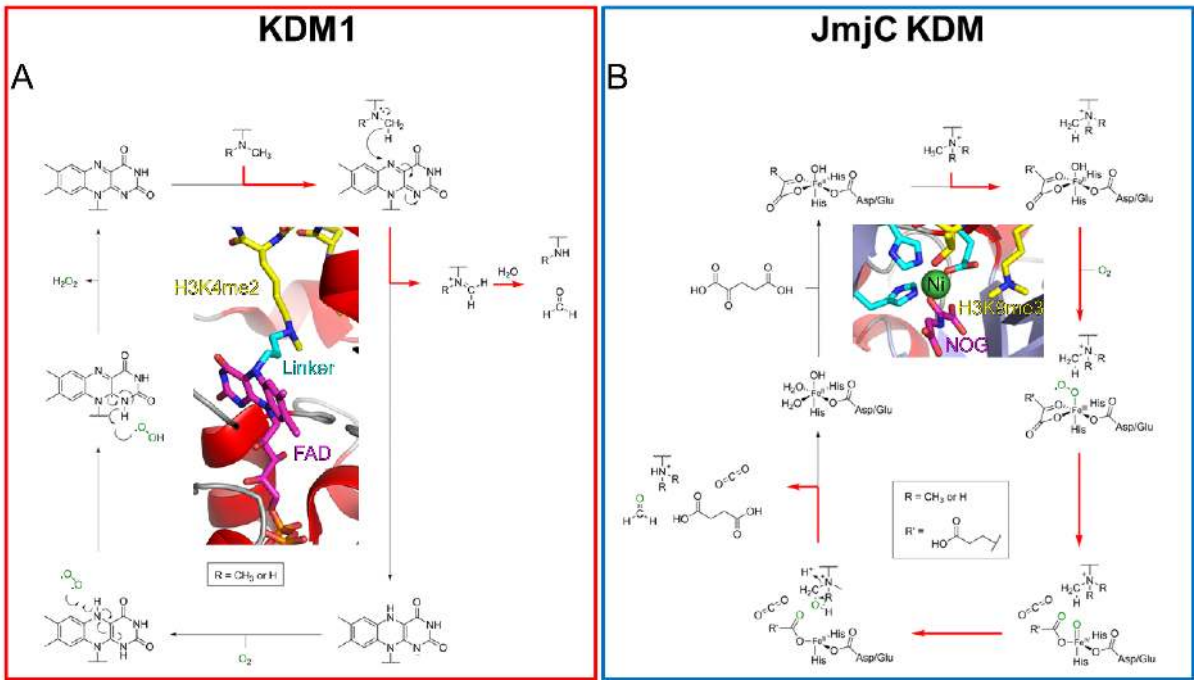


Figure 1. Outline of the demethylation mechanisms for the KDM1 (LSD) and JmjC KDM enzymes. (A) The KDM1 enzymes (KDM1A and KDM1B) are members of the amine oxidase superfamily that couple oxidation of the methyl group to reduction of the cosubstrate flavin adenine dinucleotide (FAD), likely via transfer of hydride. The resultant iminium ion intermediate is unstable and reacts with water to give the demethylated product and formaldehyde. The reduced FAD is reoxidised by molecular oxygen, forming hydrogen peroxide. The insert shows a view from a crystal structure of KDM1A (PDB 2UXN); (B) JmjC KDM catalysis proceeds via oxidative decarboxylation of 2-oxoglutarate to give succinate, carbon dioxide and a Fe(IV)=O species which catalyses methyl group oxidation to give a

hemiaminal which fragments to give the demethylated product and formaldehyde. The insert shows a view from a crystal structure of KDM4A complexed with H3K9me3, *N*-oxalylglycine susbtituting for 2-oxoglutarate, and nickel for iron (PDB 2OQ6).

Most, but not all, assigned KDM substrates occur on the *N*-terminal tail of histone H3; however, there is emerging evidence that some KDMs are able to catalyse demethylation of methyl-lysine residues on other proteins, including non-histone proteins.[20-21] There is thus the possibility that KDM catalysis is important in multiple biological processes, including, but not limited to, transcriptional regulation.

It is important to note that the KDM demethylation activities occur within the context of multicomponent and dynamic complexes. Indeed, all the KDM domains occur in proteins containing other domains, of which some have been assigned functional roles.[5] Notably, to date these 'adjunct' domains are thought to involve non-covalent interactions, sometimes directly involving the regulation of KDM activity, as exemplified in the case of the KDMs KIAA1718 (KDM7A) and PHF8 (KDM7B), where plant homeobox domains (PHD) bind N^{ϵ}-trimethylated lysine at H3K4 and direct the KDM domains to H3K27 and H3K9 respectively.[22] Further, the KDM activity of KDM1A is regulated *in vitro* and *in vivo* by interactions with CoREST (RE1-silencing transcriptional factor corepressor 1), which is proposed to increase binding of KDM1A to its nucleosomal substrates as well as to protect it from proteasomal degradation.[23] The roles of these adjunct domains coupled with the inherently context-dependent nature of the biochemistry underlying epigenetic regulation, have likely contributed to uncertainties regarding the assigned biochemical selectivities of the KDMs. For example, there has been debate whether the KDM1s are able to catalyse demethylation at both H3K4 and H3K9, and as to the precise roles of their interactions with CoREST.[24]

Recent biochemical work has indicated that the substrate selectivity of some JmjC KDMs may be broader than initially proposed, e.g. isolated KDM4 subfamily enzymes can accept H3K27-methylated substrates as well as their established substrate, i.e. methylated lysines at H3K9 and H3K36.[20] With respect to inhibitor selectivity, it is also important to note that the emerging nature of the field means that reported values may need to be revised as new assignments / data emerges, as exemplified by a discussion on the selectivity of the reported KDM6 inhibitor 3-((6-(4,5-dihydro-1H-benzo[d]azepin-3(2H)-yl)-2-(pyridin-2-yl)pyrimidin-4-yl)amino)propanoic acid (GSK-J1).[25]

In addition to the sites of demethylation, there has been a recent discussion over the type of reactions catalysed by some of the JmjC 2OG oxygenases. Early bioinformatics studies identified ~20 human JmjC proteins with predicted oxygenase/demethylase activity.[5, 26] Although many of these predictions have proven to be correct, some are controversial. The JmjC KDMs are part of the wider family of 2OG oxygenases, some of which catalyse the formation of stable protein hydroxylations, as compared to the apparently unstable hemi-aminal products of KDM catalysis, as initially shown in work on collagen prolyl- and lysyl-hydroxylation as well as epidermal growth factor-like protein aspartyl- and asparaginyl-hydroxylation.[27]

The hypoxia-inducible transcription factor (HIF) undergoes both prolyl- and asparaginyl-hydroxylation as catalysed by the HIF prolyl hydroxylases (PHDs1-3) and Factor Inhibiting HIF (FIH), respectively.[27-28] These modifications are central to the sensing mechanism of the hypoxic response, and as an aside, should likely be part of selectivity screens for JmjC KDM inhibitors. The HIF asparaginyl hydroxylase FIH was likely the first JmjC 2OG oxygenase to be identified;[26b, 29] FIH does not have KDM activity, but does accept multiple other protein substrates from the Ankyrin repeat domain family.[30] A subset of JmjC proteins are more

closely related to FIH by sequence compared to other JmjC proteins. These include MYC-induced nuclear antigen 53 (MINA53) and nucleolar protein 66 (NO66), which have been assigned in the literature as JmjC KDMs.[31] Recent work including biochemical, cellular and crystallographic analyses have provided strong evidence (though arguably we are biased in this regard) that MINA53 and NO66 are not KDMs, but are hydroxylases (i.e. catalyse stable hydroxylation) acting at the 2-position of histidine residues in the ribosomal proteins L27a and L8 respectively.[32] Work with isolated proteins, including NMR assignments of products, and qualitative cellular studies support these assignments though the full biological roles of MINA53 and NO66 are yet to be unravelled.

JMJD4 has also been recently identified as a 'hydroxylase' catalysing hydroxylation at the 4-position of a lysine residue in elongation release factor 1.[33] The most controversial of the JmjC enzymes is JMJD6, which has been the subject of a recent focused review.[34] JMJD6 has been assigned as both an *N*-methyl-arginine demethylase[35] and as a C5-lysyl-hydroxylase,[36] in the latter case within arginine-serine-rich regions of splicing regulatory proteins and on the histone H3 *N*-terminal tail. From a biochemical perspective the assignment of lysine hydroxylase activity of JMJD6 appears secure (on the basis of NMR evidence),[36b] but its *N*-methyl-arginine demethylase activity is controversial; whatever its biochemical/cellular roles, JMJD6 does appear to have important physiological roles.[34]

It should also be noted that some JmjC domains may not have any catalytic activity, due to substitution at key residues in their active sites. These include PHF2, JARID2 and Hairless, which are all predicted to have altered metal binding sites. In particular, JARID2 is predicted to retain only one residue (a histidine) of the typical metal binding triad (usually consisting of two histidines and one aspartate/glutamate in an HxD/E…H motif) and is therefore unlikely to bind iron (unless nearby residues are able to assume iron-binding).[5] PHF2 and

Hairless contain both one histidine residue and one carboxylate residue (an aspartate in PHF2, a glutamate in Hairless), but the third iron-binding residue is different; in PHF2, the second histidine residue is substituted for a tyrosine residue (HXD…Y), whereas Hairless has a cysteine residue in place of the first histidine of the HxD motif (i.e. CXE…H).[37] It is possible that these enzymes can bind iron, and may therefore be catalytically active (indeed, structural studies on PHF2 indicate metal binding and there are some reports of KDM activity for both PHF2 and Hairless acting on N^{ϵ}-dimethyl-lysine at position 9 of histone H3).[37] It is important to note that even if these 'pseudo' enzymes do not have catalytic activity, they may still bind histone methylation marks and/or inhibitors targeted at JmjC KDMs at the metal centre or in substrate binding pockets, with potential biological consequences.

Finally, one JmjC protein that was previously reported not to have KDM activity has been recently found to catalyse demethylation. UTY (KDM6C), the male-specific homologue of JmjC KDM KDM6A (UTX), was reported to be inactive based on cell-based work,[38] but its catalytic domain is near-identical in sequence to that of KDM6A, including its iron-binding residues, suggesting KDM activity may be possible. Biochemical analyses using isolated protein and histone fragment peptides have revealed KDM activity for UTY, acting on the same methylated residue as KDM6A (lysine 27 of histone H3);[39] however, the activity of UTY in current assays is greatly reduced relative to KDM6A and KDM6B, which was attributed predominantly to the presence of a proline residue in the substrate binding pocket of UTY (which is an isoleucine residue in KDM6A and KDM6B). Notably, for inhibition purposes, UTY activity is inhibited by the same types of inhibitors developed as KDM6A/B inhibitors.[25, 39]

In the following sections we describe recent progress on KDM inhibitors focusing on studies over the last 2-3 years, i.e. since previous reviews. To date, reported work has

almost exclusively focused on separately developing KDM1 and JmjC KDM inhibitors; hence we have separated descriptions of the two enzyme types. However, a recent report describes compounds with bifunctional KDM1 and JmjC KDM activities,[40] and more work on such compounds is of interest. In each case, we have separated descriptions of work reported in the academic and patent literature (the term patent is used with respect to both patent applications and granted patents), giving examples of exemplary chemotypes in the latter case. All inhibition data presented in this review are from original materials, including patents where data have not been subject to peer review.

2 KDM1 inhibitors

Most work on KDM1 inhibitors has focused on development of the established 'mechanism-based' monoamine oxidase (MAO) inhibitors: tranylcypromine **±1**, phenelzine **2** and pargyline **3**, which act by covalently binding to the FAD co-factor (Figure 2). While tranylcypromine derivatives dominate the literature (including hybrid compounds **±4** and **±5**,[40] dual LSD/JmjC KDM inhibitors; Figure 2) there have also been a few examples of development of phenelzine- and pargyline-based compounds. However, in recent years work on reversibly binding inhibitors based on heteroaromatic scaffolds have been increasing - possibly driven by concerns about selectivity *in vivo* for covalently reacting inhibitors. Excitingly for the KDM field, one of these compounds **6** (GSK2879552; Figure 3)[41] is currently in phase I clinical trials for two types of cancer.[42] It should also be noted that most of the compounds have been developed for selectivity profiles for KDM1A over MAOs, with little focus on KDM1B inhibition. Given that there are only two human KDM1s (A and B) this is likely to change in the future. Furthermore a recent report details the development of tranylcypromine derivatives as selective dopamine D3 receptor agonists, highlighting the need to rigorously establish and distinguish between on target and off target effects.[43]

2.1 Covalently Reacting KDM1 Inhibitors

Academic Literature

Prusevich *et al.* have described a series of compounds with different substitutions on the phenelzine core; compound **7** was the most potent KDM1A inhibitor (K_i of 59 nM). **7** was > 20-fold selective for KDM1A over the mechanistically related human enzymes MAO A and MAO B.[44] Schmitt *et al.* have reported pargyline derived KDM1 inhibitors that display cellular activity. The biphenyl derivative **±8** is reported to inhibit MCF7 tumour proliferation growth by 54% at 100 µM and also increased levels of H3K4me2. However, *in vitro* assays did show that this compound was a more potent inhibitor of the related enzymes MAO A and MAO B than KDM1A itself; with IC_{50} values of 0.55, 0.06, and 44 µM, respectively (Figure 2).[45]

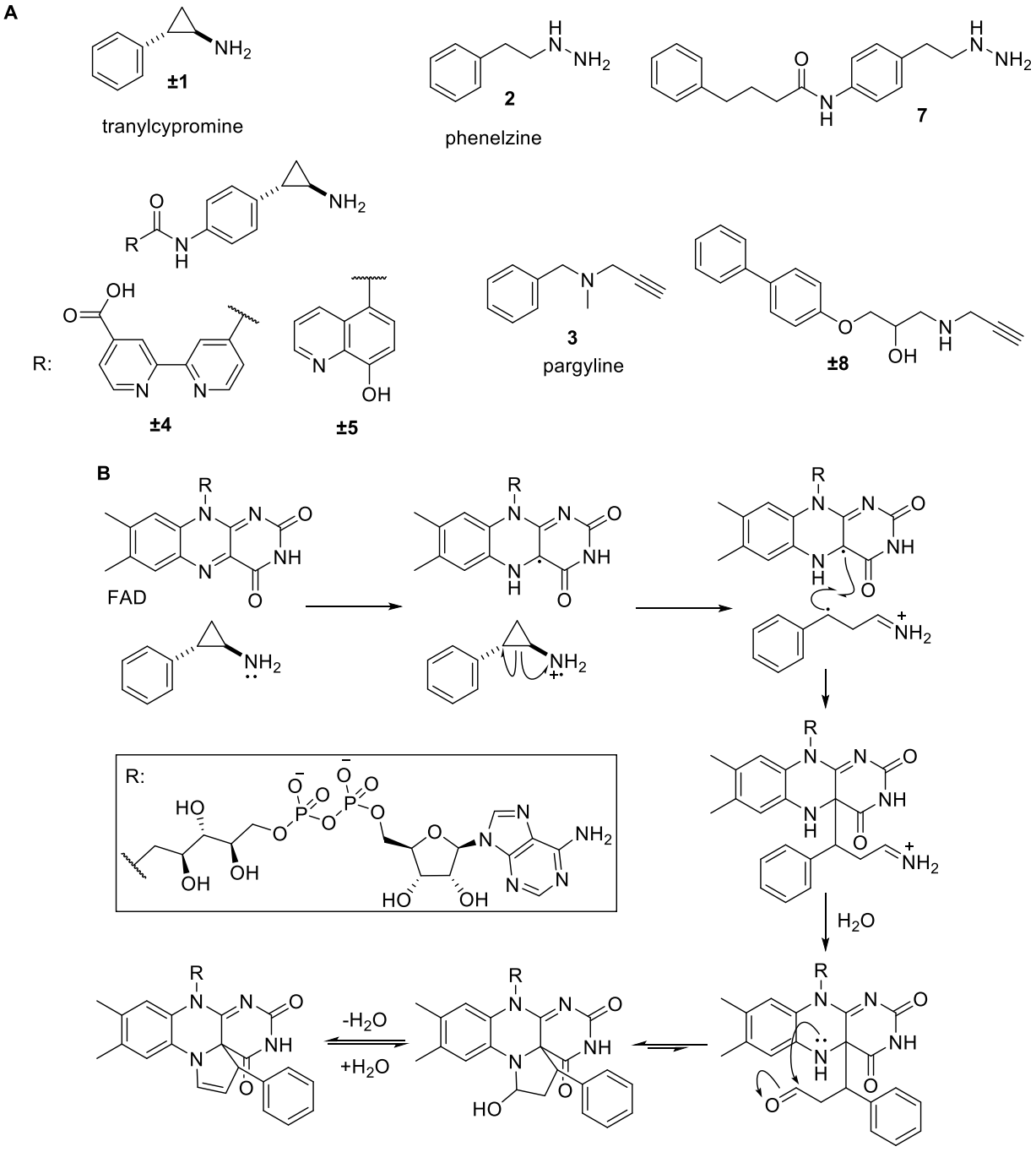


Figure 2. Representative structures and mechanism of established types of monoamine oxidase inhibitors. (A) Tranylcypromine **±1**, phenelzine **2** and pargyline **3** with tranylcypromine-derived pan-KDM inhibitors **±4** and **±5**, phenelzine derivative **7** and pargyline derivative **8**; (B) proposed mechanism of covalent adduct formation between the FAD cofactor of KDM1A and tranylcypromine **±1**.[46]

There has been extensive work on tranylcypromine derivatives, modified on either or both of the phenyl group and the amino group. It is important to note that tranylcypromine **±1** itself and many of the reported derivatives have been prepared as racemic mixtures of *trans*-substituted cyclopropanes and not as single enantiomers. Throughout this review, racemic mixtures of compounds have the relative stereochemistry of the groups indicated by bold hashed and solid bonds and the compound number is prefixed with "±" (as in tranylcypromine **±1**). For single enantiomers the stereochemistry is indicated by wedge-shaped hashed and solid bonds (as in compound **9**).

Early work from Benelkebir *et al.* with phenyl-substituted analogues, revealed that the incorporation of a *para*-bromo substituent into the (*1R,2S*) isomer **9** (Figure 3) could increase the *in vitro* K_{inact} by around 7-fold and reduce IC_{50} by 1000-fold in cell-based assays compared to tranylcypromine **±1**.[47] Derivative **±10** was more recently described and has an ethyl group *cis* to a *meta*-bromophenyl substituent on the tranylcypromine core.[48] Racemic **±10** had an IC_{50} of 31 nM (25-fold more potent than the corresponding compound lacking the bromine), the most potent of the compounds tested. Vianello *et al.* previously produced single enantiomers of tranylcypromine compounds with a quarternary carbon adjacent to the amine; of the two ethyl substituted isomers, **11** the (*1S,2R*) was more potent against MAO B (88 nM) than KDM1A (608 nM) while **12** (*1R,2S*) was less potent but displayed better MAO selectivity (IC_{50} 975 nM against KDM1A, 30-fold selective compared to MAO B). The phenyl substituted compounds **13** (*1R,2S*) and **14** (*1S,2R*) have IC_{50} values of 584 nM and 131 nM respectively for KDM1A, with good selectivity over MAO B; however, both **13** and **14** showed higher potency against MAO A than KDM1A. Modification of **14** to the benzyl derivative **15** created a less potent but a more selective inhibitor with IC_{50} 335 nM against

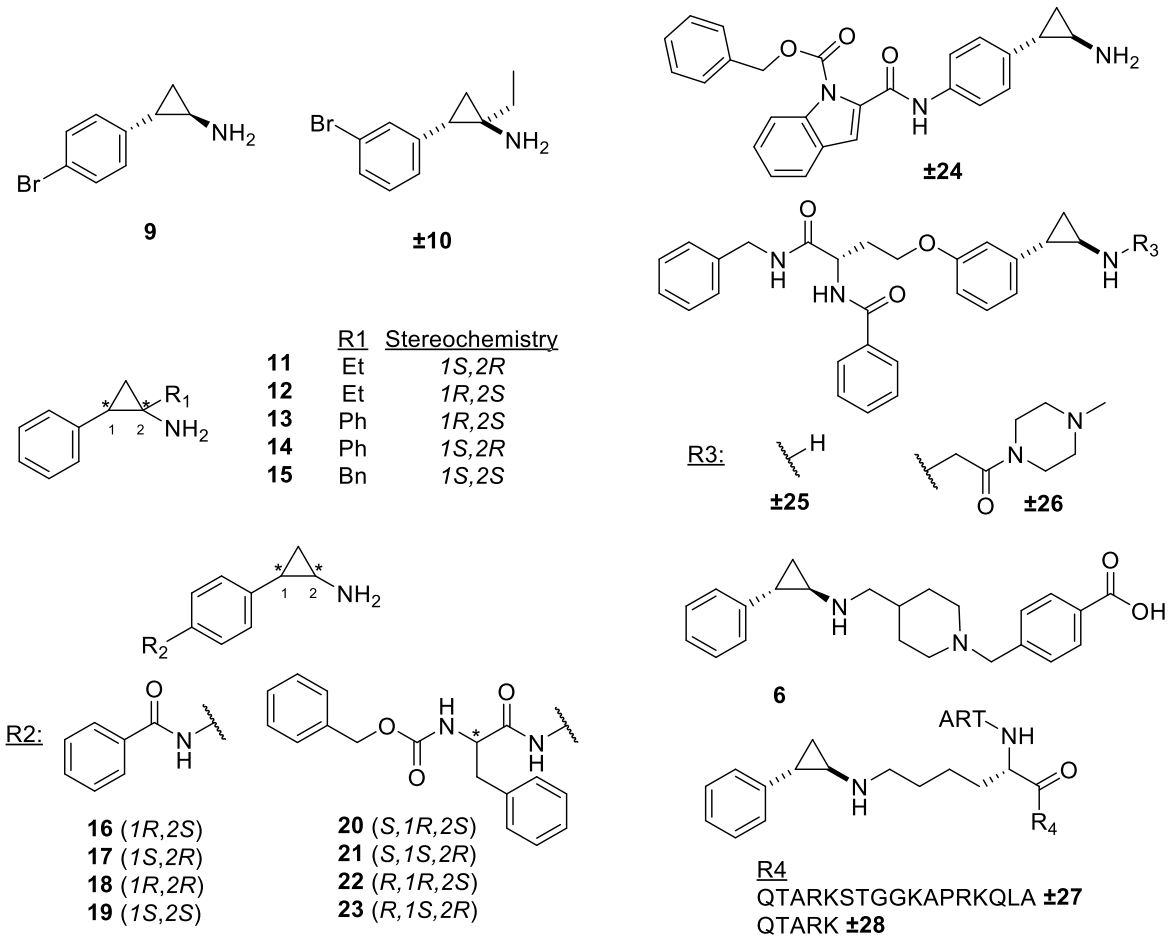
KDM1A and 2.59 μM against MAO A. The opposite enantiomer was also synthesised but an IC_{50} value for KDM1A could not be determined as it interfered with the assay used.[15]

Two series of enantiomerically pure compounds **16** - **19**[49] and **20** - **23**[50] were made to explore the influence of tranylcypromine stereochemistry on KDM1A inhibition. The *para*-benzoylamino series of *trans*- (**16** and **17**) and *cis*-isomers (**18** and **19**) were all potent KDM1A inhibitors, but exhibited little difference between isomers in the *in vitro* assay (all around 20 nM) and all four compounds also exhibited similar inhibition levels against MAO A. The second series of compounds (**20** - **23**)[50] with a phenylalanine fused side chain showed that the stereochemistry of the phenylalanine is not important, but that the (*1S*,*2R*) arrangement (compounds **21** and **23**) around the cyclopropane ring gave inhibitors 10 times more potent than the (*1R,2S*) stereoisomer. Interestingly, this trend was not repeated in the compounds' respective inhibition of MAO A.

Other recently reported KDM1 inhibitors based on tranylcypromine **±1** are relatively diverse. A racemic series of differently substituted pyrrole and indole-containing derivatives, of which **±24** was most potent (IC_{50} KDM1A : 40 nM, MAO A: 160 nM, MAO B: > 60 μM) has been reported.[16] Ahmed Khan *et al.* used the known inhibitor *N*-[(1*S*)-3-[3-(trans-2-aminocyclopropyl)phenoxy]-1-(benzylcarbamoyl)propyl]benzamide **±25** (NCL1) as a starting point and by extending from the amino group they produced **±26**, which had an IC_{50} value of 380 nM against isolated KDM1A (compared with 2.5 μM for **±25**).[51]. **±26** displayed excellent selectivity (IC_{50} > 100 μM) against MAO A and MAO B and cellular activity with GI_{50}s of 109 and 42 μM using HeLa and SH-SY5Y cells. Recently published work from GlaxoSmithKline and Johns Hopkins University revealed that dialkylamine **6** with an unmodified phenyl group on the cyclopropylamine and substantial additional modification to the amino group had a K_i value of 1.7 ± 0.5 μM *in vitro*. Furthermore the data presented for **6** show that small cell

lung carcinoma and acute myeloid leukaemia tumour cell lines responded to catalytic inhibition of KDM1A by **6** as demonstrated by cytostatic growth inhibition[41b] and phase I clinical trials are underway for both conditions.[42]

Kakizawa *et al.* took a novel approach by linking a histone 3 substrate peptide sequence with a KDM1 inhibitor through a modified lysine residue at position 4 (analogous approaches have been pursued with JmjC KDMs)[52] several inhibitors were explored with tranylcypromine being the most effective.[53] The longest peptide adduct **±27** was the most potent inhibitor; the peptide length could be reduced from 21 to 9 residues (to yield **±28**) without substantial loss of potency (compounds **±27** and **±28** are racemic mixtures at the tranylcypromine but all other stereo centres are single isomers). Further truncation significantly reduced potency against KDM1A and selectivity against MAO A/B was very high for both **±27** and **±28** (Figure 3).



Compound	IC_{50} (μM)			Compound	IC_{50} (μM)		
	KDM1A	MAO A	MAO B		KDM1A	MAO A	MAO B
9[a]	8.9	N/R	N/R	**19**	0.022	0.025	21.20
±10	0.031	N/R	N/R	**20**	0.36	0.11	42.65
11	0.608	1.43	0.088	**21**	0.03	0.14	32.02
12	0.975	6.8	33.3	**22**	0.39	0.20	14%[b]
13	0.584	0.235	5.55	**23**	0.05	0.22	33%[b]
14	0.131	0.094	11.5	**±24**	0.04	0.16	>60
15	0.335	2.59	28.1	**±26**	0.38	>100	>100
16	0.013	0.039	12.29	**6**[a]	1.7	N/D	N/D
17	0.021	0.024	16.81	**±28**	0.148	>100	>100
18	0.026	0.037	19.17	**±28**	0.443	>100	>100

Figure 3. Development of KDM1A inhibitors based on the tranylcypromine scaffold reported in the academic literature. Sources for IC_{50} values are cited in the main text. N/R –

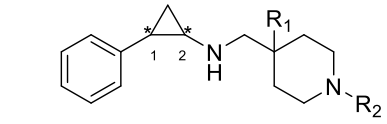
not reported; N/D not determined – inhibition level too low to quantify.[a] *Value reported is* K_i; [b] *value is percentage inhibition at 100 μM compound.*

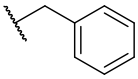
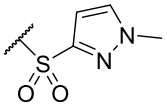
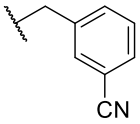
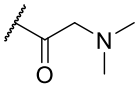
Patent literature

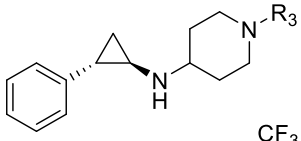
A GlaxoSmithKline patent describes KDM1A inhibitors predominantly extended on the cyclopropylamino group with C-4 methylene and *N*-alkylated piperidine substituted compounds (including **6,** covered in the previous section).[41a] Some of the most potent examples include the relatively simple enantiomerically pure compound **29** and racemic mixtures **±30** and **±31** (Figure 4). All three showed excellent selectivity against KDM1A over MAO B in an *in vitro* assay with IC_{50} values for KDM1A all below 10 nM (no data on MAO A was reported). A racemic version of **29** has been made available as a chemical probe for KDM1, as part of the Structural Genomics Consortium initiative.[54] The Incyte Corporation used a closely related scaffold in patents detailing extensively functionalised piperidine rings with two examples being **32** and **33**, both of which showed *in vitro* IC_{50} values of less than 100 nM.[55] Another series from Incyte contained variants lacking the methylene linkage between the tranylcypromine amine and the piperidine, with the piperidine ring further appended with diverse groups. Heterocylic amine-containing **34** and carboxylate **35** both had IC_{50} values below 100 nM against KDM1A; for these and **32**/**33** no activity against other enzymes (e.g. MAO A and B) was described.[56]

Compound **36**, one of a series described in a Kyoto Prefectural Public University patent, has an amino modification based on lysine with aromatic groups derivatising the α-amino acid. These modifications confer selectivity and potency with IC_{50} values of 770 nM against KDM1A and > 100 μM MAO A/B *in vitro*.[57] A similar approach was taken by Imago Biosciences who synthesised a range of compounds around a substituted lysine scaffold. An

exemplar compound from this series, **37**, with a *para*-fluoro substituent on the tranylcypromine, a *C*-terminal *para*-fluorobenzylamine-derived amide and *N*-terminal benzamide has an IC_{50} value of < 1 μM against KDM1A; no selectivity data against MAO A and B were reported.[58] This approach of modifying both the phenyl- and amino-groups of tranylcypromine has been described in several other patents. Takeda Pharmaceutical Company report a series of modified tranylcypromine derivatives with **±38** having an IC_{50} value below 1 μM against KDM1A and showing > 10-fold selectivity over MAO A/B *in vitro*. In a cellular assay, exposure of cells to **±38** at 1 μM concentration caused H3K4 methylation levels to increase by over 2-fold.[59] A similar series of KDM1A inhibitors bearing an additional group in the cyclopropane ring are described in a Istituto Europeo di Oncologia patent, of which racemic compound **±39** is a promising example with an IC_{50} value below 100 nM and greater than 100-fold selectivity against MAO B.[60] A further series of compounds was reported in a patent from the University of Nevada; compound **40** had an IC_{50} of 21.25 μM in a cell based assay.[61] Oryzon Genomics have shown compounds such as **41** with a biphenyl group further substituted with an isopropylsulfonamide are KDM1A inhibitors. **41** showed an *in vitro* IC_{50} value below 70 nM against KDM1A with over 10- and a 100-fold selectivity over MAO A and MAO B respectively (Figure 4).[62]



		R1	R2
29	(*1S,2R*)	H	H
±30	(*1S,2R*) & (*1R,2S*)	H	
±31	(*1S,2R*) & (*1R,2S*)	H	Me
32	(*1S,2R*)		
33	(*1S,2R*)		



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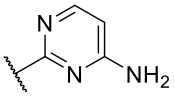

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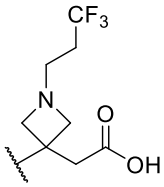

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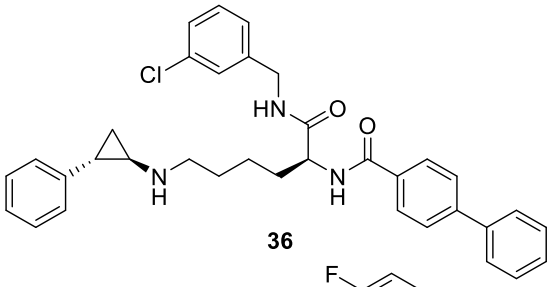

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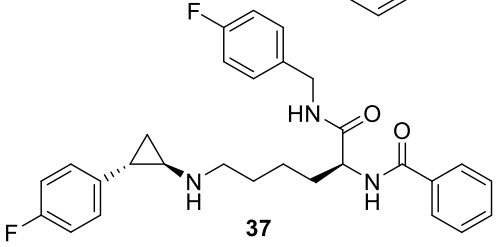

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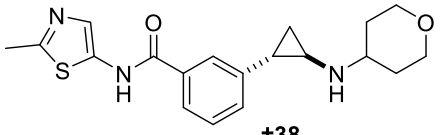

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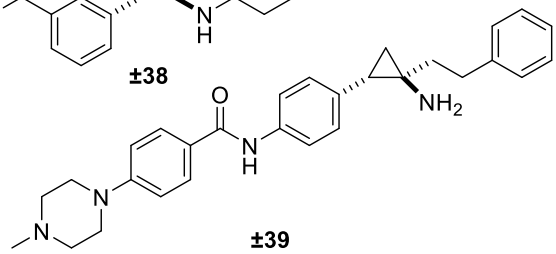

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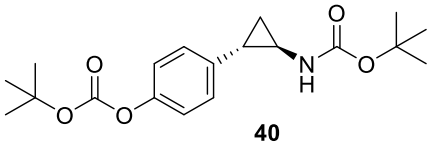

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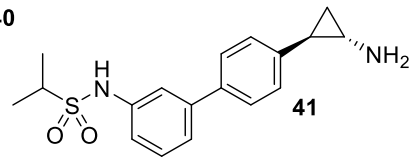

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Compound	IC$_{50}$ (μM)			Compound	IC$_{50}$ (μM)		
	KDM1A	MAO A	MAO B		KDM1A	MAO A	MAO B
29 ex5[59]	0.005	N/R	79	**36** ex21[57]	0.77	>100	>100
±30 ex8[59]	0.006	N/R	25	**37** ex4[58]	<1	N/R	N/R
±31 ex20[59]	0.006	N/R	25	**±38** ex212[59]	<1	>10	>10
32 ex116[55a]	<0.1	N/R	N/R	**±39** exA116[60]	<0.1	>10	>10
33 ex57[55b]	<0.1	N/R	N/R	**40** ex10[61b,a]	21.25	N/R	N/R
34 ex30[56b]	<0.1	N/R	N/R	**41** ex8[62]	0.07	>1	>10
35 ex130[56a]	<0.1	N/R	N/R				

Figure 4. Developments of KDM1A inhibitors based on the tranylcypromine scaffold reported in the patent literature. ex - compound example; N/R – not reported; [a] cell-based assay.

2.2 Reversible KDM1 Inhibitors

Recent work in the academic literature has reported on the development of new types of KDM1 inhibitor, in particular those not employing mechanism-based covalent modification. The first non-covalently binding KDM1 inhibitors to be reported were probably polyamine derivatives, which, like tranylcypromine **±1** and phenelzine **2** were first identified as inhibitors of the related monoamine oxidases. Several reports, including some describing cell-based work, reveal KDM1 inhibition by a variety of related polyamines including representative octa/decamines, bisguanidines, bisbiguandinines, bisureas and bisthioureas. Nowotarski *et al.* investigated the effect of chain-lengths and linking groups (e.g. guanidines, ureas, thioureas etc.) on a series of oligoamines.[63] The results indicate that the 3-5-3 backbone geometry (of methylene units between linking groups and amines) is beneficial for KDM1A inhibition, as are the inclusion of thiourea linking groups relative to ureas/guanidines. The most active of these inhibitors have diphenyl groups and their potencies appeared dependent on the distance between the thiourea and the diphenyl moieties with the longer link (2 methylene units) **42** (Figure 5) showing most potent KDM1A inhibition (IC_{50} = 5 μM). MAO B was also inhibited by **42**, with IC_{50} value of 19 μM though MAO A was not obviously affected (IC_{50} > 100 μM). **42** also showed activity in cell viability assays using Calu-6 lung tumour cells and MCF7 cells with IC_{50} values below 10 μM. There have been limited efforts to determine the mechanism(s) of action of these polyamine compounds with respect to KDM1 inhibition or their cellular selectivities and their

pharmaceutical utility is probably limited. However, from a biological target validation perspective, these results are interesting because of the cellular roles of polyamines, including in binding DNA and RNA.

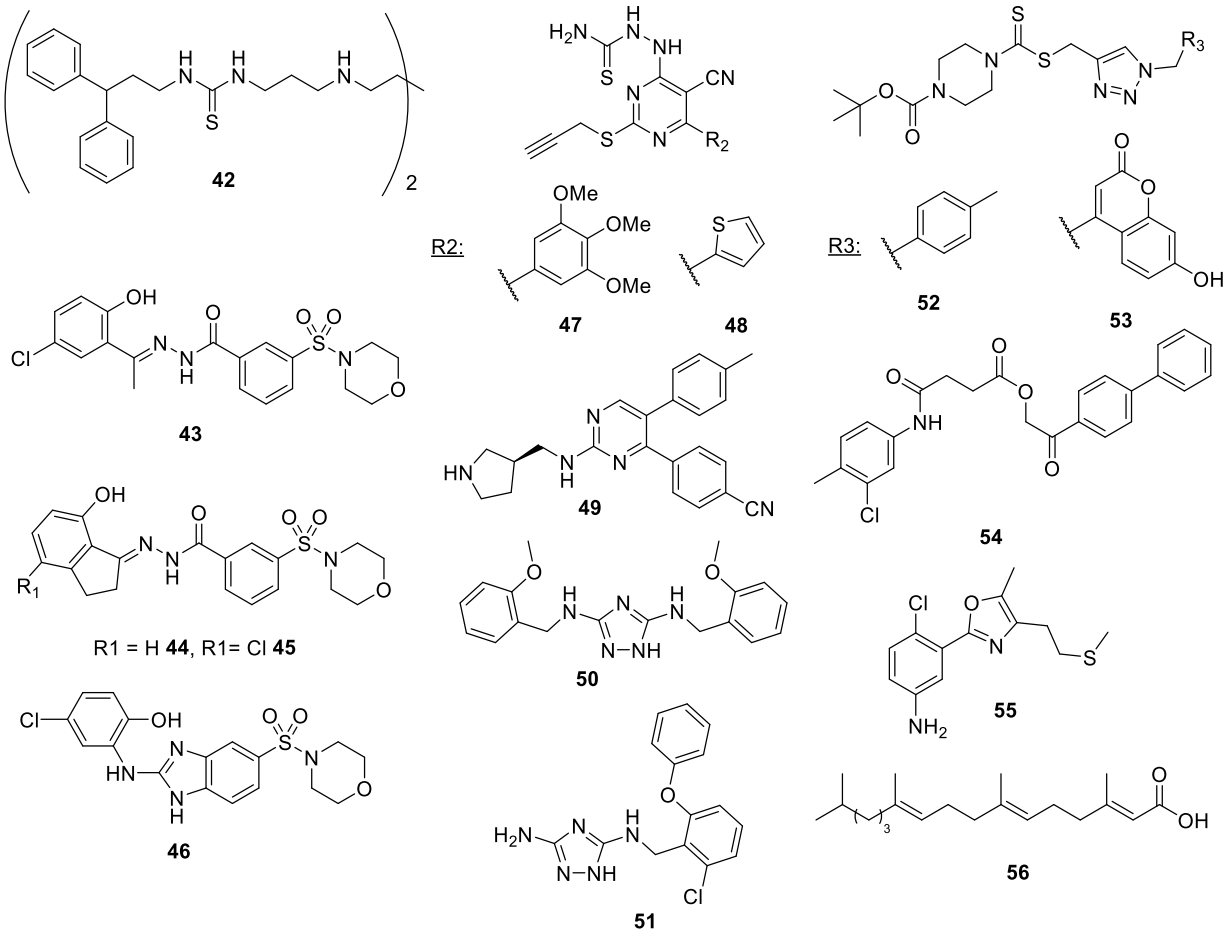
Other work has focussed on more classical types of reversibly binding KDM1 inhibitors, but as there are relatively few examples, the following section covers reports found within both the patent and academic literature. A series of indene-containing benzohydrazides has been reported by Zhou *et al.* who focused on restricting the conformational flexibility of their previously described inhibitor **43**.[64] The results reveal that indenes **44** and **45** were around 10-fold more potent than **43** with IC_{50} values of 1.4 nM and 1.7 nM compared to 13 nM for **43**. **43** - **45** showed good activity in tumour cell viability assays with IC_{50} values typically 3 µM or less. A similar series of compounds replacing the benzohydrazide moiety with an amino benzimidazole was described in a further patent and morpholino derivative **46** had an IC_{50} value of 90 nM against KDM1A.[65]

Various pyrimidine-based scaffolds are reported as potent reversible KDM1A inhibitors. First described in a patent in 2014[66] and later by a journal article from Ma *et al.*,[67] thiosemicarbazide-containing pyrimidines such as **47** and **48** have sub-micromolar *in vitro* IC_{50} values, alter H3K3me1/2 levels in and are cytotoxic across a range of gastric cancer cell lines. Through synthesis of a series of analogues the thiosemicarbazide was shown to be crucial in many cases, though substitution of this in **47** for a chloro- group only reduced potency 5-fold. Compound **47** was extensively evaluated *in vitro* with excellent (over 1000-fold) selectivity for KDM1A over MAO A and MAO B and a binding affinity (K_d) for KDM1A of 3.7 µM determined by biolayer interferometry. Quanticel pharmaceuticals reported more pyrimidine-based inhibitors in a patent, of which **49** is a typical example, with IC_{50} values of 100 nM *in vitro* and in cells (using a CD11b expression assay in THP-1 cells).[68]

Kutz *et al.* describe a series of 3,5-diamino-(1,2,4)-triazole inhibitors with the 2-methoxybenzyl derivatives **50** and **51** having the best *in vitro* IC_{50} values (1.19 and 2.22 μM, respectively) as well as selectivity over MAO A/B.[69] **50** and **51** caused an increase in H3K4me2 levels in Calu-6 cells but were not cytotoxic up to 100 μM. Zheng *et al.* utilised a (1,2,3)-triazole ring in their thiocarbamate compound **52**,[70] which displayed an IC_{50} value of 2 μM and a K_d value of 250 nM, which was determined using micro-scale thermophoresis. A series of biochemical experiments showed it to be competitive with the FAD cofactor. Selectivity was good against MAO A and B (IC_{50} > 1250 μM for both) though the compound was found to also inhibit KDM1B (IC_{50} 36 μM). Further development of this compound led to **53**, which contains a coumarin group in place of the *para*-tolyl group in **52**. **53** displayed 5-fold better potency against KDM1A and SAR revealed that substitution on the coumarin at the 7- or 8-position could increase potency while modification at the 6-postion was very detrimental.[71] Both **52** and **53** stimulated increase in H3K4me1/2 levels in gastric cancer cells. Zhou *et al.* employed an *in silico* screening step to reduce the number of potential KDM1A inhibitors in their panel from > 100,000 to around 10.[72] Of those subsequently tested *in vitro*, compound **54** was the most potent with an IC_{50} value of 2.4 μM for KDM1A and with weak inhibition of MAO A (IC_{50} 685 μM); MAO B, however, was also significantly inhibited (IC_{50} 27.5 μM).[73] Dulla *et al.* have also described a small molecule inhibitor, **55**, designed using available X-ray crystal structures of known non-covalent inhibitors bound to KDM1A. Their phenyl oxazole **55** has an *in vitro* IC_{50} value of 10 μM and IC_{50} values of 1.2 nM in cell viability assays against HeLa and MDA-MD-231 cells.[74]

An unexpected inhibitor was recently reported by Sakane *et al.* who investigated a range of terpenes as inhibitors of KDM1A.[75] In an *in vitro* assay, 14,15-dihydro geranylgeranoic acid **56** had the same IC_{50} value as tranylcypromine **±1** (around 20 μM). There are no

comparative data for **56** with MAO A/B but the lead compound from this study, farnesol, inhibited MAO B 60-times more effectively than KDM1A.



Compound	IC_{50} (μM)		
	KDM1A	MAO A	MAO B
42	5	>100	19
43	0.013	>300	>300
45	0.0014	N/R	N/R
45	0.0017	N/R	N/R
46	90	N/R	N/R
47	0.65	>1250	>1250
48	0.65	N/R	N/R
49	<0.01	N/R	N/R

Compound	IC_{50} (μM)		
	KDM1A	MAO A	MAO B
50	1.19	>100	>100
51	2.22	>100	>100
52	2	>1250	>1250
53	0.39	>1250	>1250
54	2.41	685	27.5
55	10.1	N/R	N/R
56	22	N/R	N/R

Figure 5. Structures of reversibly binding inhibitors of KDM1A and their inhibitory activities.

Sources for IC_{50} values are cited in the main text. N/R – not reported

2.3 Peptide-based KDM1 inhibitors

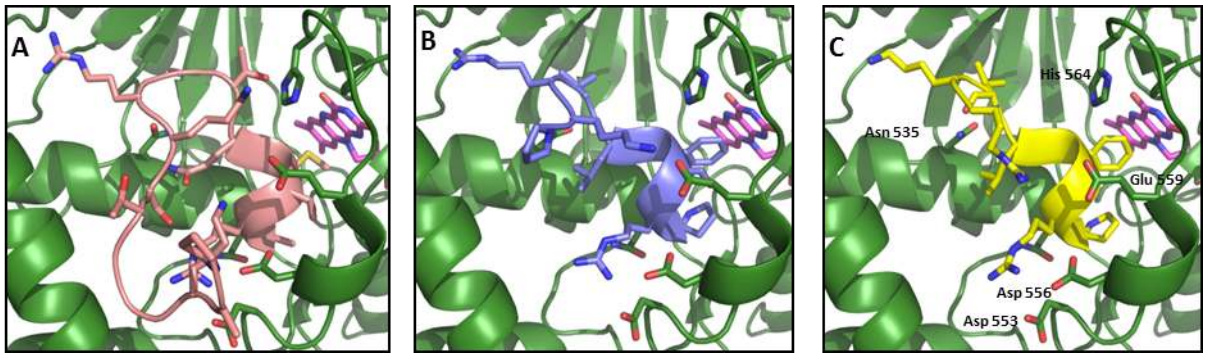
It has been reported that substitution of lysine-4 of a histone H3 peptide to methionine is sufficient to generate a potent competitive inhibitor (**57**) of KDM1A (K_i 40 nM).[76] A crystal structure of this inhibitor peptide bound to KDM1A-CoREST complex (Figure 6A) showed that in the bound conformation several side chains of **57** were in close proximity. Kumarasinghe and Woster set out to create cyclic peptides of the same sequence by linking proximal side chains together in various orientations.[77] The most potent of these features two additional substitutions, Q5K and S10E, which were condensed to form a cyclic peptide, **58**. The IC_{50} value for **58** against KDM1A was determined to be 2.1 μM and the K_i 385 nM, making **58** a slightly less potent inhibitor than **57**. Cyclic **58** displayed better metabolic stability than its linear counterpart, though the two compounds had almost identical activity in MCF-7 and Calu-6 cell viability assays (IC_{50} values around 120 μM).

Leurs *et al.* identified a linear peptide sequence (SHSEFWDWGPGGG) from a phage display screen that appeared to bind to KDM1A and inhibit; however, further investigation revealed that in fact this peptide bound to the GST-tag that had been used to purify the protein and not KDM1A itself.[78]

Tortorici *et al*. reported that linear peptides derived from SNAIL1 and INSM1 sequences could also act as KDM1A inhibitors.[79] SNAIL1 is a transcription factor that binds to the KDM1A active site through its SNAG (Snail/GFI) domain with the *N*-terminal 21 residues **59** adopting a similar conformation to the H3 substrate and acts as a competitive inhibitor (K_i = 210 nM). X-ray crystal structures show that, at least, the first 16 residues of H3 are involved

in binding to KDM1A (Figure 6B) whereas only the first 9 are resolved for SNAIL1 (Figure 6C). It was found that the *N*-terminal peptide **60** of another member of the same family of transcription factors, (Insulinoma-associated protein) INSM1, also bound to KDM1A with similar affinity (K_i 240 nM); crystallographic analysis (3ZMS) revealed that only the first 8 residues of **60** bind in an ordered conformation. Subsequent truncation of the SNAIL1 peptide revealed that the sequence could be truncated to a nonamer **61** which was slightly more potent than the longer 21-mer **59**. However, further truncation to the hexamer **62** (3ZMT) led to 100-fold loss in potency compared to the 21-mer **59**, despite the analogous residues binding in an almost identical manner. **61** was used as a starting point for further investigation; alanine-scanning substitution revealed that the *N*-terminal Pro-Arg dyad is important for tight binding while the other 4 residues are less important. Incorporation of a methionine at position 4 (as in the previously identified inhibitor peptide **57**) to yield **63** significantly improved potency relative to the endogenous phenylalanine (**62**), with an K_i value of 2.6 μM, only 10-fold less than the longer 21-mer peptide **59**. These data also provide an interesting insight into the binding of the H3-substrate to KDM1A; the first two residues of both SNAIL1 and INSM1 are a proline and an arginine while in H3 the analogous residues are an alanine and an arginine. The observed loss of affinity of the peptides with an *N*-terminal alanine compared to *N*-terminal proline may reflect the relatively poor binding affinity of short H3 peptides to KDM1A, as they lack the initial proline. It has been established that more residues of the longer H3 peptides (the first 16 residues as mentioned above) form binding interactions compared to the 9 and 8 ordered residues respectively found for these SNAIL1 **59** and INSM1 **60** peptides; perhaps this is to compensate for the weakly binding alanine at the *N*-terminus. A hybrid heptamer, **64** containing the first 4

residues of SNAIL1 and residues 5-7 of H3 showed an improved K_i value of 8 μM compared to the original SNAIL1 hexamer **62** (28 μM).



Peptide	Sequence	K_i (μM) KDM1A	Peptide	Sequence	K_i (μM) KDM1A
H3	ARTKQTARKSTGGKAPRKQLA	1.8	**61**	PRSFLVRKP	0.14
57	ARTMQTARKSTGGKAPRKQLA[a]	0.04	**62**	PRSFLV	28.4
58	ARTMK̲TARKE̲TGGKAPRKQLA[b]	0.385	**63**	PRSMLV	2.6
59	PRSFLVRKPSDPNRKPNYSE	0.21	**64**	PRSFQTV[c]	8.0
60	PRGFLVKRSKKSTPVSYRVR	0.24			

Figure 6. Peptidic inhibitors of KDM1A. Panels A-C show views from crystal structures of KDM1A in complex with various peptides; KDM1A is shown in green with the FAD cofactor in magenta. Key side chains of KDM1A residues are shown as sticks and explicitly labelled in panel C. Peptides are shown in different colours; panel A shows **58** in pink (2V1D), B shows **59** in purple (2Y48), C shows **60** in yellow (3ZMS). Sources for K_i values are cited in the main text. All peptides are N-terminal amines and C-terminal carboxamides except **57** which is a C-terminal carboxylate. No inhibition data against related enzymes MAO A/B are reported for any of the peptides; [a] C-terminal carboxylate; [b] peptide **58** has an amide formed by condensation of the side chains of K5 and E10 which are underlined; [c] this is the reported sequence though it does not correspond to the H3 peptide sequence in the manner described in the text which suggests it should be PRSFQTA.[79]

3. JmjC KDM inhibitors

The review period has seen a rapid increase in reports of JmjC KDM inhibitors. These efforts are being supported by advances in our understanding of JmjC KDM structures, principally informed by crystallography. The vast majority of reported inhibitors are likely 2OG competitors. Whilst it is clear that this approach is inherently not disfavoured (2OG competitive inhibitors of HIF hydroxylase enzymes are in late-stage clinical trials for the treatment of anaemia),[80] the complex molecular roles of the JmjC KDMs and experience with other enzyme families (e.g. kinases), suggests that new types of inhibitors are highly desirable. These may include inhibitors competing with the histone substrate(s), binding in 'allosteric' sites (the crystallographic analysis suggest uncompetitive inhibitors should be possible in some cases), or targeting non-catalytic domains of the JmjC KDMs, as has been done for a PHD domain of KDM5A.[81] In these regards, the development of new assays for inhibitor screening is an interesting development (e.g. yeast based screens).[82]

3.1 JmjC-KDM inhibitors – Academic Literature

Hydroxamic Acid Scaffolds

It has been reported that hydroxamic acids linked to tertiary amines or cyclopropane groups through alkyl-chains can efficiently inhibit KDM4 and KDM2A activity respectively.[83] Screening and development of this series against KDM5A, together with *in silico* docking studies, resulted in **65**, a selective inhibitor for KDM5A over other tested JmjC-KDMs.[84] The pro-drug **66** induced increased H3K4me3 levels in A549 lung cancer cells in a dose-dependent manner, and demonstrated synergy with vorinostat, a HDAC inhibitor, in inhibiting A549 cell growth.

Methyl (E)-4-(hydroxy(4-((4-(((naphthalen-1-ylcarbamoyl)oxy)methyl)benzyl)amino)butyl)amino)-4-oxobut-2-enoate **67** (Methylstat) is a

broad-spectrum JmjC-KDM inhibitor,[85] which was designed based on an HDAC inhibitor MS275 (Entinostat) and linked with a 2OG substrate binding mimicking hydroxamic acid. Marholz *et al.* linked the substrate mimic of **67** with a histone H3K36 peptide sequence and a photo-crosslinking group to generate a peptidic affinity probe **68** for histone demethylases.[86] This probe was used to purify KDM2A, a H3K36 targeting JmjC KDM, from a mixture of purified enzymes and histone proteins, and enrich other H3K36 targeting JmjC-KDMs from HeLa cell extracts.

Hydroxyquinoline based inhibitors

A high-throughput screen identified a range of substituted 8HQs as KDM4E inhibitors.[87] 5-Carboxyl-8-hydroxyquinoline **69** (IOX1), was the most potent inhibitor identified, with an IC_{50} value of 0.2 μM (FDH) / 1.4 μM (MS) against KDM4E and cellular activity of EC_{50} value of 87 μM in KDM4A overexpressing HeLa cells. Further profiling with 2OG oxygenases demonstrated **69** to be a broad-spectrum inhibitor, including against all JmjC-KDMs tested (KDM2A, KDM3A, KDM4A/C-E, KDM5, KDM6A/B, PHF8) both in vitro and in cells.[88] **69** chelates the active site Fe(II) via pyridinyl nitrogen and phenolic hydroxyl in a bidentate manner; the C-5 carboxylate interacts with the active-site Lys (e.g. K206 in KDM4A) and Tyr/Thr similar to binding of the 2OG C-5 carboxylate. To improve the cell permeability of **69**, esters with various alkyl-chain lengths at 5-positions were synthesized as pro-drugs.[89] The *N*-octyl-ester, **70** exhibited enhanced cell permeability and cellular activity (30-fold) with EC_{50} value of 3.8 μM for KDM4A overexpressing HeLa cells. Interestingly **70** showed reduced but appreciable activity *in vitro* against KDM4s and remained largely unhydrolysed in HeLa cells, suggesting that the cellular activity may be a compound effect of the pro-drug and the parent **69**. More recently, a series of 2-substituted 8HQs were produced by Feng *et al.* and the benzimidazole-containing analogue **71** had an in vitro IC_{50} value of 19 μM for

KDM4A though also inhibited PHD2 to a small extent (25% at 50 μM).[90] Further experiments showed **71** was highly cell permeable, HCT-116 cells treated with **71** had increased levels of H3Kme2/3 and **71** was active in an anti-proliferative assay using HCT-116, MCF-7 and A549 tumour cell lines.

A series of 8HQs derivatives were prepared by Rai *et al.* in order to enhance the selectivity of the 8HQ scaffold towards KDM4s.[91] Three compounds, **72** – **74**, from this series (IC_{50} < 1 μM against KDM4s and selectivity over KDM5A) were reported to have promising anti-tumour activities against prostate and cancer cell lines.[92] **72** was potent against both AR negative (IC_{50} 40 nM, PC3) and positive (LNCaP and VCaP; IC_{50} < 1 μM) prostate cancer cell lines but showed no effect against normal prostate cell lines. Anti-proliferative activity was also observed in xenograft model injected with PC3 *in vivo* and the expression of AR and genes critical for cell cycle progression in solid human prostate tumours were also inhibited.

In an exemplification of modulation of an inhibitor template, a modified Betti reaction was used to synthesise a series of C-7 substituted 8HQs as KDM4 inhibitors.[93] **±75** demonstrated moderate potency (IC_{50} 5 μM (KDM4C/4E)) and selectivity (> 20-fold, except against KDM2A) across the JmjC-KDMs and other 2OG oxygenases *in vitro.* **±75** inhibited KDM4A in HeLa cells overexpressing KDM4A (EC_{50} 9 μM), and demonstrated anti-proliferative effect and increased H3K9me3 levels (EC_{50} 12 μM) on MCF7 breast cancer cells in a dose dependent manner. Treatment of patient-matched cells from normal and cancerous lung regions with **±75** showed cancer specific anti-proliferative effect. Intriguingly, while **±75** did not inhibit HIF hydroxylases *in vitro*, strong HIF up-regulation was observed in RCC4 cells treated with **±75**. This observation highlights the potential disruption of iron-haemostasis by metal-chelating compounds, and of the potentially complex biological outcomes of potential KDM inhibitors in a cellular context.

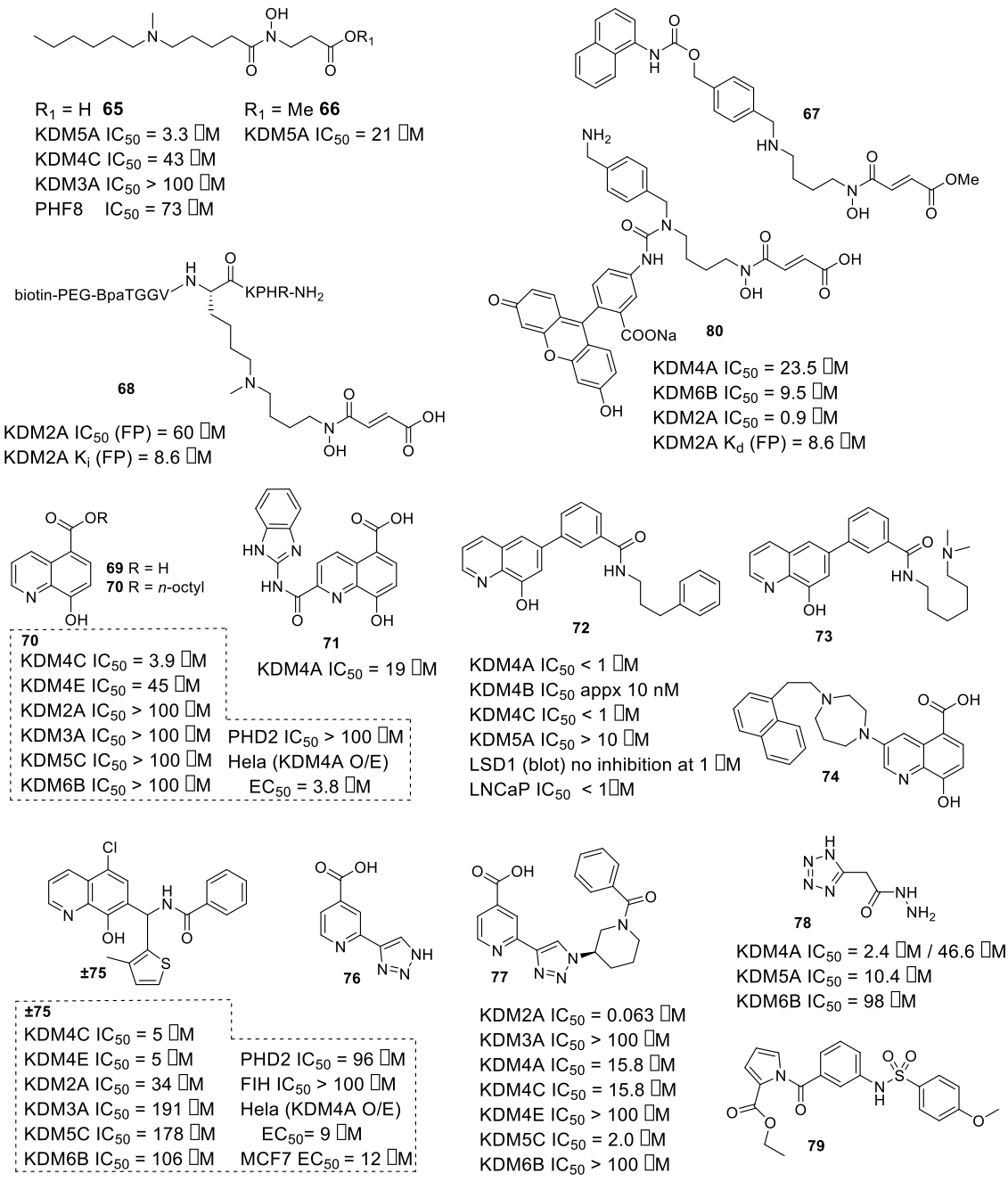


Figure 7. Metal chelating inhibitors of JmJC-KDMs. The shown inhibitors likely all bind *via* a mechanism involving chelation of the active site ferrous iron.

Other metal-chelating JmjC KDM inhibitor scaffolds

Triazolopyridines have recently been reported as a new scaffold for JmjC-KDM inhibition.[19] A co-crystal structure of KDM4A with **76** revealed that the triazole- and the pyridine-nitrogens coordinate the active site metal and occupy the 2OG binding site;

inhibitor binding is stabilized by aromatic stacking between its pyridine ring and Phe185 of KDM4A (Figure 8). Structure guided SAR analysis enabled the identification of **77**, a highly selective KDM2A inhibitor (IC_{50} 58 nM) with > 25 fold selectivity over other JmjC-KDM subfamilies.

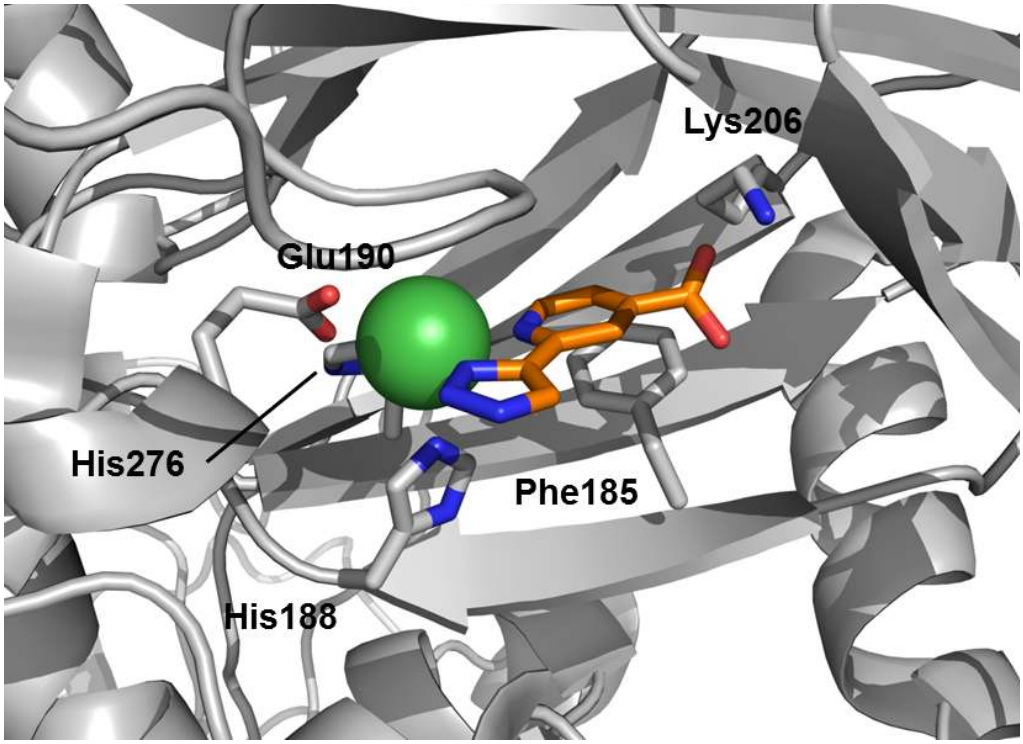


Figure. 8. View from a crystal structure of KDM4A in complex with the fused pyridine carboxylate triazole compound 76. KDM4A is shown in grey with the side chains of selected active site residues shown in sticks, **76** is shown as orange sticks (PDB:4URA). Nickel substitutes for iron and is shown as a green sphere. **76** binds in the 2OG binding pocket – compare with Figure 1B.

Tetrazoylhydrazide **78** has been identified to be a KDM4A inhibitor (IC_{50} 47 µM (FDH assay)/2.4 µM (antibody-based assay)), with 4-fold and 41 fold selectivity over KDM5A and KDM6B respectively.[94] **78** is competitive with respect to 2OG (K_i 1.94 µM), and the enzyme selectivity is notable given the size of this compound. The tetrazole group likely acts as an

isostere of C5-carboxylic acid for 2OG. Interestingly, the terminal hydrazide substitution was not tolerated, however, the alkyl chain could be extended to two or three carbon atoms with less than 2-fold loss in potency.

Many of the 2OG-mimic inhibitors reported for the JmjC-KDMs to date have poor cellular permeability; hence, pro-drug strategies have been used to mask the polar groups for enhanced cellular permeability and activity (e.g. **67**[85], GSK-J4[25a, 25c]). In order to select for cell-active compounds, Mannioroni *et al.* developed a yeast cell-based system to screen for H3K4 demethylase inhibitors as Jhd2 is the only identified H3K4 KDM in *S. cervisiae* and its catalytic domain is homologous to the human KDM5s.[82] A strain of *S. cervisiae* engineered to have an absolute requirement of Jhd2 activity grown in the presence of rapamycin (*SDBY1066*-pDPM2) was used to screen 45 putative 2OG competitors. **79** inhibited growth of the strain at 15 μM, and inhibited H3K4 KDM activity in *S. cerevisiae* and HeLa cell extracts *in vitro*. Treatment of HeLa cells with **79** manifested cytostatic and mild cytotoxicity at 30 μM with half of observed cells remaining blocked at the G2/M phase; an increase in global H3K4me3 levels was observed. While the direct mechanism of action of **79** is to be confirmed, this study demonstrates the potential for the yeast cell screening system (and by implication other non-animal eukaryotes) to be used to identify potent and cell active KDM inhibitors.

3.1.2. Non-metal chelating inhibitors

Conjugated arylalkenes

A probe-based fluorescence polarization (FP) assay has been developed in an effort to identify novel scaffolds competing with binding of the probe **80**.[95] A high throughput screen against KDM2A identified three compounds (**81** - **83**), which displace **80** and increase H3K36me2 levels in cells (Figure 9).[96] Interestingly, these compounds are apparently non-

metal chelating scaffolds, with **81** and **82** sharing a similar dimethylamino-styryl pyridine core and **83** being structurally distinct. Whilst these three compounds displaced **80** with K_i in the μM to sub μM range in both KDM2A and KDM4A, their catalytic inhibition with isolated enzymes was not reported. However, global H3K36me2 and H3K9me3 levels were increased in a dose-dependent manner when HeLa and human pancreatic ductal adenocarcinoma (MiaPaCa2) cells were treated with these compounds for 48 hrs, demonstrating the potential strength for such screening approaches in identifying new types of inhibitors.

Chu *et al.* utilized a virtual screening approach to identify putative inhibitor scaffolds targeting the active site pockets of KDM4A and KDM4B and highly conjugated arylalkene **84** was identified as a hit.[97] Interestingly, **84** was found to be a competitive inhibitor with respect to histone H3K9me3, with K_i values of 5.5 μM and 3 μM against KDM4A and KDM4B respectively, but weaker inhibition against KDM4D/4E (IC_{50} > 100 μM) (Figure 9). **84** induced apoptosis in LNCaP prostate cancer cells and negatively regulated androgen receptor (AR)-responsive genes. In a different study, a series of curcuminoids, which induce apoptosis in colon colorectal cancer cells HCT-116, were tested for inhibition of KDM4A, 4C and 4D. **85** and **86** inhibited the activity of KDM4s at 1mM as determined by histone western blot immune assays.[98] However, curcumins are known to be promiscuous inhibitors (including against other epigenetic enzyme targets such as HDACs and DNMTs)[99] and often highlighted as pan-assay interference (PAINS) compounds.[100]

Cyclic peptides

In an effort to identify novel inhibitor scaffolds for the KDM4s, Leurs *et al.* applied a cyclic peptide-based phage display method to select for KDM4A/C binding peptides.[78b] Two cyclic peptide sequences with EC_{50} values in the μM range were selected from the display against KDM4C, but resynthesized peptides had moderate inhibitory activities. Further development

through SAR analysis generated KDM4C inhibitors with much improved *in vitro* activities (**87** (IC_{50} 8.5 μM) and **88** (IC_{50} 0.6 μM), Figure 9) but no activity in cells. Interestingly, hydrogen/deuterium exchange MS analysis indicated that these peptides bind in 'allosteric sites', potentially involving cooperative binding in two distinct binding surfaces.[78a]

Metal-containing JmjC-KDM inhibitors

Recently, rather than targeting the active site metal a metal-containing inhibitor of JmjC-KDM was reported.[101] Compound **89** is an iridium(III) complex, containing 4,7-dmobpy N^N ligand and two 1-phenylisoquinoline C^N ligands (Figure 9). **89** was reported to be substitutionally inert, i.e. Ir(III) is not readily replaced by Fe(II), indicating that iron sequestering / chelation may not be the mode of inhibition. **89** was shown to inhibit KDM4D with an IC_{50} value of 15 μM. In chromatin immunoprecipitation (ChIP) assays on human lung adenocarcinoma epithelial (A549) cells, compound **89** treated cells showed increased H3K9me3 levels at the p21 gene promoter relative to the untreated cells, and the interaction of KDM4D to H3K9me3 was disrupted in a dose dependent manner. Using nuclear extracts, compound **89** was found to selectively inhibit H3K9me3 demethylation, and suppress A549 cancer cell growth with an IC_{50} value of 0.85 μM. Although it is not easily possible to determine if **89** remains intact in its cellular inhibition, or whether the cellular effect is KDM4D specific (due to the disparity between *in vitro* and cellular IC_{50} values and given similar compounds have been previously demonstrated to target other proteins)[102] the work does raise the possibility that metal-chelation might be used to deliver complexed inhibitors.

Non-catalytic domain targeting JmjC-KDM inhibitors

PHD-fingers are histone-binding domains, which, along with other 'binding domains' are present in many JmjC KDMs (e.g. KDM2s, KDM4A-C, KDM5s and KDM7s). While relatively

little is known about the functions of many JmjC-KDM associated PHD-fingers, some appear important in recruiting / targeting the JmjC-KDMs to certain histone marks (e.g. in KDM7A/B).[22b] Recently, small molecule inhibitors targeting the third PHD-finger of KDM5A (KDM5A-PHD3) were identified through application of a HaloTag® assay by screening for molecules that displaced histone H3K4me3 binding to PHD3.[81] Screening of a NIH Clinical Collection library identified compounds such disulfiram, phenothiazine, aminodarone and tegaserod maleate as inhibitors. The compounds were further tested through affinity pull-downs, fluorescence polarisation and histone reader specificity studies. A series based on aminodarone derivatives (**90** - **94**) were identified to be potent against KDM5A-PHD3, with IC_{50} values in the 25-40 μM range. This study demonstrates, for the first time, that the binding / targeting ('reader') domains of JmjC KDMs are tractable targets, and provides a promising lead for development of inhibitors targeting non-catalytic domains of JmjC-KDMs.

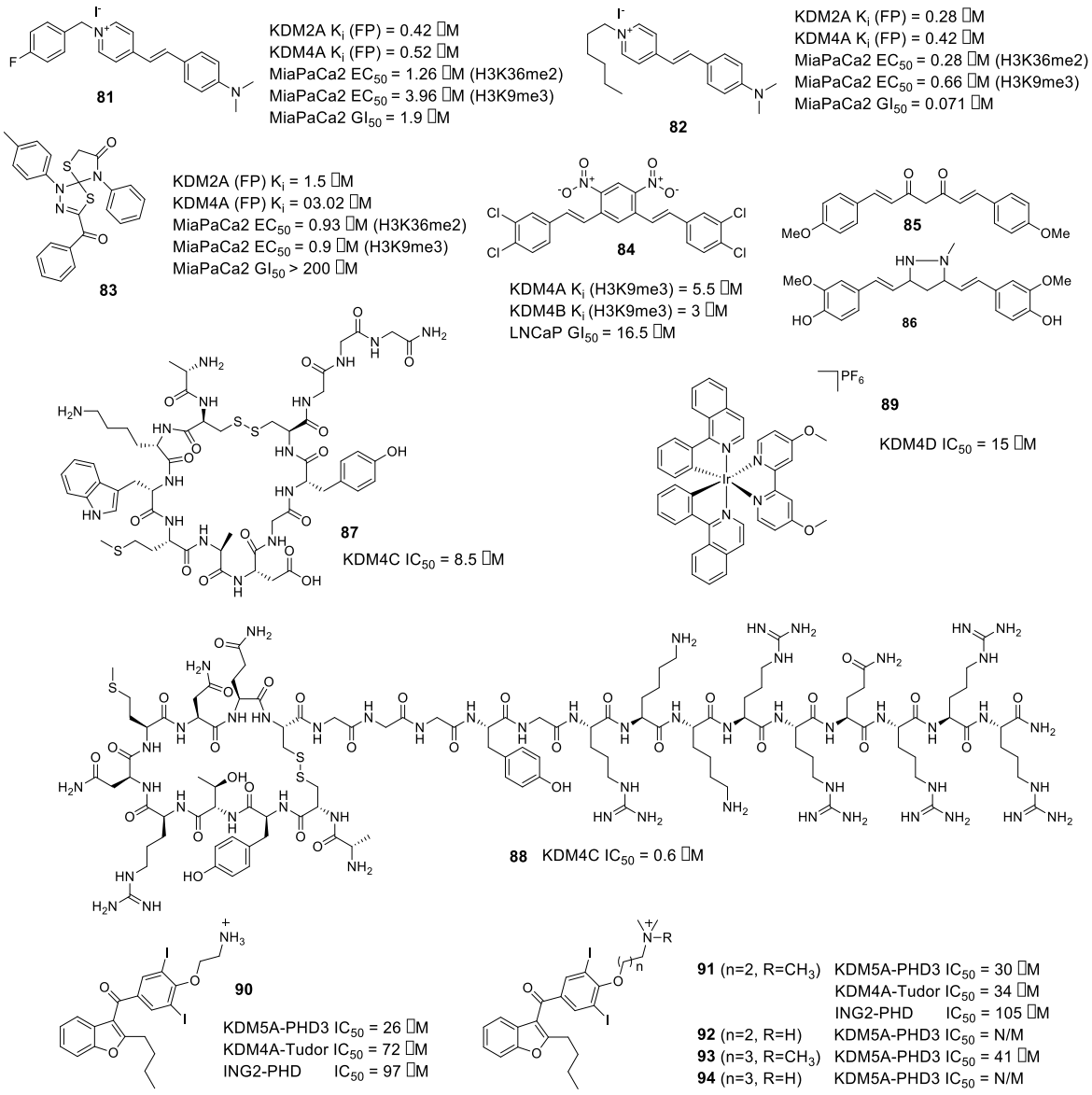


Figure 9. Non-metal chelating inhibitors of JmjC-KDMs. Compounds **81** – **89** are likely bind to the catalytic domains of JmjC-KDMs in a manner competing with histone substrate and which apparently does not involve chelation of the active site metal – though note in some cases this cannot be ruled out. Compounds **90** – **94** are not expected to chelate the active-site metal as they were developed to bind to a reader domain. N/M – not measurable within solubility limit of compond

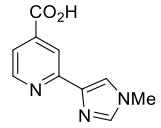
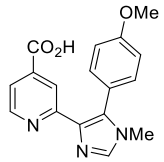
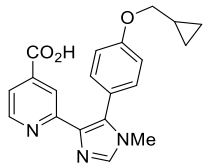
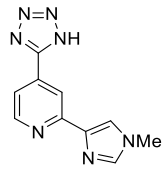
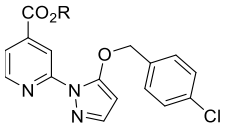
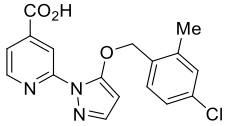
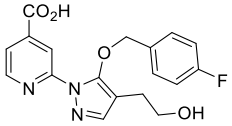
3.2. Patent Literature on JmjC KDM Inhibitors.

2014–2015 has seen a surge in patent literature concerning JmjC KDM inhibitors. Patent applications have been filed from both small and large pharmaceutical companies, including some that specialise in the field of epigenetics. These patents primarily describe KDM4 and KDM5 subfamily inhibitors for cancer treatment. The inhibitors largely incorporate a metal-binding template, usually containing a substituted pyridine with an acid or acid isostere at the C-4 position (e.g. **95**–**100**) but other heterocyclic scaffolds are also described (e.g. **119**–**125**).

4-Carboxy-2-heterocyclic pyridine derivatives are reported as KDM4/5 inhibitors in patents from Quanticel Pharmaceuticals (Table 1).[103] The methylimidazole derivative **95** is a potent (IC_{50} < 0.1 μM) inhibitor of KDM2B, 4A, 4C, 5A and 5B. Selectivity for KDM5A/B was improved by imidazole ring substitution (e.g. **96** and **97**). A loss in potency was found when the compounds were tested in a ZR-75-1 breast cancer cell-based assay measuring inhibition of H3K4me3 demethylation. Although the cellular activity did not correlate well with the *in vitro* activity, **96** and **97** were found to be some of the most potent inhibitors in the cellular assay with IC_{50} values in the 1–10 μM range. A tetrazole analogue (compound **98**) was reported; interestingly when tested in the cellular assay this was found to be inactive (IC_{50} > 10 μM). Pyrazole derivatives **99** – **102** also proved to be useful scaffolds for KDM5A/B inhibition; inhibitor **101** was found to have a cellular IC_{50} of less than 0.1 μM. **102** exhibited the best selectivity over KDM4C (10–100 fold) although its cellular efficacy was not reported.

Table 1 *In vitro* and cell-based activity of 2-imidazolyl and 2-pyrazolylpyridine derivatives reported by Quanticel Pharmaceuticals[103]

	KDM IC_{50} (μM)	Cellular

Structure	Cmpd	R	2B	4A	4C	5A	5B	IC_{50} (μM)
	**95** Ex. 1	N/A	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	> 10
	**96** Ex. 12	N/A	0.1–1.0	N/R	1.0–10	0.1–1.0	0.1–1.0	1.0–10
	**97** Ex. 16	N/A	0.1–1.0	N/R	0.1–1.0	< 0.1	< 0.1	1.0–10
	**98** Ex. 62	N/A	< 0.1	> 10	> 10	0.1–1.0	0.1–1.0	> 10
	**99** Ex. 53	H	N/R	N/R	< 0.1	< 0.1	< 0.1	0.1–1.0
	100 Ex. 64	Me	N/R	N/R	N/R	N/R	N/R	0.1–1.0
	**101** Ex. 89	N/A	0.1–1.0	N/R	0.1–1.0	< 0.1	< 0.1	< 0.1
	**102** Ex. 109	N/A	N/R	N/R	1.0–10	< 0.1	< 0.1	N/R

Cellular assays measured demethylation of H3K4me3 in ZR-75-1 cells. N/R – not reported

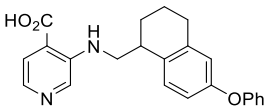
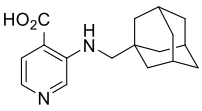
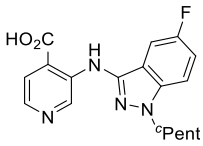
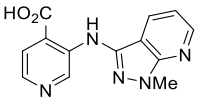
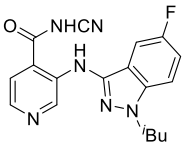
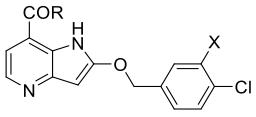
Amino-4-carboxy pyridine derivatives are reported as KDM4/5 inhibitors by Quanticel Pharmaceuticals (Table 2)[104] and GlaxoSmithKline.[105] *N*-Alkyl derivatives (e.g. **103**) were found to inhibit cell proliferation in the sub-micromolar range in KYSE-150 cells (which overexpresses KDM4C).[106] The most active compounds in the cellular assay did not exhibit a drop-off in potency compared to the *in vitro* assay, for example **103** was the most active

compound in cellular assays measuring H3K9 demethylation (IC_{50} < 0.10 μM), although **103** was only found to inhibit KDM4C with IC_{50} values between 0.1–1.0 μM in assays with isolated enzymes. The selectivity of **103** over KDM5A/B was not reported. One of the other potent inhibitors in the series (**104**: KDM4C IC_{50} < 0.10 μM) was found to be approximately 10-fold selective over KDM5A and B but this compound had disappointing activity in the cellular assays (IC_{50} > 10 μM). Several examples of *N*-indazole derivatives, for example **105** and **106**, were found to be potent inhibitors of KDM5A/B (IC_{50} < 0.10 μM) with approximately 10-fold selectivity over KDM4C and activity in the H3K4me3 ZR-75-1 breast cancer cell-based assay (IC_{50} 0.1–1.0 μM). Some C-4 pyridine substituted cyanamide derivatives resulted in greater selectivity over KDM4C in assays with isolated enzyme but their activity in the cell-based assay was not reported (e.g. **107**). Two series of inhibitors where a pyridine C-3 amino group forms part of a fused heterocycle have also been reported in patents filed by Quanticel Pharmaceuticals (Table 2 and Table 3 **108** - **118**).

Azaindole derivatives are reported as potent KDM4C inhibitors (IC_{50} < 0.10 μM) with greater than 10-fold selectivity over KDM5A/B or KDM4A (e.g. **108**, **110** and **112**).[107] Some of these azaindole-based inhibitors gave excellent cellular activity (IC_{50} < 0.10 μM) against H3K9 demethylation, both as the methyl ester (**109**, **111** and **113**) and methyl amide (**114**) derivatives of the corresponding carboxylic acids in a KYSE-150 cell-proliferation assay.

Table 2 *In vitro* and cell-based activity of 3-aminopyridine derivatives reported by Quanticel Pharmaceuticals.[104-105, 107]

Structure	Cmpd	R	X	KDM IC_{50} (μM)				Cellular IC_{50} (μM)
				4A	4C	5A	5B	

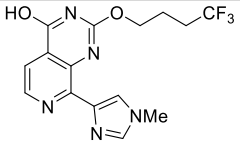
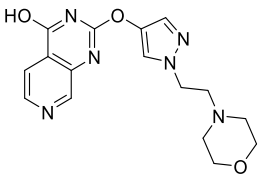
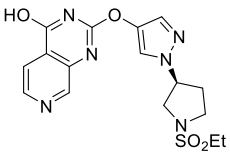
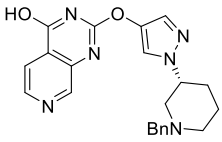
	**103** Ex. 80	N/A	N/A	N/R	0.1–1.0	N/R	N/R	< 0.1[a]
	**104** Ex. 42	N/A	N/A	N/R	< 0.1	0.1–1.0	0.1–1.0	> 10[a]
	**105** Ex. 29	N/A	N/A	N/R	0.1–1.0	< 0.1	< 0.1	0.1–1.0[b]
	**106** Ex. 52	N/A	N/A	N/R	0.1–1.0	< 0.1	< 0.1	0.1–1.0[b]
	**107** Ex. 75	N/A	N/A	N/R	1.0–10	< 0.1	< 0.1	N/R
	108 Ex. 128	OH	OMe	0.1–1.0	< 0.1	1.0–10	1.0–10	N/R
	109 Ex. 127	OMe	OMe	N/R	N/R	N/R	N/R	< 0.1[a]
	110 Ex. 138	OH	$OCH_2{}^cPr$	1.0–10	< 0.1	0.1–1.0	< 0.1	N/R
	**111** Ex. 137	OMe	$OCH_2{}^cPr$	N/R	N/R	N/R	N/R	< 0.1[a]
	112 Ex. 142	OH	OCF_3	0.1–1.0	< 0.1	0.1–1.0	< 0.1	N/R
	113 Ex. 141	OMe	OCF_3	N/R	N/R	N/R	N/R	< 0.1[a]
	114 Ex. 144	NHMe	OCF_3	N/R	N/R	N/R	N/R	< 0.1[a]

Cellular assays measured [a]inhibition of KYSE-150 cell proliferation (KDM4C) or [b]demethylation of H3K4me3 in ZR-75-1 cells (KDM5A/B). N/R – not reported

A pyridopyrimidinone series where the fused ring incorporates both the 3-amino substituent and the 4-carbonyl group has also been described by Quanticel Pharmaceuticals (Table 3).[104b] Some of these compounds showed selectivity for KDM5A/B over KDM4C in assays with isolated enzymes, but with the exception of the methylimidazole derivative **115**,

the most potent inhibitors of H3K4me3 demethylation in the ZR-75-1 breast cancer cell-based assay (e.g. **116** – **118**) were found to inhibit KDM5A/B and KDM4C in the same range (IC_{50} < 0.10 μM) against isolated enzymes.

Table 3 *In vitro* and cell-based activity of pyridopyrimidinone derivatives reported by Quanticel Pharmaceuticals.[104b]

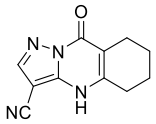
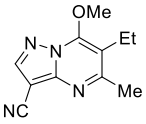
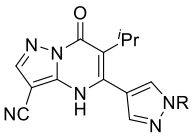
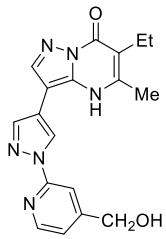
Structure	Cmpd	KDM IC_{50} (μM)			Cellular IC_{50} (μM)
		4C	5A	5B	
	**115** Ex. 74	0.1–1.0	< 0.1	< 0.1	0.1–1.0
	**116** Ex. 124	< 0.1	< 0.1	< 0.1	0.1–1.0
	**117** Ex. 136	< 0.1	< 0.1	< 0.1	0.1–1.0
	**118** Ex. 158	< 0.1	< 0.1	< 0.1	0.1–1.0

Cellular assays measured inhibition of KYSE-150 cell proliferation (KDM4C). N/R – not reported

A series of cyanopyrazole KDM4/5 inhibitors has been reported by Constellation Pharmaceuticals (Table 4).[108] Isolated enzyme activity data was reported for KDM4C, 5A and 5B, and several compounds were found to be potent inhibitors (IC_{50} < 0.1 μM). Some degree of selectivity for KDM4C was reported for compound **119** with other compounds (for example **120**) exhibiting more activity against KDM5A/B. Two further patents filed jointly by

Genentech Inc. and Constellation Pharmaceuticals describe highly potent KDM5A inhibitors where the core scaffold bears a second pyrazole ring at the 3- or 5-position (Table 4).[109] Potent KDM5A inhibitors (e.g. **121**–**123**) are described with activity in the nanomolar range. Excellent cellular activity has been achieved with this series, for example compound **121** was found to be a potent inhibitor of the demethylation of H3K4me3 in PC9 cells (EC_{50} 180 nM).

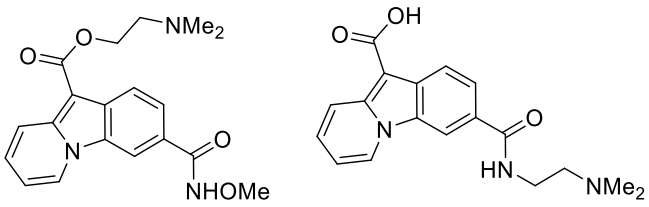
Table 4 *In vitro* and cell-based activity of pyrazole derivatives reported by Constellation Pharmaceuticals and Genentech Inc. [108-109]

Structure	Cmpd	R	KDM IC_{50} (μM)			Cellular EC_{50} (μM)
			4C	5A	5B	
	**119** Ex. 4	N/A	< 1.0	1.0–10	1.0–10	N/R
	**120** Ex. 30	N/A	1.0–10	< 1.0	< 1.0	N/R
	**121** Ex. 117	^{t}Bu	N/R	0.014	N/R	0.18
	122 Ex. 158	^{i}Bu	N/R	0.015	N/R	0.53
	**123** Ex. 11	N/A	N/R	0.0023	N/R	N/R

Cellular assays measured demethylation of H3K4me3 in PC9 cells. N/R – not reported

Pyrido[1,2-a]indole derivatives are reported as inhibitors of the demethylation of H3K9me3 by KDM4C *in vitro* by EpiTherapeutics (Figure 10).[110] The dimethylaminoethyl

ester derivative **124** and the carboxylic acid **125** are examples of inhibitors described with IC_{50} values below 0.5 μM against KDM4C; however, the cellular efficacy of these compounds has not been reported.



124 Ex. 22
KDM4C IC_{50} < 0.50 □M

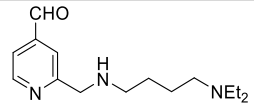
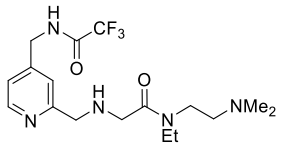
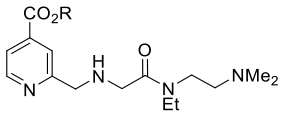
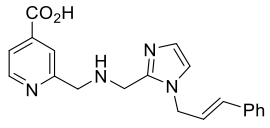
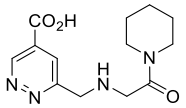
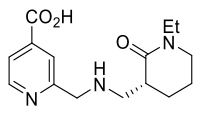
125 Ex. 14
KDM4C IC_{50} < 0.50 □M

Figure 10 *In vitro* KDM4C activity of pyrido[1,2-a]indole derivatives reported by EpiTherapeutics

EpiTherapeutics has disclosed a series of aminomethylpyridine-based KDM4/5 inhibitors (Table 5).[111] Compounds **126** – **133** were all found to inhibit the KDM5 subfamily (**126**, **128** – **133**: IC_{50} < 0.25 μM; **127**: IC_{50} 0.25–2.5 μM), however, with the exception of compounds **127** and **131**, these compounds were found to inhibit other JmjC KDMs in the same potency range (**126**, **128** – **130** and **132** were also found to inhibit the KDM4 subfamily, and **133** was also found to inhibit KDM2B). Compounds in this series were found to be active in cell proliferation assays. For example, **126** and **127** were found to inhibit the proliferation of MCF7 breast cancer cells (EC_{50} < 0.25 μM); these compounds were found to be active in other cell proliferation assays; **126** was active (EC_{50} < 0.25 μM) in BT474 (mammary duct carcinoma) and NALM6 (lymphoblastic leukaemia) cell proliferation assays and **127** inhibited cell proliferation (EC_{50} < 0.25 μM) in SU DHL6 (B cell lymphoma) and KMS 12 BM and MM1S (myeloma) assays. **128**, the carboxylic acid analogue of **127**, was found to be active (IC_{50} 1.0–50 μM) in cellular assays, including an H3K4me3 demethylation assay in U2OS cells and an MCF7 cellular proliferation assay, however, the methyl (**129**) and ethyl (**130**) esters of

128 resulted in greater activity in these assays (IC_{50} < 1.0 μM). Esters **129** and **130** inhibited KDM4C and 5B in a similar range to **128** in enzyme assays; however, in cellular systems they may act as prodrugs of **128** so the inhibitory activity of both the ester and carboxylic acid forms of **129** and **130** may contribute to their cellular efficacy.

Table 5 *In vitro* activity of aminomethylpyridine derivatives reported by EpiTherapeutics. [111]

Structure	Cmpd	R	KDM IC_{50} (μM)									
			2B	3A	3B	4A	4B	4C	5B	5C	6A	6B
	**126** Ex. 107	N/A	> 2.5	> 2.5	> 2.5	> 2.5	< 0.25	> 2.5	< 0.25	< 0.25	> 2.5	> 2.5
	**127** Ex. 25	N/A	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	> 2.5	0.25–2.5	0.25–2.5	> 2.5	> 2.5
	**128** Ex. 49	H	0.1–1.0	N/R	> 1.0	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	> 1.0	> 1.0
	129 Ex. 70	Me	> 1.0	N/R	N/R	N/R	N/R	< 0.1	< 0.1	N/R	< 0.1	N/R
	130 Ex. 65	Et	N/R	N/R	N/R	N/R	N/R	0.1–1.0	0.1–1.0	N/R	< 0.1	N/R
	**131** Ex. 13	N/A	N/R	N/R	N/R	N/R	N/R	> 2.5	< 0.25	N/R	> 2.5	N/R
	**132** Ex. 26	N/A	N/R	N/R	N/R	N/R	N/R	< 0.25	< 0.25	N/R	> 2.5	N/R
	**133** Ex. 107	N/A	< 0.25	N/R	N/R	N/R	N/R	N/R	< 0.25	N/R	> 2.5	N/R

N/R – not reported

4. Conclusions and Future Prospects

The reporting period has seen a substantial increase in academic publications and patents on KDM1 inhibitors. An important development has been the inclusion of a KDM1 inhibitor (**6**) into clinical trials for the treatment of small cell lung carcinoma and acute myeloid leukaemia.[41-42] Although there is considerable scope for further work, it is now apparent that potent inhibitors selective for small groups of KDMs will be possible. In the case of the KDM1s, however, little work appears to have been carried out on inhibitors selective for KDM1A over KDM1B. The vast majority of the reported inhibitors target the catalytic machinery of the KDM1s and the JmjC KDMs with relatively few targeting 'non-catalytic' domains. In the case of the JmjC KDMs future work focused on improving selectivity is of interest, at least from the perspective of developing inhibitors for use as probes for biological function/drug target validation.

There is a substantial need for developing new types of inhibitors, likely aided by our improving understanding of the structures (although, given the likely conformational changes during catalysis, there is a need for the crystallographic analyses to be augmented by solution studies) and biochemical selectivities of the KDMs (and related human hydroxylases/amine oxidases). Such new types of inhibitor might target the KDM catalytic domains by e.g. competing with histone (or probably other) substrates, or by co-substrate uncompetitive type mechanisms. However, given that the activity of most, if not all, KDMs is likely regulated by domains other than the catalytic ones, which may or may not be covalently linked to the catalytic domains, further studies on the inhibition of these domains, some of which are likely to have equal importance in biological function as the catalytic domains, may be an interesting avenue to follow. It is highly desirable that work continues to develop cell-based assays that are representative of the physiological roles of the KDMs – in very few cases (if any) is JmjC KDM catalysis quantitatively correlated with a

biological role in an intact adult organism, though multiple reports correlate JmjC KDM mutations with developmental defects.[112]

Overall, it would seem that within the relatively near future inhibitors of the catalytic JmjC domains of KDMs of sufficient quality (in conjugation with genetic mutants and analysis of clinical data) to probe biological functions and carry out preliminary target investigation studies will be available. To date it seems that cancer, and possibly immune-related diseases, will be the likely initial targets for KDM inhibitors. Thus is it important that early stage consideration is given as to how inhibitors will be developed/used, particularly with respect to what other treatments they might be partnered with in order to achieve a desired medicinal outcome, how they will be targeted to tumour/cancer cells and how resistance to them might develop. Especially with respect to the development of highly selective inhibitors and resistance; we think it is important that consideration be given to the apparent plasticity of epigenetic regulation i.e. resistance may be a problem with inhibition *via* a single mechanism of action.

With respect to the application of KDM inhibitors, consideration needs to be given to the desired outcome; whilst simple inhibition of cell growth/cytotoxicity will likely be a property of some types of KDM inhibition, given that many compounds clearly exhibit these effects, whether this will lead to a breakthrough in cancer treatment is unclear. Instead, for example, it may be that KDM inhibitors find application in combatting resistance to already established chemotherapeutic agents. The rational application of KDM inhibitors to non-cancer diseases would seem more distant – potential targets may emerge from genome wide association studies/work on genetic diseases, but will likely require clearer connections to be made between the biochemical and physiological roles of KDMs.

Corresponding Authors

* E-mail: christopher.schofield@chem.ox.ac.uk or akane.kawamura@chem.ox.ac.uk, Phone: +44 (0)1865 285000. Fax: +44 (0)1865 275674

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Abbreviations

2OG, 2-oxoglutarate; 8HQ, 8-hydroxyquinoline; AR, androgen receptor; ChIP, chromatin immune-precipitation; CoREST, RE1-silencing transcriptional factor corepressor 1; FAD, flavin adenine dinucleotide; FDH, formaldehyde dehydrogenase; FIH, factor inhibiting HIF; HDAC, Histone deacetylase; HIF, hypoxia inducible factor; INSM1, Insulinoma-associated protein 1; JARID, Jumonji and ARID-domain containing protein; Jhd2, Jumonji/ARID domain-containing protein 2; JmjC, Jumonji C Domain; JMJD, Jumonji domain-containing protein; KDM, *N*-methyl-lysine demethylases; LSD, Lysine Specific Demethylase; MAO, monoamine oxidase; MINA 53, MYC-induced nuclear antigen 53; NO66, nucleolar protein 66; PADI, protein arginine deiminase; PDB, Protein Data Bank; PHD, plant homeobox domain;

PHD1/2/3, HIF prolyl hydroxylase 1/2/3; PHF, PHD finger protein; SNAG, SNAIL/GFI; SNAIL1, zinc finger protein SNAI 1.

Biographies

Tom E. McAllister

Tom completed his PhD in chemical biology at the University of Leeds in 2013, under the supervision of Dr Michael E. Webb, developing methods to study labile protein post-translational modifications. He then worked as a post-doctoral fellow with Assoc. Prof. W. Bruce Turnbull, also at Leeds, to produce virus-like particles through defined protein-carbohydrate interactions. After a brief spell as a teaching fellow at the University of York, Tom began his postdoctoral position with Dr Akane Kawamura and Prof Christopher J. Schofield FRS at the University of Oxford in 2014. His current work focusses on the development of highly selective inhibitors for histone demethylases.

Katherine S. England

Katherine England completed an M.Chem. degree at the University of Oxford, working under the supervision of Prof. Jeremy Robertson for her final year research project. In 2002 she joined the Medicinal Chemistry department at Pfizer in Sandwich, UK where she worked in drug discovery across a variety of disease areas and drug classes. Katherine joined the Structural Genomics Consortium at the University of Oxford in 2011 where she designed and synthesised inhibitors of epigenetic proteins and was awarded a D.Phil. in Organic Chemistry under the supervision of Prof. Christopher Schofield and Prof. Paul Brennan. In 2015 she joined the newly formed Alzheimer's Research UK Oxford Drug Discovery Institute where she is engaged in the design and synthesis of new molecules as potential treatments for dementia.

Richard J. Hopkinson

Richard received his DPhil in organic chemistry (2012) from the University of Oxford under the supervision of Prof. Christopher J. Schofield FRS, where his work focused on studying the mechanisms of histone demethylases. Following postdoctoral work on demethylase inhibition, he was elected to the William R. Miller Junior Research Fellowship in Molecular Aspects of Biology at St. Edmund Hall, Oxford, where his current research investigates the (bio)chemistry of formaldehyde in cellular systems.

Paul E. Brennan

Paul Brennan received his PhD in organic chemistry from UC Berkeley working on combinatorial chemistry and antibiotics. Following post-doctoral research in Cambridge University on total synthesis, Paul returned to California to take a position at Amgen. His research was focussed on kinase inhibitors for oncology. After two years at Amgen, Paul moved to Pfizer in Sandwich, UK. In 2011, Paul joined the Structural Genomics Consortium as the Associate Professor of Medicinal Chemistry to discover chemical probes for epigenetic proteins. Since 2015, Paul has been the Head of Chemistry at the ARUK ODDI. His current research is focused on epigenetic proteins and new dementia targets.

Akane Kawamura

Akane Kawamura completed her MChem in Chemistry in 2000 and received her DPhil in Pharmacology from University of Oxford in 2005. She spent three years at Summit PLC, a biotechnology company, where she led a number of drug discovery projects across multiple therapeutic areas. In 2009 she joined Professor Chris Schofield's laboratory to work on developing chemical probes for epigenetic proteins. She was awarded a BHF CRE Senior Fellowship in 2012 and a Royal Society Dorothy Hodgkin Research Fellowship in 2013. Her current research focuses on understanding the molecular mechanisms of epigenetic regulation by chromatin modifying enzymes.

Christopher J. Schofield

Chris Schofield's research is driven by a desire to apply chemical principles and techniques to understanding biology. His research focuses on the functions, mechanisms, and structures of 'chemically interesting' metallo-enzymes with roles ranging from antibiotic resistance to the oxygen dependent regulation of protein biosynthesis in humans.

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