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Original article

Synthesis, biological activity and mechanistic insights of 1-substituted cyclopropylamine derivatives: A novel class of irreversible inhibitors of histone demethylase KDM1A

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ABSTRACT

Histone demethylase KDM1A (also known as LSD1) has become an attractive therapeutic target for the treatment of cancer as well as other disorders such as viral infections. We report on the synthesis of compounds derived from the expansion of tranylcypromine as a chemical scaffold for the design of novel demethylase inhibitors. These compounds, which are substituted on the cyclopropyl core moiety, were evaluated for their ability to inhibit KDM1A *in vitro* as well as to function in cells by modulating the expression of Gfi-1b, a well recognized KDM1A target gene. The molecules were all found to covalently inhibit KDM1A and to become increasingly selective against human monoamine oxidases MAO A and MAO B through the introduction of bulkier substituents on the cyclopropylamine ring. Structural and biochemical analysis of selected *trans* isomers showed that the two stereoisomers are endowed with similar inhibitory activities against KDM1A, but form different covalent adducts with the FAD co-enzyme.

1. Introduction

In the past decades, it has become evident that an altered epigenetic control of gene expression is associated to several diseases, including cancer. Posttranslational modifications of the histone tails are among the most important regulatory factors for structural changes in chromatin, both on the global scale and at specific genome loci [1–6]. Histone lysine acetylation and deacetylation are the best characterized histone modifications and currently histone deacetylase inhibitors are used in the treatment of cutaneous and peripheral T-cell lymphoma [7–10]. Contrary to histone acetylation, methylation does not lead to a change of the charge of the histone tail, but it affects its basicity, hydrophobicity and the affinity towards other proteins [11,12]. Histone lysine methylation, previously considered to be an irreversible process, has been shown to be dynamically regulated by the controlled addition and removal of one, two, or three methyl groups on the amino group of the amino acid side chain. Histone lysine demethylases (called KDMs) exert their activity through two types of mechanisms. The Jumonji domain-containing enzymes are iron and 2-oxoglutarate dependent oxygenases, which act on mono-, di- and tri-methylated lysines [11–14]. On the other hand, the flavin-dependent (FAD) histone demethylases can only act on mono and dimethylated lysine residues [15–17]. So far, two human FAD-dependent demethylases have been identified: LSD1, also known as KDM1A [18] and LSD2, also known as KDM1B [19,20]. Histone 3 dimethyl-lysine 4 (H3K4me2) is a positive chromatin mark, which has been localized to the regions of transcriptionally active human genes [21,22]. Erasure of this mark by KDM1A results in a transcriptional repression [21,23]. KDM1A has been also proposed to be

Abbreviations: LSD, lysine-specific histone demethylase; MAO, monoamine oxidase; DIPEA, *N*,*N*-diisopropylethylamine.

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involved in the demethylation of the repressive mono- and dimethyl H3K9, thus promoting gene activation [24]. In addition it has been recently found that several non-histone proteins are substrates of KDM1A, such as p53 [25], DNA methyltransferase 1 [26,27], E2F1 [28] and MYPT1 [29].

KDM1A is up-regulated in various cancers [30–33], including acute myeloid leukaemia [34], prostate cancer [35], ER-negative breast cancer [36,37], and neuroblastoma [38]. The association of KDM1A overexpression and tumours as well as the discovery of the monoamine oxidase (MAO) inhibitor tranylcypromine **1** (see Fig. 1) as KDM1A inhibitor in 2006 [39] were starting key events, which have triggered intensive research in the development of new KDM1A-inhibiting agents [40]. The majority of these activities were directed towards functionalized tranylcypromine-based compounds by the introduction either of substituents on the amino and/or phenyl moieties of the inhibitor. A series of patent applications were disclosed by Oryzon and GSK describing *N*-substituted tranylcypromine derivatives [41,42], several of them being highly selective for KDM1A over MAO A and MAO B. Two compounds from these series have been reported to have entered clinical studies [43,44]. Neelamegam et al. found that compound **RN-7** (Fig. 1) was over 300 times more potent against KDM1A over MAO A and B [45]. The authors were able to demonstrate an involvement of KDM1A as regulator of long-term memory formation implying a key function in the central nervous system. Also the introduction of different substituents on the aryl group of tranylcypromine resulted in highly potent and selective compounds [46,47]. For example, **MC2580** [47] (Fig. 1) was found to be 100 time more active against KDM1A than compound **1**, while being inactive against MAO B. Contrary to the above described research activities, modifications on the cyclopropyl moiety have been so far less explored. This moiety is essential for inhibition because it adds irreversibly to the FAD cofactor, thus inactivating the enzymes [16,48,49]. A preliminary study of the cyclopropyl ring modifications was described by Gooden et al., demonstrating that 2-diphenyl-cyclopropylamine was significantly less potent than **1** [50]. Given the growing importance of tranylcypromine as lead compound for the development of inhibitors of flavin-dependent amine oxidases such as KDM1A and MAOs, we decided to further investigate the effect of substituents on the cyclopropyl moiety. Our aim was twofold: (i) to enhance our knowledge on pharmacological properties of tranylcypromine-derived compounds, and (ii) to obtain mechanistic insights into the formation of the covalent adduct with the flavin, which causes the irreversible inhibition. We have synthesized a series of cyclopropylamino derivatives substituted in position 1 and characterized them, discovering new KDM1A inhibitors and broadening our understanding of the selectivity and stereochemistry of the inhibition mechanism (see Fig. 1).

2. Results and discussion

The reaction between styrene oxide and the corresponding phosphonate was carried according to the Wadsworth–Emmons cyclopropanation [51–54]. In specific, styrene oxide **6**, as well their 2-S (**7**) and 2-R (**8**) analogues, were treated with the phosphonates **9a**–**g** in the presence of butyl lithium in dimethoxyethane at 130 °C under microwave irradiation (Scheme 1). Subsequent hydrolysis of the ethyl esters with LiOH provided the carboxylic acids **13a**–**c**, **13e**–**g**, **14a**,**b**,**d**,**e** and **15a**,**b**,**d**,**e** which were then converted into the *N*-BOC derivatives according to a Curtius rearrangement with diphenyl phosphorazidate in presence triethylamine and in *tert*-butanol at 90 °C. Final hydrolysis of the BOC protecting group with hydrochloric acid gave the desired amines **19a**–**c**, **19e**–**g**, **20a**,**b**,**d**,**e** and **21a**,**b**,**d**,**e**. Compound **19d** was synthesized as previously reported [55].

The amino group of compound **22** [56] was protected with *tert*-butoxycarbonyl-*tert*-butyl carbonate in the presence of DIPEA and the *trans* isomer was then separated from the *cis* analogue by column chromatography with a mixture of hexane and EtOAc as eluent (Scheme 2). Basic hydrolysis of compound **23** with NaOH in ethanol resulted in the corresponding carboxylic acid **25**, while the hydroxymethyl derivative **27** was obtained by reduction of the ester **23** with $LiAlH_4$ in THF at room temperature. Finally, deprotection of the *tert*-butoxycarbonyl moieties of **23**, **25** and **27** in HCl provided the desired cyclopropyl derivatives **24**, **26** and **28**.

KDM1A inhibitory activity was assessed using human recombinant KDM1A/CoREST protein as enzymatic source and a synthetic mono-methylated H3-K4 peptide containing 21 amino acids as substrate. The compounds were incubated with the enzymatic complex and the results are expressed either as IC_{50} or in case of poor potency as percentage of inhibition at the highest dose tested.

Initially we prepared a series of analogues of compound **1**, all of them already known for their MAO inhibitory activity. As shown in Table 1, replacement of the cyclopropylamine by a $-COCH_2NH_2$ group (**2**), which is present in some highly potent MAO inhibitors [57,58] resulted in a decreased activity against KDM1A. Introduction of a CH_2 spacer as in example **3** had been described to be detrimental for any MAO inhibitory activity [59]. Similarly, compound **3** resulted to be inactive against KDM1A when tested *in vitro*. The *cis* phenoxycyclopropyl derivative **4** had been described to be

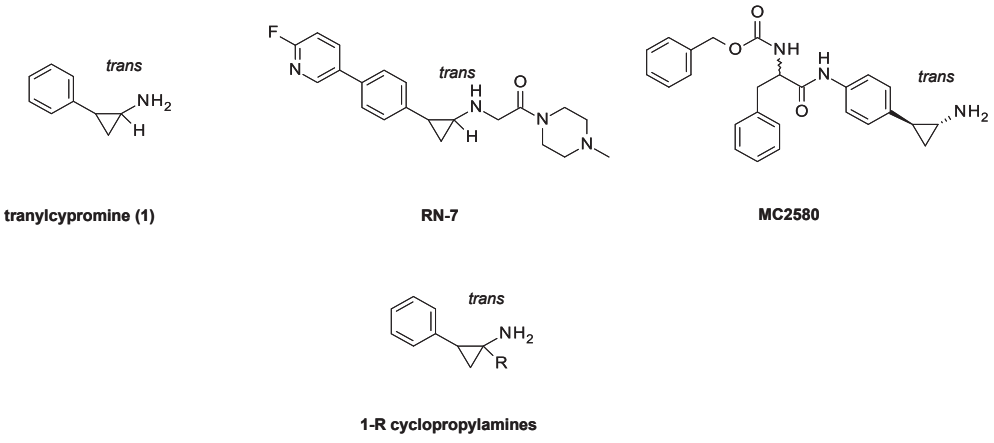
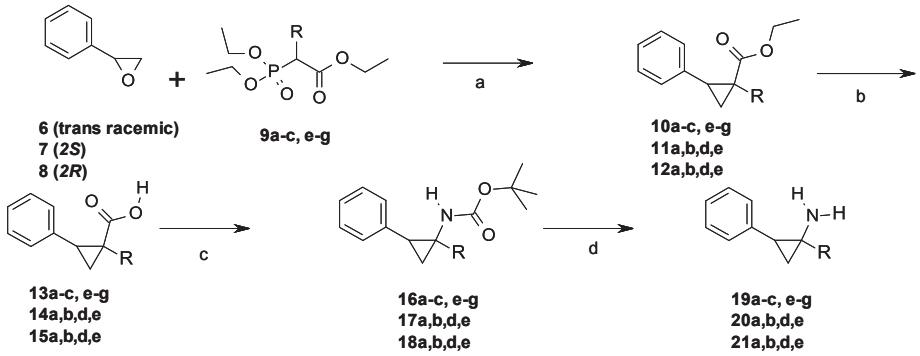
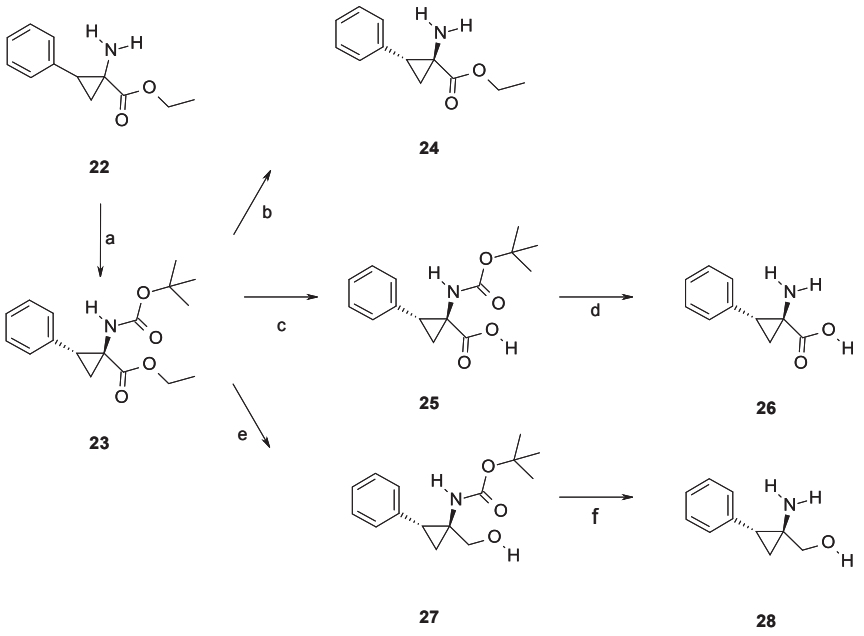


Fig. 1. Cyclopropylamines derivatives as LSD1 inhibitors.



Scheme 1. *Reagents and conditions*: (a) 1,2-dimethoxyethane, BuLi, room temperature, then MW, 130 °C, 90 min; (b) H_2O/THF (1:1), LiOH, MW, 115 °C, 1 h; (c) *tert*-BuOH, diphenyl phosphorazidate, TEA, 90 °C, 18 h; (d) Et_2O/dioxane, HCl, room temperature, 20 h.



Scheme 2. *Reagents and conditions*: (a) CH_2Cl_2, $(BOC)_2O$, DIPEA room temperature, 20 h; (b + d) MeOH, HCl, room temperature, 7 h; (c) EtOH, NaOH, 80 °C, 24 h; (e) THF, $LiAlH_4$, room temperature, 6 h.

more potent than compound **1** as MAO inhibitor [59]. However, in our hands compound **4** as well as its *trans* analogue **5** was completely inactive against KDM1A. Based on these results, we directed our efforts towards derivatives substituted on the cyclopropylamine ring. According to the literature, substitution of the α-hydrogen in **1** by a methyl group results in a MAO inhibitor **19a** with a good *in vitro* potency, which was confirmed by our studies (see Table 1) [60]. Moreover, we were able to demonstrate a positive contribution to the KDM1A inhibitory activity by this replacement. Specifically, inhibitor **19a** exhibited an IC_{50} value of 1.72 μM and was around 10 times more potent than the unsubstituted analogue **1**.

These results prompted us to investigate whether further substituents on the α-position are tolerated or may provide even more effective compounds in inhibiting KDM1A. For this purpose, a set of *trans* isomers with hydrophobic and hydrophilic α-substituents was prepared and tested. As shown in Table 2, we observed that increasing the alkyl chain from methyl to ethyl and *n*-propyl resulted in increasingly more potent inhibitors. In particular, the ethyl analogue **19b** and the *n*-propyl derivative **19c** were around 2 and 4 times more potent than compound **1**, with IC_{50} values of 0.78 and 0.36 μM, respectively. Bulkier hydrophobic substituents provided even more potent KDM1A inhibitors: the phenyl derivative **19d** exhibited an IC_{50} value of 0.16 μM and the benzyl, the 2-naphthylmethyl and the 2-phenylethyl derivatives **19e–g** had similar potencies in the submicromolar range. On the other hand, the $-CH_2OH$ analogue **28** exhibited similar potency compared to parent compound **1**. Similarly, other hydrophilic substituents such as a carboxylic acid and an ethyl ester group proved to be detrimental for the KDM1A inhibitory activity: the cyclopropanecarboxylic acid **26** and the ethyl ester **24** showed at 100 μM only minimal potency by inhibiting 21% and 11% of the enzyme activity, respectively.

Based on these data, we decided to further characterize the 2-phenylcyclopropyl derivatives which showed an increased potency compared to the parent compound **1**. For this purpose, we profiled the compounds for their MAO A and MAO B inhibition [61]. The data summarized in Table 2 show that the α-methyl derivative **19a** was around 10 times more potent against MAO A ($IC_{50} = 0.17$ μM) and 100 times against MAO B ($IC_{50} = 0.01$ μM) than the unsubstituted standard **1**. However, larger substituents on the α-position diminished the inhibitory efficiency against MAO B: the

Table 1
Hit identification of tranylcyclopropylamine analogues.

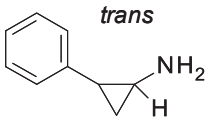
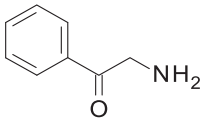
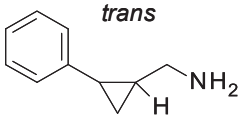
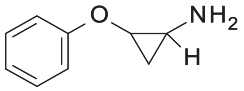
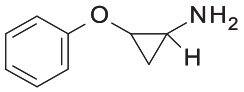
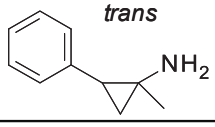
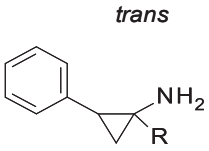
	R	KDM1A IC$_{50}$ (μM) or percentage of inhibition
1		11.6
2		30% at 22 μM
3		0% at 100 μM
4		0% at 100 μM
5		0% at 100 μM
19a		1.72

Table 2
Biological characterization of compounds **1**, **19a**–**g**, **24**, **26** and **28**.[a]



	R	KDM1A IC$_{50}$ (μM)	MAO A IC$_{50}$ (μM)	MAO B IC$_{50}$ (μM)	Target modulation Gfi-1b[b] (Fold increase vs. vehicle treated cells)
1	H	11.6	1.19	1.05	5.3
19a	CH_3	1.72	0.17	0.010	6.4
19b	C_2H_5	0.779	1.92	0.190	6.6
19c	*n*-C_3H_8	0.358	0.527	0.600	5.4
19d	Phenyl	0.161	0.0828	10.4	7.9
19e	Benzyl	0.617	3.51	50.5	6.5
19f	2-Naphthyl-(CH_2)	0.218	1.30	12.7	6.2
19g	Ph-(CH_2)$_2$	0.202	0.0214	0.941	5.7
24	$COOC_2H_5$	>100	nd.	nd.	nd.
26	COOH	>100	nd.	nd.	nd.
28	CH_2OH	9.132	nd.	nd.	nd.

[a] Assays done in replicates ($n \geq 2$). Mean values are shown and the standard deviations are <30% of the mean.
[b] The experiment was carried out at the concentration of the inhibitor equal to equal to their respective biochemical IC$_{50}$ value.

ethyl and the *n*-propyl derivatives **19b** and **19c** exhibiting IC$_{50}$ values of 0.19 and 0.60 μM, were around 20 and 60 times less active than the corresponding methyl analogue **19a**. With exception of the 2-phenylethyl analogue **19g**, all other compounds with bulkier α-substituents were much less active and the IC$_{50}$ values were higher than 10 μM. The active site of MAO A is shorter in length and wider than the narrower cavity in MAO B and thus allows to accommodate larger molecules [62]. Indeed, we found that the phenyl derivative **19d** and the 2-phenylethyl analogues **19g** were more potent against MAO A than the methyl derivative **19a**, whereas the IC$_{50}$ values for the ethyl, benzyl and 2-naphthyl derivatives **19b**, **19e** and **19f** were in the micromolar range with IC$_{50}$ values of 1.92, 3.51 and 1.30 μM, respectively. Taken together, these data indicate that inhibition by the herein presented small tranylcypromine analogues follows similar trends for MAO A and KDM1A, with MAO B featuring more distinct properties. Similar results were also observed with other trancylpromine derivatives substituted on their aryl moiety, such as for **MC2580** (Fig. 1) and similar compounds [47]. However, it has become evident that bulky α-substituents generally increase selectivity for KDM1A, as exemplified by the benzyl- and 2-naphthyl-(CH_2)-substituted **19e** and **19f** compounds (Table 2).

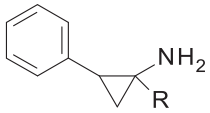
Compounds **19a**–**g** were further characterized for their KDM1A inhibitory activities in cells. Previous data [47] indicated the biological relevance in inhibiting KDM1A both in murine primary promyelocytic blasts and human promyelocytic leukaemia cell lines (NB4). Based on these results we selected NB4 cells as our privileged cellular model and we incubated them with the inhibitors at a concentration equal to their respective biochemical IC$_{50}$ value. After 24 h, Gfi-1b mRNA levels were measured by quantitative RT-PCR. The choice to follow the effect on Gfi-1b, a gene associated with haematopoietic differentiation, was based on the observation that Gf1-b levels are increased following KDM1A deletion and that the gene is a direct transcriptional target of KDM1A [63]. The selective MAO-A inhibitor, chlorgyline, which was inactive on KDM1A, in our hands was used as negative control and as expected it does not lead to any Gfi-1b mRNA level variation at doses up to 100 μM. On the other hand, the known KDM1A inhibitor MC2580 led to an increase the Gfi-1b levels respect the vehicle treated cells by six times.

As shown in Table 2, all tested compounds were able to increase the mRNA expression of Gfi-1b by at least 5 times compared to cells treated with the vehicle and thus demonstrated their ability to block KDM1A activity in cellular systems.

Next, we investigated the KDM1A inhibitory activity of some selected stereoisomers. For this scope, we prepared the *trans* enantiomers of the methyl (**19a**), ethyl (**19b**), phenyl (**19d**), and benzyl (**19e**) derivatives. As summarized in Table 3, there were no significant differences in the KDM1A inhibitory activity based on the chirality of the stereogenic centres. Only in the case of the bulkier phenyl series the differences were somewhat greater: the *1R,2S* derivative **20d** was almost 5 times less potent than the other isomer **21d**, whereas the *1S,2R* benzyl derivative **21e** was less potent than compound **20e**. Consistent with these biochemical data, all tested compounds inhibited KDM1A in NB4 cells to a rather similar extent as shown by a ~5–6 fold up-regulation of the Gfi-1b mRNA expression compared to the vehicle treated control cells.

More relevant differences were instead found for MAO A and MAO B inhibition. For both enzymes, the *1S,2R* isomer **21a** resulted to be the most active compound being 20 and 140 times more potent against MAO A and MAO B than the corresponding *1R,2S* analogue **20a**. High differences in the MAO B inhibitory activity was also found for the ethyl series: the *1S,2R* isomer **21b** exhibited an IC$_{50}$ value of 0.088 μM and was almost 400 times more potent than compound **20b**. Weak inhibitory activity against MAO B was confirmed for the molecules with the larger phenyl or benzyl

Table 3
KDM1A, MAO A and MAO B inhibition as well as gene target modulation of compounds **19a**–**b**, **19d**–**e**, **20a**–**b**, **20d**–**e**, **21a**–**b** and **21d**–**e**.[a]



	R	Stereo-chemistry	KDM1A	MAO A	MAO B	Target modulation Gfi-1b[b]
			IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	(Fold increase vs. vehicle treated cells)
19a	CH_3	*trans*	1.72	0.17	0.010	6.4
20a	CH_3	*1R,2S*	1.78	1.28	0.998	5.0
21a	CH_3	*1S,2R*	3.26	0.070	0.007	4.7
19b	C_2H_5	*trans*	0.779	1.92	0.190	6.6
20b	C_2H_5	*1R,2S*	0.975	6.8	33.3	4.9
21b	C_2H_5	*1S,2R*	0.608	1.43	0.088	4.5
19d	Phenyl	*trans*	0.161	0.083	10.4	7.9
20d	Phenyl	*1R,2S*	0.584	0.235	5.55	4.7
21d	Phenyl	*1S,2R*	0.131	0.094	11.5	5.7
19e	Benzyl	*trans*	0.617	3.51	50.5	6.5
20e	Benzyl	*1S, 2S*	0.335	2.59	28.1	9.7
21e	Benzyl	*1R, 2R*	nd[a]	8.31	74.3	9.8

[a] The IC_{50} value could not be determined due to a significant dose dependent interferences with the fluorescent signal of enzymatic assay.
[b] The experiment was carried out at the concentration of the inhibitor equal to equal to their respective biochemical IC_{50} value.

both varying α substituents and chiralities. For five inhibitors (**20a**, **20b**, **20e**, **21a**, and **21d**) the quality of the diffraction allowed us to solve the structures at resolutions sufficient for structural analysis (Fig. 2; Table 4). A first observation is the ring opening of the cyclopropylamine moiety in all compounds and the formation of

Table 4
Crystallographic statistics.

	20e	**20b**	**20a**	**21a**	**21d**
PDB code	4uv8	4uv9	4uva	4uvb	4uvc
Resolution (Å)	2.8	3.0	2.9	2.8	3.1
R_{merge} (%)[a,b,c]	7.5 (83.3)	14.3 (106.0)	6.7 (56.5)	8.7 (54.4)	19.4 (132.1)
Completeness (%)[c]	99.3 (98.4)	99.5 (99.8)	99.6 (99.7)	99.9 (99.8)	100.0 (99.9)
Unique reflections	62,089	49,581	55,688	62,197	45,854
Redundancy[c]	3.7 (3.6)	3.4 (3.6)	4.1 (4.2)	4.0 (4.0)	6.1 (5.6)
$I/\sigma c$	12.5 (1.5)	7.5 (1.3)	13.3 (2.8)	9.3 (2.5)	9.0 (1.2)
N of atoms	6367	6358	6358	6357	6361
R_{factor} (%)[d]	22.6	22.6	22.0	22.2	21.0
R_{free} (%)[d]	23.7	24.6	23.2	23.6	24.1
Rms bond length (Å)	0.005	0.005	0.005	0.005	0.006
Rms bond angle (°)	0.913	0.976	0.905	0.888	1.097

[a] Space group is I222.
[b] $R_{merge} = \Sigma|I_i - <I>|/\Sigma I_i$, where I_i is the intensity of *i*th observation and $<I>$ is the mean intensity of the reflection.
[c] Values in parentheses are for reflections in the highest resolution shell.
[d] $R_{cryst} = \Sigma|F_{obs} - F_{calc}|/\Sigma F_{obs}$ where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes, respectively.

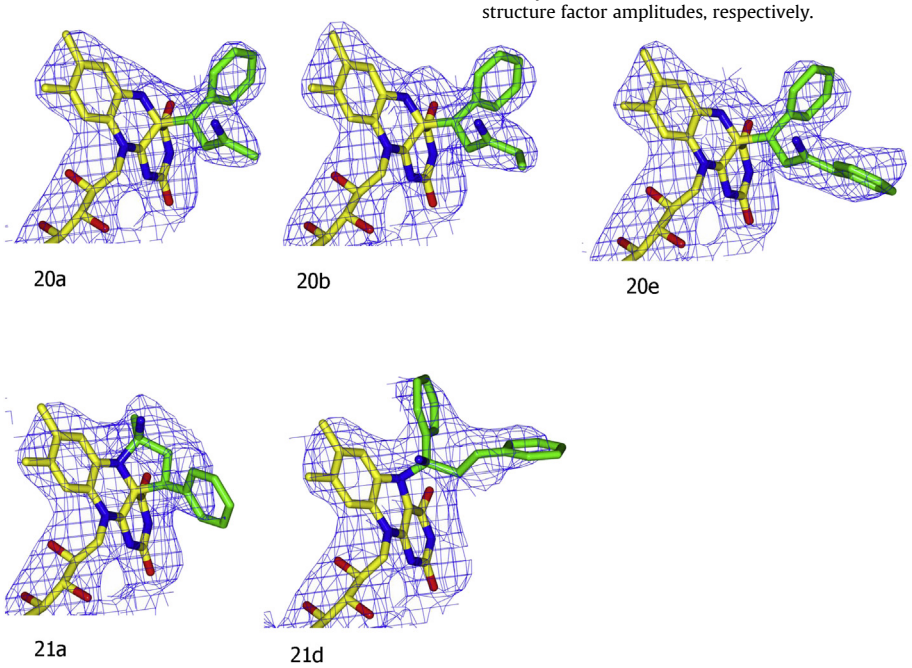


Fig. 2. Crystallographic data on binding of the inhibitors **20a**, **20b**, **20e**, **21a** and **21d** to human LSD1/CoREST. The weighted 2Fo-FC electron density maps (blue mesh) were calculated before the inclusion of the inhibitor atoms in the refinement to avoid model bias. The contour level is 1.2–1.3 σ. Carbon atoms of flavin and inhibitor are in yellow and green, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

substituents. In the case of MAO A, the methyl and phenyl *1S,2R* analogues **21a** and **21d** were the most potent stereoisomers with IC_{50} values of 0.07 and 0.094 μM, respectively. These data indicate that the two enantiomers exhibit different inhibitory activities, which are more pronounced for MAOs.

These observations prompted us to investigate the three-dimensional structures of KDM1A/CoREST in complex with the inhibitors using X-ray crystallography. The compounds used for these studies were chosen with the goal to investigate the effect of covalent adducts with the FAD by adding to the N5 or C4a atoms of the flavin or to both. A second indication is that the inhibitors are hosted in the wide active-site cleft, which forms the binding site for the *N*-terminal H3 peptide. As observed for other tranycylpromine analogues [47,49], the width of the cleft enables the various substituents on the α carbon to remain mostly exposed and accessible to the solvent, even when bound to the flavin and with no specific interactions (i.e. H-bonds) between the ligand and protein atoms. Therefore, the differences in inhibitor potencies appear to be

mainly due to non-specific van der Waals protein-inhibitor interactions and small changes in the reactivity of the tranylcypromine ring induced by the variations in the bulkiness and chemical nature of the α substituents.

These data are of particular interest in respect to the mechanism of FAD inactivation by these irreversible inhibitors. Indeed, α-substitution prevents that the C–NH_2 bonds of the inhibitors are enzymatically oxidized to an imine product (Fig. 3). Therefore, a "suicide substrate" mechanism can be ruled out, in which the inhibitor amine group has to be first oxidized to an imine before binding covalently to the flavin. Instead, our studies support the hypothesis that the ring opening occurs directly, possibly through a radical mechanism, without prior enzymatic oxidation of the inhibitor amino group [64]. Further evidences for such a mechanism is given by the finding that three types adducts are observed, with a pattern consistent with the chirality of the investigated inhibitors. The electron density for compound **21a** (α-methyl substitution with *1S,2R* chirality) is consistent with a cyclic adduct in which the 1 and 2 carbons are respectively attached to the N5 and C4a flavin atoms, as originally found for tranylcypromine bound to KDM1A [49]. Conversely, **21d** (*1S,2R*; α-phenyl) forms an adduct in which there is a single linkage between carbon 1 of the ring-opened inhibitor and the flavin N5, while the three other inhibitors **20a**, **20b**, and **20e** feature a covalent linkage between flavin C4a and inhibitor carbon 2. Collectively, these data support an inhibition mechanism, in which a cyclic adduct is initially formed. This can be followed by opening of the bond to either the N5 or C4a atoms, depending (preferentially) on the chirality of the cyclopropyl groups (Fig. 3). We do not rule out other possibilities such as initial formation of a "single-linkage" adduct followed by chemical recombination or migration of the group that initially adds to the flavin. However, independently from the exact mechanism, these data demonstrate that α-substitution (e.g. absence of an "oxidizable" C–NH bond) does not prevent covalent inhibition and that both enantiomeric forms of the inhibitor retain inhibitory reactivity although (preferentially) giving rise to different adducts, which might correlate with the observed slight differences in potencies.

3. Conclusion

A novel series of tranylcypromine analogues containing a substituted cyclopropyl core moiety have been described as demethylase inhibitors. We found that the inhibitory activity was strongly influenced by the type of the substitution on the cyclopropyl group. Introduction of hydrophobic groups, among them alkyls, phenyl, or benzyl, resulted in compounds with a higher KDM1A inhibitory potency, compared to the standard tranylcypromine (**1**), while a carboxylic acid and an ethyl ester group proved to be detrimental. Introduction of a methyl group resulted also in an increased potency against the MAO enzymes, while the introduction of larger substituents increased the selectivity for KDM1A substantially over MAO B and to a minor extent over MAO

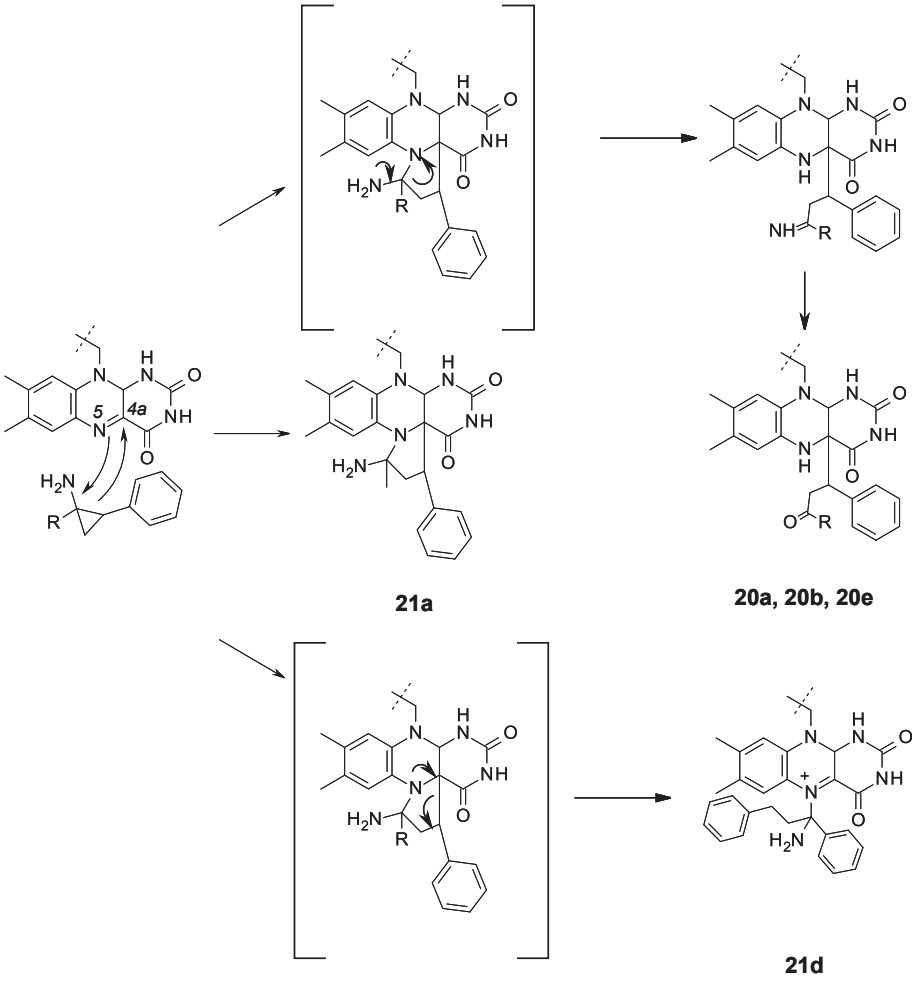


Fig. 3. Proposed mechanism for the formation of different cyclopropylamine adducts to the FAD cofactor.

A. These trends can be explained by the overall larger active site of KDM1A compared to the other two enzymes. This may also explain why the single *trans* enantiomers of some selected derivatives showed major differences in their MAO inhibitory activity and only minor ones for KDM1A, even though the crystal structure of KDM1A with the *1R,2S* enantiomers **20a**, **20b** and **20d** revealed the formation of different covalent adducts of the inhibitors to the FAD cofactor than with the *1S,2R* enantiomers **21a** and **21d**. Taken together, all these findings highlight the potential of the modifications on the carbon 1 position on the tranylcypromine scaffold as a feasible route to achieve highly potent KDM1A inhibitors with an increased selectivity over MAO enzymes.

4. Experimental section

4.1. Chemistry

Reagents and solvents used, unless stated otherwise, were of commercially available reagent grade quality and were used without further purification. Flash chromatography purifications were performed on Merck silica gel 60 (0.04–0.063 mm). Nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian 500 MHz spectrometer at 300 K and are referenced in ppm (δ) relative to TMS. Coupling constants (J) are expressed in hertz (Hz). HPLC–MS experiments were performed on an Acquity UPLC apparatus, equipped with a diode array and a Micromass SQD single quadruple (Waters). Purity was monitored at 220 nm and the purities of the compounds used for biological tests were found to be at least 95%. Ethyl 1-amino-2-phenyl-cyclopropanecarboxylate was prepared as described by Wurtz et al. [56]. Triethyl 2-phosphonopropionate (**9a**, Sigma Aldrich, Cat No. 174653), triethyl 2-phosphonobutanoate (**9b**, Sigma Aldrich, Cat No. 417467), and triethyl 2-phosphonopentanoate (**9c**, Alfa Aesar, Cat. No. 30413) are commercially available. Ethyl 2-(diethoxyphosphoryl)-3-phenylpropanoate (**9e**) was prepared as described in Eur. J. Org. Chem. [65], triethyl phosphonophenylacetate (**9d**) as in J. Org. Chem [66]. *trans*-1,2-Diphenylcyclopropanamine hydrochloride (**19d**) was prepared as described in Acta Chem. Scand. [55].

4.1.1. (1S,2R)-1-Ethyl-2-phenyl-cyclopropanamine hydrochloride (**20a**)

*4.1.1.1. Ethyl (1R,2S)-1-methyl-2-phenyl-cyclopropanecarboxylate (**11a**).* 5.6 mL (2.5 mol/L) butyl lithium was added to a solution of 3.4 g (14 mmol) ethyl 2-diethoxyphosphorylbutanoate (Sigma Aldrich) in 12 mL dry DME under N_2 atmosphere at RT. After 5 min, 1.3 g (11 mmol) (*2S*)-2-phenyloxirane (Sigma Aldrich) was added dropwise. The mixture was stirred for 20 min at RT and then heated at 130 °C under MW irradiation for 90 min. Aqueous NH_4Cl was added and the product was extracted with Et_2O. The combined organic layers were dried over Na_2SO_4 and concentrated. The dry residue was purified by column chromatography (eluent: EtOAc/hexane, 0:100–10:100) to give the ethyl cyclopropanecarboxylate **11a** (2.9 g, 90%). ^{1}H NMR ($CDCl_3$) δ (ppm): 7.25–7.20 (m, 2H), 7.19–7.13 (m, 1H), 7.12 (d, J = 7.3 Hz, 2H), 4.13–4.06 (m, 2H), 2.75–2.71 (m, 1H), 1.63–1.59 (m, 1H), 1.24–1.19 (m, 3H), 1.12–1.07 (m, 1H), 0.91 (s, 3H). MS (ESI): m/z: 205 $[M+H]^+$.

*4.1.1.2. (1R,2S)-1-Methyl-2-phenyl-cyclopropanecarboxylic acid (**14a**).* 2.9 g (9.9 mmol) ethyl ester **11a** was dissolved in 12 mL of a water/Ethanol/THF (1:1:1, v:v:v) mixture. The solution was cooled down to 0 °C, 4 g (40 mmol) of LiOH added and the mixture stirred under microwave irradiation at 115 °C for 1 h. The solution was concentrated, quenched with 2 M HCl, and the formed precipitate filtered off, washed with water and dried, yielding 1.55 g (89%) of the cyclopropanecarboxylic acid **14a**. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.34–7.27 (m, 2H), 7.26–7.18 (m, 2H), 2.67–2.64 (m, 1H), 1.51–1.48 (m, 1H), 1.32–1.22 (m, 1H), 0.85 (s, 3H). MS (ESI): m/z: 175 $[M-H]^-$.

*4.1.1.3. tert-Butyl N-[(1R,2S)-1-methyl-2-phenyl-cyclopropyl]carbamate (**17a**).* 2.4 g (8.7 mmol) diphenyl phosphorazidate (Sigma Aldrich) and 1.0 g (10 mmol) TEA were added to a solution of 1.4 g (7.9 mmol) carboxylic acid **14a** in 25 mL dry *tert*-Butanol, the resulting solution was stirred at 90 °C for 18 h. The mixture was concentrated and the residue partitioned between 10% aqueous Na_2CO_3 and Et_2O. The combined organic layers were dried (Na_2SO_4), filtered, concentrated *in vacuo* and purified by column chromatography (eluent: EtOAc/cyclohexane, 1:100–10:100) to give 1.37 g (69%) of the carbamate intermediate **17a**. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.28–7.10 (m, 5H), 4.96 (bs, 1H), 2.29–2.26 (m, 1H), 1.40 (bs, 9H), 1.16–1.05 (m, 1H), 0.98 (s, 3H), 0.96–0.93 (m, 1H). MS (ESI): m/z: 248 $[M+H]^+$

*4.1.1.4. (1R,2S)-1-Methyl-2-phenyl-cyclopropanamine hydrochloride (**20a**).* A solution of 60 mg (0.24 mmol) of *tert*-Butyl *N*-[(*1R,2S*)-1-methyl-2-phenyl-cyclopropyl]carbamate 17a, in 2 mL Et_2O was cooled down to 0 °C. 4 M HCl in dioxane (1.2 mL) was added and the solution stirred at RT for 20 h. The solvent was evaporated and the resulting residue triturated twice with Et_2O giving 32 mg (72%) of the desired cyclopropanamine hydrochloride **20a** as a white solid. ^{1}H NMR (DMSO-d_6) δ (ppm): 8.45 (s, 3H), 7.36–7.30 (m, 2H), 7.27–7.21 (m, 3H), 2.50–2.46 (m, 1H), 1.41–1.35 (m, 1H), 1.26–1.21 (m, 1H), 1.02 (s, 3H). MS (ESI): m/z: 148 $[M+H]^+$.

According to the procedure described for example **11a** the following compounds were synthesized starting from the appropriate styrene oxides and phosphonoacetates:

4.1.2. Ethyl trans-1-methyl-2-phenyl-cyclopropanecarboxylate (**10a**)

Yield: 84%. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.34–7.28 (m, 2H), 7.26–7.22 (m, 1H), 7.22–7.18 (m, 2H), 4.22–4.15 (m, 2H), 2.85–2.77 (m, 1H), 1.72–1.65 (m, 1H), 1.30 (t, J = 7.1 Hz, 3H), 1.19–1.13 (m, 1H), 0.99 (s, 3H).

4.1.3. Ethyl trans-1-ethyl-2-phenyl-cyclopropanecarboxylate (**10b**)

Yield: 79%. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.34–7.17 (m, 5H), 4.27–4.14 (m, 2H), 2.86–2.77 (m, 1H), 1.69–1.61 (m, 2H), 1.31 (t, J = 7.1 Hz, 3H), 1.22–1.14 (m, 1H), 0.94–0.83 (m, 4H).

4.1.4. Ethyl trans-2-phenyl-1-propyl-cyclopropanecarboxylate (**10c**)

Yield: 89%. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.39–7.07 (m, 5H), 4.30–4.08 (m, 2H), 2.83–2.69 (m, 1H), 1.72–1.67 (m, 1H), 1.66–1.58 (m, 1H), 1.45–1.26 (m, 5H), 1.21–1.16 (m, 1H), 0.83–0.77 (m, 1H), 0.75 (t, J = 1.0 Hz, 3H).

4.1.5. Ethyl trans-1-benzyl-2-phenyl-cyclopropanecarboxylate (**10e**)

Yield 72%. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.44–7.10 (m, 10H), 4.20–4.03 (m, 2H), 3.23–3.15 (m, 1H), 2.99–2.93 (m, 1H), 2.03–1.98 (m, 1H), 1.92–1.87 (m, 1H), 1.45–1.42 (m, 1H), 1.20 (t, J = 7.1 Hz, 3H).

4.1.6. Ethyl trans-1-(2-naphthylmethyl)-2-phenyl-cyclopropanecarboxylate (**10f**)

Yield 80%. ^{1}H NMR ($CDCl_3$) δ (ppm) 7.81–7.77 (m, 1H), 7.76–7.69 (m, 2H), 7.56–7.53 (m, 1H), 7.48–7.35 (m, 4H), 7.33–7.28 (m, 4H), 4.23–4.07 (m, 2H), 3.37 (d, J = 15.7 Hz, 1H), 2.95–2.89 (m, 1H), 2.23–2.16 (m, 1H), 1.96–1.91 (m, 1H), 1.53–1.47 (m, 1H), 1.20 (t, J = 1.0 Hz, 3H).

*4.1.7. Ethyl trans-1-(2-phenylethyl)-2-phenyl-cyclopropanecarboxylate (**10g**)*

Yield 68%. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.41–6.84 (m, 10H), 4.29–4.15 (m, 2H), 2.92–2.82 (m, 1H), 2.68–2.49 (m, 2H), 1.84–1.74 (m, 1H), 1.71–1.65 (m, 1H), 1.42–1.30 (m, 4H), 1.22 (dd, J = 4.9, 7.3 Hz, 1H).

*4.1.8. Ethyl (1S,2R)-1-methyl-2-phenyl-cyclopropanecarboxylate (**12a**)*

Yield 73%. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.35–7.15 (m, 5H), 4.23–4.13 (m, 2H), 2.86–2.76 (m, 1H), 1.73–1.66 (m, 1H), 1.30 (t, J = 7.1 Hz, 3H), 1.21–1.14 (m, 1H), 0.99 (s, 3H).

*4.1.9. Ethyl (1R,2S)-1-ethyl-2-phenyl-cyclopropanecarboxylic acid (**11b**)*

Compound **11b** was prepared as described by Bray et al. [54].

*4.1.10. Ethyl (1S,2R)-1-ethyl-2-phenyl-cyclopropanecarboxylate (**12b**)*

Yield 63%. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.35–7.16 (m, 5H), 4.32–4.05 (m, 2H), 2.89–2.75 (m, 1H), 1.73–1.58 (m, 2H), 1.30 (t, J = 7.1 Hz, 3H), 1.20–1.14 (m, 1H), 1.06–0.79 (m, 4H).

*4.1.11. Ethyl (1R,2S)-1,2-diphenylcyclopropanecarboxylate (**11d**)*

Yield: quantitative. MS (ESI): m/z: 267 $[M+H]^+$.

*4.1.12. Ethyl (1S,2R)-1,2-diphenylcyclopropanecarboxylate (**12d**)*

Yield: 70% MS (ESI): m/z: 267 $[M+H]^+$.

*4.1.13. Ethyl (1S,2S)-1-benzyl-2-phenyl-cyclopropanecarboxylic acid (**11e**)*

Compound **11e** was prepared as described by Bray et al. [54].

*4.1.14. Ethyl (1R,2R)-1-benzyl-2-phenyl-cyclopropanecarboxylate (**12e**)*

Yield 63%. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.42–7.03 (m, 10H), 4.21–4.01 (m, 2H), 3.24–3.17 (m, 1H), 2.89–2.84 (m, 1H), 2.04–1.97 (m, 1H), 1.92–1.87 (m, 1H), 1.46–1.40 (m, 1H), 1.20 (t, J = 7.1 Hz, 3H). MS (ESI): m/z: 281 $[M+H]^+$.

The following ethyl esters were hydrolysed following the procedure for the acrylic acid **14a**:

*4.1.15. trans-1-Methyl-2-phenyl-cyclopropanecarboxylic acid (**13a**)*

Yield 57%. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.40 (bs, 1H), 7.33–7.16 (m, 5H), 2.70–2.59 (m, 1H), 1.53–1.41 (m, 1H), 1.29–1.21 (m, 1H), 0.85 (s, 3H). MS (ESI): m/z: 175 $[M-H]^-$.

*4.1.16. trans-1-Ethyl-2-phenyl-cyclopropanecarboxylic acid (**13b**)*

Yield 96%. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.26 (bs, 1H), 7.34–7.16 (m, 5H), 2.69 (m, 1H), 1.51–1.36 (m, 2H), 1.31 (m, 1H), 1.00–0.86 (m, 1H), 0.76 (t, J = 7.1 Hz, 3H). MS (ESI): m/z: 189 $[M-H]^-$.

*4.1.17. trans-2-Phenyl-1-propyl-cyclopropanecarboxylic acid (**13c**)*

Yield 57%. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.23 (s, 1H), 7.34–7.26 (m, 2H), 7.25–7.17 (m, 3H), 2.68–2.61 (m, 1H), 1.51–1.45 (m, 1H), 1.44–1.36 (m, 1H), 1.32 (dd, J = 4.6, 7.1 Hz, 1H), 1.30–1.18 (m, 2H), 0.86–0.75 (m, 1H), 0.65 (t, J = 7.3 Hz, 3H). MS (ESI): m/z: 203 $[M-H]^-$.

*4.1.18. trans-1-Benzyl-2-phenyl-cyclopropanecarboxylic acid (**13e**)*

Yield 70%. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.4–12.2 (m, 1H), 7.35–6.10 (m, 10H), 3.00–2.92 (m, 1H), 2.78–2.71 (m, 1H), 2.00–1.94 (m, 1H), 1.69–1.64 (m, 1H), 1.57–1.52 (m, 1H). MS (ESI): m/z: 237 $[M-H]^-$.

*4.1.19. trans-1-Ethyl-2-(2-naphthyl)cyclopropanecarboxylic acid (**13f**)*

Yield 94%. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.42 (s, 1H), 7.97–7.04 (m, 12H), 3.11 (d, J = 15.7 Hz, 1H), 2.85–2.76 (m, 1H), 2.20 (d, J = 15.7 Hz, 1H), 1.75–1.69 (m, 1H), 1.67–1.61 (m, 1H). MS (ESI): m/z: 301 $[M-H]^-$.

*4.1.20. trans-1-(2-Phenylethyl)-2-phenyl-cyclopropanecarboxylic acid (**13g**)*

Yield 54%. ^{1}H NMR (DMSO-d_6) δ (ppm): 13.53–11.44 (m, 1H), 7.35–6.78 (m, 10H), 2.76–2.70 (m, 1H), 2.64–2.54 (m, 1H), 2.43–2.34 (m, 1H), 1.60–1.23 (m, 4H). MS (ESI): m/z: 265 $[M-H]^-$.

*4.1.21. (1S,2R)-1-Methyl-2-phenyl-cyclopropanecarboxylic acid (**15a**)*

Yield 94%. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.36–7.30 (m, 2H), 7.29–7.24 (m, 1H), 7.23–7.19 (m, 2H), 2.92 (dd, J = 7.3, 9.3 Hz, 1H), 1.80 (dd, J = 4.6, 9.0 Hz, 1H), 1.30–1.25 (m, 1H), 1.01 (s, 3H). MS (ESI): m/z: 175 $[M-H]^-$.

*4.1.22. (1R,2S)-1-Ethyl-2-phenyl-cyclopropanecarboxylic acid (**14b**)*

Yield 95%. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.28 (bs, 1H), 7.37–7.12 (m, 5H), 2.69 (dd, J = 7.3, 8.8 Hz, 1H), 1.53–1.35 (m, 2H), 1.31 (dd, J = 4.4, 6.8 Hz, 1H), 1.02–0.84 (m, 1H), 0.76 (t, J = 7.3 Hz, 3H). MS (ESI): m/z: 189 $[M-H]^-$.

*4.1.23. (1S,2R)-1-Ethyl-2-phenyl-cyclopropanecarboxylic acid (**15b**)*

Yield 66%. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.25 (s, 1H), 7.34–7.26 (m, 2H), 7.25–7.17 (m, 3H), 2.74–2.65 (m, 1H), 1.50–1.35 (m, 2H), 1.34–1.25 (m, 1H), 0.98–0.85 (m, 1H), 0.76 (t, J = 1.0 Hz, 3H). MS (ESI): m/z: 189 $[M-H]^-$.

*4.1.24. (1R,2S)-1,2-Diphenylcyclopropanecarboxylic acid (**14d**)*

Yield 67%. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.6–12.3 (m, 1H), 7.12–6.96 (m, 8H), 6.86–6.81 (m, 2H), 3.05–2.98 (m, 1H), 2.27–2.15 (m, 1H), 2.05–1.88 (m, 1H). MS (ESI): m/z: 237 $[M-H]^-$.

*4.1.25. (1S,2R)-1,2-Diphenylcyclopropanecarboxylic acid (**15d**)*

Yield 80%. ^{1}H NMR ($CDCl_3$) δ (ppm): 11.96–8.25 (m, 1H), 7.12–7.17 (m, 3H), 7.02–7.10 (m, 5H), 6.75–6.82 (m, 2H), 3.24–3.10 (m, 1H), 2.27–2.15 (m, 1H), 2.05–1.88 (m, 1H). MS (ESI): m/z: 237 $[M-H]^-$.

*4.1.26. (1S,2S)-1-Benzyl-2-phenyl-cyclopropanecarboxylic acid (**14e**)*

Yield 67%. ^{1}H NMR (DMSO-d_6) δ (ppm): 12.34 (s, 1H), 7.50–7.49 (m, 1H), 7.54–7.05 (m, 10H), 6.97–6.96 (m, 1H), AB System: VA = 2.96, VB = 1.97, JAB = 15.4 Hz, 2.81–2.68 (m, 1H), 1.77–1.61 (m, 1H), 1.58–1.47 (m, 1H). MS (ESI): m/z: 251 $[M-H]^-$.

*4.1.27. (1R,2R)-1-Benzyl-2-phenyl-cyclopropanecarboxylic acid (**15e**)*

Yield 78%. ^{1}H NMR ($CDCl_3$) δ (ppm):12.07–9.85 (m, 1H), 7.44–7.09 (m, 10H), 3.30–3.19 (m, 1H), 3.02–2.95 (m, 1H), 2.00–1.91 (m, 2H), 1.55–1.46 (m, 1H). MS (ESI): m/z: 251 $[M-H]^-$.

Curtius rearrangement with diphenyl phosphorazidate was performed for the following derivatives according to the procedure for intermediate **17a**:

*4.1.28. tert-Butyl-N-[trans-1-methyl-2-phenyl-cyclopropyl] carbamate (**16a**)*

Yield 60%. ^{1}H NMR (DMSO-d_6) δ (ppm): 7.34 (bs, 1H), 7.31–7.15 (m, 5H), 2.22–2.08 (m, 1H), 1.39 (s, 9H), 1.10–0.98 (m, 2H), 0.86 (s, 3H). MS (ESI): m/z: 192 $[MH-56]^+$.

*4.1.29. tert-Butyl-N-[trans-1-ethyl-2-phenyl-cyclopropyl] carbamate (**16b**)*

Yield 50%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 7.41–7.17 (m, 5H), 5.02 (bs, 1H), 2.51–2.39 (m, 1H), 1.66–1.40 (m, 10H), 1.20–1.09 (m, 1H), 1.05–0.99 (m, 1H), 0.97–0.79 (m, 4H). MS (ESI): *m*/*z*: 206 [MH-56]$^+$.

*4.1.30. tert-Butyl-N-[trans-1-propyl-2-phenyl-cyclopropyl] carbamate (**16c**)*

Yield 60%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.45–7.06 (m, 5H), 5.01 (s, 1H), 2.47–2.32 (m, 1H), 1.65–1.26 (m, 12H), 1.19–1.11 (m, 1H), 1.07–1.00 (m, 1H), 0.78 (t, J = 7.3 Hz, 4H). MS (ESI): *m*/*z*: 276 [MH]$^+$.

*4.1.31. tert-Butyl-N-[trans-1-benzyl-2-phenyl-cyclopropyl] carbamate (**16e**)*

Yield 33%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.50–7.18 (m, 8H), 7.05–7.00 (m, 2H), 4.82 (s, 1H), 3.05–2.95 (m, 1H), 2.56–2.51 (m, 1H), 2.11–1.96 (m, 1H), 1.70–1.40 (m, 9H), 1.33–1.28 (m, 1H), 1.18–1.13 (m, 1H). MS (ESI): *m*/*z*: 224 [MH-100]$^+$.

*4.1.32. tert-Butyl-N-[trans-1-(2-naphthylmethyl)-2-phenyl-cyclopropyl]carbamate (**16f**)*

Yield 36%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.83–7.79 (m, 1H), 7.76–7.70 (m, 2H), 7.54–7.36 (m, 7H), 7.32–7.26 (m, 1H), 7.20–7.15 (m, 1H), 4.80 (s, 1H), 3.36–2.96 (m1 H), 2.60–2.52 (m, 1H), 2.31–2.17 (m, 1H), 1.65–1.45 (m, 8H), 1.44–1.38 (m, 1H), 1.24–1.16 (m, 1H), 1.25–1.12 (m, 1H): *m*/*z*: 274 [MH-100]$^+$.

*4.1.33. tert-Butyl-N-[trans-1-(2-phenylethyl)-2-phenyl-cyclopropyl]carbamate (**16g**)*

Yield 49%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.54–6.77 (m, 10H), 4.99 (s, 1H), 2.72–2.55 (m, 2H), 2.48–2.40 (m, 1H), 1.89–1.77 (m, 1H), 1.66–1.42 (m, 9H), 1.35–1.24 (m, 1H), 1.19–1.13 (m, 1H), 1.09–1.01 (m, 1H).

*4.1.34. tert-Butyl-N-[(1S,2R)-1-methyl-2-phenyl-cyclopropyl] carbamate (**18a**)*

Yield 59%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.51–7.01 (m, 5H), 5.02 (s, 1H), 2.49–2.28 (m, 1H), 1.74–1.36 (m, 9H), 1.21–1.15 (m, 1H), 1.11–0.98 (m, 4H).

*4.1.35. tert-Butyl-N-[(1R,2S)-1-ethyl-2-phenyl-cyclopropyl] carbamate (**17b**)*

Yield 51%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.45–7.12 (m, 5H), 5.02 (bs, 1H), 2.44 (t, J = 8.1 Hz, 1H), 1.72–1.39 (m, 10H), 1.02 (dd, J = 5.9, 6.8 Hz, 1H), 0.96–0.77 (m, 5H).

*4.1.36. tert-Butyl-N-[(1S,2R)-1-ethyl-2-phenyl-cyclopropyl] carbamate (**18b**)*

Yield 43%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.45–7.12 (m, 5H), 5.02 (bs, 1H), 2.44 (t, J = 8.1 Hz, 1H), 1.72–1.39 (m, 10H), 1.02 (dd, J = 5.9, 6.8 Hz, 1H), 0.96–0.77 (m, 5H). MS (ESI): *m*/*z*: 162 [MH-100]$^+$.

*4.1.37. tert-Butyl-N-[(1R,2S)-1,2-diphenylcyclopropyl]carbamate (**17d**)*

Yield 67%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.57–6.79 (m, 10H), 5.57–5.11 (m, 1H), 2.96–2.59 (m, 1H), 1.93–1.74 (m, 1H), 1.71–1.58 (m, 1H), 1.46 (s, 9H). MS (ESI): *m*/*z*: 310 [MH]$^+$.

*4.1.38. tert-Butyl-N-[(1S,2R)-1,2-diphenylcyclopropyl]carbamate (**18d**)*

Yield 53%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.45–6.83 (m, 10H), 5.56–5.34 (m, 1H), 2.94–2.68 (m, 1H), 1.90–1.78 (m, 1H), 1.69–1.63 (m, 1H), 1.45 (s, 9H). MS (ESI): *m*/*z*: 310 [MH]$^+$.

*4.1.39. tert-Butyl-N-[(1S,2S)-1-benzyl-2-phenyl-cyclopropyl] carbamate (**17e**)*

Yield 33%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.62–7.31 (m, 4H), 7.29–7.15 (m, 4H), 7.09–6.95 (m, 2H), 4.82 (s, 1H), AB System: VA = 3.01, VB = 2.02, JAB = 13.9 Hz, 2.62–2.41 (m, 1H), 1.56 (s, 9H), 1.36–1.25 (m, 1H), 1.20–0.95 (m, 1H).

*4.1.40. tert-Butyl-N-[(1R,2R)-1-benzyl-2-phenyl-cyclopropyl] carbamate (**18e**)*

Yield 35%. ^{1}H NMR (CDCl$_3$) δ (ppm): 7.62–6.92 (m, 10H), 4.82 (s, 1H), 3.01 (m, 1H), 2.61–2.47 (m, 1H), 2.16–1.96 (m, 1H), 1.68–1.40 (m, 9H), 1.34–1.28 (m, 1H), 1.18–1.13 (m, 1H). MS (ESI): *m*/*z*: 224 [MH-100]$^+$.

The following cyclopropylamines were deprotected according to the procedure for the amine **20a**:

*4.1.41. trans-1-Methyl-2-phenyl-cyclopropanamine hydrochloride (**19a**)*

Yield 96%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.42 (bs, 3H), 7.40–7.13 (m, 5H), 2.48–2.45 (m, 1H), 1.41–1.34 (m, 1H), 1.28–1.20 (m, 1H), 1.02 (s, 3H). MS (ESI): *m*/*z*: 148 ([M+H]$^+$). Purity: 96%.

*4.1.42. trans-1-Ethyl-2-phenyl-cyclopropanamine hydrochloride (**19b**)*

Yield 97%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.28 (s, 3H), 7.36–7.29 (m, 2H), 7.28–7.21 (m, 3H), 2.59–2.44 (m, 1H), 1.39–1.27 (m, 3H), 1.27–1.16 (m, 1H), 0.79 (t, J = 7.6 Hz, 3H). MS (ESI): *m*/*z*: 162 ([M+H]$^+$). Purity: 100%.

*4.1.43. trans-1-Propyl-2-phenyl-cyclopropanamine hydrochloride (**19c**)*

Yield 78%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.46 (s, 3H), 7.41–7.28 (m, 2H), 7.28–7.14 (m, 3H), 2.58–2.51 (m, 1H), 1.43–1.18 (m, 5H), 1.12–0.99 (m, 1H), 0.67 (t, J = 1.0 Hz, 3H). MS (ESI): *m*/*z*: 176 ([M+H]$^+$). Purity: 99%.

*4.1.44. trans-1-Benzyl-2-phenyl-cyclopropanamine hydrochloride (**19e**)*

Yield 78%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.30 (s, 3H), 7.45–7.20 (m, 8H), 7.14–6.99 (m, 2H), 2.82 (d, 1H), 2.69–2.57 (m, 1H), 2.33 (d, 1H), 1.79–1.58 (m, 1H), 1.49–1.32 (m, 1H). MS (ESI): *m*/*z*: 224 ([M+H]$^+$). Purity: 99%.

*4.1.45. trans-1-(2-Naphthylmethyl)-2-phenyl-cyclopropanamine hydrochloride (**19f**)*

Yield 91%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.28 (s, 3H), 7.93–7.69 (m, 3H), 7.62–7.12 (m, 9H), AB System: VA = 2.96, VB = 2.53, J_{AB} = 15.2 Hz, 2.75–2.59 (m, 1H), 1.87–1.72 (m, 1H), 1.51–1.37 (m, 1H). MS (ESI): *m*/*z*: 274 ([M+H]$^+$). Purity: 99%.

*4.1.46. trans-1-(2-Phenylethyl)-2-phenyl-cyclopropanamine hydrochloride (**19g**)*

Yield 66%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.33 (s, 3H), 7.40–7.32 (m, 2H), 7.31–7.23 (m, 3H), 7.22–7.14 (m, 2H), 7.13–7.06 (m, 1H), 6.87–6.76 (m, 2H), 2.66–2.53 (m, 2H), 2.48–2.40 (m, 1H), 1.58–1.45 (m, 2H), 1.44–1.33 (m, 2H). MS (ESI): *m*/*z*: 238 ([M+H]$^+$). Purity: 99%.

*4.1.47. (1R,2S)-1-Ethyl-2-phenyl-cyclopropanamine hydrochloride (**20b**)*

Yield 69%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.28 (s, 3H), 7.36–7.29 (m, 2H), 7.28–7.21 (m, 3H), 2.59–2.44 (m, 1H), 1.39–1.27 (m, 3H), 1.27–1.16 (m, 1H), 0.79 (t, J = 7.6 Hz, 3H). MS (ESI): *m*/*z*: 162 ([M+H]$^+$). Purity: 99%.

*4.1.48. (1S,2R)-1-Ethyl-2-phenyl-cyclopropanamine hydrochloride (**21b**)*

Yield 69%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.35 (s, 3H), 7.36–7.29 (m, 2H), 7.27–7.18 (m, 3H), 2.58–2.50 (m, 1H), 1.43–1.28 (m, 3H), 1.26–1.13 (m, 1H), 0.79 (t, J = 7.6 Hz, 3H). MS (ESI): m/z: 162 ([M+H]$^+$). Purity: 99%.

*4.1.49. (1R,2S)-1,2-Diphenylcyclopropanamine hydrochloride (**20d**)*

Yield 71%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.95 (s, 3H), 7.37–7.31 (m, 2H), 7.26–7.18 (m, 3H), 7.12–7.00 (m, 3H), 6.97–6.93 (m, 2H), 2.87 (dd, J = 7.6, 10.0 Hz, 1H), 2.13–2.06 (m, 1H), 1.85–1.79 (m, 1H). MS (ESI): m/z: 210 ([M+H]$^+$). Purity: 99%.

*4.1.50. (1S,2R)-1,2-Diphenylcyclopropanamine hydrochloride (**21d**)*

Yield 47%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.96 (s, 3H), 7.43–6.76 (m, 10H), 2.93–2.81 (m, J 1H), 2.15–2.00 (m, 1H), 1.91–1.72 (m, 1H). MS (ESI): m/z: 210 ([M+H]$^+$). Purity: 99%.

*4.1.51. (1S,2S)-1-Benzyl-2-phenyl-cyclopropanamine hydrochloride (**20e**)*

Yield 69%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.30 (s, 3H), 7.45–7.20 (m, 8H), 7.14–6.99 (m, 2H), 2.82 (d, 1H), 2.69–2.57 (m, 1H), 2.33 (d, 1H), 1.79–1.58 (m, 1H), 1.49–1.32 (m, 1H). MS (ESI): m/z: 224 ([M+H]$^+$). Purity: 100%.

*4.1.52. (1R,2R)-1-Benzyl-2-phenyl-cyclopropanamine hydrochloride (**21e**)*

Yield 72%. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.32 (s, 3H), 7.42–7.20 (m, 8H), 7.14–7.05 (m, 2H), 2.82 (d, 1H), 2.70–2.60 (m, 1H), 2.33 (d, 1H), 1.74–1.65 (m, 1H), 1.47–1.34 (m, 1H). MS (ESI): m/z: 224 ([M+H]$^+$). Purity: 99%.

*4.1.53. (1S,2R)-1-Methyl-2-phenyl-cyclopropanamine 2,2,2-trifluoroacetic acid (**21a**)*

310 mg (1.24 mmol) of the BOC protected compound **18a** was dissolved in 10 mL of a mixture of CH_2Cl_2/TFA (96:4, v:v) and was stirred overnight at room temperature. The solution was concentrated, and the solid was dissolved twice in hexane and dried in order to eliminate the residual TFA providing the requisite cyclopropylamine **21a** as trifluoroacetate salt (322 mg, 99%). ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.30 (s, 2H), 7.26–7.14 (m, 3H), 7.13–7.07 (m, 2H), 2.65–2.54 (m, 1H), 1.54–1.40 (m, 1H), 1.14 (s, 3H), 1.11–1.03 (m, 1H). MS (ESI): m/z: 148 ([M+H]$^+$). Purity: 96%.

*4.1.54. trans-Ethyl 1-amino-2-phenyl-cyclopropanecarboxylate (**24**)*

*4.1.54.1. Ethyl-1-(tert-butoxycarbonylamino)-2-phenyl-cyclopropanecarboxylate (**23**).* 380 mg (1.85 mmol) Ethyl 1-amino-2-phenyl-cyclopropanecarboxylate [56] and 0.239 g (1.85 mmol mmol) DIPEA were added to a solution of 0.404 g (1.85 mmol) *tert*-butoxycarbonyl-*tert*-butyl carbonate in 5 mL CH_2Cl_2. The mixture was stirred for 20 h at RT. Water was added and the product was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and evaporated. The crude mixture was purified by column chromatography (hexane/EtOAc 9:1) to give 210 mg of the *trans* isomer **23** as colourless oil (37%). ^{1}H NMR (DMSO-d$_6$) δ (ppm): 7.79 (s, 1H), 7.36–7.09 (m, 5H), 3.81–3.55 (m, 2H), 2.72 (t, J = 8.8 Hz, 1H), 2.08–1.92 (m, 1H), 1.47–1.20 (m, 10H), 0.90–0.65 (m, 3H). MS (ESI): m/z: 206 [M-100+H]$^+$.

*4.1.54.2. trans-Ethyl 1-amino-2-phenyl-cyclopropanecarboxylate (**24**).* 20 mg (0.065 mmol) of the ethyl ester **23** was dissolved in 1.5 mL of HCl 1.25 M in MeOH and the mixture was stirred at room temperature for 7 h. After the evaporation of the solvent, the solid product was rinsed with Et_2O and evaporated under vacuum to give 12 mg (76%) of the desired ethyl ester **24** as yellow solid. MS (ESI): m/z: 206 [M+H]$^+$. ^{1}H NMR (MeOH-d$_4$) δ (ppm): 7.39–7.23 (m, 5H), 3.87 (dd, J = 3.4, 7.3 Hz, 2H), 3.00 (s, 1H), 2.27 (dd, J = 6.8, 8.8 Hz, 1H), 1.82 (dd, J = 6.8, 10.3 Hz, 1H), 0.78 (t, J = 7.3 Hz, 3H).

*4.1.55. trans-1-Amino-2-phenyl-cyclopropanecarboxylic acid (**26**)*

*4.1.55.1. trans-1-(tert-Butoxycarbonylamino)-2-phenyl-cyclopropanecarboxylic acid (**25**).* 0.44 mL of 2 M NaOH was added to a solution of 0.18 g (0.59 mmol) ethyl ester **23** in 10 mL EtOH. The mixture was stirred for 24 h at 80 °C. The solution was then concentrated and the crude mixture was treated with water and ethyl acetate. The aqueous phase was separated and acidified with HCl and the obtained suspension was extracted with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4 and evaporated to give 140 mg (86%) of the desired carboxylic acid **25** as white solid. MS (ESI): m/z: 300 [M+Na]$^+$. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.39–7.20 (m, 5H), 5.33 (bs, 1H), 2.88–2.75 (m, 1H), 2.27–2.13 (m, 1H), 1.57 (dd, J = 5.6, 9.5 Hz, 1H), 1.51 (s, 9H).

*4.1.55.2. trans-1-Amino-2-phenyl-cyclopropanecarboxylic acid (**26**).* 50 mg (0.18 mmol) of the BOC-protected cyclopropanecarboxylic acid **25** was dissolved in 1.5 mL HCl 1.25 M in MeOH and the mixture was stirred at room temperature. for 7 h. After the evaporation of the solvent, the solid product was rinsed with Et_2O and evaporated under vacuum to give 30 mg (78%) of the carboxylic acid **26** as a light brown solid. MS (ESI): m/z: 178 [M+H]$^+$. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 13.63–13.10 (m, 1H), 9.02–8.67 (m, 2H), 7.40–7.07 (m, 5H), 2.94 (t, J = 9.3 Hz, 1H), 2.03 (dd, J = 6.1, 8.6 Hz, 1H), 1.79 (dd, J = 5.9, 10.3 Hz, 1H).

*4.1.56. 1-Amino-(trans)-2-phenyl-cyclopropyl]methanol (**28**)*

*4.1.56.1. tert-Butyl-N-[1-(hydroxymethyl)-(trans)-2-phenyl-cyclopropyl]carbamate (**27**).* 0.100 g (0.327 mmol) of compound **23** was dissolved in 1 mL THF and added dropwise to a suspension of 0.017 g (0.46 mmol) $LiAlH_4$ in 2 mL THF at 0 °C. The reaction mixture was allowed to reach RT and stirred for 6 h. The suspension was cooled down to 0 °C and quenched with saturated sodium bisulfate. The suspension was diluted with EtOAc and filtered through a celite pad. The solution was dried, concentrated, and the crude mixture was purified by column chromatography (hexane/EtOAc from 8:2 to 1:1) to give 38 mg (44%) of the *tert*-butyl-carbamate **27** as a colourless oil. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.44–7.15 (m, 5H), 5.28 (bs, 1H), 3.54–3.28 (m, 2H), 2.53 (dd, J = 7.6, 9.0 Hz, 1H), 1.62–1.42 (m, 9H), 1.35 (t, J = 6.4 Hz, 1H), 1.31–1.23 (m, 1H). MS (ESI): m/z: 286 [M+Na]$^+$.

*4.1.56.2. [1-Amino-(trans)-2-phenyl-cyclopropyl]methanol (**28**).* 0.5 mL 4 M HCl in dioxane was added to a solution of 0.03 g (0.1 mmol) compound **27** in 0.5 mL dioxane. The reaction mixture was stirred at RT for 4 h, then the solvent was evaporated under vacuum and the product was triturated with Et_2O. Crystallization from MeOH/Et_2O gave 16 mg (70%, hydrochloride) of the *trans*-cyclopropylmethanol **28** as white solid. ^{1}H NMR (DMSO-d$_6$) δ (ppm): 8.41 (bs, 3H), 7.39–7.16 (m, 5H), 5.16 (bs, 1H), 3.29 (dd, J = 4.6, 12.0 Hz, 1H), 3.21–3.07 (m, 1H), 2.57 (t, J = 8.6 Hz, 1H), 1.37 (d, J = 7.3 Hz, 2H). MS (ESI): m/z: 164 [M+H]$^+$.

4.2. Biological assays

4.2.1. KDM1A (LSD1) enzyme inhibition assay

The complex of human recombinant KDM1A/CoREST protein was produced in *Escherichia coli* as separate proteins and co-purified following previously reported procedures [16,67]. The experiments were performed in 96 well half area white plates (cat. 3693, Corning, Corning, NY) using a mono-methylated H3-K4

peptide containing 21 amino acids (custom synthesis done by Thermo Scientific) as substrate and in a in 40 μl volume of 50 mM TRIS-HCl, pH 8.0 and 0.05 mg/ml BSA buffer. The peptide purity was >95% as checked by analytical high-pressure liquid chromatography and mass spectrometry.

The demethylase activity was estimated under aerobic conditions and at RT by measuring the release of H_2O_2 produced during the catalytic process by the Amplex® UltraRed detection system coupled with horseradish peroxidase (HRP). Briefly, 20 nM of KDM1A/CoREST complex was incubated at RT for 15 min in the absence and/or the presence of various concentrations of the inhibitors, 50 μM Amplex® UltraRed (Life Technologies) and 0.023 μM HRP (Sigma) in 50 mM Tris–HCl pH 8.0 and 0.05 mg/ml BSA. The inhibitors were tested twice in duplicates at each concentration. Tranylcypromine (Sigma) was used as control. After preincubation of the enzyme with the inhibitor, the reaction was initiated by addition of 4.5 μM of mono-methylated H3-K4 peptide. The conversion of the Amplex® Ultra Red reagent to Amplex® UltroxRed was monitored by fluorescence (excitation at 510 nm, emission at 595 nm) for 12 min and by using a microplate reader (Infinite 200, Tecan Group, Switzerland). Arbitrary units were used to measure the level of H_2O_2 produced in the absence and/or in the presence of inhibition. The maximum demethylase activity of KDM1A/CoREST was obtained in the absence of inhibitors and corrected for background fluorescence in the absence of KDM1A/CoREST. The IC_{50} was calculated using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

4.2.2. Structural studies

Crystals of KDM1A/CoREST were obtained as described [47]. For structure determination of enzyme-inhibitor complexes, crystals were soaked for 3–8 h in a solution consisting of consisting of 1.3 M sodium/potassium tartrate, 100 mM *N*-(2-acetamido)-2-iminodiacetic acid pH 6.5, 12% (v/v) glycerol, and 0.5–1 mM inhibitor. Diffraction data were measured at beamlines of the European Synchrotron Radiation Facility (Grenoble, France) and the Swiss Light Source (Villigen, Switzerland). Data processing and coordinate refinement were performed with programs of the CCP4 package [68]. Pictures were produced with CCP4mg [69] (Table 4).

4.2.3. Bioluminescent-coupled assay for monoamine oxidases (MAO Glo Assay)

The MAO Glo Assay from Promega (cat. V1402, Promega, Madison, WI) was used to measure the effect of inhibitors on MAO A and MAO B activity. Human recombinant MAO A and MAO B were expressed in *Pichia pastoris* and purified as published [70]. The assay was performed at RT in 50 μL (25 μL reaction solution + 25 μL detection reagent) in 96 well half area white plates (cat. 3693, Corning, Corning, NY). Luminescence was measured after 20 min incubation in the dark using a microplate reader (Infinite F200, Tecan Group, Switzerland) with an integration time of 0.25 s per well. 50 nM MAO A or 125 nM MAO B were incubated with five different inhibitor concentrations (from 0.004 μM to 100 μM) for 15 min at RT in Promega MAO Buffer or Promega MAO B Buffer (MAO Glo Assay kit, catalogue number V1402, Promega, Madison, WI). After 30 min of incubation the reaction was stopped with the Promega detection reagent. All compounds were tested twice and IC_{50} values were calculated using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

4.2.4. Cell studies (transcriptional assay)

Human APL NB4 cells were grown in RPMI supplemented with 10% foetal bovine serum, 2 mM L-glutamine, and antibiotics and maintained in a humidified tissue culture incubator at 37 °C in 5% CO_2. Cells were treated at the biochemical IC_{50} or with vehicle (DMSO). After 24 h the cells were collected for RNA analysis. Total RNA was purified using RNeasy Mini Kit (Qiagen, Valencia, CA), quantified and reverse transcribed. mRNA levels were measured by quantitative RT-PCR (Fast SYBR Green Master mix, Applied Biosystems Foster City, CA) using specific primers and normalized against TBP mRNA. Results are presented as fold induction relative to vehicle treated cells (DMSO). Primers used in this study were:

Gfi1b, TCTGGCCTCATGCCCTTA – TCTGGCCTCATGCCCTTA;

TBP, GCTGGCCCATAGTGATCTTT – CTTCACACGCCAAGAAACAGT.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.068.

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