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Pure enantiomers of benzoylamino-tranylcypromine: LSD1 inhibition, gene modulation in human leukemia cells and effects on clonogenic potential of murine promyelocytic blasts

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ABSTRACT

The pure enantiomers of the *N*-(2-, 3-, and 4-(2-aminocyclopropyl)phenyl)benzamides hydrochlorides **11a**–**j** were prepared and tested against LSD1 and MAO enzymes. The evaluation of the regioisomers **11a**–**j** highlighted a net increase of the anti-LSD1 potency by shifting the benzamide moiety from *ortho* to *meta* and mainly to *para* position of tranylcypromine phenyl ring, independently from their *trans* or *cis* stereochemistry. In particular, the *para*-substituted **11a,b** (*trans*) and **11g,h** (*cis*) compounds displayed LSD1 and MAO-A inhibition at low nanomolar levels, while were less potent against MAO-B. The *meta* analogs **11c,d** (*trans*) and **11i,j** (*cis*) were in general less potent, but more efficient against MAO-A than against LSD1. In cellular assays, all the *para* and *meta* enantiomers were able to inhibit LSD1 by inducing Gfi-1b and ITGAM gene expression, with **11b,c** and **11g**–**i** giving the highest effects. Moreover, **11b** and **11g,h** strongly inhibited the clonogenic potential of murine promyelocytic blasts.

1. Introduction

Post-translational methylation of histones is a covalent modification intimately linked with either transcriptional activation or repression. The histone lysine (Lys, K) residues can undergo mono-, di- and trimethylation, and each methylation state represents a specific epigenetic marker with a precise biological meaning and a well-defined chromatin localization [1–4]. In particular, methylation at the histone 3 lysine 4 (H3K4), H3K36, or H3K79 residue leads to transcriptional activation, whereas methylation at H3K9, H3K27, or H4K20 are markers of gene silencing [1–4]. Lys methylations are reversible marks due to the action of histone Lys demethylases (KDMs), a large family of "erasers" that catalyze the removal of methyl groups from (poly)methylated Lys substrates [5–7].

There are two known classes of KDMs, differing in their reaction chemistry, co-factor/co-substrate used, and Lys-substrate specificity [8]. The flavin-dependent KDMs, lysine-specific demethylase 1 and 2 (LSD1 and LSD2), only act on mono- and di-methylated Lys and produce formaldehyde and hydrogen peroxide in addition to unmethylated or monomethylated Lys, whereas the Jumonji-containing KDMs, utilizing Fe(II) and α-ketoglutarate as co-substrates, can act on mono-, di- and tri-methylated Lys side chains [6–9]. LSD1 typically acts on mono- and di-methylated

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H3K4 (H3K4me1/2), by catalyzing the oxidation of the methylamino group to an iminium cation, which was in turn hydrolyzed to a carbinolamine that spontaneously give formaldehyde and the demethylated amine [9]. Since H3K4me1/2 is an activation mark, the removal of methyl groups from it leads to gene silencing. In some contexts (prostate cancer), LSD1 demethylates H3K9me1/2, a repressive mark, regulating androgen receptor-mediated transcription, and thus changing its action by gene repression to gene activation [10]. LSD1 was originally identified as a component of transcriptional repressor complexes [11,12], being typically associated with the transcriptional corepressor protein CoREST and with the histone deacetylases (HDAC) 1 or 2. Such stable core complex is recruited by many chromatin remodeling multiprotein complexes [11–15], suggesting that the LSD1 activity itself can be modulated by its interacting proteins. LSD1 participates in several growth-promoting pathways, and LSD1 levels are up-regulated in certain high-risk tumors [16,17]. LSD1 is over-expressed in several cancer cells and tissues: neuroblastoma [18], retinoblastoma [19], prostate cancer [10,20], breast cancer [21–23], lung and bladder cancer cells [24]. The potential link between cancer and LSD1 activity is underlined by the finding that loss of H3K4 methylation and enrichment of H3K9 methylation are associated with several types of tumors [16]. Moreover, a clear role of LSD1 in sustaining the oncogenic potential of MLL-AF9 translocation in leukemia stem cells [25] as well as in the stem-like tumor propagating cells of human glioblastoma [26] has been recently reported.

The targets of LSD1 regulatory demethylation are not limited to histone H3 lysines, indeed LSD1 also demethylates Lys residues of non-histone proteins such as p53 [27], DNA methyltransferase 1 [28], STAT3 [29], E2F1 [30], MYPT1 [31], and thereby regulates their cellular functions. It has been reported that LSD1 binds several transcription factors and negatively regulates the expression of a number of genes, such as members of the secreted frizzle-related proteins (SFRPs) and the GATA family, Gfi-1b, SYN1, RARβ2, CYP26, p21 WAF1, E-cadherine, cyclin A1, Notch1 [32,33]. Increased methylation at the H3K4 mark through either LSD1 knockdown or pharmacological inhibition was shown to reactivate the expression of tumor suppressor genes (TSG) in breast, bladder, lung, and colorectal cancers [24,34]. Thus, inhibition of LSD1 is an effective strategy to switch on epigenetically silenced TSG, to up-regulate genes involved in cancer cell differentiation and/or invasion process as well as to down-regulate important proliferative pathways implicated in multiple cancer types.

Starting from the known monoamine oxidase (MAO) inhibitors, several molecules have been developed as irreversible and, more recently, as reversible LSD1 inhibitors (Fig. 1). In particular, the MAO-A/B inhibitor tranylcypromine (TCP) is a known irreversible LSD1 inhibitor recently described as able to reactivate the all-*trans*-retinoic acid differentiation pathway in acute myeloid leukemia [35], and to block repression of the E-cadherin promoter thus inhibiting the motility and invasion of tumor cell lines [36]. Several other TCP-containing derivatives (such as OG-L002, S-2101, NCL-1 and -2, GSK2879552, **1**, and pan-demethylase inhibitors such as **2**) have been reported with increased selectivity for LSD1 in comparison with TCP, and active in cancer and non-cancer diseases [37–48]. Very recently a series of phenelzine analogs, such as bizine [49], has been described as potent and selective, irreversible LSD1 inhibitors inducing cell growth arrest in H460 and LNCaP cancer cell lines. Polyamine derivatives, such as (bis)guanidines **3** [32], (bis)biguanides **4** [32], polyamines **5** [34], and (bis)ureas and (bis) thioureas **6** [50], were first evaluated as reversible LSD1 inhibitors, and some of them showed biochemical activity in the low micromolar range and growth inhibition in an human colon cancer model *in vivo* or in Calu-6 cells [34,50]. Similarly, (bis)amidines such as CBB1007 [51] have been reported as reversible LSD1 inhibitors to selectively target cancer stem cells derepressing epigenetically suppressed genes *in vivo*. Also peptides were described as LSD1 inhibitors, such as the pLys4Met peptide [52], some synthetic cyclic peptides [53], and a series of short peptides based on the SNAIL sequence exhibiting antiproliferative activities [54]. Among reversible LSD1 inhibitors, amidoximes **7** [55], dithiocarbamates **8** [56], substituted triazoles **9** [57], and benzohydrazides **10** [58] have been recently reported. More importantly, TCP and two TCP-based inhibitors, Ory1001 and GSK2869552, have entered into clinical studies for treatment of cancer diseases [59].

Pursuing our researches on design, synthesis, and biological evaluation of small molecule modulators of histone methylation [60–73], in 2010 we described some TCP-based compounds as novel LSD1 inhibitors [74]. Among them, the *trans* *N*-(4-((2-aminocyclopropyl)phenyl)benzamide hydrochloride **11** (Fig. 2) was found to be the most potent inhibitor with a K_i value of 1.1 μM [74].

To gain insight into structure–activity relationship (SAR) on this benzamide-TCP prototype, we synthesized the *ortho*, *meta* and *para* regioisomers of compound **11** as well as the *meta* and *para* analogs of its corresponding *cis* isomer, and we did enantioseparation of all the related stereoisomers (compounds **11a**–**j**, Fig. 2). All our attempts to prepare the *ortho* regioisomers with the *cis* geometry failed, and thus these last two enantiomers are not presented.

Compounds **11a**–**j** were tested against LSD1, and also evaluated against MAO-A and MAO-B to assess their selectivity profile. Selected compounds were evaluated in human leukemia NB4 cells to explore their capability to increase transcription of genes related to LSD1 activity and/or cell differentiation. Finally, the most potent enantiomers **11a**,**b**, and **11g**,**h** were tested to determine their effects on the clonogenic potential of murine promyelocytic blasts.

2. Results

2.1. Chemistry

The synthetic route for the synthesis of the *trans* regioisomers of **11** (compounds **11a**–**f**) started from the 2-, 3-, or 4-nitrostyrene which underwent a cyclopropanation reaction by treatment with ethyl diazoacetate (EDA) and catalytic copper (I) chloride in anhydrous chloroform, to afford the ester intermediates **12a**–**c**. Hydrolysis of the mentioned esters with 2 N potassium hydroxide solution in ethanol afforded the corresponding carboxylic acids **13a**–**c**, which were in turn converted into the related carbamates **14a**–**c** through a Curtius rearrangement in the presence of diphenylphosphoryl azide (DPPA), triethylamine and *tert*-butanol in refluxing anhydrous benzene. Further hydrogenation reaction performed on **14a**–**c** with 10% palladium on carbon in a Parr apparatus at 32 psi led to the aniline derivatives **15a**,**b** and the known **15c** [74], which were treated with benzoyl chloride in presence of triethylamine in anhydrous dichloromethane at 0 °C to give the intermediates **16a**–**c**. The enantioseparation of each isomer of **16a**–**c** furnished the pure stereoisomers **17c**–**f** together with **17a**,**b**, the two *ortho*-substituted enantiomers for which it was no possible to assign the absolute configuration (see below), which were cleaved at the Boc protection by using 4 N hydrochloric acid in dioxane/tetrahydrofuran affording the final compounds **11a**–**f** (Scheme 1).

The synthetic route to obtain the *cis* regioisomers of **11** (compounds **11g**–**j**) began from a very highly *cis*-selective, rhodium (I)-catalyzed cyclopropanation reaction. In particular, 3- or 4-nitrostyrene was treated with EDA in the presence of a rodium (I) complex [75,76] and sodium tetrakis[3,5-bis(trifluoromethyl) phenyl]borate (NaBArf) at 0 °C to obtain the *cis* nitroesters **18a**,**b**, which were reduced by stannous chloride in presence of 37%

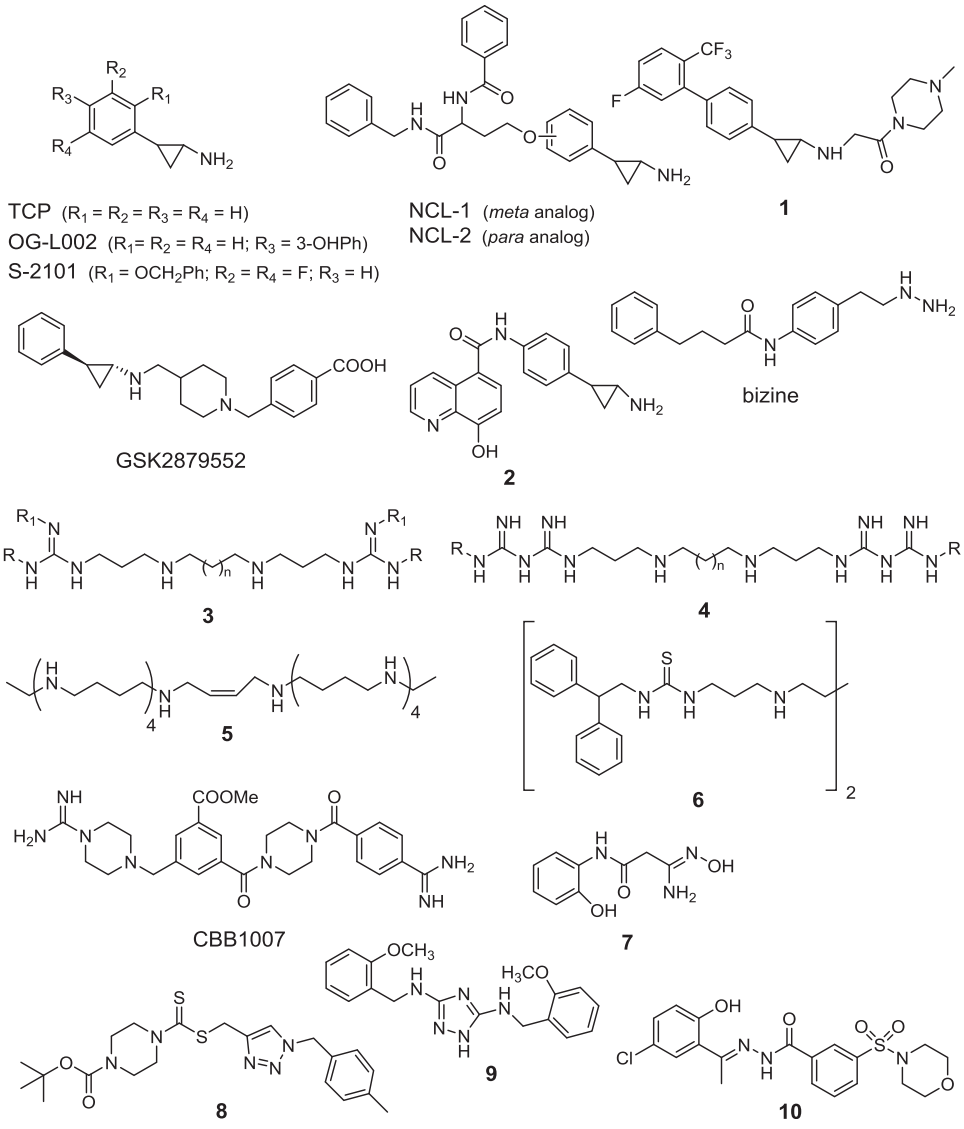


Fig. 1. Known irreversible and reversible LSD1 inhibitors.

hydrochloric acid in ethanol to the corresponding amino esters **19a**,**b**. The acylation reaction with benzoyl chloride, using the same conditions as for **16a**–**c**, provided the amide esters **20a**,**b**, which were in turn hydrolyzed by 2 N potassium hydroxide in ethanol to afford the corresponding carboxylic acids **21a**,**b**. Subsequent Curtius reaction on **21a**,**b** with DDPA, triethylamine and *tert*-butanol followed by enantioseparation of the obtained amidocarbamates **22a,b** and removal of the Boc protection with 4 N hydrochloric acid afforded the final compounds **11g**–**j** (Scheme 2).

2.2. Preparation and absolute configuration assignment of the trans ortho-, meta-, and para-benzamidoTCP stereoisomers *11a–f*, and of their cis meta- and para-analogs *11g–j*

The enantiomers shown in Fig. 2 were prepared by a procedure divided into three phases: 1) synthesis of the racemic form of the corresponding Boc derivatives; 2) direct HPLC resolution of the chiral Boc derivatives on a semipreparative scale; 3) removal of the Boc group and conversion to the corresponding hydrochlorides by treatment of the collected enantiomers with 4 N hydrochloric acid. The enantiomeric separations were carried out on immobilized polysaccharide-based chiral stationary phases (CSPs) under the operating conditions summarised in Table 1. Resolution was good in all cases and several tens of mg of both enantiomers were collected in a pure state (enantiomeric excess (ee) >98%). The stereochemical characterization procedure was based on the determination of the absolute configuration of the Boc intermediates by CD correlation using the enantiomers of Br-tPCPA and Br-cPCPA as reference samples [66]. Comparison of the CD spectra shown in Fig. S1 and Fig. S2 (Supporting Information) allowed us to assign the (1*R*,2*S*) and (1*R*,2*R*) absolute configuration to the first eluting enantiomer of the Boc-protected derivatives **16b**,**c** and **22a**,**b**, respectively (Table 1).

Only in the case of the *trans ortho*-Boc intermediate (**16a**), the replacing of the *p*-Br atom by the *o*-benzoylamino group led to a significant and unpredictable change in the spectral location of the representative CD bands (CD spectra not shown) which did not permit the empirical absolute configuration assignment. Thus, we called **17a** the first and **17b** the latter eluted enantiomer.

The stereochemical properties of the corresponding hydrochlorides of all the Boc intermediates were easily determined by chemical correlation method.

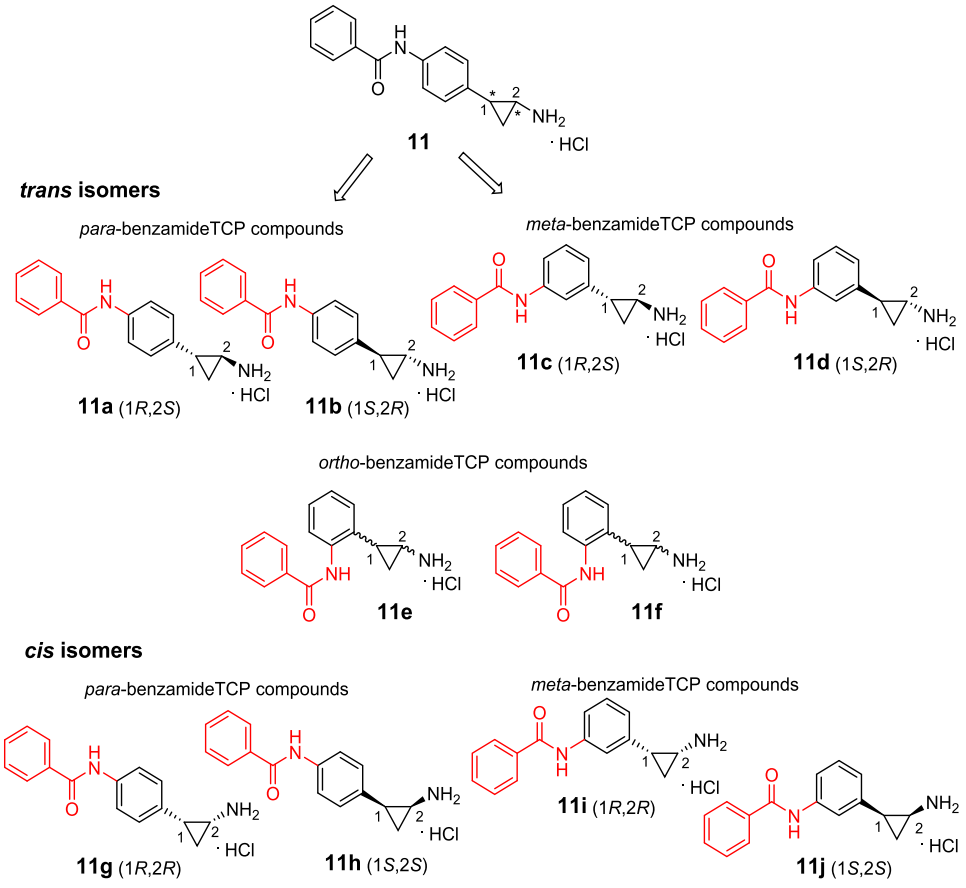
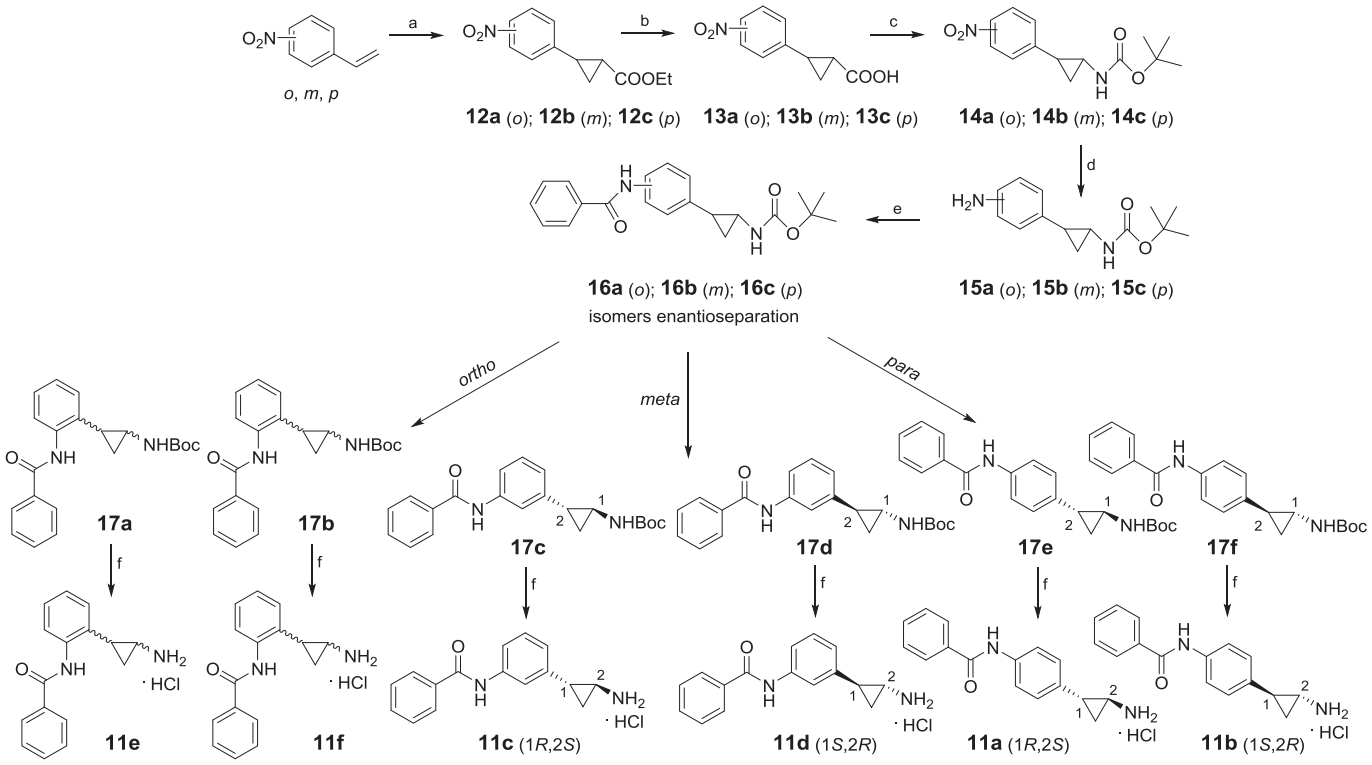
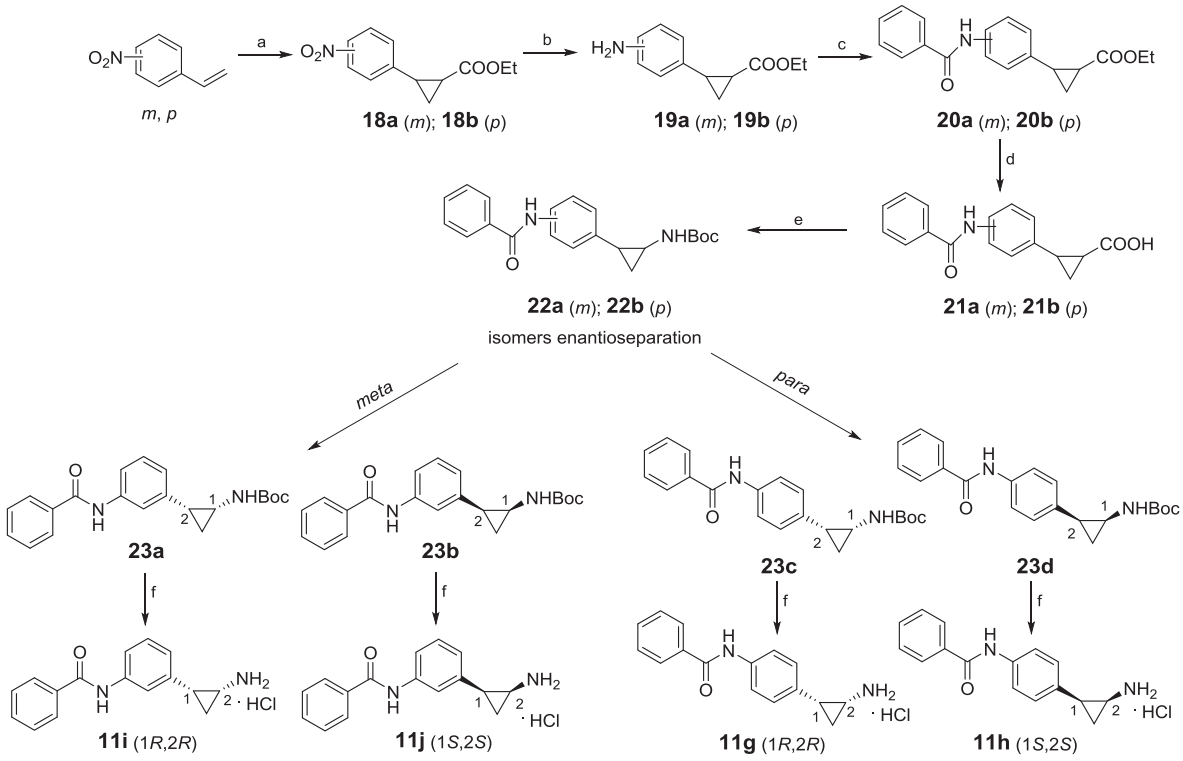


Fig. 2. Regioisomers and relative enantiomers based on the structure of **11**, compounds **11a–j**.



Scheme 1. Reagents and conditions: (a) EDA, CuCl (cat.), anhydrous $CHCl_3$, N_2 atmosphere, 2–5 h, 60 °C; (b) 2 N KOH, EtOH, overnight, rt; (c) DPPA, Et_3N, anhydrous *tert*-BuOH, anhydrous benzene, N_2 atmosphere, 16 h, 95 °C; (d) H_2, 10% Pd/C, anhydrous MeOH, 1 h, rt; (e) benzoyl cloride, Et_3N, anhydrous CH_2Cl_2, 1.5 h, 0 °C; (f) 4 N HCl, anhydrous dioxane/THF, overnight, rt.



Scheme 2. Reagents and conditions: (a) Rhodium (I)-complex, NaBArf, EDA, anhydrous CH_2Cl_2, N_2 atmosphere, 3 h, 0 °C; (b) $SnCl_2$, 37% HCl, EtOH, overnight, 0 °C; (c) benzoyl cloride, Et_3N, anhydrous CH_2Cl_2, 1.5 h, 0 °C; (d) 2 N KOH, EtOH, overnight, rt; (e) DPPA, Et_3N, anhydrous *tert*-BuOH, anhydrous benzene, N_2 atmosphere, 16 h, 95 °C; (f) 4 N HCl, anhydrous dioxane/THF, overnight, rt.

2.3. In vitro LSD1, MAO-A and MAO-B biochemical assays

The pure enantiomers **11a**–**j** were tested against LSD1, in comparison with the related *para*-substituted racemic mixture **11** and with TCP (Table 2). The same compounds were also tested against MAO-A and MAO-B, to assess their selectivity (Table 2).

Against LSD1 as well as against MAO-A, the *para*-substituted compounds displayed the highest inhibitory activity in the low nanomolar range, regardless to their *trans* (**11a,b**) or *cis* (**11g,h**) geometry. Among them, **11a** and **11g** showed slightly increased effects against LSD1 than against MAO-A. Against MAO-B, the four isomers were always less potent, with inhibition at micromolar level.

The insertion of the benzamide moiety at the *meta* position of the TCP phenyl ring led to compounds with decreased inhibitory potency against both LSD1 and MAO-A; nevertheless, this drop of potency is much more evident with the *trans* **11c,d** than with the *cis* **11i,j** isomers, and more marked for LSD1 than for MAO-A (**11c,d** were 520/900-fold less potent against LSD1, and 21/105-fold less potent against MAO-A, than **11a,b**; **11i,j** were 34/64-fold less potent against LSD1, and 3/17-fold less potent against MAO-A, than **11g,h**). In fact, the *meta*-substituted *trans* enantiomers **11c,d** displayed similar inhibition towards LSD1 and MAO-A as TCP, whereas the *cis* analogs **1i,j** were more potent. In addition, similarly to TCP, both the *trans* (**11c,d**) and *cis* (**11i,j**) enantiomers were more potent against MAO-A than against LSD1, and among them **11i,j** were more

Table 1
HPLC conditions for the enantioseparation of the chiral boc-derivatives **16a**–**c** and **22a,b** on a semipreparative scale.

Cpd	Column[a]	Eluent	k_1[b]	k_2[c]	A[d] (mg)	F1[e]		F2[e]	
						ee (%)[f]	AC[g]	ee (%)[f]	AC[g]
16a	IB	*n*-hexane-ethyl acetate -2-propanol 60:40:5	0.58	1.20	5.0	>99	n.d.	>99	n.d.
16b	IC	dicloromethane-methanol 100:1	0.96	1.88	2.3	98	(1*R*,2*S*)	98	(1*S*,2*R*)
16c	IC	*n*-hexane-ethyl acetate 50:50	1.19	2.05	1.5	98	(1*R*,2*S*)	99	(1*S*,2*R*)
22a	IA	*n*-hexane-ethyl acetate 50:50	1.18	2.95	5.0	>99	(1*R*,2*R*)	>99	(1*S*,2*S*)
22b	IC	*n*-hexane -2-propanol 70:30	1.91	3.85	1.7	>99	(1*R*,2*R*)	>99	(1*S*,2*S*)

[a] Columns: chiralpak IA, IB and IC 250 mm × 10 mm (i.d.); flow rate: 4.5 mL min^{-1}; detector: UV at 300 nm; temperature: 25 °C.
[b] Retention factor of the first eluted enantiomer.
[c] Retention factor of the second eluted enantiomer.
[d] A, amount of racemic sample resolved in a single run.
[e] F1 and F2, first (F1) and second (F2) eluted enantiomer.
[f] Yields ranged from about 65 to 80%.
[g] AC, absolute configuration.

Table 2
LSD1, MAO-A and MAO-B enzyme inhibiting activity of the pure enantiomers **11a–j** in comparison with the *para*-substituted *trans* racemic mixture **11**.

Compd	Regioisomer	AC[a]	IC_{50}, μM[b]		
			LSD1	MAO-A	MAO-B
11	*para*	racemate	0.019 ± 0.004	0.031 ± 0.0001	8.98 ± 0.51
11a	*para*	1*R*,2*S*	0.013 ± 0.005	0.039 ± 0.01	12.29 ± 6.27
11b	*para*	1*S*,2*R*	0.021 ± 0.006	0.024 ± 0.0001	16.81 ± 4.95
11c	*meta*	1*R*,2*S*	11.69 ± 2.84	1.37 ± 0.16	29 ± 5%[c]
11d	*meta*	1*S*,2*R*	10.89 ± 1.92	1.54 ± 0.18	84.7 ± 7.0
11e	*ortho*	NE_1[d]	74.65 ± 0.50	ND[e]	ND
11f	*ortho*	NE_2[d]	0%[c]	ND	ND
11g	*para*	1*R*,2*R*	0.026 ± 0.001	0.037 ± 0.01	19.17 ± 5.11
11h	*para*	1*S*,2*S*	0.022 ± 0.001	0.025 ± 0.01	21.20 ± 3.88
11i	*meta*	1*R*,2*R*	2.73 ± 0.67	0.65 ± 0.03	34 ± 4%[c]
11j	*meta*	1*S*,2*S*	0.45 ± 0.10	0.070 ± 0.01	80.07 ± 9.35
TCP		racemate	13.40 ± 2.99	1.07 ± 0.4	0.89 ± 0.36

[a] AC, absolute configuration.
[b] Data represent mean values of at least two separate experiments in duplicate. Standard deviations for **11a** (MAO-A and −B), **11g** and **11h** (MAO-A) are higher respect the other reported data, but the mean data, with the exception of MAO-B data of **11a**, are more than two times the standard deviation that, assuming a normal distribution data, it is a threshold generally accepted to consider the obtained data statistical significant.
[c] Percentage of inhibition at 100 μM.
[d] NE_1 and NE_2, not established, first and second eluted enantiomer.
[e] ND, not determined.

effective than **11c,d** by inhibiting MAO-A at nanomolar/submicromolar level. Against MAO-B, all the four *meta*-substituted analogs **11c,d** and **11i,j** showed very low inhibition.

Finally, the *ortho* substituted compounds (only the *trans* isomers **11e,f** were prepared and tested) displayed very low or no LSD1 inhibition, and were not tested against MAOs.

2.4. Gene expression modulation

Growth factor independence 1 (Gfi-1) is a transcription factor required for multilineage blood cell development, from stem and progenitor cells to differentiated lymphoid and myeloid cells. In erythroid cells, Gfi-1b is part of a multiprotein complex containing LSD1 and CoREST via the SNAG repression domain [77], thus regulating differential transcription of some target genes such as *meis1* [78]. Consistently, LSD1 depletion derepresses Gfi-1b targets in lineage-specific patterns [73,77]. Gfi-1 itself represses the expression of genes implicated in cell survival, proliferation and differentiation. Changes in Gfi-1 expression and function have not only been implicated in neutropenia, allergy, autoimmunity and hyper inflammatory responses, but also in lymphoma and more recently in the development of leukemia [79]. Integrin alpha M (ITGAM), also known as CR3A and cluster of differentiation molecule 11B (CD11B), is a typical protein marker used to assess induction of differentiation such as in leukemia cells.

All these findings, joined to the fact that by ChIPseq experiments (data not shown) we confirmed Gfi-1b and identified ITGAM as direct transcriptional targets of LSD1, and that LSD1 inhibition can cause the activation of epigenetically suppressed genes by maintaining methylation at the H3K4 mark, prompted us to assess the capability of selected **11a–j** enantiomers to induce Gfi-1b and ITGAM genes expression in NB4 cells, in comparison with the *para*-substituted racemic mixture **11**. For this scope, human promyelocytic leukemia NB4 cells were incubated with the inhibitors at a concentration equal to their respective biochemical IC_{50} value. After 24 h, the mRNA expression of the Gfi-1b and ITGAM genes was measured by quantitative RT-PCR and expressed as fold-induction respect to the control (DMSO).

Data depicted in Fig. 3 show that all the tested compounds were

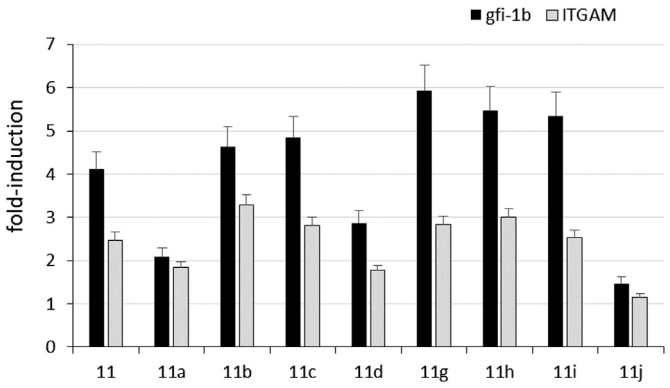


Fig. 3. Gfi-1b and ITGAM gene expression modulation by **11a–d,g–j** in NB4 cells. Compounds were tested at their own anti-LSD1 IC_{50} values. Data are expressed as average fold induction versus the vehicle (DMSO).

able to induce Gfi-1b as well as ITGAM gene expressions respect to DMSO as a control. The strongest effects were detected with the two *para*-substituted *cis* enantiomers **11g** and **11h** and with the *meta*-substituted *cis* 1*R*,2*R* analog **11i**, which displayed up to 6-fold induction. Other high effects were shown by the *para*- and *meta*-substituted isomers **11b** and **11c**, respectively, both having a *trans* geometry.

2.5. Pharmacological inhibition of LSD1 affects the clonogenic potential of murine acute myeloid leukemia blasts

Finally, the most potent compounds **11a**,**b** and **11g**,**h** were tested for their ability to inhibit colony formation of murine acute myeloid leukemia blasts (originated by a murine model of acute promyelocytic leukemia [80]) at the fixed concentration of 0.25 μM. Importantly, following seven days of semi-solid culture, all the tested compounds displayed strong inhibition on colony formation, ranging from 64% for **11a** to 72% for **11g** and 83% for both **11b** and **11h** (Table 3).

3. Discussion and conclusion

Among TCP-based compounds, the *trans* *N*-(4-((2-aminocyclopropyl)phenyl)benzamide hydrochloride **11** was previously described by us as an efficient inhibitor of LSD1 [74]. Since **11** is a *trans* racemic mixture of two enantiomers, and in **11** the TCP fragment is substituted at the *para* phenyl position with the benzamide moiety, we prepared the *meta* and *ortho* regioisomers of **11** as well as its *cis para* and *meta* analogs, and we separated all the related pure *trans* and *cis* enantiomers (**11a–f** and **11g–j**, respectively). Compounds **11a–j** were tested against LSD1 and MAO enzymes, and then in leukemia NB4 cells to assess their effect on transcription of genes related to LSD1 (such as Gfi-1b) and differentiation (such as ITGAM).

Table 3
Anti-clonogenic activity of selected **11a,b** and **11g,h** derivatives in primary murine APL blasts.

Compd	AC[a]	Colony forming unit assay, % of inhibition at 0.25 μM[b]
11a	1*R*,2*S*	64.0
11b	1*S*,2*R*	83.4
11g	1*R*,2*R*	72.0
11h	1*S*,2*S*	83.8

[a] AC, absolute configuration.
[b] Data represent mean values of at least two separate experiments in duplicate; the error is within ±10%.

Through the various regioisomers, the *para*-benzamido substituted derivatives **11a,b** and **11g,h**, regardless to their *trans* or *cis* geometry, displayed the highest LSD1 as well as MAO-A inhibition at nanomolar levels. The same compounds showed less potency, at micromolar levels, against MAO-B. The *meta*-benzamido substituted compounds **11c,d** and **11i,j** exerted micromolar or submicromolar LSD1 and MAO-A inhibition, and scarce inhibition of MAO-B, but in this case the *cis* enantiomers **11i,j** were 22/26-times more potent than the corresponding *trans* derivatives **11c,d** against both LSD1 and MAO-A enzymes, with a 4/6-times preference for MAO-A. Thus, the shift of the benzamide moiety from *para* to *meta* position in these derivatives led to less potent inhibitors (more evident with *trans* than with *cis* enantiomers), they being in any case more potent against MAO-A than against LSD1. Finally, the *ortho*-benzamide compounds **11e,f** displayed very low, if any, LSD1 inhibition: in this case, only the *trans* stereoisomers have been obtained and tested.

All the tested (*trans* and *cis*) *para* and *meta* enantiomers were able to highly induce Gfi-1b and ITGAM gene expression in NB4 cells, with the highest effects observed with **11b,c** and **11g**–**i**. The *para-trans* (**11a**,**b**) and –*cis* (**11g**,**h**) enantiomers, the most potent nanomolar LSD1 inhibitors of this series, when tested in murine promyelocytic blasts, displayed strong inhibition of their clonogenic potential.

The high biochemical potency as well as the ability to inhibit LSD1 in cells in the same range of concentration, associated to the excellent inhibitory effect on murine promyelocytic blasts lead to consider both **11b** and **11h** as lead compounds to be considered for future *in vivo* efficacy studies.

4. Experimental section

4.1. Chemistry

Melting points were determined on a Buchi 530 melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker AC 400 spectrometer; chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane (Me_4Si). EIMS spectra were recorded with a Fisons Trio 1000 spectrometer; only molecular ions (M^+) and base peaks are given. All compounds were routinely checked by TLC, ^{1}H NMR and ^{13}C NMR spectra. TLC was performed on aluminum-backed silica gel plates (Merck DC, Alufolien Kieselgel 60 F254) with spots visualized by UV light. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at reduced pressure of ca. 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Elemental analysis has been used to determine purity of the described compounds, that is >95%. Analytical results are within ±0.40% of the theoretical values. All chemicals were purchased from Aldrich Chimica, Milan (Italy), or from Alfa Aesar, Milan (Italy), and were of the highest purity.

4.1.1. General procedure for the synthesis of trans ethyl 2-(2-, 3-, and 4- nitrophenyl)cyclopropanecarboxylates *12a*–*c*. Example: trans ethyl 2-(3-nitrophenyl)cyclopropanecarboxylate (*12b*)

To a stirring solution of 3-nitrostyrene (16.76 mmol; 2.34 mL) in dry chloroform (30 mL), copper (I) chloride (0.014 mmol; 1.38 mg) and a solution of ethyl diazoacetate (13.96 mmol; 1.47 mL) in dry chloroform (5 mL) were added dropwise and under nitrogen atmosphere. The resulting mixture was then stirred at 60 °C for 4 h. After this time, the solvent was removed and the residue chromatographed on silica gel 60 and eluted with ethyl acetate/*n*-hexane 1:20 to obtain as first eluted the *trans* isomer (**12b**) and as second eluted the *cis* isomer (**18a**) in 5/1 ratio. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.06 (t, 3H, $-CH_2CH_3$), δ 1.48 (m, 1H, –C*H*H- cyclopropane), δ 1.79 (m, 1H, –CH*H*- cyclopropane), δ 2.20 (m, 1H, PhC*H*- cyclopropane), 2.65 (m, 1H, C*H*COOEt cyclopropane), 3.93 (q, 2H, $-CH_2CH_3$), 7.46 (t, 1H, benzene proton), 7.63 (d, 1H, benzene proton), 8.10 (d, 1H, benzene proton), 8.17 (s, 1H, benzene proton) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 16.9, 24.2, 25.2, 61.6, 120.0, 120.3, 129.0, 131.1, 144.2, 147.3, 171.2 ppm; MS (EI) *m/z* $[M]^+$ $C_{12}H_{13}NO_4$ calcd 235.0845, found 235.0848.

4.1.2. General procedure for the synthesis of cis ethyl 2-(3- and 4-nitrophenyl)cyclopropanecarboxylates *18a,b*. Example: cis ethyl 2-(4-nitrophenyl)cyclopropanecarboxylate (*18b*)

Rh(I) complex [75,76] (0.025 mmol, 0.0114 g) and sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaBArf) (0.025 mmol, 0.0221 g) were stirred in dry dichloromethane (13 mL) at room temperature for 1 h under an argon atmosphere. The reaction mixture was cooled to 0 °C, then 4-nitrostyrene and ethyl diazoacetate (1.0 mmol, 0.12 mL) in dry dichloromethane (7 mL) were added in one portion. The reaction mixture was stirred at 0 °C for 3 h, then the solvent was removed under vacuum and the crude product was purified by chromatography on silica gel 60 eluting with ethyl acetate:*n*-hexane (1:6) to afford the pure **18b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.06 (t, 3H, $-CH_2CH_3$), 1.49 (m, 1H, –C*H*H-cyclopropane), 1.79 (m, 1H, –CH*H*- cyclopropane), 2.22 (m, 1H, –C*H*COOEt cyclopropane), 2.64 (m, 1H, PhC*H* cyclopropane), 3.94 (q, 2H, $-CH_2CH_3$), 7.45 (d, 2H, benzene protons), 8.16 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 16.9, 24.2, 26.2, 61.6, 123.3 (2C), 125.9 (2C), 144.3, 147.9, 171.2 ppm; MS (EI) *m/z* $[M]^+$ $C_{12}H_{13}NO_4$ calcd 235.0845, found 235.0848.

4.1.3. General procedure for the synthesis of cis ethyl 2-(3- and 4-aminophenyl)cyclopropanecarboxylates *19a,b*. Example: synthesis of cis ethyl 2-(3-aminophenyl)cyclopropanecarboxylate (*19a*)

To a solution of *cis* ethyl 2-(3-nitrophenyl)cyclopropanecarboxylate (**18a**) (2.98 mmol, 0.70 g) in ethanol (3 mL), stannous chloride dihydrate (2.38 g, 10.55 mmol) and 37% hydrochloric acid (1.5 mL) were slowly added at 0 °C. The resulting mixture was stirred at room temperature overnight. The reaction was quenched by 2 N sodium carbonate solution (30 mL) and extracted with dichloromethane (3 × 30 mL), washed with saturated sodium chloride solution, and dried with sodium sulfate. The solvent was removed under vacuum and the residue purified by chromatography on silica gel 60 eluting with ethyl acetate:chloroform (1:30) to provide the pure **19a**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.02–1.05 (t, 3H, $-CH_2CH_3$), 1.27–1.32 (m, 1H, –C*H*H- cyclopropane), 1.66–1.70 (m, 1H, –CH*H*- cyclopropane), 2.03–2.09 (m, 1H, –C*H*COOEt cyclopropane), 2.49–2.55 (m, 1H, PhC*H*- cyclopropane), 3.60 (bs, 2H, $-NH_2$), 3.89–3.94 (q, 2H, $-CH_2CH_3$), 6.54–6.56 (d, 1H, benzene proton), 6.63 (s, 1H, benzene proton), 6.68–6.70 (d, 1H, benzene proton), 7.04–7.08 (t, 1H, benzene proton) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 16.9, 24.2, 26.2, 61.6, 111.4, 112.0, 115.0, 128.9, 144.1, 147.8, 171.2 ppm; MS (EI) *m/z* $[M]^+$ $C_{12}H_{15}NO_2$ calcd 205.1103, found 205.1108.

4.1.4. General procedure for the synthesis of trans tert-butyl 2-(2-, 3- and 4-benzamidophenyl)cyclopropylcarbamates (*16a*–*c*) and cis ethyl 2-(3- and 4-benzamidophenyl)cyclopropanecarboxylates (*20a,b*). Example: cis ethyl 2-(4-benzamidophenyl) cyclopropanecarboxylate (*20b*)

Triethylamine (0.59 mmol; 0.08 mL) and benzoyl cloride (0.59 mmol; 0.07 mL) were added dropwise to a cooled (0 °C) solution of *cis* ethyl 2-(4-aminophenyl)cyclopropanecarboxylate (**19b**) (0.49 mmol, 0.1 g) in dry dichloromethane (5 mL). The mixture was stirred at room temperature for 1 h, afterwards the

reaction was quenched by water (20 mL) and extracted with dichloromethane (3 × 30 mL), washed with saturated sodium chloride solution and dried with sodium sulfate. The solvent was removed under vacuum and the residue purified by chromatography on silica gel 60 eluting with ethyl acetate:chloroform (1:40) to provide pure **20b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.04–1.08 (t, 3H, $-CH_2CH_3$), 1.33–1.37 (m, 1H, −C*H*- cyclopropane), 1.70–1.74 (m, 1H, −CH*H*- cyclopropane), 2.09–2.13 (m, 1H, −C*H*COOEt cyclopropane), 2.49–2.55 (m, 1H, PhC*H*- cyclopropane), 3.90–3.96 (q, 2H, $-CH_2CH_3$), 7.28–7.30 (d, 2H, benzene protons), 7.49–7.58 (m, 5H, benzene protons), 7.81 (bs, 1H, −N*H*COPh), 7.86–7.88 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 16.9, 24.2, 26.2, 61.6, 121.0 (2C), 125.2 (2C), 127.5 (2C), 128.8 (2C), 132.1, 134.2, 134.3, 137.4, 164.7, 171.2 ppm; MS (EI) *m/z* $[M]^+$ $C_{19}H_{19}NO_3$ calcd 309.1365, found 309.1370.

*4.1.5. General procedure for the synthesis of trans 2-(2-, 3-, and 4-nitrophenyl)cyclopropanecarboxylic acids (**13a–c**) and cis 2-(3- and 4-benzamidophenyl)cyclopropanecarboxylic acids (**21a,b**). Example: trans 2-(4-nitrophenyl)cyclopropylcarboxylic acid (**13c**)*

A solution of **12c** (9.95 mmol, 2.34 g) and 2 N KOH (19.9 mmol, 1.12 g) in ethanol (30 mL) was kept in stirring overnight at room temperature. The reaction was quenched by addition of 2 N HCl until pH 2; afterwards the precipitate was filtered, washed with water (3 × 30 mL) and dried to obtain the pure **13c** as a pale yellow solid. ^{1}H NMR (DMSO-d_6, 400 MHz, δ; ppm) δ 1.52–1.56 (m, 2H, CH_2 cyclopropane), 1.97–1.99 (m, 1H, −C*H*COOH cyclopropane), 2.57–2.59 (m, 1H, PhC*H*-cyclopropane), 7.44–7.45 (d, 2H, benzene protons), 8.11–8.13 (d, 2H, benzene protons), 12.50 (bs, 1H, COO*H*) ppm; ^{13}C NMR (DMSO-d_6, 400 MHz, δ; ppm) δ 16.6, 23.1, 25.9, 123.3 (2C), 125.9 (2C), 144.3, 147.9, 172.3 ppm; MS (EI) *m/z* $[M]^+$ $C_{10}H_9NO_4$ calcd 207.0532, found 207.0538.

*4.1.6. General procedure for the synthesis of trans tert-butyl 2-(2-, 3- and 4- nitrophenyl)cyclopropylcarbamates (**14a–c**) and cis tert-butyl 2-(3- and 4-benzamidophenyl)cyclopropylcarbamates (**22a,b**). Example: cis tert-butyl 2-(4-benzamidophenyl) cyclopropylcarbamate (**22b**)*

A solution of **21b** (0.71 mmol, 0.2 g) in dry benzene (10 mL), triethylamine (0.78 mmol, 0.11 mL), diphenylphosphoryl azide (0.78 mmol; 0.17 mL) and *tert*-butanol (53 mmol, 5 mL) was stirred at 90 °C under N_2 atmosphere for 16 h. Afterwards, di-*tert*-butyldicarbonate (8 mmol, 1.7 g) was added, and the reaction was continued at 90 °C for further 2 h. The solvent was removed under vacuum and the residue was chromatographed by silica gel 60 eluting with ethyl acetate:*n*-hexane (1:3) to give pure **22b** as a pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) δ 1.00–1.02 (m, 1H, −CH*H*- cyclopropane), 1.32–1.34 (m, 1H, −C*H*H-cyclopropane), 2.23–2.25 (m, 1H, −C*H*COOH), 2.93–2.96 (m, 1H, PhC*H*-cyclopropane), 4.28 (bs, 1H, −N*H*COO*t*But), 7.22–7.25 (m, 2H, benzene protons), 7.50–7.62 (m, 5H, benzene protons), 7.83 (bs, 1H, −N*H*COPh), 7.89–7.91 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.4, 22.8, 28.4 (3C), 32.6, 79.5, 121.0 (2C), 125.2 (2C), 127.5 (2C), 128.8 (2C), 132.1, 134.2, 134.3, 137.3, 155.6, 164.7 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1791.

*4.1.7. Spectral data for the carbamates **17a–f** and **23a–d***

*4.1.7.1. Tert-butyl-2-(2-benzamidophenyl)cyclopropylcarbamate (**17a**, first eluted).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.20–1.24 (m, 1H, −C*H*H- cyclopropane), 1.29–1.33 (m, 1H, −CH*H*- cyclopropane), 1.48 (s, 9H, $-(CH_3)_3$), 2.49–2.53 (m, 1H, −C*H*NHCOO$(CH_3)_3$ cyclopropane), 2.90–2.94 (m, 1H, PhC*H*- cyclopropane), 5.00 (bs, −N*H*COO$(CH_3)_3$), 6.93–6.95 (d, 1H, benzene proton), 7.36–7.42 (m, 2H, benzene protons), 7.53–7.57 (t, 1H, benzene proton), 7.90–7.93 (d, 1H, benzene proton), 7.90 (bs, 1H, −N*H*COPh) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 17.8, 28.5 (3C), 32.7, 79.7, 114.3, 123.8, 125.2, 125.4, 127.5 (2C), 129.0 (2C), 130.9, 132.4, 135.1, 136.7, 155.8, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1790.

*4.1.7.2. Tert-butyl-2-(2-benzamidophenyl)cyclopropylcarbamate (**17b**, second eluted).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.20–1.24 (m, 1H, −C*H*H- cyclopropane), 1.29–1.33 (m, 1H, −CH*H*- cyclopropane), 1.48 (s, 9H, $-(CH_3)_3$), 2.49–2.53 (m, 1H, −C*H*NHCOO$(CH_3)_3$ cyclopropane), 2.90–2.94 (m, 1H, PhC*H*- cyclopropane), 5.00 (bs, −N*H*COO$(CH_3)_3$), 6.93–6.95 (d, 1H, benzene proton), 7.36–7.42 (m, 2H, benzene protons), 7.53–7.57 (t, 1H, benzene proton), 7.90–7.93 (d, 1H, benzene proton), 7.90 (bs, 1H, −N*H*COPh) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 17.8, 28.5 (3C), 32.7, 79.7, 114.3, 123.8, 125.2, 125.4, 127.5 (2C), 129.0 (2C), 130.9, 132.4, 135.1, 136.7, 155.8, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1790.

*4.1.7.3. Tert-butyl (1S,2R)-2-(3-benzamidophenyl)cyclopropylcarbamate (**17c**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.16–1.20 (m, 2H, $-CH_2$- cyclopropane), 1.48 (s, 9H, $-(CH_3)_3$), 2.06–2.09 (m, 1H, −C*H*NHCOO$(CH_3)_3$ cyclopropane), 2.74–2.77 (m, 1H, PhC*H*- cyclopropane), 4.89 (bs, −N*H*COO$(CH_3)_3$), 6.93–6.95 (d, 1H, benzene proton), 7.26–7.30 (t, 1H, benzene protons), 7.47–7.58 (m, 5H, benzene protons), 7.85 (bs, 1H, −N*H*COPh), 7.88–7.80 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 22.9, 28.6 (3C), 32.4, 79.7, 117.6, 118.1, 120.5, 127.5 (2C), 128.5, 129.0 (2C), 132.2, 134.3, 135.6, 143.7, 155.8, 164.9 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1790.

*4.1.7.4. Tert-butyl (1R,2S)-2-(3-benzamidophenyl)cyclopropylcarbamate (**17d**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.16–1.20 (m, 2H, $-CH_2$- cyclopropane), 1.48 (s, 9H, $-(CH_3)_3$), 2.06–2.09 (m, 1H, −C*H*NHCOO$(CH_3)_3$ cyclopropane), 2.74–2.77 (m, 1H, PhC*H*- cyclopropane), 4.89 (bs, −N*H*COO$(CH_3)_3$), 6.93–6.95 (d, 1H, benzene proton), 7.26–7.30 (t, 1H, benzene protons), 7.47–7.58 (m, 5H, benzene protons), 7.85 (bs, 1H, −N*H*COPh), 7.88–7.90 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 22.9, 28.6 (3C), 32.4, 79.7, 117.6, 118.1, 120.5, 127.5 (2C), 128.5, 129.0 (2C), 132.2, 134.3, 135.6, 143.7, 155.8, 164.9 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1790.

*4.1.7.5. Tert-butyl (1S,2R)-2-(4-benzamidophenyl)cyclopropylcarbamate (**17e**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.16–1.20 (m, 2H, $-CH_2$- cyclopropane), 1.48 (s, 9H, $-(CH_3)_3$), 2.05–2.08 (m, 1H, −C*H*NHCOO$(CH_3)_3$ cyclopropane), 2.71–2.74 (m, 1H, PhC*H*- cyclopropane), 4.85 (bs, −N*H*COO$(CH_3)_3$), 7.16–7.18 (d, 2H, benzene proton), 7.49–7.57 (m, 5H, benzene protons), 7.78 (bs, 1H, −N*H*COPh), 7.88–7.90 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 22.9, 28.6 (3C), 32.8, 79.7, 121.2 (2C), 125.3 (2C), 127.9 (2C), 128.8 (2C), 132.2, 134.3, 134.4, 137.6, 155.8, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1789.

*4.1.7.6. Tert-butyl (1R,2S)-2-(4-benzamidophenyl)cyclopropylcarbamate (**17f**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.16–1.20 (m, 2H, $-CH_2$- cyclopropane), 1.48 (s, 9H, $-(CH_3)_3$), 2.05–2.08 (m, 1H, −C*H*NHCOO$(CH_3)_3$ cyclopropane), 2.71–2.74 (m, 1H, PhC*H*- cyclopropane), 4.85 (bs, −N*H*COO$(CH_3)_3$), 7.16–7.18 (d, 2H, benzene proton), 7.49–7.57 (m, 5H, benzene protons), 7.78 (bs, 1H, −N*H*COPh), 7.88–7.90 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 22.9, 28.6 (3C), 32.8, 79.7, 121.2 (2C), 125.3 (2C), 127.9 (2C), 128.8 (2C), 132.2, 134.3, 134.4, 137.6, 155.8, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found

352.1789.

*4.1.7.7. Tert-butyl (1R,2R)-2-(3-benzamidophenyl)cyclopropylcarbamate (**23a**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.02–1.06 (m, 1H, –C*H*H- cyclopropane), 1.31–1.35 (m, 1H, –CH*H*- cyclopropane), 1.36 (s, 9H, –(CH_3)$_3$), 2.25–2.29 (m, 1H, –C*H*NHCOO(CH_3)$_3$ cyclopropane), 2.92–2.96 (m, 1H, PhC*H*- cyclopropane), 4.31 (bs, –N*H*COO(CH_3)$_3$), 7.02–7.04 (d, 1H, benzene proton), 7.31–7.35 (m, 1H, benzene proton), 7.51-7.63 (m, 5H, benzene protons), 7.82 (bs, 1H, –N*H*COPh), 7.88–7.90 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 22.9, 28.5 (3C), 32.7, 79.7, 117.3, 118.1, 120.6, 127.5 (2C), 128.3, 128.8 (2C), 132.1, 134.3, 135.3, 143.6, 155.7, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1791.

*4.1.7.8. Tert-butyl (1S,2S)-2-(3-benzamidophenyl)cyclopropylcarbamate (**23b**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.02–1.06 (m, 1H, –C*H*H- cyclopropane), 1.31–1.35 (m, 1H, –CH*H*- cyclopropane), 1.36 (s, 9H, –(CH_3)$_3$), 2.25–2.29 (m, 1H, –C*H*NHCOO(CH_3)$_3$ cyclopropane), 2.92–2.96 (m, 1H, PhC*H*- cyclopropane), 4.31 (bs, –N*H*COO(CH_3)$_3$), 7.02–7.04 (d, 1H, benzene proton), 7.31–7.35 (m, 1H, benzene proton), 7.51–7.63 (m, 5H, benzene protons), 7.82 (bs, 1H, –N*H*COPh), 7.88–7.90 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 22.9, 28.5 (3C), 32.7, 79.7, 117.3, 118.1, 120.6, 127.5 (2C), 128.3, 128.8 (2C), 132.1, 134.3, 135.3, 143.6, 155.7, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1791.

*4.1.7.9. Tert-butyl (1R,2R)-2-(3-benzamidophenyl)cyclopropylcarbamate (**23c**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.98–1.02 (m, 1H, –C*H*H- cyclopropane), 1.31–1.35 (m, 1H, –CH*H*- cyclopropane), 1.38 (s, 9H, –(CH_3)$_3$), 2.21–2.25 (m, 1H, –C*H*NHCOO(CH_3)$_3$ cyclopropane), 2.93-2.97 (m, 1H, PhC*H*- cyclopropane), 4.27 (bs, –N*H*COO(CH_3)$_3$), 7.22–7.25 (d, 2H, benzene protons), 7.50–7.62 (m, 5H, benzene protons), 7.83 (bs, 1H, –N*H*COPh), 7.87–7.91 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 22.9, 28.5 (3C), 32.7, 79.6, 121.0 (2C), 125.3 (2C), 127.5 (2C), 128.8 (2C), 132.1, 134.3, 137.4, 143.6, 155.7, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1790.

*4.1.7.10. Tert-butyl (1R,2R)-2-(3-benzamidophenyl)cyclopropylcarbamate (**23d**).* ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.98–1.02 (m, 1H, –C*H*H- cyclopropane), 1.31–1.35 (m, 1H, –CH*H*- cyclopropane), 1.38 (s, 9H, –(CH_3)$_3$), 2.21–2.25 (m, 1H, –C*H*NHCOO(CH_3)$_3$ cyclopropane), 2.93–2.97 (m, 1H, PhC*H*- cyclopropane), 4.27 (bs, –N*H*COO(CH_3)$_3$), 7.22–7.25 (d, 2H, benzene protons), 7.50–7.62 (m, 5H, benzene protons), 7.83 (bs, 1H, –N*H*COPh), 7.87–7.91 (d, 2H, benzene protons) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.6, 22.9, 28.5 (3C), 32.7, 79.6, 121.0 (2C), 125.3 (2C), 127.5 (2C), 128.8 (2C), 132.1, 134.3, 137.4, 143.6, 155.7, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{21}H_{24}N_2O_3$ calcd 352.1787, found 352.1790.

*4.1.8. General procedure for the synthesis of the TCP-based enantiomers **11a**–**j** as hydrochloride salts. Example: N-(4-((1R,2S)-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11a**)*

A solution of *tert*-butyl (1*S*,2*R*)-2-(4-benzamidophenyl)cyclopropyl)carbamate **17e** (0.08 mmol, 0.03 g) and 4 N hydrochloric acid/dioxane (0.8 mmol, 0.2 mL) in tetrahydrofuran (3 mL) was stirred at room temperature overnight. The precipitated colorless solid was filtered, washed with diethyl ether (3 × 5 mL) and dried to afford **11a** as a hydrochloride salt. ^{1}H NMR (DMSO-d_6, 400 MHz, δ; ppm) δ 1.17–1.21 (m, 1H, CH*H* cyclopropane), 1.35–1.39 (m, 1H, C*H*H cyclopropane), 2.29–2.33 (m, 1H, C*H*NH_2), 2.75–2.79 (m, 1H, PhC*H*), 7.14–7.16 (m, 2H, benzene protons), 7.51–7.59 (m, 3H, benzene protons), 7.71–7.73 (d, 2H, benzene protons), 7.94–7.96 (d, 2H, benzene protons), 8.43 (bs, 3H, NH_3^+), 10.25 (bs, 1H, CON*H*) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz, δ; ppm) δ 17.3, 25.6, 34.4, 121.2 (2C), 125.3 (2C), 127.6 (2C), 128.8 (2C), 132.1, 134.3, 134.5, 137.4, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1268.

*4.1.8.1. N-(4-((1S,2R)-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11b**).* ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.16–1.20 (m, 1H, C*H*H cyclopropane), 1.34–1.38 (m, 1H, CH*H* cyclopropane), 2.29–2.33 (m, 1H, C*H*NH_3^+ cyclopropane), 2.76–2.80 (m, 1H, PhC*H* cyclopropane), 7.14–7.16 (d, 2H, benzene protons), 7.51–7.59 (m, 3H, benzene protons), 7.71–7.73 (d, 2H, benzene protons), 7.94–7.96 (d, 2H, benzene protons), 8.43 (bs, 3H, NH_3^+), 10.25 (bs, 1H, N*H*COPh) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.1, 22.0, 28.2, 121.1 (2C), 125.3 (2C), 127.5 (2C), 128.8 (2C), 132.1, 134.3, 134.4, 138.9, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1268.

*4.1.8.2. N-(3-((1R,2S)-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11c**).* ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.16–1.20 (m, 2H, CH_2 cyclopropane), 2.25–2.29 (m, 1H, C*H*NH_3^+ cyclopropane), 2.74–2.77 (m, 1H, PhC*H* cyclopropane), 6.93–6.95 (d, 1H, benzene proton), 7.26–7.30 (t, 1H, benzene protons), 7.47–7.58 (m, 5H, benzene protons), 7.88–7.80 (d, 2H, benzene protons), 8.43 (bs, 3H, NH_3^+), 10.25 (bs, 1H, N*H*COPh) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.1, 22.0, 28.2, 117.3, 118.1, 120.6, 127.5 (2C), 128.3, 128.9 (2C), 132.2, 134.3, 135.4, 143.5, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1268.

*4.1.8.3. N-(3-((1S,2R)-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11d**).* ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.16–1.20 (m, 2H, CH_2 cyclopropane), 2.25–2.29 (m, 1H, C*H*NH_3^+ cyclopropane), 2.74–2.77 (m, 1H, PhC*H* cyclopropane), 6.93–6.95 (d, 1H, benzene proton), 7.26–7.30 (t, 1H, benzene protons), 7.47–7.58 (m, 5H, benzene protons), 7.88–7.80 (d, 2H, benzene protons), 8.43 (bs, 3H, NH_3^+), 10.25 (bs, 1H, N*H*COPh) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.1, 22.0, 28.2, 117.3, 118.1, 120.6, 127.5 (2C), 128.3, 128.9 (2C), 132.2, 134.3, 135.4, 143.5, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1268.

*4.1.8.4. N-(2-(-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11e**, first eluted).* ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.20–1.24 (m, 1H, C*H*H cyclopropane), 1.29–1.33 (m, 1H, CH*H* cyclopropane), 2.29–2.53 (m, 1H, C*H*NH_3^+ cyclopropane), 2.90–2.94 (m, 1H, PhC*H* cyclopropane), 6.93–6.95 (d, 1H, benzene proton), 7.36–7.42 (m, 2H, benzene protons), 7.53–7.57 (t, 1H, benzene proton), 7.90–7.93 (d, 1H, benzene proton), 8.43 (bs, 3H, NH_3^+), 10.25 (bs, 1H, N*H*COPh) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.0, 16.9, 28.1, 114.2, 123.8, 125.2, 125.3, 127.5 (2C), 128.8 (2C), 130.8, 132.1, 134.3, 136.6, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1267.

*4.1.8.5. N-(2-(-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11f**, second eluted).* ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.20–1.24 (m, 1H, C*H*H cyclopropane), 1.29–1.33 (m, 1H, CH*H* cyclopropane), 2.29–2.53 (m, 1H, C*H*NH_3^+ cyclopropane), 2.90–2.94 (m, 1H, PhC*H* cyclopropane), 6.93–6.95 (d, 1H, benzene proton), 7.36–7.42 (m, 2H, benzene protons), 7.53–7.57 (t, 1H, benzene proton), 7.90–7.93 (d, 1H, benzene proton), 8.43 (bs, 3H, NH_3^+), 10.25 (bs, 1H, N*H*COPh) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.0, 16.9, 28.1, 114.2, 123.8, 125.2, 125.3, 127.5 (2C), 128.8 (2C), 130.8, 132.1, 134.3, 136.6, 164.8 ppm; MS (EI) *m/z* $[M]^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1267.

*4.1.8.6. N-(4-((1R,2R)-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11g**).* ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.98–1.02 (m, 1H, C*H*H cyclopropane), 1.31–1.35 (m, 1H, CH*H* cyclopropane), 2.23–2.27 (m, 1H, C*H*NH$_3^+$ cyclopropane), 2.93–2.97 (m, 1H, PhC*H* cyclopropane), 7.22–7.25 (d, 2H, benzene protons), 7.50–7.62 (m, 5H, benzene protons), 7.87–7.91 (d, 2H, benzene protons), 8.43 (bs, 3H, N*H*$_3^+$), 10.25 (bs, 1H, N*H*COPh) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.1, 22.0, 28.2, 121.1 (2C), 125.3 (2C), 127.5 (2C), 128.8 (2C), 132.1, 134.3, 134.4, 138.9, 164.8 ppm; MS (EI) *m/z* [M]$^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1269.

*4.1.8.7. N-(4-((1S,2S)-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11h**).* ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.98–1.02 (m, 1H, C*H*H cyclopropane), 1.31–1.35 (m, 1H, CH*H* cyclopropane), 2.23–2.27 (m, 1H, C*H*NH$_3^+$ cyclopropane), 2.93–2.97 (m, 1H, PhC*H* cyclopropane), 7.22–7.25 (d, 2H, benzene protons), 7.50–7.62 (m, 5H, benzene protons), 7.87–7.91 (d, 2H, benzene protons), 8.43 (bs, 3H, N*H*$_3^+$), 10.25 (bs, 1H, N*H*COPh) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.1, 22.0, 28.2, 121.1 (2C), 125.3 (2C), 127.5 (2C), 128.8 (2C), 132.1, 134.3, 134.4, 138.9, 164.8 ppm; MS (EI) *m/z* [M]$^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1269.

*4.1.8.8. N-(3-((1R,2R)-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11i**).* ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.02–1.06 (m, 1H, C*H*H cyclopropane), 1.31–1.35 (m, 1H, CH*H* cyclopropane), 2.26–2.30 (m, 1H, C*H*NH$_3^+$ cyclopropane), 2.92–2.96 (m, 1H, PhC*H* cyclopropane), 7.02–7.04 (d, 1H, benzene proton), 7.31–7.35 (m, 1H, benzene proton), 7.51–7.63 (m, 5H, benzene protons), 7.88–7.90 (d, 2H, benzene protons), 8.43 (bs, 3H, N*H*$_3^+$), 10.25 (bs, 1H, N*H*COPh) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.1, 22.0, 28.2, 117.3, 118.1, 120.6, 127.5 (2C), 128.3, 128.9 (2C), 132.2, 134.3, 135.4, 143.5, 164.8 ppm; MS (EI) *m/z* [M]$^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1268 ppm; MS (EI) *m/z* [M]$^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1267.

*4.1.8.9. N-(3-((1S,2S)-2-aminocyclopropyl)phenyl)benzamide hydrochloride (**11j**).* ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.02–1.06 (m, 1H, C*H*H cyclopropane), 1.31–1.35 (m, 1H, CH*H* cyclopropane), 2.26–2.30 (m, 1H, C*H*NH$_3^+$ cyclopropane), 2.92–2.96 (m, 1H, PhC*H* cyclopropane), 7.02–7.04 (d, 1H, benzene proton), 7.31–7.35 (m, 1H, benzene proton), 7.51–7.63 (m, 5H, benzene protons), 7.88–7.90 (d, 2H, benzene protons), 8.43 (bs, 3H, N*H*$_3^+$), 10.25 (bs, 1H, N*H*COPh) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.1, 22.0, 28.2, 117.3, 118.1, 120.6, 127.5 (2C), 128.3, 128.9 (2C), 132.2, 134.3, 135.4, 143.5, 164.8 ppm; MS (EI) *m/z* [M]$^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1268 ppm; MS (EI) *m/z* [M]$^+$ $C_{16}H_{16}N_2O$ calcd 252.1263, found 252.1267.

4.1.9. HPLC enantioseparation

HPLC enantioseparations were performed by using the stainless-steel Chiralpak IA (250 mm × 4.6 mm i.d. and 250 × 10 mm i.d.), Chiralpak IB (250 mm × 4.6 mm i.d. and 250 × 10 mm i.d.), Chiralpak IC (250 mm × 4.6 mm i.d. and 250 × 10 mm i.d.) and Chiralcel OD (250 mm × 4.6 mm i.d. and 250 × 10 mm i.d.) (Chiral Technologies Europe, Illkirch, France) columns, respectively. All chemicals solvents for HPLC were purchased from Aldrich (Italy) and used without further purification. The analytical HPLC apparatus consisted of a Perkin–Elmer (Norwalk, CT, USA) 200 lc pump equipped with a Rheodyne (Cotati, CA, USA) injector, a 20-μL sample loop, an HPLC Dionex CC-100 oven (Sunnyvale, CA, USA) and a Jasco (Jasco, Tokyo, Japan) Model CD 2095 Plus UV/CD detector. For semipreparative separations a Perkin–Elmer 200 LC pump equipped with a Rheodyne injector, a 1 mL sample loop, a Perkin–Elmer LC 101 oven and Waters 484 detector (Waters Corporation, Milford, MA, USA) were used. The signal was acquired and processed by Clarity software (DataApex, Prague, The Czech Republic).

4.1.10. Circular dichroism spectra

The CD spectra were measured by using a Jasco Model J-700 spectropolarimeter. The optical path and temperature were set at 0.1 mm and 25 °C, respectively. The spectra are average computed over three instrumental scans and the intensities are presented in terms of ellipticity values (mdeg).

4.1.11. Enzyme assay. LSD1 inhibition

The complex of human recombinant LSD1/CoREST protein was produced in *E. coli* as separate proteins and co-purified following previously reported procedures [81,82]. 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 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 room temperature 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 LSD1/CoREST complex was incubated at room temperature 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 monomethylated 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 LSD1/CoREST was obtained in the absence of inhibitors and corrected for background fluorescence in the absence of the substrate. The IC_{50} values were calculated using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

4.1.12. 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 [83]. The assay was performed at room temperature in 50 μL (25 μL reaction solution + 25 μL detection reagent) in 96 well half area white plates (cat. 3693, Corning, Corning, NY) on a Tecan Freedom EVO liquid handler (Tecan Group Ltd. Månnedorf, Germany). 50 nM MAO A or 125 nM MAO B were incubated with ten 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). Reaction was started with the addition of 40 μM Promega MAO substrate for MAO-A or 14 μM for MAO-B. The reaction was stopped after 30 min with the Promega detection reagent. 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. After 30 min of incubation the reaction was stopped with the Promega detection reagent. All compounds were tested twice in duplicate and IC_{50} values were calculated using GraphPad Prism

version 4.0 (GraphPad Software, San Diego, CA).

4.1.13. Biology. Gene modulation assay

Human APL NB4 cells were grown in RPMI supplemented with 10% fetal 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; ITGAM, AACCCCTGGTTCACCTCCT – CATGACATAAGGTCAAGGCTGT; TBP, GCTGGCCCATAGTGATCTTT – CTTCACACGCCAAGAAACAGT.

4.1.14. Clonogenic assay

Murine Acute myeloid leukemia blasts has been recovered from spleen of Acute Promyelocitic Leukemic mice sacrificed once that the spleen was completed blast infiltrated. Specifically, the model was previously characterized and obtained by injection in 129sv mice of lineage-depleted cells from 129sv mice purified and transduced with PML-RARA-expressing retroviral vector [83]. One million of leukemic blast cells (from 129sv mice) injected intravenously into non-irradiated syngeneic recipients are able to induce leukemia development leading to mice death 21 days after blast injection. For the clonogenic assay, murine Acute Myeloid Leukemic cells (20,000 cells) originated from spleen of leukemic mice were seeded in duplicate in methylcellulose medium (MethoCultTM GF M3434, Stem Cell Technology, Vancouver, BC, Canada) in the presence of fixed concentration (0.25 μM) of compounds. After 7 days, colonies (C.F.U.) were counted. Percentage of inhibition is referred versus the vehicle (DMSO) treated cells.

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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.2015.02.060.

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