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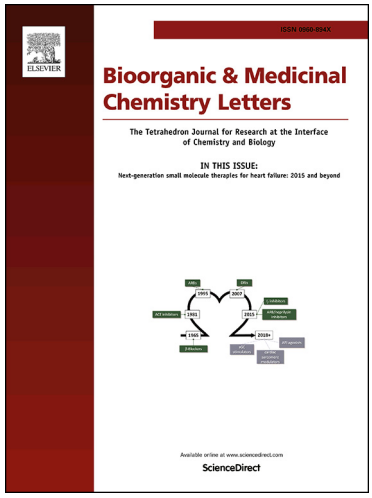

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Graphical Abstract

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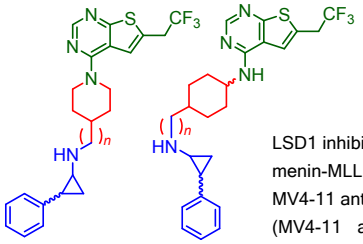

LSD1 inhibitory IC_{50} up to 66.13±6.58 nM;
menin-MLL1 PPI inhibitory IC_{50} up to 2.13±0.86 μM;
MV4-11 antiproliferative IC_{50} up to 0.29±0.11 μM.
(MV4-11 antiproliferative IC_{50} of GSK2879552: 1.16±0.14 μM)

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Tranylcypromine and 6-trifluoroethyl thienopyrimidine hybrid as LSD1 inhibitor

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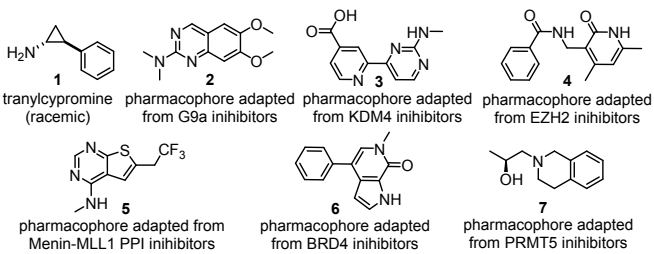
ABSTRACT

Tranylcypromine moiety extracted from LSD1 inhibitors and 6-trifluoroethyl thienopyrimidine moiety from menin-MLL1 PPI inhibitors were merged to give new chemotypes for medicinal chemistry study. Among 15 new compounds prepared in this work, some exhibited nanomolar LSD1 activity and good selectivity over MAO-A/B, low micromolar menin-MLL1 PPI inhibitory activity, as well as submicromolar MV4-11 antiprofilative activities. Intracellular LSD1 engagement of compounds with higher enzymatic and antiproliferative activities was confirmed by CD86 *m*RNA up-regulation experiments.

LSD1 emerges as an important therapeutic target against certain cancer and nervous system diseases.[1] Some LSD1 inhibitors demonstrated substantial therapeutic value and have been advanced into phase 2 clinical study.[2] Most of these clinical drugs bear tranylcypromine (TCP, **1**) structure as key pharmacophore, which irreversibly binds to flavin adenine dinucleotide (FAD) moiety sat in LSD1 catalytic cavity. [1c] A large body of SAR study indicated that TCP-containing LSD1 inhibitors tolerate wide variety of structure modification on the amino group side. [1]

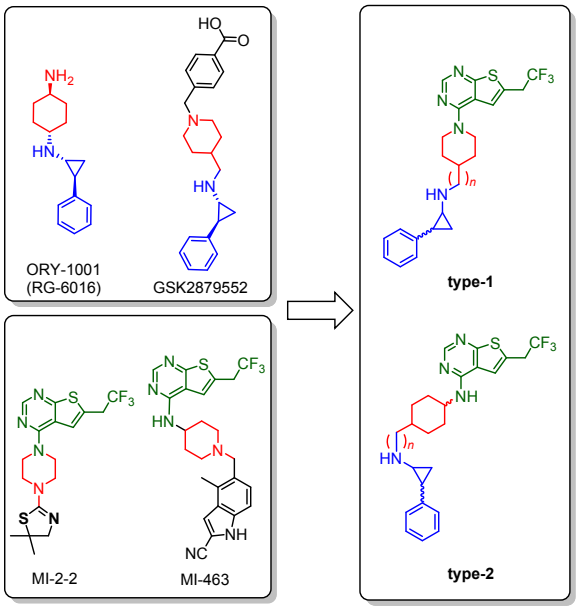
Meanwhile, LSD1 inhibitors showed synergistic antiproliferative effect with inhibition of multiple epigenetic targets, respectively.[3] It is not surprising since these targets consist of a network regulating the fate of cell. Combining above information, we ask if new activity features could be achieved by merging TCP structure and the key pharmacophores of inhibitors targeting other epigenetic enzymes. [4] A pilot computational study was made using the known pharmacophores **2**~**7** from G9a,[1c] KDM4,[1c] EZH2,[1c] menin-MLL1 protein-protein interaction (PPI),[5] BRD4,[6] and PRMT5 inhibitors[1c] (scheme 1), respectively. These pharmacophores were docked into the binding site of LSD1 prepared from the crystal structure (PDB code: 2UXX), and the binding energies were scored for each. It was found that PRMT5 inhibitor pharmacophore was the best (Glide score -5.59 kcal) and the menin-MLL1 PPI inhibitor pharmcophore ranks second (Glide score -5.09 kcal). [7]



Scheme 1. Structure of tranylcypromine (**1**) and pharmacophores **2**~**7** used in virtual screening.

A closer analysis of known menin-MLL1 PPI inhibitor MI-2-2[5e] and MI-463[5b], as well as known LSD1 inhibitor ORY-1001[2a] and GSK-2879552[2b] (scheme 2) indicated that the six-membered saturated ring (scheme 2, moieties in red) could be used as a hinge connecting the pharmacophores of menin-MLL1 PPI inhibitor (scheme 2, moieties in green) and LSD1 inhibitor

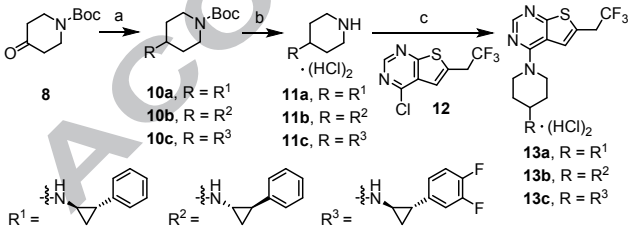
(scheme 2, moieties in blue). Thus, two types of new structures (scheme 2, type-1 and type-2) were designed using hinges containing different six-membered saturated rings. In such design, TCP moiety should be located at the solvent-exposed region of the menin-MLL1 interaction site, when the new inhibitor engaged with menin-MLL1 complex. Similarly, 6-trifluoroethyl thienopyrimidine moiety should stay outside of the enzyme when the molecule interacts with LSD1.



Scheme 2. Two types of new structures were designed by merging the tranylcypromine and 6-trifluoroethyl thienopyrimidine pharmacophores.

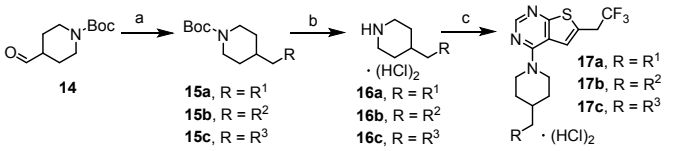
To implement above design, 6 type-1 and 9 type-2 molecules were prepared, subjected to biological evaluation. Several molecules were revealed, showing good LSD1 inhibitory activity, moderate menin-MLL1 PPI inhibitory activity, as well as promising submicromolar antiproliferative activities. The details will be reported herein.

Ketone **8** was subjected to reductive amination with several TCP derivatives including R^1H (**9a**, *1R, 2S*-isomer), R^2H (**9b**, *1S, 2R*-isomer), and R^3H (**9c**, *1R, 2S*-isomer) (scheme 3) to give products **10a**, **b**, **c**, respectively. After removal of *N*-Boc protection, **11a**, **b**, **c** were condensed with compound **12** to give type-1 target products **13a**, **b**, **c**, respectively.



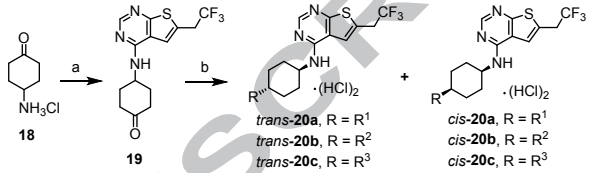
Scheme 3. Preparation of type-1 compounds **13a**,**b**,**c**. Reaction conditions: a) R^1H *R*-mandelate (**9a**), or R^2H *S*-mandelate (**9b**), or R^3H *R*-mandelate (**9c**), $NaBH_3CN$, HOAc, MeOH, 0 °C, 2 h, 30-82%; b) 4 M HCl in 1,4-dioxane, DCM, RT, 2 h, 91-99%; c) Compound **12**, DIPEA, water, isopropanol, RT, 2-3 h; then DCM, 2 M HCl in ether, RT, 0.5 h, 58-86%.

In a similar fashion, aldehyde **14** underwent reductive amination with **9a**, **b**, **c** to give intermediates **15a**, **b**, **c**, respectively, which were again deblocked and condensed with **12** to give type-1 target products **17a**, **b**, **c**, respectively (scheme 4).



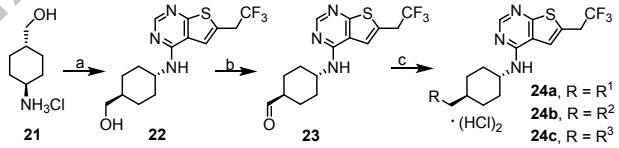
Scheme 4. Preparation of type-1 compounds **17a**, **b**, **c**. Reaction conditions: a) **9a**, or **9b**, or **9c**, $NaBH_3CN$, HOAc, MeOH, 0 °C, 2 h, 25-45%; b) 4 M HCl in 1,4-dioxane, DCM, RT, 2 h, 82-99%; c) Compound **12**, DIPEA, water, isopropanol, RT, 2-3 h; then DCM, 2 M HCl in ether, RT, 0.5 h, 18-55%.

For type-2 structures, amine **18** was condensed directly with chloride **12**, which was subjected to reductive amination with **9a**, **b**, **c** to give *trans*-**20a**, **b**, **c** and *cis*-**20a**, **b**, **c**, respectively (scheme 5).



Scheme 5. Preparation of type-2 compounds *trans*-**20a**,**b**,**c**. and *cis*-**20a**,**b**,**c**. Reaction conditions: a) Compound **12**, DIPEA, water, isopropanol, 60 °C, overnight, 68%; b) Compound **19**, $NaBH_3CN$, HOAc, MeOH, 0 °C, 2.5 h; then DCM, 2 M HCl in ether, RT, 0.5 h, 5-54%.

Similarly, amine **21** was condensed with chloride **12** and then oxidized to aldehyde **23**, which underwent reductive amination with **9a**, **b**, **c** to give **24a**, **b**, **c**, respectively (scheme 6).



Scheme 6. Preparation of type-2 compounds **24a, b, c**. Reaction conditions: a) Compound **12**, DIPEA, water, isopropanol, 60 °C, overnight, 72%; b) Dess-Martin periodinane, DCM, RT, 4 h, 44%; c) **9a**, or **9b**, or **9c**, $NaBH(OAc)_3$, HOAc, 4 Å molecular sieve, 1,2-dichloroethane, RT, 3 h; then DCM, 2 M HCl in ether, RT, 0.5 h, 15-79%.

All target products were subjected to biological evaluation and the results were shown in table 1. Type-1 compounds **13a**, **b**, **c** showed good LSD1 inhibitory activity ranging from 211.35±27.79 nM to 692.15±126.36 nM and the selectivity over MAO-A and MAO-B were moderate to good. Meanwhile, compounds **13a**, **b** showed low micromolar menin-MLL1 PPI inhibitory activity. On MV4-11 cells, compound **13a** showed good antiproliferative activity of 0.29±0.11 μM, better than that of MI-2-2 and GSK2879552. Compound **13b** containing *1S, 2R*-TCP moiety showed decreased cellular activity, and compound **13c** bearing difluorophenyl substituted TCP lost its activity on MV4-11 for unknown reasons (table 1, entries 4~6). Type-1 compounds **17a**, **b**, **c** showed improved LSD1 inhibitory activity ranging from 61.04±9.79 nM to 252.33±75.75 nM and slightly improved selectivity for LSD1 over MAO-A and MAO-B. Against menin-MLL1 PPI, compounds **17a**, **c** showed low micromolar activity, while **17b** was inactive. MV4-11 Cellular activities were moderate for **17a**,**b** and poor again for **17c** (table 1, entries 7~9).

Type-2 compounds *trans*-**20a**, **b**, **c** and *cis*-**20a**, **b**, **c** showed good LSD1 activity ranging from 66.13±6.58 nM to 303.35±40.23 nM and their selectivity for LSD1 over MAO-A and MAO-B were moderate to good. Against menin-MLL1 PPI, *cis*-**20c** showed improved activity of 2.13±0.86 μM, *trans*-**20a** and *cis*-**20a**, **b** showed low micromolar activity, while *trans*-**20b**,

c were inactive. For MV4-11 cellular activities, *trans*-**20a** and *cis*-**20c** displayed submicromolar activities, while other compounds showed low micromolar activities (table 1, entries 10~15). Type-2 compounds **24a**, **b**, **c** also showed good LSD1 activity ranging from 109.35±11.67 nM to 359.7±60.95 nM and their selectivity for LSD1 over MAO-A and MAO-B were moderate to good. Perhaps due to the longer hinge moiety, **24a**, **b**, **c** showed significantly decreased menin-MLL1 PPI inhibitory activities. Their cellular activity remained at low micromolar, largely caused by the LSD1 activities (table 1, entries 16~18).

Table 1. Type-1 and type-2 compounds were assayed for their enzymatic and cellular activity, in comparison with positive controls.

No.	compd.	LSD1 inhibitory activity (IC_{50})	menin-MLL1 PPI inhibitory activity (IC_{50})	MAO-A/B inhibitory activity (IC_{50})	MV4-11 antiproliferative activity (IC_{50}) [a]
1	MI-2-2	*n.a.*	118.30±4.53 nM	*n.a.*	2.60±0.21 μM
2	GSK2879552	1200.00±33.94 nM	*n.a.*	>100 / >100 μM	1.16±0.14 μM
3	Tranylcypromine (**1**)	21.85±3.19 μM	*n.a.*	1.02±0.10 / 0.56±0.13 μM	> 100 μM
4	**13a**	211.35±27.79 nM	10.24±4.30 μM	12.46±3.40 / 40.25±11.75 μM	0.29±0.11 μM
5	**13b**	692.15±126.36 nM	34.54±5.18 μM	8.27±2.92 / 36.70±13.69 μM	7.31±0.09 μM
6	**13c**	240.50±34.08 nM	> 100 μM	56.44±6.86 / 95.61±20.07 μM	> 100 μM
7	**17a**	84.38±6.34 nM	17.33±3.25 μM	2.23±0.22 / 1.16±0.21 μM	11.36±0.53 μM
8	**17b**	61.04±9.79 nM	> 100 μM	9.19±1.39 / 9.63±1.16 μM	5.97±1.42 μM
9	**17c**	252.33±75.75 nM	30.94±17.71 μM	20.79±7.02 / 49.51±9.79 μM	> 100 μM
10	*trans*-**20a**	303.35±40.23 nM	36.93±14.05 μM	65.88±12.66 / >100 μM	0.49±0.19 μM
11	*cis*-**20a**	124.05±15.49 nM	50.75±21.40 μM	4.55±1.33 / 6.05±0.91 μM	2.49±0.17 μM
12	*trans*-**20b**	187.00±14.00 nM	> 100 μM	12.89±6.81 / 28.77±4.84 μM	3.60±0.33 μM
13	*cis*-**20b**	241.95±27.22 nM	24.73±4.49 μM	>100 / 75.63±23.43 μM	3.42±0.37 μM
14	*trans*-**20c**	146.35±31.61 nM	> 100 μM	47.47±5.76 / 71.61±24.49 μM	2.01±0.37 μM
15	*cis*-**20c**	66.13±6.58 nM	2.13±0.86 μM	40.86±17.09 / 1.21±0.12 μM	0.51±0.16 μM
16	**24a**	109.35±11.67 nM	>100 μM	4.10±0.65 / 4.81±0.27 μM	2.96±0.19 μM
17	**24b**	184.15±22.56 nM	> 100 μM	8.57±2.29 / 21.98±0.57 μM	5.55±0.90 μM
18	**24c**	359.7±60.95 nM	> 100 μM	20.99±2.84 / 20.35±0.64 μM	6.96±0.51 μM

[a] except for entry 2, all cellular activities were measured at 7th day. Cellular activity of entry 2 was measured at 10th day.

Since CD86 up-regulation serves as a surrogate cellular biomarker for LSD1 pharmacological inhibition,[8] LSD1 inhibition effect of type-1 compound **13a** as well as type-2 compounds *trans*-**20a** and *cis*-**20c** was further evaluated for their CD86 *m*RNA expression enhancement in MV-4-11 cells (table 2). Compared with GSK2879552, our compounds showed more potential to induce the CD86 *m*RNA expression, consistent with their enzymatic and cellular activities.

Table 2. CD86 *m*RNA expression analysis of selected compounds.

Entry	Compound	CD86 expression fold on MV-4-11 [a] (IC_{50})
1	GSK2879552	15.22±1.44 @ 1000 nM
2	**13a**	22.32±0.41 @ 500 nM
3	*trans*-**20a**	16.39±0.78 @ 500 nM
4	*cis*-**20c**	7.59±0.74 @ 100 nM

[a] Q-PCR measurements were performed in triplicate.

According to above results, on biochemical assays, *1R, 2S*-TCP, *1S, 2R*-TCP, and *1R, 2S*-difluorophenyl cyclopropanamine derivatives showed no significant difference in terms of their LSD1 inhibitory activities. Incorporation of 6-trifluoroethyl thienopyrimidine moiety resulted in higher LSD1 inhibitory activity compared with GSK2879552. With 6-trifluoroethyl thienopyrimidine, the selectivity over MAO-A/B was also improved compared with that of tranylcypromine (**1**). Nevertheless, the menin-MLL1 PPI inhibitory activity of new compounds was compromised and only low micromolar value could be recorded. At cellular level, *1R, 2S*-TCP and *1S, 2R*-TCP derivatives showed also no major difference in their antiproliferative activities. For several cases, *1R, 2S*-difluorophenyl cyclopropanamine derivatives showed no cellular activities, although they have similar physicochemical properties to other analogues. The antiproliferative effect of above new compounds is not likely caused by synergistic effect due to the low menin-MLL1 PPI inhibitory activity. Meanwhile, for compounds showing higher enzymatic and antiproliferative activities, their LSD1 target engagement in MV4-11 cell was further confirmed by CD86 *m*RNA up-regulation experiments.

In summary, we have merged TCP moiety from LSD1 inhibitors and 6-trifluoroethyl thienopyrimidine moiety from menin-MLL1 PPI inhibitors to create new chemotypes showing good LSD1 activity and selectivity over MAO-A/B, moderate menin-MLL1 PPI inhibitory activity, as well as moderate to good MV4-11 antiprofilative activities. In particular, compounds **13a**, *trans*-**20a**, and *cis*-**20c** demonstrated promising submicromolar cellular activity, several folds higher than that of MI-2-2 and GSK2870552 and could be used for further medicinal chemistry study.

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Supplementary data

Experimental procedures as well as copies of the ^{1}H NMR and ^{13}C NMR spectra of new compounds are provided.

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