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Novel polyamine-based Histone deacetylases-Lysine demethylase 1 dual binding inhibitors

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ABSTRACT

Epigenetic modulators Histone deacetylases (HDACs) and Lysine demethylase (LSD1) are validated targets for anticancer therapy. Both HDAC1/2 and LSD1 are found in association with the repressor protein CoREST in a transcriptional co-repressor complex, which is responsible for gene silencing. Combined modulation of both targets results in a synergistic antiproliferative activity. In the present investigation, we report about the design and synthesis of a series of polyamine-based HDACs-LSD1 dual binding inhibitors obtained by coupling Vorinostat and Tranylcypromine. Compound **4** emerged as the most promising of the synthesized series, showing good inhibitory activity towards HDAC1 and LSD1 either *in vitro* and in cell-based assay (Ki = 42.52 ± 8.94 nM and IC_{50} = 3.85 μM, respectively). Furthermore, at 70.0 μM compound **4** induced a more pronounced cytotoxic effect than Vorinostat (68.6% *vs* 56.6% of dead cells) in MCF7 cancer cell line.

Cancer is a multifactorial disease whose onset derives from both genetic and epigenetic events; in recent years, epigenetic has gained notable consideration and epigenetic targets have been validated in therapy.[1] Epigenetic changes are reversible chromatin modifications that lead to activation or suppression of gene expressions. The epigenetic control of gene expressions takes place through modification of the DNA itself, i.e. methylation, or DNA-associated proteins, i.e. methylation and acetylation. According to the subject and the type of modification, chromatin can adopt conformational changes that lead to the activation or suppression of gene expression.[2] Histone deacetylases (HDACs) are one of the most important classes of epigenetic enzymes responsible for removing acetyl groups from histone tails, determining a more closed, transcriptionally repressed, chromatin structure.[3] Abnormal HDACs activity has been found to be associated with the aberrant gene expression and the development of several kinds of cancer.[4] Inhibition of HDACs activities restore normal gene expression resulting in cell cycle arrest, apoptotic cell death and cell differentiation.[5] Different HDAC inhibitors (HDACis) have been approved by the FDA, i.e. vorinostat, romidepsin, panobinostat and belinostat, and chidamide in China, and many others are currently under evaluation in clinical trials (Fig. 1).[6] Over the years, several different classes of HDACis have been identified;[7] among them hydroxamate-based compounds hold a special interest in spite of their unfavourable ADMET properties.[8] Hydroxamate-based inhibitors, among the most potent but rather unselective HDACis, bear the hydroxamic acid group able to strongly coordinate the Zn^{2+} located in the active site of the enzyme. The general structure for hydroxamate-HDACis comprises three main motifs: a Zn^{2+} binding group, a hydrophobic spacer and a cap group able to interact with the surface of the enzyme. Several clinical evidences reported that HDACis might be more effective as antiproliferative agents when used in combination with other chemotherapeutic or epigenetic drugs. For instance, HDACis potentiate the antiproliferative activities of Topoisomerase I and II inhibitors;[9,10] further, HDACis in combination with the EGFR inhibitor Gefitinib synergistically induce growth inhibition and apoptosis induction in gefitinib-resistant cancer cell lines.[11,12] Recently, Bhalla and coworkers reported that combined inhibition of HDACs and Lysine demethylase LSD1 is lethal in human AML cells.[13] Furthermore, the same combination resulted in a synergistic increase of apoptotic cell death in glioblastoma multiforme cell line.[14] LSD1 is a histone demethylase, member of the greater amine oxidase superfamily, that is responsible, among other activities, for the specific demethylation of mono- and dimethylated histone H3-Lys4.[15]

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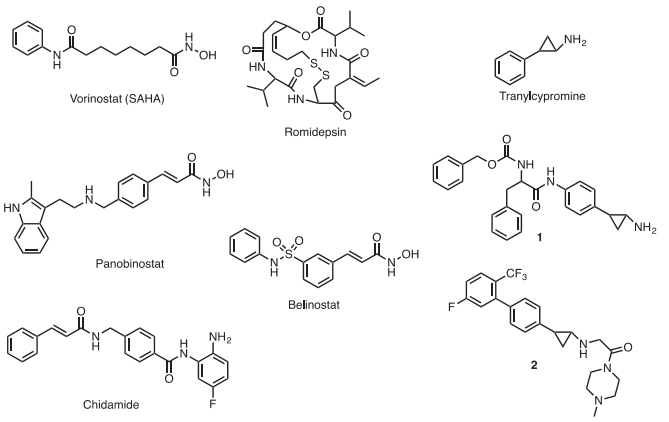

Fig. 1. Structures of known HDACis, Vorinostat, Romidepsin, Panobinostat, Belinostat and Chidamide, and LSD1 inhibitors, Tranylcypromine, **1** and **2**.

LSD1 is overexpressed in various cancers and binds to several transcription factors, regulating the expression of a vast array of genes.[16] LSD1 is found in association with the repressor protein CoREST and HDAC1/2, in a transcriptional co-repressor complex that is responsible for gene silencing.[17] Most of the known LSD1 inhibitors developed share structural features with monoamine oxidase inhibitors. In particular, Tranylcypromine is a known irreversible LSD1 inhibitor and several Tranylcypromine-based derivatives have been reported so far (Fig. 1). The synergistic activity obtained combining HDACs and LSD1 inhibitors together with the finding that LSD1 is associated with CoREST and HDAC1/2 provided the rational for designing dual binding agents able to simultaneously modulate HDACs and LSD1. Herein, we report the design, the synthesis and the preliminary biological evaluation of a series of polyamine-based HDAC/LSD1 dual binding inhibitors.

The design of these new compounds began with the observation that various substituents may be introduced at the para position of the aromatic ring of both Vorinostat and Tranylcypromine without a dramatic drop in the inhibitory activity. The two pharmacophores have been linked through a polyamine chain since it has been previously demonstrated that a) protonated nitrogen atoms can establish interactions with negatively charged amino acids[18,19] and b) polyamines interact with both HDACs and LSD1 proteins.[20–22] Three different polyamine chains have been chosen differing in the number of the nitrogen atoms and in the distance between them; indeed, compound **3** has a spermidine-like linker (3-3), compound **4** has a spermine linker (3-4-3) and compound **5** carries a spermine-like connecting unit characterized by longer (6-8-6) polymethylenes chain between the nitrogen atoms (Fig. 2).

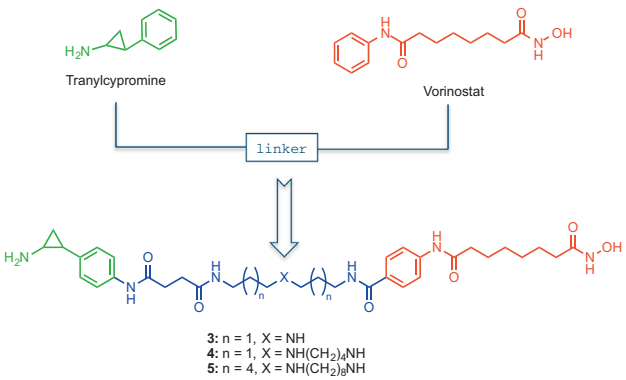

Fig. 2. Drug design leading to dual binding agents **3**–**5**.

During the drafting of this manuscript, two papers reporting about novel dual HDAC-LSD1 inhibitors appeared.[23,24] However, these compounds turned out to be structurally slightly different than those reported in the present manuscript.

For the synthesis of target compounds **3**–**5** a stepwise linear synthetic approach was developed (Scheme 1). Suberic anhydride **7** was condensed with methyl 4-aminobenzoate **6** to generate the corresponding acid **8**. This was coupled to O-(Tetrahydro-2*H*-pyran-2-yl)hydroxylamine in order to obtain the protected hydroxamic acid **9**, using EDC as coupling agent. Basic hydrolysis of the methyl ester of **9** furnished the carboxylic acid **10** that was coupled with the suitable protected polyamines **11**–**13**.[25,26] Many efforts were carried out in order to optimize the coupling conditions of **10** with **11**–**13**: after variation of solvents (DCM, DMF, THF), reaction times and coupling auxiliary (EtOCOCl and Et_3N, IBCF and Et_3N, DCC and HOBt, DCC and DMAP, EDC and HOBt) it was found that the use of EDC and DMAP gave the best yields. Basic deprotection of the trifluoromethyl protecting group led to the primary amines **17**–**19**, which further reacted with succinic anhydride leading to the corresponding acids **20**–**22**. Coupling of the latter with Boc-protected tranylcypromine[27] generated the fully-protected dimers **24**–**26**. Also in this case, several reaction conditions were evaluated with the aim to optimize the coupling reaction and again better yields were obtained using EDC as coupling agent. Final acidic hydrolysis of the Boc-protecting groups gave the target compounds **3**–**5** as hydrochloride salts.

As summarized in Table 1, the target compounds **3**–**5** as well as the reference compounds Vorinostat and Tranylcypromine were profiled for their HDAC1-CoREST3 and LSD1-CoREST3 inhibitory activities *in vitro*. Unfortunately, compound **5** was endowed with poor solubility in the media and, therefore, could not be evaluated. Compounds **3** and **4** retain the activity of the parent compounds being both active in the nanomolar and in the micromolar range of concentrations against HDAC1 and LSD1, respectively. However, by a closer look to the HDAC1 inhibitory activity, it appears that introduction of a polyamine chain and a second pharmacophore in the para position of the aromatic ring induces a slightly decrease in the activity compared to Vorinostat. Indeed, compounds **3** and **4** are respectively 9 and 14- fold less active than Vorinostat (**3**: Ki = 26.87 ± 7.87 nM; **4**: Ki = 42.52 ± 8.94 nM; Vorinostat: Ki = 3.04 ± 0.6 nM). An opposite trend was observed considering the inhibitory activity towards LSD1; in this case, the introduction of the polyamine chain and a second pharmacophore has a positive effect on the inhibitory activity with compounds **3** and **4** being respectively 37 and 23-fold more active than Tranylcypromine (**3**: IC_{50} = 2.40 μM; **4**: IC_{50} = 3.85 μM; Tranylcypromine: IC_{50} = 89.08 μM).

Compounds **3** and **4** were then evaluated in cell-based assay. First, compounds **3** and **4**, together with Vorinostat and Tranylcypromine, were evaluated for their cytotoxic activity in MCF7 breast cancer cell line. Vorinostat induced a dose-dependent decrease in cell viability with an IC_{50} of 38.2 μM (data not shown). Higher concentrations of Tranylcypromine were necessary to decrease MCF-7 viability and the concentration required to reach the IC_{50} value was 2518.9 μM (data not shown).

Compounds **3** and **4** significantly decreased MCF-7 viability starting from the lowest tested concentration and in a dose-dependent manner. The concentration required to reach the IC_{50} value was 60.2 μM for compound **3** and 39.6 μM for compound **4** (Fig. 3). The cytotoxic activity of compound **4** was similar to that of Vorinostat: at 4.0 μM, Vorinostat and compound **4** induced 20.8% and 16.3% decrease in cell viability, respectively, compared to control cells. Increasing treatment concentrations showed an analogue trend. A 49.3% and 52.7% decrease in cell viability was recorded after 24 h incubation with either Vorinostat or compound **4** at 16.0 μM and 35.0 μM, respectively. At the highest concentration tested (70.0 μM), **4** induced a more pronounced cytotoxic