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Synthesis of a selective HDAC6 inhibitor active in neuroblasts

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ABSTRACT

In recent years, the role of HDAC6 in neurodegeneration has been partially elucidated, which led some authors to propose HDAC6 inhibitors as a therapeutic strategy to treat neurodegenerative diseases. In an effort to develop a selective HDAC6 inhibitor which can cross the blood brain barrier (BBB), a modified hydroxamate derivative (compound **3**) was designed and synthetized. This compound was predicted to have potential for BBB penetration based on in silico and in vitro evaluation of passive permeability. When tested for its HDAC inhibitory activity, the IC_{50} value of compound **3** towards HDAC6 was in the nM range in both enzymatic and cell-based assays. Compound **3** showed a cell-based selectivity profile close to that of tubastatin A in SH-SY5Y human neuroblastoma cells, and a good BBB permeability profile.

Abnormalities in protein acetylation levels caused by deregulation of HDAC/HAT activities are proposed to be involved in the pathogenesis of several diseases such as cancer, cardiovascular, and neurodegenerative disorders.[1–7] In light of this, HDACs have been considered to be pharmaceutical targets against these diseases. The search for HDAC inhibitors resulted in the identification and development of an increasing number of structurally diverse compounds able to inhibit HDAC isoforms with various potency and selectivity. This effort led to the FDA approval of the pan-HDAC inhibitors romidepsin, vorinostat, belinostat, and panobinostat as therapies for cancer treatment.[8,9]

The hypothesis that the acetylation status plays an important role in learning and memory processes, and seems to be impaired in neurodegeneration, led some authors to propose HDAC inhibitors as a therapeutic strategy to treat neurodegenerative diseases.[10] Among HDACs, HDAC6 presents several important roles in cell biology, especially its implication in the control of tubulin acetylation levels, which can make it a target for the discovery of drugs against neurodegeneration. The use of tubacin, a well-known HDAC6 inhibitor, increased the acetylation level of tubulin, which improved mitochondrial transport in hippocampal neurons.[11] This improvement restored learning and memory in a mouse model of Alzheimer's disease lacking HDAC6.[4] In this disease, HDAC6 inhibition also led to beneficial effects by acting on other protein targets such as tau, a protein which promotes assembly and stabilizes microtubules. In Alzheimer's disease, hyperphosphorylation of tau affects the regulation of axonal transport and results in the accumulation of neurofibrillary tangles causing neuronal dysfunction.[12] In 2014, Cook et al.[13] demonstrated that the use of ACY-738 (Fig. 1), a selective HDAC6 inhibitor able to cross the blood brain barrier (BBB), could decrease tau pathogenic hyperphosphorylation and aggregation by increasing its acetylation level in mice.

Therefore, the development of selective HDAC6 inhibitors is of great interest and the aim of the present study was to identify compounds able to selectively target HDAC6 in neuroblasts. Previously, the key role of the *tert*-butyloxycarbonyl (BOC) group in HDAC6 selectivity was demonstrated, suggesting the impact of this large lipophilic moiety on HDAC6 surface recognition. While compound **1** (Fig. 1) did not show a significant selectivity for HDAC6, its BOC derivative **2** selectively inhibited this isoform.[14] Considering these data and the growing interest in identifying HDAC6 selective inhibitors, compounds **1** and **2** were first studied by using an in silico approach to assess their potential ability to cross the BBB and to match optimal CNS physicochemical features according to the

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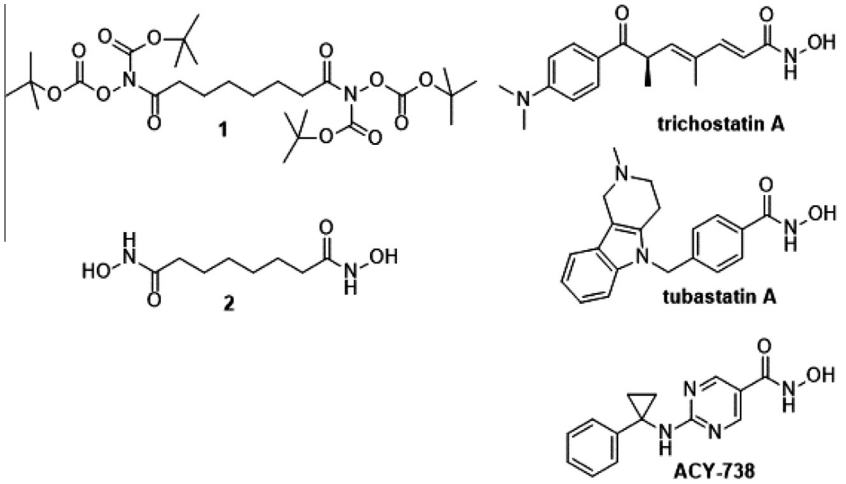
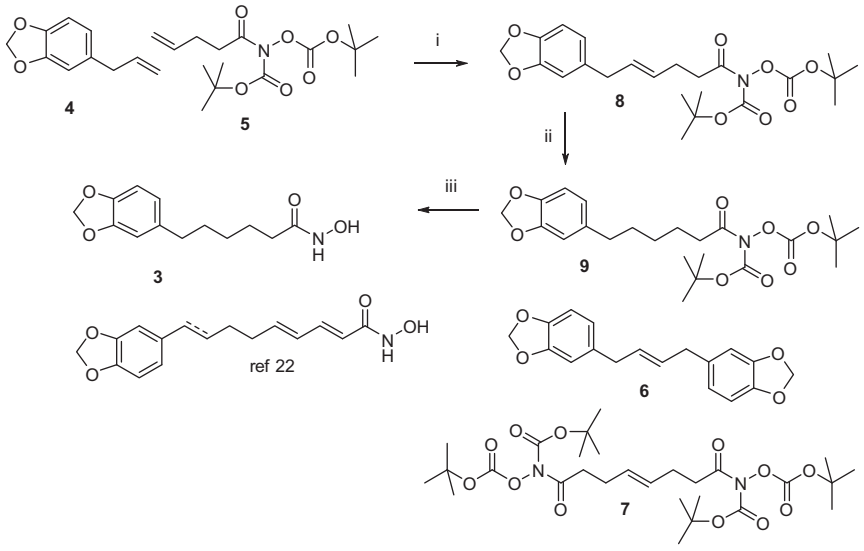


Figure 1. Chemical structures of compounds **1** and **2**, and other compounds used in this work for comparison purposes.

rules described by Pajouhesh et al.,[15] and by Wager et al. (Table S1).[16]

By combining information coming from those studies, it is clear that BBB penetration is strongly influenced by six fundamental physicochemical properties: *C*log*P*, *C*log*D*, molecular weight (MW), topological polar surface area (TPSA), number of hydrogen bond donors (HBD), and pK_a. For each of them, a desirable range is reported. Whereas compounds **1** and **2** did not satisfy the described criteria for BBB crossing, compound **3** was the most promising one (Table S1). Indeed, pK_a values reflect the difficulty of strong acids and bases to penetrate the BBB. Compound **3** is characterized by a hydroxamate chelating group with a predicted pK_a of 8.9. Pajouhesh et al.[15] proposed to limit pK_a for BBB penetration between 4 and 10, whereas pK_a values equal or lower than 8 were the limits proposed by Wager et al.[16] Hydroxamates have already been reported in the literature to be active on CNS. For example, tubastatin A (predicted pK_a of 9.8), is one of the most promising HDAC6 selective inhibitors developed so far with strong neuroprotective properties (Fig. 1).[17–20]

The use of cross metathesis has been previously validated to synthesize compounds **1** and **2**,[21] and was applied to synthetize derivative **3** (Scheme 1), aiming at achieving both HDAC6 selectivity and optimal CNS targeting features. In compound **3**, one of the two hydroxamate moieties was kept for assuring zinc chelation, while the other one was replaced by a bulky 1,3-benzodioxole as a cap group to address HDAC6 selectivity. Finally, the linker was shortened to mimic tubastatin A features. Cross metathesis was used to synthesize compound **3** from safrol (**4**) and the known intermediate **5** (Scheme 1).[21] A multistep synthesis of benzo[1,3]-dioxole with polyunsaturated alkene chain was described to give micromolar HDAC inhibitors (Scheme 1),[22] but saturated ones are unknown. Optimized condition for cross metathesis with Grubbs 1st generation catalyst was thus used to produce new HDAC inhibitors bearing this benzo[1,3]dioxole from safrol (**4**). The method consisted in preparing two CH_2Cl_2, solutions: one containing the two alkenes **4** and **5**, and the other the Grubbs 1st generation catalyst. The catalyst solution was added slowly at 0.5 ml/h in an already refluxing solution of alkenes. Once the catalyst addition was over, reflux was maintained for one more hour. The ratio of compounds **4**:**5** was first set to 1:2. In the previously optimized conditions for symmetric metathesis, the two possible symmetric cross products **6** and **7** and the desired dissymmetric one **8** were



Scheme 1. Regents and conditions: (i) 7.5% mol. Grubbs 1st generation catalyst, refluxing CH_2Cl_2, 7 h, 59% (ii) H_2, Pd/C 10%, EtOAc, room temperature, 66%, (iii) TFA, CH_2Cl_2, room temperature, 95%.

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