CHEMISTRY
A European Journal

A Journal of
ChemPubSoc Europe

Accepted Article

Title: Synthesis of potent and selective HDAC6 inhibitors bearing a cyclohexane- or cycloheptane-annulated 1,5-benzothiazepine scaffold

Authors: Rob De Vreese, Lisa Galle, Yves Depetter, Jorick Franceus, Tom Desmet, Kristof Van Hecke, Veronick Benoy, Ludo Van Den Bosch, and Matthias D'hooghe

To be cited as: *Chem. Eur. J.* 10.1002/chem.201604167

Link to VoR: http://dx.doi.org/10.1002/chem.201604167

Supported by
ACES

WILEY-VCH

10.1002/chem.201604167

Synthesis of potent and selective HDAC6 inhibitors bearing a cyclohexane- or cycloheptane-annulated 1,5-benzothiazepine scaffold

Rob De Vreese,[a] Lisa Galle,[a] Yves Depetter,[a] Jorick Franceus,[b] Tom Desmet,[b] Kristof Van Hecke,[c] Veronick Benoy,[d] Ludo Van Den Bosch[d] and Matthias D'hooghe*[a]

[a] *R. De Vreese, L. Galle, Y. Depetter, Prof. Dr. M. D'hooghe. SynBioC Research Group, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium. E-mail: matthias.dhooghe@UGent.be*

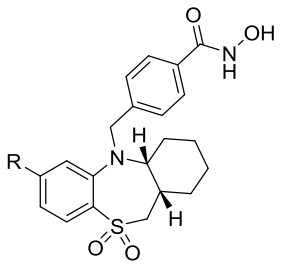
[b] *J. Franceus, Prof. Dr. T. Desmet. Centre for Industrial Biotechnology and Biocatalysis, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium*

[c] *Prof. Dr. K. Van Hecke. Xstruct, Department of Inorganic and Physical Chemistry, Faculty of Sciences, Ghent University, Krijgslaan 281-S3, B-9000 Ghent, Belgium*

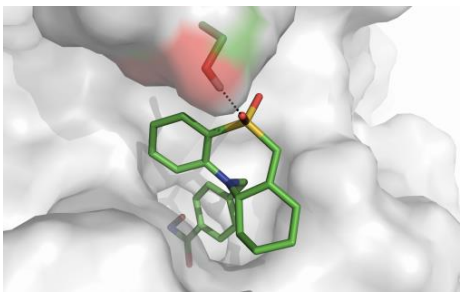
[d] *Dr. V. Benoy, Prof. Dr. L. Van Den Bosch. KU Leuven - University of Leuven, Department of Neurosciences, Experimental Neurology; VIB - Vesalius Research Center, Laboratory of Neurobiology, B-3000 Leuven, Belgium*

Abstract:

Histone deacetylase 6 (HDAC6) selective inhibitors represent an emerging class of pharmaceuticals due to the involvement of HDAC6 in different pathways related to neurodegenerative diseases, cancer and immunology. In this paper, the synthesis of a series of ten new benzohydroxamic acids, constructed employing the benzothiazepine core as a privileged pharmacophoric unit, is described. This is the first report on the synthesis and isolation of octahydrodibenzothiazepines and octahydro-6*H*-benzocycloheptathiazepines as novel heterocyclic scaffolds, which were consecutively used to develop a new class of HDAC6 inhibitors. These compounds were then evaluated for their HDAC inhibitory activity, resulting in the identification of *cis*-*N*-(4-hydroxycarbamoylbenzyl)-1,2,3,4,4a,5,11,11a-octahydrodibenzo[b,e][1,4]thiazepine-10,10-dioxide and *cis*-*N*-(4-hydroxycarbamoylbenzyl)-7-trifluoromethyl-1,2,3,4,4a,5,11,11a-octahydrodibenzo[*b*,*e*][1,4]thiazepine-10,10-dioxide as highly potent and selective HDAC6 inhibitors with activity in the low nanomolar range, also displaying excellent selectivity on an enzymatic and a cellular level. Furthermore, four promising inhibitors were subjected to an Ames fluctuation assay, revealing no mutagenic effects associated with these structures.



IC_{50} (HDAC6) = 8.3 nM (R = H)
IC_{50} (HDAC6) = 11 nM (R = CF_3)



10.1002/chem.201604167

Introduction

Histone deacetylases (HDACs), together with histone acetyltransferases (HATs), regulate the acetylation status of histones and other proteins through lysine acetylation and deacetylation.[1] This property to modify the ε-amino tail of lysine residues provides the ability to change the net charge of proteins, which makes HDACs valuable regulatory enzymes and explains the broad biological relevance of HDAC inhibitors (with potential applications in the treatment of cancer, neurodegenerative diseases, depression, inflammatory diseases,…).[2] Unfortunately, commercially available pan-HDAC inhibitors, known to inhibit multiple classes of zinc-dependent HDACs (class I: HDAC1, 2, 3 and 8, class IIa: HDAC4, 5, 7 and 9, class IIb: HDAC6 and 10, class IV: HDAC11), have been reported to demonstrate toxic side effects as well, hampering their broad clinical usability.[3] Therefore, many efforts are now devoted to the design and discovery of isozyme-selective HDAC inhibitors, with possibly less toxic side effects while maintaining a pronounced specific activity. In that regard, HDAC6 (belonging to HDAC class IIb) has been identified as an interesting pharmaceutical target since its activity is associated with biological pathways operating in neurodegenerative diseases, cancer and immunology.[4] Because of its cytoplasmic location, HDAC6 has several non-histone substrates (α-tubulin, cortactin,…), and this feature renders it an interesting protein to study the acetylation status of proteins in cells. Several research groups have thus embarked on a journey to discover selective HDAC6 inhibitors, which resulted in a variety of new compounds with promising potencies, as exemplified by inhibitors **1-8** (Figure 1).[5]

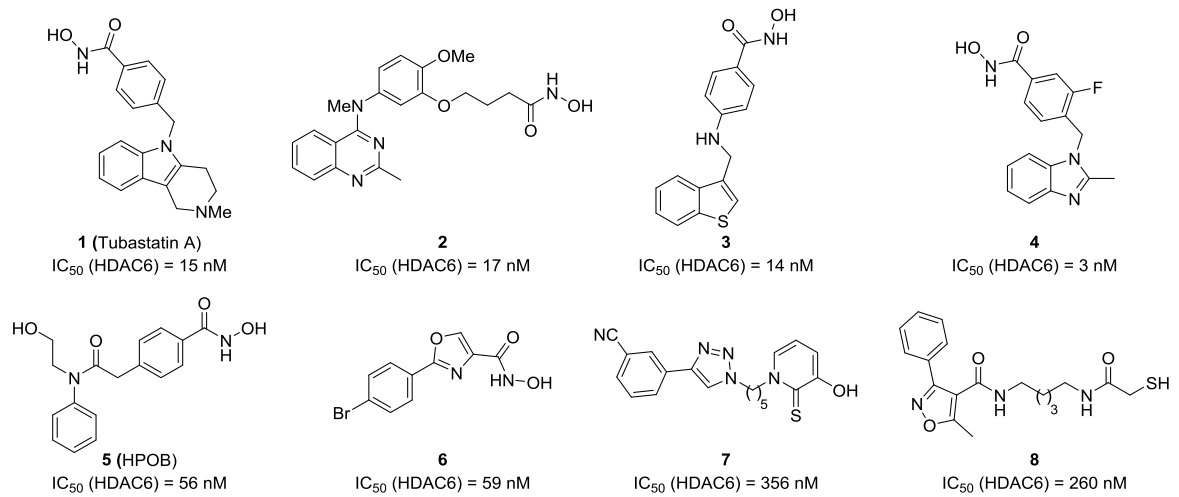
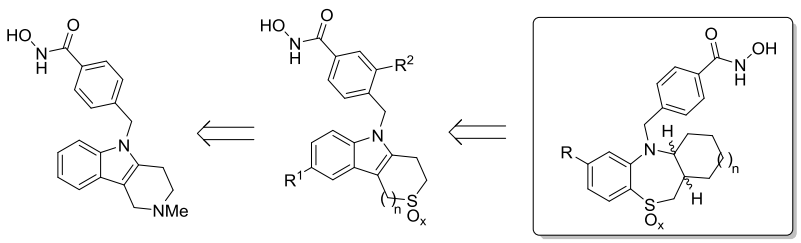


Figure 1. A selection of selective HDAC6 inhibitors reported in the literature.[5a-h]

From this list of compounds, our attention was initially drawn by Tubastatin A (**1**), a highly potent and selective HDAC6 inhibitor accommodating a tricyclic protein surface recognition group (cap group) and a benzohydroxamic acid linker/zinc binding group.

10.1002/chem.201604167

In our first attempts to pursue novel potent and selective HDAC6 inhibitors, the nitrogen-containing tricyclic pyridoindole group in Tubastatin A (**1**) was replaced by a sulfur-containing thiopyranoindole framework in compounds **9** (Figure 2), culminating in several *S*-oxidized analogs (denoted as Tubathians) demonstrating excellent *in vitro* potency, selectivity and pharmacokinetics.[6] The higher potency demonstrated by these *S*-oxidized analogs with respect to HDAC6 was rationalized *in silico* through ligand docking studies, showing that sulfoxides (x = 1) **9** and sulfones (x = 2) **9** can establish an additional hydrogen bond with the surface of HDAC6. Inspired by these interesting findings, the present work aimed at expanding our thiaheterocyclic library of HDAC6 inhibitors through the design of new structures bearing a benzohydroxamic acid functionality and an unprecedented sulfur-containing tricyclic cap group. In that respect, 1,5-benzothiazepine was identified and selected as a suitable privileged scaffold for elaboration into a new class of HDAC6 inhibitors. Indeed, 1,5-benzothiazepine is a well-known pharmacophore exhibiting a broad range of biological activities (Ca^{2+} channel blockers, CNS acting agents, anti-platelet aggregation, anti-HIV, angiotensin converting enzyme inhibitors, antimicrobial, antifungal, calmodulin antagonist, bradykinin receptor agonist, anticancer) and is present in several FDA-approved drugs (diltiazem, clentiazem, thiazesim, quetiapine hemifumaraat and clothiapine).[7] Moreover, 1,5-benzothiazepine contains a secondary amino group and an oxidizable sulfur atom, which makes it an ideal building block for further synthetic elaboration into functionalized target structures. Considering the fact that the cap group in previously developed HDAC6 inhibitors consist of a tricyclic structure bearing an aromatic A ring, an azaheterocyclic B ring and a saturated C ring, the main objective of the present study involved the development of a new tricyclic scaffold based on the annulation of a cyclohexane or cycloheptane ring to the celebrated 1,5-benzothiazepine unit *en route* to the synthesis of a novel series of octahydrodibenzo- (n = 1) or octahydro-6*H*-benzocycloheptathiazepine- (n = 2) based HDAC6 inhibitors **10**.



1 Tubastatin A

9
R^1 = H, F, Br
R^2 = H, OMe
n = 0, 1
x = 0, 1, 2

10
R = H, Cl, CF_3
x = 0, 1, 2
n = 1, 2

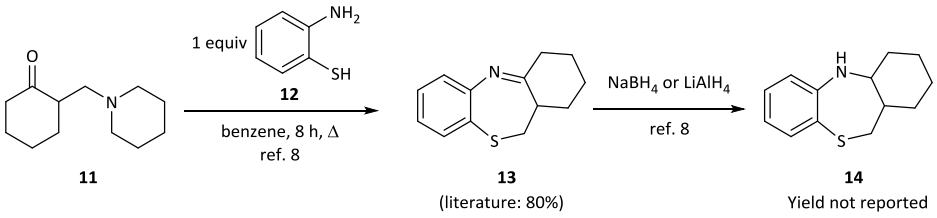
Figure 2. Synthesis rationale for the present study.

10.1002/chem.201604167

Results and discussion

Chemistry

Only one report on the synthesis of tricyclic benzothiazepine **14** is available in the literature, starting from 2-(piperidin-1-ylmethyl)cyclohexan-1-one **11** or its HCl-salt (Scheme 1).[8] This Mannich base **11** has been treated with 2-aminothiophenol **12** in benzene at reflux temperature, furnishing tricyclic imine **13** in 80% yield. After hydride reduction of cyclic imine **13**, the corresponding thiazepine **14** was obtained (although no reaction details were provided in the original report). Several attempts were made by us to reproduce these results by using the same or slightly adapted protocols, however, cyclic imine **13** could never be obtained in our hands.

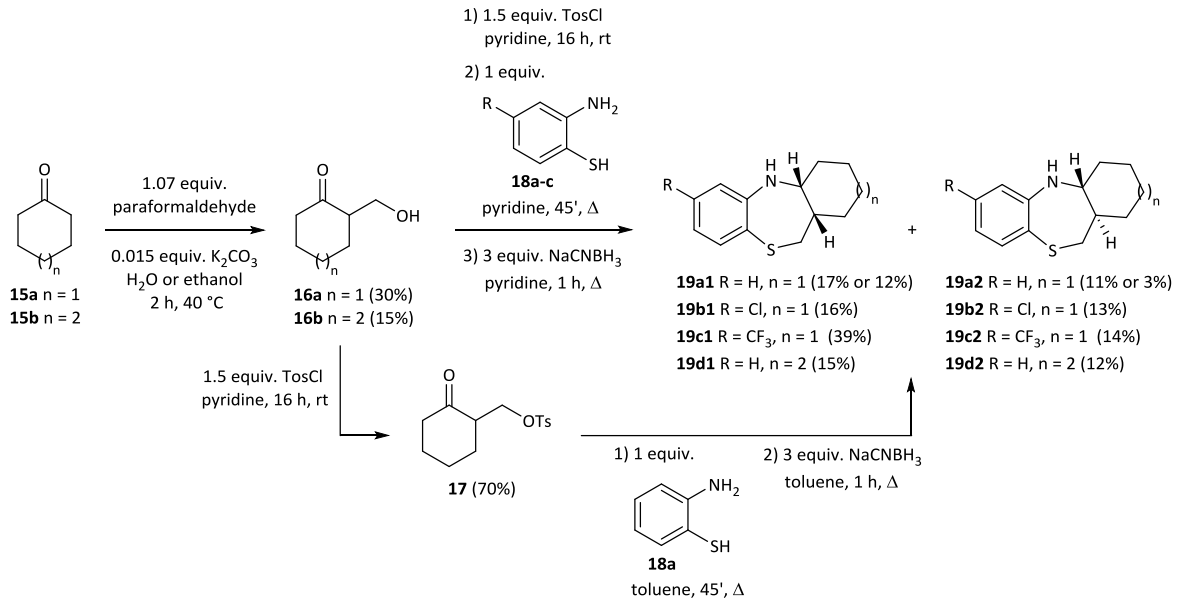


Scheme 1. Synthesis of octahydrodibenzo[*b*,*e*][1,4]thiazepine **14** reported by Hideg *et al.*[8]

Therefore, the original literature procedure was modified and 2-(tosyloxymethyl)cyclohexanone **17** was evaluated as starting product for the synthesis of secondary amines **19a-c**, bearing in mind the better leaving group potential of the tosyloxy group as compared to the piperidine ring (Scheme 2). To synthesize 2-(tosyloxymethyl)cyclohexanone **17**, β-hydroxyketone **16a** first had to be prepared out of cyclohexanone **15a** (n = 1). To that end, a literature procedure was applied (using paraformaldehyde instead of 37% aq. formaldehyde), and 2-(hydroxymethyl)cyclohexanone **16a** was thus obtained in 30% yield after column chromatography.[9] The latter ketone **16a** was subsequently tosylated in pyridine using 1.5 equiv. of *p*-toluenesulfonyl chloride, providing 2-(tosyloxymethyl)cyclohexanone **17** in 70% yield. With this β-tosyloxyketone **17** in hand, an attempt was made to produce imine **13**, and tosylate **17** was heated under reflux in toluene in the presence of 2-aminothiophenol **18a**. Although the formation of tricyclic imine **13** could be observed via LC-MS, only a mixture of products was obtained after work-up. To circumvent this problem, a 'one pot' reductive amination was performed upon treatment of tosyloxyketone **17** with 2-aminothiophenol **18a** in toluene under reflux for 45 minutes, after which the mixture was cooled to room temperature and three equiv. of sodium cyanoborohydride were added. Successively, the reaction medium was heated to boiling temperature, and after one hour a mixture of diastereomers **19a1**,**a2** was formed in a ratio **19a1**/**19a2** : 65/35 (determined via ^{1}H NMR and based on the correct assignment of the relative stereochemistry of diastereomer **19a1** through X- ray crystallography).

10.1002/chem.201604167

Separation and purification through column chromatography provided pure samples of both diastereomers **19a1**,**a2** in 12% and 3% yield, respectively. The same protocol was used for the attempted synthesis of chloro- and trifluoromethyl-substituted benzothiazepines **19b**,**c**, however, no conversion toward products **19b**,**c** could be realized. Moreover, during the synthesis of compounds **19a1**,**a2**, β-tosyloxyketone **17** appeared to be unstable at elevated temperatures; therefore, the 'one pot' approach was expanded (scheme 2) and β-hydroxyketone **16a** was converted to 1,5-benzothiazepine **19a** through the *in situ* preparation of β-tosyloxyketone **17**. Note that it was necessary to quench the excess of *p*-toluenesulfonyl chloride with water to prevent side reactions with 2-aminothiophenol. In this way, and after column chromatographic purification, all six compounds **19a1-c2** (n = 1) were obtained in pure form and acceptable yields (11 - 39%), taking into account the losses during chromatography resulting from similar R_f-values for all diastereomers. Also a cycloheptanone derivative **19d** (n = 2) was assembled from seven-membered β-hydroxyketone **16b**, which was synthesized in 15% yield from cycloheptanone **15b** (n = 2) in the same manner as β-hydroxyketone **16a** (although the reaction solvent was changed from water to ethanol). In total, four couples of diastereomers **19a1-d2** were thus prepared and isolated, with the *cis*-derivatives **19a1-c1** being formed as the major isomers (dr *cis*/*trans* = 60-70/30-40, determined via ^{1}H NMR), except for diastereomers **19d** obtained in a 1/1 ratio. The relative stereochemistry of these unprecedented heterotricyclic architectures **19a1-d2** was secured through X-ray crystallography of 1,5-benzothiazepine **19a1** (for more information, see ESI) and based on the characteristic signals present in the ^{1}H NMR spectra ($CDCl_3$) of compounds **19a1-d2**.



Scheme 2. Synthesis of 1,5-benzothiazepines **19a1**-**d2**.

10.1002/chem.201604167

In a following step, this new tricyclic core fragment was connected to methyl 4-(bromomethyl)benzoate via a nucleophilic substitution reaction at nitrogen (Scheme 3). To efficiently perform this transformation a broad range of reaction conditions was tested, including the use of different bases (K_2CO_3, Cs_2CO_3, triethylamine, NaH, KHMDS, LiHMDS, butyllithium), solvents (THF, CH_3CN, DMF, DMSO), varying amounts of electrophile, and methyl 4-(iodomethyl)benzoate as an substitute electrophile; however, none of the tested conditions could effect the desired *N*-functionalization of 1,5-benzothiazepines **19a1-d2** for more than 50% (determined by ^{1}H NMR, $CDCl_3$). The highest conversion was obtained using five equiv. of potassium carbonate in DMF at 120°C after 16 hours of reaction. To improve this degree of conversion, one equiv. of methyl 4-(bromomethyl)benzoate was treated with 1,5-benzothiazepine **19a1** under neat conditions, forming a melt at a temperature of 120°C. After two hours of reaction at 120°C, 85% conversion was observed via ^{1}H NMR ($CDCl_3$). Unfortunately, due to the release of hydrogen bromide, traces of carboxylic acid were formed as well. To obviate this problem, the released hydrogen bromide was trapped by means of three equiv. of potassium carbonate. Finally, an excellent conversion of 99% could be achieved, without the formation of any carboxylic acid, using neat reaction conditions for three hours at 120°C. Utilising a similar strategy, esters **20a-d** and **23a**,**b** were also obtained from secondary amines **19a1-d1**,**b2** and **d2** in acceptable yields after column chromatography by varying the reaction time and temperature (37-66%, Scheme 3).

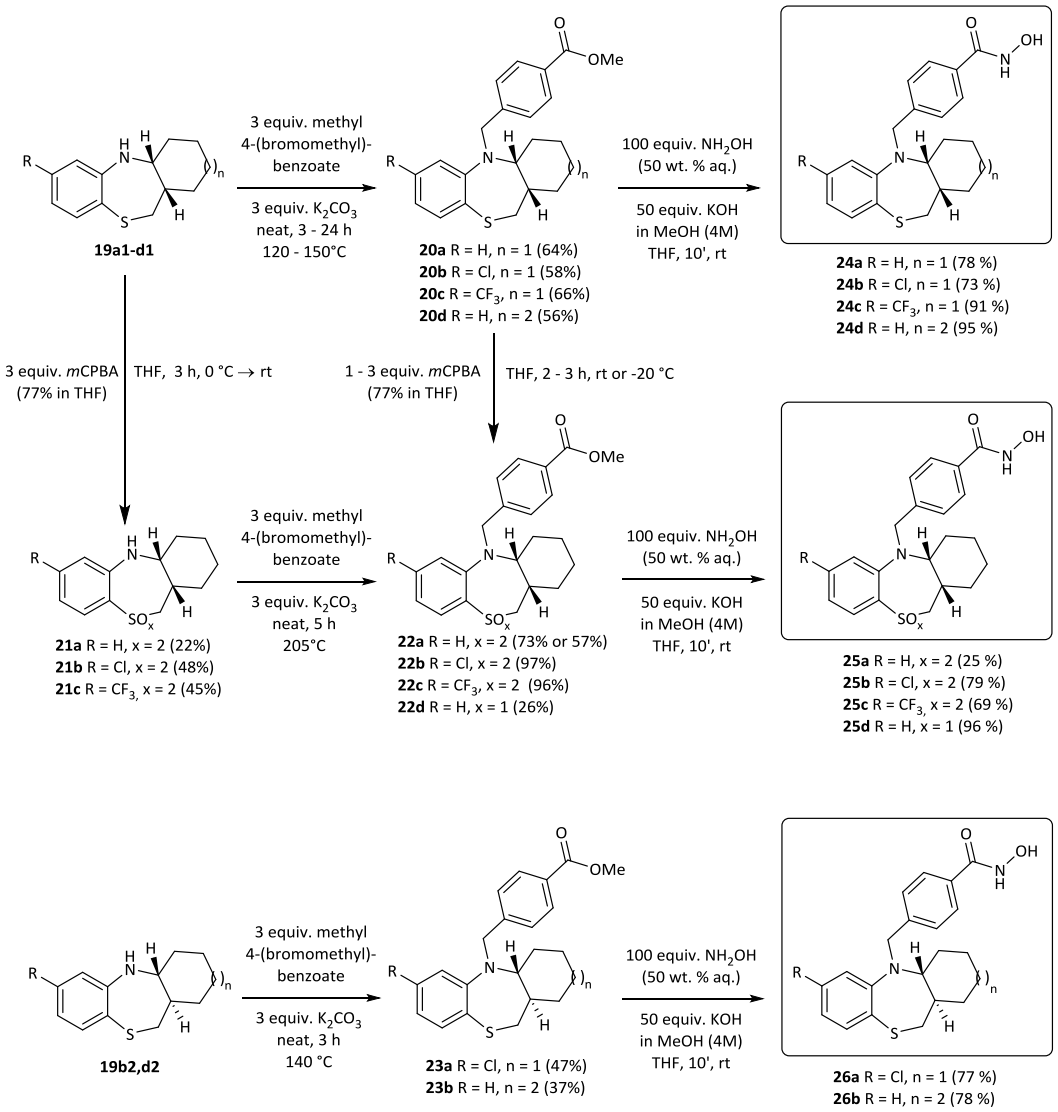
As mentioned in the introduction, oxidized sulfur analogs (e.g., sulfoxides and sulfones) of hydroxamic acids **10** could be of great value, taking into account our previous observations that sulfur-oxidized analogs displayed a higher affinity for HDAC6 than their non-oxidized counterparts due to additional hydrogen bonding.[6a] In a first attempt, compounds **19a1-c1** were converted to the corresponding sulfones using three equiv. of *m*-chloroperbenzoic acid (*m*CPBA, Scheme 3). In this way, three sulfones **21a-c** were obtained in low to moderate yields (22 - 48%) after crystallization from ethanol. However, only one of these sulfones **21a** could successively be transformed into *N*-benzylated molecule **22a** by using a very high reaction temperature (205°C). This could be attributed to the high melting points of products **21a**-**c** (234, 260 and 252°C, respectively), and the fact that compounds **21b**,**c**, as opposed to sulfone **21a**, did not form a liquefied reaction mixture at 205°C. In addition, the introduction of a strong electron-withdrawing sulfonyl group in *ortho* position with respect to the aromatic amino group results in a significant decrease in nucleophilicity of the nitrogen lone pair, hindering a smooth nucleophilic substitution. Higher reaction temperatures could possibly overcome this problem, but we chose to investigate the possibility to obtain sulfones **22b**,**c** via direct oxidation of esters **20b**,**c** instead. In that respect, esters **20a-c** were subjected to the same conditions as cyclic sulfides **19a1-c1**, and as a result sulfones **22a-c** (x = 2) were produced and

10.1002/chem.201604167

isolated in high to excellent yields after crystallisation from ethanol (73 - 97%, Scheme 3). This strategy is clearly superior over the previous approach, since higher yields were obtained for the oxidation step (22 - 48% versus 73 - 97%), and the sulfur derivatisation takes place in a later stage of the synthesis pathway. When only one equiv. of *m*CPBA was added to sulfide **20a** at a temperature of -20°C and a reaction time of two hours was applied, sulfoxide **22d** (x = 1) was obtained in 26% yield after crystallisation from ethanol. The selective synthesis of these *S*-oxidized analogs provides the opportunity to compare the influence of the oxidation state of sulfur (sulfide, sulfoxide or sulfone) on the biological profile of these compounds.

In the section above, the synthesis of the cap group and the formation of the linker unit was described to furnish methyl esters **20**/**22**/**23** starting from the diastereomerically pure cyclohexane- or cycloheptane-annulated 1,5-benzothiazepine scaffolds **19a1-d1** and **19b2,d2**. Hence, only the zinc-complexing hydroxamic acid moiety had to be introduced through functional group conversion of these esters **20**/**22**/**23** to complete the synthesis (Scheme 3). By using an excess of hydroxylamine and potassium hydroxide, methyl carboxylates **20**/**22**/**23** were converted into the target hydroxamic acids **24**-**26** in good yields (69-96%, except 25% for **25a**). In total, ten new 1,5-benzothiazepine-containing benzohydroxamic acids **24**-**26** were successfully prepared, starting from the unprecedented synthesis of tricyclic 1,5-benzothiazepines **19a1-d2**. These structures were then taken forward to biological screenings for their capability to selectively inhibit HDAC6.

10.1002/chem.201604167



Scheme 3. Synthesis of the target hydroxamic acids **24**-**26**.

Biology

First, hydroxamic acids **24-26** were tested for their *in vitro* potential to inhibit HDAC6 at a concentration of 10 µM. This preliminary evaluation revealed that all compounds **24-26** strongly inhibited HDAC6 at this concentration (96 - 100% inhibition), and thus their IC_{50}-values toward HDAC6 were determined (Table 1). From this Table, it can be concluded that these structures **24-26** are highly potent inhibitors with IC_{50}-values in the nanomolar range, and that the *S*-oxidized systems **25** trigger an even more pronounced HDAC6 inhibitory activity as compared to their non-oxidized analogs **24** (6.3 - 68 nM for **25** and 33 - 650 nM for **24**). The non-substituted compounds **24a** and **25a** (R = H) have better IC_{50}-values (36 and 8.3 nM, respectively) then the trifluoromethyl-substituted ones **24c** and **25c** (R = CF_3) (200 and 11 nM, respectively), and the chlorinated scaffolds **24b** and **25b** (R = Cl) show the lowest – yet still sub-micromolar – activity (650 and 68 nM, respectively). Seven-

10.1002/chem.201604167

membered ring-containing hydroxamic acid **24d** displays the strongest inhibition of all sulfides (33 nM), and *trans*-derivatives **26** show intermediate potency with respect to the other compounds.

Table 1. *In vitro* enzyme inhibition data: IC_{50}-values toward HDAC6.

Compound:	**24a**	**24b**	**24c**	**24d**	**25a**	**25b**	**25c**	**25d**	**26a**	**26b**
HDAC6 IC_{50} (nM):	36	650	200	33	8.3	68	11	6.3	160	92

Next, the selectivity of the five most potent HDAC6 inhibitors **24a,d** and **25a,c,d** was evaluated through determining the IC_{50}-values toward the other zinc-dependent HDAC isoforms (HDAC1-11, Table 2). Both sulfides **24a** and **d** display a similar selectivity profile, with high micromolar IC_{50}-values for HDACs 1-5, 7, 9 and 10 ($IC_{50} \geq 25$ µM) and low micromolar activities for HDAC8 and 11 (5.3 – 6.7 µM and 1.2 - 1.5 µM, respectively). Sulfones **25a**,**c** and sulfoxide **25d** show a somewhat lower selectivity profile as compared to compounds **24a**,**d**, but still should be considered as highly selective HDAC6 inhibitors. These molecules display low micromolar IC_{50}-values for HDAC8 and 11 (1.1 - 2.9 and 0.54 - 2.4 µM, respectively), and reveal some affinity for HDAC1 (4.9 – 8.8 µM). For HDAC2-5, 7, 9 and 10, higher IC_{50}-values were obtained ($IC_{50} \geq 8.9$ µM). In general, taking the low nanomolar (toward HDAC6) and (high) micromolar (toward all other HDAC isozymes) IC_{50}-values into account, it is fair to conclude that the newly developed tricyclic benzothiazepine-based hydroxamic acids **24a,d** and **25a,c,d** prepared in this work can be regarded as highly potent and selective HDAC6 inhibitors suitable for further assessment.

Table 2. HDAC1-11 screening of selected compounds **24a,d** and **25a,c,d** (IC_{50}-values in µM)[1,2].

HDAC1-11: **compound**	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***
24a	>30	N.C.	N.C.	>30	27	**0.036**	>30	5.3	25	>30	1.5
24d	>30	N.C.	>30	>30	>30	**0.033**	N.C.	6.7	>30	>30	1.2
25a	8.1	24	24	>30	9.1	**0.008**	22	1.1	13	10	2.4
25c	8.8	16	18	N.C.	22	**0.011**	15	2.0	>30	9.4	0.82
25d	4.9	26	>30	>30	12	**0.006**	>30	2.9	8.9	14	0.54

[1] Reference compound: Trichostatin A (HDAC6 IC_{50} = 0.0093 µM). [2] NC: IC_{50}-value not calculable. Concentration-response curve shows less than 25% effect at the highest validated testing concentration (30 µM). >30: IC_{50}-value above the highest test concentration. Concentration-response curve shows less than 50% effect at the highest validated testing concentration (30 µM).

The obtained *in vitro* HDAC6 inhibition data also confirmed the suggested improved affinity of oxidized analogs **25** in contrast to their non-oxidized counterparts **24** and **26**. This was further

10.1002/chem.201604167

rationalized through *in silico* ligand docking and molecular dynamics simulation. Ligand docking was performed using a homology model of the functional domain of HDAC6 (Gly482-Gly800). Three initial models were built from different templates (pdb entry 2VQW, 2VQQ, 3C10), after which the best parts of each were combined into one hybrid model. The most likely conformation for both compounds was found to have the hydroxamate group positioned near the zinc ion, the linker in the tubular access channel and the cap group contacting the protein surface, which is in agreement with previous docking studies. The positions of the hydroxamate and linker groups in the docked structures of **24a** and **25a** are very similar, while the cap groups are rotated compared to each other and form a few different apolar interactions. However, these do not result in a significantly different binding energy (binding energy 8.4 ± 0.5 kcal/mol and 8.5 ± 0.5 kcal/mol). Because docking alone could not explain the preference of HDAC6 for inhibitors carrying a sulfone moiety in the cap group, a molecular dynamics simulation was run. Indeed, the entrance to the active site is surrounded by a few highly flexible loops that may influence binding, but this dynamic structure was not taken into account during the ligand docking experiment. The simulation of the complex with HDAC6 inhibitor **25a** revealed that a serine at position 564 has a clear tendency to move toward one of the oxygens of the sulfone group, forming a hydrogen bond (Figure 3). This additional interaction might therefore increase the affinity of HDAC6 for sulfone **25a** and other sulfone ligands, accounting for experimentally observed lower IC_{50}-values.

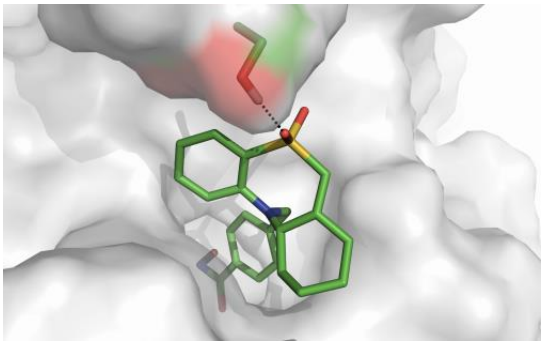


Figure 3. Molecular dynamics simulation of compound **25a** in HDAC6.

To evaluate the effectiveness of these compounds on a cellular level, HDAC6 inhibitors **24a,d** and **25a,c,d** were tested in N2a cells, a neuronal cell line, to determine their potency toward HDAC6 and their selectivity against class I HDACs. This was done via Western Blots to detect the acetylation status of known substrates of HDAC6 and class I HDACs, i.e. α-tubulin and histones, respectively. In vehicle-treated cells, α-tubulin is mainly present in its non-acetylated form (Figure 4A). Tubastatin A **1** is used as a positive control, as it increased the acetylation of α-tubulin at 1 µM (Figure 4A). Additionally, a sub-optimal concentration of 10 nM was chosen for the further characterization of the potency of the compounds. HDAC6 inhibitors **24a,d** and **25a,c,d** induced a significant increase in α-

10.1002/chem.201604167

tubulin acetylation as shown by Western Blot (Figure 4A and B). At the lower concentration of 10 nM, **25a** and **25c** induced a significant increase in the acetylation of α-tubulin (Figure 4A and C). Although for **25a,c** and **d** a low nanomolar potency toward HDAC6 was measured in the enzymatic assay, **25d** failed to induce a similar increase in α-tubulin acetylation at 10 nM, as compared to **25a** and **25c**. This indicates that in this more complex cellular environment additional cues, such as cell permeability, may lower the inhibitory capacity of **25d**.

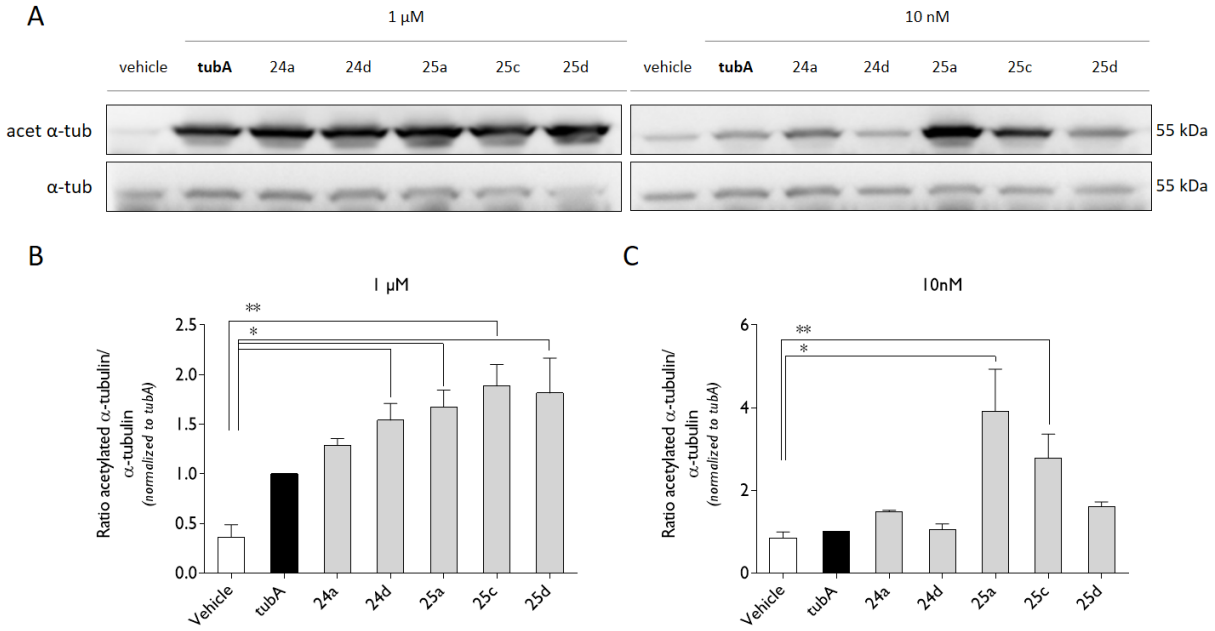


Figure 4. Assessment of the potency of the HDAC6 inhibitors **24a,d** and **25a,c,d** in a neuronal cell line (N2a cells).

A. Using Western Blot, the acetylation of α-tubulin was checked in N2a cells treated with different HDAC6 inhibitors. Tubastatin A (tubA) was used as a positive control. (**B**,**C**). Densitometry was used to quantify the levels of acetylated α-tubulin relative to the amount of total α-tubulin present in the cells, treated with 1 µM or 10 nM of the HDAC6 inhibitors or tubastatin A. All values were normalized to the tubA-samples. N = 4. One-way Analysis-of-Covariance. *p<0.05, **p<0.01.

To evaluate the specificity of these compounds, the acetylation of histone 3 was also determined by Western Blot as an indicator of class I HDAC inhibition. The rationale for this experiment relates to the fact that Tubastatin A, as a known selective HDAC6 inhibitor, does not affect the acetylation of histones (Figure 5A). From all HDAC6 inhibitors tested, none of the compounds were shown to interfere with histone acetylation, as expected and desired (Figure 5A and B). This observation further confirms the selectivity of the produced compounds toward HDAC6 substrates.

10.1002/chem.201604167

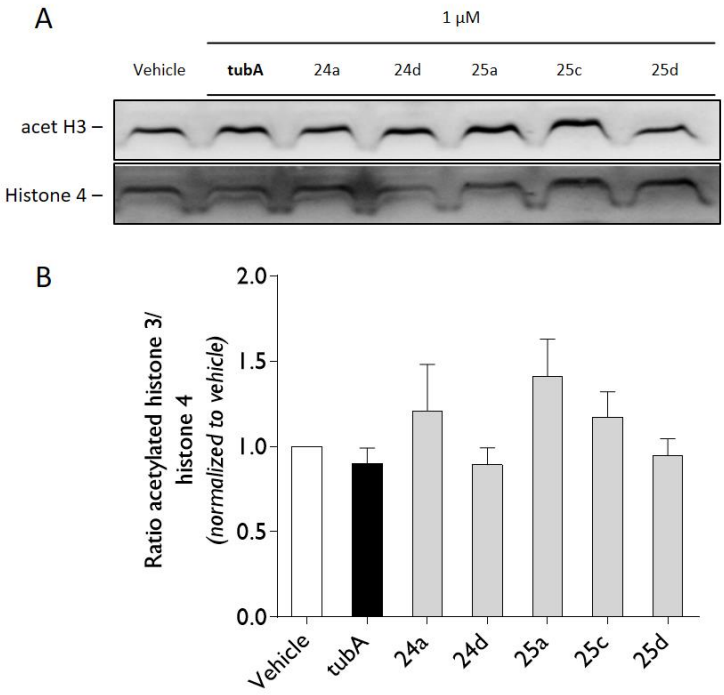


Figure 5. Assessment of the selectivity of the HDAC6 inhibitors **24a,d** and **25a,c,d** in a neuronal cell line.

A. Using Western Blot, the acetylation of histone 3 (H3) was checked in N2a cells treated with different HDAC6 inhibitors (1μM). Tubastatin A (tubA) was used as a control and an antibody directed against histone 4 was used as a loading control. (**B**). Densitometry was used to quantify the levels of acetylated histone 3 relative to the amount histone 4 present in the cells, treated with 1 μM of the HDAC6 inhibitors or tubastatin A. All values were normalized to the vehicle-samples. N = 5. One-way Analysis-of-Covariance.

Finally, it should be noted that there is an increasing concern about the potential genotoxicity of hydroxamic acids and their clinical use beyond oncology. Indeed, already in 1977 hydroxamic acids have been reported to possibly exert genotoxic effects,[10] and mutagenic activities have been documented for three approved hydroxamic acid HDAC inhibitors (Vorinostat, Belinostat and Panobinostat) – which is less of an issue in cancer therapy.[11] In that respect, compounds **24a**,**d** and **25a,c** were tested in the Ames fluctuation test against four strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537), with and without the addition of rat liver S9 fraction. Surprisingly, no statistically significant positive effects could be detected in this *in vitro* reverse mutation assay at the concentrations tested (5, 10, 50 and 100 μM), pointing to the conclusion that our tricyclic benzothiazepine-based hydroxamic acids – in contrast to other hydroxamic acids described in the literature – might have a beneficial profile for further optimization studies toward new HDAC6 inhibitors for oncology and non-oncology applications. It should also be noted that no bacterial cytotoxicity was observed at these concentrations. Of course, other and more elaborate genotoxic tests, e.g. *in vitro* micronucleus and comet assays, should be performed in the future to exclude any potential genotoxicity of these compounds.

10.1002/chem.201604167

Conclusions

This is the first report on a detailed synthesis and isolation of both diastereomers of tricyclic cyclohexane- and cycloheptane-fused benzothiazepines as new heterocyclic scaffolds, for which the correct structure was secured by X-ray crystallography. Starting from these novel benzothiazepine building blocks **19a1-d2**, ten benzohydroxamic acids **24-26** were efficiently synthesized and tested for their ability to inhibit HDAC6. In accordance with previous observations with regard to the effect of *S*-oxidation, the oxidized sulfur analogs **25** proved to be more potent HDAC6 inhibitors than their non-oxidized counterparts **24** and **26**. This superior HDAC6 inhibitory activity of sulfoxide and sulfones **25** was supported by a molecular dynamics simulation, indicating an additional hydrogen bond between the oxygen at sulfur and a serine residue. The most promising HDAC6 inhibitors **24a,d** and **25a,c,d** were further tested to assess their selectivity on both an enzymatic and a cellular level, and these studies revealed that compound **25a** (*cis*-*N*-(4-hydroxycarbamoylbenzyl)-1,2,3,4,4a,5,11,11a-octahydrodibenzo[b,e][1,4]thiazepine-10,10-dioxide) and **25c** (*cis*-*N*-(4-hydroxy-carbamoylbenzyl)-7-trifluoromethyl-1,2,3,4,4a,5,11,11a-octahydrodibenzo[*b*,*e*][1,4]thiazepine-10,10-dioxide) demonstrated very potent activity and selectivity in both assays. Concerning the reported genotoxicity associated with hydroxamic acids, four representatives **24a**,**d** and **25a,c** were tested in an Ames fluctuation assay, pointing to a safe profile in that respect. This new class of tricyclic benzothiazepine hydroxamic acids can thus be considered as a valuable pool of new lead structures for further medicinal chemistry optimization studies in the pursuit of novel therapeutic HDAC6 inhibitors.

Experimental Section

^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were recorded at 400, 100.6 or 376.5 MHz (Bruker Avance III) with $CDCl_3$ or [D_6]DMSO as solvent and tetramethylsilane as the internal standard. Mass spectra were obtained with a mass spectrometer Agilent 1100, 70 eV. IR spectra were measured with a Spectrum One FT-IR spectrophotometer. High resolution electron spray (ES) mass spectra were obtained with an Agilent Technologies 6210 series time-of-flight instrument. Melting points of crystalline compounds were measured with a Kofler Bench, type WME Heizbank of Wagner & Munz. Column chromatography was performed over silica gel (SiO_2), using TLC plates and a UV lamp to identify the correct products. The purity of all tested compounds was assessed by ^{1}H NMR analysis and/or HPLC analysis, confirming a purity of ≥ 95%.

10.1002/chem.201604167

General synthesis of benzothiazepines 19: The synthesis of *cis*- and *trans*-1,2,3,4,4a,5,11,11a-octahydrodibenzo[b,e][1,4]thiazepines **19a1**,**a2** serves as an example for the synthesis of compounds **19**. In a flask (250 mL), α-(hydroxymethyl)cyclohexanone **16a** (6.70 g, 52 mmol) was dissolved in pyridine (67 mL). *p*-Toluenesulfonyl chloride (14.87 g, 78 mmol, 1.5 equiv.) was added to this solution, and the reaction mixture was stirred at room temperature for 16 hours. Then, the reaction mixture was quenched with ice water (20 mL), after which 2-aminothiophenol (5.6 mL, 52 mmol, 1 equiv.) was added. After stirring for 45 minutes under reflux conditions, the reaction mixture was cooled, and sodium cyanoborohydride (9.80 g, 0.156 mol, 3 equiv.) was added portionwise. After a reaction time of one hour under reflux conditions, the reaction mixture was quenched with water (20 mL) and then dissolved in ethyl acetate (100 mL). The organic phase was washed with water (100 mL), a NaCl-solution (100 mL), and then dried over magnesium sulfate, filtered and evaporated. Purification by means of column chromatography yielded **19a1** (1.94 g, 8.84 mmol, 17%) and **19a2** (1.25 g, 5.72 mmol, 11%).

19a1: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.20-1.89 (9H, m), 2.77 (1H, d × d, J = 14.3, 5.4 Hz), 3.11 (1H, d × d, J = 14.3, 9.6 Hz), 3.18 (1H, s(br)), 4.35 (1H, s(br)), 6.55 (1H, d × d, J = 7.7, 1.4 Hz), 6.66 (1H, t × d, J = 7.7, 1.4 Hz), 6.94 (1H, t × d, J = 7.7, 1.4 Hz), 7.16 (1H, d × d, J = 7.7, 1.4 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 20.8, 25.4, 27.8, 32.6, 37.4, 40.2, 52.7, 118.6, 119.4, 122.6, 126.9, 131.7, 148.9. MS (70eV): m/z (%) 220 ($[M + 1]^+$, 100). HRMS (ESI) Anal. Calcd. for $C_{13}H_{18}NS$ 220.1155 $[M + H]^+$, Found 220.1162. Yellow crystals. Purification by column chromatography (EtOAc/PE 3/97, R_f = 0.19), yield 17%. Mp = 82 °C.

19a2: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09-1.19, 1.24-1.40, 1.44-1.54 and 1.64-1.90 (1H, 2H, 1H and 5H, 4 × m), 2.36 (1H, d × d, J = 14.4, 7.2 Hz), 2.92-2.98 (1H, m), 3.14 (1H, d × d, J = 14.4, 3.8 Hz), 3.36 (1H, s(br)), 6.71 (1H, d, J = 7.6 Hz), 6.78 (1H, t, J = 7.6 Hz), 7.04 (1H, t × d, J = 7.6, 1.2 Hz), 7.34 (1H, d × d, J = 7.6, 1.2 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 25.5, 25.6, 32.0, 34.6, 38.3, 47.4, 60.5, 120.2, 120.8, 125.5, 127.7, 132.4, 150.2. MS (70eV): m/z (%) = 220 ($[M + 1]^+$, 100). HRMS (ESI) Anal. Calcd. for $C_{13}H_{18}NS$ 220.1155 $[M + H]^+$, Found 220.1162. White crystals. Purification by column chromatography (EtOAc/PE 3/97, R_f = 0.11), yield 11%. Mp = 97 °C.

General synthesis of sulfones and sulfoxide 21 and 22: The synthesis of *cis*-1,2,3,4,4a,5,11,11a-octahydrodibenzo[*b*,*e*][1,4]thiazepine-10,10-dioxide **21a** serves as an example for the synthesis of compounds **21** and **22**. To a solution of **19a1** (1.10 g, 5 mmol) in tetrahydrofuran (50 mL) was added *m*-chloroperbenzoic acid (≤ 77%, 3.36 g, 15 mmol) in tetrahydrofuran at 0 °C, and the mixture was stirred at room temperature for 2 h. The solvent was then removed in vacuo and the residue was dissolved in ethyl acetate (100 mL). Afterwards, the solution was washed with saturated aqueous sodium sulfite (30 mL), water (30 mL), brine (2 × 30 mL), and dried over anhydrous magnesium

10.1002/chem.201604167

sulfate. Filtration of the drying agent and removal of the solvent in vacuo afforded the crude sulfone **21a**, which was purified by recrystallization from EtOH to provide pure **21a** (0.27 g, 1.05 mmol, 22%). For the synthesis of sulfoxide **22d**, only one equiv. of *m*-chloroperbenzoic acid was added and the reaction temperature was maintained at -20 °C.

21a: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30-1.69 and 1.75-1.90 (5H and 3H, 2 × m), 2.29-2.38 (1H, m), 3.19 (1H, d × d, *J* = 15.0, 10.6 Hz), 3.37 (1H, d × d, *J* = 15.0, 6.2 Hz), 3.99 (1H, s(br)), 4.22 (1H, s(br)), 6.75 (1H, d × d, *J* = 7.9, 0.7 Hz), 6.92 (1H, t × d , *J* = 7.9, 0.7 Hz), 7.28 (1H, t × d, *J* = 7.9, 1.4 Hz), 7.91 (1H, d × d, *J* = 7.9, 1.4 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 20.8, 24.7, 27.2, 31.3, 37.7, 54.2, 58.1, 118.7, 119.2, 126.9, 128.8, 132.8, 146.8. MS (70eV): m/z (%) = 252 ($[M + 1]^+$, 100). HRMS (ESI) Anal. Calcd. for $C_{13}H_{18}NO_2S$ 252.1053 $[M + H]^+$, Found 252.1042. Light brown crystals. Recrystallization from EtOH, yield 22%. Mp = 234 °C.

General synthesis of esters 20 and 23: The synthesis of *cis*-*N*-(4-methoxycarbonylbenzyl)-1,2,3,4,4a,5,11,11a-octahydrodibenzo[*b*,*e*][1,4]thiazepine **20a** serves as an example for the synthesis of compounds **20** and **23**. A flask (25 mL) was charged with **19a1** (219 mg, 1 mmol), potassium carbonate (415 mg, 3 mmol, 3 equiv) and methyl 4-(bromomethyl)benzoate (687 mg, 3 mmol, 3 equiv.). The reaction mixture (neat) was placed under nitrogen atmosphere and stirred for three hours at 120 °C, after which the mixture was cooled to room temperature and dissolved in ethyl acetate (20 mL). Then an extraction was performed with water (20 mL) and a saturated brine solution (15 mL), and the organic fraction was dried over magnesium sulfate, filtered and evaporated. The excess of methyl 4-bromomethylbenzoate was removed via a high vacuum distillation (0.5 mbar, 120 °C), and further purification was done by means of column chromatography, which afforded pure **20a** (235 mg, 0.64 mmol, yield = 64%). When reversed phase column chromatography was used, no initial high vacuum distillation had to be performed. Different reaction temperatures were necessary to synthesize the different compounds to obtain a liquefied melt (120 – 150 °C).

20a: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86-0.97, 1.07-1.32, 1.36-1.45, 1.49-1.53, 1.57-1.63 and 1.67-1.72 (1H, 3H, 1H, 1H, 1H and 1H, 6 × m), 2.29-2.36 (1H, m), 2.42 (1H, d × d, *J* = 14.1, 3.2 Hz), 2.93 (1H, d × d, *J* = 14.1, 12.8 Hz), 3.02 (1H, d × t, *J* = 12.6, 3.9 Hz), 3.93 (3H, s), 4.42 and 4.56 (2 × 1H, 2 × d, *J* = 14.4 Hz), 6.95 (1H, t × d, *J* = 7.7, 1.3 Hz), 7.08 (1H, d × d, *J* = 7.7, 1.3 Hz), 7.22 (1H, t × d, *J* = 7.7, 1.6 Hz), 7.59 (1H, d × d, *J* = 7.7, 1.6 Hz), 7.65 and 8.02 (2 × 2H, 2 × d, *J* = 8.3 Hz). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 20.3, 24.2, 26.6, 30.6, 32.7, 42.5, 52.0, 59.3, 61.3, 122.8, 123.6, 128.2, 128.3, 128.9, 129.6, 132.1, 133.3, 145.7, 152.0, 167.1. MS (70eV): m/z (%) 368 ($[M + 1]^+$, 100). HRMS (ESI) Anal. Calcd. for $C_{22}H_{26}NO_2S$ 368.1679 $[M + H]^+$, Found 368.1680. Very viscous colorless liquid. Purification by column chromatography (EtOAc/PE 3/97, R_f = 0.10), yield 64%.

General synthesis of hydroxamic acids 24-26: The synthesis of *cis*-*N*-(4-hydroxycarbamoylbenzyl)-1,2,3,4,4a,5,11,11a-octahydrodibenzo[*b,e*][1,4]thiazepine **24a** serves as an example for the synthesis of hydroxamic acids **24-26**. **20a** (0.367 g, 1 mmol) was dissolved in THF (10 mL), and to this solution was added hydroxylamine (50% in water, 6.1 mL, 100 mmol, 100 equiv.) followed by potassium hydroxide in methanol (4M, 12.5 mL, 50 mmol). The resulting mixture was stirred for an additional 10 minutes at room temperature before it was poured into a saturated aqueous solution of $NaHCO_3$ (10 mL). This aqueous solution was extracted two times with ethyl acetate, after which the combined organic fractions were washed with water (10 mL) and a saturated brine solution (10 mL), dried ($MgSO_4$), filtered and evaporated. Purification through crystallization from EtOH yielded **24a** (0.287 g, 0.78 mmol, 78%) as a white powder.

24a: ^{1}H NMR (400 MHz, [D_6]DMSO): δ 0.73-0.83, 1.11-1.21, 1.30-1.38, 1.44-1.47 and 1.59-1.62 (1H, 3H, 1H, 1H and 2H, 5 × m), 2.19-2.23 (1H, m), 2.47 (1H, d × d, J = 13.8, 3.4 Hz), 2.83 (1H, t, J = 13.8 Hz), 3.04 (1H, d × t, J = 12.5, 3.6 Hz), 4.43 and 4.53 (2 × 1H, 2 × d, J = 14.8 Hz), 6.90 (1H, t × d, J = 7.6, 1.3 Hz), 7.15 (1H, d × d, J = 7.6, 1.3 Hz), 7.22 (1H, t × d, J = 7.6, 1.3 Hz), 7.47 (1H, d × d, J = 7.6, 1.3 Hz), 7.54 and 7.68 (2 × 2H, 2 × d, J = 8.1 Hz), 9.03 (1H, s(br)), 11.13 (1H, s(br)). ^{13}C NMR (100.6 MHz, [D_6]DMSO): δ 20.2, 24.4, 26.5, 29.9, 32.6, 42.6, 58.8, 61.6, 122.9, 124.0, 127.2, 128.2, 128.8, 131.5, 131.9, 133.3, 143.8, 152.0, 164.4. MS (70eV): m/z (%) 369 ([M + 1]$^+$, 100). HRMS (ESI) Anal. Calcd. for $C_{21}H_{25}N_2O_2S$ 369.1631 [M + H]$^+$, Found 369.1638. White powder. Crystallization from ethanol, yield 78%. Mp = 117 °C.

Acknowledgements

This research was supported by the Ghent University Special Research Fund (BOF) and the Ghent University Industrial Research Fund (IOF, F2014 IOF StarTT 029). K.V.H. thanks the Hercules Foundation (project AUGE/11/029 "3D-SPACE: 3D Structural Platform Aiming for Chemical Excellence") and the Research Foundation - Flanders (FWO) for funding.

References

[1] a) C. Choudhary, C. Kumar, F. Gnad, M. L. Nielsen, M. Rehman, T. C. Walther, J. V. Olsen, M. Mann, *Science* **2009**, *325*, 834-840; b) X. J. Yang, E. Seto, *Mol. Cell* **2008**, *31*, 449-461; c) M. A. Glozak, N. Sengupta, X. Zhang, E. Seto, *Gene* **2005**, *363*, 15-23.

[2] a) J. E. Bolden, M. J. Peart, R. W. Johnstone, *Nat. Rev. Drug Discov.* **2006**, *5*, 769-784; b) M. Haberland, R. L. Montgomery, E. N. Olson, *Nat. Rev. Genet.* **2009**, *10*, 32-42; c) D. M. Chuang, Y. Leng, Z. Marinova, H. J. Kim, C. T. Chiu, *Trends Neurosci.* **2009**, *32*, 591-601; d) H. E.

Covington, 3rd, I. Maze, Q. C. LaPlant, V. F. Vialou, Y. N. Ohnishi, O. Berton, D. M. Fass, W. Renthal, A. J. Rush, 3rd, E. Y. Wu, S. Ghose, V. Krishnan, S. J. Russo, C. Tamminga, S. J. Haggarty, E. J. Nestler, *J. Neurosci.* **2009**, *29*, 11451-11460; e) F. Blanchard, C. Chipoy, *Drug Discovery Today* **2005**, *10*, 197-204; f) M. A. Halili, M. R. Andrews, M. J. Sweet, D. P. Fairlie, *Curr. Top. Med. Chem.* **2009**, *9*, 309-319.

[3] a) S. Subramanian, S. E. Bates, J. J. Wright, I. Espinoza-Delgado, R. L. Piekarz, *Pharmaceuticals* **2010**, *3*, 2751-2767; b) Ø. Bruserud, C. Stapnes, E. Ersvær, B. T. Gjertsen, A. Ryningen, *Curr. Pharm. Biotechno.*, *8*, 388-400.

[4] a) Y. Li, D. Shin, S. H. Kwon, *FEBS J.* **2013**, *280*, 775-793; b) G. I. Aldana-Masangkay, K. M. Sakamoto, *J. Biomed. Biotechnol.* **2011**, *2011*, 1-10; c) J. H. Kalin, J. A. Bergman, *J. Med. Chem.* **2013**, *56*, 6297-6313; d) L. Van Helleputte, V. Benoy, L. Van Den Bosch, *Res. Rep. Biol.* **2014**, *5*, 1-13; e) S. N. Batchu, A. S. Brijmohan, A. Advani, *Clin. Sci.* **2016**, *130*, 987-1003; f) F. Thaler, C. Mercurio, *ChemMedChem* **2014**, *9*, 523-536.

[5] a) K. V. Butler, J. Kalin, C. Brochier, G. Vistoli, B. Langley, A. P. Kozikowski, *J. Am. Chem. Soc.* **2010**, *132*, 10842-10846; b) Z. Yang, T. Wang, F. Wang, T. Niu, Z. Liu, X. Chen, C. Long, M. Tang, D. Cao, X. Wang, W. Xiang, Y. Yi, L. Ma, J. You, L. Chen, *J. Med. Chem.* **2016**, *59*, 1455-1470; c) R. De Vreese, N. Van Steen, T. Verhaeghe, T. Desmet, N. Bougarne, K. De Bosscher, V. Benoy, W. Haeck, L. Van Den Bosch, M. D'hooghe, *Chem. Commun.* **2015**, *51*, 9868-9871; d) S. Shen, V. Benoy, J. A. Bergman, J. H. Kalin, M. Frojuello, G. Vistoli, W. Haeck, L. Van Den Bosch, A. P. Kozikowski, *ACS Chem. Neurosci.* **2016**, *7*, 240-258; e) J. H. Lee, A. Mahendran, Y. Yao, L. Ngo, G. Venta-Perez, M. L. Choy, N. Kim, W. S. Ham, R. Breslow, P. A. Marks, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 15704-15709; f) J. Senger, J. Melesina, M. Marek, C. Romier, I. Oehme, O. Witt, W. Sippl, M. Jung, *J. Med. Chem.* **2016**, *59*, 1545-1555; g) Q. H. Sodji, V. Patil, J. R. Kornacki, M. Mrksich, A. K. Oyelere, *J. Med. Chem.* **2013**, *56*, 9969-9981; h) J. H. Kalin, H. Zhang, S. Gaudrel-Grosay, G. Vistoli, A. P. Kozikowski, *ChemMedChem* **2012**, *7*, 425-439; i) L. Wang, M. Kofler, G. Brosch, J. Melesina, W. Sippl, E. D. Martinez, J. Easmon, *PloS one* **2015**, *10*, 1-22; j) D. Diedrich, A. Hamacher, C. G. Gertzen, L. A. Alves Avelar, G. J. Reiss, T. Kurz, H. Gohlke, M. U. Kassack, F. K. Hansen, *Chem. Commun.* **2016**, *52*, 3219-3222; k) X. Lin, W. Chen, Z. Qiu, L. Guo, W. Zhu, W. Li, Z. Wang, W. Zhang, Z. Zhang, Y. Rong, M. Zhang, L. Yu, S. Zhong, R. Zhao, X. Wu, J. C. Wong, G. Tang, *J. Med. Chem.* **2015**, *58*, 2809-2820; l) I. N. Gaisina, W. Tueckmantel, A. Ugolkov, S. Shen, J. Hoffen, O. Dubrovskyi, A. Mazar, R. A. Schoon, D. Billadeau, A. P. Kozikowski, *ChemMedChem* **2016**, *11*, 81-92; m) J. Jochems, J. Boulden, B. G. Lee, J. A. Blendy, M. Jarpe, R. Mazitschek, J. H. Van Duzer, S. Jones, O. Berton, *Neuropsychopharmacol.* **2014**, *39*, 389-400; n) F. F. Wagner, D. E. Olson, J. P. Gale, T. Kaya, M. Weiwer, N. Aidoud, M. Thomas, E. L. Davoine, B. C. Lemercier, Y. L. Zhang, E. B. Holson, *J. Med. Chem.* **2013**, *56*, 1772-1776; o) J. Yoo, S. J. Kim, D. Son, H. Seo, S. Y. Baek, C. Y. Maeng, C. Lee, I. S. Kim, Y. H. Jung, S. M. Lee, H. J. Park, *Eur. J. Med. Chem.* **2016**, *116*, 126-135.

[6] a) R. De Vreese, T. Verhaeghe, T. Desmet, M. D'hooghe, *Chem. Commun.* **2013**, *49*, 3775-3777; b) R. De Vreese, Y. Depetter, T. Verhaeghe, T. Desmet, V. Benoy, W. Haeck, L. Van Den Bosch, D. H. M, *Org. Biomol. Chem.* **2016**, *14*, 2537-2549.

[7] a) J. B. Bariwal, K. D. Upadhyay, A. T. Manvar, J. C. Trivedi, J. S. Singh, K. S. Jain, A. K. Shah, *Eur. J. Med. Chem.* **2008**, *43*, 2279-2290; b) R. K. Gill, N. Aggarwal, J. Kumari, M. Kumari, P. Kaur, M. Kaur, A. Rani, A. Bansal, A. Shah, J. Bariwal, *Chem. Biol. Interface* **2013**, *3*, 146-163; c) K. A. M. El-Bayouki, *Org. Chem. Int.* **2013**, *2013*, 1-71.

[8] K. Hideg, H. O. Hankovszky, *Acta Chim. Hung.* **1968**, *56*, 405-411.

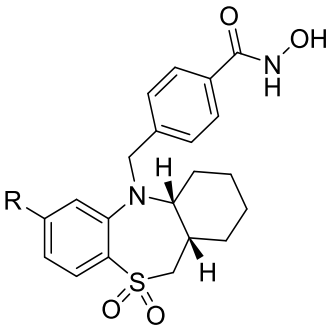
[9] P. Wipf, D. C. Aslan, *J. Org. Chem.* **2001**, *66*, 337-343.

[10] a) C. Y. Wang, *Mutat. Res.* **1977**, *56*, 7-12; b) P. L. Skipper, S. R. Tannenbaum, W. G. Thilly, E. E. Furth, W. W. Bishop, *Cancer Res.* **1980**, *40*, 4704-4708.

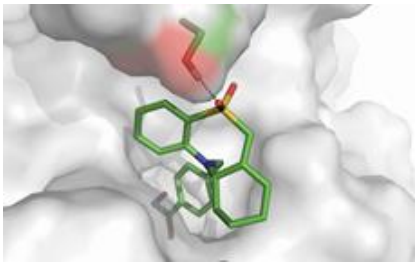
[11] S. Shen, A. P. Kozikowski, *ChemMedChem* **2016**, *11*, 15-21.

10.1002/chem.201604167

Table of Contents



IC_{50} (HDAC6) = 8.3 nM (R = H)
IC_{50} (HDAC6) = 11 nM (R = CF_3)



Selective HDAC6 inhibitors represent an emerging class of pharmaceuticals. In this paper, the synthesis of a series of ten new benzohydroxamic acids, constructed around the benzothiazepine core as a privileged pharmacophoric unit, is described, resulting in the identification o several highly potent and selective HDAC6 inhibitors with activity in the low nanomolar range, also displaying excellen selectivity on an enzymatic and a cellular level.

Keywords

HDAC6, Hydroxamic acids, Benzothiazepines, Enzyme inhibition