RESEARCH ARTICLE

View Article Online
View Journal | View Issue Cite this: *Med. Chem. Commun.*,
2019, 10, 1109Design, synthesis and biological evaluation of
 β -peptoid-capped HDAC inhibitors with anti-
neuroblastoma and anti-glioblastoma activity†Nina Reßing,^{ab} Viktoria Marquardt,^{bcde} Christoph G. W. Gertzen,^{id bf}
Andrea Schöler,^a Alexander Schramm,^g Thomas Kurz,^b Holger Gohlke,^{id bf}
Achim Aigner,^h Marc Remke^{cde} and Finn K. Hansen^{id *ab}

Histone deacetylases (HDACs) have been identified as promising epigenetic drug targets for the treatment of neuroblastoma and glioblastoma. In this work, we have rationally designed a novel class of peptoid-based histone deacetylase inhibitors (HDACi). A mini library of β -peptoid-capped HDACi was synthesized using a four-step protocol. All compounds were screened in biochemical assays for their inhibition of HDAC1 and HDAC6 and docking studies were performed to rationalize the observed selectivity profile. The synthesized compounds were further examined for tumor cell-inhibitory activity against a panel of neuroblastoma and glioblastoma cell lines. In particular, non-selective compounds with potent activity against HDAC1 and HDAC6 showed strong antiproliferative effects. The most promising HDACi, compound **6i**, displayed submicromolar tumor cell-inhibitory potential (IC_{50} : 0.21–0.67 μ M) against all five cancer cell lines investigated and exceeded the activity of the FDA-approved HDACi vorinostat.

Received 10th September 2018,
Accepted 18th October 2018

DOI: 10.1039/c8md00454d

rsc.li/medchemcomm

Introduction

Over the last decade, modification of epigenetic mechanisms has emerged as an important new approach in cancer therapy.¹ Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are enzymes that modulate the chromatin structure by controlling the acetylation levels of histones

and thus play a key role in the regulation of gene transcription.¹ In accordance with their homology to yeast proteins, HDACs are generally divided into classes I to IV, of which classes I, II (further divided into IIa and IIb) and IV contain zinc-dependent hydrolases.² Class III comprises NAD⁺-dependent homologs, the sirtuins.² The class I enzymes, HDACs 1, 2, 3 and 8 were found to be mostly localized in the nucleus

^a Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Leipzig University, Medical Faculty, Brüderstr. 34, 04103 Leipzig, Germany.

E-mail: finn.hansen@medizin.uni-leipzig.de

^b Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany^c Department of Pediatric Oncology, Hematology, and Clinical Immunology, Medical Faculty, Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf, Germany^d Department of Neuropathology, Medical Faculty, Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf, Germany^e Division of Pediatric Neuro-Oncogenomics, German Cancer Research Center (DKFZ) and German Cancer Consortium (DKTK), Partner site Essen/Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany^f John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC) and Institute for Complex Systems – Structural Biochemistry (ICS-6), Forschungszentrum Jülich GmbH, 52425 Jülich, Germany^g Department of Pediatric Oncology and Hematology, University Children's Hospital Essen, University of Duisburg-Essen, Hufelandstr. 55, 45122 Essen, Germany^h Rudolf-Boehm-Institute for Pharmacology and Toxicology, Independent Division for Clinical Pharmacology, Leipzig University, Medical Faculty, Härtelstr. 16-18, 04107 Leipzig, Germany

† Electronic supplementary information (ESI) available: Experimental details (synthetic protocols, bioassays, and computational methods) and characterization of new compounds. See DOI: 10.1039/c8md00454d

Fig. 1 (A) Selected HDAC inhibitors; (B) design of β -peptoids as HDAC inhibitors.

whereas the subgroup IIa HDACs 4, 5, 7 and 9 as well as the class IV isoform HDAC11, which is a fatty acid deacylase,³ appear to be signal proteins shuttling between the nucleus and the cytoplasm.⁴ The class IIb HDAC6, on the other hand, is responsible for protein regulation in the cytoplasm and recognizes a wider range of substrates, including the chaperone protein Hsp90.⁵ Interestingly, HDAC10 (class IIb) was recently identified as a polyamine deacetylase.⁶

Even though the exact mechanisms remain unclear, overexpression of HDACs in different cancer types is generally observed and linked to tumor growth while HDAC inhibition is related to anticancer effects such as reduced cell migration and angiogenesis, inhibition of DNA repair and induction of apoptosis.^{2,5,7} Consequently, HDAC inhibitors (HDACi) have become an important group of novel cancer drugs. Based on the highly-conserved structures of the different isoforms, a pharmacophore model for HDACi comprising a zinc-binding group (ZBG), typically a hydroxamate unit, a linker occupying the narrow channel inside the enzyme, and an aromatic cap group has been established (Fig. 1A).⁸ Since 2006, the non-selective HDACi vorinostat and belinostat as well as the class I preferential cyclic depsipeptide romidepsin (Fig. 1A) have been FDA-approved for the treatment of cutaneous T-cell lymphoma (CTCL) and/or peripheral T-cell lymphoma (PTCL). The non-selective inhibitor panobinostat (Fig. 1A) is FDA- and EMA-approved to treat multiple myeloma. Further clinical trials investigating a large number of HDACi acting against several types of solid and hematological tumors as well as other conditions such as neurodegenerative diseases, immune disorders, inflammatory disorders, and HIV are currently ongoing. However, despite the promising results in treating a range of diseases, unwanted adverse effects, such as fatigue, diarrhea, weight loss, bone marrow depression, and cardiac arrhythmias are major drawbacks that, so far, limit the potential of HDACi towards oncological applications.^{2,5} To reduce these effects, the design of isoform-selective drugs with potentially improved safety profiles is currently a major focus and some examples, *e.g.* the HDAC6 preferential inhibitor ricolinostat (ACY-1215, phase II; Fig. 1A) and its analog citarinostat (ACY-241, phase I), are being evaluated at the clinical stage.^{5,7,9} Also, a first benzamide-based inhibitor with selectivity for class I, tucidinostat (chidamide; Fig. 1A), has already achieved approval for the treatment of relapsed or refractory PTCL in China.¹⁰ It is apparent that among the eleven isoforms, HDACs 1–3 and 6 receive particular interest as relevant cancer targets due to their different modes of action.¹¹ Unlike HDAC class I inhibition, which results in anticancer activity owing to impeded cell cycle progression and cell differentiation, reduced HDAC6 activity affects Hsp90, α -tubulin, and aggresome activity, thus inducing apoptosis through protein accumulation in malignant cells.¹² As there is still no clinical evidence that isoform-selective drugs are superior in terms of their antitumor activity and side effects, it might be more beneficial to combine the anticancer activity asso-

ciated with inhibiting HDAC6 while simultaneously enhancing the cytotoxicity by additionally targeting class I isoforms.¹⁰

Non-selective HDACi have shown therapeutic potential in many malignancies.^{5,8,11b} For instance, there is increasing evidence that HDACi are promising drugs to treat cancers of the peripheral sympathetic nervous system including neuroblastoma, the most common extracranial solid tumor in early childhood.¹³ Following initial results, several phase II studies elucidating the use of the pan-HDACi vorinostat as a part of combination therapies against childhood neuroblastoma are currently active.

Further studies suggest the utility of HDAC inhibitors in the treatment of brain tumors such as glioblastoma, which still has an intolerably poor prognosis.¹⁴ More than 15 clinical trials have been initiated to investigate the activity of HDACi in high grade gliomas (anaplastic astrocytoma and glioblastoma)^{14a} and further early-stage studies using vorinostat in the treatment of medulloblastoma (phase I) are ongoing. Recent results suggest that HDACi can revert temozolomide resistance in glioblastoma cells, which indicates that HDACi can be a new treatment option for patients with temozolomide-resistant glioblastoma.¹⁵ However, no HDACi thus far has exceeded phase II to treat brain cancers.^{14a} Hence, there is an urgent need to develop new HDACi with improved properties.

In this context, aiming to develop HDACi with high activity against class I HDACs and HDAC6 as well as potent anti-proliferative properties, we herein present the rational design, synthesis and bioactivity of a new class of peptoid-based HDACi.

Results and discussion

Design and synthesis

We have recently described two series of α -peptoid-capped HDACi.¹⁶ Derivatives with a benzyl linker showed HDAC6 preferential inhibition,^{16a} whereas the corresponding analogues with an alkyl linker displayed preferential inhibition of HDAC1–3.^{16b} Compared to their peptidic analogs, peptoids feature some notable advantages, particularly proteolytic stability and increased cell permeability.¹⁷ The well-described isomerization of peptoid scaffolds into *cis*- and *trans*-amide bond rotamers further offers increased conformational space depending on the side chain residues.¹⁸ In this work, we aimed at altering the peptoid backbone in order to design highly potent pan-HDACi with significant anticancer activities (Fig. 1B).

While maintaining the well-established benzyl moiety as a linker region and the hydroxamate unit as a zinc-binding group, we decided to extend the *N*-substituted glycine unit of the α -peptoids^{16a} by one methylene group to build β -alanine scaffolds (Fig. 1B), thus enabling more flexibility.¹⁶ Since preferential inhibition of HDAC6 typically requires a bulky and rigid cap group, we expected this modification to yield

compounds with increased class I activity compared to their less flexible HDAC6 preferential α -peptoid counterparts.^{16a}

The synthesis of the resulting β -peptoids (Scheme 1) was realized in four steps starting from several commercially available amines **1** that were treated with acryloyl chloride to give the Michael acceptors **2** in excellent yields. Next, aza-Michael addition of the linker unit yielding secondary amines **3** was followed by acylation using either acyl chlorides or carboxylic acids and PyBOP as a coupling agent. The esters **5** thus obtained were then transformed into the corresponding hydroxamic acids **6** by treatment with hydroxylamine in the presence of sodium hydroxide.

Inhibition of HDAC1 and HDAC6

All synthesized compounds were first tested for their activity against HDAC6 in a biochemical assay using ZMAL (Z-Lys(Ac)-AMC) as a substrate. The FDA-approved pan-HDACi vorinostat and the HDAC6 preferential compound ricolinostat were used as reference compounds. The results are summarized in Table 1. All β -peptoid-based HDACi **6a–k** displayed potent double-digit nanomolar activity with IC₅₀ values ranging from 10 to 31 nM.

Notably, all compounds exceeded the activity of vorinostat (IC₅₀: 34 nM) against HDAC6. The highest activity was observed for compound **6k** (IC₅₀: 10 nM) bearing a 4-chlorobenzyl group in the R¹ position and a *p*-(*N,N*-dimethylamino)phenyl group as the R² residue. To investigate the selectivity profile of compounds **6a–k** for HDAC6, all compounds were subsequently screened against HDAC1 as a representative class I isoform (Table 1). This screening provided interesting data on structure–activity and structure–selectivity relationships. Most compounds (**6b**, **6d–e**, and **6g–k**) possess similar selectivity indices (SI: 1–3) to vorinostat (SI: 3). However, the benzyl-substituted (R¹) compounds **6a** (SI: 11), **6c**

(SI: 6) and **6f** (SI: 6) displayed a slight preference for HDAC6 and particularly reduced activities against HDAC1. Interestingly, **6a** revealed a similar selectivity profile to the HDAC6 preferential inhibitor ricolinostat (SI: 10, Table 1), which is currently in phase II clinical trials for the treatment of multiple myeloma and lymphoid malignancies. However, **6a** is less selective than the HDAC6 selective compounds HPOB (SI: 25) and Tubastatin A (SI: 178, Table 1). Comparison of the R² residues of these compounds indicates that for this scaffold, any substitution except in the *para*-position of the R² phenyl ring leads to a decline in HDAC1 affinity while the effects on HDAC6 inhibition are less significant. In turn, no notable decrease in HDAC1 activity was observed for elongation of the R¹ benzyl group in the *para*-position as in compounds **6j** and **6k**. In the case of the tolyl derivatives **6g–i**, it is apparent that all examples are unselective but potent inhibitors of both isoforms.

NMR spectroscopy

Given that restricted rotation around tertiary amide bonds is a well-observed phenomenon, it was expected that some compounds would form *cis*- and *trans*-rotamers.^{15,16,19} Careful inspection of ¹H and ¹³C spectra revealed that some esters **5** and hydroxamic acids **6** indeed showed two distinct sets of signals whereas all *p*-(*N,N*-dimethylamino)phenyl derivatives (**6b**, **6h**, **6j** and **6k**) as well as compound **6e** carrying a *p*-methoxyphenyl group did not suggest the formation of rotamers at 20 °C. Also, it was observed that the ratios of the two isomers differ in dependence of the chosen solvent. In order to further elucidate the possible coalescence of signals and the preferred amide bond geometry, ¹H NMR studies of the selected representative examples **6a** and **6b** in DMSO-*d*₆ and MeOH-*d*₄ at different temperatures were carried out (Fig. 2). Comparison of the ¹H NMR spectra of compound **6a** indicates that the methylene protons appear in a 1:1 ratio at 20 °C but they coalesce at temperatures between 40–60 °C. Unlike **6a**, compound **6b** starts from one set of signals at 20 °C and was therefore subjected to variable temperature (VT) experiments at lower temperatures, which revealed that the peaks appear to split and display the presence of rotamers in a 1:0.5 ratio between 0 and –15 °C. With regard to the preferred configuration of the amide bond, it is assumed that the upfield shift of the β -methylene protons, which is striking in the spectra of compound **6b**, implies a preference for the *cis*-rotamer as only this conformation would allow the shielding of the two adjacent methylene groups by aromatic substituents.^{16a,20} Taken together, our VT-NMR experiments (Fig. 2) confirm the presence of rotamers in our target compounds. Thus, it is important to consider both rotameric species when investigating the binding modes of β -peptoid-capped HDACi by means of docking studies.

Antiproliferative effects

Compounds **6a–k** were subsequently tested for their antiproliferative properties against four neuroblastoma cell lines

Scheme 1 i) Acryloyl chloride, K₂CO₃, H₂O, acetone, 0 °C, 2 h, 79–97% yields; ii) methyl 4-(aminomethyl)benzoate hydrochloride, Et₃N, MeOH, reflux, 24 h, 62–92% yields; iii) carboxylic acid, PyBOP, DIPEA, CH₂Cl₂, rt, 72 h, 55–89% yields; vi) acyl chlorides, Et₃N, CH₂Cl₂, 0 °C to rt, 16 h 43–85% yields; v) 50% hydroxylamine solution in H₂O, NaOH, CH₂Cl₂, MeOH, 0 °C to rt, 16 h, 15–78% yields.

Table 1 Inhibitory activities of compounds **6a–k** and different references against HDAC isoforms 1 and 6

Entry	R ¹	R ²	HDAC1 IC ₅₀ [nM]	HDAC6 IC ₅₀ [nM]	SI ^a
6a	Bn	Ph	134.1 ± 12.5	12.5 ± 1.6	11
6b	Bn	4-Me ₂ N-Ph	14.7 ± 8.8	14.8 ± 1.9	1
6c	Bn	3,5-Me-Ph	128.4 ± 13.6	20.5 ± 2.6	6
6d	Bn	4-Tolyl	16.1 ± 15.9	16.7 ± 0.4	1
6e	Bn	4-OMe-Ph	41.9 ± 5.9	25.3 ± 1.4	2
6f	Bn	3,5-OMe-Ph	147.5 ± 4.9	26.0 ± 1.8	6
6g	4-Tolyl	Ph	27.5 ± 6.9	10.7 ± 0.2	3
6h	4-Tolyl	4-Me ₂ N-Ph	12.9 ± 14.1	19.5 ± 2.5	1
6i	4-Tolyl	3,5-Me-Ph	43.0 ± 15.5	31.1 ± 1.3	1
6j	4-Me-Bn	4-Me ₂ N-Ph	27.8 ± 5.6	14.4 ± 0.9	2
6k	4-Cl-Bn	4-Me ₂ N-Ph	21.2 ± 6.1	10.0 ± 0.4	2
Vorinostat	—	—	101.0 ± 5.8	34.3 ± 2.1	3
Ricolinostat ^a	—	—	187.9 ± 22.4	17.9 ± 3.0	10
Tubastatin A	—	—	2490 ± 140	14 ± 0.6	178
HPOB	—	—	2100 ± 230	85 ± 9	25

^a Data from ref. 21. **Fig. 2** Variable temperature NMR spectra of **6a** in DMSO-*d*₆ and **6b** in MeOH-*d*₄ showing the coalescence of *cis* and *trans* rotamers at different temperatures.

(CHP-134, IMR-32, SK-N-AS and NB-1) using vorinostat as a positive control. The cell viability was determined after a 72 h incubation by the Celltiter-Glo assay (Promega). The results of this screening are presented in Table 2. In general, the IMR-32 and SK-N-AS cell lines were more sensitive to our HDACi than the CHP-134 and NB-1 cells. Compounds **6h–k** showed submicromolar activity against all four cell lines and exceeded the activity of the reference HDACi vorinostat (IC₅₀: 0.62–2.71 μM). In contrast, compounds **6a**, **6c** and **6f** showed

the lowest activity against all four cell lines. Interestingly, these three compounds showed the lowest activity against HDAC1 and also a preference for HDAC6 over HDAC1 (SI: 6–11, Table 1). Thus, in the case of β-peptoid-capped HDACi potent inhibition of class I HDAC and HDAC6 activity might be crucial for a high activity against neuroblastoma cell lines.

In order to investigate their growth inhibitory activity against a brain cancer entity, **6a–k** were further tested for

Table 2 Antiproliferative activity of **6a–k** and vorinostat against neuroblastoma (CHP-134, IMR-32, SK-N-AS, and NB-1) and glioblastoma (G55T2) cell lines

Entry	CHP-134 IC ₅₀ [μM]	IMR-32 IC ₅₀ [μM]	SK-N-AS IC ₅₀ [μM]	NB-1 IC ₅₀ [μM]	G55T2 IC ₅₀ [μM]
6a	4.68 ± 0.49	2.36 ± 0.17	3.31 ± 0.04	6.66 ± 0.60	5.94 ± 0.05
6b	1.16 ± 0.11	0.84 ± 0.10	1.42 ± 0.03	1.72 ± 0.07	1.15 ± 0.55
6c	2.03 ± 0.08	1.39 ± 0.13	1.86 ± 0.19	3.87 ± 0.29	4.25 ± 0.34
6d	1.10 ± 0.06	0.63 ± 0.07	0.91 ± 0.09	1.61 ± 0.08	0.69 ± 0.84
6e	1.90 ± 0.14	1.09 ± 0.08	1.74 ± 0.04	2.52 ± 0.06	3.49 ± 0.23
6f	12.29 ± 1.62	6.04 ± 0.83	9.47 ± 0.08	13.34 ± 0.31	14.71 ± 1.03
6g	1.16 ± 0.16	0.55 ± 0.06	0.62 ± 0.13	1.48 ± 0.03	0.54 ± 0.08
6h	0.54 ± 0.06	0.30 ± 0.03	0.40 ± 0.07	0.66 ± 0.01	0.33 ± 0.14
6i	0.38 ± 0.04	0.21 ± 0.03	0.22 ± 0.03	0.67 ± 0.01	0.37 ± 0.04
6j	0.42 ± 0.05	0.26 ± 0.03	0.34 ± 0.04	0.70 ± 0.07	0.43 ± 0.01
6k	0.61 ± 0.06	0.31 ± 0.04	0.39 ± 0.03	0.85 ± 0.12	0.46 ± 0.08
Vorinostat	1.74 ± 0.35	0.62 ± 0.10	0.70 ± 0.18	2.71 ± 0.31	0.78 ± 0.12

Table 3 Quantitation of the cell cycle distribution upon treatment with different concentrations of vorinostat, **6i**, and DMSO as a solvent control

Cell cycle phase	Compound 6i 1 μ M [%]	Compound 6i 3 μ M [%]	Vorinostat 3 μ M [%]	DMSO 3 μ M [%]
G0/1	52.3	30.2	31.3	49.9
S	13.2	9.8	8.1	14.7
G2/M	29.1	31.1	28.9	26.8
subG0	3.5	23.3	24.8	4.5

their antiproliferative properties against the glioblastoma cell line G55T2 in a WST-8 assay (Table 2). This screening provided similar results to those observed for the neuroblastoma cell lines. Again, **6h–k** showed the highest activity (IC_{50} : 0.33–0.46 μ M), exceeding the activity of vorinostat (IC_{50} : 0.78 μ M), while compounds **6a**, **6c**, and **6f** (IC_{50} : 4.25–14.71 μ M) displayed the lowest antiproliferative activity.

Cellular effects of compound **6i**

On average, compound **6i** (R^1 : 4-tolyl; R^2 : 3,5-Me-Ph) displayed the highest cell-inhibitory potential (IC_{50} : 0.21–0.67 μ M; Table 2) against the five cancer cell lines investigated. Therefore, compound **6i** was analyzed in more detail by flow cytometry and compared to vorinostat. Upon treatment of G55T2 cells for 72 h, DMSO, as a solvent control (Fig. 3A), did not alter the cell cycle distribution when compared to untreated cells (not shown), with a prominent G0/G1 peak and a less profound G2/M peak. The same was true for treatment with compound **6i** at 1 μ M (Fig. 3B), while increasing the concentration to 3 μ M (Fig. 3C) resulted in alterations of the cell cycle distribution. This pattern was largely comparable to the treatment with vorinostat at the same concentration (Fig. 3D; see Table 3 for quantitation). Notably, the treatment with compound **6i** resulted in the occurrence of apoptotic cells as a sub-G0 peak (Fig. 3C).

Fig. 3 Effects of 3 μ M DMSO (A), 1 μ M **6i** (B), 3 μ M **6i** (C), and 3 μ M vorinostat (D) on the cell cycle distribution.**Table 4** Docking energies of the compound **6i** rotamers in HDAC1 and HDAC6

Compound	HDAC1 ^a	HDAC6 ^a
<i>cis</i> - 6i	−12.56	−12.90
<i>trans</i> - 6i	−11.27	−12.79

^a In kcal mol^{−1}.

Docking studies

Compound **6i**, which had the highest anti proliferative potential and showed a good activity towards both HDAC1 and HDAC6, was docked into the X-ray crystal structures of the respective HDAC isoforms in both the *cis*- and *trans*-rotamer forms. **6i** only displays a slightly better activity towards HDAC6 than HDAC1. These results were replicated in our docking study as overall **6i** showed slightly more favourable docking energies in HDAC6 (Table 4). Here, the *cis*-rotamer, forming π -stacking interactions with each of its non-linker aromatic rings in HDAC1 and HDAC6 (Fig. 4A and B), displays a small difference in docking energies towards both HDAC isoforms. The *trans*-rotamer shows a ~ 1.3 kcal mol^{−1} less favourable docking energy towards HDAC1 than the *cis*-rotamer. The difference in docking energies of the *trans*-rotamer towards HDAC1 (Fig. 4C) versus HDAC6 (Fig. 4D) may be caused by more favourable interactions of its tolyl ring with F680 and F620 in HDAC6. In HDAC1, the tolyl ring merely interacts with H28. To conclude, our

Fig. 4 Predicted binding modes of the *cis*- (green, A and B) and *trans*-rotamer (blue, C and D) of compound **6i** to the X-ray crystal structures of HDAC1 (orange, A and C) and HDAC6 (navy, B and D). Important interacting residues are shown as sticks; Zn^{2+} is shown as a gray sphere. Both rotamers are complexing the Zn^{2+} ions with their ZBGs in HDAC1 and HDAC6. All predicted binding modes, excluding *trans*-**6i** in HDAC1 (C), utilize both of their non-linker aromatic rings to form π -stacking interactions with the respective HDAC isoforms.

docking results qualitatively confirm the weak selectivity profile of **6i** towards HDAC1 and HDAC6 and yield predicted binding modes (Fig. 4). Due to the inherent uncertainty of docking energies and the small difference in experimental IC₅₀ a reliable prediction of the preferred rotamer of **6i** is precluded, however. In recent X-ray crystal structures, *trans*-rotamers of similar compounds were shown to preferentially bind to zebrafish HDAC6.^{16c} However, there the aryl ring of the *trans*-rotamer binds to the L1 loop pocket formed by H500 and P501, while here the *cis*-rotamer of **6i** undergoes this interaction (Fig. 4B).

Conclusions

In this work, we have designed and synthesized a mini library of β -peptoids as a new class of HDACi. Biochemical assays of all final compounds using the two representative HDAC isoforms **1** and **6** confirmed their high affinities to both enzymes. As NMR studies disclosed the existence of *cis*- and *trans*-rotamers, docking studies were undertaken to predict and compare the binding modes of the respective conformers to HDAC1 and HDAC6. Furthermore, several compounds revealed profound activity in cytotoxicity assays against selected neuroblastoma and glioblastoma cell lines. Based on its remarkable antiproliferative effects, **6i** was singled out as a valuable lead structure for future attempts towards designing HDAC inhibitors with further improved anticancer properties.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The Deutsche Forschungsgemeinschaft (DFG) is acknowledged for funds used to purchase the UHR-TOF maXis 4G, Bruker Daltonics, Bremen HRMS instrument used in this research. FKH acknowledges financial support from the Fonds der Chemischen Industrie. We are grateful to Gabriele Oehme and Hannes Borchardt for expert technical assistance in tissue culture and cell cycle analysis. VM is supported by the Düsseldorf School of Oncology (funded by the Comprehensive Cancer Center Düsseldorf/Deutsche Krebshilfe and the Medical Faculty HHU Düsseldorf). H. G. is grateful to the Jülich Supercomputing Centre at the Forschungszentrum Jülich for computing time on the supercomputer JURECA and JUWELS (NIC project ID HKF7) and to the “Zentrum für Informations- und Medientechnologie” (ZIM) at the Heinrich Heine University Düsseldorf for providing computational support.

Notes and references

- M. Biel, V. Wascholowski and A. Giannis, *Angew. Chem., Int. Ed.*, 2005, **44**, 3186–3216.
- (a) M. New, H. Olzscha and N. B. La Thangue, *Mol. Oncol.*, 2012, **6**, 637–656; (b) I. V. Gregoret, Y.-M. Lee and H. V. Goodson, *J. Mol. Biol.*, 2004, **338**, 17–31.
- Z. Kutil, Z. Novakova, M. Meleshin, J. Mikesova, M. Schutkowski and C. Barinka, *ACS Chem. Biol.*, 2018, **13**, 685–693.
- C. M. Grozinger, C. A. Hassig and S. L. Schreiber, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 4868–4873.
- O. Witt, H. E. Deubzer, T. Milde and I. Oehme, *Cancer Lett.*, 2009, **277**, 8–21.
- Y. Hai, S. A. Shinsky, N. J. Porter and D. W. Christianson, *Nat. Commun.*, 2017, **8**, 15368.
- A. C. West and R. W. Johnstone, *J. Clin. Invest.*, 2014, **124**, 30–39.
- (a) P. Bertrand, *Eur. J. Med. Chem.*, 2010, **45**, 2095–2116; (b) J. Roche and P. Bertrand, *Eur. J. Med. Chem.*, 2016, **121**, 451–483; (c) S. Balasubramanian, E. Verner and J. J. Buggy, *Cancer Lett.*, 2009, **280**, 211–221.
- L. Santo, T. Hideshima, A. L. Kung, J. C. Tseng, D. Tamang, M. Yang, M. Jarpe, J. H. van Duzer, R. Mazitschek, W. C. Ogier, D. Cirstea, S. Rodig, H. Eda, T. Scullen, M. Canavese, J. Bradner, K. C. Anderson, S. S. Jones and N. Raje, *Blood*, 2012, **119**, 2579–2589.
- (a) Y. Shi, M. Dong, X. Hong, W. Zhang, J. Feng, J. Zhu, L. Yu, X. Ke, H. Huang, Z. Shen, Y. Fan, W. Li, X. Zhao, J. Qi, H. Huang, D. Zhou, Z. Ning and X. Lu, *Ann. Oncol.*, 2015, **26**, 1766–1771; (b) D.-S. Pan, Q.-J. Yang, X. Fu, S. Shan, J.-Z. Zhu, K. Zhang, Z.-B. Li, Z.-Q. Ning and X.-P. Lu, *MedChemComm*, 2014, **5**, 1789–1796.
- (a) C. M. Marson, C. J. Matthews, S. J. Atkinson, N. Lamadema and N. S. B. Thomas, *J. Med. Chem.*, 2015, **58**, 6803–6818; (b) J. H. Kalin and J. A. Bergman, *J. Med. Chem.*, 2013, **56**, 6297–6313.
- (a) P. Bali, M. Pranpat, J. Bradner, M. Balasis, W. Fiskus, F. Guo, K. Rocha, S. Kumaraswamy, S. Boyapalle, P. Atadja, E. Seto and K. Bhalla, *J. Biol. Chem.*, 2005, **280**, 26729–26734; (b) C. Seidel, M. Schnekenburger, A. Mazumder, M.-H. Teiten, G. Kirsch, M. Dicato and M. Diederich, *Biochem. Pharmacol.*, 2016, **99**, 31–52; (c) Y. Kawaguchi, J. J. Kovacs, A. McLaurin, J. M. Vance, A. Ito and T.-P. Yao, *Cell*, 2003, **115**, 727–738.
- (a) O. Witt, H. E. Deubzer, M. Lodrini, T. Milde and I. Oehme, *Curr. Pharm. Des.*, 2009, **15**, 436–447; (b) I. Rettig, E. Koeneke, F. Trippel, W. C. Mueller, J. Burhenne, A. Kopp-Schneider, J. Fabian, A. Schober, U. Fernekorn, A. von Deimling, H. E. Deubzer, T. Milde, O. Witt and I. Oehme, *Cell Death Dis.*, 2015, **6**, e1657; (c) T. Heimbürg, F. R. Kolbinger, P. Zeyen, E. Ghazy, D. Herp, K. Schmidtkunz, J. Melesina, T. B. Shaik, F. Erdmann, M. Schmidt, C. Romier, D. Robaa, O. Witt, I. Oehme, M. Jung and W. Sippl, *J. Med. Chem.*, 2017, **60**, 10188–10204; (d) D. C. Coffey, M. C. Kutko, R. D. Glick, S. L. Swendeman, L. Butler, R. Rifkind, P. A. Marks, V. M. Richon and M. P. LaQuaglia, *Med. Pediatr. Oncol.*, 2000, **35**, 577–581; (e) S. M. Frumm, Z. P. Fan, K. N. Ross, J. R. Duvall, S. Gupta, L. VerPlank, B.-C. Suh, E. Holson, F. F. Wagner, W. B. Smith, R. M. Paranal, C. F. Bassil, J. Qi, G. Roti, A. L. Kung, J. E. Bradner, N. Tolliday and K. Stegmaier, *Chem. Biol.*, 2013, **20**, 713–725.

- 14 (a) M. J. Williams, W. G. B. Singleton, S. P. Lowis, K. Malik and K. M. Kurian, *Front. Oncol.*, 2017, 7, 45; (b) P. Lee, B. Murphy, R. Miller, V. Menon, N. L. Manik, P. Giglio, S. M. Lindhorst, A. K. Varma, W. A. Vandergift III, S. J. Patel and A. Das, *Anticancer Res.*, 2015, 35, 615–626; (c) D. H. Lee, H.-W. Ryu, H.-R. Won and S. H. Kwon, *Oncotarget*, 2017, 8, 18577–18589.
- 15 Z. Li, Q. Li, L. Chen, B. Chen, B. Wang, X. Zhang and W. Li, *Neurochem. Res.*, 2016, 41, 3192–3205.
- 16 (a) D. Diedrich, A. Hamacher, C. G. W. Gertzen, L. A. Alves Avelar, G. J. Reiss, T. Kurz, H. Gohlke, M. U. Kassack and F. K. Hansen, *Chem. Commun.*, 2016, 52, 3219–3222; (b) V. Krieger, A. Hamacher, C. G. W. Gertzen, J. Senger, M. R. H. Zwinderman, M. Marek, C. Romier, F. J. Dekker, T. Kurz, M. Jung, H. Gohlke, M. U. Kassack and F. K. Hansen, *J. Med. Chem.*, 2017, 60, 5493–5506; (c) N. J. Porter, J. D. Osko, D. Diedrich, T. Kurz, J. M. Hooker, F. K. Hansen and D. W. Christianson, *J. Med. Chem.*, 2018, 61, 8054–8060.
- 17 (a) S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr and W. H. Moos, *Bioorg. Med. Chem. Lett.*, 1994, 4, 2657–2662; (b) Y.-U. Kwon and T. Kodadek, *J. Am. Chem. Soc.*, 2007, 129, 1508–1509; (c) B. Yoo and K. Kirshenbaum, *Curr. Opin. Chem. Biol.*, 2008, 12, 714–721.
- 18 J. S. Laursen, J. Engel-Andreasen, P. Fristrup, P. Harris and C. A. Olsen, *J. Am. Chem. Soc.*, 2013, 135, 2835–2844.
- 19 V. Krieger, E. Ciglia, R. Thoma, V. Vasylyeva, B. Frieg, N. de Sousa Amadeu, T. Kurz, C. Janiak, H. Gohlke and F. K. Hansen, *Chem. – Eur. J.*, 2017, 23, 3699–3707.
- 20 D. Marcovici-Mizrahi, H. E. Gottlieb, V. Marks and A. Nudelman, *J. Org. Chem.*, 1996, 61, 8402–8406.
- 21 M. K. W. Mackwitz, A. Hamacher, J. D. Osko, J. Held, A. Schöler, D. W. Christianson, M. U. Kassack and F. K. Hansen, *Org. Lett.*, 2018, 20, 3255–3258.