

Synthesis of thiazolyl-based hydroxamic acids as histone deacetylase inhibitors

Yodita Asfaha, Alexander Jan Skerhut, Leandro A. Alves-Avelar, Nadine Horstick-Muche,
Beate Lungerich, Stefan Klinken, Matthias U. Kassack,* and Thomas Kurz*

*Institut für Pharmazeutische und Medizinische Chemie, Heinrich-Heine-Universität Düsseldorf,
Universitätsstr.1, 40225 Düsseldorf, Germany*

Email: thomas.kurz@hhu.de, matthias.kassack@hhu.de

Dedicated to Professor Jan Bergman with respect and best wishes for the occasion of his 80th birthday

Received mm-dd-yyyy

Accepted mm-dd-yyyy

Published on line mm-dd-yyyy

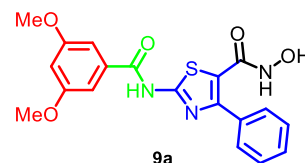
Dates to be inserted by editorial office

Abstract

Herein we report the synthesis of 4-phenyl substituted thiazolyl-based hydroxamates as histone deacetylase inhibitors. The synthesis of the target compounds is comprised of a Hantzsch-thiazole reaction, a HATU-mediated acylation and a hydroxamic acid synthesis. Preliminary docking results indicated an isozyme selectivity by addressing the lower pocket of HDAC4 with 4-phenyl thiazoles. Surprisingly, this new series of thiazolyl based hydroxamates revealed a moderate HDAC6 inhibitory activity in the low micromolar range. Two of the hydroxamic acids did not show an HDAC4 inhibition up to 100 μ M, whilst a third with a 4-pyridinyl CAP moiety displayed a moderate HDAC4 inhibitory activity in the micromolar range.



Pharmacophore model of HDAC6



IC_{50} (HDAC6) = 3.85 μ M
SI (HDAC2/6) > 26
SI (HDAC4/6) > 26
SI (HDAC8/6) = 11

Keywords: Thiazole synthesis, histone deacetylase inhibitors, ester cleavage, hydroxamic acids

Introduction

The term *epigenetic* refers to inheritable changes in gene expression without altering the underlying DNA sequence.¹ Epigenetic mechanisms are subdivided into four different classes: (1) posttranslational modifications of histones, (2) chemical modifications of DNA (3) chromatin-remodeling complexes, and (4) regulatory non-coding RNAs. An essential post-translational modification is protein acetylation/deacetylation, which is regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs). The human zinc dependent HDACs are classified into three classes: class I (HDACs 1-3, 8), class II (IIa: HDACs 4, 5, 7, 9; IIb: HDACs 6, 10) and class IV (HDAC 11).² Various studies demonstrated that HDACs are overexpressed in a broad spectrum of cancer cells.³⁻⁶ Their inhibition provides a promising strategy for the development of novel epigenetic anticancer drugs. Until now, the natural product romidepsin and four trichostatin A derived HDAC inhibitors (vorinostat, belinostat, panobinostat, tucidinostat) are approved for the treatment of lymphoma and myeloma (Figure 1).⁷⁻¹² The common pharmacophore model for HDACi consists of the following four elements: a zinc binding group (ZBG), a linker and a connecting unit (CU), that interact with the substrate binding tunnel and a cap, also known as surface recognition domain.^{13,14} According to their ZBG, HDACi can be subdivided into 4 main chemical classes: hydroxamic acids, thiols, carboxylic acids and *o*-amino anilides.¹⁵

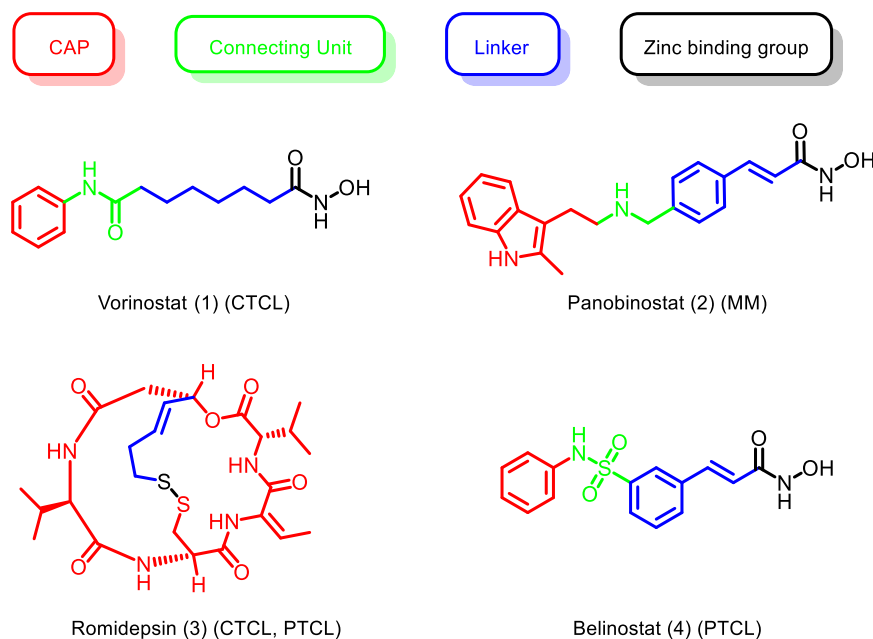


Figure 1: General pharmacophore model of HDACi and chemical structures of FDA approved HDAC inhibitors **1-4** and their indications. CTCL: cutaneous T-cell lymphoma, MM: multiple myeloma, PTCL: peripheral T-cell lymphoma⁷⁻¹²

Thiazoles are privileged structures in medicinal chemistry. Its core scaffold is found in many natural products such as alkaloids, secondary metabolites and cyclopeptides.¹⁶ The thiazole moiety is also present in several active pharmaceutical ingredients with anticancer, anti-inflammatory, antibacterial, CNS-regulatory, anti-Parkinson, and antidiuretic properties (**Figure 2**).¹⁷⁻²⁰

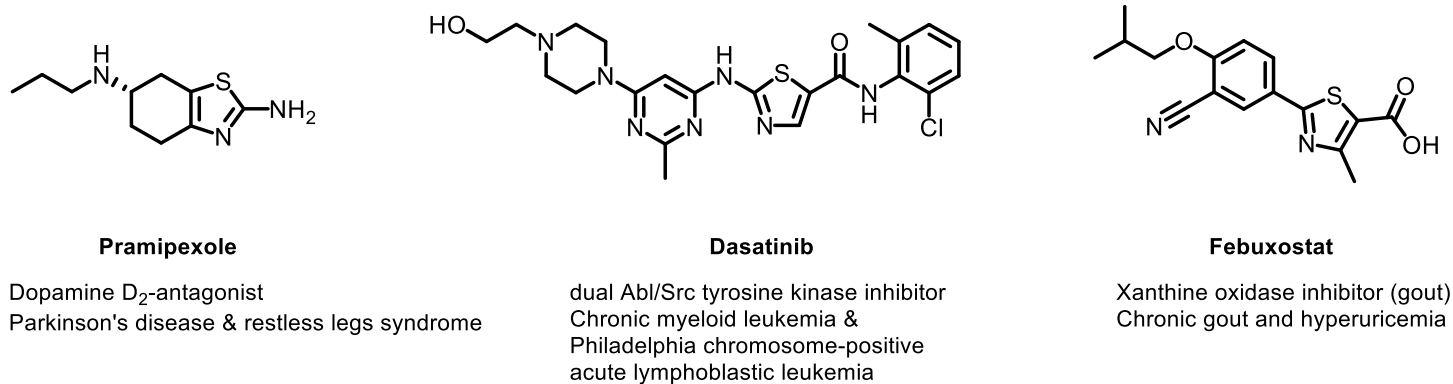


Figure 2. Selected thiazolyl-based drugs ²¹⁻²³

Initial studies for the development of thiazolyl-based HDACi were performed by Anandan *et al.* (Figure 3).²⁴ In the reported HDACi, the thiazole moiety acts as a linker that bridges the ZBG with the cap group. These HDACi displayed *in vitro* HDAC inhibitory activity in HeLa cell nuclear extracts and antiproliferative effects towards the breast cancer cell line MCF7 in the micromolar range.

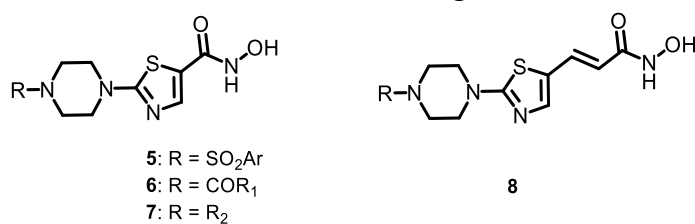
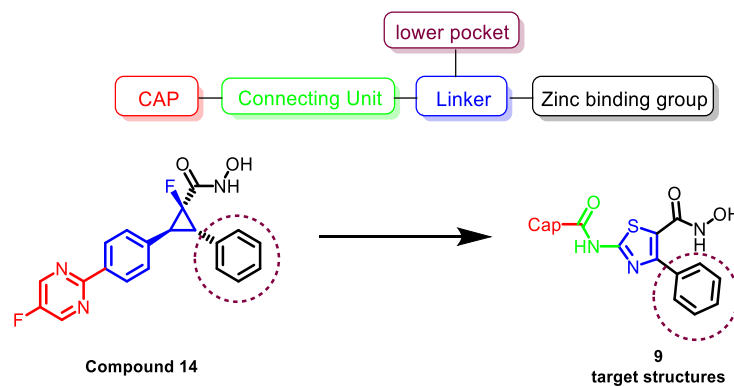


Figure 3. Thiazolyl-based hydroxamic acids HDACi **5-8** ²⁴

Results and Discussion

Design of 4-phenyl substituted thiazolyl hydroxamates as HDAC inhibitors

Recent studies have shown that HDAC class IIa enzymes might be associated with neurodegenerative diseases and cancer.²⁵⁻²⁸ So far, only trifluoromethyloxadiazoles (TFMO), cyclopropane hydroxamates as well as benzhydryl based class IIa HDAC inhibitors have been reported. In published crystal structures of class IIa HDACs, the so-called lower pocket was identified as a distinctive structural feature.^{29,30} Bürli *et al.* and Luckhurst *et al.* showed that tri- and tetrasubstituted cyclopropanes, providing a scaffold with a suitable 3D-geometry that occupy the lower pocket with their phenyl moiety (**Scheme 1**, circled in red).^{25,30} Here, we report the synthesis of trisubstituted thiazolyl-based hydroxamates as potential class IIa HDACi.



Scheme 1. Pharmacophore model of class IIa selective HDACi (above) and design of target structures as class IIa selective HDAC³⁰

In order to evaluate whether the 4-phenyl-substituent of compound **9a** might occupy the lower pocket of class IIa HDACs, we performed docking studies. Molecular docking studies of the thiazole derivative **9a**, in various HDAC-isozymes were performed using a previously validated docking protocol employing Autodock 4.2 as docking engine.³¹ **9a**, as thiazolyl-based HDACi representative, was docked in HDAC2 (PDB 5IWG)³², HDAC8 (PDB 5CFW)³³, HDAC4 (PDB 5A2S)³⁰ and HDAC6 (PDB 5EDU)³⁴.

The analysis of the docking conformation of compound **9a** in HDAC4 suggests that, as envisioned, the hydroxamate moiety of **9a** can coordinate with the zinc cation in a bidentate manner (Figure 4). In addition, the phenyl substituent of **9a** occupied the lower pocket in a similar binding mode as the phenyl moiety of compound **14** (Figure 4). The thiazolyl-based hydroxamate **9a** did not generate valid docking poses in the other HDACs as no docking pose was observed that showed a complexation of the zinc ion by the hydroxamic acid moiety.

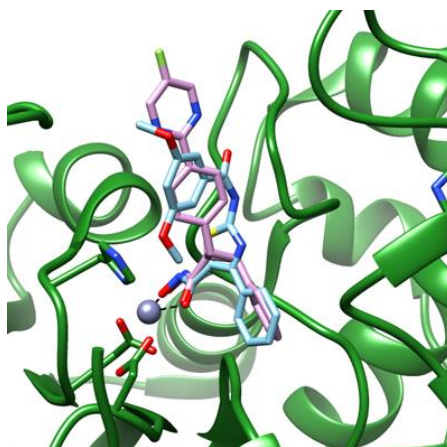
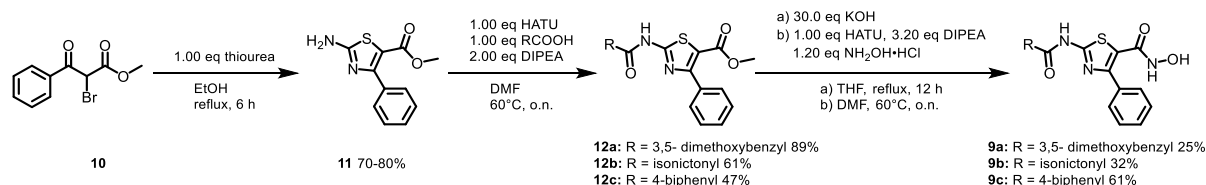


Figure 4. Proposed binding mode of compound **9a** (light blue) in HDAC4 (PDB 5A2S)³⁰. The docking pose of compound **9a** is superimposed with the HDAC4 cocrystallised compound **14** (pink).

Chemistry

The starting material of the Hantzsch thiazole synthesis was the α -bromoester **10** that was prepared according to Borowiecki *et al.*³⁵ After the treatment of **10** with thiourea, the thiazole **11** was formed. In the following step, **11** and the respective carboxylic acids were coupled using HATU under basic conditions yielding the corresponding amides **12a-c**. The direct hydroxylaminolysis of the esters **12a-c** to the corresponding hydroxamic acids **9a-c** applying various literature procedures did not provide the expected products.^{36–40} Therefore, the hydroxamic acids **9a-c** were synthesized by ester hydrolysis and subsequent conversion of the respective carboxylic acids with HATU, DIPEA and hydroxylamine hydrochloride (**Scheme 2**). The ester

hydrolysis was achieved by the addition of an excess of potassium hydroxide. However, the same protocol was unsuccessful when applied for the cleavage of the corresponding ethyl esters. This finding underscores that the hydrolysis depends on the nature of the alkyl substituent of the ester moiety.



Scheme 2. Synthesis of the target structures **9a-c**

Biological Evaluation

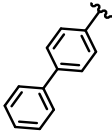
The synthesised compounds **9a-c** were assessed for their antiproliferative activity and for their HDAC inhibitory activity in the human monocytic cell line THP-1 using class-distinguishing substrates (Boc-Lys(Ac)-AMC: class I and IIb HDACs; Boc-Lys(TFAc)-AMC: class IIa, HDAC8). The results are depicted in Table 1 with vorinostat as reference compound. Among the tested compounds, **9b** exhibited the highest antiproliferative activity with 61.5% of growth inhibition at 100 μ M concentration. The introduction of a 3,5-dimethoxyphenyl (**9a**) or biphenyl (**9c**) CAP moiety led to a decreased cytotoxicity with antiproliferative effects below 50% at 100 μ M. In a whole-cell HDAC inhibition assay using the Boc-Lys(Ac)-AMC-HDAC substrate, all screened hydroxamic acids **9a-c** displayed inhibitory activities below 40% at 100 μ M. However, the cellular HDAC inhibition assay using the Boc-Lys(TFAc)-AMC-HDAC substrate revealed a slightly increased inhibition with over 45% at 100 μ M for **9a** and **9b**.

Overall there is a good correlation between the antiproliferative activity and the cellular HDAC inhibition. All tested compounds **9a-c** showed a moderate cytotoxicity and cellular HDAC inhibition.

Table 1. Cell viability (MTT assay) and whole-cell HDAC inhibition assay in the human monocytic cell line THP-1.

9a-c

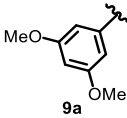
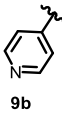
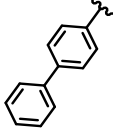
R in Cpd 9	cell viability (MTT)		HDAC inhibition			
	% inhibition of 100 μ M	IC ₅₀ [μ M] pIC ₅₀ \pm SEM)	Boc-Lys(Ac)-AMC		Boc-Lys(TFAc)-AMC	
			% inhibition of 100 μ M	IC ₅₀ [μ M] pIC ₅₀ \pm SEM)	% inhibition of 100 μ M	IC ₅₀ [μ M] pIC ₅₀ \pm SEM)
	47.1%	>100	31.2%	>100	45.2%	>100
	61.5%	82.1 4.09 \pm 0.11)	36.9%	>100	48.1%	>100

 9c	44.5%	>100	38.4%	>100	37.1%	>100
Vorinostat	96.6%	0.18 (6.74 ± 0.06)	98%	0,75 6.13 ± 0.054)	62%	41.43 4.38 ± 0.04)

Presented data are calculated from at least two experiments each performed in duplicates. IC₅₀ values were calculated for compounds with an inhibition of more than 50 %. Standard deviation of percent inhibition values is less than 14 %. Vehicle control was defined as 0 % inhibition.

For further evaluation, the synthesized compounds **9a-c** were screened against recombinant human HDAC2, HDAC4, HDAC6 and HDAC8 (**Table 2**). Vorinostat was included as a pan-HDACi reference compound. Interestingly, the new thiazolyl-based hydroxamates **9a-c** were identified as moderate HDAC6 inhibitors in the low micromolar range. In contrast to our preliminary *in-silico* data, the hydroxamic acids **9a** and **9c** displayed no HDAC4 inhibition in the tested concentration range ($\leq 100 \mu\text{M}$). However, the hydroxamic acid **9b** exhibiting a 4-pyridinyl CAP moiety demonstrated a moderate micromolar activity against HDAC4 (IC₅₀(HDAC4) = 48.8 μM).

Table 2. Inhibitory activity of **9a-c** and Vorinostat on recombinant human HDAC2, HDAC4, HDAC6 and HDAC8

Cpd.	IC ₅₀ [μM] (pIC ₅₀ ± SEM)			
	HDAC2	HDAC4	HDAC6	HDAC8
 9a	>100	>100	3.85 (5.41 ± 0.04)	43.1 (4.37 ± 0.05)
 9b	>100	48.8 4.31 ± 0.08)	3.6 (5.44 ± 0.04)	21.4 (4.67 ± 0.04)
 9c	>100	>100	9.94 (5.0 ± 0.08)	>100
Vorinostat	0.096 7.02 ± 0.04)	96.5 4.02 ± 0.05)	0.04 7.63 ± 0.05)	4.44 5.35 ± 0.05)

Data shown are from at least two experiments each performed at least as duplicates and the IC₅₀ value of pooled data is reported when IC₅₀ < 100 μM .

Conclusions

Histone deacetylases are zinc-dependent metalloproteases that catalyse the removal of acetyl functional groups from lysine residues of both histone and nonhistone proteins. HDACs are involved in a multitude of biological processes e.g. in cell cycle progression, cell survival, apoptosis and differentiation. They are clinically validated targets for the treatment of cancer. Selective HDAC inhibitors may serve as important tools for elucidating the role of specific HDACs in certain diseases. Contrary to our preliminary qualitative *in-silico* screening, all synthesized compounds **9a-c** exhibited a moderate HDAC6 inhibitory activity in the low micromolar range. Only the hydroxamic acid **9b** exhibiting a 4-pyridinyl CAP moiety displayed a moderate micromolar activity against HDAC4 ($IC_{50}(\text{HDAC4}) = 48.8 \mu\text{M}$).

Experimental Section

General methods

All chemicals and solvents were purchased from commercial suppliers (Sigma Aldrich, Alfa Aesar, Fluorochem, TCI, abcr and Acros Organics) and used without further purification. All anhydrous reactions were carried out in flame-dried Schlenk-flasks and under argon atmosphere. Dry solvents were used directly from Seal® bottles from Acros Organics. Analytic Thin Layer Chromatography (TLC) was carried out with Macherey Nagel precoated silica gel plates (ALUGRAM® Xtra SIL G/UV₂₅₄). Detection was achieved with ultraviolet irradiation (254 nm) and/or staining with potassium permanganate solution (9 g KMnO₄, 60 g K₂CO₃, 15 mL of a 5% aqueous NaOH-solution, and 900 mL demineralised water). Flash column chromatography was performed with CombiFlashRf200 (TeleDynelco) with the solvent mixtures specified in the corresponding procedure.

Physical data

Proton (¹H) and carbon (¹³C) NMR spectra were recorded on Bruker Avance III – 300, Bruker Avance DRX – 500 or Bruker Avance III – 600. Spectra were referenced to the residual non-deuterated solvent signal (¹H-NMR: DMSO-*d*₆ (2.50 ppm), ¹³C-NMR: DMSO-*d*₆ (39.52 ppm); ¹H-NMR: CDCl₃ (7.26 ppm), ¹³C-NMR: CDCl₃ (77.16 ppm)). Chemical shifts are quoted in parts per million (ppm). Signal patterns are indicated as: singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m). Coupling constants, *J*, are measured in Hz. Proton (¹H) and carbon (¹³C) NMR spectra were recorded by the NMR-Divisions of the Department of Chemistry (Heinrich-Heine-University Düsseldorf). Electrospray Ionisation (ESI) mass spectra were carried out by the Mass spectrometry-Division of the Heinrich-Heine-University Düsseldorf, using Bruker Daltonics UHR-QTOF maXis 4G (Bruker Daltonics). Melting points (mp.) were determined using a Büchi M-565 melting point apparatus and are uncorrected.

Analytical HPLC analysis were carried out on a Knauer HPLC system comprising an Azura P 6.1L pump, an Optimas 800 autosampler, a Fast Scanning Spectro-Photometer K-2600 and Knauer Reversed Phase column (SN: FK36). UV absorption was detected at 254 nm. The solvent gradient table is shown below. The purity of all final compounds was 95% or higher.

Table 3. The solvent gradient table for analytic HPLC analysis

Time / min	Water + 0.1% TFA	ACN + 0.1% TFA
Initial	90	10
0.50	90	10
20.0	0	100

30.0	0	100
31.0	90	10
40.0	90	10

Synthesis of methyl 2-bromo-3-oxo-3-phenylpropanoate (**10**)

A solution of methyl 3-oxo-3-propanoate³⁵ (2.67 g, 15.0 mmol, 1.00 eq) in methanol (40.0 mL) was cooled to 0 °C and sodium methoxide (30 wt. % in methanol, 3.43 mL, 18.0 mmol, 1.20 eq) was added dropwise. After stirring for further 5 min, bromine (0.92 mL, 18.0 mmol, 1.20 eq) in methanol (20.0 mL) was added dropwise to the reaction. The resulting mixture was stirred overnight at rt. The solvent was removed *in vacuo*, and the crude solid was resuspended in ethyl acetate. The organic layer was washed with water (3 × 25 mL), dried over Na₂SO₄ and filtered. After removing the solvent, the crude product was purified by flash column chromatography (prepacked silica cartridge, *n*-hexane/ethyl acetate) to provide 2-bromo-3-oxo-3-phenylpropanoate (**10**) (2.85 g, 11.1 mmol, 74%) as a yellow oil. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 3.75 (s, 3H), 6.70 (s, 1H), 7.60 (t, *J* 7.6 Hz, 2H), 7.69 – 7.78 (m, 1H), 8.04 (dd, *J* 8.4, 1.3 Hz, 2H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆) δ = 46.86, 53.67, 129.10, 133.08, 134.58, 165.89, 189.37 ppm. HPLC analysis: R_t = 12.150 min, 98.2%. HRMS (ESI+) = calcd. for C₁₀H₁₀BrO₃[M+H]⁺ = 256.9808, found: 256.9806.

Synthesis of methyl 2-amino-4-phenylthiazole-5-carboxylate (**11**)

To a solution of methyl 2-bromo-3-oxo-3-phenylpropanoate (**10**) (2.53 g, 6.70 mmol, 1.00 eq) in methanol (40.0 mL), thiourea (0.52 g, 6.70 mmol, 1.00 eq) was added and the resulting solution was refluxed for 6 h. The solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc, washed with saturated NaHCO₃ solution (3x 50 mL) and dried over Na₂SO₄. After filtration, the solvent was removed *in vacuo* to yield the crude product which was purified by flash column chromatography (prepacked silica cartridge, *n*-hexane/ethyl acetate) to provide methyl 2-amino-4-phenylthiazole-5-carboxylate (**11**) (0.70 g, 2.90 mmol, 43%) as a yellow solid. M.p.: 183-186 °C. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 3.62 (s, 3H), 7.29 – 7.46 (m, 3H), 7.60 – 7.68 (m, 2H), 7.86 (s, 2H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆) δ = 51.40, 107.65, 127.33, 128.69, 129.58, 134.51, 159.02, 161.53, 169.89 ppm. HPLC analysis: R_t = 7.533 min, 97.2%. HRMS (ESI+) = calcd. for C₁₁H₁₁N₂O₂S [M+H]⁺ = 235.0536, found: 235.0535.

General procedure 1. HATU-coupling

For the synthesis of the amides **12a-c**, 1.00 eq of the respective carboxylic acid was dissolved in dry DMF (0.1 mmol/mL) and 1.00 eq HATU and 2.00 eq DIPEA were added. After stirring for 15 min at rt, methyl 2-amino-4-phenylthiazole-5-carboxylate (**11**) (1.00 eq) was added and the resulting mixture was stirred for 16h at 60 °C. The solvent was removed *in vacuo* and the residue was diluted with EtOAc. The organic layer was washed with saturated NaHCO₃ (3x 50 mL), brine (1x 50 mL), dried over Na₂SO₄ and filtered. After the removal of the solvent, the crude product was purified by flash column chromatography (prepacked silica cartridge, *n*-hexane/ethyl acetate) to provide the amides **12a-c**.

Methyl 2-(3,5-dimethoxybenzamido)-4-phenyl-thiazole-5-carboxylate (12a). 3,5-Dimethoxybenzoic acid (1.01 g, 5.50 mmol, 1.00 eq) was subjected to General Procedure 1. The crude product was purified by flash column

chromatography (*n*-hexane/EtOAc) to furnish methyl 2-(3,5-dimethoxybenzamido)-4-phenyl-thiazole-5-carboxylate (**12a**) (1.95 g, 4.90 mmol, 98%) as a white solid. M.p.: 170-174°C. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 3.74 (s, 3H), 3.83 (s, 6H), 6.76 (t, *J* 2.2 Hz, 1H), 7.33 (d, *J* 2.3 Hz, 2H), 7.41 – 7.51 (m, 3H), 7.74 (dd, *J* 6.6, 3.0 Hz, 2H), 13.15 (s, 1H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆) δ = 51.90, 55.59, 105.49, 106.02, 127.55, 128.91, 129.63, 133.15, 134.01, 155.96, 160.19, 160.52, 161.80, 165.33 ppm. HPLC analysis: R_t = 15.883 min, >99%. HRMS (ESI⁺): calcd. for C₂₀H₁₉N₂O₅S [M+H]⁺ = 399.1009, found: 399.1008.

Methyl 2-(isonicotinamido)-4-phenylthiazole-5-carboxylate (12b). Isonicotinic acid (373 mg, 3.00 mmol, 1.00 eq) was subjected to General Procedure 1. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc) to furnish methyl 2-(isonicotinamido)-4-phenylthiazole-5-carboxylate (**12b**) (0.62 g, 1.80 mmol, 61%) as a white solid. M.p.: 225-229°C. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 3.73 (s, 3H), 7.30 – 7.53 (m, 3H), 7.69 – 7.79 (m, 2H), 7.97 – 8.04 (m, 2H), 8.75 – 8.85 (m, 2H), 13.47 (s, 1H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆) δ = 52.03, 115.13, 121.81, 127.62, 129.04, 129.69, 133.78, 138.51, 150.46, 155.87, 159.81, 161.68, 164.67 ppm. HPLC analysis: R_t = 9.667 min, >99%. HRMS (ESI⁺): calcd. for C₁₇H₁₄N₃O₃S [M+H]⁺ = 340.0750, found: 340.0753.

Methyl 2-([1,1'-biphenyl]-4-carboxamido)-4-phenylthiazole-5-carboxylate (12c). Biphenyl-4-carboxylic acid (595 mg, 3.00 mmol, 1.00 eq) was subjected to General Procedure 1. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc) to furnish methyl 2-([1,1'-biphenyl]-4-carboxamido)-4-phenylthiazole-5-carboxylate (**12c**) (0.58 g, 1.40 mmol, 47%) as a white solid. M.p.: 267-270°C. ¹H NMR (600 MHz, DMSO-*d*₆) δ = 3.75 (s, 3H), 7.41 – 7.47 (m, 4H), 7.51 (dd, *J* 8.4, 6.9 Hz, 2H), 7.71 – 7.82 (m, 4H), 7.83 – 7.90 (m, 2H), 8.19 – 8.27 (m, 2H), 13.24 (s, 1H) ppm. ¹³C NMR (151 MHz, DMSO-*d*₆) δ = 52.01, 114.70, 126.80, 126.99, 127.63, 128.43, 129.00, 129.10, 129.12, 129.70, 130.12, 134.01, 138.74, 144.40, 156.06, 160.34, 161.85, 165.56 ppm. HPLC analysis: R_t = 17.983 min, 98.3%. HRMS (ESI⁺): calcd. for C₂₄H₁₉N₂O₃S [M+H]⁺ = 415.1111, found: 415.1114.

General procedure 2: Methyl ester hydrolysis

For the synthesis of the carboxylic acids **13a-c**, 1.00 eq of the methyl ester **12a-c** was dissolved in THF (0.1 mmol/mL) and 30.0 eq potassium hydroxide was added. The resulting mixture was refluxed for 16 h. The solvent was removed *in vacuo* and the residue was resuspended with EtOAc. The organic layer was washed with saturated NaHCO₃ (3x 50 mL). The combined aqueous phases were acidified (pH = 2-3) and the resulting precipitate (product) was filtered off and washed with water.

2-(3,5-Dimethoxybenzamido)-4-phenylthiazole-5-carboxylic acid (13a). Methyl 2-(3,5-dimethoxybenzamido)-4-phenyl-thiazole-5-carboxylate (**12a**) (0.51 g, 1.28 mmol, 1.00 eq) was subjected to General Procedure 2. The acid **13a** (0.27 g, 0.71 mmol, 55%) was obtained as a white solid. M.p.: 187-191°C. ¹H NMR (600 MHz, DMSO-*d*₆) δ = 3.83 (s, 6H), 6.76 (t, *J* 2.3 Hz, 1H), 7.33 (d, *J* 2.3 Hz, 2H), 7.39 – 7.45 (m, 3H), 7.71 – 7.78 (m, 2H), 13.05 (s, 2H) ppm. ¹³C NMR (151 MHz, DMSO-*d*₆) δ = 55.62, 105.46, 105.94, 116.78, 127.53, 128.73, 129.78, 133.24, 134.25, 155.10, 159.73, 160.49, 162.83, 165.22 ppm. HPLC analysis: R_t = 13.00 min, 96.4%. HRMS (ESI⁺) = calcd. for C₁₉H₁₇N₂O₅S [M+H]⁺ = 385.0853, found: 385.0852.

2-(Isonicotinamido)-4-phenylthiazole-5-carboxylic acid (13b). Methyl 2-(isonicotinamido)-4-phenylthiazole-5-carboxylate (**12b**) (338 mg, 1.04 mmol, 1.00 eq) was subjected to General Procedure 2. The acid **13b** (228 mg, 0.70 mmol, 67%) was obtained as a white solid. M.p.: 246-250°C. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 1.23 (t, *J* 7.1 Hz, 3H), 4.22 (q, *J* 7.1 Hz, 2H), 7.40 – 7.55 (m, 3H), 7.68 – 7.81 (m, 2H), 7.98 – 8.08 (m, 2H), 8.78 – 8.90 (m, 2H), 13.50 (s, 1H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆) δ = 13.91, 60.85, 115.62, 121.78, 127.56, 128.99, 129.71,

133.80, 138.50, 150.46, 155.66, 159.74, 161.24, 164.64 ppm. HPLC analysis: $R_t = 6.883$ min, 98.4%. HRMS (ESI⁺): calcd. for C₁₆H₁₂N₃O₃S [M+H]⁺ = 326.0594, found: 326.0597.

2-([1,1'-Biphenyl]-4-carboxamido)-4-phenylthiazole-5-carboxylic acid (13c). Methyl 2-([1,1'-biphenyl]-4-carboxamido)-4-phenylthiazole-5-carboxylate (**12c**) (0.35 g, 0.84 mmol, 1.00 eq) was subjected to General Procedure 2. The acid **13c** (0.31 g, 0.77 mmol, 92%) was obtained as a white solid. M.p.: 248 °C (decomp.). ¹H NMR (600 MHz, DMSO-*d*₆) δ = 7.40 – 7.46 (m, 4H), 7.51 (t, *J* 7.7 Hz, 2H), 7.72 – 7.81 (m, 4H), 7.85 – 7.89 (m, 2H), 8.22 – 8.26 (m, 2H), 13.13 (s, 1H) ppm. ¹³C NMR (151 MHz, DMSO-*d*₆) δ = 126.83, 127.01, 127.56, 128.44, 128.77, 129.09, 129.12, 129.81, 138.78, 144.39, 162.86, 165.44 ppm.

HPLC analysis: $R_t = 14.933$ min, 92.0%. HRMS (ESI⁺) = calcd. for C₂₃H₁₇N₂O₃S [M+H]⁺ = 401.0954, found: 401.0953.

General procedure 3. Hydroxamic acid formation

For the synthesis of the hydroxamic acids **9a-c**, 1.00 eq of the respective carboxylic acid **13a-c** was dissolved in dry DMF (0.1 mmol/mL), 1.00 eq HATU and 3.20 eq DIPEA were added. After stirring for 15 min at rt, 1.20 eq hydroxylamine hydrochloride was added and the resulting mixture was stirred for further 16 h at 60 °C. The reaction mixture was then poured into water and the resulting particulate was then filtered and washed with aqueous HCl-solution (pH = 4) and water. The crude product was purified by flash column chromatography (prepacked silica cartridge, DCM/30% methanol in DCM) to provide the corresponding hydroxamic acids.

2-(3,5-Dimethoxybenzamido)-*N*-hydroxy-4-phenylthiazole-5-carboxamide (9a). 2-(3,5-Dimethoxybenzamido)-4-phenylthiazole-5-carboxylic acid (**13a**) (199 mg, 0.50 mmol, 1.00 eq) was subjected to General Procedure 3. The hydroxamic acid **9a** (50.0 mg, 0.12 mmol, 25%) was obtained as a white solid. M.p. 225 °C (decomp.) ¹H NMR (300 MHz, DMSO-*d*₆) δ = 3.84 (s, 6H), 6.76 (s, 1H), 7.34 (d, *J* 2.2 Hz, 2H), 7.38 – 7.52 (m, 3H), 7.77 (d, *J* 6.8 Hz, 2H), 9.28 (s, 1H), 11.07 (s, 1H), 12.98 (s, 1H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆) δ = 55.61, 105.35, 105.96, 128.13, 128.28, 128.70, 129.70, 129.71, 133.40, 134.10, 154.81, 160.52, 161.78, 165.01. HPLC analysis: $R_t = t = 10.383$ min, 95.0%. HRMS (ESI⁺) = calcd. for C₁₉H₁₈N₃O₅S [M+H]⁺ = 400.0962, found: 400.0960.

***N*-Hydroxy-2-(isonicotinamido)-4-phenylthiazole-5-carboxamide (9b).** 2-(Isonicotinamido)-4-phenylthiazole-5-carboxylic acid (**13b**) (595 mg, 1.83 mmol, 1.00 eq) was subjected to General Procedure 3. The hydroxamic acid **9b** (0.200 g, 0.59 mmol, 32%) was obtained as an orange solid. M.p. 210-214°C. ¹H NMR (300 MHz, DMSO-*d*₆) δ = 7.42 (dq, *J* 13.5, 6.8 Hz, 3H), 7.76 (d, *J* 7.3 Hz, 2H), 8.01 (d, *J* 5.2 Hz, 2H), 8.82 (d, *J* 5.1 Hz, 2H), 9.34 (s, 1H), 11.12 (s, 1H) ppm. ¹³C NMR (75 MHz, DMSO-*d*₆) δ = 117.92, 121.95, 128.40, 128.59, 133.95, 138.88, 150.59, 156.94, 164.49 ppm. HPLC analysis: $R_t = 4.817$ min, 97.3%. HRMS (ESI⁺) = calcd. for C₁₆H₁₃N₄O₃S [M+H]⁺ = 341.0703, found: 304.0706..

2-([1,1'-Biphenyl]-4-carboxamido)-*N*-hydroxy-4-phenylthiazole-5-carboxamide (9c). 2-([1,1'-Biphenyl]-4-carboxamido)-4-phenylthiazole-5-carboxylic acid (**13c**) (260 mg, 0.65 mmol, 1.00 eq) was subjected to General Procedure 3. The hydroxamic acid **9c** (0.165 g, 0.40 mmol, 61%) was obtained as a white solid. M.p. 225 °C (decomp.) ¹H NMR (600 MHz, DMSO-*d*₆) δ = 7.38 – 7.47 (m, 4H), 7.52 (t, *J* 7.6 Hz, 2H), 7.79 (d, *J* 7.6 Hz, 4H), 7.88 (d, *J* 8.0 Hz, 2H), 8.25 (d, *J* 8.1 Hz, 2H), 9.29 (s, 1H), 11.09 (s, 1H), 13.05 (s, 1H) ppm. ¹³C NMR (151 MHz, DMSO-*d*₆) δ = 126.81, 127.00, 127.55, 128.22, 128.32, 128.41, 129.02, 129.11, 130.26, 134.05, 138.79, 144.30, 148.59, 157.20, 159.67, 165.16 ppm. HPLC analysis : $R_t = t = 12.633$ min, 95.7%. HRMS (ESI⁺) = calcd. for C₂₃H₁₈N₃O₃S O₃ [M+H]⁺ = 416.1063, found: 416.1055.

Acknowledgements

YA, AJS, MUK, and TK gratefully acknowledge support from the Deutsche Forschungsgemeinschaft (DFG) for grant number KA 1942/1-1 to MUK and grant number KU 1577/2-1 to TK, and the support from the DFG research training group 270650915/GRK2158. We further acknowledge the CHDI Foundation Inc. for providing the compound CHDI-00390576-0000-004.

Supplementary Material

The performed docking studies and the experimental procedures for the biological evaluation are provided as supplementary material.

References

- 1 Bird, A. *Nature* **2007**, *447* (7143), 396–398.
<https://doi.org/10.1038/nature05913>
- 2 Fischle, W.; Dequiedt, F.; Hendzel, M. J.; Guenther, M. G.; Lazar, M. A.; Voelter, W.; Verdin, E. *Mol. Cell* **2002**, *9*, 45–57.
[https://doi.org/10.1016/s1097-2765\(01\)00429-4](https://doi.org/10.1016/s1097-2765(01)00429-4)
- 3 Gryder, B. E.; Sodji, Q. H.; Oyelere, A. K. *Future Med. Chem.* **2012**, *4*, 505–524.
<https://doi.org/10.4155/fmc.12.3>
- 4 Witt, O.; Deubzer, H. E.; Milde, T.; Oehme, I. *Cancer Lett.* **2009**, *277*, 8–21.
<https://doi.org/10.1016/j.canlet.2008.08.016>
- 5 Ropero, S.; Esteller, M. *Mol. Oncol.* **2007**, *1*, 19–25.
<https://doi.org/10.1016/j.molonc.2007.01.001>
- 6 Jung, M., *Curr. Med. Chem.* **2001**, *8*, 1505–1511.
<https://doi.org/10.2174/0929867013372058>
- 7 Marks, P. A.; Breslow, R., *Nat. Biotechnol.* **2007**, *25*, 84–90.
<https://doi.org/10.1038/nbt1272>
- 8 Duvic, M.; Talpur, R.; Ni, X.; Zhang, C.; Hazarika, P.; Kelly, C.; Chiao, J. H.; Reilly, J. F.; Ricker, J. L.; Richon, V. M.; Frankel, S. R. *Blood* **2007**, *109*, 31–39.
<https://doi.org/10.1182/blood-2006-06-025999>
- 9 Minucci, S.; Pelicci, P. G. *Nat. Rev. Cancer* **2006**, *6*, 38–51.
<https://doi.org/10.1038/nrc1779>
- 10 Richon, V. M.; Emiliani, S.; Verdin, E.; Webb, Y.; Breslow, R.; Rifkind, R. A.; Marks, P. A. *Proc. Natl. Acad. Sci.* **1998**, *95*, 3003–3007.
<https://doi.org/10.1073/pnas.95.6.3003>
- 11 Hose, C. D.; Petersen, K. D.; Shoemaker, R. H.; Kondapaka, S.; Pezzoli, P.; Monforte, J.; Monks, A.; Sehested, M.; Vansant, G. *Anticancer. Drugs* **2009**, *20*, 682–692.
<https://doi.org/10.1097/cad.0b013e32832e14e1>
- 12 Atadja, P. *Cancer Lett.* **2009**, *280*, 233–241.
<https://doi.org/10.1016/j.canlet.2009.02.019>
- 13 Melesina, J.; Praetorius, L.; Simoben, C. V.; Robaa, D.; Sippl, W. *Future Med. Chem.* **2018**, *10*, 1537–1540.
<https://doi.org/10.4155/fmc-2018-0125>

- 14 Bolden, J. E.; Peart, M. J.; Johnstone, R. W. *Nat. Rev. Drug Discov.* **2006**, *5*, 769–784.
<https://doi.org/10.1038/nrd2133>
- 15 Seidel, C.; Schnekenburger, M.; Dicato, M.; Diederich, M. *Genes Nutr.* **2012**, *7* (3), 357–367.
<https://doi.org/10.1007/s12263-012-0283-9>
- 16 Mishra, R.; Sharma, P. K.; Verma, P. K.; Tomer, I.; Mathur, G.; Dhakad, P. K. *J. Heterocycl. Chem.* **2017**, *54*, 2103–2116.
<https://doi.org/10.1002/jhet.2827>
- 17 Rashad, A. E.; Mahmoud, A. E.; Ali, M. M., *Eur. J. Med. Chem.* **2011**, *46*, 1019–1026.
<https://doi.org/10.1016/j.ejmech.2011.01.013>
- 18 Karegoudar, P.; Karthikeyan, M. S.; Prasad, D. J.; Mahalinga, M.; Holla, B. S.; Kumari, N. S. *Eur. J. Med. Chem.* **2008**, *43*, 261–267.
<https://doi.org/10.1016/j.ejmech.2007.03.014>
- 19 Cukurovali, A.; Yilmaz, I.; Gur, S.; Kazaz, C. *Eur. J. Med. Chem.* **2006**, *41*, 201–207.
<https://doi.org/10.1016/j.ejmech.2005.01.013>
- 20 Wu, X.; Kassie, F.; Mersch-Sundermann, V., *Mutat. Res. - Rev. Mutat. Res.* **2005**, *589*, 81–102.
<https://doi.org/10.1016/j.mrrev.2004.11.001>
- 21 Barone, P.; Poewe, W.; Albrecht, S.; Debieuvre, C.; Massey, D.; Rascol, O.; Tolosa, E.; Weintraub, D. *Lancet Neurol.* **2010**, *9*, 573–580.
[https://doi.org/10.1016/S1474-4422\(10\)70106-X](https://doi.org/10.1016/S1474-4422(10)70106-X)
- 22 Mukaihara, K.; Tanabe, Y.; Kubota, D.; Akaike, K.; Hayashi, T.; Mogushi, K.; Hosoya, M.; Sato, S.; Kobayashi, E.; Okubo, T.; Kim, Y.; Kohsaka, S.; Saito, T.; Kaneko, K.; Suehara, Y. *PLoS One* **2017**, *12*, e0185321.
<https://doi.org/10.1371/journal.pone.0185321>
- 23 White, W. B.; Chohan, S.; Dabholkar, A.; Hunt, B.; Jackson, R. *Am. Heart J.* **2012**, *164*, 14–20.
<https://doi.org/10.1016/j.ahj.2012.04.011>
- 24 Anandan, S.; Ward, J. S.; Brokx, R. D.; Denny, T.; Bray, M. R.; Patel, D. V.; Xiao, X. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5995–5999.
<https://doi.org/10.1016/j.bmcl.2007.07.050>
- 25 Bürli, R. W.; Luckhurst, C. A.; Aziz, O.; Matthews, K. L.; Yates, D.; Lyons, K. A.; Beconi, M.; McAllister, G.; Breccia, P.; Stott, A. J.; et al. *J. Med. Chem.* **2013**, *56*, 9934–9954.
<https://doi.org/10.1021/jm4011884>
- 26 Hobara, T.; Uchida, S.; Otsuki, K.; Matsubara, T.; Funato, H.; Matsuo, K.; Suetsugi, M.; Watanabe, Y. *J. Psychiatr. Res.* **2010**, *44* (5), 263–270.
<https://doi.org/10.1016/j.ipsychores.2009.08.015>
- 27 Sung, Y. M.; Lee, T.; Yoon, H.; DiBattista, A. M.; Song, J. M.; Sohn, Y.; Moffat, E. I.; Turner, R. S.; Jung, M.; Kim, J.; Hoe, H.-S. *Exp. Neurol.* **2013**, *239*, 192–201.
<https://doi.org/10.1016/j.expneurol.2012.10.005>
- 28 Tsankova, N.; Renthal, W.; Kumar, A.; Nestler, E. J. *Nat. Rev. Neurosci.* **2007**, *8*, 355–367.
<https://doi.org/10.1038/nrn2132>
- 29 Lobera, M.; Madauss, K. P.; Pohlhaus, D. T.; Wright, Q. G.; Trocha, M.; Schmidt, D. R.; Baloglu, E.; Trump, R. P.; Head, M. S.; Hofmann, G. A.; Murray-Thompson, M.; Schwartz, B.; Chakravorty, S.; Wu, Z.; Mander, P. K.; Kruidenier, L.; Reid, R. A.; Burkhardt, W.; Turunen, B. J.; Rong, J. X.; Wagner, C.; Moyer, M. B.; Wells, C.; Hong, X.; Moore, J. T.; Williams, J. D.; Soler, D.; Ghosh, S.; Nolan, M. A. *Nat. Chem. Biol.* **2013**, *9*, 319–325.

- <https://doi.org/10.1038/nchembio.1223>
- 30 Luckhurst, C. A.; Breccia, P.; Stott, A. J.; Aziz, O.; Birch, H. L.; Bürli, R. W.; Hughes, S. J.; Jarvis, R. E.; Lamers, M.; Leonard, P. M.; Matthews, K. L.; McAllister, G.; Pollack, S.; Saville-Stones, E.; Wishart, G.; Yates, D.; Dominguez, C. *ACS Med. Chem. Lett.* **2016**, *7*, 34–39.
<https://doi.org/10.1021/acsmchemlett.5b00302>
- 31 Asfaha, Y.; Schrenk, C.; Alves Avelar, L. A.; Lange, F.; Wang, C.; Bandolik, J. J.; Hamacher, A.; Kassack, M. U.; Kurz, T. *Bioorg. Med. Chem.* **2020**, *28*, 115108.
<https://doi.org/10.1016/j.bmc.2019.115108>
- 32 Wagner, F. F.; Weïwer, M.; Steinbacher, S.; Schomburg, A.; Reinemer, P.; Gale, J. P.; Campbell, A. J.; Fisher, S. L.; Zhao, W.-N.; Reis, S. A.; et al. *Bioorg. Med. Chem.* **2016**, *24*, 4008–4015.
<https://doi.org/10.1016/j.bmc.2016.06.040>
- 33 Chekler, E. L. P.; Pellegrino, J. A.; Lanz, T. A.; Denny, R. A.; Flick, A. C.; Coe, J.; Langille, J.; Basak, A.; Liu, S.; Stock, I. A.; Sahasrabudhe, P.; Bonin, P. D.; Lee, K.; Pletcher, M. T.; Jones, H. L. *Chem. Biol.* **2015**, *22*, 1588–1596. <https://doi.org/10.1016/j.chembiol.2015.10.013>
- 34 Hai, Y.; Christianson, D. W., *Nat. Chem. Biol.* **2016**, *12*, 741–747. <https://doi.org/10.1038/nchembio.2134>
- 35 Borowiecki, P.; Bretner, M. *Tetrahedron Asymmetry* **2013**, *24*, 925–936.
<https://doi.org/10.1016/j.tetasy.2013.06.004>
- 36 Gradilone, S. A.; LaRusso, N. F. Treatment of polycystic diseases with an HDAC6 inhibitor. US2015/0119413A1, 2015.
- 37 Diedrich, D.; Hamacher, A.; Gertzen, C. G. W.; Alves Avelar, L. A.; Reiss, G. J.; Kurz, T.; Gohlke, H.; Kassack, M. U.; Hansen, F. K. *Chem. Commun.* **2016**, 3219–3222.
<https://doi.org/10.1039/C5CC10301K>
- 38 Marson, C. M.; Savy, P.; Rioja, A. S.; Mahadevan, T.; Mikol, C.; Veerupillai, A.; Nsubuga, E.; Chahwan, A.; Joel, S. P. *J. Med. Chem.* **2006**, *49*, 800–805.
<https://doi.org/10.1021/jm051010j>
- 39 Liguori, A.; Sindona, G.; Romeo, G.; Uccella, N., *Synthesis (Stuttg)*. **1987**, 168–168.
<https://doi.org/10.1055/s-1987-27874>
- 40 Dines, J. A.; Marson, C. M. *Tetrahedron* **2016**, *72*, 8584–8592.
<https://doi.org/10.1016/j.tet.2016.11.039>

This paper is an open access article distributed under the terms of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>)