Supporting Information

Thailandepsins: Bacterial Products with Potent Histone Deacetylase Inhibitory Activities and Broad-Spectrum Antiproliferative Activities

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SI Methods

Reverse transcription (RT)-PCR detection of gene expression conditions (Figure S2a and S2b**).** *B. thailandensis* E264 wild type strain was cultured in nine different growth media (Table S1; without resin) at 30°C for 3 days under constant agitation (200 rpm) in flasks with a loose aluminum foilcap; aliquots of cell cultures were harvested at 12, 24, 48 and 72-hr time points and quickly mixed with 2 vol. of RNAprotect Bacteria Reagent (Qiagen). Total RNA samples were prepared from stabilized culture aliquots using RNeasy Mini Kit (Qiagen) according to manufacturer's instruction. The quality and quantity of RNA samples was analyzed by UV/Vis spectrophotometry and by denaturing gel electrophoresis. RNA samples were further treated with RNase-free DNase (New England BioLabs) to eliminate any DNA contamination. Absence of residual DNA in RNA samples was verified by negative amplification of 16S rDNA by 28 cycles of typical PCR reaction. Transcript detection was carried out with OneStep RT-PCR Kit (Qiagen) as follows. DNase-treated RNA sample (1.0 μg) was used as template for reverse transcription at 50°C for 30 min with OmniScript and SensiScript Reverse Transcriptases. PCR amplification were performed per manufacturer's instruction as follows: 15 min of incubation at 95°C for initial activation of HotStarTaq DNA polymerase, followed by 28 cycles of amplification with denaturing at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min. Final extension was carried out at 72°C for 5 min. PCR primer sets used to amplify the transcripts of two representative genes were as follows: *tdpA*-RT-PCR-FP, 5'-GCTTCCGTCGGCTCATCGG-3', and *tdpA*-RT-PCR-RP, 5'-CGCGAGCATCGTGTCGCGG-3'; *tdpJ*-RT-PCR-FP, 5'-GATCGAGCCGTCACCGTCG-3', and *tdpJ*-RT-PCR-RP, 5'-GGCCTGGATGCGTGACGCC-3'. Each primer set was designed to generate a PCR product of approximately 200 bp. RNA samples that had not been subjected to reverse transcription were used as negative controls; a positive control was set to amplify from genomic DNA template.

Construction of a targeted gene-deletion mutant of *B. thailandensis* E264 (Figure S2c**).** A multiplex PCR method, as described elsewhere,[1-3] was used for gene inactivation. This method utilized a broad host-range Flp-*FRT* recombination system for site-specific gene deletion/disruption and subsequent marker removal.[2,4] Specifically, a 375-bp DNA fragment toward the 3'-end of *tdpA* (Amplicon 1) was amplified from *B. thailandensis* genomic DNA with the following PCR primer set: KpnI-*tdpAB*-UpF, 5'-AGGTACCgtcgatcgtgtcggtcgtc-3', containing a KpnI site (underlined); and *FRT*-F-*tdpAB*-UpR, 5'-TCAGAGCGCTTTTGAAGCTAATTCGatctcgcccagctcgatc-3'. A 395-bp DNA fragment toward the 5'-end of *tdpB* (Amplicon 2) was amplified from *B. thailandensis* genomic DNA with the

following PCR primer set: *FRT*-R-*tdpAB*-DnF: 5'-AGGAACTTCAAGATCCCCAATTCGacaaggactatctcgcgac-3', and BamHI-*tdpAB*-DnR, 5'-AGGATCCgtcgttgttgatcgcgcc-3' containing a BamHI site (underlined).

Meanwhile, a selective marker donor plasmid, pBS854-Tp, was constructed by subcloning the trimethoprim-resistance gene (*Tp*^{r}; 617-bp EcoRI fragment) from p34E-Tp[5] into the EcoRI site of pBS854.[4] Subsequently a 0.7-kb *FRT* cassette containing the *Tp*^{r} marker gene flanked by two *FRT* recognition sequences (Amplicon 3) was amplified from pPS854-Tp with the following PCR primer set: *FRT*-F, 5'-CGAATTAGCTTCAAAAGCGCTCTGA-3', and *FRT*-R, 5'-CGAATTGGGGATCTTGAAGTTCCT-3'.

Amplicons 1–3 were assembled into a 1.45-kb Amplicon 4 by multiplex PCR using Long Amp DNA polymerase (New England Biolabs). Amplicon 4 was digested with KpnI/BamHI and the insert was subsequently cloned into suicide vector pEX10Tc (*Tc*^{r} *oriT*$^+$ *sacB*$^+$, conjugative)[4] to make a *tdpAB*-gene inactivation vector pYC05-57.

To create a *tdpAB*-gene inactivation mutant of *B. thailandensis*, pYC05-57 was first transformed into *E. coli* S17-1 strain which subsequently passed the vector to *B. thailandensis* cells via interspecies conjugation. Mutant strains of *B. thailandensis* with *tdpAB* partially replaced by the *FRT* cassette were selected on LB agar supplemented with 50 μg/ml apramycin (Am), 100 μg/ml Tp and 5% (w/v) sucrose at 30°C. The genotype of independent mutants was verified by colony PCR using the following primer sets: *tdpAB*-PCR-FP1 (5'-GCGTTCCCGAACGTCCAGC-3') and *tdpAB*-PCR-RP1 (5'- CGTGACGGATCACCTCGCG-3'), and *tdpAB*-PCR-FP2 (5'-CGGTGATTCAGTTGCACGTGG-3') and *tdpAB*-PCR-RP2 (5'-GCTGCAGGTAACGGTTCGGC-3'). One representative mutant strain was saved and named BthΔ*tdpAB*::*FRT*.

To create a marker-free mutant by removing the *FRT* cassette from BthΔ*tdpAB*::*FRT*, a broad host-range Flp-expression vector pBMTL3-Flp2[1] was introduced into BthΔ*tdpAB*::*FRT* by conjugation and the marker-free mutants were selected on LB agar supplemented with 50 μg/ml Am and 25 μg/ml chloramphenicol (Cm) at 37°C. Vector pBMTL3-Flp2 was subsequently cured from the mutants by streaking for two rounds on LB agar supplemented with 50 μg/ml Am and 5% (w/v) sucrose at 30°C. The genotype of independent gene-deletion mutants was verified by colony PCR. One final representative marker-free mutant strain was saved and named BthΔ*tdpAB*.

Purification and identification of thailandepsins A (6) and B (7). *B. thailandensis* E264 wild type strain was fermented in 50 flasks (500 ml medium in 2-L flask) for a total volume of 25 L of M9 supplemented with 1% (w/v) of Diaion HP-20 resin (Sigma-Aldrich) at 30°C for 3 days. Resins and cells were collected at the end of fermentation by filtration and centrifugation and subsequently lypholized to dryness. The dry mass was extracted five times, each with two volumes of ethyl acetate (v/w). The crude organic extract was concentrated under reduced pressure to remove ethyl acetate and yielded an oily residue. This residue was applied to a silica gel column and washed extensively with hexane-ethyl acetate (1:2). Materials remained in the column was eluted by ethyl acetate, which was subsequently concentrated from the extract under educed pressure to yield an oily residue. This oily sample was resuspended in acetonitrile and subjected to preparative HPLC (ProStar 210 model with a Prep-C_{18} column, 10 μm particle size, 21.2 x 250 mm, from Agilent) under isocratic condition with a mobile phase of 40% (v/v) acetonitrile/water, a flow rate of 8 ml/min and UV detection at 200 nm. **6** was eluted at 28-32 min and **7** was eluted at 45-49 min. The overall yield was approximately 1 mg/L for **6** and 0.1 mg/L for **7**. Fermentation optimization is in progress.

The physical chemical properties of **6** and **7** were determined as follows: CD measurement was performed on a JASCO DIP-370 digital polarimeter (University of Wisconsin-Milwaukee Department of Chemistry and Biochemistry); IR spectra were obtained with a Quantum Microscope (ATI Mattson at the University of Wisconsin-Milwaukee Advanced Analysis Facility) with resolution of 4 cm^{-1}; HR-MS spectra were obtained on a 7.0T Fourier Transform Ion Cyclotron Resonance mass spectrometer (FT-ICR-MS at the Medical College of Wisconsin MassSpec Facility) with an electrospray ionization source; amino acid analyses were performed on a Hitachi L8900 Amino Acid Analyzers (AAA Service Laboratory, Inc. Damascus, OR); ^{1}H, ^{13}C and two dimensional NMR spectra were collected on a Bruker DRX 500MHz NMR spectrometer (University of Wisconsin-Milwaukee Department of Chemistry and Biochemistry).

Determination of the absolute configuration of the amino acid moieties in thailandepsin A (6) and thailandepsin B (7). One mg of **6** or **7** was hydrolyzed in 0.2 ml of 6 N HCl at 110°C for 24 h and the HCl was removed under vacuum. The hydrolysate was dissolved in 100 μl of 50% (v/v) acetonitrile/water and split evenly into two parts. One hundred μl of 1M $NaHCO_3$ was added into each part. Fifty μl of 10 mg/ml (w/v) of L-FDLA (1-fluoro-2,4-dinitrophenyl-5-L-leucine-amide) or D-FDLA (1-fluoro-2,4-dinitrophenyl-5-D-leucine-amide) in acetone solution was added to each part and the reaction mixture was incubated at 37°C for 1

hour before quenched by neutralization with 50 μl of 2 N HCl. Three hundred μl of 50% acetonitrile was added to the suspension to dissolve the precipitant. Standard amino acids, L-Met or L-NLeu, was reacted with L-FDLA and D-FDLA, respectively. Twenty μl of reaction product was analyzed by LC-MS with a gradient solvent system from 20% to 70% (v/v) acetonitrile/water (with 0.1% formic acid added) over 50 min (column: Agilent Zorbax Eclipse XOB-C18 3.5 μm, 3.0 mm x 100 mm, 0.3 ml/min flow rate and UV 340 nm detection).

Separately 1 mg of **6** or **7** was firstly oxidized by 0.2 ml of performic acid (made fresh with formic acid and 30% hydrogen peroxide at 9:1 ratio) at 55°C for 45 min. After oxidization, individual samples were hydrolyzed with HCl and then reacted with L-FDLA and D-FDLA, respectively. L-Cys was treated by the same procedure. The final produces were analyzed by LC-MS with a linear gradient from 25% to 65% (v/v) acetonitrile/water (with 0.1% formic acid) over 50 min.

Determination of the *S*- and *R*-MPA esters of thailandepsin A (6). Eight mg of **6** was divided in two separate vials and several dry crystals of dimethylaminopyridine were added to the two reaction vials. The mixture was dissolved in freshly dry pyridine (2 ml) and stirred for 15 min at room temperature. Two hundred μl of *R*- and *S*-MPA chloride (5 μmol/μl) was added to each vials and the reaction mixtures were stirred at room temperature for 1 h. Then the reaction vials were heated at 65°C for 3 h. After, 100 μl of MeOH were added to each reaction and the acylated products were purified by preparative HPLC (gradient from 100% water to 100% acetonitrile in 60 min). *S*-MPA and *R*-MPA ester of **6** were eluted at 53 min and 55 min respectively. The products were confirmed by LC-MS ($[M + H]^+$ 696 *m/z*). The NMR spectra of *S*-MPA and *R*-MPA esters were collected in $CDCl_3$ and the ^1H chemical shifts of these esters were assigned by ^1H, gCOSY, and TOCSY NMR spectral analysis.

Computational Methods. The majority of published docking studies for HDAC-ligand complexes were concentrated on acyclic compounds.[6] In order to consider the conformational flexibility of the macrocyclic ring in **2** and **6**, conformational samplings were performed prior to docking study, using a MacroModel program (Schrödinger Inc.). The initial structure of **2** was acquired from the Cambridge Structure Database (http://www.ccdc.cam.ac.uk/). The initial structure of **6** was generated based on the X-ray structure of **2**.[7] These two initial structures were subjected to minimization and systematic torsional Monte Carlo sampling with 200 steps per rotational bond. Amber* force field was applied with water solvent model and the dielectric

constant was set at 1. Finally 593 and 873 unique conformers for **2** and **6** respectively were obtained and stored for further docking study.

A two-stage charge fitting procedure implemented inside the Antechamber module of the Amber 9 package (http://ambermd.org/) was then used to calculate the charges of **2*** and **6***. Due to a large size and the bicyclic depsipeptide property of the ligands, the structures of **2** and **6** were divided into five or four subunits, respectively. Each subunit was minimized with the HF/6-31G* method using the Gaussina 03 package (http://www.gaussian.com/) and electrostatic surface potentials were calculated based on the minimized structures. Finally, the AutoDock 4.2 package[8] was used to perform the docking study of **2*** and **6*** to human HDAC2, one of the class I HDACs whose X-ray structure was become available very recently (PDB: 3MAX).[9] The size of the grid was set at 80 × 80 × 80 points with a spacing of 0.375 Å between the grid points. It sufficiently covered the active site of HDAC2. The structure of HDAC2 was treated as rigid during docking calculations in order to reduce computational time. Other docking parameters used here were described in our previous studies.[10,11] The top 100 unique conformers of **2*** and **6*** generated from the conformational search were docked individually to HDAC2. Finally, the top-ranked docking complex was selected for presentation.

SI Results

Basic physical chemical properties of thailandepsin A (6) and thailandepsin B (7). Compound **6** was isolated as white amorphous powder and its molecular formula was established as $C_{23}H_{37}N_3O_6S_3$ with a calculated MW of 547.75; **7** was isolated also as white amorphous powder (often with a hint of yellowish impurity) and its molecular formula was established as $C_{24}H_{39}N_3O_6S_2$ with a calculated MW of 529.71. Preliminary tests found that both compounds are readily soluble and stable in acetonitrile, chloroform ($CHCl_3$) and dimethyl sulfoxide (DMSO), moderately soluble and stable in ethyl acetate, moderately soluble but less stable in methanol or alcohol, and slightly soluble but unstable in water. The circular dichroism (CD) of **6** and **7** was determined as $[\alpha]_D^{24}$ -40.7 (c 2.5, in acetonitrile) and $[\alpha]_D^{24}$ -22.8 (c 1, in acetonitrile), respectively. Both **6** and **7** have major infra-red (IR) absorption peaks at 3370, 2937, 1740, 1676, 1540, 1266, 1156 cm^{-1}.

The high resolution (HR) mass values of **6** and **7** in positive mode ($[M + H]^+$) were determined to be 548.2135 *m*/*z* and 530.2432 *m*/*z*, respectively. A dehydrated derivative of **6** and **7** was routinely detected during MS measurement, and their mass values in positive mode ($[M - H_2O + H]^+$) were determined to be 530.2027 *m*/*z* and 512.2337 *m*/*z*, respectively.

Structural determination of thailandepsin A (6) and thailandepsin B (7). The structures of **6** and **7**, including stereochemistry (Figure S4), were determined by a combination of MS analysis (above), amino acid analysis, 1D and 2D NMR analyses, degradation analysis and chemical derivatization.

Amino acid analysis (data not shown). Norleucine (Nleu) was used as an internal control in amino acid analysis, which has a retention time (RT) of 33.8 min in standard experiments. Hydrolysis of **6** (in 6N HCl/2% phenol at 110ºC for 22 hr) released free methionine (Met) monomer with an RT of 26.5 min, whereas hydrolysis of **7** under the same conditions did not produce any new major peak but the Nleu peak was elevated. Furthermore, when **6** was oxidized (in performic acid – made fresh with formic acid and 30% hydrogen peroxide at 9:1 ratio – at 55ºC for 45 min) and then hydrolyzed, it produced a major peak of cysteic acid (Cyso2) and another major peak labeled as aspartic acid (Asp), at approximately 1:1 molar ratio. This "Asp" is believed to be Met-sulfone converted from Met by oxidation, which happens to have an RT identical to that of Asp. Oxidation and hydrolysis of **7** under the same conditions produced a major peak of Cyso2 only but the Nleu peak was again elevated. Collectively those observations indicated the presence of Met and cysteine (Cys) moieties in **6** and NLeu and Cys moieties in **7**.

NMR analysis of thailandepsin A (6) in DMSO-D6. The ^{1}H NMR spectrum of **6** displayed 3 downfield doublet protons at δ 8.41, 7.29 and 6.68 ppm (Figure S5*a*). The ^{13}C NMR spectrum (Figure S5*b*) and HSQC spectrum (Figure S5*e*) showed 4 amide or ester carbonyl carbons at δ 172.5, 171.4, 170.5 and 169.2 ppm. All these NMR and IR data suggested **6** a peptide derivative. Detailed analysis of gCOSY, TOCSY, HSQC and HMBC NMR spectra data (Figure S5*c-f*) allowed assignment of all signals from the proton and carbon NMR spectra (Table S2) and revealed a structural framework consisting of peptide and polyketide moieties (Figure S4*a*).

Analysis of the gCOSY spectrum revealed four spin systems. The sequence from an amide proton at 6.68 ppm to methylene protons 4-H through an α-methine 2-H and the methylene protons 3-H was detected. An amide carbonyl carbon C1 was long-range coupled to 2-H in HMBC spectrum. The protons at 2.08 ppm were from the methylthio group (C-5) and C-4 was linked to the sulfur atom supported by the chemical shift of 41.1 ppm. Based on the amino acid analysis, this moiety was determined as Met.

The proton spin system from an amide proton at 8.41 ppm to methylene protons 3'-H through an α-methine proton 2'-H was detected. The presence of sulfur atom in this partial

structure was substantiated by the chemical shift C-3' (30.5 ppm) and the detection of Cys in the amino acid analysis. These results established a Cys moiety in 6.

Further analysis of the gCOSY spectrum revealed the sequence from 2"-H to 7"-H through 3"-H, 4"-H, 5"-H and 6"-H, which was also coupled to 3"-OH, 4"-NH and a methyl proton 8"-H, respectively. The 2"-H and 3"-H were long-range coupled to an ester carbonyl carbon C-1" to prove a 4-amino-3-hydroxy-5-methylheptanoic acid moiety.

The residual moieties were revealed by proton spin systems from 2"'-H to 7"'-H. The long-range coupling from 2"'-H and 3"'-H to an amide carbonyl carbon C-1"' as well as the chemical shift of C-3"' (70.5 ppm) revealed the presence of 3-oxy-4-hepenoyl-7-yl. The coupling constant of 4"'-H and 5"'-H from ^{1}H spectrums of *R*-MPA ester and *S*-MPA ester of **6** showed 13 Hz and revealed the (*E*) geometry of the double bond in this acyl component.

The connections among these amide and ester functional units were established by long-range couplings from the amide protons to their neighboring carbonyl carbons in HMBC, i.e. the amide protons at 2-NH, 2'-NH and 4"-NH to C-1"', C-1 and C-1' respectively. The oxymethine proton 3"'-H was long-range coupled with C-1" in HMBC experiment. The remaining two methylenes, C-3' and C-7"', were connected through a disulfide. In combination of stereochemical analysis (see a section below) the structure of **6** was determined to be a bicyclic depsipeptide, with a 15-membered macrocyclic lactone and a 15-membered side ring containing a signature disulfide bond.

NMR analysis of thailandepsin B (7) in $CDCl_3$. The ^{1}H and ^{13}C NMR spectra as well as gCOSY, HSQC and HMBC NMR spectra of **7** (Figure S6*a*-*e*) revealed an extended proton spin system from 2-H to 6-H through the methylene protons 3-H, 4-H and 5-H. All other residues and their connectivities were the same as those of **6** (Table S4). In combination of stereochemical analysis (see next section) the structure of **7** was therefore determined as shown in Figure S4*b*.

Stereochemical analysis of thailandepsin A (6) and thailandepsin B (7). We further determined the stereochemistry of **6** and **7** using a combination of spectroscopic analysis, degradation reactions and chemical derivatization (data not shown).

The relative configuration of the two adjacent stereogenic centers in 4-amino-3-hydroxy-5-methylheptanoic acid was established by *J*-based configuration analysis[12] as 3"*S* and 4"*R*. Stereochemistry of amino acid residues was revealed using the advanced Marfey's reagents [L-FDLA and D-FDLA, *N*α-(2,4-dinitro-5-fluorophenyl)-leucineamide].[13-16] Flash hydrolysis of **6** and **7** released Met and NLeu, respectively. The L- and D-FDLA derivatives of this Met were

eluted at 32.5 min and 27.5 min respectively. The Met in **6** was determined as D-form based on the retention times of the L- and D-FDLA derivatives of standard L-Met [LL (or DD): 27.0 min and LD (or DL): 32.5 min] and the absolute configuration of C-2 was determined as *R* in **6**. Similarly, the L- and D-FDLA derivatives of NLeu in **7** were eluted at 38.5 min and 31.5 min respectively. The NLeu in **7** was determined as D-form as well based on the retention times of the L- and D-FDLA derivatives with the standard L-NLeu (LL: 31.5 min and LD: 38.5 min). Thereafter C-2 was established as *R* configuration in **7**.

Similarly **6** and **7** produced identical L- and D-FDLA derivatives of cysteic acid after oxidization and hydrolysis, and the retention times of which were 12.3 and 11.7 min, respectively. The cysteic acid was determined as D-form according the retention times of the L- and D-FDLA derivatives with standard L-cysteic acid [LL (or DD): 11.7 min and LD (or DL): 12.3 min]. The absolute configuration of C-2' was thus determined as *S*.

We used the modified Mosher method[17,18] to examine the absolute configuration of the secondary alcohol in C3". This only secondary alcohol (at C3") in **6** was treated with *R*- and *S*-α-methoxy-α-phenylacetyl chloride (MPA-Cl) to yield *R*-MPA ester and *S*-MPA ester of **6**, respectively. We assigned the proton chemical shifts of the -OH by analysis of ^{1}H, gCOSY and TOCSY NMR spectra. The values of $\Delta\delta^{R-S}$ determined the absolute configuration of the secondary alcohol at C3" as *S*. C-4" was determined as *R* configuration from the relative stereochemistry and C-5"*S* configuration was determined from NOE NMR spectral data. *S*-MPA or *R*-MPA ester of **6** was eluted at 53-55 min, respectively. The products of *S*-MPA and *R*-MPA esters were confirmed by LC-MS ($[M + H]^+ = 696$ *m*/*z*). The NMR data of *S*-MPA and *R*-MPA esters of **6** were collected in $CDCl_3$ and the ^{1}H chemical shifts of these esters were assigned by ^{1}H, gCOSY, and TOCSY NMR analyses. *S*-MPA ester of **6**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.18 (4"-NH), 5.68 (4"'-H), 5.60 (3"'-H), 3.21 (2"'-H), 3.07 (4"-H), 2.93 (2"-H), 2.70 (2"-H), 1.14 (5"-H), 1.04 (6"-H), 0.80 (6"-H), 0.63 (8"-H_3), 0.61 (7"-H_3). *R*-MPA ester of **6**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 (4"-NH), 5.67 (4"'-H), 5.55 (3"'-H), 3.27 (4"-H), 3.20 (2"'-H_2), 2.65 (2"-H_2), 1.63 (5"-H), 1.34 (6"-H), 1.13 (6"-H), 0.87 (8"-H_3), 0.84 (7"-H_3).

The stereochemistry at 3"' of **6** and **7** was extrapolated from the structures of FK228, FR901375 and spiruchostatins which all exclusively have an *S*-configuration[7,19-21] (Scheme 1).

Molecular modeling of docking between human HDAC2 and activated/reduced FK228 (2*) or thailandepsin A (6*). Docking studies indicated that both **2*** and **6*** adopt a very similar conformation in the active site of HDAC2 (Figure S8). The long aliphatic chain with a

thiol group at the end is inserted in the 11 Å long channel of HDAC2. Sulfur atom coordinates with the zinc ion and forms a tetrahedral geometry. In addition, the cyclic substructure of compounds interacts with the key residues (yellow stick) on the HDAC2 surface. Interestingly and importantly, both compounds form hydrogen bonds (magenta dot lines) with Asp104. Such interactions were not detected in other HDAC-ligand complexes in previous studies.[6,9,10] The rest interactions were also found similarly in both complexes. For example, two isopropyl groups in **2*** are close to Phe210 and Leu276 while in **6*** the 2-methylsulfanyl-ethyl group and the sec-butyl group are close to those two residues. The only interaction that exists in HDAC2-**6*** complex but not in HDAC2-**2*** complex is a hydrogen bond formed between the hydroxyl group in **6*** with the carbonyl oxygen of Leu276 in HDAC2. In summary, the similar interaction map and orientation for **2*** and **6*** in the active site of HDAC2 is consistent with their similar in vitro inhibition activities against HDAC2 (Table 2).

Table S1. Nine production media tested for the cultivation of *B. thailandensis* E264. All media were supplemented with 1% (w/v) XAD16 resin and 1% (w/v) Diaion HP-20 resin (Sigma-Aldrich). Both types of resin were presoaked in 50% ethanol and rinsed with water.

Medium no.	Medium Name	Composition, g per liter	pH at 25°C	Reference and Note
M1	Modified NB Medium	Glucose 10.0g Nutrient Broth 10.0g NaCl 5.0g $CaCO_3$ 1.0g	7.0	Originally used for FK228 production by *Chromobacterium violaceum* no. 968[22]
M2	Modified YM-254890 Medium	Glycerol 20.0g Glucose 5.0g Peptone 5.0g Yeast extract 1.0g NaCl 1.0g $CaCO_3$ 1.0g	7.0	Originally used for YM-254890 production by *Chromobacterium* sp. QS3666[23]
M3	Modified spiruchostatin Production Medium	Glycerol 30.0g Glucose 10.0g Peptone 5.0g Beef extract 5.0g NaCl 5.0g $CaCO_3$ 1.0g	7.0	Originally used for spiruchostatin production by *Pseudomonas* sp. Q71576[24]
M4	*Pseudomonas pickettii* Medium	Peptone 5.0g NaCl 5.0g $Na_2HPO_4 \cdot 12H_2O$ 2.39g Yeast extract 2.0g Beef extract 1.0g K_2HPO_4 0.45g	6.8	Page1148[25]; for the cultivation of *Burkholderia pickettii*
M5	*Pseudomonas* Phage Medium	Nutrient broth 10.0g Glucose 1.0g KH_2PO_4 0.49g K_2HPO_4 0.45g	7.0	Page1148[25]; for the cultivation and maintenance of *Pseudomonas fluorescens*
M6	*Pseudomonas* Medium A	Peptone 20.0g Glycerol 10.0g K_2SO_4 10.0g $MgCl_2$ 1.4g	7.2	Page1147[25]; for the cultivation and production of pyocyanin by *Pseudomonas species*
M7	ATCC Medium 226	Yeast extract 10.0g Glucose 5.0g Sodium acetate 0.5g	7.0	Page1146[25]; for the cultivation and maintenance of *Pseudomonas indigofera*
M8	Modified PPGA Medium	Glucose 5.0g Peptone 5.0g NaCl 3.0g Na_2HPO_4 1.2g KH_2PO_4 0.5g	7.0	Page1124[25]; for the cultivation of *Burkholderia glumea* and *Bukhoderia pantarii*
M9	Modified Minimal Broth, Davis	Glucose 10.0g K_2HPO_4 7.0g KH_2PO_4 2.0g $(NH4)_2SO_4$ 1.0g Sodium citrate 0.5g $MgSO_4 \cdot 7H_2O$ 0.1g	7.0	Page948[25]; for the isolation, cultivation and characterization of nutritional mutants

Table S2. ^{1}H and ^{13}C NMR data (500 MHz, DMSO-D6) for thailandepsin A (6).

residue	position	δ_C	δ_H (J in Hz)	type
Met	1	171.4		C
	2	55.1	4.69, dt (3.2, 9.5)	CH
	2-N		6.68, d (9.5)	NH
	3	30.9	2.72, m	CH_2
			2.63, m	
	4	41.1	3.36, m	CH_2
			3.04, m	
	5	15.3	2.08, s	CH_3
Cys	1'	169.2		C
	2'	56.0	4.12, dt (3.1, 6.9)	CH
	2'-N		8.41, d (3.1)	NH
	3'	30.5	1.94, m	CH_2
			2.63, m	
Ahhp[a]	1"	170.5		C
	2"	41.8	2.61, m	CH_2
			2.57, m	
	3"	66.9	4.49, dt (3.7, 9.6)	CH
	3"-O		4.92, d (5.9)	OH
	4"	60.3	2.97, m (4.0, 7.5, 9.6)	CH
	4"-N		7.29, d (7.5)	NH
	5"	36.6	1.98, m	CH
	6"	27.9	1.42, m	CH_2
			1.15, m	
	7"	12.5	0.88, t (7.4)	CH_3
	8"	15.9	0.84, d (6.9)	CH_3
Acyl[b]	1"'	172.5		C
	2"'	40.8	2.71, m	CH_2
			2.75, m	
	3"'	70.5	5.59, m	CH
	4"'	132.2	6.02, s	CH
	5"'	130.7	6.02, s	CH
	6"'	31.9	2.42, m	CH_2
			2.55, m	
	7"'	40.6	3.17, m	CH_2
			2.87, m	

[a]Ahhp: 4-amino-3-hydroxy-5-methylheptanoic acid;

[b]Acyl: 3-oxy-4-heptenoyl-7-yl.

Table S3. ^{1}H and ^{13}C NMR data (500 MHz, $CDCl_3$) for thailandepsin B (7).

residue	position	δ_C	δ_H (J in Hz)	type
Norleucine	1	170.8		C
	2	57.0	4.27, m	CH
	2-N		5.81, d (3.5)	NH
	3	31.2	2.03, m	CH_2
			1.78, m	
	4	22.6	1.48, m	CH_2
	5	28.9	1.47, m	CH_2
	6	14.1	1.00, m	CH_3
Cys	1’	169.5		C
	2’	54.4	5.02, td (9.5, 3.7)	CH
	2’-N		6.74, d (9.5)	NH
	3’	42.8	3.56, m	CH_2
			3.13, m	
Ahhp[a]	1”	172.2		C
	2”	40.1	2.78, m	CH_2
			2.76, m	
	3”	68.8	4.72, w	CH
	3”-O		2.84, m	OH
	4”	62.4	2.99, m	CH
	4”-N		7.26, d (6.8)	NH
	5”	36.8	2.18, m	CH
	6”	27.6	1.62, m	CH_2
			1.33, m	
	7”	11.9	1.01, m	CH_3
	8”	15.7	0.99, m	CH_3
Acyl[b]	1”’	170.9		C
	2”’	41.3	3.42, m	CH_2
			2.62, m	
	3”’	70.7	5.57, d (1.7)	CH
	4”’	128.7	5.69, t (13.2)	CH
	5”’	134.3	6.59, t (13.2)	CH
	6”’	34.4	2.82, m	CH_2
			2.56, m	
	7”’	40.8	3.25, m	CH_2
			2.81, m	

[a]Ahhp: 4-amino-3-hydroxy-5-methylheptanoic acid;
[b]Acyl: 3-oxy-4-heptenoyl-7-yl.

Figure S1. Predicted structures of and proposed model for the biosynthesis of thailandepsins A and B by a hybrid polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS) pathway in *Burkholderia thailandensis* E264.

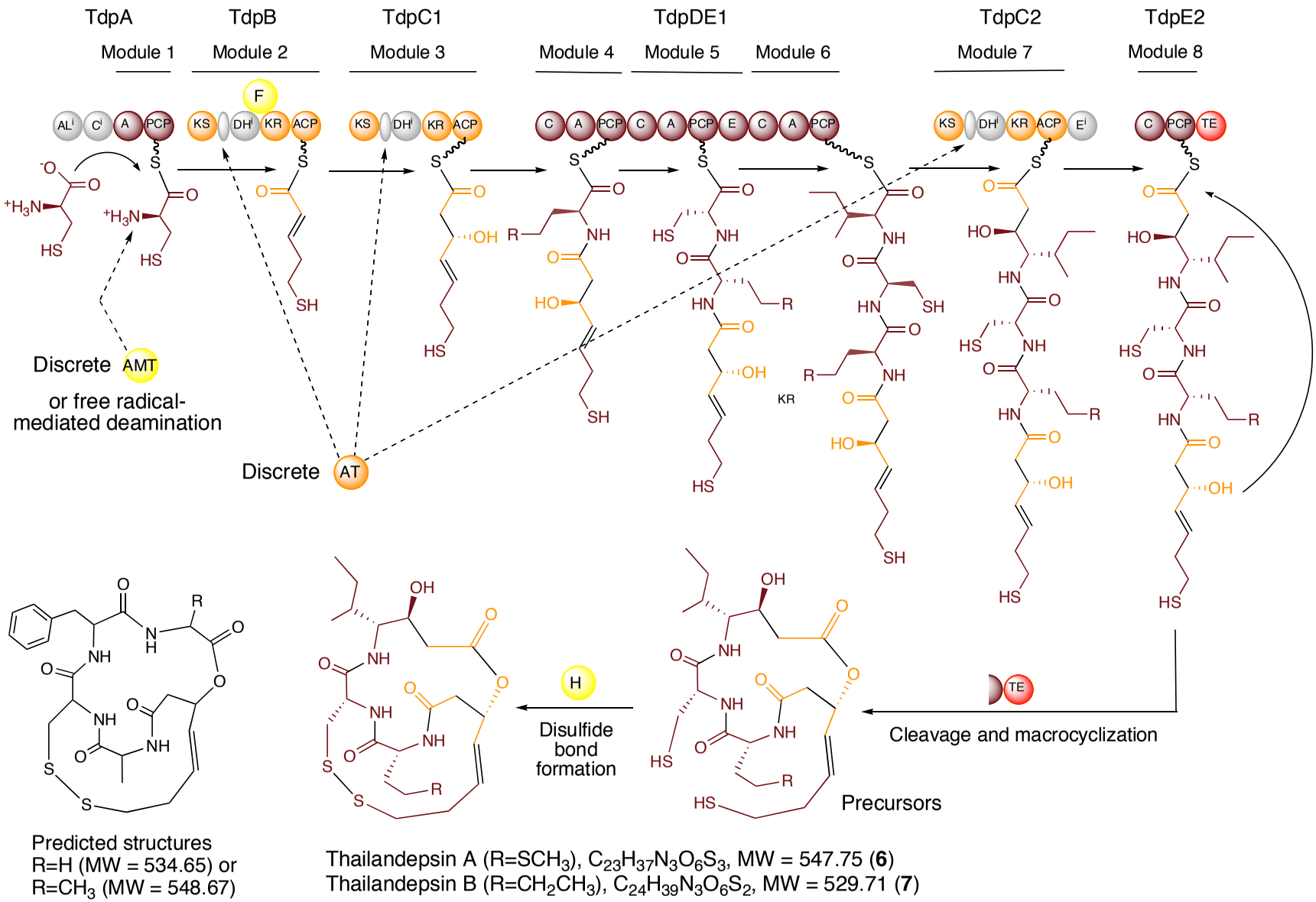
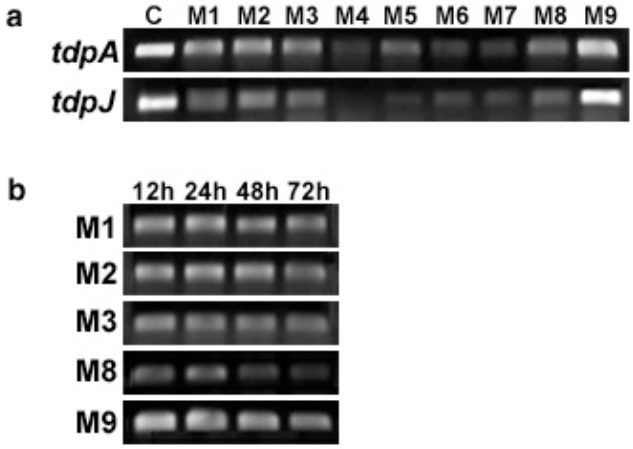
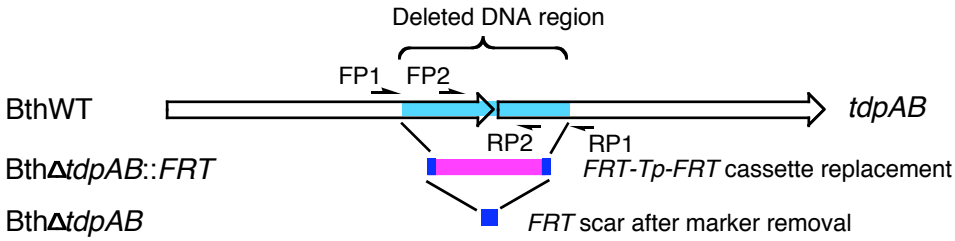


Figure S2. Molecular and genetic anlyses of critical *tdp*-genes in *Burkholderia thailandensis* E264. (a) Semi-quantitative RT-PCR analysis of the expression of two representative structural genes (*tdpA* and *tdpJ*) in nine different growth media (M1 through M9) at 24 hr. C, positive control reaction of respective gene amplification from genomic DNA template. (**b**) RT-PCR analysis of the expression of representative *tdpA* gene in five media at four different time points.



(c) Partial deletion of *tdpAB* verified by PCR analysis and gel electrophoresis. Lane 1: 1-kb DNA ladder; Lane 2: 100-bp DNA ladder; Lanes 3 (1.9 kb from BthWT), 4 (1.5 kb from Bth**Δ***tdpAB*::*FRT*) and 5 (0.9 kb from Bth**Δ***tdpAB*) show products of PCR1 (with FP1/RP1 primer set); Lanes 6 (1.0 kb from BthWT), 7 (no amplification from Bth**Δ***tdpAB*::*FRT*) and 8 (no amplification from Bth**Δ***tdpAB*) show products of PCR2 (with FP2/RP2 primer set).



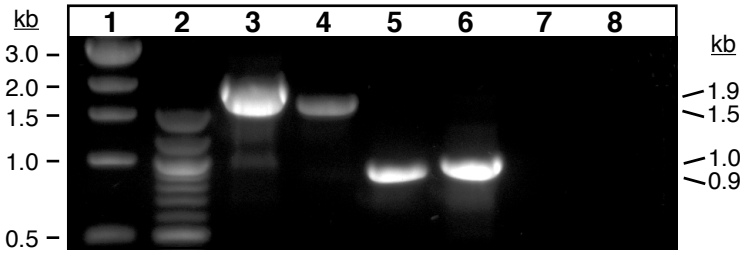


Figure S3. Enzyme inhibition assays. (a) Dose-response curves of each natural compound (in oxidized prodrug form) vs. HDAC1–4 and HDAC6–9. **(b)** Dose-response curves of each reduced compound (*, activated form) vs. HDAC1–4 and HDAC6–9. **(c)** Dose-response curves of compound **2** vs. HDAC3/N-CoR2 complex. Different H_2O_2 concentrations were applied to exclude reducing events. The dataset is normalized in respect to the standard dose-response curve of **2** in the absence of H_2O_2.

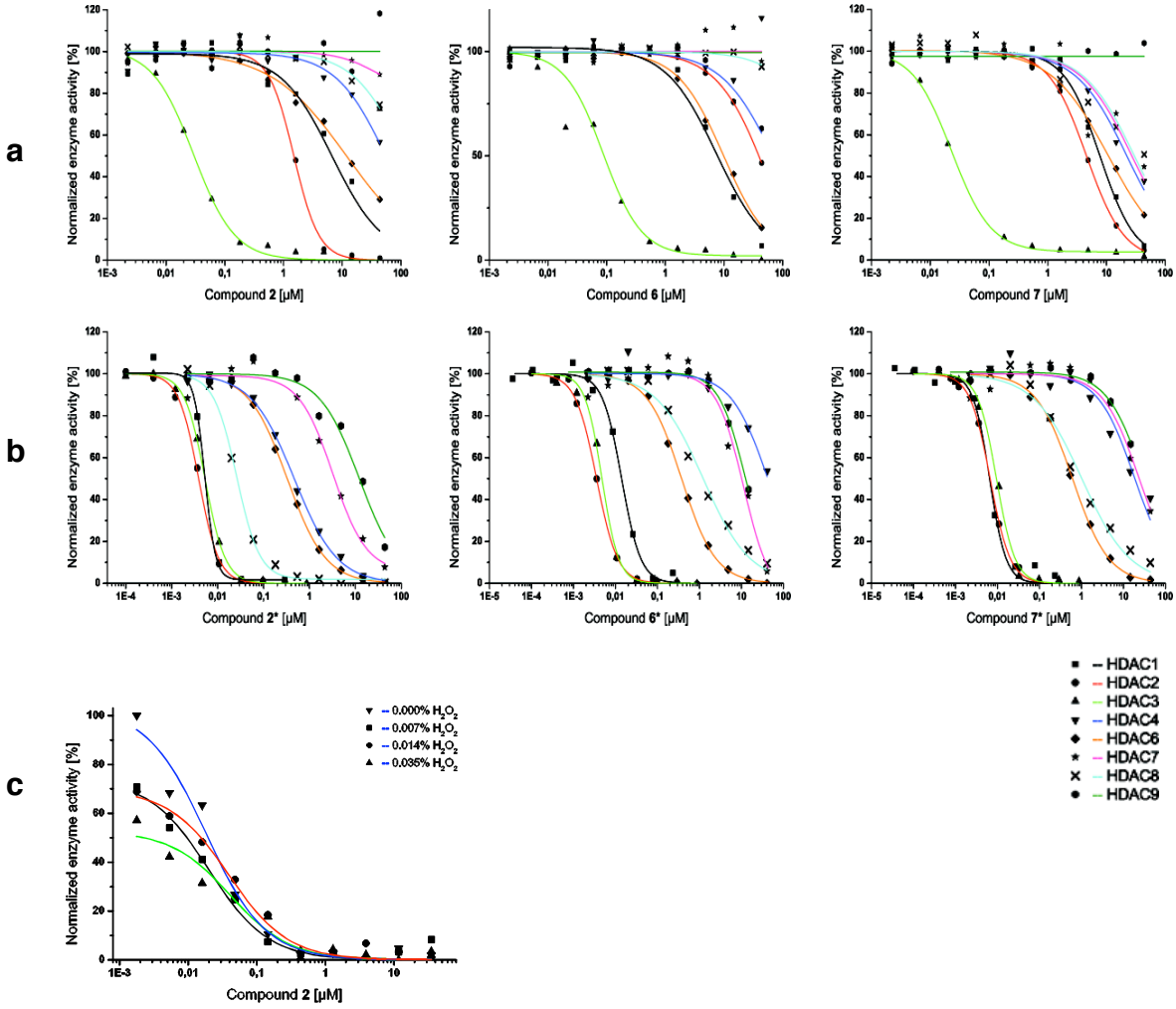


Figure S4. Chemical structure of thailandepsin A (6) (a) and thailandepsin B (7) (b) determined by NMR spectral analyses, and their key COSY and HMBC correlations.

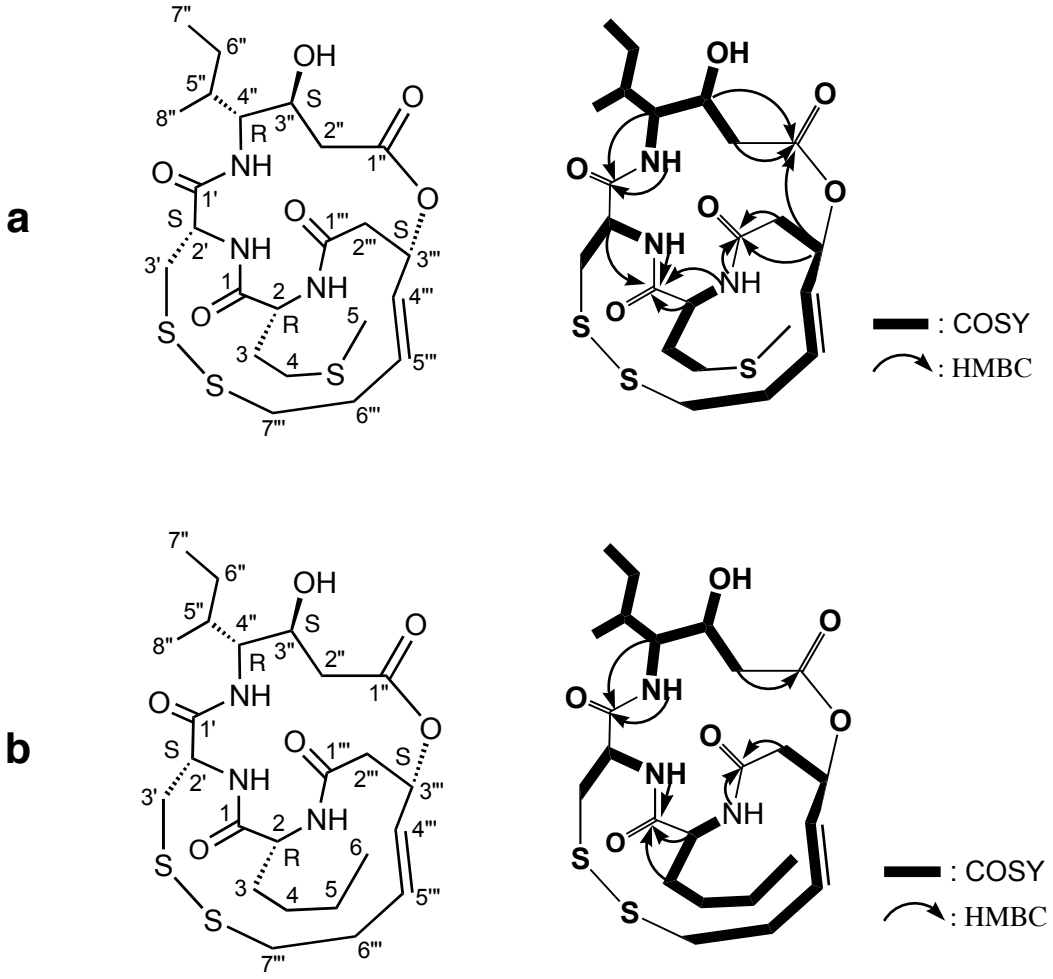
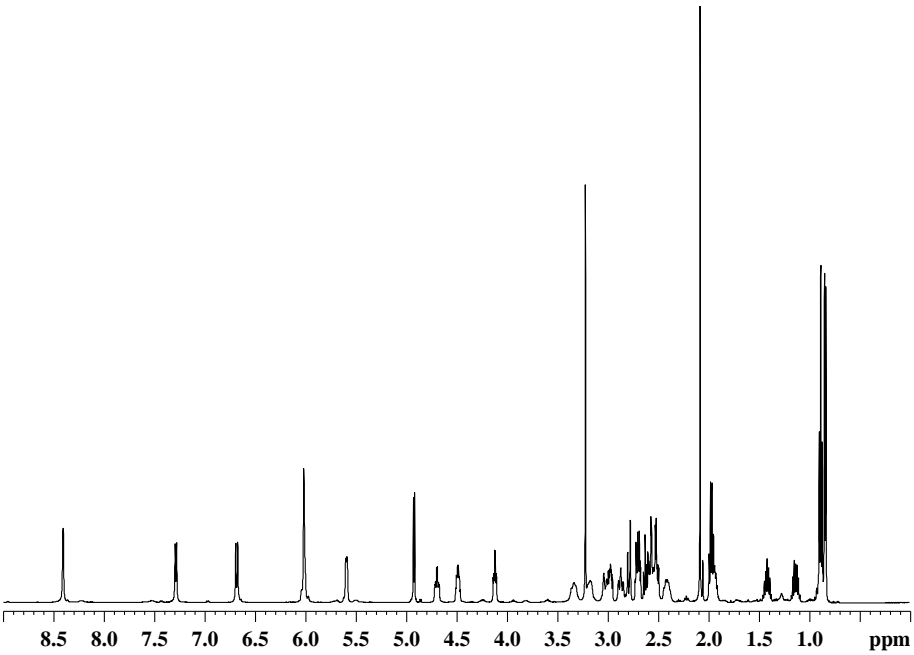
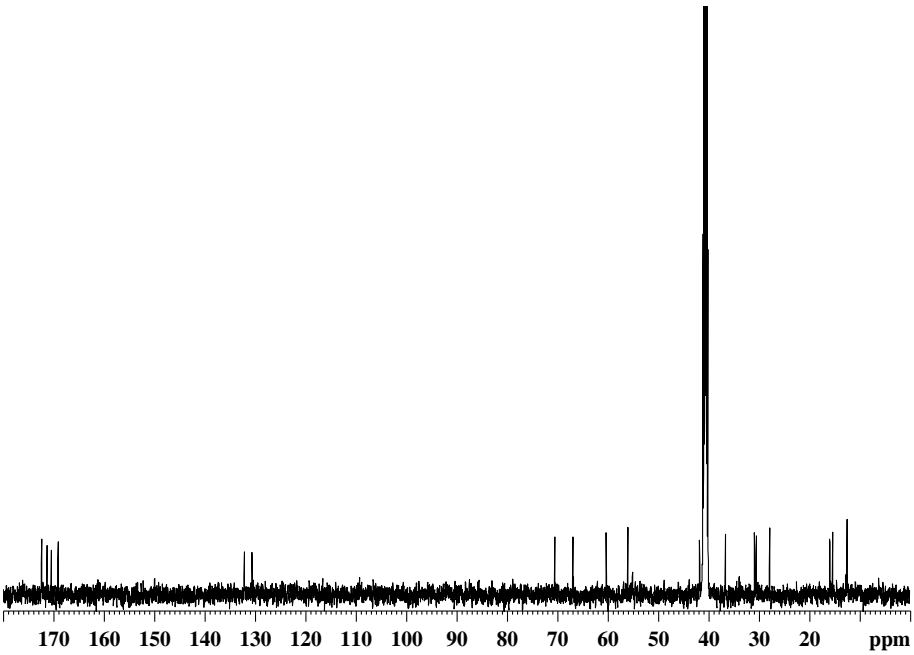


Fig. S6. NMR spectra of thailandepsin A

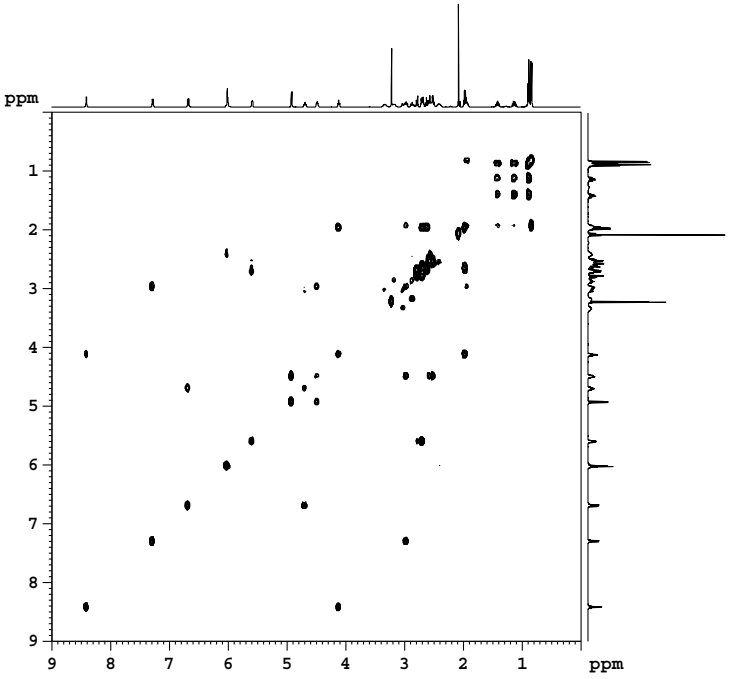


a ^{1}H NMR spectrum of thailandepsin A

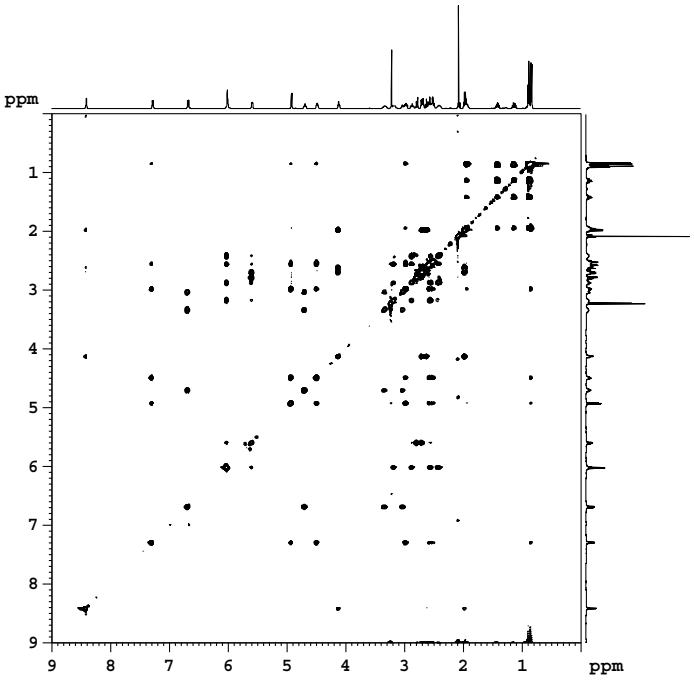


b ^{13}C NMR spectrum of thailandepsin A

Fig. S6. NMR spectra of thailandepsin A (continued)

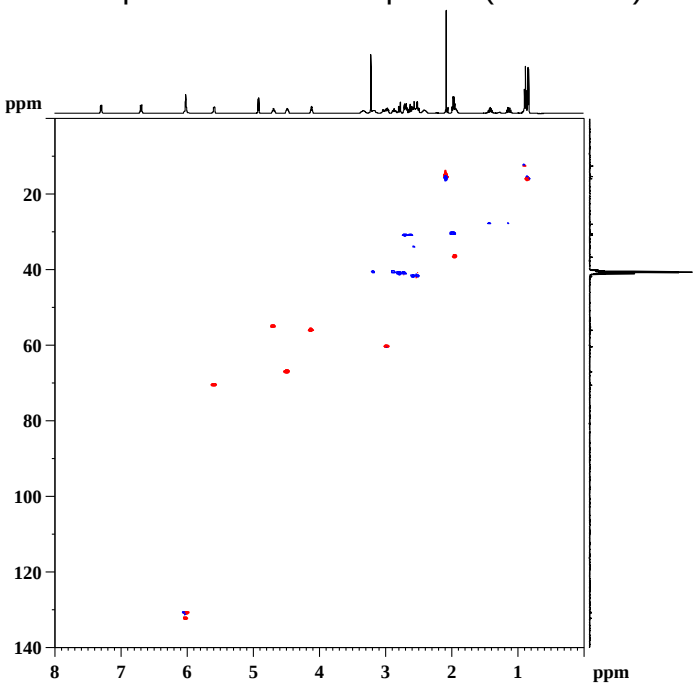


c gCOSY NMR spectrum of thailandepsin A

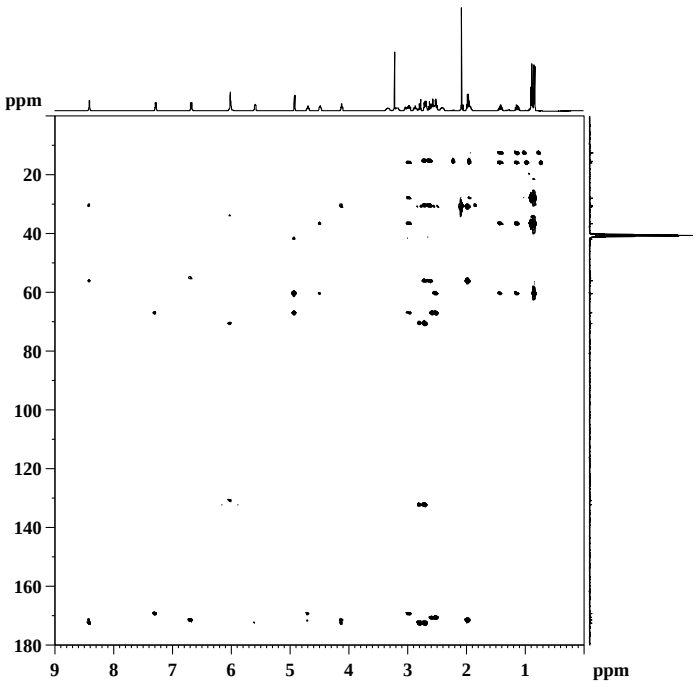


d TOCSY NMR spectrum of thailandepsin A

Fig. S6. NMR spectra of thailandepsin A (continued)

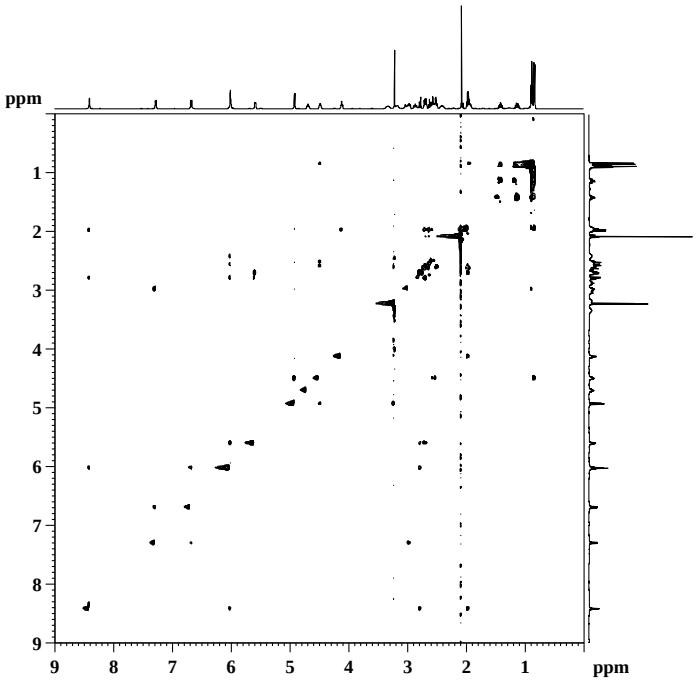


e edited HSQC NMR spectrum of thailandepsin A



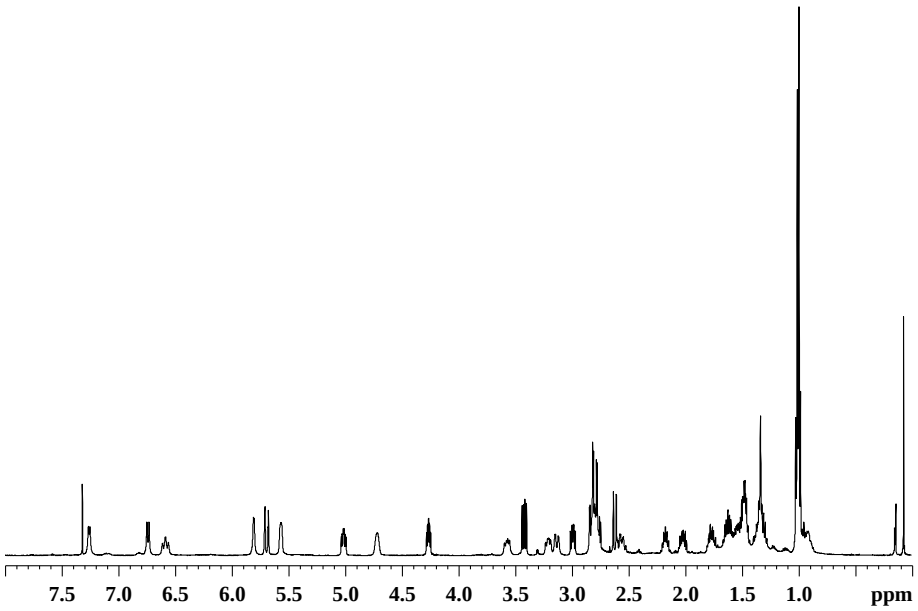
f HMBC NMR spectrum of thailandepsin A

Fig. S6. NMR sepctra of thailandepsin A (continued)

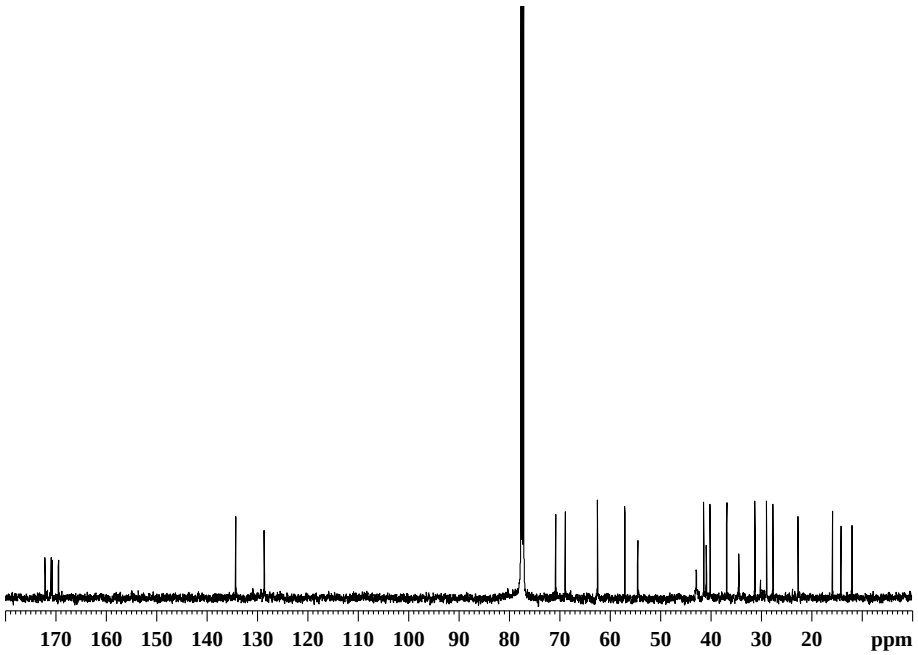


g NOESY NMR spectrum of thailandepsin A

Fig. S8. NMR sepctra of thailandepsin B

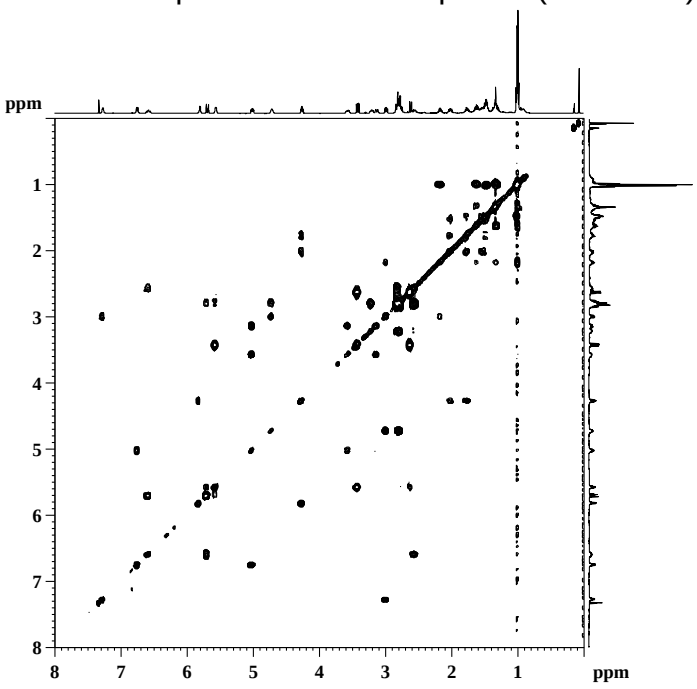


a ^{1}H NMR spectrum of thailandepsin B

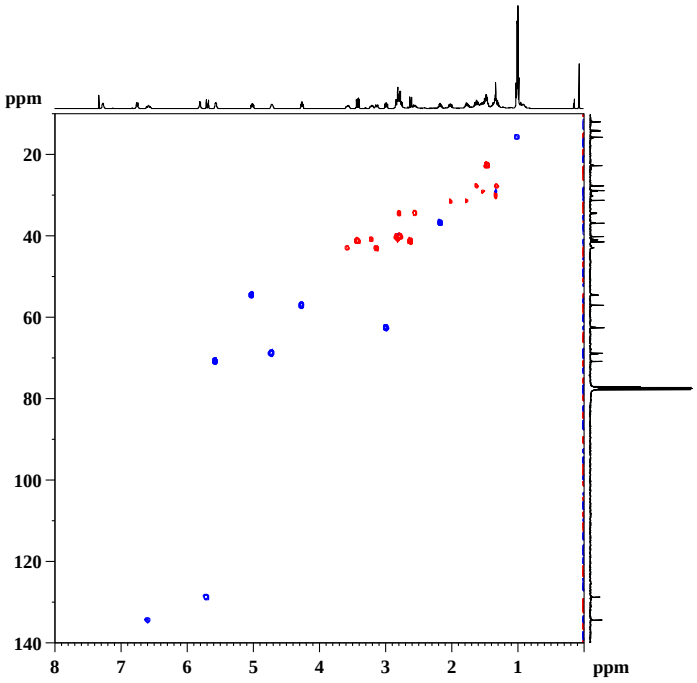


b ^{13}C NMR spectrum of thailandepsin B

Fig. S8. NMR spectra of thailandepsin B (continued)

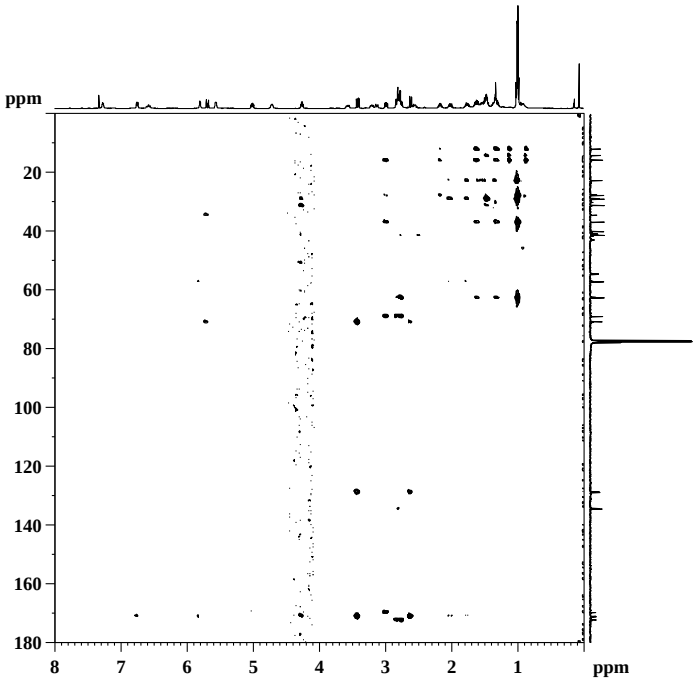


c gCOSY NMR spectrum of thailandepsin B



d edited HSQC NMR spectrum of thailandepsin B

Fig. S8. NMR spectra of thailandepsin B (continued)



e HMBC NMR spectrum of thailandepsin B

Figure S7. Phylogenetic relationship of human HDAC1–10. The phylogenetic tree was generated with ClustalW2 and edited with tree-viewing programs. Only the active-site regions (corresponding amino acids are in brackets) of HDACs were used for tree calculation. HDAC6 has two active-site regions that were considered separately. Different classes of HDACs are color-coded (class I = red; class IIa = dark blue; class IIb = light blue).

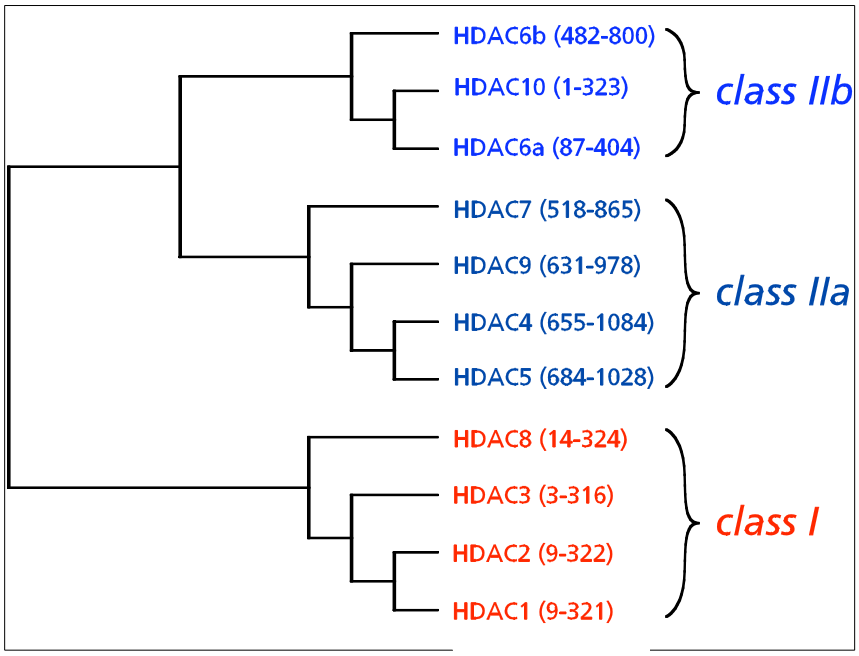
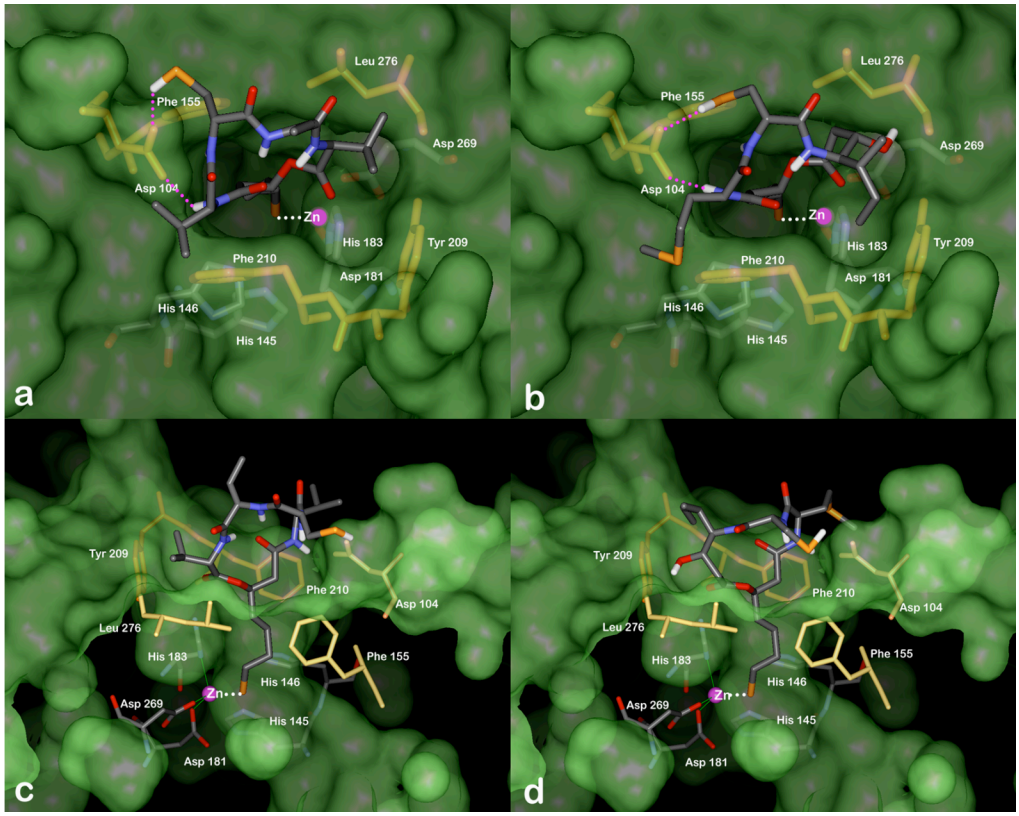


Figure S8. Top and side views of activated FK228 (2*; a and c) and thailandepsin A (6*; b and d) docking to the active site of HDAC2. Protein surface is shown in green, surface residues are marked in yellow. For clarity only the active site residues are annotated. Hydrogen bonds are shown in magenta dot line. For both compounds the thiol group binds to zinc atom located at the bottom of the 11 Å long channel. Activated thailandepsin B (**7***) has a docking conformation closely resembling **6*** therefore is omitted.



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