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Full Paper

Three-Component Aminoalkylations Yielding Dihydronaphthoxazine-Based Sirtuin Inhibitors: Scaffold Modification and Exploration of Space for Polar Side-Chains

Steffen Vojacek[1], Katja Beese[1], Zayan Alhalabi[2], Sören Swyter[3], Anja Bodtke[1], Carola Schulzke[4], Manfred Jung[3], Wolfgang Sippl[2], and Andreas Link[1]

[1] Institute of Pharmacy, University of Greifswald, Greifswald, Germany
[2] Institute of Pharmacy, Martin Luther University of Halle-Wittenberg, Halle/Saale, Germany
[3] Institute of Pharmaceutical Sciences, University of Freiburg, Freiburg, Germany
[4] Institute of Biochemistry, University of Greifswald, Greifswald, Germany

Nonpolar derivatives of heterocyclic aromatic screening hits like the non-selective sirtuin inhibitor splitomicin tend to be poorly soluble in biological fluids. Unlike sp^3-rich natural products, flat aromatic compounds are prone to stacking and often difficult to optimize into leads with activity in cellular systems. The aim of this work was to identify anchor points for the introduction of sp^3-rich fragments with polar functional groups into the newly discovered active ($IC_{50} = 5\ \mu M$) but nonpolar scaffold 1,2-dihydro-3*H*-naphth[1,2-*e*][1,3]oxazine-3-thione by a molecular modeling approach. Docking studies were conducted with structural data from crystallized human SIRT2 enzyme. Subsequent evaluation of the *in silico* hypotheses through synthesis and biological evaluation of the designed structures was accomplished with the aim to discover new SIRT2 inhibitors with improved aqueous solubility. Derivatives of 8-bromo-1,2-dihydro-3*H*-naphth[1,2-*e*][1,3]oxazine-3-thione N-alkylated with a hydrophilic morpholino-alkyl chain at the thiocarbamate group intended for binding in the acetyl-lysine pocket of the enzyme appeared to be promising. Both the sulfur of the thiocarbamate and the bromo substituent were assumed to result in favorable hydrophobic interactions and the basic morpholino-nitrogen was predicted to build a hydrogen bond with the backbone Ile196. While the brominated scaffold showed moderately improved activity ($IC_{50} = 1.8\ \mu M$), none of the new compounds displayed submicromolar activity. Synthesis and characterization of the new compounds are reported and the possible reasons for the outcome are discussed.

Keywords: Docking studies / Enzyme inhibitors / Epigenetics / Mannich reaction / Sirtuins

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Introduction

Human sirtuin-2 (SIRT2) is a ubiquitously expressed class III histone deacetylase (HDAC). HDACs of this class require nicotinamide adenine dinucleotide (NAD^+) for deacetylation of substrates [1]. In spite of the name histone deacetylases, sirtuins deacylate various other proteins than histones [2]. In the case of SIRT2, important substrates are transcription factors (FOXO1 [3] and FOXO3a [4]), α-tubulin [5], and glucose-6-phosphate dehydrogenase (G6PD) [6].

Even though the role of SIRT2 as a drug target is far from being settled, a recent study revealed that regulation of G6PD acetylation by SIRT2 is involved in the metabolic reprogramming of acute myeloid leukemia (AML) cells. Inhibition of SIRT2 through small-molecules or genetic

Correspondence: Prof. Andreas Link, Institute of Pharmacy, University of Greifswald, Friedrich-Ludwig-Jahn-Str. 17, 17487 Greifswald, Germany.
E-mail: link@uni-greifswald.de
Fax: +49 3834 4204895

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ablation seems beneficial for the treatment of a number of neurodegenerative diseases and AML. The molecular basis for interference with leukemia cell proliferation is based upon the pronounced dependence on the oxidative branch of the pentose phosphate pathway (PPP) in these malignant cells. The first and rate-limiting enzyme of the PPP is G6PD. It has been reported, that SIRT2 is overexpressed in clinical AML samples, while acetylation at lysine 403 of G6PD is downregulated and G6PD catalytic activity is increased in comparison to normal controls. Because SIRT2 deacetylates G6PD at K403, it promotes production of NADPH. Pharmacological inhibition of SIRT2 thus leads to reduced cell proliferation of leukemia cells, leaving normal hematopoietic stem and progenitor cells unaffected. For this and other reasons, SIRT2 may serve as a promising target for further therapeutic investigations [6].

Results and discussion

The non-selective sirtuin inhibitor splitomicin [7] (**1**) is a screening hit that was used as starting point for hit-to-lead optimization seeking novel chemotypes inhibiting SIRT2. Splitomicin (**1**) is a fragment-like, flat molecule with $M_r < 200$. The hydrolysis-sensitive lactone is inactive in cellular systems and was analyzed in structure–activity studies, resulting in β-phenyl-8-bromo derivatives such as compound **2** that shows a manifest improvement in terms of stability and target inhibition (Fig. 1). Because docking studies as well as biological tests with recombinant human SIRT2 indicated that the (*R*)-1-phenyl splitomicin derivative **3R** was markedly more active than its (*S*)-enantiomer **3S**, the individual investigation of enantiomers was regarded mandatory in order to obtain valid SAR data within this series of compounds [8].

Replacement of the hydrogen-bond acceptor oxygen atom of the lactone series by a nitrogen atom resulted in non-hydrolysable, biologically active lactames such as **4**. Again, separation of enantiomers was necessary in order to investigate the activity of the racemic mixture in more depth. In a different approach toward less hydrolysable analogs we made use of a variant of the Betti reaction [1], a classical three-component reaction. Instead of aromatic amines, urea or thiourea were reacted with suitable β-naphthols and aromatic aldehydes. In an operationally simple one-pot reaction, we were able to obtain compounds such as **5** and **6** in racemic form. While both of these new splitomicin analogs displayed the desired activity against the target enzyme in low micromolar concentrations in a trypsin-coupled homogeneous assay ($IC_{50} = 2.6 \pm 0.3\ \mu M$ for **5** and $IC_{50} = 6.7 \pm 0.9\ \mu M$ for **6**), the molecules still require laborious chiral separation and are characterized by pronounced lipophilicity. In order to tackle both of these issues at the same time, we designed achiral naphtho[1,3]oxazin-2-one **7** and naphtho[1,3]oxazine-2-thione **8** derivatives (Fig. 1) and synthesized these two compound series via solvent-free one-pot syntheses.

While these molecular frameworks are closely related to **3** in terms of hydrogen-bond-acceptor properties, the carbamate and thiocarbamate structures are much less sensitive to hydrolysis than the lactone ring of splitomicin (**1**). The shift of the substituent from the benzylic carbon atom to the nitrogen atom resulted in a still non-flat pattern but without the formation of a chiral center. The exchange of aromatic substituents in position R^2 for aliphatic side-chains with polar hetero atoms as solubility enhancing motifs was intended to cure the problems associated with poor solubility.

We started our docking study using hSIRT2 in complex with the inhibitor EX-243 ((1*S*)-6-chloro-2,3,4,9-tetrahydro-1*H*-carbazole-1-carboxamide, PDB ID 5D7P). Interestingly, two inhibitor molecules are interacting in the C- and extended-C

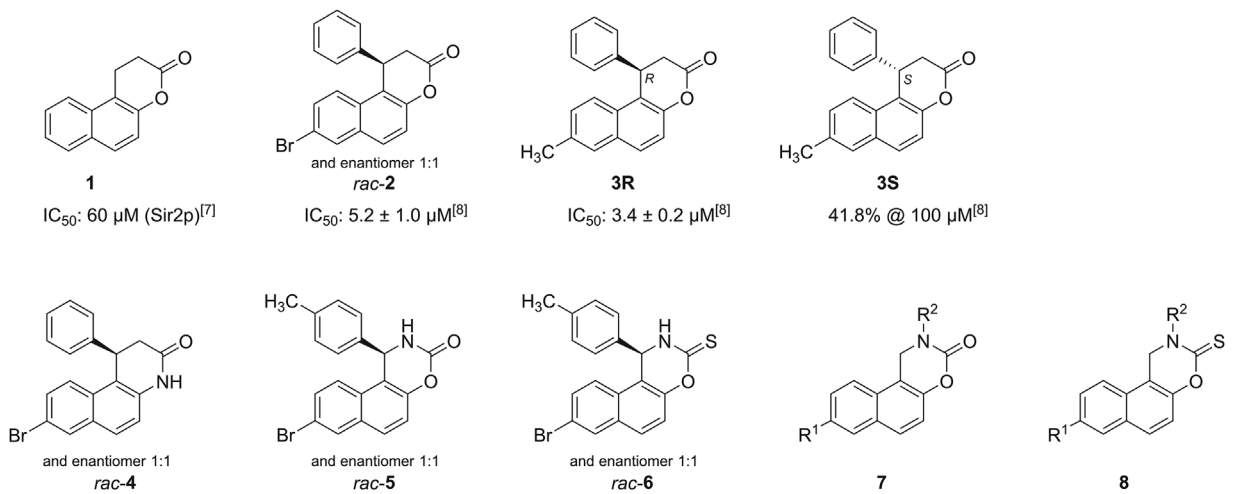


Figure 1. Splitomicin (**1**), hydrolysable derivatives (**2**–**3**) and less hydrolysable analogs (**4**–**8**).

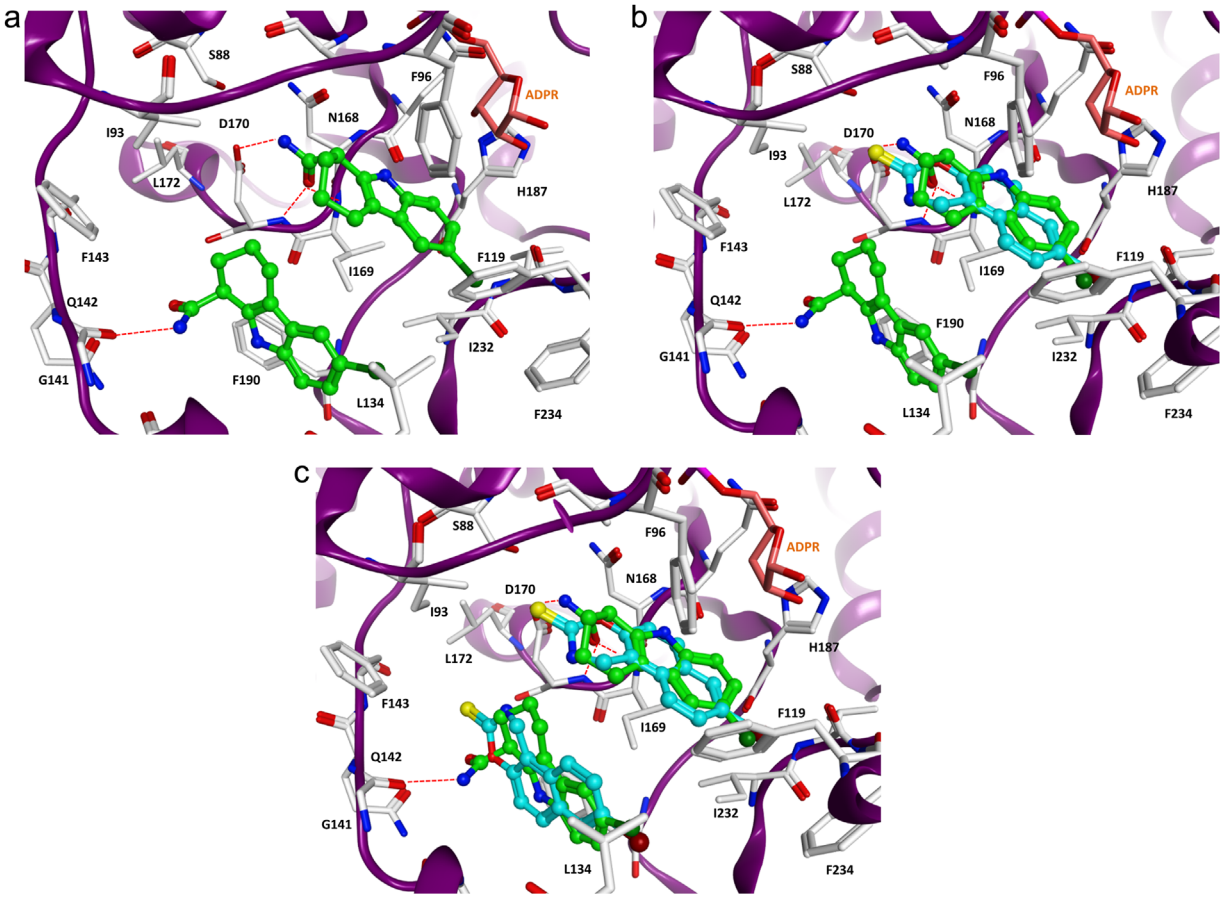


Figure 2. (a) Crystal structure of hSIRT2 complexed with the potent inhibitor EX-243 (green carbon atoms). Two inhibitor molecules are observed in the crystal structure, one bound to the nicotinamide site and one bound in the extended-C site of hSIRT2. Hydrogen bonds are shown as dashed lines. The cocrystallized ADPR molecule is colored in salmon. (b) Top-ranked docking solution for **8b** (cyan carbon atoms) in comparison to the cocrystallized inhibitor EX-243 (same coloring scheme as in a). (c) Docking solutions for **8b** (cyan carbon atoms) in comparison to the cocrystallized inhibitor EX-243 (same coloring scheme as in b).

site of hSIRT2 [9] (Fig. 2a). The docked 1,2-dihydro-*3H*-naphth[1,2-e][1,3]oxazine-3-thiones **8a** and **8b** were found to interact similarly as observed for Ex-243 (Fig. 2b). The sulfur atom of the thiocarbamate makes favorable hydrophobic interactions with Ile193 that might explain the loss of activity for the more polar carbamate derivatives. Moreover, the 8-bromo substituent indicated good hydrophobic interactions with the enzyme (Phe119, Leu134, Ile232). The tricyclic moieties of both inhibitors are nicely superimposed. We also observed that as in case of EX-243 (PDB ID 5D7P) and CHIC35 (PDB ID 5D7Q) two inhibitor molecules of **8a** and **8b** can be bound simultaneously in the hSIRT2 pocket. The top-ranked docking solution is found for the nicotinamide binding site whereas the second molecule interacts at the extended C-site. The high structural similarity to the cocrystallized EX-243 is obvious (Fig. 2c).

In case of the substituted derivatives, the hydrophilic morpholino-alkyl-chain in position R^2 was proposed to bind at the exit of the acetyl-lysine pocket of the enzyme (Fig. 3) where the second inhibitor molecule of EX-243 is bound in the hSIRT2 crystal structure.

Our initial synthetic strategy to access the target compound was to prepare an unsubstituted naphthoxazine thione (e.g., **8a**) with subsequent alkylation of the nitrogen atom. Key reaction of this approach was the formation of an electrophilic aromatic quinone methide *in situ* and subsequent oxazinthione-ring closure employing inorganic potassium thiocyanate [10].

Dimethylmethylidene ammonium iodide, a dimethylaminomethylation agent known as Eschenmoser's salt, was reacted with 2-naphthol under basic conditions, resulting in the formation of the corresponding Mannich bases **9** and **10**, respectively (Scheme 1). Using Eschenmoser's salt, superior regioselectivity and predominating mono-substitution compared to the classical Mannich reaction with formaldehyde and dimethyl amine, was anticipated [11]. In the next step, the

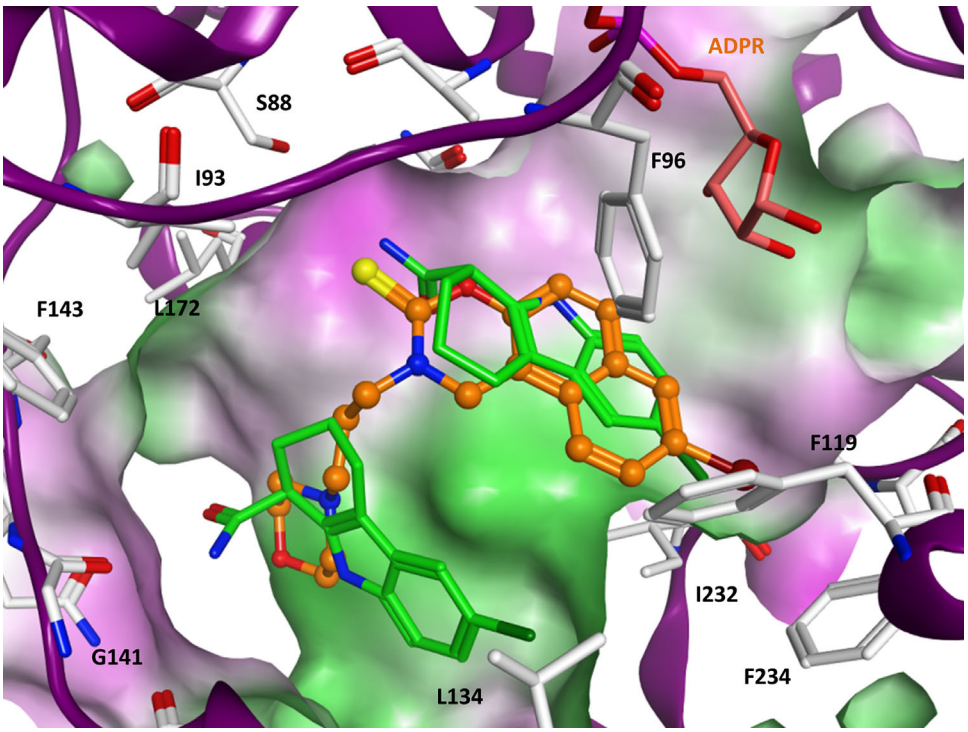
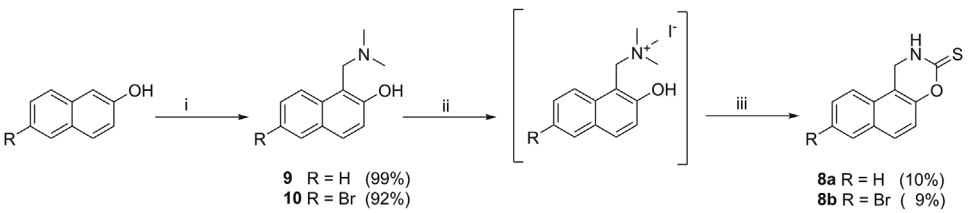
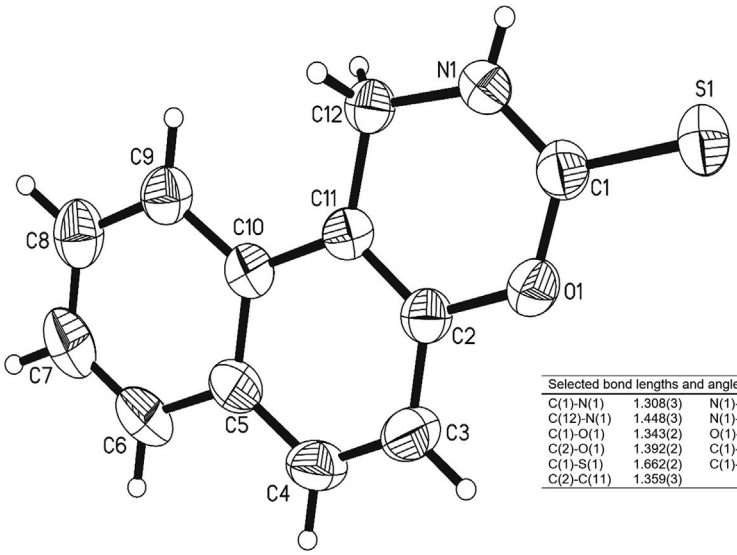


Figure 3. Docking result for the substituted derivative **8d** (orange carbon atoms). The two cocrystallized inhibitor molecules of EX-243 are shown as green sticks. The molecular surface of the binding pocket is displayed and colored according to the hydrophobicity (polar regions are colored magenta, hydrophobic regions are colored green).

resulting Mannich product was exhaustively alkylated with methyl iodide, and subsequently reacted with potassium thiocyanate [10]. Despite repeated attempts to purify the quaternized intermediate resulting from exhaustive alkylation by established procedures, we were unable to obtain the ammonium salts in pure form. While the progress of the reaction could be deduced from the precipitation observed, purification of the precipitate was neither possible via silica gel chromatography on normal nor on reversed phase, because of compound polarity. On normal silica gel phases, the compounds could not be eluted, because of the very strong interactions with the stationary phase. Quite to the contrary, preparative endcapped RP-18 HPLC columns were unsuitable, because of very weak retention of the compounds. Due to a lack of thermal- and photo-stability of the quaternary products, recrystallization was not effective, either. So the intermediate was collected by filtration, washed, dried, and used without further purifications for the next step. A mechanistic reason for the failure to isolate the quaternary compounds can be attributed to the possible formation of quinone methide species. Most probably, highly reactive and short-lived quinone methide intermediates were generated. While these quinone methides formed *in situ* were too unstable to be isolated, they could efficiently be trapped by reaction with thiocyanate ions to yield the desired isolated products [12]. In addition to analytical characterization by



Scheme 1. First synthetic strategy. Dimethylaminomethylation followed by exhaustive methylation and subsequent thiocarbamylation.



Selected bond lengths and angles in Å and °, respectively

C(1)-N(1)	1.308(3)	N(1)-C(1)-O(1)	118.81(18)
C(12)-N(1)	1.448(3)	N(1)-C(1)-S(1)	124.76(17)
C(1)-O(1)	1.343(2)	O(1)-C(1)-S(1)	116.43(16)
C(2)-O(1)	1.392(2)	C(1)-N(1)-C(12)	128.05(19)
C(1)-S(1)	1.662(2)	C(1)-O(1)-C(2)	120.38(16)
C(2)-C(11)	1.359(3)		

Figure 4. X-ray crystallography confirmed structure of compound **8a**. Ellipsoids are shown at the 50% probability level.

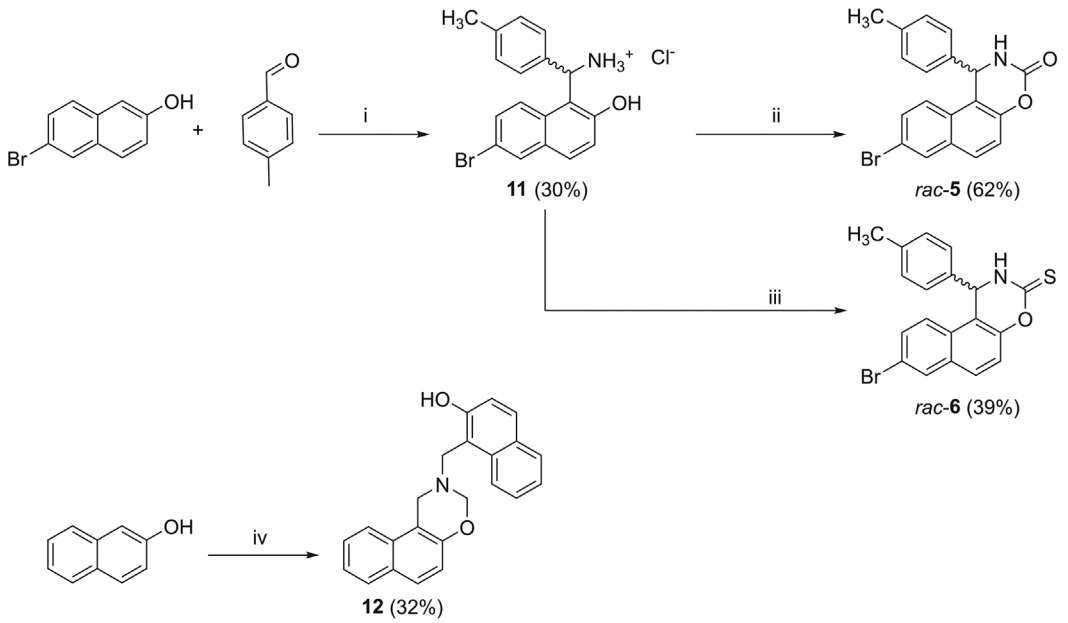
NMR and MS spectroscopy, the formation of the desired product **8a** was confirmed by X-ray crystallography (Fig. 4). The ring system under investigation contains a thiocarbamate with an exocyclic carbon sulfur double bond ROC(=S)NR_2, which can be called *O*-organyl thiocarbamate or thionourethane in order to indicate the difference to another isomeric thiocarbamate, with an exocyclic carbonyl group RSC(=O)NR_2, which could be called an *S*-organyl thiocarbamate or thiolurethane.

Because of the modest overall yield of the 1,2-dihydro-3*H*-naphth[1,2-*e*][1,3]oxazine-3-thiones **8a** and **8b** of not more than 10% (Scheme 1), an alternative route to the desired test compounds **8d** was investigated. In contrast to the strategy described above, we pursued a synthesis with 1-aminomethyl-2-naphthols as intermediates. It was known from the synthesis of **6**, that there are a number of suitable reagents, for instance thiophosgene [13], 1,1′-thiocarbonyldiimidazole [14] or carbon disulfide [15] available that are suited to transform the synthesized intermediate aminomethyl-2-naphthols into the desired heterocyclic carbamates **7** and thiocarbamates **8**.

The electrophilic aromatic substitution of an *in situ* formed benzaldimine with 2-naphthol was described by Betti even before the related 3-component reaction was reported by Mannich [16]. In a typical 3-component Betti reaction, 2-naphthol reacts in the presence of an ammonia source with benzaldehyde derivatives to give 1,3-diphenyl-2,3-dihydro-1*H*-naphth-[1,2-*e*][1,3]-oxazines. After hydrolysis of the intermediately formed N,O-acetals, the racemic Betti bases **11** with primary amino groups are formed (Scheme 2) [17]. Cyclization of compounds of type **11** yielded compounds **5** and **6**. To our understanding this 3-component aminoalkylation reaction can be interpreted as a Mannich reaction in the broadest sense, or with respect to the historical chronology, vice versa. However, despite mechanistic similarities, Mannich and Betti reactions differ by the use of a secondary amine in place of ammonia and an enolizable CH-acidic compound instead of an electron-rich 2-naphthol [18]. In certain cases, however, for instance in the synthesis of the alkaloid gramine by aminomethylation of indole, it could be argued that this atypical Mannich reaction could as well or even better be classified as atypical Betti reaction [19].

In order to synthesize compounds of classes **7** and **8**, benzaldehyde had to be replaced by paraformaldehyde in order to obtain 1-aminomethyl-2-naphthols with two benzylic protons as intermediates under comparable 3-component Betti reaction conditions. However, in our case this did not result in the anticipated formation of primary amines, but yielded the bis-Betti-product **12**. The attempted hydrolysis of the N,O-acetals **12** would not cleave the C–N single bond to the required primary amines. Thus the 1-aminomethyl-2-naphthol intermediate **17** was synthesized by reaction of 2-naphthol with methenamine in the presence of acetic acid with subsequent hydrolysis [20]. This so-called Duff reaction can be regarded as related or similar to the observation published by Mannich and Krösche [18], that methenamine reacted in acidic solution with antipyrine, an electron-rich aromatic drug, which led Mannich to the development of the synthetic use of this phenomenon. Whereas the unwanted reaction of methenamine and antipyrine results in an aminomethylene bonded trimeric pyrazolone compound [21], in the Duff reaction the bis-naphtol-derivative **13** (respectively, **14**) with an iminomethyl group is formed.

In the first step of this reaction, protonated methenamine, acting as an electrophile, forms the aminomethyl group as condensation product with naphthol. Remarkably, in the course of the reaction methenamine acts as an oxidizing agent, as well [22]. The N,N-acetal-carbon of methenamine is reduced to methylamine, meanwhile the compound is



i: 1: NH_4HCO_2, solvent free, 15 min, 110°C; 2: H_2O, HCl, 2 h, reflux; ii: CDI, THF, 10 min, μw 100°C; iii: TCDI, THF, 10 min, μw 100°C; iv: NH_4HCO_2, paraformaldehyde, DMF, 20 min, 120°C

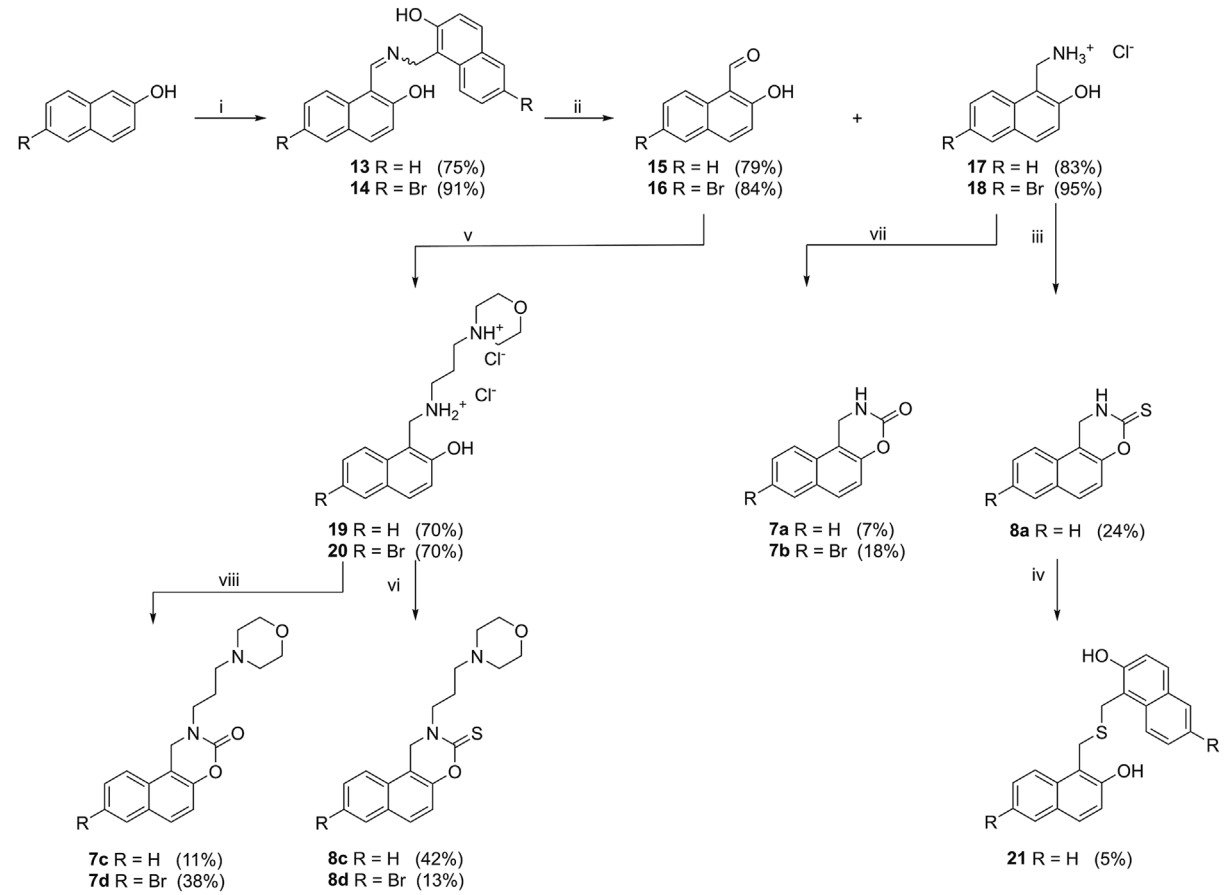
Scheme 2. Synthetic strategy. Betti reaction employing paraformadehyde instead of the benzaldehyde derivative.

oxidized to an imine [23]. In the next step, the imine concurrently reacts with another methanal molecule and a second molecule naphthol to form the anticipated dimer. Hydrolysis of this intermediate results in two different naphthol derivatives: the primary amine and the aldehyde (Scheme 3). Consequently, the Duff synthesis suffers from the fact, that only half of the starting 2-naphthol can be converted into the aldehyde. On the other hand, it is reported that the yield of the respective aldehyde could be improved by hydrolysis of the intermediate imine **13** in aqueous acetic acid. One hydrolysis product, the primary amine, becomes oxidized by remaining methenamine yielding 1-iminomethyl-2-naphthol which is hydrolyzed to the aldehyde, directly. Although the drawback of the Duff reaction to yield the aldehyde in only 50% could thus be overcome by the above operation [23], we pursued an alternate synthesis employing the Gattermann–Adams reaction (Scheme 4) [24]. Hence, the 2-naphthol derivative was stirred with zinc cyanide in diethyl ether. Subsequent treatment with hydrochloric acid gas released zinc chloride and likewise HCN, which was protonated to form the intermediate iminium ion. Additionally, the zinc ions catalyzed due to their Lewis acid character the aromatic substitution of the electrophilic iminium yielding more than 85% of the aldehyde (based on recovered starting material).

In the second step, formation of the 1,2-dihydro-3*H*-naphth[1,2-*e*][1,3]oxazine-3-thione ring was envisioned. In order to achieve this goal, the thiocarbamate synthesis using carbon disulfide under basic conditions seemed to be the most promising strategy. The 1-aminomethyl-2-naphthol reacted with CS_2 in basified methanol to a dithiocarbamate anion. The following addition of hydrogen peroxide caused the ring closure reaction to the thiocarbamate, presumably via an isothiocyanate intermediate [15]. Most likely, an intermediary dithiocarbamate disulfide [25] species emerged and subsequently disintegrated by ring closure reaction to the cyclic thiocarbamate.

In an analogous approach reported by Keck et al. [15], 2-hydroxymethyl aniline derivatives treated with one equivalent of triethylamine, three equivalents of carbon disulfide and two equivalents of hydrogen peroxide for disintegration yielded the inverse thionourethane in 78% yield. Under the same conditions but starting from aminomethyl-naphthol instead of 2-hydroxymethyl aniline, we were only able to gain the desired product in 24% yield despite performing several attempts. Possibly, the reason for the much lower yield in our case compared to the inverse oxazine-thione is the dissimilarity in acidity of the different starting materials. In contrast to the weakly basic aniline derivative with only one nucleophilic center employed by Keck et al. [15], 1-aminomethyl-naphth-2-ol possesses two potentially nucleophilic groups, a basic aliphatic amino group and a phenolic OH-group. Most likely in our hands the phenol was partly deprotonated, thus forming a strong nucleophile giving rise to the formation of xanthates as side-products and hampering the ring closure reaction.

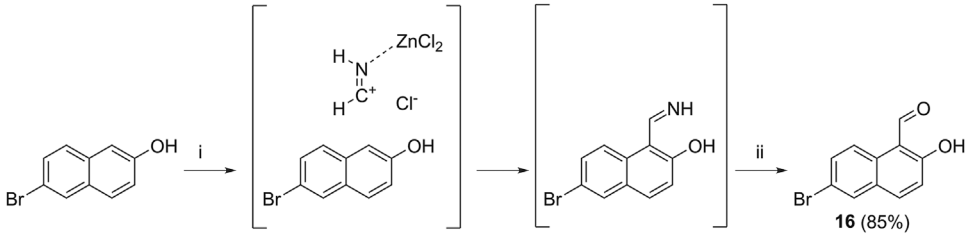
Once the envisioned thiocarbamate was synthesized in moderate yield, the derivatization was to be conducted with a strong base and subsequent addition of a alkyl halide in a



i: Methenamine, AcOH, 1h, 100°C; ii: EtOH, H_2O, HCl, 2h, reflux; iii: CS_2, NEt_3, MeOH, 1h, r.t.; H_2O_2, 1h; iv: NEt_3, THF, ethyl-3-bromopropionate, 0°C → r.t.; v: 3-morpholino-propylamine, MeOH, 2h, r.t.; $NaBH_4$, 1h, r.t.; HCl; vi: thiophosgene, NEt_3, DCM, 3h, 0°C → r.t.; vii: TEA, 4-nitrophenyl chloroformate, dioxane, 10 min, r.t.; viii: phosgene, TEA, toluene/water, **7c**: 6 h, r.t.; **7d**: 4 h, r.t. (**7d** was isolated as HCl salt)

Scheme 3. Third synthetic strategy. Duff reaction with different attempts toward derivatization.

third step [26]. In order to deprotonate the N–H group, triethylamine was added [27]. Despite cooling or changes in solvent properties, the addition of the alkyl halide (e.g., chloride, iodide, or bromide) caused decomposition of the thiocarbamate in all attempts. Most of the degradation products emerged as amorphous solid, insoluble in common solvents like DMSO or DMF. Therefore only the supernatant could be purified, yielding 5.5% of the 1,1′-[thiobis-(methylene)]bis(naphthalen-2-ol) as yellowish crystals. The isolated by-product indicated that once the sulfur was



i: $Zn(CN)_2$, HCl, DEE, 2h, r.t.; ii: H_2O, reflux

Scheme 4. Alternative synthesis route. Gattermann–Adams reaction.

alkylated, the sulfonium ion forced the cleavage to occur. Probably, a thiocyanante derivative was eliminated, yielding a naphthoquinone methide. The intermediate quinone methide as a strong nucleophile could react with another thiocarbamate as sulfur donating molecule. Eventually an intramolecular rearrangement caused the connection to the accessible thiomethyl-naphthol as nucleophile with subsequent elimination of a cyano-group containing molecule, explaining the formation of a thioether.

The rearrangement of substituted thiourea derivatives via methyl mercaptan elimination is described in the literature [28]. According to these reports, the methyl iodide reacts with the sulfur atom of thiourea derivatives. Upon basification, methyl mercaptan can be eliminated. Depending on the nucleophile, the compounds under investigation rearrange to cyclic isourea [13] or guanidine derivatives [29], respectively. Although the transformations discussed here are aimed at the formation of thionourethanes, from our preliminary observations together with the knowledge available from reports on thiourea rearrangement reactions, we draw the conclusion, that a different route toward these thiocarbamate derivatives had to be pursued. Based on our analysis of this problem, derivatization of carbamates appeared more suitable than alkylation of thiocarbonyl containing compounds. Thus, step three was reformed and divided into the synthesis of substituted carbamates and an additional step four, where the oxo groups should be exchanged for a thiono group with the help of Lawesson's reagent [30].

The conceptual basis for the intended carbamylation was the successful model reaction of **17** and **18** with 1-chloro-1-(4-nitrophenyl)formate (NPCF) in dioxane yielding **7a** and **8a**, respectively [31]. This transformation had allowed us to avoid highly toxic and hazardous reagents such as phosgene. On the other hand, the reaction produced only small amounts of the desired product, e.g., **7a** in 7% yield (Scheme 3). Hence, further experiments like derivatization or thionylation of **7a**, could not be pursued due to a lack of material.

Because the ring closure reaction of the 1-aminomethyl-2-naphthol to the thiocarbamate was effective but derivatization failed, and the alternative carbamylation route suffers from poor yields, synthetic access was attempted via derivatization of the 2-hydroxy-naphth-1-yl-methanal **15** using a modified reductive amination [32] with subsequent thiocarbamylation of the secondary amine. The aldehyde **15** was stirred with 3-morpholinopropan-1-amine in methanolic solution to form a yellowish imine. After a short reaction time, the reducing agent sodium borohydride was added [32].

Addition of solid borohydride in portions to the cooled solution led to a sluggish reaction with the formation of multiple by-products lowering yield and thwarting purification. With the aim of improving the outcome of this reaction, $NaBH_4$ was dissolved in a small amount of water and added as aqueous solution. After extraction and subsequent addition of HCl gas, the secondary amine could thus be obtained as its hydrochloride salt in almost 70% yield.

In the next step, thiophosgene was used to acquire the desired cyclic thiocarbamate in dichloromethane in good yields using triethylamine to neutralize released HCl. Alternatively, 1,1′-thiocarbonyldiimidazole (TCDI) was used as solid reactant. Advantageously, besides the low toxicity of TCDI, the released imidazole molecules made the addition of triethylamine unnecessary.

Overall, the synthesis of new test compounds with naphthoxazinethione scaffold and a hydrophilic side chain intended as water-soluble SIRT2 inhibitors was conducted and reactions were optimized. Key of the most suitable reaction was the formation of 2-hydroxy-1-naphthaldehyde by the Duff reaction, yielding more than 75% product referring to the use of two equivalents of 2-naphthol. Although the Gattermann–Adams reaction led to even higher yields (based on recovered starting material) of aldehyde **15** compared to the Duff synthesis, the Duff reaction using comparatively cheap and non-hazardous methenamine was advantageous with respect to improved safety, reduced expenditure and lower costs.

The following reductive amination was performed by combining both the formation of the imine and the reduction with sodium borohydride in one pot. The secondary amines **19** and **20** were gained as HCl-salt by treatment of extracted organic phases with hydrochloric acid gas. The conversion to the thionocarbamates was realized by the addition of thiophosgene and an auxiliary base. Compound **8c** was also synthesized from **19** by an alternative route using TCDI. Advantageously, the synthesis could be conducted without adding further bases, because released imidazole accepts protons from the hydrochloride salt **19**.

The 4-step synthesis for the bromo-substituted product **8d** could be achieved in a satisfactory overall yield of 26.6% using TCDI as ring closure reagent.

Selected compounds were tested for inhibition of human NAD-dependent deacetylase hSIRT2 in a fluorescence assay, with IC_{50} determined for the best inhibitors (Table 1, Fig. 5) [33]. Unexpectedly the thionocarbamates **8c** and **8d**, the most promising compounds revealed by prior docking studies on hSIRT2, showed only moderate inhibition. To the contrary, both the non-derivatized naphtho[1,3]oxazine-2-thione **8a** and its bromo derivative **8b** exhibited good inhibition. An explanation for the only moderate inhibition of **8c/8d** is the high hydrophobicity of the extended C-site (Leu138, Ile169, Phe190, Leu206). We calculated the hydrophobic interaction between a methyl probe and the extended C-site using the GRID approach [34]. The hydrophobicity of the extended-C site is also favorable for the binding of the lipophilic myristoyl chain of a substrate peptide (PDB ID 4Y6L) [35] (Fig. 6a and b).

The results of the biochemical assay revealed that both small molecules inhibited the enzyme with IC_{50} values of 5.0 and 1.8 μM, respectively. Apparently, the hydrophilic sp^3-rich side chain elongations hinder binding in the catalytic cleft, which was not expected from the anticipated docking pose. Nevertheless, the sulfur appeared to be important for

Table 1. Biological test data of some selected compounds against hSIRT2 in an assay with a fluorescence-labeled *N*-acetyl-lysine derivative (ZMAL) [36] trypsin-coupled homogeneous assay.

Entry	IC_{50} or percentual inhibition of hSirt2
Nicotinamide[a)]	49.8 ± 4.6 μM
rac-**5**	2.6 ± 0.3 μM
rac-**6**	6.7 ± 0.9 μM
7a	n.i.[b)] @ 25 μM
7b	n.i. @ 25 μM
7c	n.i. @ 25 μM
7d	n.s.[c)]
8a	5.0 ± 0.4 μM
8b	1.8 ± 0.3 μM
8c	11% @ 25 μM
8d	n.i. @ 25 μM
19	33% @ 25 μM
20	33% @ 25 μM
22	n.i.% @ 25 μM
23	19% @ 25 μM

[a)] Nicotinamide is shown as a reference inhibitor. [b)] n.i. = no inhibition (<10% inhibition). [c)] n.s. = not sufficiently soluble in DMSO.

inhibition whereas the oxo-derivatives, e.g., **7a** and **7b** showed no inhibition under the same conditions. Most likely the more space-filling and lipophilic sulfur-containing compounds could fit more suitably in the binding pocket with increased efficacy by interacting with Ile93 which is mainly driven by van-der-Waals interaction. In addition, the sulfur atom is known to be much more polarizable than the oxygen atom, which may account for the observed differences of closely related analogs as well.

Interestingly the amino-naphthol derivatives **19** and **20** appeared to be also moderate inhibitors of Sirt2. Possibly these derivatives with potential chelating properties were able to complex the allosteric zinc ion of the enzyme forcing a change in conformation resulting in decreasing its efficacy.

Conclusion

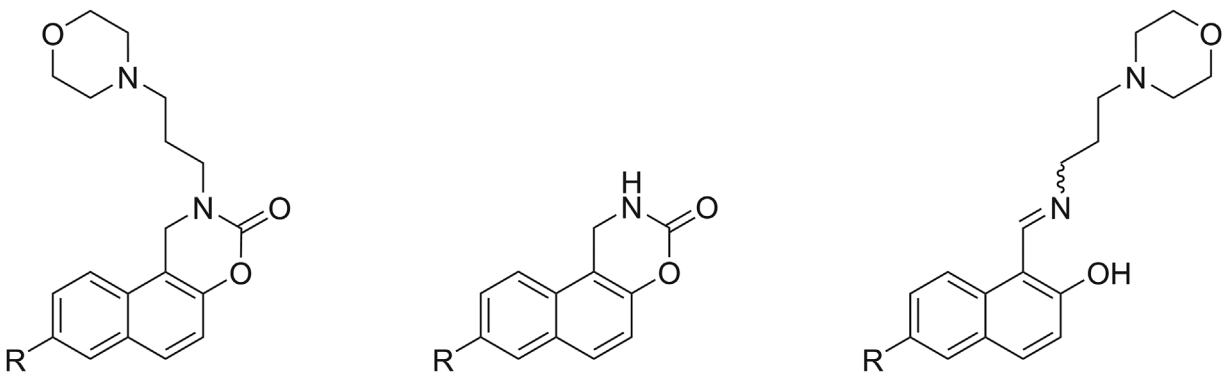
The synthesis of 1,2-dihydro-3*H*-naphth[1,2-e][1,3]oxazine-3-thiones **8** intended as intermediate led to the discovery of achiral splitomicin analogs with improved stability and significant biological activity. The most active new compound was the brominated scaffold 8-bromo-1,2-dihydro-3*H*-naphth[1,2-e][1,3]oxazine-3-thione **8b** [IC_{50} = 1.8 μM]. Because these novel scaffolds for diversity showed promising results, the designed N-alkylated derivatives with a hydrophilic morpholino-alkyl chain at the thiocarbamate that appeared to be promising were synthesized and tested. A thorough investigation of classical chemical name reactions was necessary in order to obtain the test candidates in practical yields for biological evaluation. While the predicted favorable hydrophobic interactions of the sulfur of the thiocarbamate and the bromo substituent resulted in the good activity of **8b**, the anticipated hydrogen bond of the basic morpholino-nitrogen with the backbone Ile196 did not, most likely due to the high hydrophobicity of this region. Soaking experiments with **8b** should give further insights and guide the way for an alternative position for the connection of additional binding motifs necessary for improved solubility and affinity.

Experimental

Chemistry

General remarks

All chemicals and solvents were purchased from commercial suppliers and used without further purification. Melting



7c R = H
7d R = Br

7a R = H
7b R = Br

22 R = H
23 R = Br

Figure 5. Additional target compounds which were tested in the biological assay.

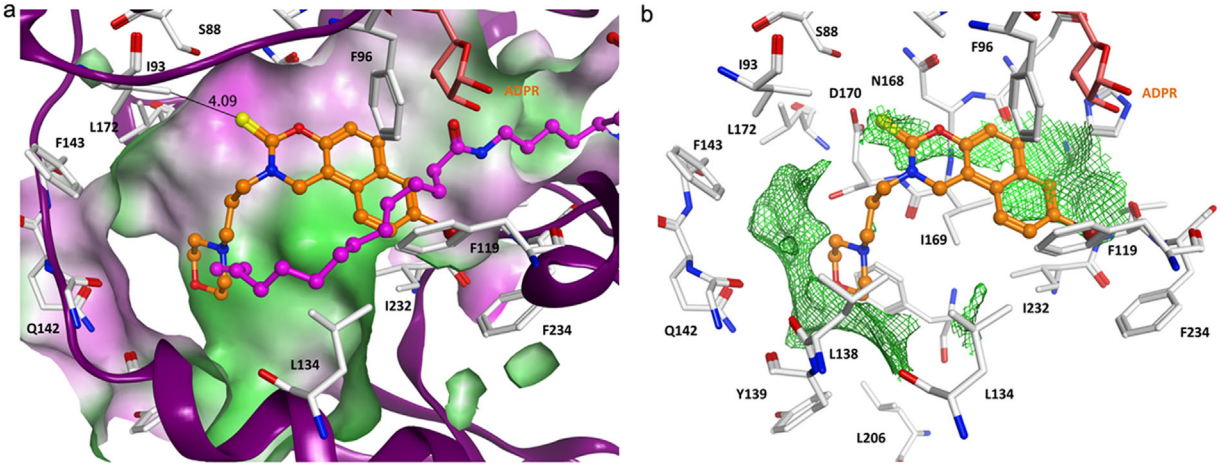


Figure 6. (a) Docking result of compound **8d** (orange carbon atoms) in comparison to the co-crystallized myristoyl-lysine peptide (magenta carbon atoms) which was found to be a substrate of hSIRT2 (PDB ID 4 × 3O). The distance between the thione sulfur and Ile93 is given in Å (same coloring scheme as in Fig. 3). (b) Hydrophobic interaction calculated for the extended C-site (shown as green contour plot, C3 probe, contour level −2.2 kcal/mol) (same coloring scheme as in panel a).

points were determined with a Büchi "Schmelzpunkt M-565" apparatus and are uncorrected. Microwave-assisted synthesis was performed using a microwave synthesis reactor Monowave 300 ("closed vessel" mode, G30-vials: 20 mL total capacity vessel, temperature control via IR sensor) from Anton Paar, while stirring at 600 rpm. NMR spectroscopic measurements were recorded with a Bruker Biospin Avance III Ultrashield 400 instrument (^{1}H: 400.2 MHz, ^{13}C: 100.6 MHz). Samples were dissolved in deuterated solvents, and chemical shifts (δ) in ^{1}H and ^{13}C NMR spectra are given in parts per million (ppm) with tetramethylsilane (TMS) signals as reference. Abbreviations are defined as follows: s = singlet, d = doublet, t = triplet, q = quartet. Mid-infrared spectra were recorded on an ALPHA FT-IR instrument from Bruker Optics with diamond ATR accessory. Elemental analyses (C, H, N, and S) were carried out with an Elementar Vario micro elemental analyzer. TLC was performed using pre-coated aluminum foil sheets silica gel 60 F354 provided by Merck. Flash chromatography and medium pressure liquid chromatography (MPLC) were performed using silica gel 60 (particle size 50–100 μm, 140–270 mesh provided by Macherey-Nagel) with Büchi devices C-630, C-601, C-615, and C-660 (column length 40 cm, column diameter 3.5 cm). High performance liquid chromatography (HPLC) was performed using Shimadzu devices CBM-20A, LC-20A P, SIL-20A, and FRC-10A with a SPD 20A UV-Vis detector and LiChrospher100 RP-18 endcapped (250 × 25 mm) HPLC columns.

The NMR spectra as well as the InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

Computational methods

3D structures of all compounds under study were generated from SMILES strings, and a subsequent energy minimization was carried out using the MMFF94× force field implemented in Molecular Operating Environment System (MOE) 2012.10 (Chemical Computing Group, Montreal, Canada). All compounds were used in their neutral form. A maximum of 100 conformations were generated for each ligand using the Conformational Search module implemented in MOE.

Sirt2 protein structures in complex with small molecule inhibitors were downloaded from the Protein Data Bank (PDB ID 5D7P and 5D7Q) [9]. Currently there are 21 crystal structures of human Sirt2 stored in the PDB. We took the protein structures cocrystallized with inhibitors structurally similar to the compounds under study. All protein structures were prepared by using the Structure Preparation module in MOE. Hydrogen atoms were added, for titratable amino acids the protonation state was calculated using the Protonate 3D module in MOE. Protein structure was energy minimized using the AMBER99 force field [37] using a tethering force constant of (3/2) kT/2 (σ = 0.5 Å) for all atoms during the minimization. AM1-BCC charges [38] were used for ligands. All molecules except the zinc ion were removed from the structures.

Protein-ligand docking was performed using program GOLD 5.2 [39]. Phe96 was used to define the size of the grid box (15 Å radius). The ligand was treated as flexible and 20 docking poses were calculated for each inhibitor. All other options were left at their default values. The top-ranked pose from each docking run was included in the final analysis and viewed graphically together with the protein structure using the program. Using the docking setup, the two cocrystallized inhibitors EX-243 and CHIR35 could be correctly docked into its crystal structure with RMSD values below 0.8 Å (PDB ID, 5D7P, and 5D7Q).

The hydrophobic interaction at the extended C-site was calculated using the GRID approach [34] implemented in the

MOE program. The interaction was calculated for the region around the docked inhibitor **8d** using the hydrophobic methyl probe (C3) and was contoured at an interaction energy level of −2.2 kcal/mol. The protein structure PDB ID 5D7P was used for the calculation.

Binding free energies for the inhibitors under study were calculated using the top-ranked docking poses. Structurally conserved water molecules included for docking studies were maintained during the geometry optimization of the complexes. The protein–inhibitor complexes were energy minimized using the AMBER PFROSST force field and the GBSA solvation model implemented in MOE 2012.10.

X-ray crystallography

A suitable single crystal of compound **8a** was mounted on a thin glass fiber coated with paraffin oil. X-ray single-crystal structural data were collected at room temperature using a STOE-IPDS 2T diffractometer equipped with a normal-focus, 2.4 kW, sealed-tube X-ray source with graphite-monochromated Mo K_α radiation ($\lambda = 0.71073$ Å). The program XArea was used for integration of diffraction profiles; a numerical absorption correction was applied with the programs X-Shape and X-Red32; all from STOE © 2010. The structure was solved by SIR92 [40] and refined by full-matrix least-squares methods using SHELXL-2013 [41]. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were refined isotropically on calculated positions using a riding model with their Uiso values constrained to 1.2 Ueq of their pivot atoms. All calculations were carried out using SHELXL-2013 and the WinGX system, Ver1.70.01.61. Basic crystallographic data and structure refinement parameters are summarized in Table 2. Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre with deposition number CCDC 1538210. Copies of the data can be obtained free of charge by contacting the CCDC via e-mail: deposit@ccdc.cam.ac.uk or its webpage https://www.ccdc.cam.ac.uk/.

Table 2. Crystal data and structure refinement parameters for 8a.

Parameters	8a
Formula	$C_{12}H_9NOS$
Formula weight	215.26
Crystal system	Monoclinic
Space group	$P2_1/n$
Z	4
a, Å	5.0351 (10)
b, Å	10.062 (2)
c, Å	19.736 (4)
β, deg	90.10 (3)
V, Å^3	999.9 (3)
T, K	293 (2)
$\lambda(Mo_{K\alpha})$, Å	0.71073
μ, mm^{-1}	0.291
D_{calcd}, g/cm^3	1.430
F (000)	448
Collected reflections	9967
Unique reflections	2690
R_{int}	0.0840
GOF on F^2	0.971
R_1[a], R_w[b] [$I > 2\sigma(I)$]	0.0467, 0.1067
R_1, R_w (all data)	0.1004, 0.1307
$\Delta\rho$ max/min (e Å^{-3})	0.260, −0.204

[a] $R_1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$. [b] $R_w = \left[\Sigma\left\{w\left(F_o^2 - F_c^2\right)^2\right\}/\Sigma\left\{w\left(F_o^2\right)^2\right\}\right]^{1-2}$.

*8-Bromo-1-(p-tolyl)-1,2-dihydro-3H-naphtho[1,2-e][1,3]-oxazin-3-one (rac-**5**)*

To a suspension of **11** (0.57 g, 1.5 mmol) in 10 mL tetrahydrofuran, carbonyl diimidazole (0.24 g, 1.5 mmol, 1.0 equiv.) was added in a G30 vial. The vial cap was closed and the vial was placed in the reaction chamber of the microwave reactor. The reaction was conducted at 250 W maximum to reach the reaction temperature of 100°C. Reaction temperature was held at 100°C for 10 min. Afterwards the solvent was evaporated and residue was crystallized from ethyl acetate/*n*-hexane, yielding compound *rac*-**5** as colorless needles. Yield: 0.34 g (62.3%); mp: 200.5°C; ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 2.23$ (s, 3H, C(14)H_3), 6.16 (s, 1H, C(9)H), 7.13 (d, 2H, C(12)H, C(12′)H, $J = 8.0$ Hz), 7.18 (d, 2H, C(11)H, C(11′)H, $J = 8.0$ Hz), 7.44 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.59 (d, 1H, C(7)H, $J = 9.2$ Hz), 7.75 (d, 1H, C(8)H, $J = 9.2$ Hz), 7.98 (d, 1H, C(4)H, $J = 8.8$ Hz), 8.24 (s, 1H, C(5)H), 8.86 (s, 1H, NH) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 20.6$ (C14), 53.3 (C9), 114.5 (C1), 118.2 (C6), 118.2 (C3), 125.4 (C8), 126.8 (C11, C11′), 127.6 (C8a), 129.4 (C4), 129.5 (C12, C12′), 130.1 (C7), 130.4 (C5), 131.7 (C4a), 137.4 (C13), 139.7 (C10), 147.7 (C2), 149.0 (C15) ppm; FT-IR ($\tilde{\nu}$): 3149 cm^{-1} (m), 1747 cm^{-1} (s), 1582 cm^{-1} (m), 1499 cm^{-1} (m), 1219 cm^{-1} (s), 1109 cm^{-1} (m); HRMS (ESI): found 368.0273, calcd. for $C_{19}H_{14}NO_2Br$ $[M+H]^+$ $m/z = 368.0281$.

*8-Bromo-1-(p-tolyl)-1,2-dihydro-3H-naphth[1,2-e][1,3]-oxazine-3-thione (rac-**6**)*

To a suspension of **11** (0.20 g, 0.5 mmol) in 10 mL tetrahydrofuran, 1,1′-thiocarbonyldiimidazole (0.13 g, 0.74 mmol, 1.4 equiv.) was added in a G30 vial. The vial cap was closed and the vial was placed in the reaction chamber of the microwave reactor. The reaction was conducted at 250 W maximum to reach the reaction temperature of 100°C within 5 min and temperature was held for 10 min. Afterwards the solvent was evaporated and residue was purified by column chromatography (*n*-hexane/ethyl acetate/acetic acid; 10:10:1). Product was crystallized from ethyl acetate/*n*-hexane, yielding compound as colorless needles. Yield: 0.08 g (38.6%); mp: 207.5°C; ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 2.23$ (s, 3H, C(14)H_3), 6.20 (s, 1H, C(9)H), 7.14 (d, 2H, C(12)H, C(12′)H, $J = 8.8$ Hz), 7.17 (d, 2H, C(11)H, C(11′)H, $J = 8.8$ Hz), 7.53 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.61 (d, 1H, C(7)H, $J = 8.8$ Hz), 7.74 (d, 1H, C(8)H, $J = 8.8$ Hz), 8.02 (d, 1H, C(4)H, $J = 9.2$ Hz), 8.28 (s, 1H, C(5)H), 11.11 (s, 1H, NH) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 20.6$ (C14), 53.5 (C9), 119.8 (C1), 117.5 (C3), 118.7 (C6), 125.4 (C8), 127.2 (C11, C11′), 127.3 (C8a), 129.5 (C12, C12′), 129.8 (C4), 130.3 (C7), 130.5 (C5), 132.2

(C4a), 137.8 (C13), 138.3 (C10), 146.3 (C2), 180.0 (C15) ppm; FT-IR ($\tilde{\nu}$): 3198 cm^{-1} (m), 1584 cm^{-1} (m), 1496 cm^{-1} (s), 1145 cm^{-1} (s), 1081 cm^{-1} (m); HRMS (ESI): found 384.0066, calcd. for $C_{19}H_{14}NOSBr$ $[M+H]^+$ $m/z = 384.0052$.

*1,2-Dihydro-3H-naphtho[1,2-e][1,3]oxazin-3-one (**7a**)*

To a solution of **17** (2.26 g, 10.8 mmol) in dioxane (20 mL) was added triethylamine (1.65 mL, 1.20 g, 11.8 mmol, 1.1 equiv.) while stirring under nitrogen atmosphere at room temperature. Subsequently, 4-nitrophenyl chloroformate (1.94 g, 9.6 mmol, 1.0 equiv.) pre-dispersed in dioxane (10 mL) was added dropwise. After addition was complete, the reaction was stirred for further 10 min and then solid was collected by filtration. Solid was dissolved in 50 mL dichloromethane and extracted 3 times with 30 mL ammonia-ammonium chloride buffer (pH 9). Addition of 5 mL acetic acid to organic phase caused a precipitation. Solid was recrystallized from toluene yielding product as white needle-shaped crystals. Yield: 0.138 g (7.2%); mp: 193.7°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 4.80$ (s, 2H, C(9)H_2), 7.23 (d, 1H, C(3)H, $J = 9.2$ Hz), 7.52 (t, 1H, C(6)H, $J = 7.6$ Hz), 7.60 (t, 1H, C(7)H, $J = 7.6$ Hz), 7.75 (d, 1H, C(8)H, $J = 8.2$ Hz), 7.91 (d, 1H, C(4)H, $J = 9.2$ Hz), 7.96 (d, 1H, C(5)H, $J = 8.0$ Hz), 8.23 (s, 1H, N(10)H) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 39.9$ (C9), 110.0 (C1), 116.6 (C3), 122.4 (C8), 125.1 (C6), 127.3 (C7), 128.4 (C5), 129.1 (C8a), 129.3 (C4), 129.9 (C4a), 147.0 (C2), 149.4 (C19) ppm; FT-IR ($\tilde{\nu}$): 3241 cm^{-1} (w), 3150 cm^{-1} (w), 1732 cm^{-1} (s), 1634 cm^{-1} (w), 1380 cm^{-1} (m), 1215 cm^{-1} (m), 1171 cm^{-1} (s), 743 cm^{-1} (s); $C_{12}H_9NO_2$ (199.06): calcd.: C, 72.35; H, 4.55; N, 7.03; found C, 72.54; H, 4.32; N, 7.41 [28].

*8-Bromo-1,2-dihydro-3H-naphtho[1,2-e][1,3]oxazin-3-one (**7b**)*

To a solution of **18** (1.13 g, 3.91 mmol) in dioxane (30 mL) was added triethylamine (2.44 mL, 1.77 g, 17.5 mmol, 4.5 equiv.) while stirring under nitrogen atmosphere at room temperature. Further 20 mL dioxane and 10 mL dimethylformamide were added because of poor solubility. Subsequently, 4-nitrophenyl chloroformate (0.75 g, 3.7 mmol, 1.0 equiv.) pre-dispersed in dioxane (10 mL) was added dropwise. After addition was complete, the reaction mixture was stirred for a further 10 min at room temperature and then heated up to reflux for 1 h. While heating reaction color had changed to orange. After reaction was stirred for further 10 h at room temperature, dioxane was removed in rotary evaporator. A solid precipitated when 30 mL dichloromethane and 30 mL aqueous sodium carbonate solution (8%) were added to oily residue. The solid was collected by filtration off and was recrystallized from toluene yielding product as beige needle-shaped crystals. Yield: 0.183 g (17.8%); mp: 231.9°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 4.78$ (s, 2H, C(9)H_2), 7.29 (d, 1H, C(3)H, $J = 9.2$ Hz), 7.68– 7.73 (m, 2H, C(7,8)H), 7.91 (d, 1H, C(4)H, $J = 8.8$ Hz), 8.24 (s, 1H, C(5)H), 8.25 (s, 1H, N(10)H) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 39.8$ (C9), 110.5 (C1), 118.0 (C3), 118.2 (C6), 124.9 (C8), 127.8 (C8a), 128.6 (C4), 130.1 (C7), 130.2 (C5), 131.2 (C4a), 147.4 (C2), 149.2 (C19) ppm; FT-IR ($\tilde{\nu}$): 3234 cm^{-1} (w), 3117 cm^{-1} (w), 2956 cm^{-1} (w), 1726 cm^{-1} (s), 1498 cm^{-1} (m), 1220 cm^{-1} (m), 808 cm^{-1} (s); $C_{12}H_8BrNO_2$ (276.97): calcd.: C, 51.83; H, 2.90; N, 5.04; found C, 51.82; H, 2.78; N, 5.43 [42].

*2-(3-Morpholinopropyl)-1,2-dihydro-3H-naphtho[1,2-e]-[1,3]oxazin-3-one (**7c**)*

To an emulsion of **19** (1.12 g, 3.0 mmol) in 40 mL toluene/water (1:1) was added triethylamine (2.51 mL, 1.82 g, 18.0 mmol) while stirring under argon atmosphere. To the ice-cooled reaction mixture, a 20% solution of phosgene in toluene (2.33 mL, 4.5 mmol) was added. Reaction was stirred for 6 h at room temperature and quenched by addition of ethyl acetate (40 mL) and 0.1 M hydrochloric acid solution (40 mL). The phases were separated and the organic phase was dried with sodium sulfate and concentrated *in vacuo*. Meanwhile, the aqueous phase was pH-adjusted to pH 9 with sodium carbonate and extracted with ethyl acetate (3×50 mL). Ethyl acetate phase was dried with sodium sulfate and hydrochloric acid gas was injected. The solid formed was collected by filtration, dissolved in 50 mL 1 M sodium hydroxide solution and extracted with ethyl acetate (3×50 mL). Organic phase was dried over sodium sulfate and concentrated *in vacuo*. Both residues were combined, purified by column chromatography (diethyl ether/isopropanol/ammonia, 10:3:0.1) and recrystallized from ethyl acetate/isopropanol (5:1) yielding product as colorless crystals. Yield: 0.11 g (11.0%); mp: 98.5°C; ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 1.83$–1.90 (m, 2H, C(12)H_2), 2.35 (t, 6H, C(13,15,18)H_2, $J = 6.8$ Hz), 3.50–3.54 (m, 6H, C(11,16,17)H_2), 4.93 (s, 2H, C(9)H_2), 7.24 (d, 1H, C(3)H, $J = 9.2$ Hz), 7.52 (t, 1H, C(6)H, $J = 7.6$ Hz), 7.63 (t, 1H, C(7)H, $J = 7.6$ Hz), 7.80 (d, 1H, C(8)H, $J = 8.0$ Hz), 7.92 (d, 1H, C(4)H, $J = 8.8$ Hz), 7.97 (d, 1H, C(5)H, $J = 8.0$ Hz) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 22.8$ (C12), 45.5 (C9), 47.3 (C11), 53.3 (C15,18), 55.5 (C13), 66.1 (C16,17), 110.3 (C1), 116.3 (C3), 122.4 (C8), 125.1 (C6), 127.3 (C7), 128.5 (C5), 128.9 (C8a), 129.3 (C4), 129.8 (C4), 129.8 (C4a), 146.6 (C2), 149.1 (C19) ppm; FT-IR ($\tilde{\nu}$): 3083 cm^{-1} (w), 2947 cm^{-1} (w), 1699 cm^{-1} (m), 1225 cm^{-1} (m), 822 cm^{-1} (m), 744 cm^{-1} (m); HRMS (ESI): found 327.1703, calcd. for $C_{19}H_{22}N_2O_3$ $[M+H]^+$ $m/z =$ 327.1703.

*8-Bromo-2-(3-morpholinopropyl)-1,2-dihydro-3H-naphtho[1,2-e][1,3]oxazin-3-one (**7d**)*

To an emulsion of **20** (0.45 g, 1.0 mmol) in 40 mL toluene/water (1:1) was added triethylamine (0.84 mL, 0.61 g, 6.0 mmol) while stirring under argon atmosphere. To the ice-cooled reaction mixture, a 20% solution of phosgene in toluene (0.77 mL, 1.5 mmol) was added and reaction was stirred for 4 h at room temperature. The emerged product as white solid was collected by filtration. More product was obtained by addition of 0.1 N hydrochloric acid solution (40 mL) to filtrate and following filtration. Then filtrate was pH adjusted to pH 11 and subsequently extracted with ethyl acetate (40 mL). Organic phase was dried with sodium sulfate and hydrochloric acid gas was injected. The emerged

white solid was collected by filtration. The solids were combined yielding product as white crystals. Yield: 0.17 g (38.4%); mp: 257.9°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 1.82–1.89$ (m, 2H, C(12)H_2), 2.35 (t, 6H, C(13,15,18)H_2, $J = 6.8$ Hz), 3.50–3.53 (m, 6H, C(11,16,17)H_2), 7.29 (d, 1H, C(3)H, $J = 8.8$ Hz), 4.91 (s, 2H, C(9)H_2), 7.43 (d, 1H, C(7)H, $J = 9.2$ Hz), 7.76 (d, 1H, C(8)H, $J = 8.8$ Hz), 7.91 (d, 1H, C(4)H, $J = 8.8$ Hz), 8.26 (s, 1H, C(5)H) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 22.7$ (C12), 45.4 (C9), 47.4 (C11), 53.3 (C15,18), 55.6 (C13), 66.2 (C16,17), 110.8 (C1), 117.6 (C3), 118.2 (C6), 124.8 (C8), 127.6 (C8a), 128.7 (C4), 130.1 (C7), 130.1 (C7), 130.3 (C5), 131.2 (C4a), 147.0 (C2), 148.9 (C19) ppm; FT-IR ($\tilde{\nu}$): 3075 cm^{-1} (w), 2981 cm^{-1} (w), 2548 cm^{-1} (w), 2468 cm^{-1} (w), 1716 cm^{-1} (m), 1108 cm^{-1} (m), 821 cm^{-1} (w); HRMS (ESI): found 405.0808, calcd. for $C_{19}H_{21}BrN_2O_3$ $[M+H]^+$ $m/z = 405.0808$.

*1,2-Dihydro-3H-naphth[1,2-e][1,3]oxazine-3-thione (**8a**)*
A suspension of **17** (3.13 g, 15.0 mmol) in methanol (40 mL) was stirred at room temperature. Successively triethylamine (3.1 mL, 2.25 g, 22.5 mmol, 1.5 equiv.) and carbon disulfide (2.72 mL, 3.43 g, 45.0 mmol, 3.0 equiv.) were added. The pale yellow solution was stirred for 1 h at room temperature. Via dropping funnel hydrogen peroxide (6.0 mL, 58.7 mmol, 3.9 equiv.) was added in such a manner as the solution boiled slightly. After addition was completed, the solution was allowed cooling to room temperature. After 1 h the formed precipitate was collected by filtration and washed with methanol (3 × 20 mL). Colorless solid was used in next step without further purification. Yield: 0.789 g (24.4%); mp: 181°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 4.80$ (s, 2H, C(9)H_2), 7.,32 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.55 (t, 1H, C(6)H, $J = 7.6$ Hz), 7.63 (t, 1H, C(7)H, $J = 7.6$ Hz), 7.75 (d, 1H, C(8)H, $J = 8.0$ Hz), 7.96 (d, 1H, C(4)H, $J = 8.8$ Hz), 7.99 (d, 1H, C(5)H, $J = 8.8$ Hz), 10.54 (s, 1H, N(10)H) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 40.06$ (C9), 109.4 (C1), 116.0 (C3), 122.6 (C8), 125.6 (C6), 127.5 (C7), 128.5 (C5), 128.9 (C8a), 129.6 (C4), 130.4 (C4a), 145.3 (C2), 180.9 (C19) ppm; FT-IR ($\tilde{\nu}$): 3164 cm^{-1} (w), 3085 cm^{-1} (w), 2993 cm^{-1} (w), 1636 cm^{-1} (w), 1580 cm^{-1} (m), 1175 cm^{-1} (s), 1149 cm^{-1} (s), 806 cm^{-1} (s), 746 cm^{-1} (s). Product was confirmed by X-ray crystallography.

*Alternative synthesis of 1,2-dihydro-3H-naphth[1,2-e][1,3]-oxazine-3-thione (**8a**)*
To a solution of **9** (1.71 g, 8.5 mmol) in dichloromethane (35 mL) was added methyl iodide (1.59 mL, 3.61 g, 25.4 mmol, 3.0 equiv.). After 7 h stirring at room temperature, the solid was collected by filtration and washed with dichloromethane (2 × 50 mL). Solid was used directly in the crude form for next step. So a suspension of crude intermediate product (1.722 g, 5.0 mmol) and potassium thiocyanate (0.99 g, 10.3 mmol) in butanone (50 mL) was heated to reflux. After 24 h, reaction mixture was allowed to cool down to room temperature and solvent was removed in rotary evaporator. Afterwards 100 mL 0.25 M aqueous hydrochloric acid solution was added and solid was collected by filtration. Crude product was washed with acetone (2 × 20 mL), H_2O (2 × 50 mL), methanol (2 × 50 mL) and was recrystallized from ethyl acetate, yielding product **8a** as colorless crystals. Yield: 0.18 g (9.8%); mp: 181°C (decomp.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 4.80$ (s, 2H, C(9)H_2), 7.,32 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.55 (t, 1H, C(6)H, $J = 7.6$ Hz), 7.63 (t, 1H, C(7)H, $J = 7.6$ Hz), 7.74 (d, 1H, C(8)H, $J = 8.4$ Hz), 7.95 (d, 1H, C(4)H, $J = 9.2$ Hz), 7.98 (d, 1H, C(5)H, $J = 8.0$ Hz), 10.56 (s, 1H, N(10)H) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 40.1$ (C9), 109.4 (C1), 116.0 (C3), 122.6 (C8), 125.6 (C6), 127.5 (C7), 128.5 (C5), 128.9 (C8a), 129.6 (C4), 130.4 (C4a), 145.3 (C2), 180.9 (C19) ppm; FT-IR ($\tilde{\nu}$): 3164 cm^{-1} (w), 3085 cm^{-1} (w), 2993 cm^{-1} (w), 1636 cm^{-1} (w), 1580 cm^{-1} (m), 1175 cm^{-1} (s), 1149 cm^{-1} (s), 806 cm^{-1} (s), 746 cm^{-1} (s). Product confirmed by X-ray crystallography.

*8-Bromo-1,2-dihydro-3H-naphth[1,2-e][1,3]oxazine-3-thione (**8b**)*
To a solution of **10** (3.08 g, 11.0 mmol) in dichloromethane (20 mL) was added methyl iodide (2.06 mL, 4.68 g, 33.0 mmol, 3.0 equiv.). After 7 h stirring at room temperature the solid was collected by filtration and washed with dichloromethane (2 × 50 mL). Solid was used directly in the crude form for next step. So a suspension of crude intermediate product (2.988 g, 7.1 mmol) and potassium thiocyanate (1.37 g, 14.1 mmol) in butanone (60 mL) was heated to reflux. After 24 h, reaction mixture was allowed to cool down to room temperature. Afterwards 10 mL 1 M aqueous hydrochloric acid solution were added and solid was collected by filtration. Crude product was washed with acetone (2 × 20 mL), H_2O (2 × 50 mL), methanol (2 × 50 mL) and was recrystallized from ethyl acetate, yielding product **8b** as colorless crystals. Yield: 0.31 g (9.4%); mp: 255°C (decomp.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 4.79$ (s, 2H, C(9)H_2), 7.38 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.72 (m, 2H, C(4)H, C(8)H), 7.95 (d, 1H, C(7)H, $J = 9.2$ Hz), 8.28 (s, 1H, C(5)H), 10.58 (s, 1H, NH) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 40.1$ (C9), 110.0 (C1), 117.4 (C3), 118.7 (C6), 125.0 (C8), 127.6 (C8a), 128.9 (C7), 130.3 (C4, C5), 131.7 (C4a), 145.6 (C2), 180.7 (C=S) ppm; FT-IR ($\tilde{\nu}$): 3343 cm^{-1} (m), 3065 cm^{-1} (w), 1635 cm^{-1} (m), 1502 cm^{-1} (s), 1173 cm^{-1} (s), 1139 cm^{-1} (s); HRMS (ESI): found 293.9569, calcd. for $C_{12}H_8BrNOS$ $[M+H]^+$ $m/z = 293.9583$.

*2-(3-Morpholinopropyl)-1,2-dihydro-3H-naphth[1,2-e]-[1,3]oxazine-3-thione (**8c**)*
To a suspension of **19** (0.75 g, 2.0 mmol) in dichloromethane (40 mL) triethylamine (1.39 mL, 1.01 g, 10.0 mmol) was added under argon atmosphere. While stirring in an ice bath, thiophosgene (0.25 mL, 0.37 g, 3.0 mmol) dissolved in 10 mL dichloromethane was added dropwise. After addition was complete, ice bath was removed and reaction was stirred for 2 h at room temperature. Reaction mixture was extracted with water (2 × 20 mL) and finally organic phase was extracted with 0.5 N hydrochloric acid (20 mL). Dichloromethane phase was dried with anhydrous sodium sulfate and concentrated *in vacuo*. Residue was diluted in less dichloromethane and extracted with aqueous sodium hydroxide solution (2 × 10 mL). Organic phase was dried with

sodium sulfate and concentrated *in vacuo*. Solid residue was recrystallized from ethyl acetate/isopropanol (5:1) yielding pale brownish crystals. Yield: 0.32 g (42.3%); mp: 144°C; ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 1.99–2.07$ (m, 2H, C(12)H_2), 2.35–2.41 (m, 6H, C(13,15,18)H_2), 3.48 (t, 4H, C(16,17)H_2, $J = 4.6$ Hz), 4.03 (t, 2H, C(11)H_2, $J = 7.2$ Hz), 4.54 (s, 2H, C(9)H_2), 7.32 (d, 1H, C(3)H, $J = 9.2$ Hz), 7.56 (t, 1H, C(6)H, $J = 7.6$ Hz), 7.65 (t, 1H, C(7)H, $J = 7.6$ Hz), 7.82 (d, 1H, C(8)H, $J = 8.0$ Hz), 7.96 (d, 1H, C(4)H, $J = 8.8$ Hz), 7.99 (d, 1H, C(5)H, $J = 8.0$ Hz) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 21.3$ (C12), 46.3 (C9), 53.2 (C15,18), 53.9 (C11), 55.4 (C13), 66.1 (C16,17), 110.2 (C1), 115.5 (C3), 115.5 (C3), 122.4 (C8), 125.6 (C6), 127.5 (C7), 128.6 (C8a,5), 129.6 (C4), 130.3 (C4a), 144.8 (C2), 179.9 (C19) ppm; FT-IR ($\tilde{\nu}$): 3054 cm^{-1} (w), 2976 cm^{-1} (w), 1200 cm^{-1} (m), 1110 cm^{-1} (s), 811 cm^{-1} (m), 745 cm^{-1} (m); HRMS (ESI): found 343.1475, calcd. for $C_{19}H_{22}N_2O_2S$ $[M+H]^+$ $m/z = 343.1475$.

*Alternative synthesis of 2-(3-morpholinopropyl)-1,2-dihydro-3H-naphth[1,2-e][1,3]oxazine-3-thione (**8c**)*

To a suspension of **19** (1.12 g, 3.0 mmol) in tetrahydrofuran (10 mL) was added 1,1′-thiocarbonyldiimidazole (0.54 g, 3.0 mmol) pre-dissolved in 5 mL tetrahydrofuran while stirring under argon atmosphere. Reaction mixture was stirred 43 h at room temperature, and then solvent was removed in rotary evaporator. Aqueous sodium hydroxide solution (50 mL, 1 N) was added to residue and solution was extracted with ethyl acetate (3 × 50 mL). Organic phases were combined and washed with water (2 × 30 mL). Ethyl acetate phase was dried over anhydrous sodium sulfate and concentrated *in vacuo*. Residue was recrystallized from ethyl acetate/isopropanol (5:1) yielding desired product as pale brownish crystals. Yield: 0.52 g (50.0%); mp: 144°C; ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 1.99–2.07$ (m, 2H, C(12)H_2), 2.35–2.41 (m, 6H, C(13,15,18)H_2), 3.48 (t, 4H, C(16,17)H_2, $J = 4.6$ Hz), 4.03 (t, 2H, C(11)H_2, $J = 7.2$ Hz), 4.54 (s, 2H, C(9)H_2), 7.32 (d, 1H, C(3)H, $J = 9.2$ Hz), 7.56 (t, 1H, C(6)H, $J = 7.6$ Hz), 7.65 (t, 1H, C(7)H, $J = 7.6$ Hz), 7.82 (d, 1H, C(8)H, $J = 8.0$ Hz), 7.96 (d, 1H, C(4)H, $J = 8.8$ Hz), 7.99 (d, 1H, C(5)H, $J = 8.0$ Hz) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 21.3$ (C12), 46.3 (C9), 53.2 (C15,18), 53.9 (C11), 55.4 (C13), 66.1 (C16,17), 110.2 (C1), 115.5 (C3), 115.5 (C3), 122.4 (C8), 125.6 (C6), 127.5 (C7), 128.6 (C8a,5), 129.6 (C4), 130.3 (C4a), 144.8 (C2), 179.9 (C19) ppm; FT-IR ($\tilde{\nu}$): 3054 cm^{-1} (w), 2976 cm^{-1} (w), 1200 cm^{-1} (m), 1110 cm^{-1} (s), 811 cm^{-1} (s), 745 cm^{-1} (s).

*8-Bromo-2-(3-morpholinopropyl)-1,2-dihydro-3H-naphth[1,2-e][1,3]oxazine-3-thione (**8d**)*

To a suspension of **20** (0.90 g, 2.0 mmol) in dichloromethane (15 mL), triethylamine (1.39 mL, 1.01 g, 10.0 mmol) was added under argon atmosphere. While stirring in an ice bath, thiophosgene (0.25 mL, 0.37 g, 3.0 mmol) dissolved in 10 mL dichloromethane was added dropwise. After addition was complete, ice bath was removed and reaction was stirred for 3 h at room temperature. Reaction mixture was extracted with water (2 × 20 mL) and afterwards organic phase was extracted with 0.5 M hydrochloric acid (20 mL). Finally the dichloromethane phase was extracted with aqueous sodium hydroxide solution (2 × 10 mL), dried with anhydrous sodium sulfate and concentrated *in vacuo*. Residue was purified with column chromatography (diethyl ether/isopropanol/25% ammonia solution, 10:3:0.1) yielding product as pale brownish platelets. Yield: 0.11 g (12.9%); mp: 167°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 1.99–2.06$ (m, 2H, C(12)H_2), 2.35–2.41 (m, 6H, C(13,15,18)H_2), 3.47 (t, 4H, C(16,17)H_2, $J = 4.4$ Hz), 4.01 (t, 2H, C(11)H_2, $J = 7.4$ Hz), 5.07 (s, 2H, C(9)H_2), 7.38 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.78 (s, 1H, C(7,8)H), 7.95 (d, 1H, C(4)H, $J = 8.8$ Hz), 8.30 (s, 1H, C(5)H) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 21.3$ (C12), 46.1 (C9), 53.2 (C15,18), 54.0 (C11), 55.4 (C13), 66.1 (C16,17), 110.7 (C1), 116.9 (C3), 118.7 (C6), 124.8 (C8), 127.3 (C8a), 128.9 (C4), 130,3 (C7), 130.4 (C5), 131.6 (C4a), 145.1 (C2), 179.7 (C19) ppm; FT-IR ($\tilde{\nu}$): 3068 cm^{-1} (w), 2950 cm^{-1} (w), 1536 cm^{-1} (m), 1353 cm^{-1} (m), 1204 cm^{-1} (m), 1109 cm^{-1} (m), 808 cm^{-1} (m); HRMS (ESI): found 443.0399, calcd. for $C_{19}H_{21}BrN_2O_2S$ $[M+Na]^+$ $m/z = 443.0399$.

*1-[(Dimethylamino)methyl]naphthalen-2-ol (**9**)*

To a suspension of 2-naphthol (5.85 g, 40.2 mmol), dimethylmethylideneammonium iodide (97%, 7.58 g, 39.7 mmol, 1.0 equiv.) and potassium carbonate (8.31 g, 60.1 mmol, 1.5 equiv.) in toluene (20 mL) was added 30 mL dichloromethane. After stirring for 24 h at room temperature, the reaction mixture was extracted with 3 × 60 mL 1 M aqueous hydrochloride acid solution. Phases were separated and potassium carbonate was added to the aqueous phase adjusting the pH to 8, whereby product precipitation emerged. The aqueous phase was stored at 5°C for 5 h and the white solid was collected by filtration, yielding **9** as white crystals. Yield: 7.98 g (98.7%); mp: 74.5°C; ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 3.98$ (s, 2H, C(9)H_2), 7.06 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.27 (t, 1H, C(6)H, $J = 8.0$ Hz), 7.43 (t, 1H, C(7)H, $J = 8.4$ Hz), 7.70 (d, 1H, C(8)H, $J = 8.8$ Hz), 7.77 (d, 1H, C(4)H, $J = 8.0$ Hz), 7.94 (d, 1H, C(5)H, $J = 8.4$ Hz) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 44.3$ (N(CH_3)$_2$), 55.4 (C9), 113.0 (C8a), 118.5 (C3), 122.2 (C5, C6), 126.1 (C7), 127.9 (C1), 128.3 (C4), 128.7 (C8), 132.9 (C4a), 155.4 (C2) ppm; FT-IR ($\tilde{\nu}$): 3380 cm^{-1} (w), 3006 cm^{-1} (w), 2950 cm^{-1} (w), 1626 cm^{-1} (s), 1439 cm^{-1} (m), 1322 cm^{-1} (m), 1245 cm^{-1} (s); HRMS (ESI): found 202.1226, calcd. for $C_{13}H_{15}NO$ $[M+H]^+$ $m/z = 202.1226$ [12].

*6-Bromo-1-[(dimethylamino)methyl]naphthalen-2-ol (**10**)*

To a suspension of 6-bromo-2-naphthol (9.20 g, 40.0 mmol), dimethylmethylideneammonium iodide (97%, 7.62 g, 40.0 mmol, 1.0 equiv.) and potassium carbonate (8.30 g, 60.0 mmol, 1.5 equiv.) in toluene (30 mL) was added 20 mL dichloromethane. The suspension was stirred 16 h at room temperature. Product was gained as a solid by filtering the reaction mixture. In addition 0.2 M hydrochloride acid solution (50 mL) was added to the filtrate. Phases were separated and 2.0 g potassium carbonate was added to the aqueous phase, whereby product **10** emerged as a voluminous precipitate. Solids were combined and washed with water (3 × 50 mL) yielding **10** as colorless solid. Yield: 10.35 g

(92.4%); mp: 178°C; ^{1}H-NMR (DMSO-d$_6$, 400 MHz): $\delta = 2.28$ (s, 6H, N(CH$_3$)$_2$), 3.96 (s, 2H, C(9)H$_2$), 7.12 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.53 (d, 1H, C(4)H, $J = 9.2$ Hz), 7.71 (d, 1H, C(8)H, $J = 8.8$ Hz), 7.91 (d, 1H, C(9)H, $J = 8.8$ Hz), 8.04 (s, 1H, C(6)H), 11.33 (s, 1H, OH) ppm; ^{13}C-NMR (DMSO-d$_6$, 100 MHz): $\delta = 44.3$ (N(CH$_3$)$_2$), 55.0 (C9), 113.5 (C1), 115.0 (C6), 119.7 (C3), 124.9 (C8), 128.0 (C7), 128.8 (C4), 129.3 (C4a), 129.9 (C5), 131.7 (C8a), 155.7 (C2) ppm; FT-IR ($\tilde{\nu}$): 3195 cm^{-1} (m), 3036 cm^{-1} (w), 2955 cm^{-1} (w), 1357 cm^{-1} (m), 1270 cm^{-1} (s); HRMS (ESI): found 280.0332, calcd. for $C_{13}H_{14}BrNO$ $[M+H]^+$ $m/z = 280.0332$.

*(6-Bromo-2-hydroxynaphthalen-1-yl)(p-tolyl)-methanaminium chloride (**11**)*

To a flask containing 6-bromo-2-naphthol (2.23 g, 10.0 mmol) and ammonium formate (1.1 g, 22.3 mmol, 2.2 equiv.) was added 4-methylbenzaldehyde (3.36 mL, 2.40 g, 2.0 eqiv). Reaction mixture was heated to 110°C for 15 min and was allowed to cool down to room temperature. To the solid residue 50 mL water and 2 mL HCl (36%) were added and the slurry was heated for 2 h to 100°C. The solid was collected by filtration and was subsequently diluted again in 50 mL water and 1 mL H_2SO_4 (96%) and then heated for 15 h to 70°C. Slurry was stored at 5°C for 1 h. Solid was collected by filtration and was refluxed in ethyl acetate. The product was collected by filtration of hot slurry. Procedure was repeated for the insoluble precipitate yielding product as colorless crystals. Yield: 1.15 g (30.4%); mp: 188°C (degrad.); ^{1}H-NMR (DMSO-d$_6$, 400 MHz): $\delta = 2.26$ (s, 3H, C(14)H$_3$), 6.20 (s, 1H, C(9)H), 7.16 (d, 2H, C(12)H, C(12′)H, $J = 8.0$ Hz), 7.37 (d, 2H, C(11)H, C(11′)H, $J = 8.4$ Hz), 7.52 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.56 (d, 1H, C(7)H, $J = 8.8$ Hz), 7.87 (d, 1H, C(4)H, $J = 8.8$ Hz), 7.97 (d, 1H, C(8)H, $J = 9.2$ Hz), 8.14 (s, 1H, C(5)H), 8.91 (s, 3H, N$^+$H$_3$), 11.22 (s, 1H, OH) ppm; ^{13}C-NMR (DMSO-d$_6$, 100 MHz): $\delta = 20.6$ (C14), 50.5 (C9), 114.3 (C1), 115.7 (C6), 119.9 (C3), 124.4 (C8), 127.1 (C11, 11′), 129.0 (C12, 12′), 129.4 (C4a), 129.7 (C4), 129.8 (C7), 130.4 (C5), 130.5 (C8a), 134.3 (C13), 137.4 (C10), 154.3 (C2) ppm; FT-IR ($\tilde{\nu}$): 3059 cm^{-1} (m), 2903 cm^{-1} (m), 1517 cm^{-1} (s), 1503 cm^{-1} (s), 1273 cm^{-1} (s), 455 cm^{-1} (s); HRMS (ESI): found 325.0209, calcd. for $C_{18}H_{13}OBr$ $[M+H]^+$ $m/z = 325.0223$.

*1-([1H-Naphtho[1,2-e][1,3]oxazin-2(3H)-yl]methyl)-naphthalen-2-ol (**12**)*

A suspension of 2-naphthol (7.26 g, 50.4 mmol), paraformaldehyde (3.07 g, 102.1 mmol, 2.0 equiv.) and ammonium formate (4.98 g, 78.9 mmol, 1.5 equiv.) in dimethylformamide (30 mL) was heated to 120°C for 20 min. Within 15 min mixture turned into a homogeneous yellow solution. Then reaction mixture was allowed to cool down to room temperature and was poured into 600 mL water. The mixture was stored 1 h at 5°C and precipitate was collected by filtration. The collected solid was recrystallized from ethanol, yielding product **12** as pale pink needle-shaped crystals. Yield: 2.76 g (32.1%); mp: 162°C; ^{1}H-NMR (DMSO-d$_6$, 400 MHz): $\delta = 4.25$ (s, 2H, C(9)H$_2$), 4.35 (s, 2H, C(9′)H$_2$), 5.02 (s, 2H, C(10)H$_2$), 7.11 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.15 (d, 1H, C(3′)H, $J = 8.8$ Hz), 7.30 (t, 1H, C(6′)H, $J = 7.6$ Hz), 7.36 (t, 1H, C(6)H, $J = 7.6$ Hz), 7.44 (m, 2H, C(7)H, C (7′)H), 7.78 (d, 1H, C(8′)H, $J = 8.4$ Hz), 7.74 (dd, 2H, C (4′)H, C(4) H, $J = 8.8$ Hz), 7.79 (d, 1H, C(5′)H, $J = 8.0$ Hz), 7.84 (d, 1H, C(5)H, $J = 8.0$ Hz), 8.06 (d, 1H, C(8)H, $J = 8.4$ Hz), 9.63 (s, 1H, OH) ppm; ^{13}C-NMR (DMSO-d$_6$, 100 MHz): $\delta = 45.1$ (C9′), 46.0 (C9), 82.0 (C10), 112.4 (C1), 114.7 (C1′), 118.0 (3′), 118.4 (C3), 121.3 (C8), 122.4 (C6), 123.2 (C6′), 123.7 (C8′), 126.1 (C7′), 126.4 (C7), 127.6 (C4), 128.1 (C5′), 128.2 (C4a), 128.3 (C5), 129.1 (C4a′), 131.7 (C8a), 134.0 (C8a′), 151.5 (C2), 153.8 (C2′) ppm; FT-IR ($\tilde{\nu}$): 3059 cm^{-1} (w), 2996 cm^{-1} (w), 1593 cm^{-1} (m), 1213 cm^{-1} (s), 807 cm^{-1} (s); HRMS (ESI): found 342.1491, calcd. for $C_{23}H_{19}NO_2$ $[M+H]^+$ $m/z = 342.1489$.

*1-({[(E)-(2-Hydroxynaphthalen-1-yl)methyl]imino}methyl)-naphthalen-2-ol (**13**)*

General procedure: To a mixture of 2-naphthol (10.0 g, 69.4 mmol) and methenamine (10.0 g, 71.3 mmol) acetic acid (40 mL) was added. The suspension was stirred at 100°C for 1 h. The yellow precipitate formed was collected by filtration and washed with ethanol (3 × 20 mL). The amorphous yellow solid was used in the next step without further purification. Yield: 8.55 g (75.3%); mp: 211°C (degrad.); ^{1}H-NMR (DMSO-d$_6$, 400 MHz): $\delta = 5.28$ (s, 2H, C(11)H$_2$), 6.63 (d, 1H, C(3)H, $J = 9.6$ Hz), 7.19 (t, 1H, C(6)H, $J = 8.0$ Hz), 7.29 (d, 1H, C(14)H, $J = 8.8$ Hz), 7.34 (t, 1H, C(17)H, $J = 8.0$ Hz), 7.46 (t, 1H, C(7)H, $J = 7.6$ Hz), 7.55 (t, 1H, C(18)H, $J = 7.6$ Hz), 7.61 (d, 1H, C(5)H, $J = 8.0$ Hz), 7.68 (d, 1H, C(4)H, $J = 9.6$ Hz), 7.87–7.83 (2d, 2H, C(15, 16)H), 8.09 (d, 1H, C(8)H, $J = 8.4$ Hz), 8.22 (d, 1H, C(19)H, $J = 8.4$ Hz), 9.39 (d, 1H, C(9)H, $J = 10.4$ Hz), 14.27 (s, 1H, =N$^+$(10)H), 10.34 (s, 1H, OH) ppm; ^{13}C-NMR (DMSO-d$_6$, 100 MHz): $\delta = 44.8$ (C11), 105.5 (C1), 113.8 (C12), 118.0 (C14), 118.2 (C8), 122.1 (C19), 122.1 (C6), 122.8 (C17), 125.1 (C4a), 125.5 (C3), 127.0 (C18), 127.9 (C7), 128.1 (C15a), 128.6 (C16), 128.9 (C5), 130.0 (C15), 132.7 (C19a), 134.3 (C8a),137.0 (C4), 153.8 (C13), 158.3 (C9), 177.3 (C2) ppm; FT-IR ($\tilde{\nu}$): 3050 cm^{-1} (w, ν_{C-H}, unsat.), 2957 cm^{-1} (w, ν_{C-H}, sat.), 2497 cm^{-1} (w, ν_{O-H}), 1639 cm^{-1} (s, $\nu_{C=N}$); HRMS (ESI): found 328.1332, calcd. for $C_{22}H_{17}NO$ $[M+H]^+$ $m/z = 328.1332$ [43].

*6-Bromo-1-({[(6-bromo-2-hydroxynaphthalen-1-yl)-methyl]imino}methyl)naphthalen-2-ol (**14**)*

To a mixture of 6-bromo-naphth-2-ol (15.9 g, 71.3 mmol) and methenamine (10.0 g, 71.3 mmol) acetic acid (40 mL) was added. The suspension was stirred at 100°C for 2 h. The yellow precipitate formed was collected by filtration and washed with acetic acid (2 × 20 mL) and finally with hot ethanol (3 × 30 mL). The amorphous yellow solid was used in the next step without further purification. Yield: 15.72 g (90.7%); mp: 222°C (degrad.); ^{1}H-NMR (DMSO-d$_6$, 400 MHz): $\delta = 5.25$ (s, 2H, C(11)H$_2$), 6.67 (d, 1H, C(3)H, $J = 9.2$ Hz), 7.32 (d, 1H, C(14)H, $J = 8.8$ Hz), 7.58 (d, 1H, C(18)H, $J = 8.8$ Hz), 7.64 (d, 1H, C(7)H, $J = 8,8$ Hz), 7.68 (d, 1H, C(4) H, $J = 9,2$ Hz), 7.83 (d, 1H, C(15)H, $J = 8.8$ Hz), 7.87 (s, 1H, C(5)H), 8.04 (d, 1H, C(8)H, $J = 9.2$ Hz), 8.13 (s, 1H, C(16)H), 8.19 (d, 1H, C(19)H, $J = 9.2$ Hz), 9.39 (s, 1H, C(9)H), 10.55 (s, 1H, OH), 14.23 (s, 1H, =N$^+$(10)H) ppm; ^{13}C-NMR (DMSO-d$_6$, 100 MHz): $\delta = 44.7$ (C11), 105.3 (C1), 114.1 (C12), 114.4 (C6), 115.6 (C17), 119.2 (C14), 120.6 (C8), 124.6 (C19), 126.7 (C4a), 126.9 (C3), 129.3 (C15), 129.4

(C15a), 129.8 (C7), 130.3 (C16,C18), 133.2 (C8a), 136.0 (C4), 154.3 (C13), 130.6 (C5), 131.3 (C19a), 158.6 (C9), 177.5 (C2) ppm; FT-IR ($\tilde{\nu}$): 3052 cm^{-1} (w), 2582 cm^{-1} (m), 1627 cm^{-1} (s), 1534 cm^{-1} (w), 1494 cm^{-1} (s), 1345 cm^{-1} (s), 1165 cm^{-1} (s), 804 cm^{-1} (s); HRMS (ESI): found 481.9401, calcd. for $C_{22}H_{15}NO_2Br_2$ $[M-H]^-$ *m/z* = 481.9397.

*2-Hydroxy-1-naphthaldehyde (**15**)*
To a suspension of **13** (12.07 g, 36.85 mmol) in ethanol (200 mL) was added 6 N hydrochloric acid (40 mL) via dropping funnel with continuous stirring. After adding was completed, mixture was refuxed for 2 h. Subsequently suspension was cooled down on an ice bath. Solid was filtered off. Hereafter cold water (400 mL) was added to filtrate, whereby a yellow precipitate was formed immediately. Suspension was stored at 5°C for 2 h and then precipitate was collected by filtration. A pale yellow solid was obtained by recrystallization in 2-propanol. Yield: 5.03 g (79.3%); mp: 82°C (degrad.). Spectroscopic data were consistent with those reported in the literature [44].

*6-Bromo-2-hydroxy-1-naphthaldehyde (**16**)*
To a suspension of **14** (12.20 g, 25.0 mmol) in ethanol (150 mL) was added 6 N hydrochloric acid (37.5 mL) via dropping funnel with continuous stirring. After adding was completed, mixture was refuxed for 2.5 h. Subsequently suspension was cooled down on an ice bath. Solid was filtered off. Hereinafter cold water (300 mL) was added to filtrate, whereby a bluish precipitate was formed immediately. Suspension was stored at 5°C for 2 h and then precipitate was collected by filtration. Solid was dissolved in diethyl ether (100 mL), insoluble materials were collected by filtration. Filtrate was dried with sodium sulfate and evaporated to dryness, by means of a rotary evaporator. Yield was improved by extracting hydrolysis filtrate with diethyl ether (3 × 30 mL). Organic phase was filtered and desiccated with sodium sulfate and evaporated to dryness, by means of a rotary evaporator. Yielding product as yellow crystals. Yield: 5.30 g (83.9%); mp: 150°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 7.31 (d, 1H, C(3)H, *J* = 9.2 Hz), 7.73 (d, 1H, C(7)H, *J* = 9.2 Hz), 8.11 (d, 1H, C(4)H, *J* = 8.8 Hz), 8.17 (s, 1H, C(5)H), 8.93 (d, 1H, C(8)H, *J* = 9.2 Hz), 10.77 (s, 1H, C(9)H), 11.91 (s, 1H, OH) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): δ = 112.6 (C1), 116,9 (C6), 120.1 (C3), 125.0 (C8), 129.0 (C4a), 130.2 (C8a), 130.5 (C5), 131.9 (C7), 137.2 (C4), 164.1 (C2), 192.1 (C9) ppm; FT-IR ($\tilde{\nu}$): 2983 cm^{-1} (w), 1630 cm^{-1} (m), 1458 cm^{-1} (m), 1302 cm^{-1} (m), 1163 cm^{-1} (m), 809 cm^{-1} (m); HRMS (ESI): found 248.9565, calcd. for $C_{11}H_7O_2Br$ $[M-H]^-$ *m/z* = 248.

*Alternative synthesis of 6-bromo-2-hydroxy-1-naphthaldehyde (**16**)*
To a suspension of 6-bromo-naphthol (30.78 g, 138,0 mmol) and zinc cyanide (20.76 g, 176.8 mmol) in 150 mL diethyl ether was injected continuously hydrochloride acid gas at room temperature. After 2 h, yellowish solid was collected by filtration and was refluxed two times in 250 mL water. The insoluble solid was refluxed in 240 mL water/methanol (4:1) and was collected by filtration, yielding product as yellow solid. The filtrates were allowed to cool to 5°C and formed precipitations were combined recovering 22.98 g starting material 6-bromo-2-naphthol. Yield: 7.49 g (21.6%, 85.3% based on recovered starting material); mp: 150°C (degrad.). Spectroscopic data were consistent with those reported in literature.

*(2-Hydroxynaphthalen-1-yl)methanaminium chloride (**17**)*
To a suspension of **13** (12.07 g, 36.85 mmol) in ethanol (200 mL) was added dropwise 6 M hydrochloric acid (40 mL) with stirring. After addition was completed, mixture was refluxed for 2 h. Subsequently, the suspension was cooled down on an ice bath. The precipitate was collected by filtration and was washed thoroughly with cold ethanol (3 × 20 mL). A colorless solid was recrystallized from 2-propanol. Yield: 6.43 g (83.2%); mp: 207°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 4.39 (s, 2H, C(9)H_2), 7.33–7.40 (m, 2H, C(3, 6)H), 7.53 (t, 1H, C(7)H, *J* = 7.6 Hz), 7.85 (d, 2H, C(5, 4)H, *J* = 8.8 Hz), 8.03 (d, 1H, C(8)H, *J* = 8.4 Hz), 8.25 (s, 3H, N(10)H_3), 10.71 (s, 1H, OH) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): δ = 32.7 (C9), 111.0 (C1), 118.0 (C3), 122.4 (C8), 122.8 (C6), 126.9 (C7), 127.9 (C4a), 128.5 (C5), 130.6 (C4), 132.7 (C8a), 154.6 (C2) ppm; FT-IR ($\tilde{\nu}$): 3020 cm^{-1} (m), 2923 cm^{-1}(m), 1597 cm^{-1} (m), 1517 cm^{-1} (m), 1286 cm^{-1} (s), 813 cm^{-1} (s), 746 cm^{-1} (s); HRMS (ESI): found 174.0913, calcd. for $C_{11}H_{12}NO$ $[M]^+$ *m/z* = 174.0913 [45].

*(6-Bromo-2-hydroxynaphthalen-1-yl)methanaminium chloride (**18**)*
To a suspension of **14** (12.20 g, 25.0 mmol) in ethanol (150 mL) was added 6 M hydrochloric acid (37.5 mL) via dropping funnel with continuous stirring. After addition was completed, the mixture was refluxed for 2.5 h. Subsequently the resulting suspension was cooled down on an ice bath for 2 h. Formed solid was collected by filtration and was washed with hot ethanol (2 × 20 mL). Yield was improved by treatment of filtrate with cold water (300 mL), whereby a bluish precipitate was formed immediately. Suspension was stored at 5°C for 2 h and then bluish precipitate was collected by filtration. The solid was dissolved in diethyl ether (100 mL), thereby an insoluble solid was collected by filtration and washed with ethanol (2 × 20 mL). Both the solid of the hydrolysis and the diethyl ether precipitate were combined yielding product as pale yellow crystals. Yield: 6.91 g (95.2%); mp: 223°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 4.37 (s, 2H, C(9)H_2), 7.41 (d, 1H, C(3)H, *J* = 8.8 Hz), 7.63 (d, 1H, C(7)H, *J* = 9.2 Hz), 7.86 (d, 1H, C(4)H, *J* = 9.2 Hz), 8.01 (d, 1H, C(8)H, *J* = 9.2 Hz), 8.12 (s, 1H, C(5)H), 8.20 (s, 3H, N^+(10)H_3), 10.85 (s, 1H, OH) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): δ = 32.7 (C9), 111.4 (C1), 115.6 (C6), 119.1 (C3), 125.0 (C8), 129.1 (C4a), 129.5 (C7), 129.9 (C4), 130.1 (C5), 131.4 (C8a), 155.1 (C2) ppm; FT-IR ($\tilde{\nu}$): 3198 cm^{-1} (m), 3022 cm^{-1} (m), 2925 cm^{-1} (m), 1578 cm^{-1} (m), 1496 cm^{-1} (s), 1271 cm^{-1} (s), 1053 cm^{-1} (w), 879 cm^{-1} (s), 811 cm^{-1} (s); HRMS (ESI): found 252.0019, calcd. for $C_{11}H_{11}BrNO$ $[M]^+$ *m/z* = 252.0019 [45].

*4-(3-{[(2-Hydroxynaphthalen-1-yl)methyl]ammonio}-propyl)morpholin-4-ium chloride (**19**)*
To a solution of **15** (3.44 g, 20.0 mmol) in methanol (50 mL) 3-(morpholino-4-yl)propan-1-amine (2.90 g, 20.1 mmol) was added under a nitrogen atmosphere. After 3 h stirring at room temperature, mixture was cooled in an ice-bath. Afterwards sodium borohydride (1.51 g, 39.9 mmol) predissolved in 20 mL water was added. After removing the ice-bath, solution was stirred 1 h at room temperature. Reaction was quenched by slow addition of 4 M hydrochloric acid (25 mL). After addition was completed, methanol was removed by means of a rotary evaporator and aqueous residue was extracted with diethyl ether (3 × 30 mL). Subsequently aqueous phase was extracted with ethyl acetate (3 × 50 mL) after pH-adjustment to pH 7–8 with ammonium chloride and sodium carbonate. Immediately aqueous phase was adjusted to pH 9 with sodium hydroxide and extracted again with ethyl acetate (3 × 50 mL). Both organic phases were dried with anhydrous sodium sulfate and then hydrochloric acid gas was introduced. The resulting precipitate was collected by filtration after storage at −20°C for 12 h. Solid was recrystallized from ethyl acetate/methanol/isopropanol yielding product as pale rose crystals. Yield: 5.19 g (69.7%); mp: 169°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 2.20$–2.27 (m, 2H, C(12)H_2), 3.03–3.19 (m, 6H, C(11,13,15/18)H_2), 3.36 (s, 2H, C(15/18)H), 3.85–3.97 (m, 4H, C(16,17)H_2), 4.54 (s, 2H, C(9)H_2), 7.36 (t, 1H, C(6)H, $J = 7.4$ Hz), 7.44 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.54 (t, 1H, C(7)H, $J = 7.6$ Hz), 7.89 (d, 1H, C(4)H, $J = 9.2$ Hz), 8.12 (d, 1H, C(8)H, $J = 8.4$ Hz), 9.14 (s, 1H, N^+(10)H_2), 10.87 (s, 1H, OH), 11.70 (s, 1H, N^+(14)H) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 19.6$ (C12), 40.7 (C9), 44.3 (C11), 50.9 (C15,18), 53.0 (C13), 63.0 (C16,17), 109.0 (C1), 117.9 (C3), 122.6 (C8), 122.9 (C6), 127.0 (C7), 127.9 (C4a), 128.5 (C5), 131.1 (C4), 133.0 (C8a), 155.3 (C2) ppm; FT-IR ($\tilde{\nu}$): 3429 cm^{-1} (w), 3358 cm^{-1} (w), 3135 cm^{-1} (w), 2934 cm^{-1} (w), 2590 cm^{-1} (w), 1626 cm^{-1} (w), 1264 cm^{-1} (m), 1136 cm^{-1} (w), 819 cm^{-1} (m), 751 cm^{-1} (m); HRMS (ESI): found 371.1299, calcd. for $C_{18}H_{26}Cl_2N_2O_2$ $[M-H]^-$ $m/z = 371.1299$.

*4-(3-{[(6-Bromo-2-hydroxynaphthalen-1-yl)methyl]-ammonio}propyl)morpholin-4-ium chloride (**20**)*
To a solution of **18** (2.51 g, 10.0 mmol) in methanol (50 mL) 3-(morpholino-4-yl)propan-1-amine (2.90 g, 20.1 mmol) was added under a nitrogen atmosphere. After 2 h stirring at room temperature, mixture was cooled in an ice-bath. Afterwards sodium borohydride (1.0 g, 26.4 mmol) pre-dissolved in 20 mL water was added. After removing the ice-bath, solution was stirred 1 h at room temperature. Reaction was quenched by slow addition of 4 M hydrochloric acid (30 mL). After addition was completed, methanol was removed on a rotary evaporator and aqueous residue was extracted with diethyl ether (3 × 30 mL). Subsequently, aqueous phase was extracted with ethyl acetate (3 × 50 mL) after pH-adjustment to pH 7–8 with sodium carbonate. Immediately aqueous phase was adjusted to pH 9 with ammonia and sodium carbonate and extracted again with ethyl acetate (3 × 50 mL). Both organic phases were dried with anhydrous sodium sulfate and then hydrochloric acid gas was introduced. The resulting precipitate was collected by filtration after storage at −20°C for 12 h. Solid was recrystallized from ethyl acetate/methanol/isopropanol yielding product as pale rose crystals. Yield: 3.16 g (69.9%); mp: 184°C (degrad.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 2.21$–2.26 (m, 2H, C(12)H_2), 3.05–3.18 (m, 6H, C(11,13,15/18)H_2), 3.37 (s, 2H, C(15/18)H), 3.85–3.97 (m, 4H, C(16,17)H_2), 4.52 (s, 2H, C(9)H_2), 7.47 (d, 1H, C(3)H, $J = 8.8$ Hz), 7.63 (d, 1H, C(7)H, $J = 8.8$ Hz), 7.89 (d, 1H, C(4)H, $J = 8.8$ Hz), 8.11 (d, 1H, C(8)H, $J = 9.2$ Hz), 9.13 (s, 1H, N^+(10)H_2), 11.03 (s, 1H, OH), 11.62 (s, 1H, N^+(14)H) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 19.6$ (C12), 40.6 (C9), 44.3 (C11), 50.9 (C15,18), 53.0 (C13), 63.0 (C16,17), 109.5 (C1), 115.7 (C6), 119.1 (C6), 125.3 (C8), 129.2 (C4a), 129.6 (C7), 130.1 (C5), 130.4 (C4), 131.7 (C8a), 155.7 (C2) ppm; FT-IR ($\tilde{\nu}$): 3444 cm^{-1} (w), 3058 cm^{-1} (w), 2935 cm^{-1} (w), 2465 cm^{-1} (w), 1595 cm^{-1} (w), 1269 cm^{-1} (m), 1087 cm^{-1} (m), 816 cm^{-1} (s); HRMS (ESI): found 449.0404, calcd. for $C_{18}H_{25}BrCl_2N_2O_2$ $[M-H]^-$ $m/z = 449.0404$.

*1,1′-[Thiobis(methylene)]bis(naphthalen-2-ol) (**21**)*
To a suspension of **8a** (1.076 g, 5.0 mmol) in 30 mL dry tetrahydrofuran was added triethylamine (1.50 mL, 1.10 g, 10.8 mmol, 2.2 equiv.). After 25 min stirring under nitrogen atmosphere, ethyl-3-bromopropionate (0.64 mL, 0.90 g, 5.0 mmol, 1.0 equiv.) was added to the yellowish solution at room temperature. Within few minutes the reaction mixture became turbid. After 9 days stirring at room temperature (TLC indicated that after 30 min the reaction was ended, composition does not change within the long reaction time) the insoluble solid was filtered off. To the filtrate 10 mL *n*-hexane was added. The slurry was stored at −20°C for 10 h. The flaky precipitation was collected by filtration and was subsequently purified by column chromatography (*n*-hexane/ethyl acetate/acetic acid; 10:5:1) yielding product as colorless crystals. Crystallization for X-ray diffraction measurement was conducted via vapor diffusion with a solution of the product in dichloromethane in *n*-hexane atmosphere. Yield: 0.10 g (5.5%); mp: 255°C (decomp.); ^{1}H-NMR (DMSO-d_6, 400 MHz): $\delta = 4.29$ (s, 4H, C(9)H_2, C(9′)H_2), 7.17 (d, 2H, C(3)H, C(3′)H, $J = 9.2$ Hz), 7.25 (t, 2H, C(6)H, C(6′)H, $J = 8.0$ Hz), 7.31 (t, 2H, C(7)H, C(7′)H, $J = 8.0$ Hz), 7.38 (d, 2H, C(4)H, C(4′)H, $J = 8.8$ Hz), 7.76 (d, 4H, C(5)H, C(8)H, C(5′)H, C(8′)H, $J = 9.2$ Hz), 9.66 (s, 2H, OH) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz): $\delta = 26.3$ (C9,9′), 115.2 (C1,1′), 117.9 (C3,3′), 122.4 (C6,6′), 123.0 (C8,8′), 126.0 (7,7′), 128.2 (C4a, C4a′), 128.2 (C5, C5′), 128.4 (C4, C4′), 132.8 (C8a, C8a′), 152.8 (C2, C2′) ppm; FT-IR ($\tilde{\nu}$): 3526 cm^{-1} (w), 3056 cm^{-1} (w), 1626 cm^{-1} (m), 1356 cm^{-1} (m), 1213 cm^{-1} (m), 809 cm^{-1} (s). Product structure was confirmed by X-ray crystallography.

Biochemistry

Expression and purification of recombinant protein
The expression and purification of $Sirt2_{56–356}$ was performed as described previously with minor modifications [46]. The enzyme was resuspended in lysis buffer (25 mM KH_2PO_4,

25 mM NaH_2PO_4, 400 mM NaCl, 5% (v/v) glycerol, 5 mM 2-mercaptoethanol, pH 8.0) and finally purified with a Superdex S75 26/60 gel filtration column (25 mM Hepes, 200 mM NaCl, 5% (v/v) glycerol, pH 8.0). Via SDS-PAGE the identity and purity of the produced enzymes was verified [47] and the protein concentration was determined by Bradford assay [48]. The catalytic reaction of hSirt2 was dependent on NAD^+ and was able to inhibit by nicotinamide.

Biochemical assay

For the activity testing of hSirt2 an established high-throughput (96-well plate) fluorescence-based assay was used [33]. $hSirt2_{56–356}$ was added to a solution of NAD^+ (final assay concentration 500 μM), the substrate Z-(Ac)Lys-AMC (ZMAL, final assay concentration 10.5 μM), DMSO as a control or the inhibitor in several concentrations dissolved in DMSO (final DMSO concentration 5% (v/v)), filled up with assay buffer (50 mM Tris, 137 mM NaCl, 2.7 mM KCl, pH 8.0) to 60 μL. The enzyme concentration was adjusted to a final substrate conversion of 15–30% to stay in a linear range. The incubation time was 4 h at 37°C and 150 rpm. The catalytic reaction was stopped by adding 60 μL of a developer solution (50 mM Tris, 100 mM NaCl, 6.7% (v/v) DMSO, trypsin 16.5 U/μL, 8 mM nicotinamide, pH 8.0) and incubated 20 min at 37°C and 150 rpm. Fluorescence intensity was measured in a microplate reader (BMG Polarstar, $\lambda_{ex} = 390$ nm, $\lambda_{em} = 460$ nm). All compounds were pretested on auto-fluorescence, aminomethylcoumarin (AMC) quenching, and trypsin inhibition under assay conditions. No interferences were observed. The inhibitory effect was determined by using the DMSO-controls as a reference. Graphpad Prism software (La Jolla, CA) was used to calculate IC_{50} values.

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Author contributions

S.V. and K.B. synthesized, purified, and isolated new compounds. A.B. performed structure elucidation by NMR and accurate mass analysis. Z.A.H. and W.S. carried out docking experiments, C.S. accomplished X-ray crystallographic structure determination, S.S. and M.J. performed the biological evaluation; W.S. and A.L. designed the study and wrote the manuscript together with S.V.

The authors have declared no conflict of interest.

References

[1] A. A. Sauve, I. Celic, J. Avalos, H. Deng, J. D. Boeke, V. L. Schramm, *Biochemistry* **2001**, *40*, 15456–15463.
[2] I. Carnevale, L. Pellegrini, P. D'Aquila, S. Saladini, E. Lococo, L. Polletta, E. Vernucci, E. Foglio, S. Coppola, L. Sansone, G. Passarino, D. Bellizzi, M. A. Russo, M. Fini, M. Tafani, *J. Cell. Physiol*. **2017**, *232*, 1835–1844.
[3] E. Jing, S. Gesta, C. Ronald Kahn, *Cell Metab*. **2007**, *6*, 105–114.
[4] F. Wang, M. Nguyen, F.X. -F. Qin, Q. Tong, *Aging Cell* **2007**, *6*, 505–514.
[5] Y. K. Yoon, T. S. Choon, *Arch. Pharm*. **2016**, *349*, 1–8.
[6] S.-N. Xu, T.-S. Wang, X. Li, Y.-P. Wang, *Sci. Rep*. **2016**, *6*, 32734.
[7] A. Bedalov, T. Gatbonton, W. P. Irvine, D. E. Gottschling, J. A. Simon, *Proc. Natl. Acad. Sci. U.S.A*. **2001**, *98*, 15113–15118.
[8] R. C. Neugebauer, U. Uchiechowska, R. Meier, H. Hruby, V. Valkov, E. Verdin, W. Sippl, M. Jung, *J. Med. Chem*. **2008**, *51*, 1203–1213.
[9] T. Rumpf, S. Gerhardt, O. Einsle, M. Jung, *Acta Crystallogr. F* **2015**, *71*, 1498–1510.
[10] J. Arct, E. Jakubska, G. Olszewska, *Synthesis* **1977**, *1977*, 314–315.
[11] A. Pochini, G. Puglia, R. Ungaro, *Synthesis* **1983**, *1983*, 906–907.
[12] A. Blade-Font, T. de Mas Rocabayera, *J. Chem. Soc., Perkin Trans*. **1982**, *1*, 841–848.
[13] M. Heydenreich, A. Koch, I. Szatmari, F. Fülöp, E. Kleinpeter, *Tetrahedron* **2008**, *64*, 7378–7385.
[14] T. Meyer, D. Geffken, *Pharmazie* **2003**, *59*, 593–596.
[15] D. Keck, S. Vanderheiden, S. Bräse, *Eur. J. Org. Chem*. **2006**, *2006*, 4916–4923.
[16] C. Cardellicchio, M. A. M. Capozzi, F. Naso, *Tetrahedron-Asymmetry* **2010**, *21*, 507–517.
[17] D. Tóth, I. Szatmári, F. Fülöp, *Eur. J. Org. Chem*. **2006**, *2006*, 4664–4669.
[18] S. B. Sapkal, K. F. Shelke, A. H. Kategaonkar, M. S. Shingare, *Green Chem. lett. Rev*. **2009**, *2*, 57–60.
[19] J.-T. Li, S.-F. Sun, M.-X. Sun, *Ultrason. Sonochem*. **2011**, *18*, 42–44.
[20] J. C. Duff, E. J. Bills, *J. Chem. Soc*. **1934**, *282*, 1305–1308.
[21] C. Mannich, W. Krösche, *Arch. Pharm*. **1912**, *250*, 647–667.
[22] I. S. Belostotskaya, N. L. Komissarova, T. I. Prokof'eva, L. N. Kurkovskaya, V. B. Vol'eva, *Russ. J. Org. Chem*. **2005**, *41*, 703–706.
[23] Y. Ogata, A. Kawasaki, F. Sugiura, *Tetrahedron* **1968**, *24*, 5001–5010.
[24] S. K. Grover, A. C. Jain, T. R. Seshadri, *J. Indian Chem. Soc*. **1962**, *39*, 301–305.
[25] D. Li, Y. Shu, P. Li, W. Zhang, H. Ni, Y. Cao, *Med. Chem. Res*. **2013**, *22*, 3119–3125.
[26] J. Girniene, S. Tardy, A. Tatibouët, A. Sačkus, P. Rollin, *Tetrahedron Lett*. **2004**, *45*, 6443–6446.
[27] Y. Nagao, K. Inoue, M. Yamaki, M. Shiro, S. Takagi, E. Fujita, *Chem. Pharm. Bull*. **1988**, *36*, 4293–4300.
[28] I. Szatmári, A. Hetényi, L. Lázár, F. Fülöp, *Heterocycl. Chem*. **2004**, *41*, 367–373.
[29] C. L. Perrin, D. B. Young, *J. Am. Chem. Soc*. **2001**, *123*, 4446–4450.

[30] M. P. Cava, M. I. Levinson, *Tetrahedron* **1985**, *41*, 5061–5087.
[31] J. Mindl, O. Hrabik, V. Sterba, J. Kavalek, *Collect. Czech. Chem. Commun*. **2000**, *65*, 1262–1272.
[32] N. Philippe, F. Denivet, J.-L. Vasse, J. Sopkova-de Olivera Santos, V. Levacher, G. Dupas, *Tetrahedron* **2003**, *59*, 8049–8056.
[33] B. Heltweg, J. Trapp, M. Jung, *Methods* **2005**, *36*, 332–337.
[34] P. J. Goodford, *J. Med. Chem*. **1985**, *28*, 849–857.
[35] J. L. Feldman, K. E. Dittenhafer-Reed, N. Kudo, J. N. Thelen, A. Ito, M. Yoshida, J. M. Denu, *Biochemistry* **2015**, *54*, 3037–3050.
[36] B. Heltweg, F. Dequiedt, E. Verdin, M. Jung, *Anal. Biochem*. **2003**, *319*, 42–48.
[37] J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, D. A. Case, *J. Comput. Chem*. **2004**, *25*, 1157–1174.
[38] A. Jakalian, D. B. Jack, C. I. Bayly, *J. Comput. Chem*. **2002**, *23*, 1623–1641.
[39] G. Jones, P. Willett, R. C. Glen, A. R. Leach, R. Taylor, *J. Mol. Biol*. **1997**, *267*, 727–748.
[40] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, *J. Appl. Crystallogr*. **1993**, *26*, 343–350.
[41] G. Sheldrick, *Acta Crystallogr. Sect. A* **2008**, *64*, 112–122.
[42] R. D. Haworth, R. MacGillivray, D. H. Peacock, *J. Chem. Soc*. **1952**, *541*, 2857–2858.
[43] W. J. Burke, M. J. Kolbezen, R. J. Reynolds, G. A. Short, *J. Am. Chem. Soc*. **1956**, *78*, 805–808.
[44] Y. Wang, M. Wang, Y. Wang, Y. Chen, L. Sun, *Synth. Commun*. **2011**, *41*, 1381–1393.
[45] R. D. Haworth, R. MacGillivray, D. H. Peacock, *J. Chem. Soc*. **1950**, *309*, 1493–1498.
[46] T. Rumpf, M. Schiedel, B. Karaman, C. Roessler, B. J. North, A. Lehotzky, J. Oláh, K. I. Ladwein, K. Schmidtkunz, M. Gajer, M. Pannek, C. Steegborn, D. A. Sinclair, S. Gerhardt, J. Ovádi, M. Schutkowski, W. Sippl, O. Einsle, M. Jung, *Nat.Commun*. **2015**, *6*, 6263.
[47] U. K. Laemmli, *Nature* **1970**, *227*, 680–685.
[48] M. M. Bradford, *Anal. Biochem*. **1976**, *72*, 248–254.