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Original article

Boehmeriasin A as new lead compound for the inhibition of topoisomerases and SIRT2

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ABSTRACT

Two synthetic approaches to boehmeriasin A are described. A gram scale racemic preparation is accompanied by an efficient preparation of both the pure enantiomers using the conformationally stable 2-piperidin-2-yl acetaldehyde as starting material. The anti-proliferative activity in three cancer cell lines (CEM, HeLa and L1210) and two endothelial cell lines (HMEC-1, BAEC) indicates promising activity at the nanomolar range. Topoisomerases and SIRT2 are identified as biological targets and the experimental data has been supported by docking studies.

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1. Introduction

Boehmeriasin A (Scheme 1), a phenanthroquinolizidine alkaloid, was first isolated from the aqueous ethanolic extract of *Boehmeria siamensis* Craib (Urticaceae) by bioassay-guided fractionation [1]. *In vitro* tests showed that boehmeriasin A possesses a strong cytotoxic activity, more potent than taxol, against 12 cell lines from 6 panels of cancer including breast, kidney, prostate, colon, lung cancer and leukaemia, with GI_{50} values between 0.2 and 100 ng/mL. Furthermore, this alkaloid restrains the expression of a series of genes related to cell proliferation and cell cycle regulation [2]. This results in G1 phase arrest of the cell cycle and differentiation in the breast cancer cell line MDA-MB-232 [3]. Consequently, we were attracted by its biological activity, the uncertainty of its biological mechanism and by the features of its structure that show boehmeriasins as possible building blocks for further structural modification. In this context, we planned a convenient synthesis to both enantiomers of boehmeriasin A with the potential extension of this methodology to create a library of analogues. Several synthetic methodologies have been developed for the elaboration of some boehmeriasin A congeners (e.g. cryptopleurine and its hydroxy derivatives) in both racemic and optically active forms [4–15].

Herein, we report two synthetic approaches to obtain the racemic mixture and the pure enantiomers of boehmeriasin A. Biological evaluation showed high anti-proliferative activity on cancer and endothelial cells, with topoisomerases and SIRT2 as involved targets. Virtual screening (Hurakan tool) [16] and docking

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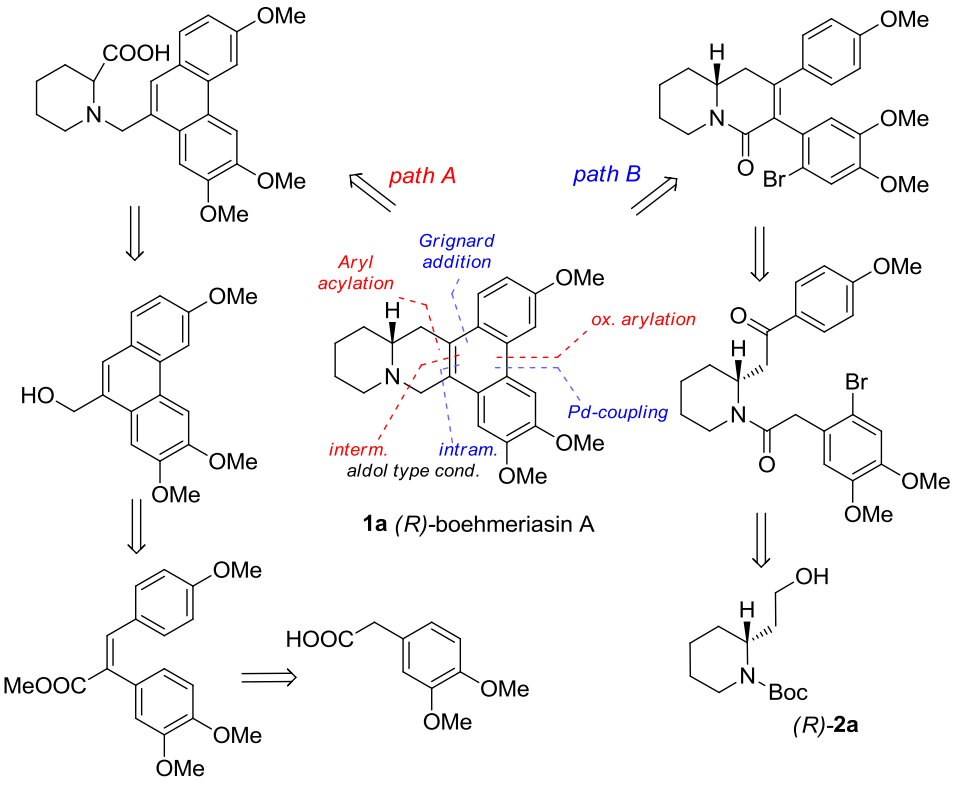


Scheme 1. Retrosynthetic plan.

studies support the experimental results.

2. Results and discussion

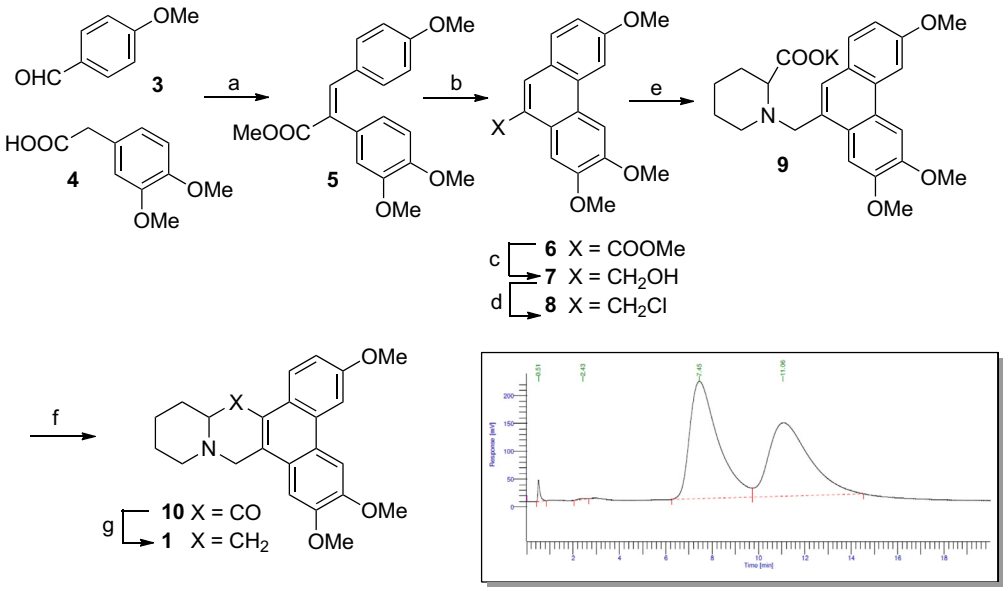
2.1. Chemistry

The retrosynthetic plan (Scheme 1) indicates the possibility to first create the phenanthrene nucleus and then conduct a further cyclization to form the quinolizidine ring (path A) or (path B). This would permit the generation of the nitrogen containing bicyclic system in a stereospecific way by an intramolecular aldol type reaction followed by an intramolecular arylation reaction.

Driven by a desire to access both enantiomers of boehmeriasin A, we initiated our synthetic efforts first through a racemic preparation of the target compound. To this end, we pursued the synthesis outlined in Scheme 2 which through its simplicity and robustness would allow gram quantities of the desired natural product to be obtained.

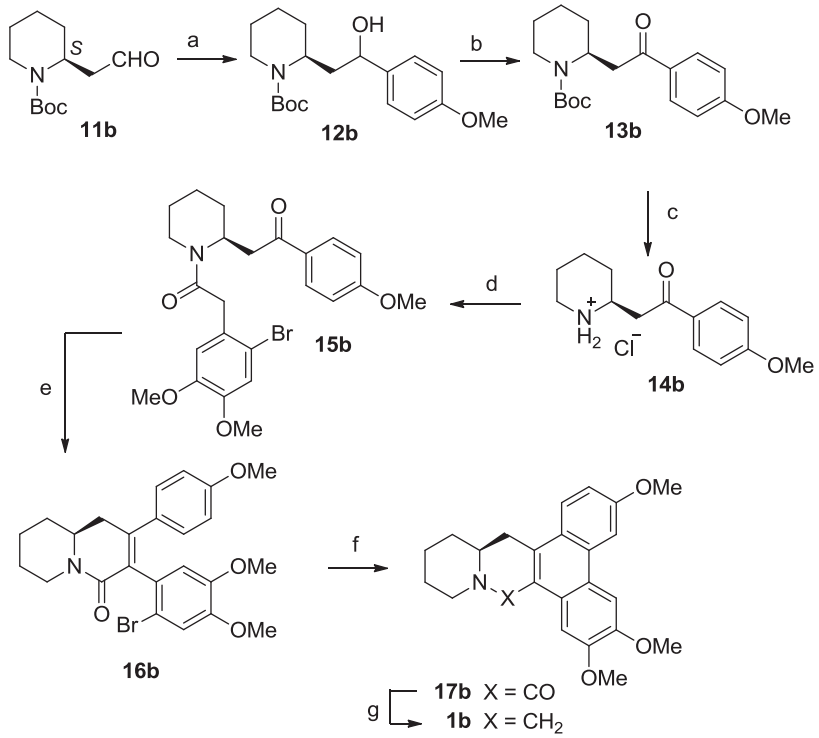
The synthesis commences with a Perkin reaction between commercially available 4-methoxybenzaldehyde (**3**) and 3,4-dimethoxyphenylacetic acid (**4**). This reaction can be easily performed at >100 mmol scale. Next, a microwave-assisted Fischer esterification delivered the corresponding ester **5** in quantitative yield through rapid flash heating. A ferrous chloride mediated oxidative biaryl coupling was then performed generating the quinolizidine ring. Again, this transformation can be performed on multigram scale in an efficient manner; however, the generation of stoichiometric amounts of insoluble inorganic by-products requires close reaction monitoring and specific work-up strategies in order to achieve a reproducible reaction outcome (see Experimental Section). Reduction of the ester functionality of intermediate **6** using $LiAlH_4$ and chlorodehydroxylation of the resulting benzylic alcohol **7** with concentrated HCl cleanly affords the benzylic chloride **8**. This material is subjected to nucleophilic substitution, with the potassium salt of racemic pipecolic acid, furnishing the desired adduct **9** without recourse to any protecting group chemistries. In order to prepare the pentacyclic scaffold of boehmeriasin A, a Friedel–Crafts acylation in neat polyphosphoric acid was used. This transformation was found to reliably furnish the desired ketone **10** in good yield, as long as the instability of this material under basic conditions is addressed by maintaining the pH during the aqueous work-up below 8. Finally, removal of the ketone functionality was accomplished in a two-step fashion firstly using $LiAlH_4$ followed by dehydroxylation under TFA/triethylsilane conditions. In summary, this sequence allowed the preparation of racemic boehmeriasin A in a short 7 step sequence and in 22% overall yield. In addition, the feasibility of separating the racemic natural product by means of chiral HPLC was evaluated. Pleasingly, it was quickly established that for analytical purposes a short chiral HPLC column (AD, 5 cm) can be used in order to achieve resolution of the racemic boehmeriasin A (see insert in Scheme 2). Consequently, we feel confident that separation of the racemate on a preparative scale using a larger AD column can be accomplished; however, before doing so we elected to carry out an asymmetric synthesis of both (*S*)- and (*R*)-boehmeriasin A.

In this second approach we developed an enantioselective synthesis using enantiopure piperidinethanol **2** as the starting material, which can be produced at gram scale [17] and used for target- [17–21] and diversity-oriented [22] synthesis. The aldehyde **11**, obtained from oxidation of the alcohol **2**, was converted to the secondary alcohol **12** by Grignard addition using (4-methoxyphenyl)magnesium bromide in high yield (90–92%, Scheme 3). DMP (Dess-Martin periodinane) oxidation of alcohol **12**, followed by deprotection of the Boc group (**13** → **14**) and *N*-acylation of the amine **14** with the easily prepared 2-bromo-4,5-dimethoxyphenylacetic acid [23] yielded compound **15** in 63% (3



[a]Conditions: : (a) i) Et_3N, Ac_2O, 120 °C, ii) MeOH, H_2SO_4, MW, 125 °C; (b) DCM, anhydr. $FeCl_3$, r.t.; (c) $LiAlH_4$, THF; (d) conc. HCl, DCM, r.t.; (e) pipecolic acid, NaOH, *i*PrOH, 40 °C; f) PPA, 90 °C; g) i) $LiAlH_4$, THF, r.t. ii) Et_3SiH, TFA, DCM, r.t..

Scheme 2. Racemic synthesis of boehmeriasin A and its resolution via chiral HPLC *(path A)*[a].



[a]Conditions: (a) (4-methoxyphenyl)magnesium bromide, THF, -78 ºC to r.t.; (b) DMP, r.t., 1 h; (c) TMSCl, MeOH, 0 ºC to r.t.; (d) 2-bromo-4,5-dimethoxyphenylacetic acid, DIPEA, HATU, THF, r.t., 1 h; (e) KOH, EtOH, reflux, 2 h; (f) $Pd(OAc)_2$, K_2CO_3, 2′-(diphenylphosphino)-*N,N*′-dimethyl-(1,1′-biphenyl)-2-amine, DMA, reflux, 5 h; (g) LiAlH4, THF, reflux, 2 h.

Scheme 3. Enantioselective synthesis of boehmeriasin A *(path B)*[a].

steps) (Scheme 3, *S*-enantiomer shown). An intramolecular aldol-type condensation in the presence of KOH furnished the desirable intermediates **16** as mixture of atropisomers (85%, 1:1 ratio).

To obtain the phenanthroquinolizidine skeleton a palladium catalysed intramolecular coupling was investigated (see Supp. Inf.). The use of $Pd(OAc)_2$ in dimethylacetamide (125 °C for 5 h), in the presence of K_2CO_3 and 2′-(diphenylphosphino)-*N*,*N*′-dimethyl-(1,1′-*biphenyl*)-2-amine as the ligand furnished **17** in 60% yield. A final reduction with $LiAlH_4$, completed the synthesis of boehmeriasin A. HPLC analysis confirmed that the purity of both enantiomers was in accordance with the e.e. of the starting materials (**2**).

2.2. Biological evaluation

2.2.1. Anti-proliferative activity

Both enantiomers of boehmeriasin A, as well as the racemic mixture were evaluated for their anti-proliferative activity (Table 1) in three cancer cell lines [human lymphoblastic leukaemia (CEM), human cervical carcinoma (HeLa) and mouse lymphocytic leukaemia (L1210) cells] and two endothelial cell lines [human microvascular endothelial cells (HMEC-1) and bovine aortic endothelial cells (BAEC)]. As a reference compound, we used the vascular-targeting agent combretastatin A4P (**CA-4P**), which inhibits the proliferation of the tumour cells, and endothelial cells with IC_{50} values around 80 nM and 3 nM, respectively. In accordance with other results reported in the literature [1–3,14] both enantiomers showed potent cytostatic activity against the different tumour cells, with IC_{50} values in the nanomolar range. Interestingly, (*R*)-**1** proved to be significantly more potent than the (*S*)-enantiomer against HeLa and L1210 cells. Both compounds also inhibited the proliferation of endothelial cells, with IC_{50} values of 23 nM in BAEC and 7 and 82 nM for the (*R*)- and (*S*)-enantiomers in HMEC-1, respectively. Together, these data confirm the inhibitory activity of (*R*)- and (*S*)-boehmeriasin A against both tumour and endothelial cells, the (*R*)-enantiomer being up to 11-fold more potent than the (*S*)-enantiomer in selected cell lines. The anti-proliferative activity of the racemic mixture was comparable to the activity of the (*R*)-enantiomer, being equally active in HeLa cells, about 2-fold more active in CEM cells and BAEC and 3–4-fold less active in L1210 cells and HMEC-1.

We were attracted by the possibility to identify the biological targets that could justify the anti-proliferative activity on different cell lines. We wanted to avoid any structural change that could be useful for the application of interesting approaches such as surface plasmon resonance, but has the drawback to require SAR information to identify the proper alterable region of the molecule.

2.2.2. Virtual screening

We planned to use virtual screening with the help of Hurakan software [16] that compares the input molecule with the structures present in the reference database using CoMSIA fields on a 3D grid. Hurakan uses ChemblDB [24] as a reference database because it contains molecules, targets and biological activities. In this way, Hurakan predicts the biological profile of an input molecule and, in our case, it predicted 13 proteins for the *R*-enantiomer and 15 proteins for the *S*-enantiomer (see Supporting Information). We were driven by the fact that topoisomerases were identified as a possible target for the (*S*)-enantiomer.

Table 1
Anti-proliferative activity of (*R*)- and (*S*)-boehmeriasin A in comparison with combretastatin A4P.

Compound	Tumour cell lines (IC_{50}[a] [nM])			Endothelial cell lines (IC_{50}[a] [nM])	
	HeLa	CEM	L1210	HMEC-1	BAEC
(*R*)-**1**	66 ± 56	185 ± 156	19 ± 10	7.4 ± 1.1	23 ± 13
(*S*)-**1**	182 ± 164	201 ± 127	111 ± 11	82 ± 66	23 ± 6
rac.-**1**	76 ± 52	119 ± 71	71 ± 23	29 ± 8	9.0 ± 2.1
CA-4P	79 ± 3	95 ± 6	82 ± 12	2.9 ± 0	3.9 ± 0.1

[a] 50% inhibitory concentration. CA-4P: combretastatin A4P. CEM: human lymphoblastic leukaemia. HeLa: human cervical carcinoma. L1210: mouse lymphocytic leukaemia cells. HMEC-1: human microvascular endothelial cells. BAEC: bovine aortic endothelial cells.

2.2.3. Topoisomerase inhibition

Based on our previous efforts in this field [25–29], both (*R*)- and (*S*)-boehmeriasin A were tested for their capacity to affect the activity of topoisomerases.

Fig. 1 shows the effect of (*R*)- and (*S*)-boehmeriasin A on the relaxation of supercoiled plasmid pBR322 DNA, mediated by topoisomerase II. The enzyme relaxes supercoiled DNA giving rise to a series of topoisomers, representing differently relaxed forms (Topo II). The test compounds were assayed at 10, 25 and 50 μM concentration, while *m*-AMSA, taken as reference drug, was used at 10 μM. Both enantiomers of boehmeriasin A exhibit a comparable and dose-dependent effect on enzymatic activity. Indeed, at 10 μM both (*R*)- and (*S*)-enantiomers are unable to exert a significant inhibitory activity, while the topoisomerase-mediated relaxation is completely inhibited at the higher concentration taken into account (50 μM).

Similar experiments were performed to assay the effect on topoisomerase I relaxation activity, and the results indicate the occurrence of a higher inhibitory effect compared to that obtained on topoisomerase II. Indeed, at 10 μM concentration both (*R*)- and (*S*)-boehmeriasin A completely inhibit the relaxation mediated by topoisomerase I (Fig. 2).

Some anti-proliferative agents, called topoisomerase poisons, interfere with topoisomerase activity by stabilizing a biological intermediate, the cleavable complex, into a lethal agent. The occurrence of the cleavable complex can be demonstrated experimentally by the enzyme-dependent formation of linear (topoisomerase II) or nicked (topoisomerase I) DNA from supercoiled DNA. Fig. 3 shows a cleavable complex assay performed on topoisomerase II in the presence of 100 μM of (*R*)- and (*S*)-boehmeriasin A and 10 μM *m*-AMSA used as reference. The results show that both derivatives do not stabilize the formation of the cleavage complex, although tested at a significantly higher concentration compared to that of the reference drug. Otherwise, as expected, *m*-AMSA, which is a well-known topoisomerase II poison, induces the formation of a detectable amount of linear DNA (Fig. 3).

With respect to topoisomerase I, the results reported in Fig. 4 also show the inability of both (*R*)- and (*S*)-boehmeriasin A to act as a poison at the considered concentration (0.5 μM). On the other hand, the topoisomerase I poison camptothecin (CPT), taken as reference, induces in the same experimental results that of occurrence of the band corresponding to nicked DNA (Fig. 4).

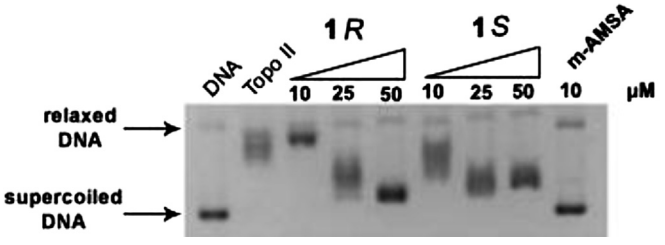


Fig. 1. Effect of (*R*)- and (*S*)-boehmeriasin A on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase II. *m*-AMSA was taken as reference.

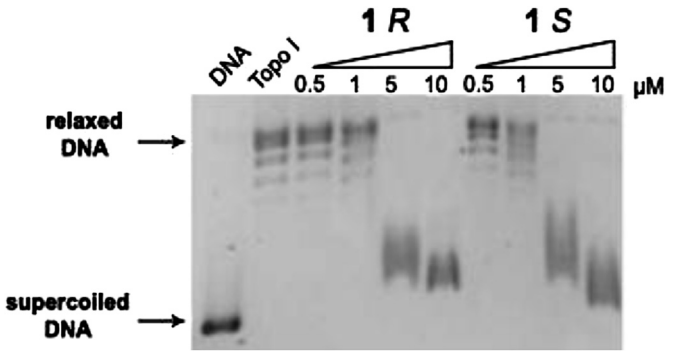


Fig. 2. Effect of (*R*)- and (*S*)-boehmeriasin A on relaxation of supercoiled pBR322 DNA by human recombinant topoisomerase I.

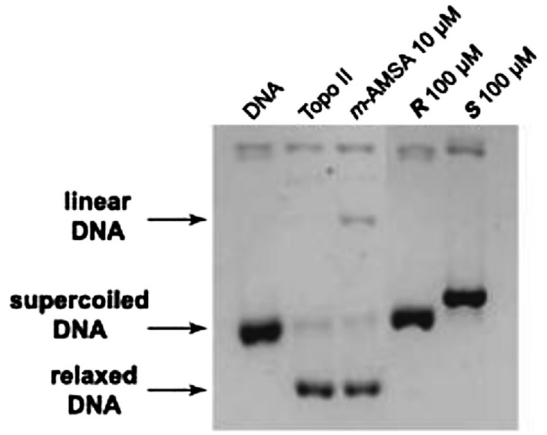


Fig. 3. Effect of (*R*)- and (*S*)-boehmeriasin A on the stabilization of covalent DNA-topoisomerase II complex. *m*-AMSA was taken as reference.

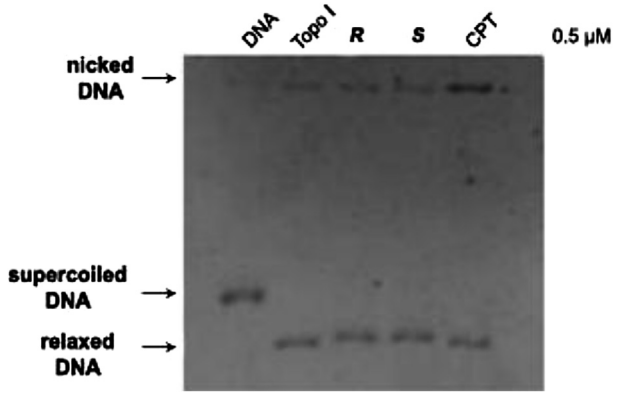


Fig. 4. Effect of (*R*)- and (*S*)-boehmeriasin A on the stabilization of covalent DNA-topoisomerase I complex. Camptothecin (CPT) was taken as reference.

Finally, the ability of (*R*)- and (*S*)-boehmeriasin A to interfere with topoisomerase-mediated relaxation activity, along with the lack of poisoning effects, suggest that the effect on the enzymatic activity could come from the capacity of the compounds to form a molecular complex with DNA.

2.2.4. Sirtuins inhibition

We were driven to study boehmeriasin A against sirtuins due to its structural similarity with known sirtuin inhibitors [30]. Both enantiomers were screened *in vitro* against SIRT1 and SIRT2 at 200 μM concentration (Table 2). For SIRT1 (*R*)- and (*S*)-boehmeriasin A showed no inhibition. Interestingly, for SIRT2 (*R*)-boehmeriasin A gave ~65% inhibition, whereas (*S*)-boehmeriasin A gave only 41% inhibition.

2.2.5. Docking studies

To gain better insight into the interactions between boehmeriasin A the topoisomerases and sirtuins, molecular dockings studies were carried out. Docking simulations on topoisomerase I showed that both boehmerasin enantiomers display good binding affinity to the target. Cluster analysis showed two major clusters, comprising 50% and 36% of the docked structures of the *S* enantiomer (Fig. 5a), and two minor clusters. Cluster analysis of the docking results on the topoisomerase II showed only one cluster for the *S* enantiomer, with the phenanthrene moiety stacked between the DNA bases (Fig. 5b). A slight preference toward topoisomerase II can, on the other hand, be inferred on the basis of the docking results. It is worth noting that in all of the docked conformations both *S* and *R* enantiomers of boehmeriasin A do not display any significant contact with either one of the topoisomerase isoforms that have been tested, but seem to mainly interact with the DNA strand through stacking interactions (see Supp. Inf.).

In SIRT1, both enantiomers of boehmeriasin A were docked into the place of the nicotinamide-moiety (so called C-pocket in sirtuins) showing π-interactions with His363 or Phe273 but they could not adopt a good orientation in the binding pocket (Fig. 5c). As for SIRT2, (*R*)-boehmeriasin A was docked into the C-pocket having an interaction of the phenyl-ring with Ile169 and showed good complementarity with the binding site. Whereas (*S*)-boehmeriasin A had no interactions with SIRT2, but showed also complementary binding. Based on the modelling there was no clear difference in the putative binding modes of the enantiomers (see Supp. Inf.).

3. Conclusion

We described two different approaches for the synthesis of boehmeriasin A which in spite of being strategically based on previous reported preparations present some novelties that result in increased efficacy and simplicity which is relevant for large-scale synthesis and analogue preparation. The high anti-proliferative activity in three endothelial and two cancer cell lines has been described. The biological evaluation accompanied by virtual screening and docking studies permitted to identify the interaction with DNA and SIRT2 as biological mechanisms that justify their activity. These results offer new suggestions for the design and practical synthesis of new topoisomerase and SIRT-2 inhibitors based on the boehmeriasin A scaffold.

4. Experimental section

4.1. Chemistry general procedures

All reactions were carried out in oven-dried glassware and dry solvents under nitrogen atmosphere. Unless otherwise stated, all

Table 2
Evaluation of (*R*)- and (*S*)-boehmeriasin A against SIRT1 and SIRT2.

Compound	SIRT1[a]	SIRT2[a]
(*R*)-**1**	12 ± 1.4	65 ± 1.6
(*S*)-**1**	10 ± 4.2	41 ± 0.56

[a] Inhibition % at 200 μM inhibitor concentration.

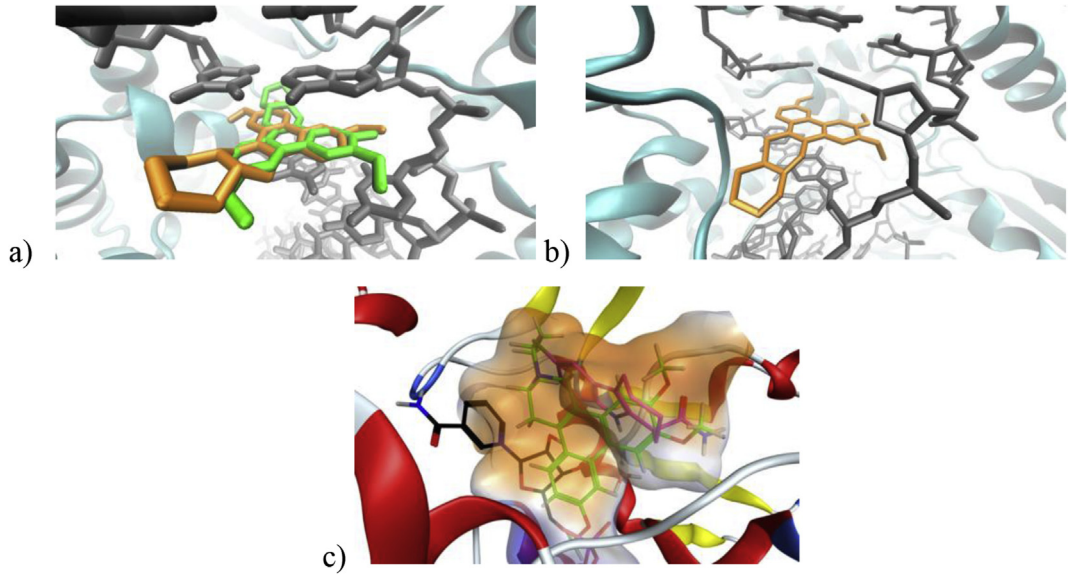


Fig. 5. a) Docked structure of (*S*)-boehmeriasin A. The best fit structure of the principal cluster is depicted in orange, while the best fit structure of secondary cluster is green. Boehmerasin is intercalated between DNA bases of the nucleic acid double helix complexed with topoisomerase I. b) Docked structure of (*S*)-boehmeriasin A (orange molecule). The phenanthrene moiety is intercalated between base pairs of the DNA in complex with topoisomerase II; c) (*R*)-Boehmeriasin A (green) in the putative binding site of SIRT2. NAD+ (black) and Ex-527(purple) is also presented based on their position in SIRT1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

solvents were purchased from Fisher Scientific and Sigma Aldrich and used without further purification. Substrates and reagents were purchased from Alfa Aesar or Sigma Aldrich and used as received. Thin layer chromatography (TLC) was performed on Merck precoated $60F_{254}$ plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or by a solution of $KMnO_4$ with heating. Flash chromatography was performed using silica gel (240–400 mesh, Merck). All tested compounds possessed a purity of >98% confirmed via elemental analyses (CHN) in Perkin Elmer 2400 instrument. Optical rotation was measured on a JAS.CO P-1030 instrument. ^{1}H NMR spectra were recorded on either Bruker DRX-400 or Bruker Avance-400 or Varian VNMRS-700 instruments and are reported relative to residual $CHCl_3$ (δ 7.26 ppm) or DMSO (δ 2.50 ppm). ^{13}C NMR spectra were recorded on the same instruments (100 MHz or 175 MHz) and are reported relative to $CDCl_3$ (δ 77.16 ppm) or d_6-DMSO (δ 39.52 ppm). Chemical shifts (δ) for proton and carbon resonances are quoted in parts per million (ppm) relative to tetramethylsilane (TMS), which was used as an internal standard. Low and high resolution mass spectrometry was performed using the indicated techniques on either Waters LCT Premier XE or Waters TQD instruments equipped with Acquity UPLC and a lock-mass electrospray ion source. EI mass spectra were recorded at an ionizing voltage of 6Kev on a VG 70-70 EQ. Melting ranges were recorded on an Optimelt automated melting point system and are uncorrected operating at a heating rate of 1 °C/min.

4.1.1. (E)-Methyl 2-(3,4-dimethoxyphenyl)-3-(4-methoxyphenyl) acrylate (**5**)

4-Methoxybenzaldehyde (**3**) (13.6 g, 100 mmol) and 3,4-dimethoxyphenylacetic acid (**4**) (19.6 g, 100 mmol) were dissolved in a mixture of triethylamine (10 mL) and acetic anhydride (20 mL) and heated at 120 °C for 30 h. After the reaction mixture was cooled to room temperature ethyl acetate (150 mL) was added leading to precipitation of a yellow solid. After filtration and drying this material was charged into 20 mL microwave vessels (~4 g each), filled with 7 mL methanol and 0.1 mL conc. sulphuric acid and sealed with the appropriate cap. Each of these samples was heated in a Biotage Microwave Synthesizer for 90 min at 125 °C. Upon cooling to rt the desired ester product precipitated as pale yellow solid and was isolated by filtration. Yield (over 2 steps) 70–75% (23–25 g); mp 106.7–107.9 °C (MeOH). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.77 (1H, s), 7.02 (2H, d, J = 8.8 Hz), 6.89 (1H, d, J = 8.0 Hz), 6.78 (1H, dd, J = 8.0 Hz J = 1.6 Hz), 6.73 (1H, d, J = 1.6 Hz), 6.69 (2H, d, J = 8.8 Hz), 3.92 (3H, s), 3.80 (3H, s), 3.78 (3H, s), 3.75 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.7, 160.3, 149.1, 148.6, 140.2, 132.4, 129.6, 128.6, 127.3, 122.1, 113.7, 112.8, 111.4, 55.88, 55.81, 55.22, 52.29. ESI-MS: 351.8 $[M+Na]^+$. HR-MS: calculated for $C_{19}H_{21}O_5$ 329.1389, found 329.1393.

4.1.2. Methyl 3,6,7-trimethoxyphenanthrene-9-carboxylate (**6**)

(*E*)-methyl 2-(3,4-dimethoxyphenyl)-3-(4″-methoxyphenyl) acrylate (**5**) (4 g, 12.2 mmol) was dissolved in DCM (40 mL) at rt. To this solution anhydrous $FeCl_3$ (5 g, 31 mmol) was added portionwise. The resulting reaction mixture was stirred at ambient temperature for 3 h after which more anhydrous $FeCl_3$ (1 g, 6 mmol) was added. After a total reaction time of 5 h complete consumption of substrate was achieved (monitored by ^{1}H NMR). Methanol (~30 mL) was added to this crude mixture resulting in a homogeneous red-brownish solution, which was subsequently extracted with DCM/water giving the desired phenanthrene product as dark brown foam after evaporation of the solvents. Filtration of the dried organic layer over a plug of silica gel (10 g) prior to solvent removal yields the desired product as light brown foam. At this scale the isolated yield commonly varied from 60 to 75% (>90% purity). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.64 (1H, s), 8.43 (1H, s), 7.85 (1H, s), 7.84 (1H, d, J = 8.8 Hz), 7.79 (1H, d, J = 2.4 Hz), 7.20 (1H, dd, J = 8.8 Hz J = 2.4 Hz), 4.10 (3H, s), 4.08 (3H, s), 4.02 (3H, s), 4.01 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 168.2, 160.2, 149.9, 148.9, 133.4, 131.8, 131.3, 125.2, 125.0, 124.2, 121.6, 116.0, 106.9, 103.7, 103.2, 55.90, 55.87, 55.53, 52.03. ESI-MS: 327.0 $[M+H]^+$. HR-MS: calculated for $C_{19}H_{19}O_5$ 327.1232, found 327.1240.

4.1.3. (3,6,7)-Trimethoxyphenanthren-9-yl)-methanol (**7**)

Methyl 3,6,7-trimethoxyphenanthrene-9-carboxylate (**6**)

(3.26 g, 10.0 mmol) was dissolved in THF (20 mL) and cooled to 0 °C. To this vigorously stirred solution, $LiAlH_4$ (880 mg, 25 mmol) was added in small portions over a period of 10 min. After 30 min the ice bath was removed allowing the reaction mixture to warm to rt where it was maintained for 2 h. Upon quenching of the reaction mixture with sat. NH_4Cl (2 mL) a greyish slurry was obtained which was filtered over a plug of silica (eluent DCM). The desired reduction product was obtained after removal of the volatiles as a pale yellow amorphous solid. Yield 95% (2.82 g). ^{1}H NMR ($CDCl_3$, 700 MHz) δ: 7.79 (1H, s), 7.73 (1H, d, J = 2.1 Hz), 7.69 (1H, d, J = 9.1 Hz), 7.51 (1H, s), 7.45 (1H, s), 7.15 (1H, dd, J = 9.1 Hz J = 1.2 Hz), 5.01 (2H, s), 4.06 (3H, s), 4.01 (3H, s), 3.99 (3H, s), 1.92 (1H, br. s). ^{13}C NMR ($CDCl_3$, 175 MHz) δ: 158.3, 149.3, 148.7, 131.3, 131.1, 130.1, 125.7, 125.5, 124.8, 124.4, 115.4, 104.8, 103.9, 103.7, 64.59, 55.91, 55.86, 55.47. ESI-MS: 281.0 $[M-OH]^+$. HR-MS: calculated for $C_{18}H_{17}O_3$ (M−OH) 281.1178, found 281.1186.

4.1.4. 10-(Chloromethyl)-2,3,6-trimethoxyphenanthrene (**8**)

To a vigorously stirred solution of (3,6,7)-trimethoxyphenanthren-9-yl-methanol (**7**) (3.0 g, 10 mmol, in DCM) was added conc. HCl (37%, 5 mL) changing the colour of the initial pale yellow solution to dark brown. After 2 h at rt the reaction is directly extracted (DCM/H_2O) giving the title compound **8** as brown solid after removal of the solvent. Yield 98% (3.1 g). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.76 (1H, s), 7.70 (1H, d, J = 2.4 Hz), 7.66 (1H, d, J = 8.4 Hz), 7.51 (1H, s), 7.40 (1H, s), 7.14 (1H, dd, J = 8.4 Hz J = 2.4 Hz), 4.92 (2H, s), 4.05 (3H, s), 4.04 (3H, s), 3.96 (3H, s). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 158.8, 149.4, 148.9, 131.7, 130.4, 127.9, 126.8, 125.2, 125.1, 125.0, 115.6, 104.7, 103.8, 55.96, 55.91, 55.47, 46.11. ESI-MS (ASAP): 316.1 [radical cation]. HR-MS: calculated for $C_{18}H_{18}O_3Cl$ 317.0944, found 317.0931.

4.1.5. Potassium 1-[(3,6,7-trimethoxyphenanthren-9-yl)methyl] piperidine-2-carboxylate (**9**)

A suspension containing *rac*-pipecolic acid (750 mg, 5.8 mmol) and potassium hydroxide (1.0 g, 17.9 mmol) in isopropanol (6 mL) is stirred at r.t. for 30 min. To this mixture 10-(chloromethyl)-2,3,6-trimethoxyphenanthrene (**8**) (1.6 g, 5 mmol) is added portionwise over 30 min creating a beige slurry. This mixture is stirred at 40 °C for 14 h before cooling to rt. Filtration and washing of this crude material, with a minimal amount of cold isopropanol (3 mL), yields the title compound **9** as beige solid which is used in the subsequent step without further purification. Yield 87% (1.9 g > 90% pure). ^{1}H NMR (d_6-DMSO, 700 MHz) δ: 8.81 (1H, s), 7.94 (2H, br. s), 7.73 (1H, d, J = 8.4 Hz), 7.39 (1H, s), 7.11 (1H, dd, J = 8.4 Hz J = 2.8 Hz), 4.58 (1H, d, J = 12.6 Hz), 3.97 (3H, s), 3.96 (3H, s), 3.92 (3H, s), 3.03 (1H, d, J = 12.6 Hz), 2.54 (1H, m), 2.49 (1H, m), 1.68–1.73 (2H, m), 1.50–1.58 (2H, m), 1.25–1.30 (1H, m), 1.10–1.18 (2H, m). ^{13}C NMR (d_6-DMSO, 175 MHz) δ: 177.7, 158.1, 149.2, 149.0, 131.3, 131.0, 130.0, 127.8, 126.0, 125.5, 124.5, 115.9, 109.6, 104.2, 103.8, 71.8, 60.5, 56.68, 56.11, 55.88, 51.41, 30.87, 25.97, 24.63.

4.1.6. 3,6,7-Trimethoxy-12,13,14,14a-tetrahydro-9H-dibenzo[f,h] pyrido[1,2-b]isoquinolin-15(11H)-one (**10**)

Potassium 1-((3,6,7-trimethoxyphenanthren-9-yl)methyl) piperidine-2-carboxylate (**9**) (900 mg, 2.0 mmol) is added to polyphosphoric acid (~3 g) and stirred at 90 °C for 4–5 h. Within 30 min a thick black solution is obtained which is maintained at this temperature until full conversion of the substrate is obtained (monitored by LC–MS). The reaction mixture is then cooled to rt and carefully quenched by addition of methanol not allowing the temperature to rise above ~40 °C (ice-bath). The resulting solution is then neutralized by careful addition of a saturated solution of potassium carbonate. Extractive work-up of this material with DCM/H_2O gives a crude product which can be purified further by column chromatography (15% EtOAc/Hex) providing the title compound **10** as a pale yellow solid. Yield 70%. ^{1}H NMR ($CDCl_3$, 700 MHz) δ: 9.26 (1H, d, J = 9.8 Hz), 7.24 (1H, s), 7.72 (1H, d, J = 1.4 Hz), 7.22 (1H, dd, J = 9.8 Hz J = 1.4 Hz), 7.11 (1H, s), 4.36 (1H, d, J = 15.4 Hz), 4.07 (3H, s), 4.01 (3H, s), 3.97 (3H, s), 3.65 (1H, d, J = 15.4 Hz), 3.19 (1H, d, J = 10.5 Hz), 2.77 (1H, d, J = 10.5 Hz), 2.47 (1H, d, J = 13.3 Hz), 2.40 (1H, t, J = 13.3 Hz), 1.96 (1H, d, J = 13.3 Hz), 1.70–1.77 (1H, m), 1.66 (1H, tq, J = 13.3 Hz J = 2.8 Hz), 1.60 (1H, q, J = 13.3 Hz), 1.45 (1H, tq, J = 13.3 Hz J = 2.8 Hz). ^{13}C NMR ($CDCl_3$, 175 MHz) δ: 197.3, 157.8, 151.0, 149.5, 139.3, 130.9, 129.3, 127.3, 123.1, 123.0, 122.7, 115.6, 104.3, 104.3, 103.7, 68.92, 55.99, 55.96, 55.89, 55.34, 54.85, 27.38, 24.98, 23.97. ESI-MS: 392.0 $[M+H]^+$. HR-MS: calculated for $C_{24}H_{26}O_4N$ 392.1861, found 392.1855.

4.1.7. 1-(4-Methoxyphenyl)-2-(1-tert-butoxycarbonylpiperidin-2-yl)ethanol (**12**)

To a solution of aldehyde **11** (0.34 g, 1.5 mmol) in THF (13 mL) at −78 °C, (4-methoxyphenyl)magnesium bromide (6 mL, 3 mmol) was added, and the new solution was stirred for 10 min at −78 °C and then overnight at rt. After the completion of the reaction, the solvent was evaporated in vacuum, sat. NH_4Cl was added and the aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with water and brine, dried with Na_2SO_4, filtered and evaporated. The residue was purified by flash column chromatography (Hex/EtOAc 7:3) to provide the two diastereomer alcohols **12** as oils. **12a** (*R*-COH) and **12b** (*S*-COH): Yield 92–95%; R_f = 0.39 (Hex/EtOAc, 7:3); **12a** (*R*-COH): $[a]_D^{13}$ = + 35.9 (c = 0.90 in $CHCl_3$), HR-MS: calculated for $C_{19}H_{30}NO_4$ 336.2175, found 336.2156; **12b** (*S*-COH): $[a]_D^{28}$ = − 36.6 (c = 0.92 in $CHCl_3$), HR-MS found 336.2179. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.32 (2H, d, J = 8.4 Hz), 6.90 (2H, d, J = 8.4 Hz), 4.61 (1H, m), 4.41 (1H, m), 4.04 (1H, m), 3.82 (3H, s), 2.80 (1H, t, J = 11.6 Hz), 2.20 (1H, td, J = 14.0 Hz J = 2.0 Hz), 1.78–1.47 (8H, m), 1.52 (9H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 159.0, 155.6, 136.9, 127.5, 114.4, 81.08, 70.16, 55.99, 47.36, 40.88, 40.25, 29.97, 29.13, 26.20, 19.87. **12a** (*S*-COH) and **12b** (*R*-COH): oils; yields 92–95 %; R_f = 0.17 (Hex/EtOAc, 7:3); **12a** (*S*-COH): $[a]_D^{13}$ = + 71.9 (c = 1.06 in $CHCl_3$), HR-MS: found 336.2160; **12b** (*R*-COH): $[a]_D^{28}$ = − 73.3 (c = 0.98 in $CHCl_3$), HR-MS: found 336.2158. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.32 (2H, d, J = 8.4 Hz), 6.89 (2H, d, J = 8.4 Hz), 4.71 (1H, m), 4.38 (1H, m), 3.94 (1H, m), 3.82 (3H, s), 2.82 (1H, td, J = 13.2 Hz J = 1.6 Hz), 2.12 (1H, dt, J = 14.4 Hz J = 6.8 Hz), 1.85 (1H, dt, J = 14.4 Hz J = 5.6 Hz), 1.62–1.51 (6H, m), 1.48 (9H, s), 1.43–1.40 (1H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 159.6, 156.2, 137.6, 127.7, 114.4, 80.41, 73.03, 55.98, 49.32, 40.86, 40.19, 30.39, 29.17, 25.96, 19.80.

4.1.8. 1-(4-Methoxyphenyl)-2-(1-tert-butoxycarbonylpiperidin-2-yl)ethanone (**13**)

To a solution of alcohol **12** (0.22 g, 0.65 mmol) in DCM (8.4 mL), Dess-Martin periodinane (0.34 g, 0.78 mmol) was added and the new mixture was stirred for 1 h at rt. After the completion of the reaction, the solvent was evaporated in vacuum, 10% K_2CO_3 was added and the aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with water and brine, dried with Na_2SO_4, filtered and evaporated. The residue was purified by flash column chromatography (Hex/EtOAc 7:3) to provide ketone **13** as oil. Yield 83%; R_f = 0.38 (Hex/EtOAc, 7:3); **13a**: $[a]_D^{22}$ = −34.2 (c = 0.95 in $CHCl_3$), HR-MS: calculated for $C_{19}H_{28}NO_4$ 334.2018, found 334.2009; **13b**: $[a]_D^{25}$ = +35.6 (c = 1.00 in $CHCl_3$), HR-MS: found 334.2006. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.00 (2H, d, J = 8.8 Hz), 6.96 (2H, d, J = 8.8 Hz), 4.82 (1H, m), 4.05 (1H, m), 3.89 (3H, s), 3.18 (1H, dd, J = 14.0 Hz J = 6.4 Hz), 3.12 (1H, dd, J = 14.0 Hz J = 6.4 Hz), 2.90 (1H, td, J = 13.0 Hz J = 2.3 Hz), 1.64 (5H, m), 1.41 (10H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 197.0, 163.6, 154.8, 130.6, 130.1, 113.8, 79.57, 55.49, 48.44, 39.44, 38.99, 28.38, 28.13, 25.34,

18.90.

4.1.9. 1-(4-Methoxyphenyl)-2-(piperidin-2-yl)ethanone hydrochloride (**14**)

To a cooled solution at 0 °C of ketone **13** (0.31 g, 0.94 mmol) in MeOH (7.5 mL), TMSCl (0.59 mL, 5.7 mmol) was added and the new solution was stirred overnight at rt. After the completion of the reaction, the solvent was evaporated in vacuum, to provide ketone **14** as oil. Yield 95%; **14a**: $[a]_D^{22} = +17.5$ ($c = 1.14$ in $CHCl_3$); **14b**: $[a]_D^{22} = -18.5$ ($c = 1.25$ in $CHCl_3$) for (*S*)-**14**. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 9.73$ (1H, s), 9.30 (1H, s), 7.92 (2H, d, $J = 8.0$ Hz), 6.86 (2H, d, $J = 8.0$ Hz), 3.84 (3H, s), 3.78–3.72 (1H, m), 3.70–3.64 (1H, m), 3.55–3.47 (2H, m), 2.97–2.91 (1H, m), 2.00–1.80 (5H, m), 1.58–1.50 (1H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 195.7$, 164.7, 131.4, 129.7, 114.6, 56.21, 54.67, 45.82, 41.62, 29.12, 23.08, 22.82.

4.1.10. 2-(2-Bromo-4,5-dimethoxyphenyl)-1-(2-(2-(4-methoxyphenyl)-2-oxoethyl)piperidin-1-yl)ethanone (**15**)

To a solution of 2-bromo-4,5-dimethoxyphenylacetic acid (0.25 g, 0.90 mmol) in THF (23 mL), HATU (0.39 g, 0.99 mmol) and DIPEA (0.31 mL, 1.8 mmol) were added and the new mixture was stirred for 30 min at rt. Then, the solution was cooled at 0 °C and a solution of compound **14** (0.24 g, 0.90 mmol) in THF (12 mL) and DIPEA (200 μL) was added and the new solution was stirred for 1 h at rt. After the completion of the reaction, the solvent was evaporated in vacuum, sat. NH_4Cl was added and the aqueous layer was extracted 3 times with DCM. The combined organic layers were washed with water and brine, dried with Na_2SO_4, filtered and evaporated. The residue was purified by flash column chromatography (Hex/EtOAc 3:7) to provide compound **15** as oil. Yield 80%; $R_f = 0.25$ (Hex/EtOAc, 3:7); **15a**: $[a]_D^{22} = -1.2$ ($c = 0.82$ in $CHCl_3$), HR-MS: calculated for $C_{24}H_{29}BrNO_5$ 490.1229, found 490.1215; **15b**: $[a]_D^{22} = +1.3$ ($c = 0.86$ in $CHCl_3$), HR-MS: found 490.1210. ^{1}H NMR ($CDCl_3$, 400 MHz, amide rotamers 1:1): rotamer a $\delta = 8.05$ (2H, d, $J = 8.8$ Hz), 7.03 (1H, s), 6.97 (2H, d, $J = 8.8$ Hz), 6.83 (1H, s), 5.33–5.28 (1H, m), 4.00 (1H, d, $J = 16.0$ Hz), 3.90 (3H, s), 3.87 (3H, s), 3.85 (3H, s), 3.78–3.72 (2H, m), 3.26 (2H, d, $J = 6.8$ Hz), 3.23–3.20 (1H, m), 1.78–1.56 (5H, m), 1.46–1.30 (1H, m) and rotamer b $\delta = 7.93$ (2H, d, $J = 8.8$ Hz), 6.96 (1H, s), 6.95 (2H, d, $J = 8.8$ Hz), 6.83 (1H, s), 4.78–4.74 (1H, m), 4.67–4.64 (1H, m), 3.87 (3H, s), 3.85 (3H, s), 3.81 (3H, s), 3.78–3.72 (2H, m), 3.18–3.09 (2H, m), 2.70 (1H, td, $J = 13.0$ Hz $J = 2.4$ Hz), 1.78–1.56 (5H, m), 1.46–1.30 (1H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): rotamer a $\delta = 197.5$, 170.4, 164.5, 149.2, 149.0, 131.5, 130.5, 128.1, 116.0, 115.3, 114.6, 114.0, 56.74, 56.21, 47.40, 42.72, 41.55, 39.65, 29.99, 26.29, 20.00 and rotamer b $\delta = 196.4$, 170.1, 164.3, 149.2, 149.0, 131.0, 130.3, 127.7, 116.0, 115.0, 114.6, 113.5, 56.74, 56.14, 50.36, 41.13, 39.25, 38.32, 27.96, 26.16, 19.35.

4.1.11. 3-(2-Bromo-4,5-dimethoxyphenyl)-2-(4-methoxyphenyl)-1,6,7,8,9,9a-hexahydroquinolizin-4-one (**16**)

A solution of compound **15** (0.37 g, 0.76 mmol) in 5% ethanolic KOH (15.8 mL) was refluxed for 2 h. After the completion of the reaction, the solvent was evaporated in vacuum, sat. NH_4Cl was added and the aqueous layer was extracted 3 times with DCM. The combined organic layers were washed with water and brine, dried with Na_2SO_4, filtered and evaporated. The residue was purified by flash column chromatography (Hex/EtOAc 4:6) to provide atropisomer compounds **16** as oils. Yield 85%; **16a (mode 1)**: $R_f = 0.35$ (Hex/EtOAc, 4:6); $[a]_D^{22} = +57.6$ ($c = 0.89$ in $CHCl_3$); HR-MS: calculated for $C_{24}H_{27}BrNO_4$ 472.1123, found 472.1110. **16b (mode 1)**: $[a]_D^{22} = -58.4$ ($c = 0.62$ in $CHCl_3$); HR-MS: found 472.1109. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.01$ (2H, d, $J = 8.4$ Hz), 7.00 (1H, s), 6.70 (2H, d, $J = 8.4$ Hz), 6.46 (1H, s), 4.56 (1H, br. d, $J = 13.6$ Hz), 3.86 (3H, s), 3.76 (3H, s), 3.68 (1H, m), 3.65 (3H, s), 2.86–2.69 (3H, m), 1.95–1.84 (3H, m), 1.58–1.44 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 166.8$, 159.8, 149.3, 148.7, 146.3, 132.2, 131.3, 131.2, 129.6, 116.1, 115.6, 115.5, 114.0, 56.74, 56.58, 55.82, 54.11, 43.46, 38.30, 34.06, 25.37, 23.97. EI-MS 392: [M−Br]. **16a (mode 2)**: $R_f = 0.23$ (Hex/EtOAc, 4:6); $[a]_D^{22} = -39.2$ ($c = 1.09$ in $CHCl_3$); HR-MS: found 472.1113. **16b (mode 2)**: $[a]_D^{22} = +40.4$ ($c = 1.10$ in $CHCl_3$); HR-MS: found 472.1111. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 7.04$ (2H, d, $J = 8.8$ Hz), 6.99 (1H, s), 6.71 (2H, d, $J = 8.8$ Hz), 6.50 (1H, s), 4.62 (1H, br. d, $J = 13.6$ Hz), 3.85 (3H, s), 3.76 (3H, s), 3.70 (3H, s), 3.67–3.60 (1H, m), 3.09 (1H, dd, $J = 17.2$ Hz $J = 6.4$ Hz), 2.69–2.63 (2H, m), 1.97–1.92 (1H, m), 1.89–1.82 (1H, m), 1.76–1.73 (2H, m), 1.70–1.59 (2H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 166.1$, 159.2, 148.6, 148.0, 145.7, 131.9, 130.5, 130.4, 128.9, 115.8, 115.2, 115.0, 113.4, 56.08, 55.95, 55.15, 53.46, 44.64, 36.09, 32.54, 25.10, 24.42.

4.1.12. 3,6,7-Trimethoxy-11,12,13,14,14a,15-hexahydro-9H-phenanthro[9,10-b]quinolizin-9-one (**17**)

To a solution of compound **16** (0.12 g, 0.24 mmol) in DMA (5.3 mL), $Pd(OAc)_2$ (13 mg, 0.058 mmol), 2′-(diphenylphosphino)-N,N′-dimethyl-(1,1′-biphenyl)-2-amine (28 mg, 0.072 mmol) and K_2CO_3 (0.066 g, 0.48 mmol) were added and the new mixture was heated at 125 °C for 5 h. After the completion of the reaction, H_2O was added and the aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with water and brine, dried with Na_2SO_4, filtered and evaporated. The residue was purified by flash column chromatography (Hex/EtOAc 4:6) to provide compound **17** as oil. Yield 60%; $R_f = 0.46$ (Hex/EtOAc, 3:7); **17a**: $[a]_D^{20} = -108.4$ ($c = 0.44$ in $CHCl_3$), HR-MS calculated for $C_{24}H_{26}NO_4$ 392.1861, found 392.1850; **17b**: $[a]_D^{19} = +110.2$ ($c = 0.65$ in $CHCl_3$), HR-MS found 392.1847. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 9.41$ (1H, s), 8.01 (1H, d, $J = 8.8$ Hz), 7.88 (1H, d, $J = 3.2$ Hz), 7.87 (1H, s), 7.23 (1H, dd, $J = 9.2$ Hz $J = 2.4$ Hz), 4.74 (1H, br. d, $J = 11.6$ Hz), 4.12 (6H, s), 4.05 (3H, s), 3.64–3.56 (1H, m), 3.52 (1H, dd, $J = 16.4$ Hz $J = 4.8$ Hz), 3.05 (1H, dd, $J = 16.0$ Hz $J = 5.2$ Hz), 2.90 (1H, td, $J = 13.2$ Hz $J = 3.2$ Hz), 2.01 (1H, br. d, $J = 10.0$ Hz), 1.93–1.91 (2H, m), 1.67–1.48 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 168.2$, 160.2, 150.2, 149.0, 135.1, 133.9, 127.2, 126.4, 125.2, 123.4, 120.3, 116.1, 109.5, 105.1, 103.7, 56.63, 56.52, 56.19, 53.54, 43.48, 36.65, 33.37, 25.39, 23.64. EI-MS: 391 [M].

4.1.13. 3,6,7-Trimethoxy-11,12,13,14,14a,15-hexahydro-9H-dibenzo[f,h]pyrido[1,2-b]isoquinoline, Rac-boehmeriasin A (**1**, path A)

To a solution of 3,6,7-trimethoxy-12,13,14,14a-tetrahydro-9*H*-dibenzo[*f*,*h*]pyrido[1,2-*b*]isoquinolin-15(11*H*)-one (**10**) (391 mg, 1.0 mmol) in THF (5 mL, cooled to 0 °C) $LiAlH_4$ (100 mg, 2.6 mmol) was added portionwise. After 10 min the mixture was allowed to warm to rt where it was stirred for 2 h, prior to careful quenching by addition of aqueous NH_4Cl solution. After aqueous extraction with DCM the combined organic layers were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the intermediate aminoalcohol product as yellow oil which was not purified further. This crude material was redissolved in DCM (2 mL) and combined with TFA (0.3 mL) and Et_3SiH (0.3 mL). After stirring for 4 h at 40 °C the reaction had reached completion (monitored by LC-MS) and was quenched by addition of aqueous $NaHCO_3$ solution. The crude product was isolated after aqueous extraction as yellow foam. Final purification was accomplished by flash column chromatography furnishing racemic boehmeriasin A (**1**) as solid (308 mg, 82%) after evaporation of the solvents. IR (neat) ν 2931.1 (m), 1610.4 (m), 1511.5 (s), 1467.9 (s), 1253.9 (s), 1201.9 (s), 1138.6 (s), 1038.7 (s), 837.8 (m), 783.1 (m), 728.9 (s) cm-1. LR-MS (ESI): 377.9 (M+H). HR-MS: calculated for $C_{24}H_{28}O_3N$ 378.2069, found 378.2055 (Δ −3.7 ppm).

HPLC: (AD 5 cm, EtOH: Hexane 1:9, 1 ml/min, 22 °C) can be used in order to achieve resolution of racemic boehmeriasin A (see Supporting Information).

*4.1.14. 3,6,7-Trimethoxy-11,12,13,14,14a,15-hexahydro-9H-dibenzo [f,h]pyrido[1,2-b]isoquinoline, boehmeriasin A (**1**, path B)*

To a cooled at 0 °C suspension of $LiAlH_4$ (0.020 g, 0.49 mmol) in THF (5 mL), a solution of compound **17** (48 mg, 0.12 mmol) in THF (2.5 mL) was added dropwise and the new mixture was refluxed for 2 h. After the completion of the reaction, the reaction mixture was cooled at 0 °C, and carefully quenched by addition of 10% NaOH aqueous solution and after the THF was evaporated in vacuum. Then, the aqueous layer was extracted 3 times with EtOAc. The combined organic layers were washed with water and brine, dried with Na_2SO_4, filtered and evaporated. The residue was purified by flash column chromatography (DCM/MeOH 9.6:0.4) to provide boehmeriasin A (**1**) as solid. Yield 80%; R_f = 0.24 (DCM/MeOH, 9.6:0.4); **1a**: $[a]_D^{22} = -79.2$ ($c = 0.12$ in MeOH), HR-MS: calculated for $C_{24}H_{28}O_3N$ 378.2069, found 378.2057. Anal. Calcd for $C_{24}H_{27}NO_3$: C, 76.36; H, 7.21; N, 3.71. Found: C, 75.58; H, 7.02; N, 3.56; **1b**: $[a]_D^{22} = +80.6$ ($c = 0.10$ in MeOH), HR-MS: found 378.2058; Found: C, 75.53; H, 7.04; N, 3.53. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.92–7.90 (3H, m), 7.22 (1H, dd, J = 8.8 Hz J = 2.4 Hz), 7.14 (1H, s), 4.64 (1H, d, J = 15.2 Hz), 4.12 (3H, s), 4.07 (3H, s), 4.03 (3H, s), 3.58 (1H, d, J = 15.2 Hz), 3.30 (1H, d, J = 11.2 Hz), 3.18 (1H, dd, J = 16.4 Hz J = 2.8 Hz), 2.94 (1H, dd, J = 16.4 Hz J = 6.0 Hz), 2.41–2.29 (2H, m), 2.03 (1H, d, J = 13.2 Hz), 1.92–1.78 (3H, m), 1.56–1.45 (2H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 158.2, 150.1, 148.9, 130.9, 126.6, 125.9, 125.7, 124.9, 123.9, 115.4, 105.3, 104.7, 103.8, 58.19, 57.05, 56.84, 56.66, 56.19, 35.35, 34.44, 26.67, 25.05.

4.2. Virtual screening

(*R*)- and (*S*)-boehmeriasin A were used as input structures for Hurakan running the jobs on default parameters.

4.3. Biological evaluation

4.3.1. Cell proliferation

4.3.1.1. Endothelial cells. Bovine aortic endothelial cells (BAEC) and human dermal microvascular endothelial cells (HMEC-1) were seeded in 48-well plates at 10,000 cells/well and 20,000 cells/well, respectively. After 24 h, 5-fold dilutions of the compounds were added. The cells were allowed to proliferate 3 days (or 4 days for HMEC-1) in the presence of the compounds, trypsinized, and counted by means of a Coulter counter (Analis, Belgium). *Tumour cells*. Human cervical carcinoma (HeLa) cells were seeded in 96-well plates at 15,000 cells/well in the presence of different concentrations of the compounds. After 4 days of incubation, the cells were trypsinized and counted in a Coulter counter. Suspension cells (Mouse leukaemia L1210 and human lymphoid Cem cells) were seeded in 96-well plates at 60,000 cells/well in the presence of different concentrations of the compounds. L1210 and Cem cells were allowed to proliferate for 48 h or 96 h, respectively and then counted in a Coulter counter. The 50% inhibitory concentration (IC_{50}) was defined as the compound concentration required to reduce cell proliferation by 50%. Combretastatin A-4 phosphate was added as reference compound.

4.3.2. Analysis of in vitro sirtuin inhibition

SIRT1 and SIRT2 in Vitro Assay. The compounds were studied using the Fluor de Lys fluorescence assays which are described in the BioMol product sheet (Enzo Life Sciences). In assays the BioMol KI177 substrate was used for SIRT1 and the KI179 substrate for SIRT2. The determined Km value of SIRT1 for KI177 was 58 μM and the Km of SIRT2 for KI179 was 198 μM [31]. The Km values of SIRT1 and SIRT2 were 558 μM and 547 μM for NAD+ reported by BioMol, respectively. Briefly, assays were carried out using the Fluor de Lys acetylated peptide substrate at 0.7 Km and NAD+ (Sigma N6522 or BioMol KI282) at 0.9 Km, recombinant GST-SIRT1/2-enzyme and SIRT assay buffer (KI286). GST-SIRT1 and GST-SIRT2 were produced as described previously [32,33]. The buffer together with Fluor de Lys acetylated peptide substrate, NAD+ and DMSO/compounds in DMSO (2.5 μL in 50 μL total reaction volume; DMSO from Sigma, D2650) were preincubated for 5 min at room temperature. Then enzyme was added to start the reaction. The reaction mixture was incubated for 1 h at 37 °C and after that, Fluor de Lys developer (KI176) and 2 mM nicotinamide (KI283) in SIRT assay buffer (total volume 50 μL) were added. The incubation was continued for 45 min at 37 °C. Finally, fluorescence readings were obtained using EnVision 2104 Multilabel Reader (PerkinElmer) with excitation wavelength 370 nm and emission 460 nm. The Fluor de Lys fluorescence assays of sirtuins are regularly performed with compounds from our own collections to calibrate data between assay runs.

4.3.3. Topoisomerase-mediated DNA relaxation

Supercoiled pBR322 plasmid DNA (0.25 μg, Fermentas Life Sciences) was incubated with 1U topoisomerase II (human recombinant topoisomerase II α, USB Corporation) or 2U topoisomerase I (human recombinant topoisomerase I, TopoGen) and the test compounds as indicated, for 60 min at 37 °C in 20 μL reaction buffer.

Reactions were stopped by adding 4 μL stop buffer (5% sodium dodecyl sulphate (SDS), 0.125% bromophenol blue, and 25% glycerol), 50 μg/mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel at room temperature. The gels were stained with ethidium bromide 1 μg/mL in TAE buffer (0.04 M Tris–acetate and 0.001 M EDTA), transilluminated by UV light, and fluorescence emission was visualized by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

4.3.4. Topoisomerase II-mediated DNA cleavage

Reaction mixtures (20 μL) containing 10 mM Tris–HCl (pH = 7.9), 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL bovine serum albumin, 1 mM ATP, 0.25 μg pBR322 plasmid DNA (Fermentas Life Sciences), 10 U topoisomerase II (human recombinant topoisomerase II α, USB Corporation) and test compounds were incubated for 60 min at 37 °C. Reactions were stopped by adding 4 μL stop buffer (5% SDS, 0.125% bromophenol blue and 25% glycerol), 50 μg/mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel containing ethidium bromide 0.5 μg/mL at room temperature in TBE buffer (0.09 M Tris–borate and 0.002 M EDTA), transilluminated by UV light, and fluorescence emission was visualized by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

4.3.5. Topoisomerase I-mediated DNA cleavage

Reaction mixtures (20 μL) containing 35 mM Tris–HCl (pH = 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 5 mM spermidine, 0.01% bovine serum albumin, 20 ng pBR322 plasmid DNA (Fermentas Life Sciences), 5 U topoisomerase I (human recombinant topoisomerase I, TopoGen) and test compounds were incubated for 60 min at 37 °C. Reactions were stopped by adding 4 μL of stop buffer (5% SDS, 0.125% bromophenol blue and 25% glycerol), 50 μg/mL proteinase K (Sigma) and incubating for a further 30 min at 37 °C. The samples were separated by electrophoresis on a 1% agarose gel containing ethidium bromide 0.5 μg/mL (Sigma) at room temperature in TBE buffer (0.09 M Tris–borate and 0.002 M EDTA), transilluminated by UV light, and fluorescence emission was visualized by a CCD camera coupled to a Bio-Rad Gel Doc XR apparatus.

4.4. Docking studies

4.4.1. Topoisomerase

(*R*)- and (*S*)-Boehmeriasin A were docked in the enzyme mediated DNA cleavage site in the crystal structure of the topoisomerase I and of the topoisomerase II-beta, both in complex with DNA using AutoDock 4.2 software. *Sirtuins*: (*R*)- and (*S*)-Boehmeriasin A were docked in the crystal structure of SIRT1 complex with Ex-527(PDB id 4I5I) and the homology model of SIRT2 using Schrödinger's Glide software.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.01.038.

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