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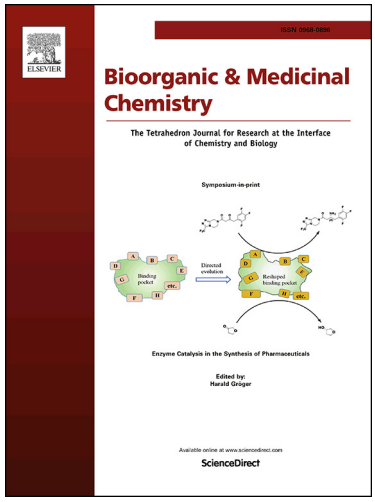

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Design, Synthesis and Antifungal Evaluation of Borrelidin Derivatives

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ABSTRACT

Borrelidin, a nitrile containing 18-membered polyketide macrolide, display potent antifungal activity. In this study, a library of borrelidin derivatives were synthesized. Their structures were elucidated by detailed spectroscopic data analysis. The antifungal activity and cytotoxicity of these target compounds were evaluated by broth microdilution and 3-(4,5-dimethylthiazol-2-yl)-3,5-phenytetrazoliumromide (MTT) methods. Among forty-seven prepared analogues, compound **3b** had the inhibitory effect on *Candida albicans* and *Candida parapsilosis* (MIC: 50 and 12.5 μg/mL, respectively). Furthermore, compounds **4n** and **4r** presented better antifungal activity against *Aspergillus fumigatus* with 12.5 μg/mL MIC value, which were insensitive to borrelidin. Preliminary structure-activity relationships (SAR) revealed that the ester analogues containing fragment -OCH_2CH_2N- had an important effect on the antifungal activity. Meanwhile, the molecular docking study indicated the carboxyl substituents in BN could provide extra interaction with pathogenic fungal threonyl-tRNA synthetase (ThrRS).

1. Introduction

Invasive candidiasis and aspergillosis serve as the mainly life-threatening fungal infections, especially in immunocompromised patients.[1] The majority of these invasive infections are mostly caused by *Candida albicans*, *Candida parapsilosis* and *Aspergillus fumigatus*.[2,3] Unfortunately, limited few classes of antifungal drugs such as polyenes and azoles, had various drawbacks in terms of toxicity, spectrum of activity, and drug resistance to increase the risk of treatment failure.[4,5] Searching for novel antifungal agents from natural products and their derivatives is a significant approach.[6] Borrelidin (BN, Fig. 1), an 18-membered macrolide polyketide, was initially isolated from *Streptomyces roche* in 1949.[7] The gross structure of BN was firstly elucidated in 1967 and subsequently refined by detailed NMR analysis.[8,9] Its absolute configuration was confirmed by X-ray crystallography in 1989.[10] Since then, the macrolide BN was continuously focused on in total synthesis,[11-14] biosynthesis,[15-17] isolation,[18-20] and bioactivity,[21-24] which highlighted its significance. Especially, BN presented strong inhibitory activity against gram-positive, few gram-negative species of bacteria, actinomyces and fungicidal activity.[19,20] However, so far BN had a narrow therapeutic window due to its strong cytotoxic activity.[25] The multiple bioactivities of BN have been confirmed to be correlated with BN suppressing the threonyl-tRNA formation in organisms.[26-29] The expression level or sequence alteration of ThrRS from bacteria to human cells was associated with the potency of inhibition of BN.[30]

The derivatization of BN was limited because of its complicated total synthesis and low output from fermentation of strains. Therefore, few literatures had evaluated the structure-activity relationships (SAR) of BN. In the process of our search for antimicrobial active natural products from actinomycetes, *Streptomyces vinaceusdrappus* (YIM 100880) with a high-yield borrelidin was isolated from the *Hylobates hoolock* feces.[31] Our preliminary research indicated that BN was active in *vitro* against *Candida parapsilosis* (MIC, 50 μg/mL) and no activity against *Candida albicans* and *Aspergillus fumigatus*. In an effort to investigate the antifungal SAR of BN and discover promising antifungal leads with low toxicity, a series of borrelidin derivatives (BNs) were designed and synthesized in the present research. Meanwhile, the docking studies were carried out to explore the new BNs-binding pocket of fungal ThrRS.

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2. Results and Discussion

2.1. Chemistry

A detailed structural and functional analysis of the binding of BN to ThrRS revealed that the plane of the 18-membered ring in BN played a central role in binding the β-sheet of the catalytic domain of ThrRS.[30] Notably, the carboxyl acid group and the cyano moiety were closely correlation with the ThrRS-specific recognition of BN in the different kingdom of life. Thus, a series of BNs with the modifications of cyano and carboxyl groups were designed and synthesized in the present research to discover the promising leads against *C. albicans*, *C. parapsilosis* and *A. fumigatus*. As showed in Fig. 1, the total design strategies of BNs include: (a) hydrolyzation of nitrile functional group to obtain compound **1**; (b) amidation of the carboxyl group at C-23 position to synthesize borrelidin amide analogues **2a-2s**; (c) esterification of the carboxyl group at C-23 position to prepare borrelidin ester analogues **3a-3i** and **4a-4r**.

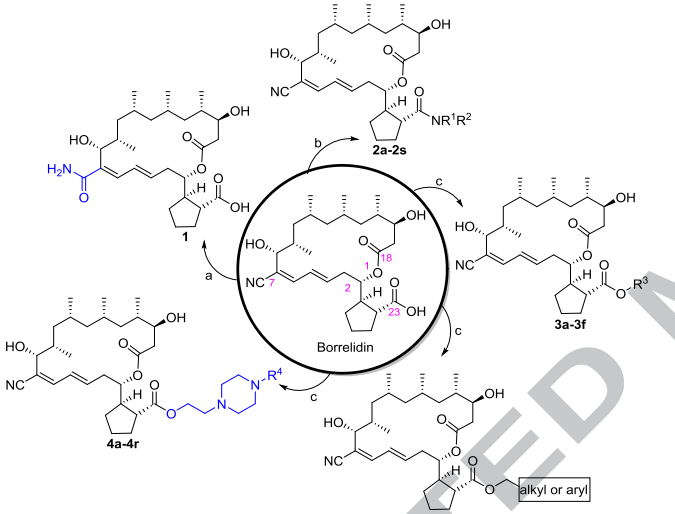
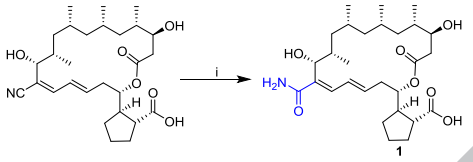
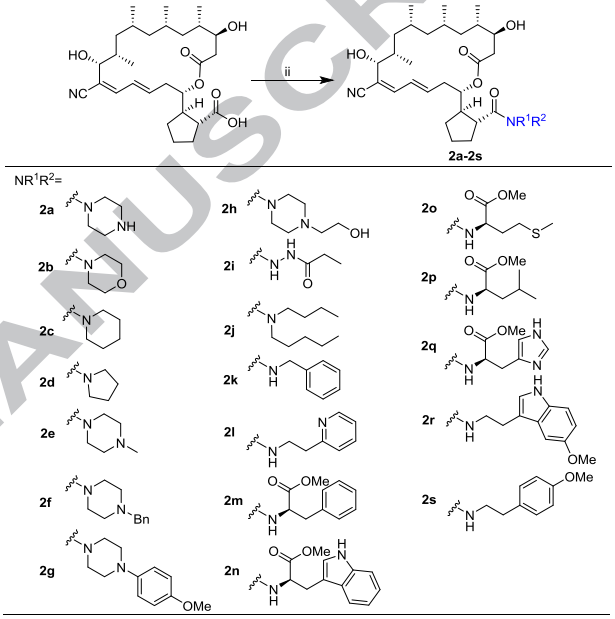


Figure 1. Total design of novel borrelidin derivatives.

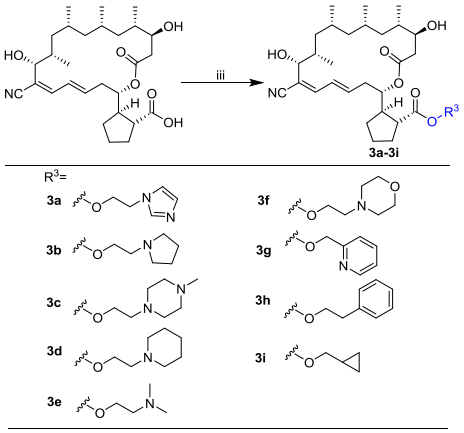
On the basis of the above design strategy, the structure and synthetic routes to these target compounds were described in Schemes 1-4. These reaction conditions were relatively mild without hydrolyzing macrocyclic lactone ring and the yields were high. There was no predecessor that had hydrolyzed the cyano group of BN by organic chemistry method. The hydration of nitriles in BN could not be performed on the strong base condition since lactone ring was instable for acids and bases. A metal catalyst method[32] was firstly used to yield compound **1** in present study (Scheme 1). Nitrogen heterocyclic played an important role in the numerous antifungal regeants to improve its pharmacokinetic properties and biological activity.[33-36] Consequently, some suitable nitrogen heterocyclic were introduced to structural skeleton *via* amidation and esterification. Compounds **2a-2s**, **3a-3i** and **4a-4r** were synthesized as depicted in Schemes 2-4. Among them, compounds **2a-2s** and **3a-3i** were prepared *via* a condensation reaction of BN and different amines and alcohols in the presence of PyBOP or DCC, respectively. Compound **4a** was obtained by condensation of BN and 1-Boc-4-(2-hydroxyethyl)-piperazine and further to give **4b** by the Boc deprotection. Compounds **4c-4r** were synthesized from **4b** by N-acylation or alkylation.



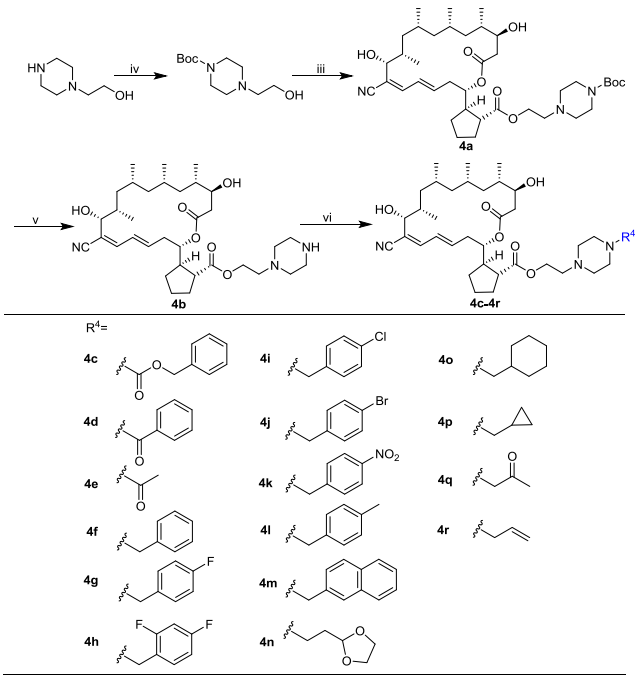
Scheme 1. Preparation of compound **1**. Reagents and conditions: (i) CH_3CH=NOH, CuO, H_2O/MeOH (1:1), 100℃, 12 h.



Scheme 2. Preparation of compound **2a**-**2s**. Reagents and conditions: (ii) PyBoP, TEA, appropriate amines, DCM, r.t, 6-24 h.



Scheme 3. Preparation of compound **3a**-**3i**. Reagents and conditions: (iii) DCC, HoBt, R^3OH (appropriate alcohols), THF, r.t, 12-24 h.



Scheme 4. Preparation of compound **4a**-**4r**. Reagents and conditions: (iv) $(Boc)_2O$, TEA, DCM, r.t, 12 h; (v) TFA/DCM (V/V = 1:1), 0℃→ r.t., 0.5 h; (vi) K_2CO_3, R^4X (appropriate acyl halides or halohydrocarbons), NaI, CH_3CN, 80℃, 2 h.

2.2. Evaluation of Antifungal Activity

The antifungal activities of all synthetic compounds were evaluated *in vitro* by a micro-dilution method to obtain their minimum inhibitory concentration (MIC) values against three pathogenic fungi, *Candida albicans* ATCC MYA-2876, *Candida parapsilosis* ATCC 22019 and *Aspergillus fumigatus* CCTCC AF 93048. Amphotericin B was used as positive control. The MIC values were summarized in Table 1.

The MIC values of compound **1** were all greater than 100 μg/mL against the tested strains, which demonstrated compound **1** with converting the cyano group to primary amide had lost antifungal activity compared with BN. Combined with the activity result and previous literature,[20] it further confirmed that the cyanide group on BN was necessary for its antifungal activity. All of borrelidin amide analogues (**2a**-**2s**), which were synthesized by introducing some nitrogen heterocyclic and amino acid compounds, were found to be no antifungal activity at the concentration of 100 μg/mL. Amide analogues were possibly unable to recognize the specific residues of the enzymatic site of fungal ThrRS, resulting in the resistance of BNs binding to ThrRS.

In order to further explore the effects of the modifications of carboxyl group in BN on the antifungal activity, we attempted to construct the derivatives (**3a**-**3i**) through ester bond in the following research. Besides, piperazine moieties as an important component of many antifungal agents[37] were also implanted into BNs (**4a**-**4r**). The results showed that analogues **3b**-**3f** displayed the inhibition effects against *C. albicans* and *C. parapsilosis* with MIC values in a range of 12.5-100 μg/mL. More significantly, ester analogues **3b**-**3f** presented some inhibition effect against *C. albicans*, while BN was inactive at 100 μg/mL concentration. In comparison with BN (MIC: 50 μg/mL), only ester analogues **3b** (MIC: 12.5 μg/mL) and **3c** (MIC: 25 μg/mL) had excellent antifungal activity against *C. parapsilosis*. Among all of BNs, compouds **3a**-**3d**, **4n**-**4p**, and **4r** showed a certain extent inhibition effect against *A. fumigatus* with 12.5-50 μg/mL MICs. Particularly, the MIC value of compound **4n** and **4r** against *A. fumigatus* could unexpectedly reach 12.5 μg/mL, whereas BN had no inhibition effect at a high concentration of 100 μg/mL.

Table 1. *In vitro* antifungal activity of some compounds [a]

Comp	MIC [b] (μg/mL)		
	C.a [c]	*C.p* [d]	*A.f* [e]
3a	– [f]	–	25
3b	50	12.5	50
3c	100	25	50
3d	100	100	50
3e	100	100	–
3f	100	100	–
4n	–	–	12.5
4o	–	–	25
4p	–	–	50
4r	–	–	12.5
Borrelidin	–	50	–
AmB [g]	2.0	1.0	2.0

[a] The compounds (**1**, **2a**-**2s**, **3g**-**3i**, **4a**-**4m** and **4q**) had no inhibitory effect on the tested pathogenic strains at the concentration 100 μg/mL.

[b] MIC was defined as the minimal inhabitory concentration of a compound. Identical values were obtained for each compound in three replicates by visual investigation as stated in the experimental section.

[c] *Candida albicans* ATCC MYA-2876

[d] *Candida parapsilosis* ATCC 22019

[e] *Aspergillus fumigatus* CCTCC AF 93048

[f] The "–" symbol represents no effect at the highest tested concentration (100 μg/mL)

[g] Amphotericin B (AmB) was used as positive control.

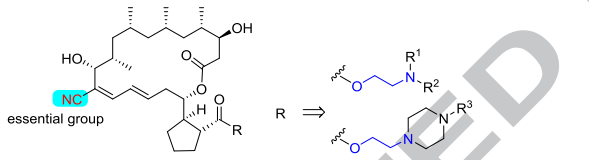
2.3. Structure-Activity Relationship Summary

In combination with the analysis of antifungal activity, the primary structure-activity relationship was summarised in Fig. 2. The loss of cyano group led to the significant decrease of activity (**1** *vs.* BN). The antifungal activity of all amide analogues (**2a**-**2s**) were dramatically decreased or disappeared. The most of ester analogues (**3a**-**3i**, **4a**-**4r**) were apparently better activities than BN, which indicated that the modification of the carboxyl group to the ester-type analogues could produce novel antifungal agents with new structural scaffolds. As can be seen from Fig. 2, the fragment (-OCH_2CH_2N-) was quite favorable for BNs to exert antifungal activity. At the same time, the strong electron donating ability of nitrogen in the fragment (-OCH_2CH_2N-) is beneficial for improving antifungal activity (**3b**-**3f** *vs.* **3a**). Although the introduction of imidazole

group was unfavorable for inhibiting *C. albicans* and *C. parapsilosis*, it was benefit to improve the activity against *A. fumigatus*. Unsubstituted piperazine ring had not change antimicrobial spectrum of borrelidin (**4b** *vs.* BN), but N-alkyl substituted piperazine ring introduced into BN favored to improve its activity against *A. fumigatus* compared with N-acyl/aromatic substituted piperazine ring (**3c**, **4n-4r** *vs.* **4b-4m**). Only N-methyl piperazine derivative of BNs (**3c**) presented the activity against *C. albicans* and *C. parapsilosis*.

2.4. Cytotoxicity and selectivity index of BNs

To create BNs' selectivity between fungi and human cells,[38] *in vitro* cytotoxicities of BNs (**3a-3f**, **4n-4p** and **4r**) were further examined using human kidney epithelial cell line (293T) by MTT method. As described in Table 2, the cytotoxicities of all the tested derivatives dropped obviously in comparation with BN. The selectivity index value of derivative **3b** with the best activity against *C. albicans*, was at least five times that of BN. Compounds **3b** and **3c** with satisfying activity against *C. parapsilosis* gave good selectivity index values (SI: 2.81, 2.34). Remarkably, The SI values of derivatives (**3c**, **4n** and **4r**) possessing the excellent activity against *A. fumigatus* were 8-40-fold superior to that of BN. Overall, these results suggested that borrelidin analogs (**3b**, **3c**, **4n** and **4r**) have a potential usefulness in development of drugs for infectious diseases and pathogenic fungi control.



- cyano group is essential for antifungal activity.
- ester analogues can improve the antimicrobial spectrum
- -OCH_2CH_2N- and the strong electron donating ability of nitrogen in the fragment are quite favorable for antifungal activity.
- unsubstituted piperazine moiety do not change the antimicrobial spectrum.
- N-alkyl substituted piperazine group is favorable to improve the activity against *A. fumigatus*, but only N-methyl piperazine group improve the activities against *C. albicans* and *C. parapsilosis*.
- Imi

Figure 2. Structure-activity relationships of borrelidin *albicans* and *C. parapsilosis*.

Table 2. IC_{50} (μg/mL) and selectivity index (SI) values of active compounds against 293T

Comp	IC_{50} [a] (μg/mL)	SI value [b]		
	293T [c]	*C.a*	*C.p*	*A.f*
3a	18.09±0.42	<0.18 [d]	<0.18	0.72
3b	35.18±0.56	0.71	2.81	0.71
3c	58.46±0.31	0.58	2.34	1.17
3d	27.62±0.28	0.28	0.28	0.55
3e	39.23±0.26	0.39	0.39	<0.39
3f	28.02±0.74	0.28	0.28	<0.28
4n	>70.15 [e]	<0.70	<0.70	>5.61
4o	23.02±1.00	<0.23	<0.23	0.92
4p	17.04±0.62	<0.17	<0.17	0.34
4r	22.04±0.70	<0.22	<0.22	1.76
Borrelidin	13.97±0.15	<0.14	0.28	<0.14

[a] IC_{50} is defined as the concentration at which 50% growth is inhibited.

[b] Selectivity index (in *vitro*) = cytotoxicity (IC_{50}: μg/mL)/antifungal activity (MIC: μg/mL)

[c] 293T: human kidney epithelial cell line

[d] The MIC values of these compounds > 100 μg/mL.

[e] The IC_{50} value of **4n** > 100 μM

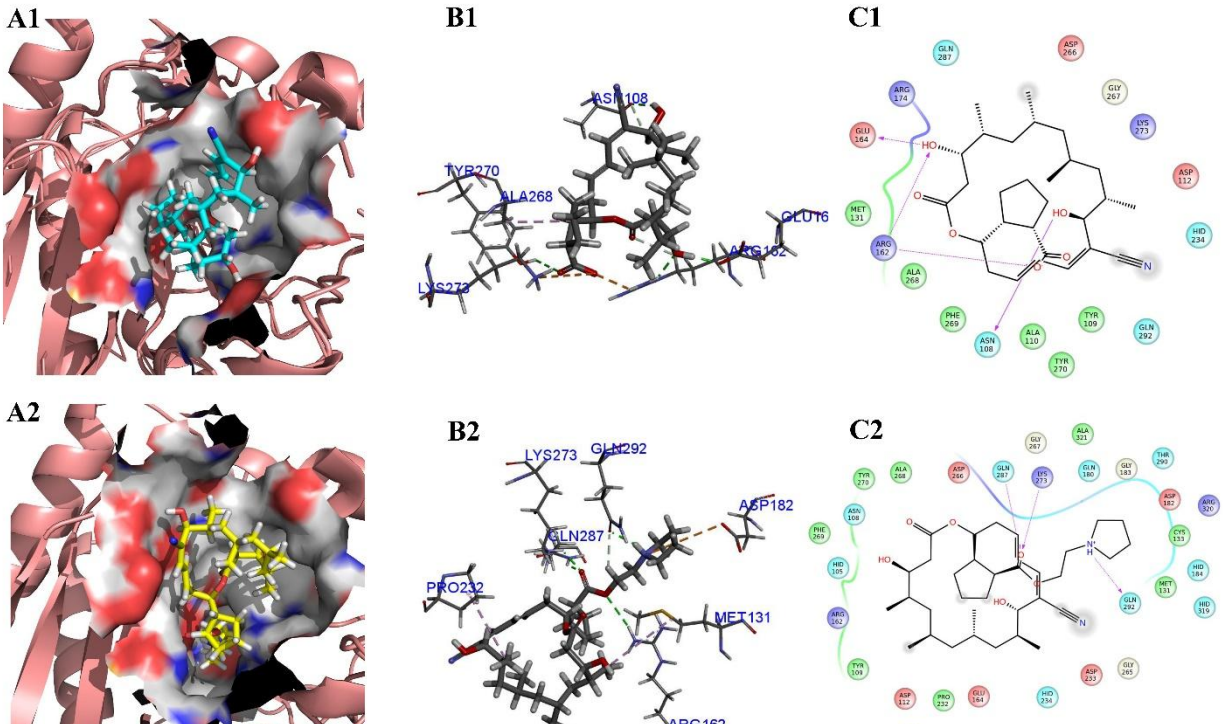


Figure 3. Molecular binding mode of BN (**A1**) and **3b** (**A2**) into the active pocket of ThrRS (protein surface diagram in software PyMOL); the bingding modes of BN (**B1**) and **3b** (**B2**) with the key residues of ThrRS (3D interaction relationship diagram in software Discovery Studio 3.0; Purple: hydrophobic interaction, Green: hydrogen bond, Red: ionic bond); hydrogen bonding interactions of BN (**C1**) and **3b** (**C2**) with the key residues of ThrRS (2D interactions in Glide module; Purple: hydrogen bond, The arrow point to the hydrogen bond acceptor).

2.5. Molecular Docking

Molecular docking is an effective and reliable tool able to locate the probable binding interactions of ligands with their target proteins.[39,40] BN is a natural inhibitor of threonyl-tRNA synthetase (ThrRS).[23] Due to the absence of crystal structure of ThrRS from *C. albicans*, *C. parapsilosis* and *A. fumigatus* in the Protein Data Bank, the docking studies were performed based on the X-ray structure of the yeast mitochondrial ThrRS (PDB ID: 4yye). Hence, BN and the potent analogue **3b** were docked in the active site of ThrRS to predict binding mode and support the antifungal results (Fig. 3). Although the compound **3b** filled the similar binding space as BN, the side chain of **3b** was projected deeper into the bottom of binding pocket than BN as shown in figure 3A1/A2. Compared to BN, **3b** did not establish hydrogen bonds with Asn108, Glu164 and Arg162 through 8/16-hydroxyl groups but form hydrophobic interactions with Pro232 and Met131 through 9/15-methyl groups (Fig. 3B1/B2). The great distinction of **3b** and BN depended on the formation of hydrogen bonds between 23-position substituted group and different amino acids in ThrRS. Gln287 and Lys273 oriented toward 23-position carbonyl of **3b** to form two hydrogen bonds. Oxygen atom in the fragment -OCH_2CH_2N- of **3b** was positioned toward amino group of guanidyl on Arg162 to make hydrogen bond. Both methylene group in the fragment -OCH_2CH_2N- and the pyrrole-NH of **3b** were also involved in the interactions with active site residue Gln292. Moreover, the binding mode revealed that the compound **3b** bearing the pyrrolidine ring created a strong ionic bond *via* interacting with the important acidic amino acid Asp182. Perhaps, this was the primary cause of its improving antifungal activity. According to the in *vitro* antifungal activity and molecular docking results, it was established that the 23-position substituted group of BNs extended into a new orthogonal pocket, which underwent induced-fit nature of BNs' interaction with ThrRS of pathogenic fungi.

3. Conclusions

In summary, a series of antifungal borrelidin derivatives were designed and synthesized *via* the rational design. The SAR indicated that the presence of nitrile group and the fragment (-OCH_2CH_2N-) introduced into carboxyl of BN were essential for antifungal activity. The biological assay displayed a good selectivity index between fungi and human cells for the active BNs (**3b**, **4n** and **4r**), which were greatly promising antifungal candidates. Furthermore, the molecular docking investigation indicated the carboxyl substituents in BN existed extra interaction with ThrRS of pathogenic fungi, which provided useful guidelines for new tRNA synthetase inhibitor.

4. Experimental Section

4.1. General Experimental Procedures

The synthetic routes to borrelidin derivatives **1**, **2a-2s**, **3a-3i** and **4a-4r** were depicted in Schemes 1-4. All the target compounds were fully analyzed and characterized by ^1H and ^{13}C NMR spectra, high resolution mass spectrometry (ESI-HRMS), optical rotations, ultraviolet spectra and IR spectra before biological screening. Column chromatography were Silica gel (100-200 mesh, 200-300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Sephadex LH-20 (GE Healthcare Biosciences AB, Uppsala, Sweden), YMC*GEL ODS-A (S-50 μm, 12 nm) (YMC Co., Ltd., Kyoto, Japan), and Amberlite XAD-16 polymeric resin (Rohm and Hass ShanghaiChemical Industry Co., Ltd., Shanghai, China) were used for columnchromatography. MTT and antimicrobial assays were analyzed using amicroplate reader (Bio Tek Synergy H1, Bio Tek Instruments, Inc., Vermont, USA).

4.1.1. Synthesis of (1*R*,2*R*)-2-[(2*S*,4*E*,6*E*,8*R*,9*S*,11*R*,13*S*,15*S*, 16*S*)-7-carbamoyl-8-hydroxy-9,11,13,15,16-pentamethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylic acid (**1**)

To a 10 mL round-bottom flask were added BN 20 mg (0.041 mmol), acetaldoxime 4.84 mg (0.082 mmol), copper oxide 1.59 mg (0.020 mmol) and H_2O/MeOH (v/v =1:1, 3 mL). The mixture was heated to reflux for 12 h. The completion of reaction was checked with monitoring TLC. After cooling to room temperature, the reaction mixture was directly evaporated to dryness and the residue was purified by column chromatography on silica gel (dichloromethane/methanol = 25:1) to give **1** as a light-yellow crystal (70% yield): $[\alpha]_D^{20}$ -3.4 (c 1.18, MeOH); UV (MeOH) λmax (log ε) 256 (4.33) nm; IR v 3409, 2952,

2925, 1718, 1658, 1578, 1454, 1384, 1267, 1182, 1023, 976 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.91 (d, J = 11.4 Hz, 1H), 6.46 (dd, J = 14.9, 11.4 Hz, 1H), 6.13 (ddd, J = 14.9, 10.5, 4.6 Hz, 1H), 4.85 (dt, J = 10.5, 3.7 Hz, 1H), 4.21 (d, J = 10.0 Hz, 1H), 3.80 (dt, J = 10.3, 2.9 Hz, 1H), 2.59 (quint, J = 9.4 Hz, 1H), 2.49 (ddd, J = 14.9, 10.6, 4.2 Hz, 1H), 2.42 (brd, J = 14.9 Hz, 1H), 2.32 (q, J = 7.7 Hz, 1H), 2.28 (dd, J = 16.0, 2.9 Hz, 1H), 2.12 (dd, J = 16.0, 10.1 Hz, 1H), 1.92-1.86 (m, 2H), 1.84 (m, 1H), 1.78-1.62 (m, 5H), 1.50 (m, 1H), 1.31 (m, 1H), 1.06 (m, 2H), 0.94 (d, J = 6.3 Hz, 3H, CH_3), 0.93 (m, 1H), 0.87 (m, 1H), 0.84 (m, 1H), 0.75 (d, J = 6.7 Hz, 3H, CH_3), 0.74 (d, J = 6.7 Hz, 3H, CH_3), 0.67 (d, J = 6.5 Hz, 3H, CH_3), 0.58 (brt, J = 13.2 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 174.9, 172.5, 171.8, 136.7, 136.3, 132.9, 128.0, 76.2, 73.2, 71.6, 48.9, 48.2, 46.1, 43.4, 38.3, 36.9, 35.6, 35.2, 34.4, 31.1, 29.2, 27.1, 26.3, 24.9, 19.6, 17.6, 17.2, 14.6. ESI-HRMS (*m/z*): calcd. for $C_{28}H_{46}NO_7$ $[M+H]^+$: 508.3274; found, 508.3273.

4.1.2. General Procedure for the Synthesis of Compounds **2a-2s**

To a stirred solution of BN (30 mg, 0.061 mmol) in DCM (1.0 mL) was added TEA (12.4 mg, 0.122 mmol) and PyBoP (35.1 mg, 0.067 mmol) at room temperature. After 30 min, the appropriate amine (0.153 mmol) was added. Keep stirring for 6-24 h, the reaction mixture was washed with 3% HCl aqueous (2 × 10 mL), saturated $NaHCO_3$ aqueous (2 × 10 mL) and brine (2 × 10 mL). The resulting organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case. The data of **2k** was found in previous literature.[41]

4.1.2.1. (2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-8,16-dihydroxy-9,11, 13,15-tetramethyl-2-[(1*R*,2*R*)-2-(piperazine-1-carbonyl)cyclo-pentyl]-18-oxooxacyclooctadeca-4,6-diene-7-carbonitrile (***2a***)

Eluent dichloromethane/methanol (40:1). light-yellow crystal. 96% yield: $[\alpha]_D^{20}$ -16 (c 0.82, MeOH); UV (MeOH) λmax (log ε) 254 (4.50) nm; IR ν 3399, 2956, 2921, 2873, 2211, 1720, 1631, 1446, 1374, 1262, 1180, 1037, 974 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.93 (d, J = 11.3 Hz, 1H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.39 (ddd, J = 14.9, 10.0, 5.3 Hz, 1H), 4.98 (dt, J = 9.8, 3.6 Hz, 1H), 4.19 (d, J = 9.8 Hz, 1H), 3.90 (m, 1H), 3.68-3.54 (m, 4H, 2 × -CH_2-N-CO-), 3.00-2.85 (m, 6H, 2 × -CH_2-N-, 2 × -CH-), 2.60-2.53 (m, 2H), 2.28 (m, 2H), 2.12-2.00 (m, 2H), 1.86-1.69 (m, 5H), 1.67 (m, 1H), 1.56 (m, 1H), 1.47 (m, 1H), 1.26-1.18 (m, 2H), 1.11 (brt, J = 12.6 Hz, 1H), 1.04 (d, J = 6.4 Hz, 3H, CH_3), 0.98-0.93 (m, 2H), 0.87 (d, J = 6.9 Hz, 3H, CH_3), 0.85 (d, J = 6.4 Hz, 3H, CH_3), 0.84 (d, J = 6.0 Hz, 3H, CH_3), 0.70 (brt, J = 12.6 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 174.7, 171.5, 144.0, 139.0, 127.1, 118.4, 115.9, 76.5, 71.6, 70.2, 45.3, 45.0, 44.7, 44.3, 44.2, 43.1, 41.3, 38.7, 37.4, 35.7, 35.6, 34.4, 30.9, 29.0, 27.0, 26.0, 24.2, 19.3, 17.0, 16.7, 13.8. ESI-HRMS (*m/z*): calcd. for $C_{32}H_{52}N_3O_5$ $[M+H]^+$: 560.3907; found, 560.3910.

4.1.2.2. (2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-8,16-dihydroxy-9,11, 13,15-tetramethyl-2-[(1*R*,2*R*)-2-(morpholine-4-carbonyl)cyclo-pentyl]-18-oxooxacyclooctadeca-4,6-diene-7-carbonitrile (***2b***)

Eluent dichloromethane/methanol (40:1). light-yellow crystal. 96% yield: $[\alpha]_D^{20}$ -16 (c 0.82, MeOH); UV (MeOH) λmax (log ε) 254 (4.50) nm; IR ν 3488, 3430, 2957, 2921, 2864, 2212, 1723, 1628, 1525, 1456, 1379, 1265, 1229, 1182, 1116, 1075, 1036, 976, 857, 790, 700 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.93 (d, J = 11.3 Hz, 1H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.39 (ddd, J = 14.9, 8.6, 6.6 Hz, 1H), 4.98 (dt, J = 9.9, 3.4 Hz, 1H), 4.18 (d, J = 9.8 Hz, 1H), 3.90 (ddd, J = 8.0, 4.5, 3.2 Hz, 1H), 3.70-3.59 (m, 5H, 2 × -CH_2-N-CO-), 3.54-3.47 (m, 3H, 2 × -CH_2O-), 2.97-2.90 (m, 2H), 2.57 (m, 2H), 2.31 (dd, J = 11.3, 3.6 Hz, 1H), 2.27 (d, J = 11.3 Hz, 1H), 2.12-2.00 (m, 2H), 1.86-1.69 (m, 5H), 1.67 (m, 1H), 1.56 (m, 1H), 1.47 (m, 1H), 1.24-1.19 (m, 2H), 1.11 (brt, J = 12.5 Hz, 1H), 1.04 (d, J = 6.4 Hz, 3H, CH_3), 0.98-0.93 (m, 2H), 0.87 (d, J = 6.9 Hz, 3H, CH_3), 0.85 (d, J = 6.4 Hz, 3H, CH_3), 0.84 (d, J = 6.0 Hz, 3H, CH_3), 0.70 (brt, J = 12.5 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 174.9, 171.5, 144.0, 139.0, 127.1, 118.4, 115.9, 76.5, 71.6, 70.4, 66.4, 66.3, 47.8, 45.9, 45.2, 44.2, 43.1, 42.3, 38.3, 37.5, 35.6, 35.6, 34.4, 31.0, 29.0, 27.0, 26.0, 24.3, 19.3, 17.0, 16.9, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{32}H_{51}N_2O_6$ $[M+H]^+$: 559.3747; found, 559.3749.

4.1.2.3. (2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-8,16-dihydroxy-9,11, 13,15-tetramethyl-2-[(1*R*,2*R*)-2-(piperidine-1-carbonyl)cyclo-pentyl]-18-oxooxacyclooctadeca-4,6-diene-7-carbonitrile (***2c***)

Eluent petroleum ether/EtOAc (3:1). Colorless crystal. 89% yield: $[\alpha]_D^{20}$ -4.4 (c 0.92, MeOH); UV (MeOH) λmax (log ε) 254 (4.56) nm; IR ν 3428, 2927, 2864, 2212, 1725, 1618, 1454, 1378, 1253, 1187, 1126, 1036, 975, 854, 812, 755, 696 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.94 (d, J = 11.3 Hz, 1H), 6.62 (brt, J = 11.3 Hz, 1H), 6.40 (m, 1H), 5.00 (dt, J = 10.6, 3.5 Hz, 1H), 4.20 (d, J = 9.8 Hz, 1H), 3.92 (dt, J = 9.8, 3.3 Hz, 1H), 3.57 (m, 2H, 1 × -CH_2-N-CO-), 3.49 (m, 2H, 1 × -CH_2-N-CO-), 2.95 (m, 2H), 2.57 (m, 2H), 2.27 (m, 2H), 2.04 (m, 2H), 1.79 (m, 5H), 1.68 (m, 2H), 1.55 (m, 6H, 3 × -CH_2-), 1.45 (m, 1H), 1.23 (m, 2H), 1.11 (m, 1H), 1.05 (d, J = 6.4 Hz, 3H, CH_3), 0.97 (m, 2H), 0.86 (brd, J = 6.4 Hz, 9H, 3 × CH_3), 0.70 (m, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 174.4, 171.6, 144.1, 139.2, 127.2, 118.5, 115.9, 76.6, 71.6, 70.6, 48.0, 46.5, 45.4, 44.3, 43.2, 43.1, 37.9, 37.5, 35.7, 35.6, 34.5, 31.2, 29.2, 26.9, 26.4, 26.1, 25.5, 24.4, 24.1, 19.4, 17.1, 17.0, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{33}H_{52}N_2O_5Na$ $[M+Na]^+$: 579.3774; found, 579.3770.

4.1.2.4. (2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-8,16-dihydroxy-9,11, 13,15-tetramethyl-2-[(1*R*,2*R*)-2-(pyrrolidine-1-carbonyl)cyclo-pentyl]-18-oxooxacyclooctadeca-4,6-diene-7-carbonitrile (***2d***)

Eluent dichloromethane/methanol (80:1). Colorless crystal. 83% yield: $[\alpha]_D^{20}$ -16.9 (c 1.06, MeOH); UV (MeOH) λmax (log ε) 252 (4.68) nm; IR ν 3400, 2954, 2878, 2212, 1723, 1619, 1452, 1380, 1260, 1180, 1087, 1040, 976, 832, 788, 737, 657 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.90 (d, J = 11.3 Hz, 1H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.35 (m, 1H), 5.01 (dt, J = 10.6, 3.5 Hz, 1H), 4.17 (d, J = 9.8 Hz, 1H), 3.88 (dt, J = 9.2, 3.3 Hz, 1H), 3.45 (t, J = 6.8 Hz, 2H, 1 × -CH_2-N-CO-), 3.38 (m, 2H, 1 × -CH_2-N-CO-), 2.82 (m, 1H), 2.72 (q, J = 9.0 Hz, 1H), 2.54 (dd, J = 7.5, 3.1 Hz, 2H), 2.23 (dd, J = 16.1, 9.5 Hz, 1H), 2.19 (dd, J = 16.1, 3.7 Hz, 1H), 2.11-2.00 (m, 2H), 1.98-1.83 (m, 3H, 2 × -CH_2-), 1.84-1.74 (m, 6H), 1.65 (m, 1H), 1.59 (m, 1H), 1.44 (m, 1H), 1.20 (m, 2H), 1.09(brt, J = 12.6 Hz, 1H), 1.03 (d, J = 6.4 Hz, 3H, CH_3), 0.93-0.98 (m, 2H), 0.84 (d, J = 6.2 Hz, 6H, CH_3), 0.83 (d, J = 6.8 Hz, 3H, CH_3), 0.67 (brt, J = 12.6 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 175.0, 171.5, 144.1, 139.0, 127.3, 118.5, 116.0, 76.5, 71.6, 70.9, 48.0, 46.5, 45.8, 44.7,43.2, 37.6, 37.3, 35.8, 35.6, 34.5, 30.5, 28.9, 27.0, 26.1, 25.5, 24.2, 24.0, 19.5, 17.4,

17.1, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{32}H_{51}N_2O_5Na$ $[M+Na]^+$: 565.3617; found, 565.3614.

4.1.2.5. (2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-8,16-dihydroxy-9,11, 13,15-tetramethyl-2-[(1*R*,2*R*)-2-(4-methylpiperazine-1-carbonyl)cyclopentyl]-18-oxooxacyclooctadeca-4,6-diene-7-carbonitrile (***2e***)

Eluent dichloromethane/methanol (80:1). light-yellow crystal. 87% yield: $[\alpha]_D^{20}$ -4.1 (c 0.97, MeOH); UV (MeOH) λmax (log ε) 256 (4.48) nm; IR v 3428, 2956, 2923, 2802, 2213, 1721, 1628, 1460, 1384, 1261, 1163, 1087, 1035, 844, 731, 656 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.95 (d, J = 11.3 Hz, 1H), 6.63 (dd, J = 14.9, 11.3 Hz, 1H), 6.41 (ddd, J = 14.9, 8.4, 6.2 Hz, 1H), 5.00 (dt, J = 10.3, 3.6 Hz, 1H), 4.20 (d, J = 9.8 Hz, 1H), 3.92 (m, 1H), 3.76-3.46 (m, 4H, 2 × -CH_2-N-CO-), 2.95 (m, 2H), 2.64-2.46 (m, 6H), 2.39 (s, 3H, -N-CH_3), 2.31 (dd, J = 11.6, 4.0 Hz, 1H), 2.27 (d, J = 11.6 Hz, 1H), 2.15-2.00 (m, 2H), 1.89-1.72 (m, 5H), 1.68 (m, 1H), 1.57 (m, 1H), 1.48 (m, 1H), 1.23 (brt, J = 11.9 Hz, 2H), 1.12 (brt, J = 11.6 Hz, 1H), 1.06 (d, J = 6.5 Hz, 3H, CH_3), 0.98-0.94 (m, 2H), 0.88 (d, J = 6.6 Hz, 3H, CH_3), 0.86 (d, J = 6.5 Hz, 3H, CH_3), 0.85 (d, J = 6.5 Hz, 3H, CH_3), 0.70 (brt, J = 11.6 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 174.8, 171.6, 144.1, 139.2, 127.2, 118.5, 115.9, 76.6, 71.7, 70.5, 54.5, 54.1, 47.9, 47.5, 45.4, 44.6, 44.3, 43.2, 41.2, 38.4, 37.5, 35.8, 35.7, 34.5, 31.1, 29.1, 27.0, 26.1, 24.3, 19.4, 17.2, 17.0, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{33}H_{54}N_3O_5$ $[M+H]^+$: 572.4063; found, 572.4063.

4.1.2.6. (2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-2-[(1*R*,2*R*)-2-(4-benzylpiperazine-1-carbonyl)cyclopentyl]-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-diene-7-carbonitrile (***2f***)

Eluent dichloromethane/methanol (80:1). light-yellow crystal. 90% yield: $[\alpha]_D^{20}$ -8.1 (c 0.99, MeOH); UV (MeOH) λmax (log ε) 200 (4.72), 256 (4.57) nm; IR v 3442, 2956, 2922, 2877, 2212, 1724, 1629, 1458, 1378, 1260, 1224, 1182, 1125, 1038, 976, 843, 742, 701, 646, 626 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.35 (m, 4H, Ar-H), 7.31 (m, 1H, Ar-H), 6.94 (d, J = 11.3 Hz, 1H), 6.62 (dd, J = 14.9, 11.3 Hz, 1H), 6.40 (m, 1H), 4.99 (brd, J = 9.5 Hz, 1H), 4.20 (d, J = 9.8 Hz, 1H), 3.92 (m, 1H), 3.73-3.49 (m, 6H, 2 × -CH_2-N-CO-, -N-CH_2-Ar), 2.94 (m, 2H), 2.58-2.48 (m, 6H), 2.28 (m, 2H), 2.05 (m, 2H), 1.85-1.75 (m, 5H), 1.68 (m, 1H), 1.56 (m, 1H), 1.47 (m, 1H), 1.23 (brt, J = 12.3 Hz, 2H), 1.12 (brt, J = 11.7 Hz, 1H), 1.06 (d, J = 6.4 Hz, 3H, CH_3), 0.99-0.95 (m, 2H), 0.90 (d, J = 6.3 Hz, 3H, CH_3), 0.86 (d, J = 6.2 Hz, 6H, CH_3), 0.70 (brt, J = 11.7 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 174.8, 171.6, 144.1, 139.2, 129.2 (2C), 128.1 (3C), 127.3, 127.2, 118.5, 115.9, 76.5, 71.6, 70.5, 52.8, 52.4, 48.0, 45.4, 44.9, 44.3, 43.2, 41.5, 38.3, 37.5, 35.8, 35.7, 34.5, 31.0, 29.1, 27.0, 26.1, 24.3, 19.4, 17.3, 17.0, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{39}H_{58}N_3O_5$ $[M+H]^+$: 648.4376; found, 648.4374.

4.1.2.7. (2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-8,16-dihydroxy-2-[(1*R*,2*R*)-2-(4-(4-methoxyphenyl)piperazine-1-carbonyl)cyclo-pentyl]-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-diene-7-carbonitrile (***2g***)

Eluent petroleum ether/EtOAc (2:1). Colorless crystal. 85% yield: $[\alpha]_D^{20}$ -20.9 (c 0.76, MeOH); UV (MeOH) λmax (log ε) 200 (4.96), 250 (4.91) nm; IR v 3427, 2918, 2214, 1724, 1626, 1512, 1453, 1379, 1240, 1097, 1033, 972, 924, 824, 754, 662 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.93 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 11.3 Hz, 1H), 6.83 (d, J = 8.8 Hz, 2H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.39 (ddd, J = 14.9, 8.8, 6.4 Hz, 1H), 4.99 (dt, J = 10.0, 3.7 Hz, 1H), 4.17 (d, J = 9.8 Hz, 1H), 3.88 (ddd, J = 7.9, 5.0, 2.9 Hz, 1H), 3.73 (s, 3H), 3.71-3.65 (m, 4H), 3.04-2.93 (m, 6H), 2.57 (dd, J = 8.4, 3.7 Hz, 2H), 2.28 (dd, J = 11.2, 3.5 Hz, 1H), 2.24 (d, J = 11.2 Hz, 1H), 2.08 (m, 1H), 2.03 (m, 1H), 1.81-1.71 (m, 5H), 1.65 (m, 1H), 1.58 (m, 1H), 1.46 (m, 1H), 1.19 (m, 2H), 1.08 (brt, J = 12.5 Hz, 1H), 1.02 (d, J = 6.4 Hz, 3H, CH_3), 0.93 (m, 2H), 0.83 (d, J = 6.3 Hz, 3H, CH_3), 0.82 (d, J = 6.2 Hz, 3H, CH_3), 0.75 (d, J = 6.2 Hz, 3H, CH_3), 0.68 (brt, J = 12.5 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 174.8, 171.6, 154.6, 145.1, 144.1, 139.2, 127.3, 118.7 (2C), 118.5, 115.9, 114.1 (2C), 76.6, 71.7, 70.7, 54.5, 51.2, 50.8, 47.9, 45.5, 44.3, 43.1, 42.0, 38.1, 37.5, 35.8, 35.7, 34.5, 31.2, 29.2, 27.0, 26.1, 24.4, 19.4, 17.3, 17.0, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{39}H_{58}N_3O_6$ $[M+H]^+$: 664.4326; found, 664.4324.

4.1.2.8. (2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-8,16-dihydroxy-2-[(1*R*,2*R*)-2-(4-(2-hydroxyethyl)piperazine-1-carbonyl)cyclo-pentyl]-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-diene-7-carbonitrile (***2h***)

Eluent dichloromethane/methanol (80:1). Colorless crystal. 80% yield: $[\alpha]_D^{20}$ -12.6 (c 0.64, MeOH); UV (MeOH) λmax (log ε) 254 (4.63) nm; IR v 3411, 2922, 2867, 2213, 1723, 1624, 1456, 1377, 1265, 1138, 1041, 978, 848, 742, 645 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.82 (d, J = 11.3 Hz, 1H), 6.50 (dd, J = 14.9, 11.3 Hz, 1H), 6.28 (ddd, J = 14.9, 9.2, 6.0 Hz, 1H), 4.88 (dt, J = 10.0, 3.6 Hz, 1H), 4.08 (d, J = 9.8 Hz, 1H), 3.80 (dt, J = 8.6, 3.8 Hz, 1H), 3.58 (t, J = 5.9 Hz, 2H, - CH_2-OH), 3.55 (m, 1H), 3.50-3.40 (m, 3H, 2 × -CH_2-N-CO-), 2.82 (m, 2H), 2.53-2.41 (m, 6H, 3 × -N-CH_2-), 2.38 (m, 2H), 2.19 (dd, J = 15.9, 5.6 Hz, 1H), 2.16 (d, J = 15.9 Hz, 1H), 1.94 (m, 2H), 1.73-1.63 (m, 5H), 1.56 (m, 1H), 1.44 (m, 1H), 1.35 (m, 1H), 1.11 (m, 2H), 1.00 (brt, J = 12.5 Hz, 1H), 0.94 (d, J = 6.4 Hz, 3H, CH_3), 0.88-0.82 (m, 2H), 0.76 (d, J = 6.4 Hz, 3H, CH_3), 0.75 (d, J = 6.4 Hz, 3H, CH_3), 0.74 (d, J = 6.2 Hz, 3H, CH_3), 0.59 (brt, J = 12.5 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 174.7, 171.6, 144.1, 139.1, 127.2, 118.5, 116.0, 76.5, 71.7, 70.6, 59.7, 58.3, 53.4, 52.9, 47.9, 45.4, 45.0, 44.3, 43.1, 41.5, 38.2, 37.5, 35.8, 35.7, 34.5, 31.1, 29.1, 27.0, 26.1, 24.4, 19.4, 17.2, 17.1, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{34}H_{56}N_3O_6$ $[M+H]^+$: 602.4169; found, 602.4169.

4.1.2.9. (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-*N*'-propionylcyclopentane-1-carbohydrazide (***2i***)

Eluent dichloromethane/methanol (100:1). Colorless crystal. 60% yield: $[\alpha]_D^{20}$ -8 (c 1.00, MeOH); UV (MeOH) λmax (log ε) 256 (4.46) nm; IR v 3431, 3375, 3258, 3034, 2957, 2924, 2213, 1714, 1619, 1463, 1377, 1262, 1209, 1181, 1107, 1038, 974, 814, 779, 626 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.89 (d, J = 11.3 Hz, 1H), 6.58 (dd, J = 14.9, 11.3 Hz, 1H), 6.31 (ddd, J = 14.9, 10.8, 4.4 Hz, 1H), 4.95 (dt, J = 10.6, 3.5 Hz, 1H), 4.17 (d, J = 9.8 Hz, 1H), 3.90 (dt, J = 10.1, 3.1 Hz, 1H), 2.77 (m, 1H), 2.62-2.52 (m, 2H), 2.45 (brq, J = 7.0 Hz, 1H), 2.30-2.22 (m, 2H), 2.23 (q, J = 7.2 Hz, 2H, NH-CO-CH_2-), 2.06-1.96 (m, 2H), 1.87-1.71 (m, 6H), 1.65 (m, 1H), 1.43 (m, 1H), 1.26-1.19 (m, 2H), 1.14 (t, J = 7.2 Hz, 3H, CH_3), 1.08 (brt, J = 12.2 Hz, 1H), 1.03 (d, J = 6.0 Hz, 3H, CH_3), 1.00-0.93 (m, 2H), 0.86 (d, J = 6.0 Hz, 3H, CH_3), 0.85 (d, J = 6.0 Hz, 6H, 2 × CH_3), 0.68 (brt, J = 12.2 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.0, 173.6, 172.1, 144.1, 138.9, 127.3, 118.5, 116.0, 76.2, 71.6, 71.5, 48.0, 47.2, 44.4, 43.2, 37.6,

37.4, 35.8, 35.3, 34.5, 31.9, 29.7, 27.1, 26.5, 26.2, 25.2, 19.4, 17.5, 17.2, 13.9, 8.6. ESI-HRMS (*m/z*): calcd. For $C_{31}H_{50}N_3O_6$ $[M+H]^+$: 560.3700; found, 560.3700.

4.1.2.10. (1*R*,2*R*)-*N*-butyl-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclo-octadeca-4,6-dien-2-yl]-*N*-pentylcyclopentane-1-carboxamide (***2j***)

Eluent dichloromethane/methanol (80:1). Colorless crystal. 85% yield: $[\alpha]_D^{20}$ -8 (c 1.00, MeOH); UV (MeOH) λmax (log ε) 256 (4.42) nm; IR v 3424, 2959, 2882, 2214, 1718, 1623, 1455, 1376, 1272, 1201, 1121, 1037, 970, 689, 651 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.93 (d, J = 11.3 Hz, 1H), 6.62 (brt, J = 12.3 Hz, 1H), 6.37 (m, 1H), 4.98 (brd, J = 10.5 Hz, 1H), 4.19 (d, J = 9.8 Hz, 1H), 3.95 (m, 1H), 3.44 (m, 1H), 3.35 (m, 1H), 3.25-3.14 (m, 2H, 1 × -CH_2-N-CO-), 2.94 (m, 1H), 2.74 (brq, J = 8.3 Hz, 1H), 2.61 (brt, J = 14.5 Hz, 1H), 2.55 (brd, J = 14.5 Hz, 1H), 2.24 (m, 2H), 2.09-2.05 (m, 2H), 1.91 (m, 2H), 1.81 (m, 3H), 1.66 (m, 1H), 1.61 (m, 1H), 1.53 (m, 4H, 2 × -CH_2-), 1.45 (m, 1H), 1.34 (m, 4H, 2 × -CH_2-), 1.26 (brt, J = 12.8 Hz, 1H), 1.17 (brt, J = 12.6 Hz, 1H), 1.11 (brt, J = 12.6 Hz, 1H), 1.05 (d, J = 6.2 Hz, 3H, CH_3), 1.02-0.92 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H, CH_3), 0.96 (t, J = 7.2 Hz, 3H, CH_3), 0.85 (d, J = 6.4 Hz, 3H), 0.84 (d, J = 6.4 Hz, 6H, 2 × CH_3), 0.68 (brt, J = 12.6 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.0, 171.6, 144.2, 139.0, 127.4, 118.5, 116.0, 76.7, 71.6, 71.0, 48.1, 47.7, 46.4, 45.8, 44.4, 43.1, 37.6, 36.4, 35.5, 35.4, 34.5, 31.8, 31.5, 29.9, 29.4, 26.8, 26.1, 24.6, 19.9, 19.7, 19.5, 17.8, 17.2, 14.0, 13.0, 12.9. ESI-HRMS (*m/z*): calcd. For $C_{36}H_{61}N_2O_5$ $[M+H]^+$: 623.4400; found, 623.4401.

4.1.2.11. (1*R*,2*R*)-*N*-benzyl-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*) -7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxa-cyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxamide (***2k***)

^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.31 (t, J = 7.3 Hz, 2H), 7.27 (d, J = 7.3 Hz, 2H), 7.24 (t, J = 7.3 Hz, 1H), 6.90 (d, J = 11.3 Hz, 1H), 6.61 (dd, J = 14.9, 11.3 Hz, 1H), 6.34 (ddd, J = 15.0, 10.7, 4.5 Hz, 1H), 4.98 (dt, J = 10.7, 3.6 Hz, 1H), 4.45 (d, J = 15.1 Hz, 1H), 4.25 (d, J= 15.1 Hz, 1H), 4.19 (d, J = 9.8 Hz, 1H), 3.86 (dt, J = 10.2, 3.0 Hz, 1H), 2.77 (m, 1H), 2.60 (ddd, J = 15.0, 10.7, 4.1 Hz, 1H), 2.54 (brd, J = 15.0 Hz, 1H), 2.45 (q, J = 7.9 Hz, 1H), 2.25 (dd, J = 16.2, 2.8 Hz, 1H), 2.18 (dd, J = 16.2, 10.2 Hz, 1H), 2.01 (m, 2H), 1.84-1.74 (m, 6H), 1.66 (m, 1H), 1.44 (m, 1H), 1.24 (brt, J = 13.1 Hz, 1H), 1.08 (m, 2H), 1.04 (d, J = 6.4 Hz, 3H, CH_3), 0.93 (m, 2H), 0.93 (m, 2H), 0.86 (d, J = 6.6 Hz, 3H, CH_3), 0.84 (d, J = 6.6 Hz, 3H), 0.77 (d, J = 7.0 Hz, 3H, CH_3), 0.71 (brt, J = 13.1 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 177.2, 171.6, 144.0, 138.9, 138.4, 128.1 (2C), 127.3, 127.0 (2C), 126.8, 118.4, 116.0, 76.2, 71.5, 71.3, 49.9, 47.9, 44.6, 43.1, 42.8, 37.6, 36.9, 35.5, 35.3, 34.5, 31.8, 29.4, 27.0, 26.1, 24.9, 19.4, 17.6, 17.1, 13.9. ESI-MS (*m/z*): 579.2000 $[M+H]^+$, 601.1000 $[M+Na]^+$, calcd. for $C_{35}H_{50}N_2O_5$.

4.1.2.12. (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-*N*-(2-(pyridin-2-yl)ethyl)cyclopentane-1-carbox-amide (***2l***)

Eluent petroleum ether/EtOAc (1:2). Colorless crystal. 88% yield: $[\alpha]_D^{20}$ -18.7 (c 1.07, MeOH); UV (MeOH) λmax (log ε) 254 (4.84) nm; IR v 3642, 3477, 3410, 3357, 2924, 2211, 1724, 1647, 1543, 1447, 1372, 1259, 1180, 1104, 1038, 975, 850, 769, 699, 638 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 8.46 (brd, J = 5.7 Hz, 1H), 7.76 (brt, J = 7.5Hz, 1H), 7.31 (brd, J = 7.5 Hz, 1H), 7.27 (brdd, J = 7.5, 5.7 Hz, 1H), 6.91 (d, J = 11.3 Hz, 1H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.32 (ddd, J = 14.9, 10.5, 4.7 Hz, 1H), 4.94 (dt, J = 10.6, 3.6 Hz, 1H), 4.18 (d, J = 9.8 Hz, 1H), 3.89 (dt, J = 10.1, 3.1 Hz, 1H), 3.68 (dt, J = 13.4, 7.3 Hz, 1H, -CH_b-NH-CO), 3.34 (dt, J = 13.4, 7.3 Hz, 1H, -CH_b-NH-CO), 2.96 (m, 2H, -CH_2-Py), 2.68 (m, 1H), 2.58 (ddd, J = 15.1, 10.5, 4.1 Hz, 1H), 2.52 (brd, J = 15.1 Hz, 1H), 2.34 (dd, J = 15.7, 3.0 Hz, 1H), 2.33 (t, J = 6.0 Hz, 1H), 2.25 (dd, J = 15.7, 10.0 Hz, 1H), 1.99 (m, 1H), 1.90 (m, 1H), 1.85-1.75 (m, 4H), 1.72 (m, 1H), 1.68-1.60 (m, 2H), 1.41 (m, 1H), 1.21 (brt, J = 13.0 Hz, 1H), 1.16 (brt, J = 13.0 Hz, 1H), 1.07 (brt, J = 12.4 Hz, 1H), 1.03 (d, J = 6.4 Hz, 3H, CH_3), 0.95 (brt, J = 12.6 Hz, 1H), 0.91 (brt, J = 12.6 Hz, 1H), 0.84 (d, J = 6.2 Hz, 3H, CH_3), 0.82 (d, J = 6.4 Hz, 3H, CH_3), 0.81 (d, J = 7.0 Hz, 3H, CH_3), 0.67 (brt, J = 12.4 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 177.1, 171.5, 158.7, 148.4, 144.0, 138.9, 137.2, 127.2, 123.6, 121.7, 118.4, 115.9, 76.2, 71.5, 71.0, 50.0, 47.8, 44.5, 43.1, 39.0, 37.6, 37.2, 35.6, 35.3, 34.4, 31.7, 37.0, 29.1, 27.0, 26.1, 24.6, 19.4, 17.4, 17.2, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{35}H_{52}N_3O_5$ $[M+H]^+$: 594.3907; found, 594.3906.

4.1.2.13. methyl{(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclo-octadeca-4,6-dien-2-yl]cyclopentane-1-carbonyl}-*D*-phenyl-alaninate (***2m***)

Eluent petroleum ether/EtOAc (3:1). Colorless crystal. 60% yield: $[\alpha]_D^{20}$ -10.1 (c 0.79, MeOH); UV (MeOH) λmax (log ε) 256 (4.55) nm; IR v 3421, 3366, 2956, 2923, 2213, 1734, 1653, 1531, 1457, 1374, 1257, 1213, 1177, 1125, 1087, 1035, 976, 738, 701, 643 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.28 (t, J = 7.2 Hz, 2H, Ar-H), 7.22 (t, J = 7.2 Hz, 1H, Ar-H), 7.20 (d, J = 7.2 Hz, 2H, Ar-H), 6.88 (d, J = 11.3 Hz, 1H), 6.59 (dd, J = 14.9, 11.3 Hz, 1H), 6.26 (ddd, J = 14.9, 10.5, 4.6 Hz, 1H), 4.94 (dt, J = 10.3, 3.6 Hz, 1H), 4.56 (dd, J = 8.0, 6.8 Hz, 1H), 4.17 (d, J = 9.8 Hz, 1H), 3.89 (dt, J = 10.1, 3.1 Hz, 1H), 3.63 (s, 3H, -OCH_3), 3.09 (dd, J = 13.8, 6.8 Hz, 1H), 3.01 (dd, J = 13.8, 8.0 Hz, 1H), 2.64 (m, 1H), 2.56-2.46 (m, 3H), 2.37 (dd, J = 16.3, 2.9 Hz, 1H), 2.21 (dd, J = 16.3, 10.1 Hz, 1H), 2.00-1.88 (m, 2H), 1.85-1.76 (m, 3H), 1.76-1.68 (m, 3H), 1.63 (m, 1H), 1.41 (m, 1H), 1.22 (m, 1H), 1.18 (m, 1H), 1.06 (brt, J = 12.5 Hz, 1H), 1.03 (d, J = 6.2 Hz, 3H, CH_3), 0.93 (m, 2H), 0.83 (d, J = 6.8 Hz, 3H, CH_3), 0.82 (d, J = 7.2 Hz, 6H, 2 × CH_3), 0.70 (brt, J = 12.5 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 177.2, 172.1, 171.7, 144.2, 139.1, 136.8, 128.8 (2C), 128.2 (2C), 127.3, 126.6, 118.5, 116.0, 75.9, 71.6, 71.1, 54.2, 51.2, 48.8, 47.9, 44.4, 43.2, 37.6, 37.4, 37.1, 35.7, 35.2, 34.6, 31.4, 29.6, 27.1, 26.2, 25.0, 19.4, 17.4, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. for $C_{38}H_{54}N_2O_7Na$ $[M+Na]^+$: 651.4009; found, 651.4012.

4.1.2.14. methyl{(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclo-octadeca-4,6-dien-2-yl]cyclopentane-1-carbonyl}-*D*-tryptophan-ate (***2n***)

Eluent dichloromethane/methanol (100:1). White crystal. 63% yield: $[\alpha]_D^{20}$ -7.4 (c 1.09, MeOH); UV (MeOH) λmax (log ε) 220 (4.69), 256 (4.63) nm; IR v 3397, 2956, 2922,

2212, 1731, 1646, 1524, 1459, 1379, 1353, 1256, 1213, 1179, 1100, 1033, 976, 844, 746, 665 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.50 (d, J = 7.9 Hz, 1H, Ar-H), 7.34 (d, J = 7.9 Hz, 1H, Ar-H), 7.09 (t, J = 7.9 Hz, 1H, Ar-H), 7.08 (s, 1H, Ar-H), 7.01 (t, J = 7.9 Hz, 1H, Ar-H), 6.80 (d, J = 11.3 Hz, 1H), 6.54 (dd, J = 14.9, 11.3 Hz, 1H), 6.15 (ddd, J = 14.9, 10.5, 4.4 Hz, 1H), 4.93 (dt, J = 10.4, 3.6 Hz, 1H), 4.66 (t, J = 6.9 Hz, 1H), 4.15 (d, J = 9.8 Hz, 1H), 3.83 (dt, J = 9.9, 3.1 Hz, 1H), 3.59 (s, 3H, -OCH_3), 3.26 (dd, J = 14.6, 6.9 Hz, 1H), 3.19 (overlapped, 1H), 2.66 (m, 1H), 2.55-2.45 (m, 3H), 2.30 (dd, J = 16.3, 3.0 Hz, 1H), 2.17 (dd, J = 16.3, 9.9 Hz, 1H), 1.99-1.90 (m, 2H), 1.82-1.67 (m, 6H), 1.62 (m, 1H), 1.40 (m, 1H), 1.19 (m, 1H), 1.14 (m, 1H), 1.05 (brt, J = 12.6 Hz, 1H), 0.93 (brt, J = 13.0 Hz, 1H), 0.87 (brt, J = 13.0 Hz, 1H), 0.67 (brt, J = 12.6 Hz, 1H), 1.02 (d, J = 6.4 Hz, 3H, CH_3), 0.81 (d, J = 6.1 Hz, 6H, 2 × CH_3), 0.71 (d, J = 6.8 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 177.1, 172.6, 171.8, 144.2, 139.0, 136.6, 127.3, 127.2, 123.0, 121.2, 118.5, 118.6, 117.8, 115.9, 111.0, 109.2, 76.0, 71.6, 71.1, 53.8, 51.2, 49.0, 47.9, 44.3, 43.0, 37.6, 37.3, 35.7, 35.2, 34.5, 31.3, 29.6, 27.3, 27.1, 26.3, 25.0, 19.4, 17.3, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. for $C_{40}H_{56}N_3O_7$ $[M+H]^+$: 690.4118; found, 690.4118.

4.1.2.15. methyl{(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclo-octadeca-4,6-dien-2-yl]cyclopentane-1-carbonyl}-*D*-methionin-ate (***2o***)

Eluent dichloromethane/methanol (100:1). Colorless crystal. 60% yield: $[\alpha]_D^{20}$ -21.9 (c 0.18, MeOH); UV (MeOH) λmax (log ε) 256 (4.93) nm; IR v 3347, 2955, 2920, 2872, 2851, 2211, 1728, 1649, 1538, 1437, 1378, 1261, 1208, 1174, 1022, 972 cm^{-1}; 1H NMR (600 MHz, CD_3OD) δ (ppm): 6.90 (d, J = 11.3 Hz, 1H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.32 (ddd, J = 14.9, 10.6, 4.8 Hz, 1H), 4.96 (dt, J = 10.7, 3.6 Hz, 1H), 4.49 (dd, J = 8.7, 5.2 Hz, 1H), 4.17 (d, J = 9.8 Hz, 1H), 3.93 (dt, J = 10.4, 2.8 Hz, 1H), 3.71 (s, 3H, -OCH_3), 2.71 (m, 1H), 2.61-2.49 (m, 5H), 2.39 (dd, J = 16.3, 2.7 Hz, 1H), 2.26 (dd, J = 16.3, 10.4 Hz, 1H), 2.10 (m, 1H), 2.08 (s, 3H, -SCH_3), 2.03-1.95 (m, 2H), 1.94 (m, 1H), 1.89-1.70 (m, 6H), 1.65 (m, 1H), 1.42 (m, 1H), 1.23 (brt, J = 12.7 Hz, 2H), 1.08 (brt, J = 12.6 Hz, 1H), 1.03 (d, J = 6.2 Hz, 3H, CH_3), 1.00-0.94 (m, 2H), 0.86 (d, J = 6.9 Hz, 3H, CH_3), 0.84 (d, J = 6.2 Hz, 3H, CH_3), 0.83 (d, J = 6.2 Hz, 3H, CH_3), 0.71 (brt, J = 12.6 Hz, 1H); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 177.6, 172.4, 171.7, 144.2, 138.9, 127.4, 118.5, 116.0, 76.2, 71.6, 71.3, 51.6, 51.3, 48.8, 47.9, 44.2, 43.1, 37.6, 37.0, 35.7, 35.3, 34.5, 31.6, 30.5, 29.9, 29.8, 27.0, 26.2, 25.1, 19.5, 17.7, 17.2, 14.0, 14.0. ESI-HRMS (*m/z*): calcd. for $C_{34}H_{55}N_2O_7S$ $[M+H]^+$: 635.3730; found, 635.3732.

4.1.2.16. methyl{(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclo-octadeca-4,6-dien-2-yl]cyclopentane-1-carbonyl}-*D*-leucinate (***2p***)

Eluent petroleum ether/EtOAc (2:1). Colorless crystal. 85% yield: $[\alpha]_D^{20}$ -36.4 (c 0.33, MeOH); UV (MeOH) λmax (log ε) 256 (4.63) nm; IR v 3371, 2957, 2925, 2875, 2854, 2213, 1727, 1655, 1534, 1463, 1378, 1281, 1260, 1204, 1176, 1095, 1041, 1023, 972, 802, 745, 651 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.90 (d, J = 11.3 Hz, 1H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.32 (ddd, J = 14.9, 10.5, 4.8 Hz, 1H), 4.96 (dt, J = 10.5, 3.6 Hz, 1H), 4.37 (dd, J = 9.0, 6.0 Hz, 1H), 4.17 (d, J = 9.8 Hz, 1H), 3.93 (dt, J =10.2, 2.8 Hz, 1H), 3.69 (s, 3H, -OCH_3), 2.74 (m, 1H), 2.60-2.50 (m, 3H), 2.36 (dd, J = 16.1, 2.9 Hz, 1H), 2.24 (dd, J = 16.1, 10.3 Hz, 1H), 2.00-1.95 (m, 2H), 1.91-1.70(m, 6H), 1.67 (m, 1H), 1.62-1.56 (m, 2H), 1.46 (m, 1H), 1.41 (m, 1H), 1.20-1.17 (m, 2H), 1.09 (brt, J = 12.5 Hz, 1H), 1.00-0.91 (m, 2H), 0.67 (brt, J = 12.5 Hz, 1H), 1.03 (d, J = 6.4 Hz, 3H, CH_3), 0.96 (d, J = 6.6 Hz, 3H, CH_3), 0.93 (d, J = 6.6 Hz, 3H, CH_3), 0.85 (d, J = 6.5 Hz, 3H, CH_3), 0.84 (d, J = 7.1 Hz, 3H, CH_3), 0.83 (d, J = 7.0 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 177.7, 173.3, 171.7, 144.1, 138.9, 127.3, 118.5, 116.0, 76.1, 71.6, 71.3, 51.2, 51.1, 48.6, 47.9, 43.9, 43.2, 40.0, 37.6, 36.9, 35.7, 35.3, 34.6, 31.7, 29.9, 27.1, 26.2, 25.2, 24.7, 21.9, 21.0, 19.5, 17.7, 17.2, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{35}H_{57}N_2O_7$ $[M+H]^+$: 617.4166; found, 617.4162.

4.1.2.17. methyl{(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclo-octadeca-4,6-dien-2-yl]cyclopentane-1-carbonyl}-*D*-histidinate (***2q***)

Eluent dichloromethane/methanol (40:1). Colorless crystal. 79% yield: $[\alpha]_D^{20}$ -33.3 (c 0.24, MeOH); UV (MeOH) λmax (log ε) 254 (4.83) nm; IR v 3323, 2956, 2924, 2854, 2212, 1730, 1651, 1535, 1461, 1377, 1261, 1209, 1176, 1093, 1041, 1024, 973, 802, 684, 650 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.60 (s, 1H, Ar-H), 6.90 (d, J = 11.1 Hz, 1H), 6.88 (s, 1H, Ar-H), 6.58 (dd, J = 14.9, 11.1 Hz, 1H), 6.30 (ddd, J = 14.9, 10.5, 4.8 Hz, 1H), 4.95 (dt, J = 10.0, 3.7 Hz, 1H), 4.57 (t, J = 4.6 Hz, 1H), 4.17 (d, J = 9.7 Hz, 1H), 3.86 (dt, J = 9.8, 3.2 Hz, 1H), 3.65 (s, 3H, -OCH_3), 3.05 (m, 2H), 2.70 (m, 1H), 2.59-2.48 (m, 3H), 2.33 (dd, J = 16.1, 3.2 Hz, 1H), 2.20 (dd, J = 16.1, 10.1 Hz, 1H), 2.00-1.95 (m, 2H), 1.84-1.68 (m, 6H), 1.63 (m, 1H), 1.42 (m, 1H), 1.20 (brt, J = 12.3 Hz, 2H), 1.08 (brt, J = 11.4 Hz, 1H), 1.00-0.88 (m, 2H), 0.69 (brt, J = 11.4 Hz, 1H), 1.03 (d, J = 6.7 Hz, 3H, CH_3), 0.83 (d, J = 6.6 Hz, 6H, 2 × CH_3), 0.79 (d, J = 6.7 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 177.3, 171.9, 171.7, 144.1, 139.1, 135.0, 127.2, 118.6, 117.2, 115.9, 76.0, 71.6, 70.9, 53.0, 51.3, 49.0, 48.2, 44.3, 43.1, 37.6, 35.7, 35.3, 34.6, 31.7, 29.5, 27.2, 26.2, 24.9, 22.3, 19.5, 17.3, 17.0, 13.9. ESI-HRMS (*m/z*): calcd. for $C_{35}H_{53}N_4O_7$ $[M+H]^+$: 641.3914; found, 641.3904.

4.1.2.18. (1*R*,2*R*)-*N*-[2-(1*H*-indol-2-yl)ethyl]-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*, 11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetrameth-yl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxamide (***2r***)

Eluent dichloromethane/methanol (80:1). Colorless crystal. 86% yield: $[\alpha]_D^{20}$ -8 (c 1.00, MeOH); UV (MeOH) λmax (log ε) 224 (4.69), 256 (4.70) nm; IR v 3412, 2926, 2213, 1721, 1642, 1539, 1462, 1381, 1289, 1217, 1178, 1085, 1034, 974, 846, 672 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.23 (d, J = 8.7 Hz, 1H, Ar-H), 7.07 (d, J = 2.4 Hz, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 6.92 (d, J = 11.3 Hz, 1H), 6.76 (dd, J = 8.7, 2.4 Hz, 1H, Ar-H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.33 (ddd, J = 14.9, 10.5, 4.8 Hz, 1H), 4.95 (dt, J = 10.7, 3.6 Hz, 1H), 4.19 (d, J = 9.8 Hz, 1H), 3.89 (dt, J =10.2, 3.0 Hz, 1H), 3.84 (s, 3H, -OCH_3), 3.61 (dt, J = 13.2, 7.5 Hz, 1H), 3.32 (dt, J = 13.2, 7.5 Hz, 1H), 2.91 (t, J = 7.5 Hz, 2H), 2.73 (m, 1H), 2.58 (dd, J = 14.9, 3.9 Hz, 1H), 2.52 (brd, J = 14.9 Hz, 1H), 2.37 (m, 1H), 2.34 (dd, J

= 16.3, 2.9 Hz, 1H), 2.25 (dd, *J* = 16.3, 10.2 Hz, 1H), 2.01 (m, 1H), 1.94 (m, 1H), 1.88-1.76 (m, 4H), 1.72 (m, 2H) 1.63 (m, 1H), 1.42 (m, 1H), 1.21(brt, *J* = 12.6 Hz, 1H), 1.13(brt, *J* = 12.1 Hz, 1H), 1.04 (m, 1H), 0.91 (m, 2H), 0.67 (brt, *J* = 11.6 Hz, 1H), 1.04 (d, *J* = 6.3 Hz, 3H, CH_3), 0.83 (d, *J* = 6.3 Hz, 3H, CH_3), 0.82 (d, *J* = 6.3 Hz, 3H, CH_3), 0.79 (d, *J* = 6.3 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 177.2, 171.6, 153.6, 144.1, 139.1, 132.0, 127.6, 127.4, 122.7, 118.5, 116.0, 111.5, 111.4, 111.2, 99.9, 76.4, 71.6, 71.3, 54.9, 50.3, 48.2, 44.6, 43.2, 40.1, 37.6, 37.0, 35.6, 35.4, 34.5, 31.8, 29.3, 27.0, 26.2, 25.2, 24.7, 19.4, 17.7, 17.2, 13.9. ESI-HRMS (*m/z*): calcd. For $C_{39}H_{56}N_3O_6$ $[M+H]^+$: 662.4169; found, 662.4167.

4.1.2.19. (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-*N*-(4-methoxyphenethyl)cyclopentane-1-carbox-amide (***2s***)

Eluent dichloromethane/methanol (120:1). Colorless crystal. 87% yield: $[\alpha]_D^{20}$ -14.5 (c 0.83, MeOH); UV (MeOH) λmax (log ε) 226 (4.18), 256 (4.47) nm; IR ν 3350, 2954, 2922, 2213, 1723, 1645, 1544, 1515, 1458, 1372, 1248, 1181, 1036, 975, 818, 643 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 7.13 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.93 (d, *J* = 11.3 Hz, 1H), 6.85 (d, *J* = 8.4 Hz, 2H), 6.61 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.34 (ddd, *J* = 14.9, 10.5, 4.8 Hz, 1H), 4.95 (dt, *J* = 10.7, 3.6 Hz, 1H), 4.19 (d, *J* = 9.8 Hz, 1H), 3.91 (dt, *J* = 10.5, 3.1 Hz, 1H), 3.78 (s, 3H, -OCH_3), 3.51 (dt, *J* = 13.2, 7.5 Hz, 1H), 3.18 (dt, *J* = 13.2, 7.5 Hz, 1H), 2.72 (m, 3H), 2.60 (dd, *J* = 14.9, 3.9 Hz, 1H), 2.54 (brd, *J* = 14.9 Hz, 1H), 2.36 (dd, *J* = 16.1, 3.0 Hz, 1H), 2.34 (m, 1H), 2.26 (dd, *J* = 16.1, 10.2 Hz, 1H), 2.01 (m, 1H), 1.94 (m, 1H), 1.82 (m, 4H), 1.74 (m, 1H), 1.70-1.60 (m, 2H), 1.43 (m, 1H), 1.22 (brt, *J* = 11.7 Hz, 1H), 1.15 (brt, *J* = 12.1 Hz, 1H), 1.06 (brt, *J* = 12.6 Hz, 1H), 0.96 (m, 1H), 0.91 (m, 1H), 0.69 (brt, *J* = 12.6 Hz, 1H), 1.04 (d, *J* = 6.3 Hz, 3H, CH_3), 0.85 (d, *J* = 6.3 Hz, 3H, CH_3), 0.84 (d, *J* = 7.0 Hz, 3H, CH_3), 0.83 (d, *J* = 6.3 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 177.1, 171.6, 158.4, 144.1, 139.0, 130.8, 129.3 (2C), 127.4, 118.5, 116.0, 113.5 (2C), 76.4, 71.6, 71.3, 54.3, 50.3, 48.2, 44.5, 43.2, 41.1, 37.6, 37.1, 35.6, 35.4, 34.5, 34.5, 31.8, 29.3, 27.0, 26.2, 24.7, 19.4, 17.7, 17.2, 13.9. ESI-HRMS (*m/z*): calcd. For $C_{37}H_{55}N_2O_6Na$ $[M+H]^+$: 623.4060; found, 623.4062.

4.1.3. General Procedure for the Synthesis of Compounds **3a-3i**

To a solution of borrelidin (40 mg, 0.082 mmol) in THF (2.0 mL) was added 1-hydroxybenzotriazole (HoBt, 22.2 mg, 0.164 mmoL) and N,N'-dicyclohexylcarbodiimide (DCC, 33.8 mg, 0.164 mmol) at room temperature. The reaction mixture was stirred at room temperature for 0.5 h after which the appropriate alcohol (0.205 mmol) was added. Keep stirring for 12-24 h, the mixture was extracted with EtOAc (20 mL). The organic layer was then washed with 3% HCl aqueous (2 × 10 mL), saturated $NaHCO_3$ aqueous (2 × 10 mL), brine (2 × 10 mL). The resulting organic layer wasdried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case. The data of compound **3e** and **3f** can be found at the literature.[41,42]

4.1.3.1. 2-(1*H*-imidazol-1-yl)ethyl(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*, 11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetra-methyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***3a***)

Eluent dichloromethane/methanol (50:1). White crystal. 75% yield: $[\alpha]_D^{20}$ -12.6 (c 0.95, MeOH); UV (MeOH) λmax (log ε) 254 (4.52) nm; IR ν 3419, 2956, 2922, 2211, 1730, 1638, 1595, 1512, 1459, 1375, 1282, 1168, 1081, 1040, 975, 871, 825, 750, 657 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 7.69 (s, 1H, Ar-H), 7.17 (s, 1H, Ar-H), 7.00 (s, 1H, Ar-H), 6.94 (d, *J* = 11.3 Hz, 1H), 6.59 (dd, *J* = 15.0, 11.3 Hz, 1H), 6.29 (ddd, *J* = 14.9, 10.4, 4.7 Hz, 1H), 4.96 (dt, *J* = 10.2, 3.6 Hz, 1H), 4.44 (m, 1H, -CO-O-CH_a-), 4.36-4.26 (m, 3H), 4.19 (d, *J* = 9.6 Hz, 1H), 3.87 (dt, *J* = 9.8, 3.3 Hz, 1H), 2.63 (m, 1H), 2.59-2.51 (m, 3H), 2.29 (dd, *J* = 15.8, 3.0 Hz, 1H), 2.19 (dd, *J* = 15.8, 10.2 Hz, 1H), 2.01-1.92 (m, 2H), 1.84-1.77 (m, 3H), 1.77-1.72 (m, 3H), 1.64 (m, 1H), 1.42 (m, 1H), 1.22 (brt, *J* = 13.0 Hz, 1H), 1.17 (brt, *J* = 11.7 Hz, 1H), 1.08 (brt, *J* = 12.4 Hz, 1H), 0.97 (m, 1H), 0.94 (m, 1H), 0.70 (brt, *J* = 12.4 Hz, 1H), 1.04 (d, *J* = 6.4 Hz, 3H, CH_3), 0.85 (d, *J* = 6.4 Hz, 3H, CH_3), 0.84 (d, *J* = 6.9 Hz, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 175.7, 171.5, 143.9, 138.5, 127.8, 127.5, 119.4, 118.4, 116.0, 75.8, 71.5, 71.1, 63.3, 48.4, 47.9, 45.5, 45.4 , 43.1, 37.6, 37.1, 35.7, 35.0, 34.5, 30.7, 28.9, 27.1, 26.2, 24.7, 19.4, 17.3, 17.3, 13.9. ESI-HRMS (*m/z*): calcd. For $C_{33}H_{51}N_3O_6$ $[M+H]^+$: 584.3700; found, 584.3700.

4.1.3.2. 2-(pyrrolidin-1-yl)ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*, 13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxyl-ate (***3b***)

Eluent petroleum ether/EtOAc (2:1, 1:3). Colorless crystal. 69% yield: $[\alpha]_D^{20}$ -18.5 (c 0.86, MeOH); UV (MeOH) λmax (log ε) 256 (4.52) nm; IR ν 3644, 3440, 2958, 2923, 2878, 2214, 1726, 1637, 1460, 1378, 1281, 1170, 1035, 975, 847, 739 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 6.91 (d, *J* = 11.3 Hz, 1H), 6.61 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.34 (ddd, *J* = 14.9, 10.4, 4.7 Hz, 1H), 4.97 (dt, *J* = 10.5, 3.7 Hz, 1H), 4.34 (ddd, *J* = 11.7, 6.4, 5.3 Hz, 1H), 4.19 (d, *J* = 9.8 Hz, 1H), 4.12 (ddd, *J* = 11.7, 6.4, 5.3 Hz, 1H), 3.91 (dt, *J* = 10.1, 3.2 Hz, 1H), 2.83-2.62 (m, 6H, 3 × -CH_2-N-), 2.64 (m, 1H), 2.60-2.50 (m, 3H), 2.33 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.21 (dd, *J* = 15.9, 10.0 Hz, 1H), 2.05-1.97 (m, 2H), 1.91-1.75 (m, 10H, 2 × -CH_2-pyrrole), 1.66 (m, 1H), 1.43 (m, 1H), 1.25 (brt, *J* = 12.0 Hz, 1H), 1.17(brt, *J* = 11.5 Hz, 1H), 1.10 (brt, *J* = 11.5 Hz, 1H), 0.99 (m, 1H), 0.96 (m, 1H), 0.70 (brt, *J* = 11.5 Hz, 1H), 1.04 (d, *J* = 6.4 Hz, 3H, CH_3), 0.86 (d, *J* = 6.4 Hz, 6H, 2 × CH_3), 0.85 (d, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.1, 171.5, 144.1, 138.8, 127.6, 118.5, 116.1, 76.0, 71.6, 71.5, 62.8, 54.3 (2C), 54.2, 48.9, 45.5, 45.3, 43.3, 37.7, 36.6, 35.6, 35.2, 34.6, 30.8, 29.1, 27.1, 26.2, 24.8, 22.9 (2C), 19.5, 17.9, 17.2, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{34}H_{55}N_2O_6$ $[M+H]^+$: 587.4060; found, 587.4062.

4.1.3.3. 2-(4-methylpiperazin-1-yl)ethyl(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*, 9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetra-methyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate ***(3c)***

Eluent dichloromethane/methanol (25:1). Yellow crystal. 65% yield: $[\alpha]_D^{20}$ -13.3 (c 0.30, MeOH); UV (MeOH) λmax (log ε) 254 (4.72) nm; IR ν 3642, 3477, 3410, 2924, 2211, 1724, 1647, 1543, 1447, 1372, 1259, 1180, 1104, 1038, 975, 850, 769, 699, 638 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 6.92 (d, *J* = 11.3 Hz, 1H), 6.60 (dd, *J* = 14.9, 11.4

Hz, 1H), 6.32 (ddd, *J* = 14.9, 10.4, 4.7 Hz, 1H), 4.97 (dt, *J* = 10.5, 3.7 Hz, 1H), 4.29 (dt, *J* = 11.6, 5.7 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.14 (dt, *J* = 11.6, 5.7 Hz, 1H), 3.89 (dt, *J* = 10.1, 3.2 Hz, 1H), 2.90-2.64 (m, 10H, 5 × -CH_2-N-), 2.60-2.50 (m, 4H), 2.32 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.21 (dd, *J* =15.9, 10.0 Hz, 1H), 2.04-1.97 (m, 2H), 1.87-1.75 (m, 6H), 1.65 (m,1H), 1.42 (m, 1H), 1.23 (brt, *J* = 11.9 Hz, 1H), 1.17 (brt, *J* = 12.3 Hz, 1H), 1.10 (brt, *J* = 11.5 Hz, 1H), 0.97 (m, 2H), 0.69 (brt, *J* = 11.5 Hz, 1H), 2.49 (s, 3H, -NCH_3), 1.03 (d, *J* = 6.4 Hz, 3H, CH_3), 0.86 (d, *J* = 6.7 Hz, 6H, 2 × CH_3), 0.85 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 143.9, 138.7, 127.6, 118.5, 116.2, 76.0, 71.5, 71.4, 61.4, 56.0, 54.1 (2C), 51.7 (2C), 48.7, 48.1, 45.6, 43.9, 43.3, 37.7, 37.0, 35.7, 35.2, 34.6, 30.8, 29.1, 27.3, 26.3, 24.8, 19.5, 17.6, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{35}H_{58}N_3O_6$ $[M+H]^+$: 616. 4326; found, 616.4327.

4.1.3.4. 2-(piperidin-1-yl)ethyl(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*, 13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxyl-ate (***3d***)

Eluent dichloromethane/methanol (80:1). Colorless crystal. 85% yield: $[\alpha]_D^{20}$ -4 (c 1.00, MeOH); UV (MeOH) λmax (log ε) 256 (4.36) nm; IR v 3500, 3409, 2923, 2859, 2215, 1727, 1630, 1459, 1379, 1275, 1171, 1087, 1033, 977, 844, 744, 709 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 6.94 (d, *J* = 11.3 Hz, 1H), 6.65 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.37 (ddd, *J* = 14.9, 10.4, 4.7 Hz, 1H), 5.01 (dt, *J* = 10.5, 3.7 Hz, 1H), 4.45 (m, 1H, -CO-O-CH_a-), 4.31 (m, 1H, -CO-O-CH_b-), 4.21 (d, *J* = 9.8 Hz, 1H), 3.92 (dt, *J* = 10.1, 3.2 Hz, 1H), 3.21-3.01 (m, 6H, 3 × -CH_2-N-), 2.75 (m, 1H), 2.62 (m, 1H), 2.60 (m, 1H), 2.58 (m, 1H), 2.36 (brd, *J* = 15.9 Hz, 1H), 2.25 (dd, *J* =15.9, 10.0 Hz, 1H), 2.10-1.98 (m, 2H), 1.94-1.76 (m, 10H, 2 × -CH_2-piperidine), 1.66 (m, 3H, 1 × -CH_2-piperidine), 1.47 (m, 1H), 1.27 (m, 1H), 1.21 (m, 1H), 1.13 (m, 1H), 0.98 (m, 1H), 0.92 (m, 1H), 0.70 (brt, *J* = 11.6 Hz, 1H), 1.06 (d, *J* = 6.4 Hz, 3H, CH_3), 0.89 (d, *J* = 6.4 Hz, 3H, CH_3), 0.87 (d, *J* = 6.4 Hz, 3H, CH_3), 0.86 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 175.6, 171.6, 143.9, 138.8, 127.5, 118.5, 116.2, 76.0, 71.6, 71.3, 59.4, 55.9, 53.8 (2C), 48.4, 45.4, 43.2, 37.6, 37.2, 35.7, 35.3, 34.5, 30.7, 29.1, 27.2, 26.2, 24.8, 23.7 (2C), 22.0, 19.5, 17.5, 17.2, 13.9. ESI-HRMS (*m/z*): calcd. For $C_{35}H_{57}N_2O_6$ $[M+H]^+$: 601. 4217; found, 601.4216.

4.1.3.5. 2-(dimethylamino)ethyl(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*, 11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetra-methyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***3e***)

^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 6.81 (d, *J* = 11.3 Hz, 1H), 6.50 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.23 (ddd, *J* = 14.9, 10.4, 4.7 Hz, 1H), 4.87 (dt, *J* = 10.5, 3.7 Hz, 1H), 4.20 (ddd, *J* = 11.2, 6.2, 5.0 Hz, 1H), 4.08 (d, *J* = 9.8 Hz, 1H), 4.02 (ddd, *J* = 11.2, 6.2, 5.0 Hz, 1H), 3.80 (dt, *J* = 10.1, 3.2 Hz, 1H), 2.64 (m, 1H), 2.58 (m, 2H), 2.50-2.41 (m, 3H), 2.25 (dd, *J* = 16.0, 2.9 Hz, 1H), 2.25 (s, 6H, 2 × -NCH_3), 2.11 (dd, *J* =16.0, 10.3 Hz, 1H), 1.92 (m, 2H), 1.77-1.66 (m, 6H), 1.56 (m, 1H), 1.33 (m, 1H), 1.14 (brt, *J* = 12.5 Hz, 1H), 1.07 (brt, *J* = 12.5 Hz, 1H), 1.00 (brt, *J* = 12.5 Hz, 1H), 0.84-0.91 (m, 2H), 0.59 (brt, *J* = 12.5 Hz, 1H), 0.93 (d, *J* = 6.4 Hz, 3H, CH_3), 0.76 (d, *J* = 6.4 Hz, 6H, 2 × CH_3), 0.74 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 144.1, 138.9, 127.6, 118.5, 116.1, 76.0, 71.5, 71.5, 61.7, 57.2, 48.7, 48.1, 45.5, 44.5 (2C), 43.3, 37.7, 36.9, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.2, 24.8, 19.5, 17.7, 17.3, 14.0. ESI-MS (*m/z*): 561.4000 $[M+H]^+$, calcd. For $C_{32}H_{53}N_2O_6$.

4.1.3.6. 2-morpholinoethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*, 15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***3f***)

Eluent dichloromethane/methanol (80:1). Colorless crystal. 83% yield: $[\alpha]_D^{20}$ -22.4 (c 1.07, MeOH); UV (MeOH) λmax (log ε) 252 (4.49) nm; IR v 3435, 2954, 2923, 2872, 2212, 1728, 1639, 1457, 1375, 1270, 1169, 1118, 1035, 976, 862, 772, 689 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 6.91 (d, *J* = 11.3 Hz, 1H), 6.61 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.32 (ddd, *J* = 14.9, 10.4, 4.7 Hz, 1H), 4.97 (dt, *J* = 10.3, 3.6 Hz, 1H), 4.35 (ddd, *J* = 11.7, 6.4, 5.1 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.11 (ddd, *J* = 11.7, 6.4, 5.1 Hz, 1H), 3.91 (dt, *J* = 10.3, 3.1 Hz, 1H), 3.69 (t, *J* = 4.7 Hz, 4H, 2 × -CH_2-O-), 2.71 (m, 1H), 2.63 (m, 2H), 2.57 (m, 1H), 2.52 (m, 6H, 3 × -CH_2-N-), 2.32 (dd, *J* = 16.0, 3.0 Hz, 1H), 2.21 (dd, *J* =16.0, 10.2 Hz, 1H), 2.00 (m, 2H), 1.85 (m, 3H), 1.79 (m, 3H), 1.65 (m, 1H), 1.42 (m, 1H), 1.23 (brt, *J* = 12.5 Hz, 1H), 1.17 (brt, *J* = 12.5 Hz, 1H), 1.09 (brt, *J* = 12.5 Hz, 1H), 1.00 (m, 1H), 0.96 (m, 1H), 0.70 (brt, *J* = 12.5 Hz, 1H), 1.03 (d, *J* = 6.4 Hz, 3H, CH_3), 0.85 (d, *J* = 6.4 Hz, 6H, 2 × CH_3), 0.84 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 144.0, 138.7, 127.6, 118.5, 116.2, 76.0, 71.5, 71.5, 66.3 (2C), 61.3, 56.9, 53.6 (2C), 53.4, 48.7, 45.6, 43.3, 37.7, 36.8, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.3, 24.9, 19.5, 17.8, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{34}H_{55}N_2O_7$ $[M+H]^+$: 603.4009; found, 603.4006.

4.1.3.7. pyridin-2-ylmethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*, 15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***3g***)

Eluent petroleum ether/EtOAc (1:3). Colorless crystal. 88% yield: $[\alpha]_D^{20}$ -20.4 (c 0.98, MeOH); UV (MeOH) λmax (log ε) 202 (4.15), 256 (4.85) nm; IR v 3469, 3421, 2954, 2923, 2213, 1729, 1640, 1593, 1453, 1378, 1270, 1167, 1096, 1037, 974, 862, 817, 764, 670 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) δ (ppm): 8.43 (d, *J* = 5.0 Hz, 1H, Ar-H), 7.75 (td, *J* = 7.7, 1.8 Hz, 1H, Ar-H), 7.39 (d, *J* = 7.7 Hz, 1H, Ar-H), 7.27 (dd, *J* = 7.7, 5.0 Hz, 1H, Ar-H), 6.82 (d, *J* = 11.3 Hz, 1H), 6.51 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.25 (ddd, *J* = 14.9, 10.4, 4.7 Hz, 1H), 5.18 (d, *J* = 13.7 Hz, 1H, -CH_a-Py), 5.03 (d, *J* = 13.7 Hz, 1H, -CH_b-Py), 4.90 (dt, *J* = 10.8, 4.2 Hz, 1H), 4.08 (d, *J* = 9.8 Hz, 1H), 3.75 (dt, *J* = 10.4, 3.1 Hz, 1H), 2.69 (m, 1H), 2.53 (m, 1H), 2.48 (m, 1H), 2.44 (m, 1H), 2.10 (dd, *J* = 15.9, 2.9 Hz, 1H), 2.00 (dd, *J* = 15.9, 10.3 Hz, 1H), 1.94 (m, 2H), 1.80 (m, 1H), 1.70 (m, 5H), 1.53 (m, 1H), 1.35 (m, 1H), 1.12 (brt, *J* = 13.5 Hz, 1H), 0.94 (m, 1H), 0.89 (m, 1H), 0.81 (m, 1H), 0.80 (m, 1H), 0.56 (brt, *J* = 12.5 Hz, 1H), 0.93 (d, *J* = 6.3 Hz, 3H, CH_3), 0.74(d, *J* = 6.7 Hz, 3H, CH_3), 0.72 (d, *J* = 6.4 Hz, 3H, CH_3), 0.63 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 175.8, 171.6, 155.7, 148.7, 144.1, 138.8, 137.6, 127.7, 123.2, 121.7, 118.5, 116.2, 76.0, 71.7, 71.5, 65.9, 48.5, 48.0, 45.8 , 43.2, 37.7, 36.6, 35.6, 35.2, 34.6, 30.9, 29.2, 27.2, 26.2, 25.0, 19.5, 17.9, 17.2, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{34}H_{49}N_2O_6$ $[M+H]^+$: 581.3591; found, 581.3592.

4.1.3.8. phenethyl(1*R*,2*S*)-2-[(1*R*,3*E*,5*Z*,7*R*,8*S*,10*R*,12*S*,14*S*,15*S*)-6-cyano-7-hydroxy-

8,10,12,14,15-pentamethyl-17-methylene-cyclooctadeca-3,5-dien-1-yl]-2-methylcyclopentane-1-carboxyl-ate (***3h***)

Eluent petroleum ether/EtOAc (1:3). Colorless crystal. 80% yield: $[\alpha]_D^{20}$ -22.9 (c 1.05, MeOH); UV (MeOH) λmax (log ε) 206 (4.13), 256 (4.50) nm; IR v 3486, 3447, 2957, 2922, 2213, 1725, 1638, 1597, 1458, 1372, 1262, 1173, 1036, 975, 746, 698, 643 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.20 (m, 2H, Ar-H), 7.14 (m, 3H, Ar-H), 6.79 (d, J = 11.3 Hz, 1H), 6.50 (dd, J = 14.9, 11.3 Hz, 1H), 6.16 (ddd, J = 14.9, 10.4, 4.7 Hz, 1H), 4.85 (dt, J = 10.7, 3.6 Hz, 1H), 4.29 (dt, J = 10.8, 6.9 Hz, 1H, -CO-O-CH_a-), 4.09 (dt, J = 10.8, 6.9 Hz, 1H, -CO-O-CH_b-), 4.08 (d, J = 9.8 Hz, 1H), 3.80 (dt, J = 10.3, 3.1 Hz, 1H), 2.83 (brt, J = 6.8 Hz, 2H), 2.54 (m, 1H), 2.46 (ddd, J = 14.7, 10.5, 4.0 Hz, 1H), 2.40 (brd, J = 14.7 Hz, 1H), 2.37 (q, J = 7.5 Hz, 1H), 2.21 (dd, J = 16.0, 2.9 Hz, 1H), 2.09 (dd, J = 16.0, 10.3 Hz, 1H), 1.86 (m, 2H), 1.72 (m, 3H), 1.65 (m, 3H), 1.52 (m, 1H), 1.30 (m, 1H), 1.11 (brt, J = 12.5 Hz, 1H), 1.03 (brt, J = 12.8 Hz, 1H), 0.90 (m, 1H), 0.86 (brt, J = 12.5 Hz, 1H), 0.78 (brt, J = 12.0 Hz, 1H), 0.57 (brt, J = 12.3 Hz, 1H), 0.92 (d, J = 6.4 Hz, 3H, CH_3), 0.74 (d, J = 6.0 Hz, 3H, CH_3), 0.73 (d, J = 6.0 Hz, 3H, CH_3), 0.69 (d, J = 6.5 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.3, 171.6, 144.1, 138.7, 137.9, 128.6 (2C), 128.2 (2C), 127.6, 126.2, 118.5, 116.1, 76.0, 71.6, 71.5, 64.9, 48.9, 47.9, 45.6, 43.2, 37.6, 36.7, 35.7, 35.1, 34.8, 34.6, 30.7, 29.1, 27.1, 26.2, 24.8, 19.4, 17.8, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{36}H_{50}NO_5Na$ $[M+Na]^+$: 616.3614; found, 616.3614.

4.1.3.9. cyclopropylmethyl(1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*, 15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***3i***)

Eluent petroleum ether/EtOAc (1:1). Colorless crystal. 75% yield: $[\alpha]_D^{20}$ -12.6 (c 0.95, MeOH); UV (MeOH) λmax (log ε) 256 (4.63) nm; IR v 3455, 2925, 2213, 1724, 1639, 1457, 1374, 1260, 1174, 1097, 1033, 976, 816 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.81 (d, J = 11.3 Hz, 1H), 6.51 (dd, J = 14.9, 11.3 Hz, 1H), 6.23 (ddd, J = 14.9, 10.4, 4.7 Hz, 1H), 4.87 (dt, J = 10.7, 3.6 Hz, 1H), 4.09 (d, J = 9.8 Hz, 1H), 3.87 (dd, J = 11.4, 7.3 Hz, 1H, -CO-O-CH_a-), 3.81 (dt, J = 10.3, 3.0 Hz, 1H), 3.75 (dd, J = 11.4, 7.3 Hz, 1H, -CO-O-CH_b-), 2.62 (m, 1H), 2.48 (ddd, J = 14.7, 10.5, 4.0 Hz, 1H), 2.43 (brd, J = 14.7 Hz, 1H), 2.38 (q, J = 8.0 Hz, 1H), 2.22 (dd, J = 16.0, 2.9 Hz, 1H), 2.09 (dd, J =16.0, 10.4 Hz, 1H), 1.91 (m, 2H), 1.76 (m, 2H), 1.70 (m, 4H), 1.54 (m, 1H), 1.32 (m, 1H), 1.14 (brt, J = 13.0 Hz, 1H), 0.96-1.06 (m, 3H), 0.90 (brt, J = 13.0 Hz, 1H), 0.84 (brt, J = 13.0 Hz, 1H), 0.59 (brt, J = 12.6 Hz, 1H), 0.46 (m, 2H), 0.19 (m, 2H), 0.93 (d, J = 6.4 Hz, 3H, CH_3), 0.76 (d, J = 6.4 Hz, 3H, CH_3), 0.75 (d, J = 6.4 Hz, 3H, CH_3), 0.74 (d, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 175.7, 170.7, 143.1, 137.8, 126.7, 117.5, 115.1, 75.0, 70.7, 70.6, 67.9, 47.9, 47.2, 44.7, 42.3, 36.7, 35.6, 34.7, 34.2, 33.6, 29.9, 28.1, 26.1, 25.3, 23.9, 18.6, 16.9, 16.3, 13.0, 8.4, 1.4, 1.3. ESI-HRMS (*m/z*): calcd. For $C_{32}H_{50}NO_6$ $[M+H]^+$: 544.3638; found, 544.3638.

4.1.4. General Procedure for the Synthesis of Compounds **4a-4r**

To a solution of 1-(2-hydroxyethyl) piperazine (1.0 g, 7.686 mmol) in dichloromethane (25 mL) were added di-*tert*butyl dicarbonate (2.013 g, 9.223 mmol) and triethylamine (TEA, 2.130 g, 15.372 mmol). The reaction mixture was stirred at room temperature for 12 h, then washed with 3% HCl aqueous (2 × 50 mL), saturated $NaHCO_3$ aqueous (2 × 50 mL), brine (2 × 50 mL). The resulting organic layer wasdried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with dichloromethane/methanol (40:1) to form 1-Boc-4-(2-hydroxyethyl)-piperazine a colorlessoil (1.592 g, 90% yield).

To a solution of BN (500 mg, 1.022 mmol) in THF (12 mL) was added 1-hydroxybenzotriazole (HoBt, 276.206 mg, 2.044 mmoL) and N,N'-dicyclohexylcarbodiimide (DCC, 421.739 mg, 2.044 mmol) at room temperature. After stirring at room temperature for 0.5 h, the 1-Boc-4-(2-hydroxyethyl)-piperazine (588.059 mg, 2.555 mmol) was added. After another for 12 h, the excess THF was evaporated, and then the mixture was extracted with EtOAc (2 × 50 mL), and washed with 3% HCl aqueous (2 × 50 mL), saturated $NaHCO_3$ aqueous (2 × 50 mL), brine (2 × 50 mL). The resulting organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with petroleum ether/EtOAc (2:1) to afford target compound **4a** as a colorless crystal (551.957 mg, 77% yield).

The solution of **4a** (500 mg, 0.713 mmol) in CH_2Cl_2 (5 mL) was cooled to 0℃ and TFA (5mL) was slowly added and stirring was continued for 30 min (monitored by TLC), then quenched by the addition of saturated aqueous Na_2CO_3. The mixture was extracted with CH_2Cl_2 (2 × 50 mL), washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was further purified by silica gel column chromatography eluting with dichloromethane /methanol (25: 1) to prepare compound **4b** as a colorless crystal (372.928 mg, 87% yield).

To a solution of **4b** (20 mg, 0.033 mmol) in CH_3CN (1.5 mL) was added potassium carbonate (4.5621 mg, 0.033 mmol), appropriate halohydrocarbon (0.028 mmol) and sodium iodide (catalytic amount). The mixture was heated at reflux for 1-2 h (monitored by TLC). Excess CH_3CN was evaporated. Then the mixture was extracted with EtOAc (2 × 10 mL), and washed with H_2O (2 × 10 mL) and brine (2 × 10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was further purified by silica gel column chromatography eluting with appropriate mixture as indicated in each case to give target compounds **4c**-**4r**.

4.1.4.1. (2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-tert-butyl-4-{2-[((1*R*, 2*R*)-2-(7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxo-oxacyclooctadeca-4,6-dien-2-yl)cyclopentane-1-carbonyl)oxy]-ethyl}piperazine-1-carboxylate (***4a***)

$[\alpha]_D^{20}$ -18.9 (c 1.06, MeOH); UV (MeOH) λmax (log ε) 256 (4.66) nm; IR v 3633, 3431, 2956, 2213, 1691, 1424, 1373, 1252, 1170, 1033, 978, 847 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.81 (d, J = 11.3 Hz, 1H), 6.51 (dd, J = 14.9, 11.3 Hz, 1H), 6.22 (ddd, J = 14.9, 10.3, 4.8 Hz, 1H), 4.88 (dt, J = 10.6, 3.7 Hz, 1H), 4.22 (dt, J = 11.7, 5.9 Hz, 1H), 4.09 (d, J = 9.8 Hz, 1H), 4.03 (dt, J = 11.7, 5.9 Hz, 1H), 3.80 (dt, J = 10.1, 3.1 Hz, 1H), 3.33 (m, 4H, 2 × -CON-CH_2-), 2.61 (m, 2H), 2.55 (t, J = 5.0 Hz, 2H), 2.51-2.41 (m, 3H, 1 × -CH_2-N-), 2.38 (m, 4H, 2 × -CH_2-N-), 2.23 (dd, J = 15.9, 3.0 Hz, 1H), 2.12 (dd, J = 16.0, 10.1 Hz, 1H), 1.90 (m, 2H), 1.80-1.60 (m, 6H), 1.55 (m, 1H), 1.36 (s, 9H), 1.32 (m, 1H), 1.14 (brt, J = 12.6 Hz, 1H), 1.07 (brt,

J = 12.6 Hz, 1H), 1.00 (brt, *J* = 12.6 Hz, 1H), 0.88 (m, 2H), 0.60 (brt, *J* = 12.6 Hz, 1H), 0.94 (d, *J* = 6.4 Hz, 3H, CH_3), 0.76 (d, *J* = 6.4 Hz, 6H, 2 × CH_3), 0.75 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) *δ* (ppm): 176.3, 171.6, 154.9, 143.9, 138.7, 127.6, 118.5, 116.2, 79.9, 76.0, 71.6, 71.4, 61.3, 56.4, 52.9 (2C), 48.6 (2C), 48.2 (2C), 48.1, 45.6, 43.3, 37.7, 37.0, 35.7, 35.2, 34.6, 30.9, 29.1, 27.3 (3C), 27.2, 26.3, 24.8, 19.6, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{39}H_{64}N_3O_8$ $[M+H]^+$: 702.4693; found, 702.4693.

4.1.4.2. 2-(piperazin-1-yl) ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*, 13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxyl-ate (***4b***)

$[\alpha]_D^{20}$ -12 (c 1.00, MeOH); UV (MeOH) λmax (log ε) 256 (4.49) nm; IR v 3419, 2926, 2219, 1681, 1451, 1379, 1199, 1142, 1036, 973, 842, 801, 723, 669 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) *δ* (ppm): 6.81 (d, *J* = 11.3 Hz, 1H), 6.51 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.22 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.88 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.24 (m, 1H, -CO-O-CH_a-), 4.11 (m, 1H, -CO-O-CH_b-), 4.09 (d, *J* = 9.7 Hz, 1H), 3.78 (dt, *J* = 9.9, 3.2 Hz, 1H), 3.19 (m, 4H, 2 × -NH-CH_2-), 2.84 (m, 2H, 1 × -N-CH_2-), 2.78 (m, 4H, 2 × -N-CH_2-), 2.59 (m, 1H), 2.53-2.43 (m, 3H), 2.23 (dd, *J* = 15.8, 3.1 Hz, 1H), 2.14 (dd, *J* = 15.8, 9.8 Hz, 1H), 1.92 (m, 2H), 1.76-1.66 (m, 6H), 1.55 (m, 1H), 1.34 (m, 1H), 1.13 (brt, *J* = 12.6 Hz, 1H), 1.08 (brt, *J* = 12.6 Hz, 1H), 1.00 (brt, *J* = 12.6 Hz, 1H), 0.87 (m, 2H), 0.60 (brt, *J* = 12.6 Hz, 1H), 0.94 (d, *J* = 6.4 Hz, 3H, CH_3), 0.76 (d, *J* = 6.4 Hz, 3H, CH_3), 0.75 (d, *J* = 6.4 Hz, 3H, CH_3), 0.74 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) *δ* (ppm): 176.2, 171.6, 144.0, 138.8, 127.5, 118.5, 116.1, 76.0, 71.6, 71.2, 60.6, 55.9, 49.6 (2C), 48.5, 48.4, 45.5, 43.2, 43.0 (2C), 37.7, 37.4, 35.8, 35.2, 34.6, 31.0, 29.1, 27.3, 26.3, 24.8, 19.5, 17.4, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{34}H_{56}N_3O_6$ $[M+H]^+$: 602.4169; found, 602.4168.

4.1.4.3. benzyl-4-{2-[((1*R*,2*R*)-2 ((2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*, 16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxo-oxacyclooctadeca-4,6-dien-2-yl)cyclopentane-1-carbonyl)oxy]-ethyl}piperazine-1-carboxylate (***4c***)

Eluent dichloromethane/methanol (80:1). Colorless crystal. 90% yield: $[\alpha]_D^{20}$ -32 (c 1.00, MeOH); UV (MeOH) λmax (log ε) 256 (4.95) nm; IR v 3421, 2918, 2213, 1715, 1446, 1372, 1244, 1171, 1029, 967, 852, 747 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) *δ* (ppm): 7.38-7.33 (m, 4H, Ar-H), 7.30 (m, 1H, Ar-H), 6.89 (d, *J* = 11.3 Hz, 1H), 6.60 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.31 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 5.12 (s, 2H, -CH_2-Ar), 4.97 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.32 (dt, *J* = 11.7, 5.7 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.11 (dt, *J* = 11.7, 5.7 Hz, 1H), 3.89 (dt, *J* = 10.2, 3.1 Hz, 1H), 3.50 (m, 4H, 2 × -CON-CH_2-), 2.68 (m, 1H), 2.64 (t, *J* = 5.9 Hz, 2H, 1 × -N-CH_2-), 2.60-2.52 (m, 3H), 2.50 (m, 4H, 2 × -N-CH_2-), 2.32 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.21 (dd, *J* = 15.9, 10.1 Hz, 1H), 1.99 (m, 2H), 1.83 (m, 4H), 1.77 (m, 2H), 1.64 (m, 1H), 1.42 (m, 1H), 1.23 (brt, *J* = 12.5 Hz, 1H), 1.16 (brt, *J* = 12.5 Hz, 1H), 1.08 (brt, *J* = 12.5 Hz, 1H), 0.97 (m, 2H), 0.69 (brt, *J* = 12.5 Hz, 1H), 1.03 (d, *J* = 6.4 Hz, 3H, CH_3), 0.85 (d, *J* = 6.4 Hz, 3H, CH_3), 0.84 (d, *J* = 6.4 Hz, 3H, CH_3), 0.83 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) *δ* (ppm): 176.2, 171.6, 155.4, 143.9, 138.7, 136.7, 128.1 (2C), 127.7, 127.6, 127.5 (2C), 118.5, 116.2, 76.0, 71.6, 71.4, 67.0, 61.3, 56.4, 52.8 (2C), 48.6, 48.1, 45.6, 43.5 (2C), 43.3, 37.7, 36.9, 35.7, 35.2, 34.6, 30.9, 29.1, 27.2, 26.3, 24.9, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{42}H_{62}N_3O_8$ $[M+H]^+$: 736. 4537; found, 736.4536.

4.1.4.4. 2-(4-benzoylpiperazin-1-yl) ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*, 8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl] cyclopentane -1-carboxylate (***4d***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 95% yield: $[\alpha]_D^{20}$ -44 (c 1.00, MeOH); UV (MeOH) λmax (log ε) 252 (4.87) nm; IR v 3391, 2925, 2213, 1728, 1626, 1448, 1375, 1273, 1168, 1025, 971, 857, 774, 712 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) *δ* (ppm): 7.37 (m, 3H, Ar-H), 7.31 (m, 2H, Ar-H), 6.80 (d, *J* = 11.3 Hz, 1H), 6.51 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.22 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.88 (dt, *J* = 10.5, 3.7 Hz, 1H), 4.22 (dt, *J* = 11.7, 5.7 Hz, 1H), 4.08 (d, *J* = 9.8 Hz, 1H), 4.05 (dt, *J* = 11.7, 5.7 Hz, 1H), 3.80 (dt, *J* = 10.1, 3.2 Hz, 1H), 3.67 (m, 2H, 1 × -CON-CH_2-), 3.36 (m, 2H, 1 × -CON-CH_2-), 2.62 (m, 1H), 2.58 (t, *J* = 5.7 Hz, 2H, 1 × -N-CH_2-), 2.51-2.41 (m, 3H), 2.39 (m, 4H, 2 × -N-CH_2-), 2.22 (dd, *J* = 15.9, 3.1 Hz, 1H), 2.12 (dd, *J* = 15.9, 10.0 Hz, 1H), 1.91 (m, 2H), 1.72 (m, 3H), 1.67 (m, 3H), 1.54 (m, 1H), 1.33 (m, 1H), 1.14 (m, 1H), 1.08 (brt, *J* = 12.5 Hz, 1H), 0.99 (brt, *J* = 12.5 Hz, 1H), 0.89 (m, 1H), 0.85 (m, 1H), 0.59 (brt, *J* = 12.5 Hz, 1H), 0.93 (d, *J* = 6.4 Hz, 3H, CH_3), 0.75 (d, *J* = 6.4 Hz, 3H, CH_3), 0.74 (d, *J* = 6.4 Hz, 3H, CH_3), 0.73 (d, *J* = 6.4 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) *δ* (ppm): 176.3, 171.6, 171.0, 143.9, 138.7, 135.3, 129.8, 128.4 (2C), 127.6, 126.7 (2C), 118.5, 116.2, 75.9, 71.6, 71.4, 61.3, 56.2, 53.3, 52.7, 48.6, 48.2, 45.6, 43.3, 41.8 (2C), 37.7, 37.0, 35.7, 35.2, 34.6, 30.9, 29.1, 27.2, 26.3, 24.8, 19.5, 17.6, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{41}H_{60}N_3O_7$ $[M+H]^+$: 706. 4431; found, 706.4432.

4.1.4.5. 2-(4-acetylpiperazin-1-yl) ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*, 9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihdihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***4e***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 95% yield: $[\alpha]_D^{20}$ -50.9 (c 0.55, MeOH); UV (MeOH) λmax (log ε) 252 (4.74) nm; IR v 3378, 2928, 2211, 1728, 1632, 1448, 1371, 1257, 1168, 1039, 987, 864, 764, 678 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) *δ* (ppm): 6.91 (d, *J* = 11.3 Hz, 1H), 6.61 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.32 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.97 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.33 (dt, *J* = 11.5, 5.7 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.13 (dt, *J* = 11.5, 5.7 Hz, 1H), 3.90 (dt, *J* = 10.1, 3.1 Hz, 1H), 3.58 (t, *J* = 5.1 Hz, 2H, 1 × -CON-CH_2-), 3.54 (t, *J* = 5.1 Hz, 2H, 1 × -CON-CH_2-), 2.70 (m, 1H), 2.65 (t, *J* = 5.7 Hz, 2H), 2.59-2.49 (m, 7H), 2.33 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.21 (dd, *J* = 15.9, 10.1 Hz, 1H), 2.09 (s, 3H), 2.00 (m, 2H), 1.87-1.77 (m, 6H), 1.65 (m, 1H), 1.42 (m, 1H), 1.23 (brt, *J* = 12.5 Hz, 1H), 1.17 (brt, *J* = 12.5 Hz, 1H), 1.10 (brt, *J* = 12.5 Hz, 1H), 1.00 (m, 1H), 0.96 (m, 1H), 0.69 (brt, *J* = 12.5 Hz, 1H), 1.03 (d, *J* = 6.4 Hz, 3H, CH_3), 0.86 (d, *J* = 6.6 Hz, 3H, CH_3), 0.85 (d, *J* = 5.4 Hz, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, CD_3OD) *δ* (ppm): 176.2, 171.6, 170.2, 143.9, 138.7, 127.6, 118.5, 116.2, 75.9, 71.5, 71.4, 61.3, 56.3, 53.2, 52.7, 48.6, 48.1, 45.9, 45.6, 43.3, 41.1, 37.7, 37.0, 35.7, 35.2, 34.6, 30.9, 29.1, 27.2, 26.3, 24.8, 19.8, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{36}H_{58}N_3O_7$ $[M+H]^+$: 644.4275; found, 644.4275.

4.1.4.6. 2-(4-benzylpiperazin-1-yl) ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*, 8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***4f***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 90% yield: $[\alpha]_D^{20}$ -18.8 (c 0.64, MeOH); UV (MeOH) λmax (log ε) 254 (4.81) nm; IR v 3427, 2933, 2212, 1728, 1636, 1457, 1373, 1284, 1166, 1033, 975, 822, 742, 694 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.36-7.29 (m, 4H, Ar-H), 7.26 (m, 1H, Ar-H), 6.93 (d, *J* = 11.3 Hz, 1H), 6.60 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.31 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.96 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.30 (dt, *J* = 11.5, 5.9 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.12 (dt, *J* = 11.5, 5.9 Hz, 1H), 3.89 (dt, *J* = 10.2, 3.1 Hz, 1H), 3.57 (d, *J* = 12.8 Hz, 1H), 3.54 (d, *J* = 12.8 Hz, 1H), 2.70 (m, 2H), 2.64 (m, 2H), 2.60 (m, 2H, 1 × -N-CH_2-), 2.56 (m, 2H, 1 × -N-CH_2-), 2.53 (m, 2H, 1 × -N-CH_2-), 2.49 (m, 4H, 2 × -N-CH_2-), 2.31 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.20 (dd, *J* = 15.9, 10.1 Hz, 1H), 1.98 (m, 2H), 1.82 (m, 4H), 1.77 (m, 2H), 1.65 (m, 1H), 1.42 (m, 1H), 1.23 (brt, *J* = 13.0 Hz, 1H), 1.16 (brt, *J* = 13.0 Hz, 1H), 1.09 (brt, *J* = 12.2 Hz, 1H), 1.00 (m, 1H), 0.97 (m, 1H), 0.69 (brt, *J* = 12.2 Hz, 1H), 1.03 (d, *J* = 6.5 Hz, 3H, CH_3), 0.85 (d, *J* = 6.0 Hz, 3H, CH_3), 0.84 (d, *J* = 6.4 Hz, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 144.0, 138.7, 136.7, 129.4 (2C), 127.9 (2C), 127.6, 127.1, 118.5, 116.2, 76.0, 71.5, 71.4, 62.5, 61.4, 56.4, 52.8 (2C), 52.2 (2C), 48.7, 48.1, 45.6, 43.3, 37.7, 36.9, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.3, 24.8, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{41}H_{62}N_3O_6$ $[M+H]^+$: 692.4639; found, 692.4641.

4.1.4.7. 2-[4-(4-fluorobenzyl) piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***4g***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 90% yield: $[\alpha]_D^{20}$ -21.8 (c 0.55, MeOH); UV (MeOH) λmax (log ε) 258 (4.40) nm; IR v 3393, 2930, 2212, 1727, 1636, 1510, 1456, 1372, 1282, 1223, 1165, 1035, 974, 841, 764 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.35 (dd, *J* = 8.5, 5.6 Hz, 2H, Ar-H), 7.04 (brt, *J* = 8.5 Hz, 2H, Ar-H), 6.93 (d, *J* = 11.3 Hz, 1H), 6.60 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.31 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.96 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.31 (dt, *J* = 11.5, 5.7 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.12 (dt, *J* = 11.5, 5.7 Hz, 1H), 3.89 (dt, *J* = 10.2, 3.1 Hz, 1H), 3.56 (d, *J* = 13.0 Hz, 1H), 3.53 (d, *J* = 13.0 Hz, 1H), 2.70 (m, 2H), 2.64 (m, 2H), 2.60 (m, 2H, 1 × -N-CH_2-), 2.57 (m, 2H, 1 × -N-CH_2-), 2.53 (m, 2H, 1 × -N-CH_2-), 2.49 (m, 4H, 2 × -N-CH_2-), 2.31 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.20 (dd, *J* = 15.9, 10.1 Hz, 1H), 1.98 (m, 2H), 1.82 (m, 4H), 1.77 (m, 2H), 1.65 (m, 1H), 1.41 (m, 1H), 1.23 (brt, *J* = 12.5 Hz, 1H), 1.16 (brt, *J* = 12.5 Hz, 1H), 1.09 (brt, *J* = 12.5 Hz, 1H), 0.99 (m, 1H), 0.97 (m, 1H), 0.69 (brt, *J* = 12.5 Hz, 1H), 1.03 (d, *J* = 6.4 Hz, 3H, CH_3), 0.85 (d, *J* = 6.0 Hz, 3H, CH_3), 0.84 (d, *J* = 7.2 Hz, 3H, CH_3), 0.83 (d, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 163.1, 161.5, 144.0, 138.7, 132.8, 131.1, 130.9, 127.6, 118.5, 116.2, 114.6, 114.5, 76.0, 71.5, 71.4, 61.5, 61.4, 56.4, 52.8 (2C), 52.1 (2C), 48.7, 48.1, 45.6, 43.3, 37.7, 36.8, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.3, 24.8, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{41}H_{61}FN_3O_6$ $[M+H]^+$: 710.4544; found, 710.4544.

4.1.4.8. 2-[4-(2,4-difluorobenzyl) piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl)cyclopentane-1-carboxylate (***4h***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 90% yield: $[\alpha]_D^{20}$ -21.8 (c 0.55, MeOH); UV (MeOH) λmax (log ε) 256 (4.53) nm; IR v 3467, 2923, 2862, 2213, 1728, 1610, 1501, 1458, 1373, 1277, 1168, 1094, 1037, 971, 850, 812, 740 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.44 (m, 1H, Ar-H), 6.94 (m, 2H, Ar-H), 6.92 (d, *J* = 11.3 Hz, 1H), 6.60 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.31 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.96 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.31 (dt, *J* = 11.5, 5.7 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.11 (dt, *J* = 11.5, 5.7 Hz, 1H), 3.89 (dt, *J* = 10.2, 3.1 Hz, 1H), 3.59 (s, 2H, -CH_2-Ar), 2.70 (m, 2H), 2.63 (m, 2H), 2.59 (m, 2H, 1 × -N-CH_2-), 2.57 (m, 2H, 1 × -N-CH_2-), 2.53 (m, 2H, 1 × -N-CH_2-), 2.49 (m, 4H, 2 × -N-CH_2-), 2.31 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.20 (dd, *J* = 15.9, 10.2 Hz, 1H), 1.99 (m, 2H), 1.82 (m, 4H), 1.77 (m, 2H), 1.65 (m, 1H), 1.42 (m, 1H), 1.23 (brt, *J* = 12.5 Hz, 1H), 1.16 (brt, *J* = 12.5 Hz, 1H), 1.09 (brt, *J* = 12.5 Hz, 1H), 0.99 (m, 1H), 0.96 (m, 1H), 0.69 (brt, *J* = 12.5 Hz, 1H), 1.03 (d, *J* = 6.4 Hz, 3H, CH_3), 0.85 (d, *J* = 6.0 Hz, 3H, CH_3), 0.84 (d, *J* = 6.0 Hz, 3H, CH_3), 0.83 (d, *J* = 6.0 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 162.3, 163.2, 162.4, 162.3, 161.7, 161.6, 160.7, 160.6, 144.0, 138.7, 133.0, 127.6, 119.7, 118.5, 116.1, 110.7, 103.0, 76.0, 71.5 (2C), 61.4, 56.4, 54.0, 52.8 (2C), 52.9 (2C), 48.7, 48.1, 45.6, 43.3, 37.7, 36.9, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.3, 24.8, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{41}H_{60}F_2N_3O_6$ $[M+H]^+$: 728.4450; found, 728.4450.

4.1.4.9. 2-[4-(4-chlorobenzyl)piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-cyclopentane-1-carboxylate (***4i***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 90% yield: $[\alpha]_D^{20}$ -23.1 (c 1.04, MeOH); UV (MeOH) λmax (log ε) 254 (4.85) nm; IR v 3434, 2926, 2213, 1728, 1636, 1595, 1457, 1374, 1291, 1167, 1092, 1031, 974, 806, 671 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.32 (m, 4H, Ar-H), 6.93 (d, *J* = 11.3 Hz, 1H), 6.60 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.31 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.96 (dt, *J* = 10.7, 3.7 Hz, 1H), 4.31 (dt, *J* = 11.5, 5.5 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.11 (dt, *J* = 11.5, 5.5 Hz, 1H), 3.89 (dt, *J* = 10.2, 3.1 Hz, 1H), 3.54 (d, *J* = 13.0 Hz, 1H), 3.51 (d, *J* = 13.0 Hz, 1H), 2.70 (m, 2H), 2.63 (m, 2H), 2.60 (m, 2H, 1 × -N-CH_2-), 2.58 (m, 2H, 1 × -N-CH_2-), 2.53 (m, 2H, 1 × -N-CH_2-), 2.49 (m, 4H, 2 × -N-CH_2-), 2.31 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.20 (dd, *J* = 15.9, 10.2 Hz, 1H), 1.98 (m, 2H), 1.83 (m, 3H), 1.77 (m, 3H), 1.65 (m, 1H), 1.41 (m, 1H), 1.23 (brt, *J* = 12.6 Hz, 1H), 1.16 (brt, *J* = 12.6 Hz, 1H), 1.08 (brt, *J* = 12.6 Hz, 1H), 0.98 (m, 1H), 0.96 (m, 1H), 0.69 (brt, *J* = 12.6 Hz, 1H), 1.03 (d, *J* = 6.4 Hz, 3H, CH_3), 0.85 (d, *J* = 6.0 Hz, 3H, CH_3), 0.84 (d, *J* = 6.0 Hz, 3H, CH_3), 0.82 (d, *J* = 6.0 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 144.0, 138.7, 135.9, 132.8, 130.8 (2C), 128.0 (2C), 127.6, 118.5, 116.2, 76.0, 71.5, 71.5, 61.6, 61.4, 56.4, 52.9 (2C), 52.2 (2C), 48.7, 48.1, 45.6, 43.3, 37.7, 36.8, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.3, 24.8, 19.5, 17.8, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{41}H_{61}ClN_3O_6$ $[M+H]^+$: 726.4249; found, 726.4248.

4.1.4.10. 2-[4-(4-bromobenzyl)piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-cyclopentane-1-carboxylate (***4j***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 85% yield: $[\alpha]_D^{20}$ -17.6 (c 0.91, MeOH); UV (MeOH) λmax (log ε) 256 (4.90) nm; IR v 3428, 2950, 2925, 2818, 2212, 1727, 1635, 1593, 1458, 1374, 1290, 1168, 1028, 972, 838, 802, 680, 654 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.47 (d, J = 8.4 Hz, 2H, Ar-H), 7.27 (d, J = 8.4 Hz, 2H, Ar-H), 6.93 (d, J = 11.3 Hz, 1H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.31 (ddd, J = 14.9, 10.1, 4.8 Hz, 1H), 4.96 (dt, J = 10.7, 3.7 Hz, 1H), 4.31 (dt, J = 11.5, 5.5 Hz, 1H), 4.18 (d, J = 9.8 Hz, 1H), 4.11 (dt, J = 11.5, 5.5 Hz, 1H), 3.89 (dt, J = 10.2, 3.1 Hz, 1H), 3.53 (d, J = 13.0 Hz, 1H), 3.50 (d, J = 13.0 Hz, 1H), 2.70 (m, 2H), 2.63 (m, 2H), 2.60 (m, 2H, 1 × -N-CH_2-), 2.58 (m, 2H, 1 × -N-CH_2-), 2.53 (m, 2H, 1 × -N-CH_2-), 2.49 (m, 4H, 2 × -N-CH_2-), 2.31 (dd, J = 15.9, 3.0 Hz, 1H), 2.20 (dd, J = 15.9, 10.2 Hz, 1H), 1.99 (m, 2H), 1.83 (m, 3H), 1.77 (m, 3H), 1.65 (m, 1H), 1.41 (m, 1H), 1.24 (brt, J = 12.6 Hz, 1H), 1.16 (brt, J = 12.6 Hz, 1H), 1.08 (brt, J = 12.6 Hz, 1H), 0.98 (m, 1H), 0.96 (m, 1H), 0.69 (brt, J = 12.6 Hz, 1H), 1.03 (d, J = 6.4 Hz, 3H, CH_3), 0.85 (d, J = 6.0 Hz, 3H, CH_3), 0.84 (d, J = 6.0 Hz, 3H, CH_3), 0.82 (d, J = 6.0 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 144.0, 138.7, 136.4, 131.1 (2C), 131.0 (2C), 127.6, 120.7, 118.5, 116.2, 76.0, 71.5, 71.5, 61.6, 61.4, 56.4, 52.9 (2C), 52.2 (2C), 48.7, 48.1, 45.6, 43.3, 37.7, 36.8, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.3, 24.8, 19.5, 17.8, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. for $C_{41}H_{63}BrN_3O_6$ $[M+H]^+$: 770.3744; found, 770.3744.

4.1.4.11. 2-[4-(4-nitrobenzyl) piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-cyclopentane-1-carboxylate (***4k***)

Eluent dichloromethane/methanol (80:1). Colorless crystal. 90% yield: $[\alpha]_D^{20}$ -22 (c 0.91, MeOH); UV (MeOH) λmax (log ε) 256 (4.60) nm; IR v 3430, 2929, 2211, 1727, 1636, 1522, 1457, 1346, 1276, 1169, 1030, 973, 857, 743 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 8.20 (d, J = 8.5 Hz, 2H, Ar-H), 7.61 (d, J = 8.5 Hz, 2H, Ar-H), 6.93 (d, J = 11.3 Hz, 1H), 6.61 (dd, J = 14.9, 11.3 Hz, 1H), 6.32 (ddd, J = 14.9, 10.1, 4.8 Hz, 1H), 4.96 (dt, J = 10.6, 3.6 Hz, 1H), 4.34 (dt, J = 11.5, 5.5 Hz, 1H), 4.18 (d, J = 9.8 Hz, 1H), 4.13 (dt, J = 11.5, 5.5 Hz, 1H), 3.90 (dt, J = 10.3, 3.1 Hz, 1H), 3.69 (d, J = 13.0 Hz, 1H), 3.66 (d, J = 13.0 Hz, 1H), 2.72-2.49 (m, 14H), 2.32 (dd, J = 15.9, 2.9 Hz, 1H), 2.20 (dd, J = 15.9, 10.2 Hz, 1H), 1.99 (m, 2H), 1.83 (m, 3H), 1.78 (m, 3H), 1.65 (m, 1H), 1.41 (m, 1H), 1.23 (m, 1H), 1.16 (brt, J = 12.6 Hz, 1H), 1.08 (brt, J = 12.6 Hz, 1H), 0.99 (m, 1H), 0.94 (m, 1H), 0.68 (brt, J = 12.6 Hz, 1H), 1.03 (d, J = 6.4 Hz, 3H, CH_3), 0.85 (d, J = 6.0 Hz, 3H, CH_3), 0.84 (d, J = 6.0 Hz, 3H, CH_3), 0.81 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.1, 171.6, 147.3, 145.5, 144.0, 138.8, 129.8 (2C), 127.6, 123.0 (2C), 118.6, 116.2, 76.0, 71.5, 71.5, 61.4, 61.2, 56.4, 53.0 (2C), 52.3 (2C), 48.8, 48.1, 45.6, 43.3, 37.7, 36.8, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.3, 24.8, 19.5, 17.8, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{41}H_{61}N_4O_8$ $[M+H]^+$: 737.4489; found, 737.4492.

4.1.4.12. 2-[4-(4-methylbenzyl)piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-cyclopentane-1-carboxylate (***4l***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 89% yield: $[\alpha]_D^{20}$ -34.8 (c 0.46, MeOH); UV (MeOH) λmax (log ε) 256 (4.40) nm; IR v 3395, 2923, 2862, 2211, 1726, 1636, 1457, 1374, 1275, 1167, 1036, 975 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.20 (d, J = 7.8 Hz, 2H, Ar-H), 7.13 (d, J = 7.8 Hz, 2H, Ar-H), 6.92 (d, J = 11.3 Hz, 1H), 6.60 (dd, J =14.9, 11.3 Hz, 1H), 6.31 (ddd, J = 14.9, 10.1, 4.8 Hz, 1H), 4.96 (dt, J = 10.6, 3.7 Hz, 1H), 4.30 (dt, J = 11.5, 5.5 Hz, 1H), 4.18 (d, J = 9.8 Hz, 1H), 4.11 (dt, J = 11.5, 5.5 Hz, 1H), 3.89 (dt, J = 10.3, 3.1 Hz, 1H), 3.53 (d, J = 13.0 Hz, 1H), 3.50 (d, J = 13.0 Hz, 1H), 2.70-2.48 (m, 14H), 2.31 (dd, J = 15.9, 3.0 Hz, 1H), 2.31 (s, 3H, -Ar-CH_3), 2.20 (dd, J = 15.9, 10.1 Hz, 1H), 2.00 (m, 2H), 1.83 (m, 3H), 1.77 (m, 3H), 1.63 (m, 1H), 1.41 (m, 1H), 1.23 (brt, J = 12.6 Hz, 1H), 1.16 (brt, J = 12.6 Hz, 1H), 1.09 (brt, J = 12.6 Hz, 1H), 0.98 (m, 1H), 0.96 (m, 1H), 0.69 (brt, J = 12.6 Hz, 1H), 1.03 (d, J = 6.4 Hz, 3H, CH_3), 0.85 (d, J = 6.6 Hz, 3H, CH_3), 0.84 (d, J = 7.2 Hz, 3H, CH_3), 0.83 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 144.0, 138.7, 136.9, 129.4 (2C), 128.5 (2C), 127.6, 118.5, 116.2, 76.0, 71.5 (2C), 62.2, 61.4, 56.4, 52.7 (2C), 52.2 (2C), 48.7, 48.1, 45.6, 43.3, 37.7, 36.9, 35.7, 35.2, 34.6, 30.9, 29.1, 27.2, 26.3, 24.8, 19.8, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{42}H_{64}N_3O_6$ $[M+H]^+$: 706.4795; found, 706.4797.

4.1.4.13. 2-[4-(naphthalen-2-ylmethyl)piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***4m***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 85% yield: $[\alpha]_D^{20}$ -28 (c 1.00, MeOH); UV (MeOH) λmax (log ε) 256 (4.74) nm; IR v 3490, 3360, 2945, 2213, 1727, 1640, 1456, 1373, 1276, 1168, 1040, 976, 819, 750, 673 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 7.83 (m, 3H, Ar-H), 7.79 (s, 1H, Ar-H), 7.51 (d, J = 7.8 Hz, 1H, Ar-H), 7.46 (m, 2H, Ar-H), 6.93 (d, J = 11.3 Hz, 1H), 6.60 (dd, J = 14.9, 11.3 Hz, 1H), 6.29 (ddd, J = 14.9, 10.1, 4.8 Hz, 1H), 4.95 (dt, J = 10.6, 3.7 Hz, 1H), 4.31 (dt, J = 11.5, 5.5 Hz, 1H), 4.18 (d, J = 9.8 Hz, 1H), 4.10 (dt, J = 11.5, 5.5 Hz, 1H), 3.88 (dt, J = 10.1, 3.1 Hz, 1H), 3.73 (m, 2H, -CH_2-Ar), 2.69 (m, 2H), 2.64 (m, 4H, 1 × -N-CH_2-), 2.57(m, 4H, 2 × -N-CH_2-), 2.50 (m, 4H, 2 × -N-CH_2-), 2.31 (dd, J = 15.9, 3.0 Hz, 1H), 2.20 (dd, J = 15.9, 10.1 Hz, 1H), 1.97 (m, 2H), 1.82 (m, 4H), 1.75 (m, 2H), 1.63 (m, 1H), 1.39 (m, 1H), 1.23 (m, 1H), 1.15 (brt, J = 12.5 Hz, 1H), 1.06 (brt, J = 12.5 Hz, 1H), 0.96 (m, 2H), 0.69 (brt, J = 12.5 Hz, 1H), 1.03 (d, J = 6.6 Hz, 3H, CH_3), 0.84 (d, J = 6.6 Hz, 3H, CH_3), 0.83 (d, J = 6.6 Hz, 3H, CH_3), 0.81 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 144.0, 138.7, 134.3, 133.4, 133.0, 128.1, 127.6 (2C), 127.4, 127.3, 127.2, 125.7, 125.5, 118.6, 116.2, 76.0, 71.5 (2C), 62.6, 61.4, 56.4, 52.8 (2C), 52.3 (2C), 48.7, 48.1, 45.6, 43.3, 37.7, 36.9, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.3, 24.8, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (m/z): calcd. for$C_{45}H_{64}N_3O_6$ $[M+H]^+$: 742.4795; found, 742.4795.

4.1.4.14. 2-{4-[2-(1,3-dioxolan-2-yl)ethyl]piperazin-1-yl} ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***4n***)

Eluent dichloromethane/methanol (40:1). Colorless crystal. 85% yield: $[\alpha]_D^{20}$ -29.4 (c 1.09, MeOH); UV (MeOH) λmax (log ε) 252 (4.72) nm; IR v 3481, 2955, 2921, 2882, 2212, 1729, 1638, 1538, 1460, 1376, 1282, 1163, 1037, 973, 815, 779, 726, 657 cm^{-1}; ^1H NMR (600 MHz, CD_3OD) δ (ppm): 6.83 (d, J = 11.3 Hz, 1H), 6.51 (dd, J = 14.9, 11.3

Hz, 1H), 6.23 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.86 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.79 (t, *J* = 4.8 Hz, 1H, -CH), 4.21 (dt, *J* = 11.5, 5.5 Hz, 1H), 4.09 (d, *J* = 9.8 Hz, 1H), 4.04 (dt, *J* = 11.5, 5.5 Hz, 1H), 3.84 (m, 2H), 3.80 (dt, *J* = 10.0, 3.2 Hz, 1H), 3.73 (m, 2H), 2.61 (m, 2H), 2.55 (m, 3H, 1 × -N-CH_2-), 2.49 (m, 4H, 2 × -N-CH_2-), 2.44 (m, 3H, 1 × -N-CH_2-), 2.42 (m, 4H, 2 × -N-CH_2-), 2.23 (dd, *J* = 15.9, 3.1 Hz, 1H), 2.11 (dd, *J* = 15.9, 10.1 Hz, 1H), 1.91 (m, 2H), 1.76 (m, 3H), 1.69 (m, 5H), 1.55 (m, 1H), 1.33 (m, 1H), 1.14 (brt, *J* = 12.5 Hz, 1H), 1.07 (brt, *J* = 12.5 Hz, 1H),1.00 (brt, *J* = 12.5 Hz, 1H), 0.90 (m, 1H), 0.86 (m, 1H), 0.60 (brt, *J* = 12.5 Hz, 1H), 0.94 (d, *J* = 6.0 Hz, 3H, CH_3), 0.77 (d, *J* = 6.6 Hz, 3H, CH_3), 0.76 (d, *J* = 6.6 Hz, 3H, CH_3), 0.75 (d, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) *δ* (ppm): 176.2, 171.6, 143.9, 138.7, 127.6, 118.5, 116.2, 102.7, 76.0, 71.5 (2C), 64.5 (2C), 61.4, 56.4, 52.8, 52.6 (2C), 52.5 (2C), 48.7, 48.1, 45.6, 43.3, 37.7, 36.9, 35.7, 35.2, 34.6, 30.8, 30.5, 29.1, 27.3, 26.3, 24.8, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{39}H_{64}N_3O_8$ $[M+H]^+$: 702.4693; found, 702.4693.

4.1.4.15. 2-[4-(cyclohexylmethyl)piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-cyclopentane-1-carboxylate (***4o***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 88% yield: $[\alpha]_D^{20}$ -25 (c 0.64, MeOH); UV (MeOH) λmax (log ε) 256 (4.68) nm; IR v 3435, 2953, 2922, 2873, 2851, 2211, 1730, 1676, 1641, 1568, 1550, 1461, 1378, 1290, 1273, 1164, 1126, 1074, 1039, 974, 847, 808, 786, 652, 619 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) *δ* (ppm): 6.83 (d, *J* = 11.3 Hz, 1H), 6.51 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.22 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.87 (dt, *J* = 10.7, 3.7 Hz, 1H), 4.22 (dt, *J* = 11.5, 5.5 Hz, 1H), 4.09 (d, *J* = 9.8 Hz, 1H), 4.02 (dt, *J* = 11.5, 5.5 Hz, 1H), 3.80 (dt, *J* = 10.2, 3.1 Hz, 1H), 3.54 (m, 2H), 2.62 (m, 2H), 2.55 (m, 2H), 2.39-2.52 (m, 10H), 2.22 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.13 (m, 2H), 2.11 (dd, *J* = 15.9, 10.1 Hz, 1H), 1.90 (m, 2H), 1.76-1.57 (m, 13H), 1.46 (m, 2H), 1.32 (m, 2H), 1.14 (m, 1H), 1.07 (brt, *J* = 12.5 Hz, 1H), 1.00 (brt, *J* = 12.5 Hz, 1H), 0.87 (m, 1H), 0.82 (m, 1H), 0.59 (brt, *J* = 12.5 Hz, 1H), 0.94 (d, *J* = 6.6 Hz, 3H, CH_3), 0.76 (d, *J* = 7.2 Hz, 6H, 2 × CH_3), 0.75 (d, *J* = 6.6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) *δ* (ppm): 176.2, 171.6, 143.9, 138.7, 127.6, 118.5, 116.2, 76.0, 71.5 (2C), 65.2, 61.4, 56.5, 52.9 (2C), 52.7 (2C), 48.8, 48.1, 45.6, 43.3, 37.7, 36.8, 35.7, 35.2, 34.6, 34.6, 31.6, 31.5, 30.8, 29.4, 29.1, 27.2, 26.3 (2C), 25.7, 24.8, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{41}H_{68}N_3O_6$ $[M+H]^+$: 698.5108; found, 698.5106.

4.1.4.16. 2-[4-(cyclopropylmethyl)piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-cyclopentane-1-carboxylate (***4p***)

Eluent dichloromethane/methanol (30:1). Colorless crystal. 89% yield: $[\alpha]_D^{20}$ -34.1 (c 0.82, MeOH); UV (MeOH) λmax (log ε) 256 (4.82) nm; IR v 3399, 2954, 2923, 2212, 1728, 1636, 1599, 1458, 1374, 1276, 1166, 1035, 976, 826, 745, 677, 637 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) *δ* (ppm): 6.92 (d, *J* = 11.3 Hz, 1H), 6.60 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.32 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.97 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.30 (dt, *J* = 11.5, 5.5 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.14 (dt, *J* = 11.5, 5.5 Hz, 1H), 3.89 (dt, *J* = 10.0, 3.2 Hz, 1H), 2.71 (m, 2H), 2.66 (m, 2H), 2.62 (m, 2H, 1 × -N-CH_2-), 2.56 (m, 4H, 2 × -N-CH_2-), 2.51 (m, 4H, 2 × -N-CH_2-), 2.34 (m, 2H, -CH_2-cyclopropane), 2.33 (m, 1H), 2.32 (dd, *J* = 15.9, 3.0 Hz, 1H), 2.20 (dd, *J* = 15.9, 10.2 Hz, 1H), 2.00 (m, 2H), 1.83 (m, 3H), 1.78 (m, 3H), 1.65 (m, 1H), 1.43 (m, 1H), 1.23 (m, 1H), 1.17 (brt, *J* = 12.5 Hz, 1H), 1.09 (brt, *J* = 12.5 Hz, 1H), 0.99 (m, 1H), 0.96 (m, 1H), 0.69 (brt, *J* = 12.5 Hz, 1H), 0.56 (m, 2H), 0.18 (m, 2H), 1.03 (d, *J* = 6.6 Hz, 3H, CH_3), 0.86 (d, *J* = 6.6 Hz, 3H, CH_3), 0.85 (d, *J* = 5.4 Hz, 6H, 2 × CH_3); ^{13}C NMR (150 MHz, CD_3OD) *δ* (ppm): 174.5, 170.0, 142.2, 137.0, 125.9, 116.8, 114.5, 74.3, 69.9, 69.8, 61.3, 59.8, 54.7, 50.8 (2C), 50.7 (2C), 47.0, 46.4, 43.9, 41.6, 36.1, 35.3, 34.0, 33.5, 32.9, 29.1, 27.4, 25.6, 24.6, 23.1, 17.8, 16.0, 15.6, 12.3, 5.5, 1.4, 1.4. ESI-HRMS (*m/z*): calcd. For $C_{38}H_{62}N_3O_6$ $[M+H]^+$: 656.4639; found, 656.4640.

4.1.4.17. 2-[4-(2-oxopropyl)piperazin-1-yl] ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*,9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetramethyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]-cyclopentane-1-carboxylate (***4q***)

Eluent dichloromethane/methanol (50:1). Colorless crystal. 89% yield: $[\alpha]_D^{20}$ -35.2 (c 0.46, MeOH). IR v 3435, 2955, 2921, 2211, 1727, 1637, 1459, 1378, 1283, 1165, 1038, 976 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) *δ* (ppm): 6.96 (d, *J* = 11.3 Hz, 1H), 6.60 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.33 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 4.97 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.32 (ddd, *J* = 11.5, 6.0, 4.8 Hz, 1H), 4.18 (d, *J* = 9.8 Hz, 1H), 4.12 (ddd, *J* = 11.5, 6.0, 4.8 Hz, 1H), 3.90 (dt, *J* = 10.1, 3.2 Hz, 1H), 3.37 (d, *J* = 18.0 Hz, 1H), 3.32 (d, *J* = 18.0 Hz, 1H), 2.72-2.48 (m, 14H), 2.32 (dd, *J* = 15.9, 3.1 Hz, 1H), 2.20 (dd, *J* = 15.9, 10.1 Hz, 1H), 2.11 (s, 3H), 2.00 (m, 2H), 1.87-1.76 (m, 6H), 1.65 (m, 1H), 1.42 (m, 1H), 1.24 (m, 1H), 1.16 (brt, *J* = 12.5 Hz, 1H), 1.10 (brt, *J* = 12.5 Hz, 1H), 0.98 (m, 2H), 0.68 (brt, *J* = 12.5 Hz, 1H), 1.03 (d, *J* = 6.6 Hz, 3H, CH_3), 0.85 (d, *J* = 6.6 Hz, 9H, 3 × CH_3); ^{13}C NMR (150 MHz, CD_3OD) *δ* (ppm): 206.3, 176.1, 171.6, 144.1, 138.7, 127.7, 118.6, 116.1, 76.0, 71.5, 71.4, 61.5, 61.1, 56.5, 52.7 (2C), 52.5 (2C), 52.4, 48.9, 48.1, 45.6, 43.3, 37.8, 36.7, 35.7, 35.2, 34.6, 30.7, 29.1, 27.2, 26.4, 26.3, 21.8, 19.5, 17.8, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{37}H_{60}N_3O_7$ $[M+H]^+$: 658.4431; found, 658.4431.

4.1.4.18. 2-(4-allylpiperazin-1-yl) ethyl (1*R*,2*R*)-2-[(2*S*,4*E*,6*Z*,8*R*, 9*S*,11*R*,13*S*,15*S*,16*S*)-7-cyano-8,16-dihydroxy-9,11,13,15-tetra-methyl-18-oxooxacyclooctadeca-4,6-dien-2-yl]cyclopentane-1-carboxylate (***4r***)

Eluent dichloromethane/methanol (40:1). Colorless crystal. 90% yield: $[\alpha]_D^{20}$ -36.4 (c 0.44, MeOH); UV (MeOH) λmax (log ε) 256 (4.31) nm; IR v 3401, 2954, 2922, 2855, 2212, 1729, 1640, 1597, 1459, 1376, 1267, 1165, 1099, 1037, 974, 926, 812, 778, 737 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD) *δ* (ppm): 6.82 (d, *J* = 11.3 Hz, 1H), 6.51 (dd, *J* = 14.9, 11.3 Hz, 1H), 6.22 (ddd, *J* = 14.9, 10.1, 4.8 Hz, 1H), 5.78 (ddt, *J* = 17.0, 10.2, 6.8 Hz, 1H), 5.15 (dd, *J* = 17.0, 1.8 Hz, 1H), 5.11 (dd, *J* = 10.2, 1.8 Hz, 1H), 4.87 (dt, *J* = 10.6, 3.7 Hz, 1H), 4.21 (dt, *J* = 11.5, 5.5 Hz, 1H), 4.09 (d, *J* = 9.8 Hz, 1H), 4.04 (dt, *J* = 11.5, 5.5 Hz, 1H), 3.80 (dt, *J* = 10.1, 3.2 Hz, 1H), 2.96 (m, 2H, -CH_2-alkene), 2.61 (m, 2H), 2.56 (m, 2H), 2.51 (m, 2H, 1 × -N-CH_2-), 2.46 (m, 4H, 2 × -N-CH_2-), 2.41 (m, 4H, 2 × -N-CH_2-), 2.23 (dd, *J* = 15.9, 3.1 Hz, 1H), 2.11 (dd, *J* = 15.9, 10.1 Hz, 1H), 1.90 (m, 2H), 1.73 (m, 3H), 1.69 (m, 3H), 1.56 (m, 1H), 1.33 (m, 1H), 1.14 (m, 1H), 1.07 (brt, *J* = 12.5 Hz, 1H), 1.00 (brt, *J* = 12.5 Hz, 1H), 0.90 (m, 1H), 0.86 (m, 1H), 0.60

(brt, J = 12.5 Hz, 1H), 0.94 (d, J = 6.6 Hz, 3H, CH_3), 0.76 (d, J = 6.6 Hz, 6H, 2 × CH_3), 0.75 (d, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, CD_3OD) δ (ppm): 176.2, 171.6, 143.9, 138.7, 133.6, 127.6, 118.5, 118.3, 116.2, 76.0, 71.5, 71.4, 61.5, 61.1, 56.4, 52.6 (2C), 52.3 (2C), 48.7, 48.1, 45.6, 43.3, 37.7, 36.9, 35.7, 35.2, 34.6, 30.8, 29.1, 27.2, 26.3, 24.8, 19.5, 17.7, 17.3, 14.0. ESI-HRMS (*m/z*): calcd. For $C_{37}H_{60}N_3O_6$ $[M+H]^+$: 642.4482; found, 642.4484.

4.2. Biological Assays

4.2.1. *In vitro* Antifungal Activity Evaluation by MIC Assay

The MIC values of borrelidin derivatives were determined by using a micro broth dilution method.[43] *C. albicans* (ATCC MYA-2876) and *C. parapsilosis* (ATCC 22019) were obtained from China General Microbiological Culture Collection Center. *A. fumigatus* (CCTCC AF 93048) was obtained from China Center for Type Culture Collection. Antifungal assay were performed in RPMI-1640 broth (10.4 g/L RPMI-1640, 2 g/L $NaHCO_3$, 34.53 g/L MOPs, pH 7.0). The tested compounds were dissolved in DMSO and 2-fold serially diluted to eight different concentrations (10-0.31 mg/mL), and the above samples (1 μL) was added to each well. Afterwards, prepared fungal suspensions containing 2 × 10^3 cfu/mL for *C. albicans*, *C. parapsilosis* and *A. fumigatus* were added to each well of 96-well microtiter plates, and the plates were incubated for 48 h at 28 °C for *C. albicans*, *C. parapsilosis* and *A. fumigatus*. The minimum concentration of sample showing no turbidity was recorded as the MIC.

4.2.2. Cytotoxicity Assay

The human kidney epithelial cell line (293T) were obtained from National Infrastructure of Cell Line Resource (Beijing, China), and cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified 5% CO_2 atmosphere incubator (Thermo Fisher Scientific, Waltham, MA, USA). The cytotoxicity and proliferation inhibition were quantified by the MTT assay. 293T (7 × 10^3 cells/well) was seeded in 96-well plates respectively and exposed to fourteen target compounds at six concentrations (0.33, 1.0, 3.3, 10.0, 33.0 and 100.0 μM). After 48 h incubation at 37°C, 20 μL MTT was added into each well and incubated another 4 h. And then the liquid in the wells was removed. Subsequently, 150 μL DMSO was added into each well. Use a microplate reader at 570 nm to measure the absorbance of each well. The results were represented as the mean ± SD from three independent experiments. The inhibition rate (%) = [(A_{570} (control) - A_{570} (compound)]/A_{570} (control) × 100% and the IC_{50} values were calculated by a non-linear regression analysis using SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

4.2.3. Molecular docking study

Download the homo threonyl-tRNA synthetase with co-crystal borrelidin (PDB: 4P3N) and threonyl-tRNA synthetase of the yeast mitochondrial (PDB: 4YYE) from the Protein Data Bank (http://www.rcsb.org/pdb). Align their structures to find out the binding site by using Discovery Studio 3.0 software. This software was also applied as for the preparation of protein, during which missing amino acid residues complemented, hydrogen atoms added, all water molecules removed and missing side chains fixed. The complex of protein structure (PDB: 4YYE) and compound borrelidin was optimized and saved for the next docking experiments.

Since the docking studies were performed by Glide in Schrodinger (version 2014) in subsequent works, the protein was prepared by Schrodinger's protein preparation wizard as well and crystallographic water molecules were deleted as there was no water molecules exist any co-ordination with the ligand. Other processes of preparation include correcting protein structure defects, building the missing side chain atoms and adding hydrogen atoms. Both the acidic and basic amino acid residues were disposed into the appropriate ionization states at pH 7.4. Ultimately, the energy of the protein was minimized till RMSD value of 0.30 Å was done using OPLS_2005 force field to relieve the steric clashes among the residues due to addition of hydrogen atoms. Next, the generated grid file from the prepared protein was used for Glide XP docking calculations. The minimized conjugate gradient output of the reference ligand. XP Glide scoring function was used to order the best ranked compounds and the specific interactions like hydrogen bonding and pi-pi stacking were analyzed by formed 2D interactions diagram using in Glide module. Finally, the docked completed file was imported into software Discovery Studio 3.0 and PyMOL to investigate the 3D interaction relationship and the conformation of the compound on the protein surface.

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Supplementary Material

Supplementary materials are available online. The ^{1}H-NMR, ^{13}C-NMR and IR spectra of target compounds.

Graphical Abstract

Design, Synthesis, and Antifungal Evaluation of Borrelidin Derivatives

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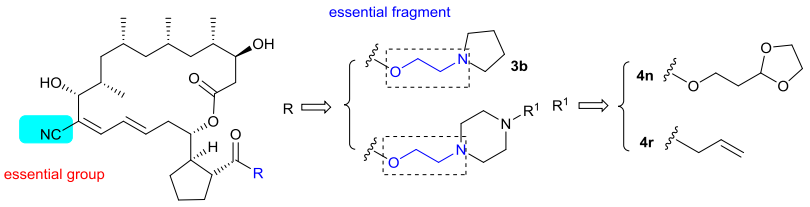

3b. MIC: 50, 12.5 μg/mL against *C.albicans* and *C. parapsilosis,* respectively

4n. MIC: 12.5 μg/mL against *A. fumigatus*
4r. MIC: 12.5 μg/mL against *A. fumigatus*

Highlights

- Synthesis and antifungal evaluation of forty-seven borrelidin analogs.
- Fragment -OCH_2CH_2N- was essential for antifungal activity.
- **3b** exhibited activity against *C. albicans* which was insensitive to borrelidin.
- Compared with borrelidin, **4n** and **4r** presented extra activity against *A. fumigatus*.
- Carboxyl substituents in borrelidin existed extra interaction with fungal ThrRS.