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Design, synthesis and biological evaluation of novel hamamelitannin analogues as potentiators for vancomycin in the treatment of biofilm related *Staphylococcus aureus* infections

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ABSTRACT

Staphylococcus aureus is a frequent cause of biofilm-related infections. Bacterial cells within a biofilm are protected from attack by the immune system and conventional antibiotics often fail to penetrate the biofilm matrix. The discovery of hamamelitannin as a potentiator for antibiotics, recently led to the design of a more drug-like lead. In the present study, we want to gain further insight into the structure–activity relationship (S.A.R.) of the 5-position of the molecule, by preparing a library of 21 hamamelitannin analogues.

1. Introduction

For a long time, bacteria have been solely regarded as simple, free living, planktonic micro-organisms. Today, however, it is generally accepted that microbes often live in communities.[1] As bacteria grow on biotic as well as abiotic surfaces, they tend to form structurally complex and dynamic systems, known as biofilms. Biofilms are sessile assemblages of cells attached to a surface and enclosed in an adhesive and self-produced extracellular matrix.[2] The latter is typically composed of polysaccharides, proteins and extracellular DNA (eDNA). The phenotype of certain bacteria subpopulations in the biofilm (especially apparent for so-called 'persisters'), together with the limited penetration of antimicrobial drugs through the matrix are two main reasons for the capacity of biofilm bacteria to escape the effect of antibiotics. Moreover, cells within a biofilm are protected from attack by the immune system.[3,4] Bacterial biofilms are implicated in many medical conditions, including periodontal disease, tuberculosis, respiratory tract infections in cystic fibrosis patients and staphylococcal wound infections. *Staphylococcus aureus* (*S. aureus*) is a Gram-positive bacterium that is a human commensal organism, but may turn into a versatile and dangerous pathogen causing infections that are difficult to eradicate.[5,6] Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are notorious for their resistance to a wide range of antibiotics.

For the last decades, antibacterial research and development has focused on drugs that kill bacteria or inhibit their growth by interfering with essential cellular processes. However, conventional antibiotics inherently impose selective pressure on bacteria and cause resistance, which constitutes a complex global public health challenge. Inadequate investment in antibiotic research and a poorly filled antibiotics pipeline add urgency to the situation.[7,8]

We decided to explore agents which potentiate the effect of existing antibiotics. Bacterial biofilm formation and virulence are associated with quorum sensing (QS) mechanisms. QS is a cell-to-cell communication system by which bacteria sense population density and control gene expression. The natural product hamamelitannin (2′,5-di-*O*-galloyl-D-hamamelose, HAM, **1**, Fig. 1) increases the susceptibility of *S. aureus* towards a wide range of antibiotics by affecting peptidoglycan thickness and eDNA release through the QS receptor TraP.[9] Structural optimization of HAM led to bisbenzamide **2** and lead compound **3** (Fig. 1). The latter increases the effect of antibiotics in two in vivo infection models, in which it is superior to the parent natural product (HAM, **1**).[10] In the present study, we want to gain further insight into the structure–activity relationship (S.A.R.) of the 5-position of **3**, by preparing a library of 21 derivatives.

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0968-0896/

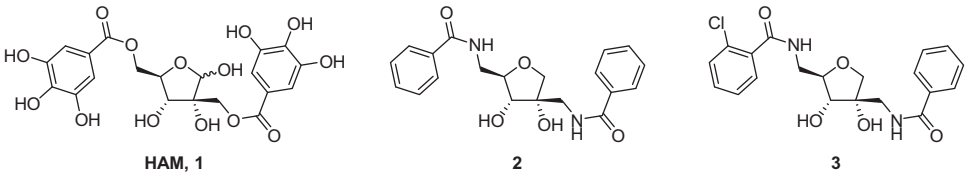


Figure 1. Structure of hamamelitannin (**1**), a more drug-like bisbenzamide derivative **2** and lead compound **3**.

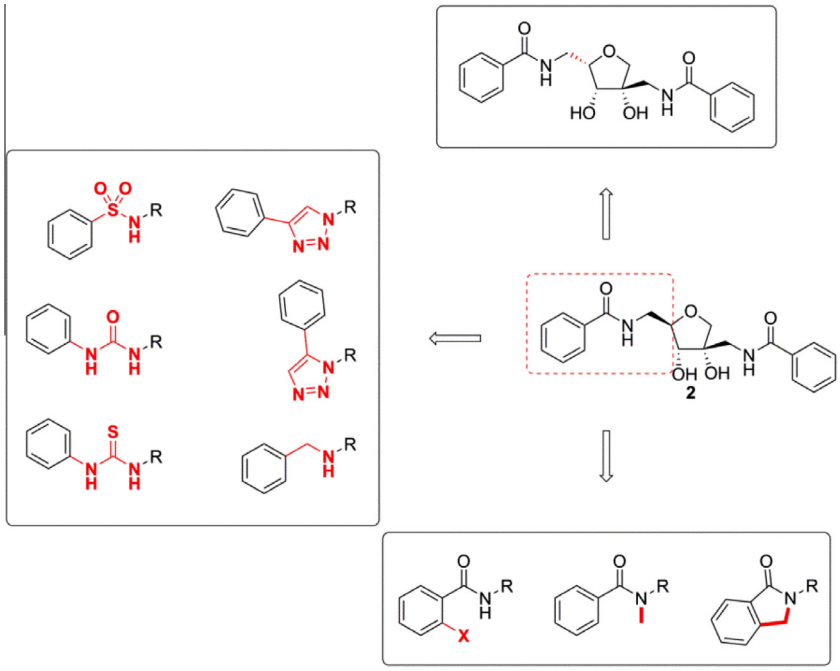


Figure 2. Overview of the structure variations described in this study.

The structure variation focuses on three aspects (Fig. 2). First, we wanted to investigate the stereochemical requirements at C_4 by synthesizing the C_4 epimer of bisbenzamide **2**. In order to investigate the role of the 5-amido group, we explored alternative nitrogen-based moieties to link the aromatic group to the central scaffold. Finally, we wanted to corroborate the hypothesis that *ortho* substitution of the 5-phenyl ring increases activity.

2. Chemistry

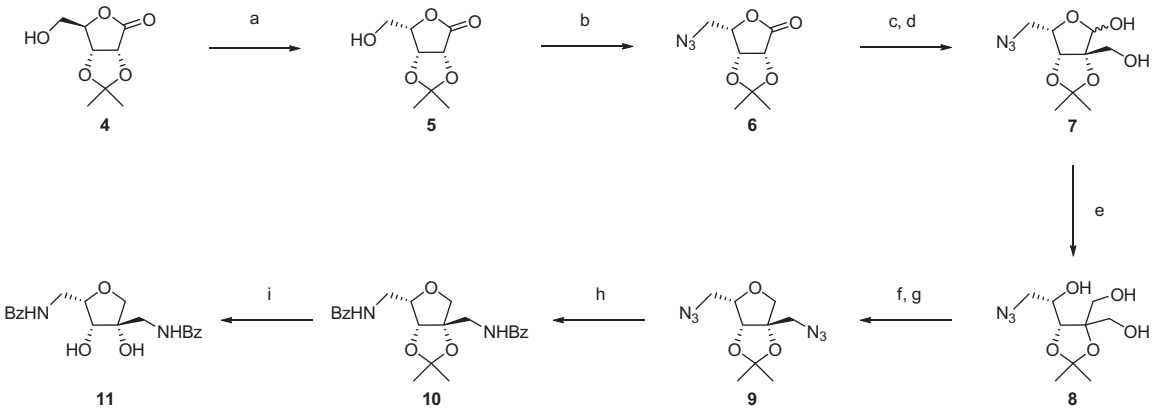
The commercially available lactone **4** was converted to its 4-epimer following the method of Batra et al.[11] (Scheme 1), which involves intramolecular opening of an intermediate epoxide in a 5-*exo*-tet process. Successful inversion of configuration at C_4 was apparent from NMR analysis of the protected lyxonolactone **5**. In the ^{1}H NMR spectrum, the H_4 signal appears at 4.65 ppm as a ddd, which couples to H_3, H_{5a} and H_{5b} in the COSY spectrum, while the H_4 signal of the starting material appears as a triplet at 4.59 ppm. The H_3–C_3–C_4–H_4 dihedral angle of nearly 90° is responsible for the seemingly reduced multiplicity of the H_4 signal in the starting material. Correspondingly, in ribonolactone **4**, H_3 appears as a doublet, where one would expect a higher multiplicity (dd) (Supporting info). Compound **5** was converted to 2-*C*-branched-chain derivative **7** according to the method of Simone et al.[12] Borohydride reduction, tosylate-promoted cyclisation and subsequent substitution with sodium azide gave intermediate **9**. Reduction of this bis-azide with trimethylphosphine, EDC-mediated coupling of the resulting amine with benzoic acid and removal of the acetonide protecting group gave the desired 4-epimer **11**.

Antimicrobial potentiator **3**, identified in a previous study, comprises two different aromatic moieties, linked via amides to the central scaffold. The known amine **13**[10] served as a valuable precursor for the synthesis of sulfonamides (**19a**–**i**), urea (**20a**) and thiourea (**20b**) derivatives (Scheme 2). Reductive amination of **13** with benzaldehyde and $NaBH_4$ gave benzylamine **21**. The triazole regioisomers **22** and **23** were synthesized via a copper- or ruthenium-catalyzed 1,3-dipolar cycloaddition reaction on the azide of precursor **12**.

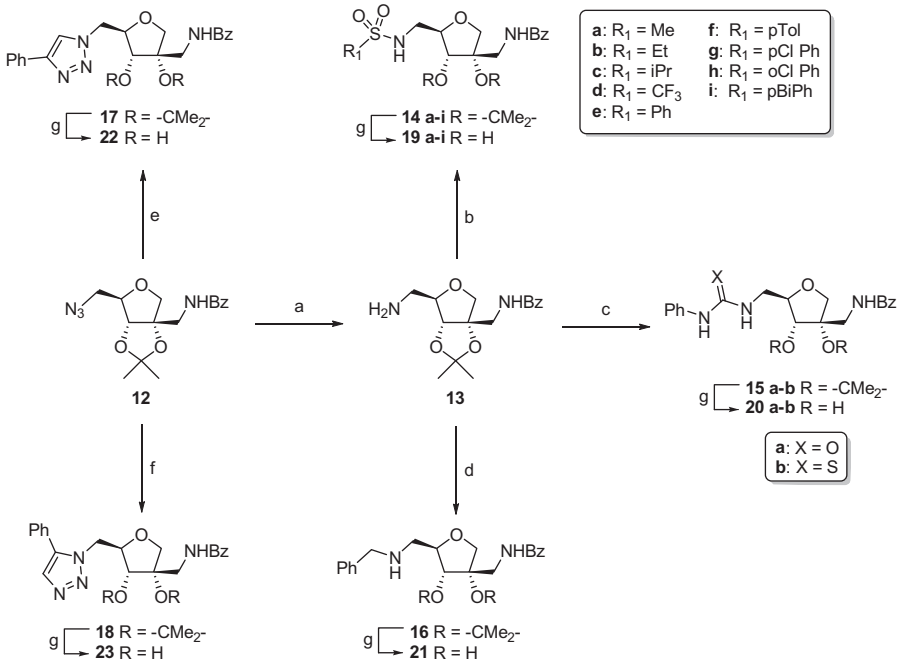
Phthalimide **24**, known from a previous study,[10] served as a versatile and orthogonally protected intermediate for the synthesis of **33**–**35** (Scheme 3). For the selective methylation of the 5-benzamide, we started with a one-pot reduction of azide **24** and acylation of the resulting amine, yielding benzamide **25**. Removal of the phthalimide with ethanolic hydrazine gave primary amine **26**, which was subjected to diazotransfer (**27**). After methylation, intermediate **28** was converted to final compound **33** via known procedures. HAM derivatives **34a**–**d** were obtained via EDC-mediated acylation of **13** with the appropriate benzoic acid. Radical bromination of commercially available methyl 2-methylbenzoate, subsequent treatment of bromide **31** with amine **13** and acidic hydrolysis of the acetonide in **32** gave isoindolinone **35**, which can be considered as a rigidified 5-benzamide analogue.

3. Results and discussion

The minimum inhibitory concentrations (MIC) of all final compounds against *S. aureus* Mu50 were higher than 500 μM, which rules out a direct effect on growth (data not shown). Subsequently,



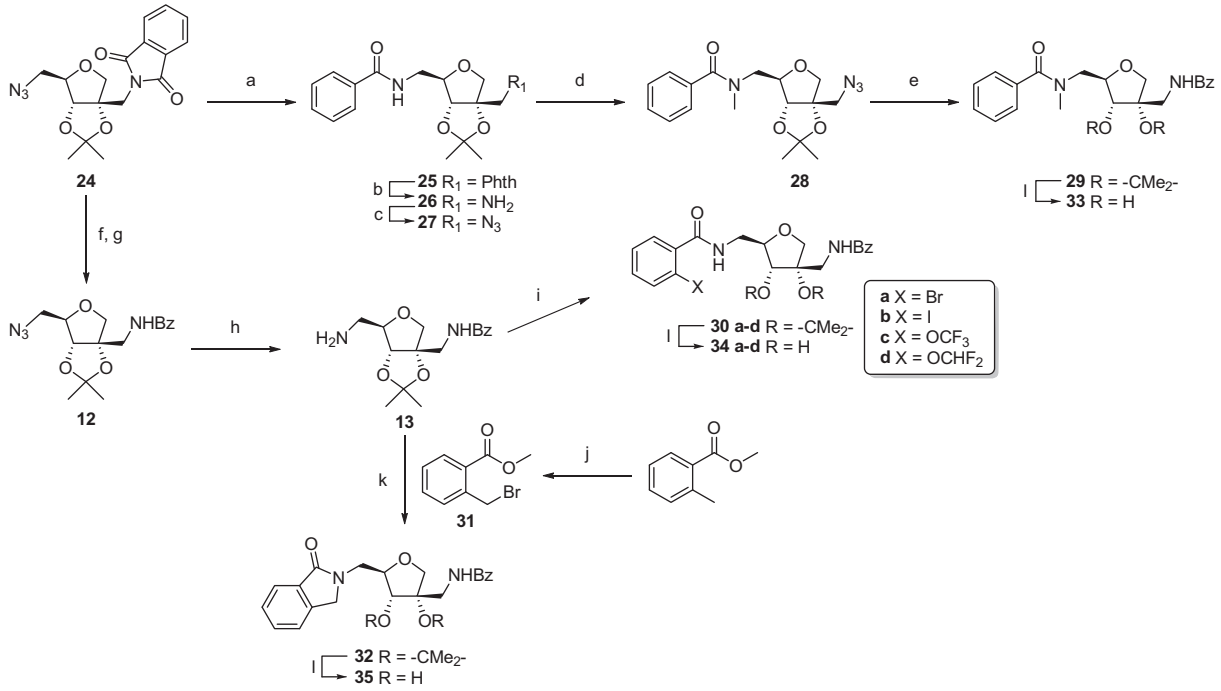
Scheme 1. Reagents and conditions: (a) [i] MsCl, Et_3N, CH_2Cl_2, rt, 3 h; [ii] KOH, dioxane/H_2O; [iii] dil. aq HCl, 65%; (b) [i] MsCl, Et_3N, CH_2Cl_2, rt, 3 h; [ii] NaN_3, DMF, 90 °C, 16 h, 81%; (c) DIBALH, CH_2Cl_2, −78 °C, 4 h; (d) aq CH_2O, K_2CO_3, MeOH, 50 °C, 24 h, 46% over two steps; (e) $NaBH_4$, MeOH, 0 °C, 16 h, 24% (82% based on recovered starting material); (f) [i] TsCl, pyridine, rt, 3 h; [ii] 60 °C, 14 h; (g) NaN_3, DMF, 80 °C, 16 h 29% over 2 steps; (h) [i] PMe_3, THF, 3 h; [ii] H_2O, 45 min; [iii] PhCOOH, EDC·HCl, DIPEA, HOBt, DMF, rt, 16 h; (i) 35% TFA, H_2O, rt, 16 h, 74% over two steps.



Scheme 2. Reagents and conditions: (a) [i] PMe_3, THF, 3 h; [ii] H_2O, 45 min; (b) RSO_2Cl, Et_3N, CH_2Cl_2, 0 °C, 3 h, 16–91%; (c) PhNCX (X = O,S), pyridine, rt, 3 h, (X = O, 76%; X = S, 65%); (d) [i] benzaldehyde, mol. sieves, MeOH; [ii] $NaBH_4$, 47%; (e) PhC≡CH, CuI, TBTA, DMF/H_2O/TEA, 75 °C, 4 h, 84%; (f) PhC≡CH, $CpRuCl(PPh_3)_2$, dioxane, 60 °C, 24 h, 83%; (g) 35% TFA, H_2O, rt, 16 h, 63%—quant.

the HAM derivatives were tested for their in vitro effect on *S. aureus* biofilm susceptibility to vancomycin (VAN), both under pretreatment and combination treatment regimens. To evaluate the effect of pretreatment, *S. aureus* Mu50 was allowed to form a biofilm in the presence of HAM analogues, after which the biofilm was treated with VAN (20 μg/ml). In the combination treatment setup, the bacteria were allowed to form a mature biofilm after which HAM, or a HAM analogue and VAN were administered simultaneously. When used alone, VAN resulted only in a minor reduction of the number of *S. aureus* sessile cells (30% ± 14% compared to an untreated control, Table 1). In contrast, combined treatment of VAN with HAM resulted in significantly more killing of bacterial biofilm cells, both under pretreatment and under combined treatment regimens. For most of the HAM-analogues, combined treatment with VAN also resulted in a significantly higher reduction of CFUs per biofilm. For the most interesting derivatives, the effect on biofilm susceptibility towards VAN was tested in lower concentrations, which allowed us to determine an EC_{50} value. The latter is defined as the concentration of the analogue needed to double the activity of vancomycin, as measured by the number of surviving cells.

A marked difference in activity was observed between **2** and its 4-epimer **11** (Table 1), with the latter exhibiting very poor activity in increasing biofilm susceptibility towards VAN (even lower than HAM), indicating that the observed potentiator activity of **2** and derivatives thereof likely involves specific target interactions. When comparing **2** with **19e**, it can be concluded that substitution of the 5-amide with a sulfonamide linker is well-tolerated (Table 1). Therefore, we extended the library of HAM derivatives with monosubstituted aromatic sulfonamides (**19f–i**) as well as alkylsulfonamide analogues (**19a–d**). In agreement with previous observations, non-aromatic derivatives displayed very poor activ-



Scheme 3. Reagents and conditions: (a) BzCl, PMe_3, rt, 16 h, 45%; (b) $N_2H_4{\cdot}H_2O$, EtOH, reflux, 5 h, 96%; (c) TfN_3, MeOH, Et_3N, $CuSO_4$, 90%; (d) [i] NaH, THF, 1 h, [ii] MeI, 88%; (e) [i] PMe_3, THF, 3 h, [ii] H_2O, 45 min, [iii] benzoic acid, EDC·HCl, DIPEA, HOBt, DMF, rt, 16 h, 93% over two steps; (f) $N_2H_4{\cdot}H_2O$, EtOH, reflux, 4 h, 93%; (g) BzCl, TEA, CH_2Cl_2, 0 °C, 3 h, 99%; (h) [i] PMe_3, THF, 3 h; [ii] H_2O, 45 min; (i) RCOOH, EDC·HCl, DIPEA, HOBt, DMF, rt, 16 h, 53–74% from 12; (j) NBS, AIBN, CCl_4, reflux, 68%; (k) TEA, MeOH, reflux, 59% from 12; (l) 35% TFA, H_2O, rt, 16 h, 84–98%.

ity and although some of the aromatic sulfonamides displayed interesting potentiator activity, they proved to be less potent than lead compound **3** (EC_{50} = 0.389 μM). None of the other derivatives with alternative linkers were exceptionally potent, with triazoles **22** and **23** being almost completely devoid of activity. Therefore, we focused our attention on amides. *Ortho*-bromo (**34a**) and *ortho*-iodo (**34b**) 5-benzamide substitution yielded compounds with 10- to 20-fold higher potencies relative to parent compound **2**. Analogues **34c**–**d** on the other hand, for which hydrogen-bonded pseudo six ring formation is possible, lose activity. Methylating the 5-benzamide also results in twisting the phenyl group out of the plane, which is reflected in the interesting effect of **33** on biofilm susceptibility. An out-of-plane distortion of the benzamide system probably enables better interaction with the binding site. Along the same lines, a proportional decrease in the potentiating activity was observed for lactam **35**, in which the amide nitrogen is covalently linked to the aromatic moiety, rendering the benzamide system planar. These observations strongly reinforce our hypothesis that twisting the phenylamide to a non-planar conformation improves activity.

4. Conclusions

In conclusion, a library of 21 HAM-analogues was designed and the compounds were evaluated for their ability to potentiate vancomycin, a drug of last resort, in the treatment of biofilm related and resistant staphylococcal infections. Starting from earlier identified bisbenzamide analogues **2** and **3**, we further investigated the S.A.R. of the substituent at position 5. Epimer **11** exhibits relatively poor potentiating activity. Although displaying moderate to good activity, compounds with different nitrogen-based linkers were less potent than lead compound **3**. Out-of-plane distortion of the 5-benzamide moiety improves activity. Further efforts to throw light on the S.A.R. of HAM and to improve the potentiating activity of HAM analogues are ongoing and will be reported in due course.

5. Experimental section

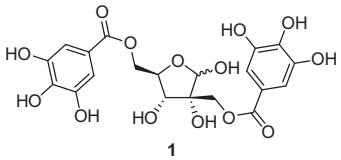
5.1. Chemistry

All reactions described were performed under an argon atmosphere and at ambient temperature unless stated otherwise. All reagents and solvents were purchased from Sigma–Aldrich (Diegem, Belgium), Acros Organics (Geel Belgium), TCI Europe (Zwijndrecht, Belgium) or Carbosynth Ltd (Compton Berkshire, United Kingdom) and used as received. NMR solvents were purchased from Eurisotop (Saint-Aubin, France). Reactions were monitored by TLC analysis using TLC aluminium sheets (Macherey–Nagel, Alugram Sil G/UV_{254}) with detection by UV or by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in H_2SO_4 (10%) followed by charring or an aqueous solution of $KMnO_7$ (20 g/L) and K_2CO_3 (10 g/L) or an ethanolic solution of ninhydrin (2 g/L) and acetic acid (1% v/v) followed by charring. Silica gel column chromatography was performed manually using Grace Davisil 60 Å silica gel (40–63 μm) or automated using a Grace Reveleris X2 system and the corresponding flash cartridges. High resolution spectra were recorded with a Waters LCT Premier XE Mass spectrometer. ^1H and ^{13}C NMR spectra were recorded with a Varian Mercury-300BB (300/75 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal standard (^1H NMR) or the NMR solvent (^{13}C NMR). In ^{19}F NMR, signals have been referred to $CDCl_3$ or DMSO-d_6 lock resonance frequency according to IUPAC referencing with $CFCl_3$ set to 0 ppm. Coupling constants are given in Hz. Preparative HPLC purifications were carried out using a Laprep preparative HPLC system equipped with a Xbridge Prep C18 column (19 × 250 mm, 5 micron) using a water/acetonitrile/formic acid gradient system.

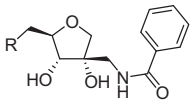
5.1.1. General procedure 1: Staudinger reduction

A solution of compound **9**, **12** or **29** in THF (10 mL/mmol) was treated with Me_3P (1 M solution, 5 equiv per azide) and the reaction mixture was stirred for 3 h. Water (13 equiv per resulting

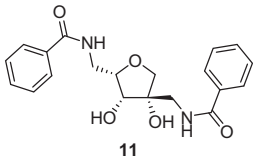
Table 1
Microbiological evaluation of HAM analogues with differentiation at 5-position



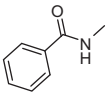
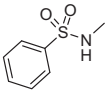
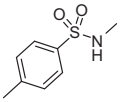
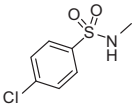
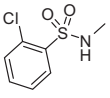
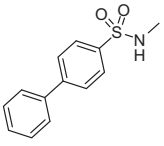
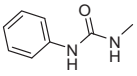
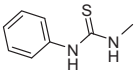
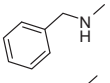
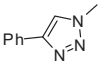
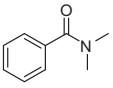
1



2, **19a-i**, **20a-b**, **21**, **23**

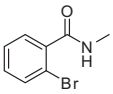
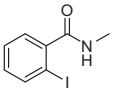
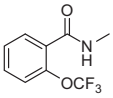
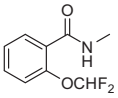
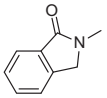


11

Compound	R	Reduction in CFU's compared to control (%)[a]		EC_{50} (μM)	
		Pretreatment	Combination treatment	Pretreatment	Combination treatment
VAN alone	–	30 ± 14*	30 ± 14*	–	–
HAM, **1**	–	57 ± 13	57 ± 22	145.5	165.1
2		66 ± 19	65 ± 14	97.0	124.0
11	–	48 ± 11	44 ± 11	224.0	>250.0
19a		69 ± 13	62 ± 9		
19b		49 ± 4	47 ± 23		
19c		72 ± 5*	67 ± 9		
19d		71 ± 12	57 ± 20		
19e		78 ± 9*	69 ± 3		
19f		83 ± 7*	74 ± 1*		
19g		86 ± 6*	85 ± 3*	23.5	27.8
19h		65 ± 6	73 ± 9		
19i		84 ± 4*	83 ± 14*		
20a		76 ± 6*	81 ± 3*		
20b		80 ± 4*	73 ± 6		
21		81 ± 8*	78 ± 11*	41.2	45.1
22		46 ± 4	66 ± 8		
23		53 ± 14	26 ± 53		
33		94 ± 2*	90 ± 3*	8.5	24.3

(*continued on next page*)

Table 1 (*continued*)

Compound	R	Reduction in CFU's compared to control (%)[a]		EC_{50} (μM)	
		Pretreatment	Combination treatment	Pretreatment	Combination treatment
34a		96 ± 2*	82 ± 2*	9.8	19.7
34b		96 ± 2*	84 ± 2	1.9	20.4
34c		78 ± 6*	69 ± 17	26.4	56.7
34d		81 ± 6*	78 ± 7	31.0	25.7
35		75 ± 10	70 ± 6	57.7	60.2

[a] Percentage reduction in Colony Forming Units (CFU's) per biofilm when biofilms are treated with VAN alone (20 μg/ml) or in combination with HAM or a HAM-analogue (100 μM) compared to the untreated (negative) control.

* Significantly different from treatment with HAM + VAN ($p < 0.05$).

iminophosphorane) was added and the solution was stirred for another hour, after which it was concentrated in vacuo. The residue was co-evaporated with toluene. The obtained crude amine was used without further purification.

5.1.2. General procedure 2: EDC-mediated amide formation

To a solution of the crude amine, obtained via general procedure 1, in DMF (25 mL/mmol) were added the appropriate organic acid (1.5 equiv per amine), EDC·HCl (2 equiv per amine), diisopropylethylamine (4 equiv per amine) and a catalytic amount of 1-hydroxybenzotriazole. The reaction mixture was stirred 16 h at rt. The mixture was concentrated and partitioned between water and EtOAc. The organic layer was dried over sodium sulphate, filtered and concentrated in vacuo. The products were purified by column chromatography with appropriate eluents.

5.1.3. General procedure 3: formation of 5-sulfonamide derivatives 14a–i from amine 13

A solution of the crude amine **13** in CH_2Cl_2 (20 mL/mmol) was cooled in an ice-bath, treated with triethylamine (2 equiv) and the appropriate sulfonyl chloride (1 equiv) and the reaction mixture was stirred for 3 h. When TLC indicated that the reaction was finished, the reaction mixture was concentrated under reduced pressure. The residue was taken up in EtOAc and washed with 0.1 M aq HCl and sat. aq $NaHCO_3$ solution. The organic layer was dried over sodium sulphate, filtered and concentrated. The residue was purified by column chromatography.

5.1.4. General procedure 4: deprotection—cleavage of acetonide

A known amount of the isopropylidene protected compound was treated with a 35% aq CF_3COOH solution (30 mL/mmol) 16 h at room temperature. For the more lipophilic derivatives the reaction mixture was put at ultra sound for 2–3 h. When TLC indicated that the deprotection was complete, the reaction mixture was concentrated and, if required, purified by column chromatography.

5.1.5. (3a*R*,6*S*,6a*R*)-6-(Hydroxymethyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-4(3a*H*)-one (5)

To a solution of 2,3-*O*-isopropylidene-D-ribonolactone **4** (24.5 g, 130 mmol) and triethylamine (36.2 mL, 260 mmol) in CH_2Cl_2 (650 mL) stirred at 0 °C, methanesulfonyl chloride (12.1 mL, 156 mmol) was added dropwise under a nitrogen atmosphere. The reaction mixture was allowed to attain ambient temperature. After 3 h, TLC analysis (toluene/EtOAc 3:2) showed complete consumption of the starting material. The reaction mixture was washed with saturated sodium bicarbonate solution and water. The organic layer was dried over sodium sulphate, filtered and concentrated in vacuo to afford the mesylate as a yellow to orange colored oil. To this crude mesylate, dissolved in 1,4-dioxane (600 mL), was added a solution of KOH (21.9 g, 390 mmol) in 300 mL of water. This solution was stirred vigorously for 3 h. When complete, the pH was adjusted to 3 by adding 1 M HCl (270 mL). The acidic solution was concentrated in vacuo to afford a solid mass, that was triturated with acetone (250 mL) and heated to reflux (20 min at 70 °C). The acetone was decanted and filtered. The remaining solid mass was triturated 2 more times and each time the boiling acetone was decanted and filtered. The combined clear filtrate was concentrated in vacuo to yield 2,3-*O*-isopropylidene-L-Lyxonolactone (**5**) as a colorless oil (65%). ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ ppm 1.34 (s, 3H), 1.37 (s, 3H), 3.83 (dd, J = 12.0, 7.0 Hz, 1H), 3.91 (dd, J = 12.0, 10.3 Hz, 1H), 4.15 (br s, 1H), 4.65 (ddd, J = 7.1, 4.9, 2.9 Hz, 1H), 4.93–5.02 (m, 2H). ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ ppm 26.0, 27.0, 60.9, 77.2, 77.3, 80.6, 113.9, 174.6. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_8H_{13}O_5^+$ 189.07575; Found 189.0766.

5.1.6. (3a*R*,6*S*,6a*R*)-6-(Azidomethyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-4(3a*H*)-one (6)

To a dry and cooled (0 °C) solution of 2,3-*O*-isopropylidene-L-Lyxonolactone **5** (4.00 g, 21.3 mmol) in CH_2Cl_2 (150 mL) were subsequently added Et_3N (5.90 mL, 42.5 mmol) and MsCl (2.00 mL, 25.5 mmol). The resulting reaction mixture was stirred for 3 h, after which time TLC analysis showed completion. The reaction mixture was transferred to a sep. fun., diluted with CH_2Cl_2, washed with sat. aq $NaHCO_3$ solution, dried (Na_2SO_4), filtered and concentrated. The residue was taken up in DMF (200 mL). Next, NaN_3 (5.50 g, 85.0 mmol) was added and the reaction mixture was stirred 16 h at 90 °C. When complete, volatile organics were evaporated. The residue was diluted with EtOAc and washed with water and brine. Purification via FCC (toluene/EtOAc 7:3) yielded the title compound

as a yellow to orange colored liquid (81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.41 (s, 3H), 1.49 (s, 3H), 3.61–3.76 (m, 2H), 4.58 (ddd, J = 7.0, 6.2, 3.2 Hz, 1H), 4.81–4.90 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 25.9, 26.8, 49.8, 75.8, 76.1, 77.3, 114.7, 173.1.

5.1.7. (3a*R*,6*S*,6a*R*)-6-(Azidomethyl)-3a-(hydroxymethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (7)

Azidolactone **6** (10.8 g, 50.7 mmol) was dissolved in CH_2Cl_2 (130 mL) and cooled to −78 °C. This solution was flushed with nitrogen gas, after which a solution of diisobutylaluminium hydride (1 M in toluene, 55.5 mL, 55.5 mmol) was added dropwise. The cooled solution was allowed to react for 4 h under nitrogen. The reaction was quenched by adding EtOAc (10 mL) and the mixture was allowed to come to room temperature over 30 min, after which a saturated Na^+/K^+ tartrate solution (300 mL) was added. The mixture was stirred for another hour and extracted with EtOAc (4 × 250 mL). The combined organic layers were dried (sodium sulphate), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (hexane/EtOAc 3:1) to afford 10.5 g of a pale oil. This azidolactole (10.0 g, 46.5 mmol) was dissolved in 350 mL of MeOH. Potassium carbonate (3.20 g, 23.3 mmol) and an aqueous solution of formaldehyde (38%, 110 mL) were added. The reaction mixture was stirred for 24 h at 50 °C. TLC analysis showed presence of a major product. The reaction mixture was cooled to ambient temperature and the MeOH was evaporated under reduced pressure. The residual aqueous solution was extracted with EtOAc (3 × 250 mL). The organic layers were combined, dried over sodium sulphate, filtered and concentrated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH 98:2) to afford compound **7** as a white powder (46% over two steps). Data in accordance with Simone et al.[12]

5.1.8. ((*R*)-5-((*S*)-2-Azido-1-hydroxyethyl)-2,2-dimethyl-1,3-dioxolane-4,4-diyl)dimethanol (8)

Sodium borohydride (0.360 g, 9.60 mmol) was added to a stirred and cooled (0 °C) solution of compound **7** (0.780 g, 3.20 mmol) in MeOH (30 mL). The reaction mixture was allowed to attain ambient temperature and was stirred 16 h. Ammonium chloride (1.85 g) was added to quench the excess of borohydride. The resulting suspension was stirred for 2 h, concentrated and adsorbed onto celite. Purification by flash chromatography afforded triol **8** as a white powder in 24.2% yield (82% based on recovered SM). ^{1}H NMR (300 MHz, CD_3OD) δ ppm 1.37 (s, 3H), 1.46 (s, 3H), 3.34–3.52 (m, 2H), 3.58–3.70 (m, 4H), 3.99–4.06 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 26.8, 28.4, 55.9, 63.0, 64.4, 69.6, 80.2, 85.1, 109.1.

5.1.9. (3a*S*,6*S*,6a*R*)-3a,6-Bis(azidomethyl)-2,2-dimethyltetrahydrofuro [3,4-*d*][1,3]dioxole (9)

To a stirred solution of triol **8** (0.320 g, 1.30 mmol) in pyridine (15.0 mL) was added *p*-toluenesulfonylchloride (0.530 g, 2.80 mmol). The reaction mixture was stirred at room temperature for 3 h and then heated to 60 °C to let it react for 14 h. The resulting suspension was filtered and the residue was concentrated under reduced pressure. The crude material was then purified by flash column chromatography (hexane/EtOAc 4:1) to afford 180 mg of a colorless oil, which was subsequently taken up in DMF (10.0 mL). Next, NaN_3 was added (0.150 g, 2.35 mmol) and the reaction mixture was stirred 16 h at 80 °C. TLC analysis (hexane/EtOAc 3:1) showed the presence of one major product. The solvent was evaporated and the residue was taken up in EtOAc. The resulting solution was washed with saturated sodium bicarbonate solution and water. The organic layer was dried over sodium sulphate, filtered and concentrated in vacuo. This material was purified by flash column chromatography (hexane/EtOAc 9:1) to afford **9** as a pale oil (29% over two steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.46 (s, 3H), 1.51 (s, 3H), 3.42–3.61 (m, 5H), 3.66–3.73 (m, 1H), 3.98–4.04 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.47 (2 C), 49.7, 54.2, 76.0, 81.2, 83.8, 92.3, 114.5.

5.1.10. *N*,*N*′-(((2*S*,3*R*,4*S*)-3,4-Dihydroxytetrahydrofuran-2,4-diyl)bis(methylene))dibenzamide (11)

Bisazide **9** was subjected to general procedure 1, followed by general procedure 2. Then general procedure 4, but purification via HPLC in this case: the acidic solution was concentrated and the residue was purified by HPLC (H_2O/MeCN 9:1 to 0:1) to yield homobisbenzamide **11** as a white foam (74% from **9**). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.33–3.69 (m, 6H), 3.96 (app. t, J = 5.0 Hz, 1H), 4.13 (app. dt, J = 7.4, 4.8 Hz, 1H), 4.99 (s, 1H), 5.24 (d, J = 5.0 Hz, 1H), 7.41–7.57 (m, 6H), 7.82–7.89 (m, 4H), 8.40–8.50 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.50 (2 C), 72.4, 73.2, 79.1, 79.6, 127.2, 127.3, 128.23 (2 C), 131.1, 131.2, 134.3, 134.5, 166.3, 167.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{23}N_2O_5^+$ 371.16015; Found 371.1588.

5.1.11. *N*-(((3a*S*,6*R*,6a*R*)-6-(Azidomethyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (12)

See reference 10. Starting from phthalimide **24** (3.10 g, 8.70 mmol), compound **12** was obtained in 86% yield over two steps. In subsequent reactions, a known amount of benzamide **12** (±0.14 g, 0.42 mmol) was used. Spectroscopy data for **12** are consistent with those published previously.

5.1.12. *N*-(((3a*S*,6*R*,6a*R*)-6-(Aminomethyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (13)

See Ref. 10. The crude amine **13** was obtained via general procedure 1 and used without further purification in subsequent reactions.

5.1.13. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-(methylsulfonamidomethyl)dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (14a)

General procedure 3. White foam, 77% ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.37 (s, 3H), 1.51 (s, 3H), 2.96 (s, 3H), 3.25–3.40 (m, 2H), 3.74 (dd, J = 14.4, 6.2 Hz, 1H), 3.82–3.94 (m, 3H), 4.19 (app. td, J = 5.6, 1.6 Hz, 1H), 4.45 (d, J = 1.8 Hz, 1H), 5.96 (t, J = 6.3 Hz, 1H), 7.02 (t, J = 6.3 Hz, 1H), 7.40–7.54 (m, 3H), 7.77–7.84 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.9, 28.1, 40.7, 43.1, 43.7, 75.4, 84.1, 84.6, 92.4, 114.1, 127.3, 128.9, 132.2, 133.8, 168.5. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd for $C_{17}H_{23}N_2O_6S^-$ 383.12823; Found 383.1270.

5.1.14. *N*-(((3a*S*,6*R*,6a*R*)-6-(Ethylsulfonamidomethyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (14b)

General procedure 3. White foam, 78% ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.32 (t, J = 7.3 Hz, 3H), 1.37 (s, 3H), 1.51 (s, 3H), 3.03 (q, J = 7.3 Hz, 2H), 3.20–3.35 (m, 2H), 3.74 (dd, J = 14.4, 6.2 Hz, 1H), 3.80–3.96 (m, 3H), 4.17 (app. td, J = 5.8, 1.3 Hz, 1H), 4.48 (d, J = 1.5 Hz, 1H), 5.89 (t, J = 6.3 Hz, 1H), 7.15 (t, J = 6.3 Hz, 1H), 7.39–7.53 (m, 3H), 7.77–7.86 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 8.2, 27.6, 27.8, 42.9, 43.4, 47.1, 75.1, 84.0, 84.4, 92.1, 113.7, 127.1, 128.6, 131.9, 133.6, 168.2. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd for $C_{18}H_{25}N_2O_6S^-$ 397.14388; Found 397.1423.

5.1.15. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-(((1-methylethyl) sulfonamido)methyl)dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl) benzamide (14c)

General procedure 3. White foam, 36% ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.28–1.42 (m, 9H), 1.52 (s, 3H), 3.18 (sep, J = 6.9 Hz, 1H), 3.25–3.41 (m, 2H), 3.74 (dd, J = 14.5, 6.0 Hz, 1H), 3.84–3.96 (m, 3H), 4.18 (app. td, J = 5.7, 1.5 Hz, 1H), 4.46 (d, J = 1.5 Hz, 1H), 5.52 (dd, J = 7.3, 5.3 Hz, 1H), 6.93 (t, J = 6.3 Hz, 1H), 7.41–7.55 (m,

3H), 7.77–7.86 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 16.66, 16.71, 27.7, 27.9, 43.0, 44.0, 53.8, 75.3, 84.2, 84.4, 92.3, 113.8, 127.1, 128.8, 132.0, 133.7, 168.2. HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{19}H_{27}N_2O_6S^-$ 411.15953; Found 411.1585.

5.1.16. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-(((trifluoromethyl) sulfonamido)methyl)dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (14d)

General procedure 3. White foam, 16% ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.33 (s, 3H), 1.54 (s, 3H), 3.43–3.57 (m, 2H), 3.60–3.70 (m, 1H), 3.90 (q, *J* = 9.7 Hz, 2H), 4.10 (dd, *J* = 14.9, 7.9 Hz, 1H), 4.24 (app. td, *J* = 4.3, 1.8 Hz, 1H), 4.36 (d, *J* = 1.8 Hz, 1H), 6.84 (t, *J* = 6.3 Hz, 1H), 7.44–7.59 (m, 3H), 7.76–7.85 (m, 2H), 7.92 (br s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm −77.9 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.5, 28.3, 42.6, 45.2, 75.7, 82.9, 83.7, 92.3, 114.2, 120.0 (q, *J* = 321.2 Hz), 127.2, 129.1, 132.6, 133.1, 169.1. HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{17}H_{20}F_3N_2O_6S^-$ 437.09997; Found 437.0984.

5.1.17. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-(phenylsulfonamidomethyl)dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (14e)

General procedure 3. White foam, 60% ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.35 (s, 3H), 1.48 (s, 3H), 3.07 (app. dt, *J* = 13.6, 5.6 Hz, 1H), 3.21 (ddd, *J* = 13.6, 8.0, 5.4 Hz, 1H), 3.65–3.88 (m, 4H), 4.13 (app. td, *J* = 5.6, 1.5 Hz, 1H), 4.44 (d, *J* = 1.8 Hz, 1H), 6.11 (dd, *J* = 7.8, 5.1 Hz, 1H), 6.96 (t, *J* = 6.2 Hz, 1H), 7.34–7.59 (m, 6H), 7.72–7.93 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.7, 27.9, 43.0, 43.6, 75.3, 83.6, 84.5, 92.2, 113.8, 127.0, 127.1, 128.8, 129.2, 132.0, 132.7, 133.7, 140.1, 168.3. HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{22}H_{25}N_2O_6S^-$ 445.14388; Found 445.1421.

5.1.18. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-(((4-methylphenyl) sulfonamido)methyl)dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl) methyl)benzamide (14f)

General procedure 3. White powder, 86% ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.34 (s, 3H), 1.47 (s, 3H), 2.38 (s, 3H), 2.98–3.21 (m, 2H), 3.67–3.85 (m, 4H), 4.12 (app. td, *J* = 5.9, 1.3 Hz, 1H), 4.47 (d, *J* = 1.5 Hz, 1H), 6.10 (dd, *J* = 7.6, 5.3 Hz, 1H), 7.09 (t, *J* = 6.2 Hz, 1H), 7.23 (d, *J* = 8.2 Hz, 2H), 7.35–7.43 (m, 2H), 7.45–7.52 (m, 1H), 7.70 (d, *J* = 8.2 Hz, 2H), 7.76–7.83 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 21.6, 27.8, 27.9, 43.2, 43.7, 75.4, 83.6, 84.6, 92.3, 113.9, 127.14 (2 C), 128.8, 129.9, 132.0, 133.8, 137.1, 143.5, 168.2. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{23}H_{29}N_2O_6S^+$ 461.17408; Found 461.1738.

5.1.19. *N*-(((3a*S*,6*R*,6a*R*)-6-(((4-Chlorophenyl)sulfonamido) methyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (14g)

General procedure 3. White foam, 91% ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.35 (s, 3H), 1.48 (s, 3H), 3.09 (app. dt, *J* = 13.7, 5.5 Hz, 1H), 3.22 (ddd, *J* = 13.6, 8.0, 5.4 Hz, 1H), 3.69 (dd, *J* = 14.4, 6.2 Hz, 1H), 3.75–3.90 (m, 3H), 4.13 (app. td, *J* = 5.5, 1.6 Hz, 1H), 4.42 (d, *J* = 1.8 Hz, 1H), 6.37 (dd, *J* = 7.8, 5.1 Hz, 1H), 6.95 (t, *J* = 6.2 Hz, 1H), 7.36–7.56 (m, 5H), 7.71–7.86 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.7, 27.9, 43.0, 43.7, 75.3, 83.5, 84.4, 92.2, 113.9, 127.1, 128.5, 128.8, 129.4, 132.1, 133.6, 138.8, 139.0, 168.4. HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{22}H_{24}ClN_2O_6S^-$ 479.10491; Found 479.1047.

5.1.20. *N*-(((3a*S*,6*R*,6a*R*)-6-(((2-Chlorophenyl)sulfonamido) methyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (14h)

General procedure 3. White foam, 76% ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.34 (s, 3H), 1.46 (s, 3H), 3.04 (ddd, *J* = 13.7, 7.0, 5.1 Hz, 1H), 3.17 (ddd, *J* = 13.6, 7.8, 5.6 Hz, 1H), 3.68 (dd, *J* = 14.4, 6.2 Hz, 1H), 3.75–3.89 (m, 3H), 4.12 (app. t, *J* = 6.3 Hz, 1H), 4.45 (d, *J* = 1.5 Hz, 1H), 6.23 (dd, *J* = 7.6, 5.0 Hz, 1H), 7.04 (t, *J* = 6.2 Hz, 1H), 7.30–7.53 (m, 6H), 7.73–7.83 (m, 2H), 7.96–8.03 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.6, 27.8, 43.0, 43.3, 75.1, 83.3, 84.4, 92.1, 113.7, 127.0, 127.2, 128.6, 131.0, 131.4, 131.6, 131.8, 133.6, 133.8, 137.1, 168.1. HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{22}H_{24}ClN_2O_6S^-$ 479.10491; Found 479.1055.

5.1.21. *N*-(((3a*S*,6*R*,6a*R*)-6-(([1,1′-Biphenyl]-4-sulfonamido) methyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (14i)

General procedure 3. White foam, 67% ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.35 (s, 3H), 1.48 (s, 3H), 3.13 (dt, *J* = 13.8, 5.6 Hz, 1H), 3.25 (ddd, *J* = 13.6, 7.8, 5.3 Hz, 1H), 3.73 (dd, *J* = 14.4, 6.2 Hz, 1H), 3.78–3.91 (m, 3H), 4.16 (app. td, *J* = 5.6, 1.5 Hz, 1H), 4.47 (d, *J* = 1.8 Hz, 1H), 6.22 (dd, *J* = 7.3, 5.3 Hz, 1H), 6.98 (t, *J* = 6.3 Hz, 1H), 7.31–7.52 (m, 6H), 7.52–7.60 (m, 2H), 7.61–7.71 (m, 2H), 7.75–7.85 (m, 2H), 7.85–7.96 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.7, 27.9, 43.1, 43.7, 75.3, 83.6, 84.5, 92.2, 113.8, 127.1, 127.4, 127.6, 127.8, 128.5, 128.7, 129.1, 132.0, 133.7, 138.7, 139.4, 145.5, 168.3. HRMS (ESI-TOF) *m/z*: $[M-H]^-$ Calcd for $C_{28}H_{29}N_2O_6S^-$ 521.17518; Found 521.1768.

5.1.22. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-((3-phenylureido) methyl) dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl) benzamide (15a)

A flask containing a solution of the crude amine **13** (originating from 0.540 mmol of azide **12** via general procedure 1) in 5 mL of pyridine was purged with nitrogen gas and treated with phenylisocyanate (65.0 μL, 0.590 mmol). After 3 h, TLC (CH_2Cl_2/MeOH 97:3) showed no starting material. Evaporation of pyridine and purification of the residue via flash column chromatography (CH_2Cl_2/MeOH 98:2) afforded the final compound in 76% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.32 (s, 3H), 1.49 (s, 3H), 3.14 (app. dt, *J* = 14.2, 4.5 Hz, 1H), 3.62 (app. dt, *J* = 14.3, 7.1 Hz, 1H), 3.74 (dd, *J* = 14.4, 5.6 Hz, 1H), 3.81 (app. s, 2H), 3.93 (dd, *J* = 14.4, 7.3 Hz, 1H), 4.11 (app. t, *J* = 5.13 Hz, 1H), 4.51 (d, *J* = 1.2 Hz, 1H), 6.39 (dd, *J* = 7.2, 4.83 Hz, 1H), 6.96 (app. t, *J* = 7.3 Hz, 1H), 7.21 (app. t, *J* = 7.9 Hz, 2H), 7.30–7.44 (m, 4H), 7.46–7.54 (m, 1H), 7.78 (t, *J* = 6.3 Hz, 1H), 7.85–7.95 (m, 2H), 8.06 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.4, 27.9, 40.1, 42.6, 75.0, 84.0, 84.4, 92.0, 113.5, 119.6, 122.6, 127.3, 128.7, 128.9, 132.0, 133.5, 139.3, 156.8, 168.6. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{23}H_{28}N_3O_5^+$ 426.20235; Found 426.2041.

5.1.23. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-((3-phenylthioureido) methyl)dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (15b)

A flask containing a solution of the crude amine **13** (originating from 0.560 mmol of azide **12** via general procedure 1) in 5 mL of pyridine was purged with nitrogen gas and treated with phenylisothiocyanate (74.0 mL, 0.620 mmol). After 3 h, TLC (CH_2Cl_2/MeOH 97:3) showed no starting material. Evaporation of pyridine and purification of the residue via flash column chromatography (CH_2Cl_2/MeOH 95:5) afforded the final compound in 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.37 (s, 3H), 1.48 (s, 3H), 3.63–3.80 (m, 3H), 3.83 (app. s, 2H), 3.97–4.07 (m, 1H), 4.30 (app. t, *J* = 6.0 Hz, 1H), 4.48 (d, *J* = 1.8 Hz, 1H), 6.84 (br s, 1H), 7.09 (br s, 1H), 7.16–7.53 (m, 8H), 7.70–7.83 (m, 2H), 8.61 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.7, 27.8, 43.1, 45.1, 75.0, 83.3, 84.4, 92.1, 113.8, 124.8, 126.6, 127.1, 128.6, 129.7, 131.9, 133.5, 136.9, 168.2, 181.2. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{23}H_{28}N_3O_4S^+$ 442.17950; Found 442.1802.

5.1.24. *N*-(((3a*S*,6*R*,6a*R*)-6-((Benzylamino)methyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (16)

A flask containing a solution of crude amine **13** (originating from 0.600 mmol of azide **12** via general procedure 1) in MeOH (9 mL) was purged with nitrogen gas. Molecular sieves (3 Å, rods) were added and the flask was flushed again. Benzaldehyde (0.180 mL, 1.80 mmol) was added and the whole was stirred for 4 h at RT. After that, the resulting aldimine was carefully treated with $NaBH_4$ (0.110 g, 3.00 mmol) for 30 min. The RM was filtered and the filtrate was adsorbed onto celite. The product was purified by column chromatography (toluene/EtOAc 100:0 → 60:40) and appeared as a pale yellow oil (47%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.41 (s, 3H), 1.54 (s, 3H), 2.04 (br s, 1H), 2.71–2.79 (m, 2H), 3.71–3.92 (m, 6H), 4.20 (app. td, J = 6.1, 1.9 Hz, 1H), 4.36 (d, J = 2.1 Hz, 1H), 6.76 (t, J = 5.7 Hz, 1H), 7.19–7.35 (m, 5H), 7.39–7.54 (m, 3H), 7.75–7.81 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.9, 28.0, 43.9, 49.4, 53.8, 75.3, 84.9, 86.2, 91.9, 113.9, 127.0, 127.1, 128.2, 128.5, 128.7, 131.8, 134.2, 139.9, 167.8. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{29}N_2O_4^+$ 397.21218; Found 397.2119.

5.1.25. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (17)

To a solution of compound **12** (0.190 g, 0.560 mmol) in 5.5 mL of a mixture of DMF/H_2O/TEA (4:1:0.5) were added a catalytic amount of Cu(I)I and TBTA, along with phenylacetylene (0.180 mL, 1.68 mmol). The reaction mixture was stirred for 4 h at 75 °C, after which time TLC (toluene/EtOAc 1:1) showed no more starting material. The reaction mixture was concentrated under reduced pressure. The residue was taken up in ethyl acetate and washed with 0.1 M aq HCl and sat. aq $NHCO_3$ solution. The organic layer was dried over sodium sulphate, filtered and concentrated. Purification by flash column chromatography (toluene/EtOAc 3:2) afforded the triazole **17** in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.36 (s, 3H), 1.50 (s, 3H), 3.67–3.80 (2× dd, J = 14.3, 6.6 Hz, J = 14.3, 6.2 Hz, 2H), 3.87–3.97 (2× d, J = 10.3 Hz, 2H), 4.46–4.69 (m, 4H), 6.90 (t, J = 6.2 Hz, 1H), 7.27–7.55 (m, 6H), 7.75–7.88 (m, 4H), 7.99 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.8, 27.9, 43.0, 50.7, 75.3, 83.5, 84.3, 92.1, 114.3, 121.3, 125.9, 127.1, 128.2, 128.8, 128.9, 130.6, 132.0, 133.7, 148.0, 168.0. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{27}N_4O_4^+$ 435.20268; Found 435.2034.

5.1.26. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-((5-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (18)

To a solution of compound **12** (0.170 g, 0.510 mmol) in 1,4-dioxane (5 mL) was added phenylacetylene (56.0 µL, 0.510 mmol). The flask was purged with nitrogen gas, sealed and heated to 60 °C. A catalytic amount of $CpRuCl(PPh_3)_2$ was dissolved in 500 µL of 1,4-dioxane and added to the reaction mixture, which was further stirred for 24 h at 60 °C. The reaction mixture was concentrated under reduced pressure and adsorbed onto celite. Purification by flash column chromatography afforded triazole **18** in 83% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.37 (s, 3H), 1.46 (s, 3H), 3.51–3.70 (m, 3H), 3.78 (d, J = 10.5 Hz, 1H), 4.38–4.57 (m, 3H), 4.66 (d, J = 1.5 Hz, 1H), 7.02 (t, J = 6.2 Hz, 1H), 7.34–7.55 (m, 8H), 7.69 (s, 1H), 7.78–7.89 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.74, 27.78, 42.9, 48.0, 74.9, 83.7, 84.7, 92.1, 113.9, 126.8, 127.09, 127.10, 128.7, 129.1, 129.2, 129.6, 131.8, 133.9, 139.0, 167.8. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{27}N_4O_4^+$ 435.20268; Found 435.2037.

5.1.27. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-(methylsulfonamidomethyl)tetrahydrofuran-3-yl)methyl)benzamide (19a)

Compound **14a** was subjected to general procedure 4. White foam, 81% ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.89 (s, 3H), 3.03 (app. dt, J = 13.4, 6.6 Hz, 1H), 3.24 (ddd, J = 13.7, 5.8, 3.1 Hz, 1H), 3.42 (m, 2H), 3.55–3.71 (m, 3H), 3.87 (d, J = 9.7 Hz, 1H), 4.81 (s, 1H), 5.01 (d, J = 6.7 Hz, 1H), 7.05 (t, J = 6.2 Hz, 1H), 7.43–7.57 (m, 3H), 7.82–7.88 (m, 2H), 8.35 (t, J = 5.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 39.6, 44.2, 44.9, 73.9, 74.8, 77.9, 81.1, 127.3, 128.2, 131.2, 134.4, 167.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{21}N_2O_6S^+$ 345.11148; Found 345.1107.

5.1.28. *N*-(((3*S*,4*R*,5*R*)-5-(Ethylsulfonamidomethyl)-3,4-dihydroxytetrahydrofuran-3-yl)methyl)benzamide (19b)

Compound **14b** was subjected to general procedure 4. White foam, 78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.17 (t, J = 7.3 Hz, 3H), 2.92–3.08 (m, 3H), 3.23 (ddd, J = 13.8, 5.8, 2.9 Hz, 1H), 3.35–3.48 (m, 2H), 3.53–3.70 (m, 3H), 3.87 (d, J = 9.7 Hz, 1H), 4.80 (s, 1H), 4.99 (d, J = 6.4 Hz, 1H), 7.10 (t, J = 5.9 Hz, 1H), 7.42–7.58 (m, 3H), 7.80–7.89 (m, 2H), 8.35 (t, J = 5.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 8.1, 44.2, 44.7, 45.7, 74.0, 74.8, 78.0, 81.2, 127.3, 128.2, 131.2, 134.4, 167.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{15}H_{23}N_2O_6S^+$ 359.12713; Found 359.1285.

5.1.29. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-(((1-methylethyl)sulfonamido)methyl)tetrahydrofuran-3-yl)methyl)benzamide (19c)

Compound **14c** was subjected to general procedure 4. White foam, 87%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.19 (d, J = 2.1 Hz, 3H), 1.21 (d, J = 2.1, 3H), 3.03 (app. dt, J = 13.6, 6.6 Hz, 1H), 3.12–3.30 (m, 2H), 3.35–3.48 (m, 2H), 3.54–3.69 (m, 3H), 3.87 (d, J = 9.4 Hz, 1H), 4.80 (s, 1H), 4.98 (d, J = 6.7 Hz, 1H), 7.08 (t, J = 5.9 Hz, 1H), 7.43–7.57 (m, 3H), 7.82–7.88 (m, 2H), 8.34 (t, J = 6.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 16.3 (2 C), 44.2, 45.0, 51.6, 74.0, 74.8, 78.0, 81.4, 127.3, 128.2, 131.2, 134.4, 167.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{25}N_2O_6S^+$ 373.14278; Found 373.1445.

5.1.30. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-(((trifluoromethyl)sulfonamido)methyl)tetrahydrofuran-3-yl)methyl)benzamide (19d)

Compound **14d** was subjected to general procedure 4. White foam, 84%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.17–3.25 (m, 1H), 3.36–3.48 (m, 3H), 3.56–3.64 (m, 2H), 3.67–3.74 (m, 1H), 3.89 (d, J = 9.7 Hz, 1H), 4.85 (s, 1H), 5.09 (d, J = 6.6 Hz, 1H), 7.43–7.57 (m, 3H), 7.81–7.88 (m, 2H), 8.36 (t, J = 6.0 Hz, 1H), 9.56 (br. s, 1H). ^{19}F NMR (282 MHz, DMSO-d_6) δ ppm −77.6 (s). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.1, 45.9, 73.9, 74.9, 78.0, 80.5, 119.7 (q, J = 322.7 Hz), 127.3, 128.2, 131.2, 134.4, 167.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{14}H_{18}F_3N_2O_6S^+$ 399.08322; Found 399.0831.

5.1.31. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-(phenylsulfonamidomethyl)tetrahydrofuran-3-yl)methyl)benzamide (19e)

Compound **14e** was subjected to general procedure 4. White foam, 93%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.82 (app. dt, J = 13.3, 6.7 Hz, 1H), 3.00 (ddd, J = 13.0, 5.7, 2.6 Hz, 1H), 3.33–3.44 (m, 2H), 3.48–3.65 (m, 3H), 3.80 (d, J = 9.7 Hz, 1H), 4.76 (s, 1H), 4.99 (d, J = 6.7 Hz, 1H), 7.36–7.68 (m, 6H), 7.72 (t, J = 6.0 Hz, 1H), 7.75–7.90 (m, 4H), 8.31 (t, J = 5.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.3, 45.1, 74.1, 74.8, 77.9, 80.7, 126.5, 127.3, 128.3, 129.1, 131.2, 132.3, 134.4, 140.5, 167.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{23}N_2O_6S^+$ 407.12713; Found 407.1266.

5.1.32. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-(((4-methylphenyl)sulfonamido)methyl)tetrahydrofuran-3-yl)methyl)benzamide (19f)

Compound **14f** was subjected to general procedure 4. White foam, 78%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.38 (s, 3H), 2.80 (app. dt, J = 13.0, 6.4 Hz, 1H), 2.93–3.02 (m, 1H), 3.35–3.47 (m, 2H), 3.50–3.66 (m, 3H), 3.82 (d, J = 9.7 Hz, 1H), 4.77 (s, 1H), 5.00 (d, J = 6.4 Hz, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.43–7.57 (m, 3H), 7.63 (t, J = 5.6 Hz, 1H), 7.69 (d, J = 8.2 Hz, 2H), 7.80–7.88 (m, 2H), 8.32 (t, J = 5.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 21.0, 44.3, 45.1, 74.1, 74.8, 77.9, 80.7, 126.6, 127.3, 128.2, 129.5, 131.2, 134.4, 137.6, 142.5, 167.1. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd for $C_{20}H_{23}N_2O_6S^-$ 419.12823; Found 419.1286.

5.1.33. *N*-(((3*S*,4*R*,5*R*)-5-(((4-Chlorophenyl)sulfonamido)methyl)-3,4-dihydroxytetrahydrofuran-3-yl)methyl)benzamide (19g)

Compound **14g** was subjected to general procedure 4. White foam, 67%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.83 (app. dd, J = 13.0, 6.6 Hz, 1H), 3.00–3.04 (m, 1H), 3.33–3.46 (m, 2H), 3.47–3.66 (m, 3H), 3.79 (d, J = 9.7 Hz, 1H), 4.77 (s, 1H), 4.99 (d, J = 5.3 Hz, 1H), 7.40–7.49 (m, 2H), 7.50–7.56 (m, 1H), 7.61–7.71 (m, 2H), 7.74–7.91 (m, 5H), 8.31 (t, J = 5.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.3, 45.1, 74.0, 74.8, 77.9, 80.6, 127.3, 128.2, 128.5, 129.2, 131.2, 134.4, 137.1, 139.5, 167.1. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{19}H_{22}ClN_2O_6S^+$ 441.08816; Found 441.0902.

5.1.34. *N*-(((3*S*,4*R*,5*R*)-5-(((2-Chlorophenyl)sulfonamido)methyl)-3,4-dihydroxytetrahydrofuran-3-yl)methyl)benzamide (19h)

Compound **14h** was subjected to general procedure 4. White foam, 65%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.97 (app. dt, J = 13.6, 6.6 Hz, 1H), 3.17 (ddd, J = 13.8, 5.6, 2.9 Hz, 1H), 3.31–3.49 (m, 3H), 3.54 (d, J = 7.9 Hz, 1H), 3.62 (app. td, J = 7.5, 2.9 Hz, 1H), 3.72 (d, J = 9.7 Hz, 1H), 4.81 (br s, 2H), 7.40–7.67 (m, 6H), 7.80–7.88 (m, 2H), 7.89–7.99 (m, 2H), 8.29 (t, J = 6.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.3, 45.0, 74.1, 74.8, 77.9, 80.6, 127.3, 127.5, 128.2, 130.2, 130.6, 131.2, 131.6, 133.7, 134.4, 138.3, 167.0. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd for $C_{19}H_{20}ClN_2O_6S^-$ 439.07361; Found 439.0734.

5.1.35. *N*-(((3*S*,4*R*,5*R*)-5-(([1,1′-Biphenyl]-4-sulfonamido)methyl)-3,4-dihydroxytetrahydrofuran-3-yl)methyl)benzamide (19i)

Compound **14i** was subjected to general procedure 4. White foam, 63%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.86 (app. dt, J = 13.2, 6.6 Hz, 1H), 3.05 (ddd, J = 13.2, 5.6, 2.9 Hz, 1H), 3.39 (m, 2H), 3.50–3.69 (m, 3H), 3.82 (d, J = 9.7 Hz, 1H), 4.77 (s, 1H), 5.01 (d, J = 6.7 Hz, 1H), 7.38–7.57 (m, 6H), 7.69–7.90 (m, 9H), 8.32 (t, J = 5.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 45.0, 45.8, 74.7, 75.5, 78.6, 81.4, 127.8, 127.9, 128.01 (2 C), 128.9, 129.1, 129.8, 131.9, 135.1, 139.3, 140.0, 144.5, 167.8. HRMS (ESI-TOF) m/z: $[M-H]^-$ Calcd for $C_{25}H_{25}N_2O_6S^-$ 481.14388; Found 481.1446.

5.1.36. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-((3-phenylureido)methyl)-tetrahydrofuran-3-yl)methyl)benzamide (20a)

Compound **15a** was subjected to general procedure 4. White foam, quant. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.19 (m, 1H), 3.35–3.50 (m, 3H), 3.53–3.63 (m, 2H), 3.63–3.70 (m, 1H), 3.89 (d, J = 9.4 Hz, 1H), 4.79 (s, 1H), 5.00 (d, J = 6.7 Hz, 1H), 6.17 (t, J = 5.4 Hz, 1H), 6.88 (app. t, J = 7.3 Hz, 1H), 7.21 (app. t, J = 7.9 Hz, 2H), 7.30–7.58 (m, 5H), 7.77–7.91 (m, 2H), 8.37 (t, J = 5.9 Hz, 1H), 8.50 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 41.2, 44.2, 73.9, 74.8, 78.2, 81.2, 117.5, 121.0, 127.3, 128.3, 128.7, 131.2, 134.5, 140.5, 155.2, 167.2. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{24}N_3O_5^+$ 386.17105; Found 386.1708.

5.1.37. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-((3-phenylthioureido)methyl)tetrahydrofuran-3-yl)methyl)benzamide (20b)

Compound **15b** was subjected to general procedure 4. White foam, 86%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.40–3.65 (m, 5H), 3.76–3.93 (m, 3H), 4.86 (br s, 2H), 7.04–7.13 (m, 1H), 7.30 (app. t, J = 7.8 Hz, 2H), 7.39–7.57 (m, 5H), 7.65 (br s, 1H), 7.80–7.91 (m, 2H), 8.39 (t, J = 6.0 Hz, 1H), 9.60 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.2, 46.2, 74.3, 74.9, 78.2, 80.2, 122.9, 124.1, 127.4, 128.3, 128.5, 131.3, 134.4, 139.4, 167.2, 180.7. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{24}N_3O_4S^+$ 402.14820; Found 402.1469.

5.1.38. *N*-(((3*S*,4*R*,5*R*)-5-((Benzylamino)methyl)-3,4-dihydroxytetrahydrofuran-3-yl)methyl)benzamide (21)

Compound **16** was subjected to general procedure 4. White foam, 84%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.08 (br s, 1H), 2.57 (dd, J = 12.2, 6.3 Hz, 1H), 2.73 (dd, J = 12.2, 3.1 Hz, 1H), 3.36–3.47 (m, 2H), 3.54 (d, J = 9.4 Hz, 1H), 3.59–3.74 (m, 4H), 3.85 (d, J = 9.4 Hz, 1H), 4.74 (s, 1H), 4.90 (br s, 1H), 7.17–7.33 (m, 5H), 7.42–7.57 (m, 3H), 7.81–7.88 (m, 2H), 8.35 (t, J = 6.0 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.4, 50.8, 53.1, 74.4, 74.8, 77.9, 81.8, 126.5, 127.3, 127.9, 128.1, 128.2, 131.2, 134.4, 140.8, 167.0. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{20}H_{25}N_2O_4^+$ 357.18088; Found 357.1804.

5.1.39. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-((4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-3-yl)methyl)benzamide (22)

Compound **17** was subjected to general procedure 4. White foam, 83%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.36 (dd, J = 13.6, 6.0 Hz, 1H), 3.44 (dd, J = 13.5, 6.2 Hz, 1H), 3.59 (d, J = 9.7 Hz, 1H), 3.65 (d, J = 8.2 Hz, 1H), 3.84 (d, J = 9.7 Hz, 1H), 4.02 (app. td, J = 7.7, 3.2 Hz, 1H), 4.52 (dd, J = 14.2, 7.5 Hz, 1H), 4.67 (dd, J = 14.2, 3.1 Hz, 1H), 7.29–7.54 (m, 6H), 7.77–7.86 (m, 4H), 8.37 (t, J = 6.0 Hz, 1H), 8.48 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 43.6, 51.9, 73.8, 74.9, 78.0, 80.2, 122.1, 125.2, 127.3, 127.8, 128.2, 128.9, 130.8, 131.2, 134.4, 146.3, 167.2. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{21}H_{23}N_4O_4^+$ 395.17138; Found 395.1730.

5.1.40. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-((5-phenyl-1*H*-1,2,3-triazol-1-yl)methyl)tetrahydrofuran-3-yl)methyl)benzamide (23)

Compound **18** was subjected to general procedure 4. Light brown colored foam, 91%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.32–3.46 (m, 2H), 3.50 (d, J = 9.7 Hz, 1H), 3.69–3.82 (m, 2H), 4.05 (app. td, J = 8.2, 2.9 Hz, 1H), 4.42 (dd, J = 14.4, 8.2 Hz, 1H), 4.64 (dd, J = 14.4, 2.9 Hz, 1H), 4.88 (br s, 1H), 5.18 (br s, 1H), 7.41–7.64 (m, 8H), 7.78–7.87 (m, 3H), 8.34 (t, J = 5.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.4, 50.8, 75.0, 75.2, 78.6, 81.0, 127.1, 127.8, 129.1, 129.4, 129.7, 130.0, 132.2, 133.2, 134.6, 139.1, 168.4. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{21}H_{23}N_4O_4^+$ 395.17138; Found 395.1724.

5.1.41. 2-(((3a*S*,6*R*,6a*R*)-6-(Azidomethyl)-2,2-dimethyldihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)isoindoline-1,3-dione (24)

See Ref. 10. Spectroscopy data for **24** are consistent with those published previously.

5.1.42. *N*-(((3a*R*,4*R*,6a*S*)-6a-((1,3-Dioxoisoindolin-2-yl)methyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)benzamide (25)

To a solution of phthalimide **24** (1.09 g, 3.04 mmol) in THF (30 mL) was added benzoyl chloride (0.710 mL, 6.08 mmol), fol-

lowed by PMe_3 (1 M solution in THF, 12.2 mL). Flocculation was observed upon addition, after which the reaction mixture turned yellow. The RM was stirred 16 h at rt. TLC analysis (toluene/EtOAc 1:1) showed complete consumption of starting material. The RM was concentrated in vacuo and adsorbed onto celite. Purification via FCC (toluene/EtOAc 1:0 → 1:1) afforded compound **25** as a white foam in 45% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.29 (s, 3H), 1.49 (s, 3H), 3.46 (ddd, J = 14.0, 8.7, 4.3 Hz, 1H), 3.85 (ddd, J = 14.0, 7.3, 5.1 Hz, 1H), 3.95 (d, J = 10.5 Hz, 1H), 3.98–4.06 (m, 3H), 4.27 (ddd, J = 8.6, 5.1, 1.8 Hz, 1H), 4.59 (d, J = 1.8 Hz, 1H), 6.59 (dd, J = 6.4, 4.4 Hz, 1H), 7.39–7.53 (m, 3H), 7.71–7.92 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.7, 27.9, 39.9, 41.7, 75.2, 83.9, 86.0, 91.8, 114.6, 123.7, 127.2, 128.7, 131.7, 131.9, 134.3, 134.5, 167.7, 168.5. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{25}N_2O_6^+$ 437.17071, found 437.1712.

5.1.43. *N*-(((3a*R*,4*R*,6a*S*)-6a-(Aminomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)benzamide (26)

To a solution of phthalimide **25** (0.520 g, 1.19 mmol) in EtOH (15 mL) was added hydrazine monohydrate (0.115 mL, 2.38 mmol). The RM was heated to 60 °C for 5 h. When the reaction was complete (TLC toluene/EtOAc 1:1 and CH_2Cl_2/MeOH 9:1), the RM was concentrated in vacuo and the residue adsorbed onto celite. Purification via FCC (CH_2Cl_2/MeOH/NH_4OH 100:0:1 → 85:15:1) afforded compound **26** as a transparent oil (96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.42 (s, 3H), 1.51–1.70 (m, 5H), 2.98 (s, 2H), 3.46 (ddd, J = 14.1, 7.9, 4.4 Hz, 1H), 3.76 (ddd, J = 14.1, 6.4, 5.3 Hz, 1H), 3.90 (d, J = 10.0 Hz, 1H), 3.95 (d, J = 10.3 Hz, 1H), 4.26 (ddd, J = 7.7, 5.5, 1.8 Hz, 1H), 4.39 (d, J = 2.1 Hz, 1H), 6.67 (br s, 1H), 7.39–7.54 (m, 3H), 7.75–7.81 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 28.0, 28.1, 40.3, 46.6, 75.5, 84.2, 85.4, 93.3, 113.9, 127.1, 128.7, 131.8, 134.4, 167.9. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{23}N_2O_4^+$ 307.16523, found 307.1658.

5.1.44. *N*-(((3a*R*,4*R*,*6aS*)-6a-(Azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)benzamide (27)

5.1.44.1. Preparation of triflyl azide (TfN_3). NaN_3 (0.680 g, 10.5 mmol) was dissolved in water (8 mL) and cooled to 0 °C. After addition of CH_2Cl_2 (8 mL) the mixture was stirred for 15 min. Tf_2O (0.352 mL, 2.09 mmol) was added dropwise over 1 min. The RM was stirred vigorously at 0 °C and then extracted with DCM (2 × 3 mL) and washed with aqueous saturated Na_2CO_3 solution (2 × 3 mL). The organic layer was saved for the diazo transfer reaction.

5.1.44.2. Diazo transfer reaction. Amine **26** (0.32 g, 1.05 mmol) was dissolved in MeOH (8 mL), giving a clear solution. Et_3N (0.291 mL, 2.09 mmol) was added. A freshly prepared solution of $CuSO_4$ (1.64 mg, 1 mol %) in water (3 mL) was added. The clear solution became turbid. The above-prepared TfN_3 solution was added and the RM was stirred vigorously for 18 h at rt. TLC analysis (toluene/EtOAc 6:4 and CH_2Cl_2/MeOH 9:1) showed complete consumption of amine and presence of 1 large higher-running spot. The RM was concentrated in vacuo and adsorbed onto celite. Purification via FCC (toluene/EtOAc 10:0 → 6:4) gave the title compound as a colourless oil in 90% yield.

^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.45 (s, 3H), 1.53 (s, 3H), 3.37–3.51 (m, 2H), 3.56 (d, J = 12.9 Hz, 1H), 3.71 (ddd, J = 14.0, 6.7, 5.4 Hz, 1H), 3.85 (d, J = 10.3 Hz, 1H), 3.93 (d, J = 10.3 Hz, 1H), 4.23 (ddd, J = 7.7, 5.5, 1.8 Hz, 1H), 4.39 (d, J = 1.8 Hz, 1H), 6.67 (t, J = 5.3 Hz, 1H), 7.37–7.53 (m, 3H), 7.74–7.81 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.6, 28.0, 39.9, 54.8, 74.9, 84.2, 85.4, 91.8, 114.9, 127.0, 128.7, 131.8, 134.1, 167.9. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{16}H_{21}N_4O_4^+$ 333.15573; Found 333.1568.

5.1.45. *N*-(((3a*R*,4*R*,6a*S*)-6a-(Azidomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-*N*-methylbenzamide (28)

A flask containing a solution of azide **27** (0.300 g, 0.900 mmol) in THF (9 mL) was purged with nitrogen gas, treated with NaH (60% dispersion in mineral oil, 72 mg, 1.81 mmol) and backflushed. After 1 h, MeI (112 μL, 1.81 mmol) was added and the whole was stirred at rt (16 h). The reaction was monitored by mass spectrometry (ESI-TOF), which showed complete consumption of SM and presence of the desired compound. The RM was concentrated in vacuo and adsorbed onto celite. Purification via flash column chromatography (toluene/EtOAc 100:0 → 70:30) gave the title compound in 88% yield (transparent oil that solidifies on standing). HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{17}H_{23}N_4O_4^+$ 347.17138; Found 347.1722.

5.1.46. *N*-(((3a*R*,4*R*,6a*S*)-6a-(Benzamidomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-*N*-methylbenzamide (29)

Azide **28** was subjected to general procedure 1, followed by procedure 2. White foam, 93%. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{24}H_{29}N_2O_5^+$ 425.20710; Found 425.2076.

5.1.47. *N*-(((3a*R*,4*R*,6a*S*)-6a-(Benzamidomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-bromobenzamide (30a)

Compound **12** was subjected to general procedure 1, followed by general procedure 2. White foam, 53%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.38 (s, 3H), 1.52 (s, 3H), 3.35 (ddd, J = 14.1, 7.2, 4.8 Hz, 1H), 3.64–3.81 (m, 2H), 3.85–3.97 (m, 3H), 4.25 (app. td, J = 7.2, 1.2 Hz, 1H), 4.53 (d, J = 1.5 Hz, 1H), 6.96 (dd, J = 7.2, 4.8 Hz, 1H), 7.20–7.34 (m, 3H), 7.36–7.59 (m, 5H), 7.79–7.85 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.76, 27.84, 39.8, 43.3, 74.9, 83.6, 85.2, 92.2, 113.6, 119.4, 127.2, 127.5, 128.7, 129.3, 131.3, 131.8, 133.4, 133.7, 137.7, 167.9, 168.5. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{26}BrN_2O_5^+$ 489,10196; Found 489.1030.

5.1.48. *N*-(((3a*R*,4*R*,6a*S*)-6a-(Benzamidomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-iodobenzamide (30b)

Compound **12** was subjected to general procedure 1, followed by general procedure 2. White powder, 74%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.37 (s, 3H), 1.51 (s, 3H), 3.32 (ddd, J = 14.0, 7.3, 4.8 Hz, 1H), 3.64–3.80 (m, 2H), 3.85–3.97 (m, 3H), 4.25 (app. td, J = 7.1, 1.0 Hz, 1H), 4.56 (d, J = 1.5 Hz, 1H), 6.94 (dd, J = 7.2, 4.8 Hz, 1H), 7.06 (ddd, J = 8.0, 6.7, 2.3 Hz, 1H), 7.29–7.43 (m, 5H), 7.45–7.52 (m, 1H), 7.79–7.86 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.76, 27.82, 39.7, 43.2, 74.9, 83.6, 85.2, 92.2, 92.6, 113.5, 127.2, 128.16, 128.22, 128.7, 131.2, 131.8, 133.7, 139.9, 141.8, 167.9, 170.2. HRMS (ESI-TOF) m/z: $[M+H]^+$ Calcd for $C_{23}H_{26}IN_2O_5^+$ 537.08809; Found 537.0892.

5.1.49. *N*-(((3a*R*,4*R*,6a*S*)-6a-(Benzamidomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(trifluoromethoxy)benzamide (30c)

Compound **12** was subjected to general procedure 1, followed by general procedure 2. White foam, 72%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.38 (s, 3H), 1.52 (s, 3H), 3.38 (ddd, J = 13.8, 7.9, 4.7 Hz, 1H), 3.63–3.84 (m, 2H), 3.84–4.00 (m, 3H), 4.20–4.30 (m, 1H), 4.48 (d, J = 1.5 Hz, 1H), 7.18–7.54 (m, 8H), 7.79–7.87 (m, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm −57.4 (d, J = 1.98 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.67, 27.73, 39.9, 43.2, 74.8, 83.5, 85.1, 92.2, 113.5, 120.3 (q, J = 259.5 Hz), 121.2, 127.1, 127.3, 128.4, 128.6, 131.1, 131.8, 132.2, 133.7, 145.9 (br. s), 165.2,

167.9. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{24}H_{26}F_3N_2O_6^+$ 495.17375; Found 495.1739.

5.1.50. *N*-(((3a*R*,4*R*,6a*S*)-6a-(Benzamidomethyl)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl)methyl)-2-(difluoromethoxy)benzamide (30d)

Compound **12** was subjected to general procedure 1, followed by general procedure 2. White foam, 69%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.38 (s, 3H), 1.52 (s, 3H), 3.40–3.52 (m, 1H), 3.66–3.79 (m, 2H), 3.83–3.99 (m, 3H), 4.25 (app. t, J = 7.0 Hz, 1H), 4.49 (d, J = 1.5 Hz, 1H), 6.66 (app. t, J = 73.4 Hz, 1H), 7.15 (d, J = 8.2 Hz, 1H), 7.19–7.32 (m, 2H), 7.36–7.54 (m, 5H), 7.78–7.89 (m, 2H), 7.94–8.00 (m, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm −81.2 (dd, J = 167.0, 72.8 Hz), −80.4 (dd, J = 166.5, 73.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.76 (2 C), 39.8, 43.3, 74.9, 83.7, 85.2, 92.2, 113.6, 116.2 (t, J = 260.5 Hz), 119.5, 125.9, 126.2, 127.1, 128.6, 131.6, 131.8, 132.6, 133.8, 148.6 (br s), 165.3, 167.9. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{24}H_{27}F_2N_2O_6^+$ 477.18317; Found 477.1820.

5.1.51. Methyl 2-(bromomethyl)benzoate (31)

A mixture of Methyl 2-methylbenzoate (1.00 mL, 7.20 mmol) and NBS (1.40 g, 7.90 mmol) in CCl_4 (28 mL) was degassed. AIBN (24.0 mg, 0.140 mmol) was added and the mixture was heated to 85 °C for 16 h. Further NBS (0.130 g, 0.710 mmol) was added and the whole was refluxed for 1 more hour. The mixture was diluted with CH_2Cl_2 and washed with sat. aq $NaHCO_3$ solution and water. The organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo. Purification of the residue by flash chromatography (hexane/EtOAc 100:0 → 95:5) gave the title compound as an orange to pink liquid that solidifies on standing (68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.92 (s, 3H), 4.95 (s, 2H), 7.30–7.39 (m, 1H), 7.41–7.53 (m, 2H), 7.89–8.01 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 31.7, 52.4, 128.7, 129.2, 131.4, 131.8, 132.7, 139.4, 167.2.

5.1.52. *N*-(((3a*S*,6*R*,6a*R*)-2,2-Dimethyl-6-((1-oxoisoindolin-2-yl)methyl)dihydrofuro[3,4-*d*][1,3]dioxol-3a(4*H*)-yl)methyl)benzamide (32)

To a flask containing a solution of the crude amine **13** (originating from 0.460 mmol of azide **12**) in 6 mL of MeOH was added TEA (70.9 μL, 0.510 mmol) and methyl 2-(bromomethyl)benzoate (**31**) (0.120 g, 0.510 mmol). The mixture was heated to reflux at 80 °C for 14 h. The reaction was monitored by mass spectrometry (ESI-TOF), which indicated the formation of the target compound. The reaction mixture was concentrated and adsorbed onto celite. Purification via flash column chromatography (toluene/EtOAc 100:0 → 30:70) yielded the title compound as a white foam (59%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.38 (s, 3H), 1.51 (s, 3H), 3.46 (dd, J = 14.4, 7.0 Hz, 1H), 3.77 (dd, J = 14.1, 5.6 Hz, 1H), 3.85–4.05 (m, 4H), 4.34 (app. t., J = 7.5, 1H), 4.42 (d, J = 17.0 Hz, 1H), 4.57 (d, J = 17.0 Hz, 1H), 4.61 (d, J = 1.2 Hz, 1H), 7.37–7.58 (m, 6H), 7.66 (t, J = 6.2 Hz, 1H), 7.81–7.87 (m, 1H), 7.91–8.04 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ ppm 27.7, 27.8, 42.5, 43.1, 51.2, 74.8, 83.4, 85.7, 92.3, 113.5, 122.9, 123.8, 127.4, 128.3, 128.6, 131.7, 131.8, 132.1, 133.9, 141.5, 167.8, 169.4. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{24}H_{27}N_2O_5^+$ 423.19145; Found 423.1908.

5.1.53. *N*-(((2*R*,3*R*,4*S*)-4-(Benzamidomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-*N*-methylbenzamide (33)

Compound **29** was subjected to general procedure 4. White foam, 98%. ^{1}H NMR (300 MHz, DMSO-d_6) at 90 °C δ ppm 2.96 (s, 3H), 3.38–3.71 (m, 6H), 3.78–3.91 (m, 2H), 4.36 (br s, 2H), 7.31–7.54 (m, 8H), 7.78–7.84 (m, 2H), 8.03 (br s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) at 90 °C δ ppm 44.3, 51.7 (weak), 52.2 (weak), 74.6, 74.9, 77.4, 80.6, 126.3, 126.7, 127.6, 127.7, 128.5, 130.6, 134.3, 136.5, 166.9, 170.2. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{22}H_{26}ClN_2O_5^+$ 385.17580; Found 385.1770.

5.1.54. *N*-(((2*R*,3*R*,4*S*)-4-(Benzamidomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-2-bromobenzamide (34a)

Compound **30a** was subjected to general procedure 4. White solid, 92%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.33–3.59 (m, 5H), 3.65–3.69 (m, 1H), 3.74–3.81 (m, 1H), 3.89 (d, J = 9.7 Hz, 1H), 4.85 (br s, 2H), 7.28–7.39 (m, 3H), 7.43–7.57 (m, 3H), 7.59–7.65 (m, 1H), 7.83–7.89 (m, 2H), 8.36 (t, J = 6.0 Hz, 1H), 8.45 (t, J = 5.6 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 41.3, 44.3, 74.4, 74.7, 78.1, 80.6, 118.9, 127.33 (2 C), 128.2, 128.8, 130.7, 131.2, 132.6, 134.4, 139.2, 167.0, 167.4. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{20}H_{22}BrN_2O_5^+$ 449.07066; Found 449.0724.

5.1.55. *N*-(((2*R*,3*R*,4*S*)-4-(Benzamidomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-2-iodobenzamide (34b)

Compound **30b** was subjected to general procedure 4. White solid, 90%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.31–3.60 (m, 5H), 3.66–3.70 (m, 1H), 3.75–3.82 (m, 1H), 3.89 (d, J = 9.7 Hz, 1H), 4.82 (br s, 2H), 7.13 (app. td, J = 7.5, 1.9 Hz, 1H), 7.27 (dd, J = 7.6, 1.8 Hz, 1H), 7.36 (app. td, J = 7.5, 1.2 Hz, 1H), 7.42–7.57 (m, 3H), 7.82–7.90 (m, 3H), 8.32–8.45 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 41.4, 44.3, 74.5, 74.8, 78.1, 80.6, 93.5, 127.3, 127.8, 128.1, 128.2, 130.6, 131.2, 134.4, 139.0, 143.1, 167.0, 169.0. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{20}H_{22}IN_2O_5^+$ 497.05679; Found 497.0583.

5.1.56. *N*-(((2*R*,3*R*,4*S*)-4-(Benzamidomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-2-(trifluoromethoxy)benzamide (34c)

Compound **30c** was subjected to general procedure 4. White foam, 97%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.28–3.66 (m, 6H), 3.69–3.81 (m, 1H), 3.89 (d, J = 9.7 Hz, 1H), 4.73 (br s, 1H), 4.86 (br s, 1H), 7.35–7.60 (m, 7H), 7.82–7.90 (m, 2H), 8.36 (t, J = 5.9 Hz, 1H), 8.44 (t, J = 5.6 Hz, 1H). ^{19}F NMR (282 MHz, DMSO-d_6) δ ppm −56.3 (s). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 41.6, 44.3, 74.5, 74.8, 78.0, 80.6, 120.0 (q, J = 257.01 Hz), 121.4, 127.3, 128.2, 129.7, 131.0, 131.2, 131.3, 134.4, 144.9 (br. s), 164.7, 167.1. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{21}H_{22}F_3N_2O_6^+$ 455.14245; Found 455.1423.

5.1.57. *N*-(((2*R*,3*R*,4*S*)-4-(Benzamidomethyl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)-2-(difluoromethoxy)benzamide (34d)

Compound **30d** was subjected to general procedure 4. White foam, 91%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.32–3.66 (m, 6H), 3.70–3.82 (m, 1H), 3.89 (d, J = 9.7 Hz, 1H), 4.83 (br s, 2H), 7.13 (app. t, J = 74.2 Hz, 1H), 7.18–7.31 (m, 2H), 7.41–7.61 (m, 5H), 7.79–7.91 (m, 2H), 8.25 (t, J = 5.6 Hz, 1H), 8.36 (t, J = 5.9 Hz, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ ppm −81.5 (app. s), −81.2 (app. s). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 41.4, 44.3, 74.4, 74.8, 78.0, 80.5, 116.7 (t, J = 258.0 Hz), 119.4, 125.3, 127.3, 128.2, 129.0, 129.7, 131.2, 131.3, 134.4, 147.8 (t, J = 2.9 Hz), 165.2, 167.1. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{21}H_{23}F_2N_2O_6^+$ 437.15187; Found 437.1528.

5.1.58. *N*-(((3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-((1-oxoisoindolin-2-yl)methyl)tetrahydrofuran-3-yl)methyl)benzamide (35)

Compound **32** was subjected to general procedure 4. White foam, 84%. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.34–3.49 (m, 2H), 3.54–3.67 (m, 3H), 3.78–3.93 (m, 3H), 4.45–4.60 (2× d, J = 18.2 Hz, 2H), 4.77 (br s, 2H), 7.40–7.62 (m, 6H), 7.65–7.71 (m, 1H), 7.78–7.85 (m, 2H), 8.38 (t, J = 5.9 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 44.1, 44.4, 51.0, 74.6, 74.8, 77.9, 81.0, 122.7, 123.3, 127.3, 127.7, 128.2, 131.18, 131.22, 132.1, 134.4, 142.1, 167.1, 167.4. HRMS (ESI-TOF) *m/z*: $[M+H]^+$ Calcd for $C_{21}H_{23}N_2O_5^+$ 383.16015; Found 383.1610.

5.2. Microbiology

5.2.1. Reagents used

Hamamelitannin (HAM) and vancomycin (VAN) were purchased from Sigma Aldrich (Bornem, Belgium). HAM was stored in DMSO at −20 °C. VAN was dissolved in ultrapure water and stored at 4 °C.

5.2.2. Strains and culture conditions

was cultured in Mueller–Hinton broth (MH, Oxoid, Basingstoke, England) at 37 °C under aerobic conditions.

5.2.3. Determination of the MIC

MICs of HAM analogues against *S. aureus* Mu50 used were determined in triplicate using flat-bottom 96-well microtiter plates (TPP, Trasadingen, Switzerland) as previously described.[13]

5.2.4. Effect of pretreatment and co-treatment on biofilm susceptibility

S. aureus Mu50 biofilms were formed and HAM analogues were evaluated as previously described.[9,10] In brief, overnight cultures in MH were centrifuged, the pellet was resuspended in double-concentrated MH (2 × MH) and diluted to an $OD_{590\ nm}$ of 0.2. Fifty microliter of the diluted bacterial suspension was transferred to the wells of a round-bottom 96-well microtiter plate (TPP). Control wells received 50 μl MilliQ. Wells used to evaluate pre-treatment received 50 μl of HAM-analogue solution. Bacteria were allowed to adhere and grow without agitation for 4 h at 37 °C. After 4 h, medium was removed, and the adhered cells were washed with sterile physiological saline (0.9% NaCl; PS). After this washing step, control wells were filled with 50 μl 2 × MH and 50 μl MilliQ. Other wells were filled with 50 μl 2 × MH and 50 μl of HAM analogue solution, and the plate was incubated for 20 h at 37 °C. To evaluate the effect of co-treatment on mature biofilms, control biofilms were formed in the absence of HAM analogues, as described above. After 24 h of biofilm formation, the medium was removed and the wells were rinsed with PS. Control wells were either filled with 100 μl PS (untreated controls) or with 50 μl PS and 50 μl antibiotic solution. Wells used to evaluate the effect of pre-treatment were also filled with 50 μl PS and 50 μl antibiotic solution while wells used to evaluate combination treatment were filled with 50 μl of a HAM analogue solution and 50 μl antibiotic solution. The plates were then incubated for an additional 24 h at 37 °C. After biofilm formation and treatment of the biofilms, the number colony forming units (CFU) per biofilm were determined by conventional plating. To collect the cells for plating, plates were rinsed with PS, sessile cells were removed from the microtiter plate by two cycles of vortexing (5 min) and sonication (5 min) and the number of CFU/biofilm was determined by plating the resulting suspensions. The number of CFU/biofilm (for plating) of the control biofilms was set to 100% and the results of the treated biofilms were compared to this. Each condition was tested in at least three wells in each assay, and each assay was carried out at least in triplicate ($n \geqslant 9$).

5.2.5. Statistical evaluation

The normal distribution of the data was checked by using the Shapiro–Wilk test. Normally distributed data were analyzed using a one-way ANOVA. Non-normally distributed data were analyzed using the Kruskal–Wallis test. Statistics were determined using SPSS software, version 22.0.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.07.058.

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