ChemMedChem

Chemistry Europe
European Chemical Societies Publishing

Accepted Article

Title: Synthesis and biological evaluation of (-) and (+)-spiroleucettadine and analogues

Authors: Michael Badart, Emma M Barnes, Andrew P Cording, Selena Gilmer, Ian D Billinghurst, Veera Edupuganti, Guillaume Lessene, Abigail Bland, Rebekah Bower, Zohaib Rana, Scott Ferguson, Helen Opel Reading, Greg Cook, Rhonda Rosengren, Kurt Krause, Allan Gamble, John Ashton, and Bill C. Hawkins

To be cited as: *ChemMedChem* 10.1002/cmdc.202000954

Link to VoR: https://doi.org/10.1002/cmdc.202000954

WILEY-VCH

WILEY-VCH

FULL PAPER

Synthesis and biological evaluation of (-) and (+)-spiroleucettadine and analogues

Michael P. Badart,[a] Emma M. Barnes,[a] Andrew P. Cording, [a] Selena C. L. Gilmer, [a] Ian D. Billinghurst, [a] Veera V. Shivaji R. Edupuganti,[b] Guillaume Lessene, [c][d][e] Abigail R. Bland, [f] Rebekah L. Bower, [f] Zohaib Rana, [f] Scott A. Ferguson, [g] Helen K. Opel Reading, [h] Gregory M. Cook,[g] Rhonda J. Rosengren,[f] Kurt L. Krause, [h] Allan B. Gamble, [b] John C. Ashton,[f] and Dr. Bill C. Hawkins*[a]

[a] Michael P. Badart, Emma M. Barnes, Andrew P. Cording, Ian Billinghurst, Selena Gilmer, Dr. Bill C. Hawkins.
Department of Chemistry
University of Otago
Dunedin 9054 (New Zealand)
E-mail: bhawkins@chemistry.otago.ac.nz

[b] Veera V. Shivaji R. Edupuganti, Dr. Allan B. Gamble.
School of Pharmacy
University of Otago
Dunedin 9054 (New Zealand)

[c] Prof. Guillaume Lessene
ACRF Chemical Biology Division
The Walter and Eliza Hall Institute of Medical Research
1G Royal Parade, Parkville, 3052 (Australia)

[d] Prof. Guillaume Lessene
Department of Medical Biology
The University of Melbourne, Parkville, VIC 3050 (Australia)

[e] Prof. Guillaume Lessene
Department of Pharmacology and Therapeutics
The University of Melbourne, Parkville, VIC 3050 (Australia)

[f] Abigail R. Bland, Rebekah L. Bower, Zohaib Rana, Prof. Rhonda J. Rosengren, Assoc. Prof. John C. Ashton
Department of Pharmacology and Toxicology
University of Otago
Dunedin 9054 (New Zealand)

[g] Scott A. Ferguson, Prof. Gregory M. Cook
Department of Microbiology and Immunology
University of Otago
Dunedin 9054 (New Zealand)

[h] Helen K. Opel Reading, Prof. Kurt L. Krause
Department of Biochemistry
University of Otago
Dunedin 9054 (New Zealand)

Supporting information for this article is given via a link at the end of the document.

Abstract: A second generation enantiospecific synthesis of spiroleucettadine is described. The original reported antibacterial activity was not observed when repeated on the synthetic samples, however, significant anti-proliferative activity was uncovered for both enantiomers of spiroleucettadine. Comparison of the optical rotational data and ORD-CD spectra of both enantiomers and the reported spectrum from the natural source has not provided a definitive answer regarding the absolute stereochemistry of naturally occurring spiroleucettadine. Efforts then focussed on alteration at the C-4 and C-5 position of the slightly more active (-)-spiroleucettadine. Ten analogues were synthesised, with three analogues found to possess similar anti-proliferative profiles to spiroleucettadine against the H522 lung cancer cell line.

Introduction

The *Leucetta* genus of calcareous sea-sponges is a rich source of bioactive alkaloids.[1] Alkaloids produced as secondary metabolites from *Leucetta chagosensis* were first outlined in 1987 and included naamidine A (**1**). The naamidine alkaloid isolated from this organism has been shown to possess inhibitory activity against the epidermal growth factor receptor (EGF), as well as anti-proliferative effects on tumours which rely on this mitogen for growth.[2] Following this discovery in 1987 more than 70 alkaloids, including spiro-fused alkaloids and napthimidazoles, which vary in complexity and biological relevance, have been isolated from sea-sponges in the *Leucetta* genus.[3] Since then more heavily oxygenated alkaloids like (+)-spironaamidine (**2**) and (-)-spiroleucettadine (**3**) have been reported (Figure 1).[4]

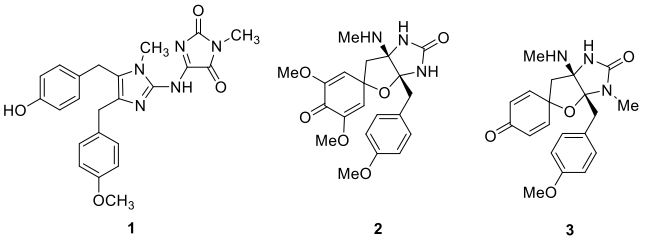
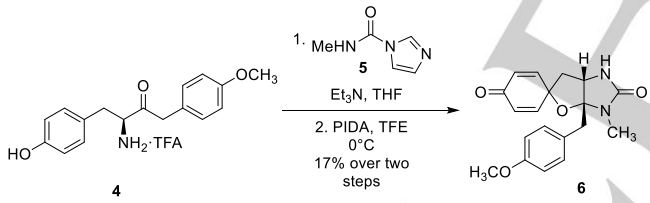


Figure 1. The structures of naamidine A (**1**), (+)-spironaamidine (**2**) and (-)-spiroleucettadine (**3**).

Discovered by the Crews group in 2004,[4a, 4b] spiroleucettadine (**3**) represents one of the most structurally complex molecules to be isolated from the *Leucetta* sponge (Figure 1). Beyond the interesting structural features, spiroleucettadine was also reported to possess good anti-bacterial activity against *Enterococcus durans* with a minimum inhibitory concentration (MIC) of less than 6.25 μg mL^{-1}.[4a] Recently, we reported the first and, to date, only synthesis of spiroleucettadine (reported as (-) spiroleucettadine, $[\alpha]_D^{25}$ = -2.6°, c 0.25, MeOH).[5] Whilst the synthesis provided access to spiroleucettadine, it suffered from a low yielding reaction sequence (Scheme 1) which hampered the ability to readily produce meaningful quantities of spiroleucettadine and analogues. Herein, we report a second-generation synthesis which has greatly enhanced access to spiroleucettadine itself and facilitated the rapid synthesis of analogues. We also revise the optical rotational data associated with spiroleucettadine derived from L-tyrosine and report its potent biological activity against lung cancer cells.



Scheme 1. Original strategy used to access the spirocyclic cyclohexadienone core of spiroleucettadine.[5a]

Results and Discussion

The original synthesis of spiroleucettadine allowed for a preliminary evaluation of its biological activity. Surprisingly, despite screening against a range of bacterial genera including Gram-negative and Gram-positive bacteria, only *Staphylococcus aureus* was inhibited by very high concentrations of (-) and (+)-spiroleucettadine. *Enterococcus faecalis* was inhibited by (+)-spiroleucettadine at high concentrations (Table 1). Neither (-) or (+)-spiroleucettadine had any effect on the growth of *Escherichia coli*. Gentamicin and ampicillin were potent inhibitors of *E. coli* (positive control) as was penicillin G against *S. aureus* and ampicillin against *E. faecalis* (Table 1).

Given that other alkaloids isolated from the *Leucetta* sponge, such as naamidine A (**1**, Figure 1), exhibited anti-proliferative properties, a synthetic sample of spiroleucettadine (**3**) was sent to the National Cancer Institute (NCI) to be tested against the NCI-60 cancer cell lines. From this, spiroleucettadine was found to possess anti-cancer activity against several cancer cell lines in the nanomolar range.[6] The highest anti-proliferative activity was reported against the non-small cell lung carcinoma line NCI-H522 (IC_{50} 0.37 μM).[6] Beyond the significant potency of spiroleucettadine, the range of activity observed also suggests a degree of selectivity between particular cancer cell types that warrants further investigation.

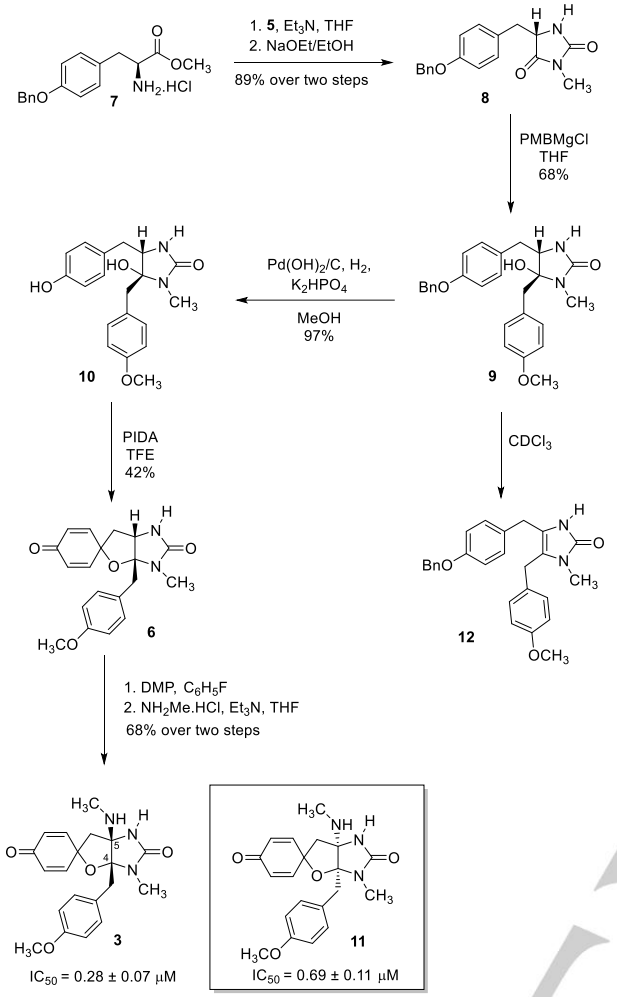
Table 1. Minimum Inhibitory Concentration (MIC)

Compound	*E. coli*	*S. aureus*	*E. faecalis*
(-) spiroleu. (3) (μM)	>256	256	256
(+) spiroleu. (11) (μM)	>512	512	-
Penicillin G (μg/mL)	-	0.06	-
Gentamycin (μg/mL)	2		-
Ampicillin	4	0.0625	1

MIC values were determined by broth microdilution in cation-adjusted Mueller-Hinton broth. The initial inoculum of bacterial cells was 5×10^5 CFU mL^{-1}. The MIC was determined after 16-24 hour at 35±2°C according to the CLSI guidelines.[7]

Inspired by the promising screening results we re-evaluated the original synthetic pathway, in particular its ability to provide rapid access to analogues. The main set back in the synthesis, from an analogue generation point-of-view, was the low yielding urea formation/oxidative spirocyclization sequence, along with limited opportunities for the incorporation of structural diversity. With these limitations in mind we designed a second generation synthesis (Scheme 2). The hydantoin **8** was accessed by treatment of the protected tyrosine derivative **7** with *N*-methyl carbamoylimidazole (**5**) in the presence of triethylamine to yield the acyclic urea (not shown), which was not isolated but rather immediately used in the next reaction. The addition of freshly prepared sodium ethoxide to the crude residue provided the hydantoin **8** in an 89% yield over two steps.[8] Subsequent treatment with *p*-methoxybenzyl magnesium chloride afforded the acid-sensitive tertiary hemiaminal **9**, after trituration with cold ether, in a 68% yield. Unsurprisingly, this compound readily dehydrated in the presence of acid to form the corresponding 2-imidazolone **12**, even with low concentrations of HCl such as those found in $CDCl_3$ (Scheme 2). Given the propensity of the alcohol to dehydrate, benzyl ether hydrogenolysis was performed under buffered conditions using 10% wt. $Pd(OH)_2$/C to afford the phenol **10**, with the spectra recorded in base washed deuterated chloroform.

Upon reaction with PIDA,[9] the phenol **10** underwent oxidative spirocyclization to afford the spirocycle **6**. From here, spiroleucettadine (**3**) was accessed in a similar fashion to that described in our original synthesis with an overall yield of 16.8% (c.f 5.3% for original route).[5a]



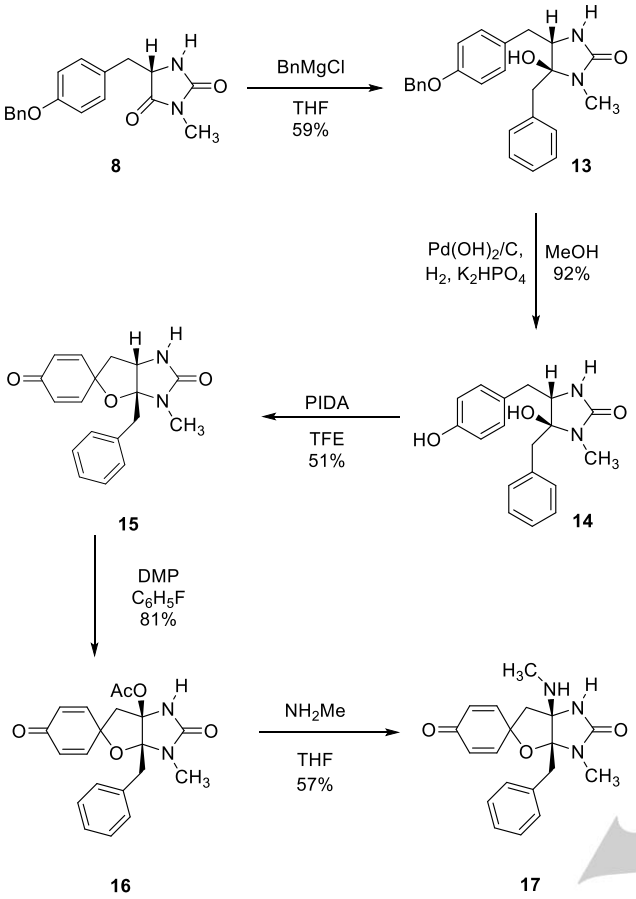
Scheme 2. The second-generation synthesis and bioactivity against H522 cancer cells of (-)-spiroleucettadine (**3**) and (+)-spiroleucettadine (**11**).

Prior to this second-generation synthesis, it was difficult to obtain meaningful quantities of the spiroleucettadine enantiomers. However, the new synthetic route allowed for the rapid, high yielding, enantiospecific preparation of both spiroleucettadine (**3,** starting from L-tyrosine) and spiroleucettadine (**11,** starting from D-tyrosine). Upon measuring the optical rotation for spiroleucettadine (**11**, derived from D-tyrosine)**,** we were surprised to find that, presumably due to the small magnitude of rotation, the values obtained were inconsistent and varied between $[\alpha]_D^{25}$ +5 to -5° when recorded in methanol. Intriguingly, varying sample concentration and temperatures appeared to have little to no effect on the rotational value obtained.[6] This prompted us to re-measure the rotation of spiroleucettadine (**3**), derived from L-tyrosine, from both the original and new syntheses and again both sign and magnitude of light rotation was varying. Notably, Crews and co-workers reported significant variation in optical rotational values between isolated samples which they attributed to isolation of scalemic mixtures of spiroleucettadine ($[\alpha]_D$ -27.1° c 0.38, MeOH,[4a] $[\alpha]_D$ -5.1° c 0.56, MeOH[4b], no temperature provided for either measurement). Furthermore, HPLC studies on the samples indicated no racemisation had occurred for either generation of syntheses.[6] This suggests our original measurement of optical rotation[5a] and potentially the measurements reported in the isolation papers[4a, 4b] were inaccurate. In an effort to obtain reproducible data we found chloroform to be a more reliable solvent for recording optical rotations of spiroleucettadine: (**3**) $[\alpha]_D^{20}$ -23° c 1.23, $CHCl_3$, 98% ee and (**11**) $[\alpha]_D^{20}$ +17.9° c 0.34, $CHCl_3$, 95% ee.

Given the relatively small magnitude, and irreproducibility (in methanol), of rotation of plane polarized light and limited access to the natural source, unambiguous determination of the absolute configuration of the spiroleucettadine remained elusive. Interestingly, the structurally similar spironaamidine (**2**, Figure 1, $[\alpha]_D^{14}$ = +7.1°, c 0.85, MeOH) was reported to be dextrorotatory.[4c] Further attempts to confirm the absolute configuration of the natural product by comparison of the ORD and CD spectra of synthesised (-) and (+)-spiroleucettadine to the data from the original isolation paper was not enlightening.[6]

At this point, (+)-spiroleucettadine (**11**) (derived from D-tyrosine) was tested against several bacterial strains with no significant antibacterial activity observed (Table 1). In addition, (+) spiroleucettadine (**11**) was also tested against the same cancer cell lines as (-)-spiroleucettadine (**3**) and found to be approximately half as potent (**11** IC_{50} = 0.69 μM vs **3** IC_{50} = 0.28 μM, Scheme 2).[6]

In order to ascertain which features of the more potent (-)-spiroleucettadine were important for biological activity, and keeping synthetic tractability in mind, we began with the modification of the methylamine and the methoxybenzyl side chain. With the desmethylamino analogue **6** already in hand, efforts were turned towards the synthesis of the other deletion analogues **15** and **17** (Scheme 3). To this end, Grignard addition of benzyl magnesium chloride to the hydantoin **8** afforded the tertiary alcohol **13**, after trituration with cold ether, in a 59% yield (Scheme 3). Benzyl ether hydrogenolysis under pH buffered reaction conditions then provided the phenol **14** in excellent yield (92%). The phenol **14** was taken up in 2,2,2-trifluoroethanol and treated with PIDA at 0 °C to afford the desmethylamino-desmethoxy analogue **15** in a 51% yield (Scheme 3). Following this, installation of the methyl amine group was achieved using the same chemistry described earlier to provide the desmethoxy analogue **17**, in a 46% yield over two steps (Scheme 3).



Scheme 3. Synthesis of the C-4 benzyl deletion analogues **15** and **17**.

The IC_{50} of **6** against the NCI-H522 cancer cell line was found to be 3.93 μM, compared to that of spiroleucettadine **3** (IC_{50} 0.28 μM). In contrast, testing found desmethoxy-spiroleucettadine **17** to be less active than **3** (IC_{50} 1.78 μM c.f. 0.28 μM). In addition to this, desmethylamino-desmethoxy-spiroleucettadine **15** was synthesised to investigate the effect of deleting both functional groups. Interestingly, **15** was the most active of the three analogues with an IC_{50} of 0.61 μM.

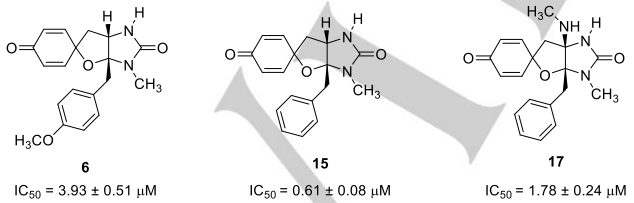


Figure 2. The deletion analogues **5**, **15** and **17**, with the IC_{50} values against the NCI-H522 lung cancer cell line.

Given that the analogues **15** and **17** possessed greater activity than the desmethoxy analogue **6**, it was postulated that the methoxy group was not essential for the activity of spiroleucettadine **3**. Thereby, simplifying the structure while also decreasing the metabolic liability posed by an electron rich aromatic system. However, at stages in the study the *para*-methoxybenzyl group will be reintroduced at the C-4 position to re-test this hypothesis. Given that the methylamino deletion analogue **6** is the least active, this position needed to be probed further to determine whether or not this moiety was required for the biological activity of spiroleucettadine. Desmethylamino-spiroleucettadine **6** was synthesised to probe whether the *N*-methylamine side chain was potentially involved in these interactions (Figure 2).

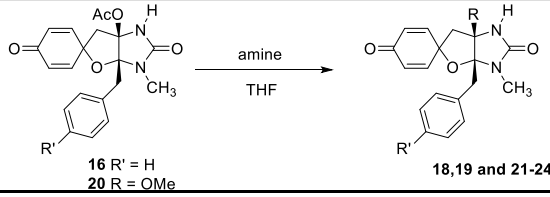
Synthesis of analogues with variation at C-5

Given the ability of the acetate **16** to undergo nucleophilic substitution with methylamine we decided to focus attention on the role of the *N*-methylamine side chain in spiroleucettadine's bioactivity. Therefore, acetate **16** was separately treated with piperidine and morpholine to afford tertiary amine adducts **18** (cLogP 4.44) and **19** (cLogP 3.37), respectively, in excellent yield (Table 2 entries 1 and 2). To examine the effect of having a hydrophobic amine side chain at C5, substitution of the acetate of the *para*-methoxybenzyl analogue **20** with n-butylamine afforded **21**, while treatment of benzyl acetate **16** with n-butyl amine afforded **22**. With the end goal of protein/enzyme pull-down studies in mind, substitution with propargyl amine gave access to **23** in excellent yield (Table 2). Finally, in order to screen for an additional pi-stacking interaction, benzyl amine was chosen for the nucleophilic substitution providing the *N*-benzyl analogue **24**, in good yield.

Biological activity of *N*-methylamine side chain analogues

Spiroleucettadine (**3**, cLogP 3.10) features a secondary amine, which can act as both a hydrogen bond donor and acceptor. Like secondary amines, tertiary amines are basic and can change ionisation status, however, they can only act as hydrogen bond acceptors. Furthermore, tertiary amines are often used in drug design because they are metabolised to less toxic metabolites than the equivalent primary and secondary amines, and hence, exhibit an improved safety profile.[10] Replacement of the methylamine side chain with the relatively hydrophobic cyclic tertiary piperidine based analogue (cLogP = 4.44, IC_{50} 0.50 μM, Table 2, entry 1) did not appear to adversely affect biological activity. Likewise, the more hydrophilic morpholine derivative also maintained a similar level of activity (cLogP = 3.37, Entry 2 IC_{50} 0.47 μM.)

Table 2. S_N1 substitution with various amines provided the analogues **18, 19 and 21-24.23** and their IC_{50} values against the NCI-H522 cancer cell line.



Entry #	Compound	*R*	*R'*	*Yield (%)*	*IC_{50} (μM)*[a]
1	**18**	piperidine	H	96	0.50 ± 0.06
2	**19**	morpholine	H	79	0.47± 0.04

3	**21**	butylamine	OCH_3	47	0.33 ± 0.03
4	**22**	butylamine	H	78	0.38 ± 0.03
5	**23**	propargylamine	H	83	0.61 ± 0.08
6	**24**	benzylamine	H	68	0.28 ± 0.02

[a] Assay was performed in triplicate (n = 3), see Supporting Information for dose response curves.

Hydrophobic interactions have long been recognised as an important biological phenomena, particularly in drug design and drug activity.[11] They are one of the governing interactions in the binding of drugs to receptors, and hence, are important in determining binding affinities and drug efficacy.[11b] However, there is a delicate balance between the hydrophobic and hydrophilic nature of a molecule. If molecules are too hydrophobic, they can become trapped in membranes and not reach their site of action.[11]

The *para*-methoxybenzyl *N*-butyl analogue **21** was found to possess comparable activity to (-)-spiroleucettadine (cLogP 4.31, Table 2, entry 3, IC_{50} 0.33 μM). The simplified benzyl *N*-butyl analogue **22** was also a potent inhibitor (cLogP 4.56, IC_{50} 0.38 μM) which is consistent with the working hypothesis that the methoxy group is not essential for the biological activity of **3**. Interestingly, the *N*-propargyl analogue **23** was approximately half as potent as **3** against the H522 cell line (entry 5, IC_{50} 0.61 μM). The *N*-benzyl analogue was found to possess equipotent activity to (-)-spiroleucettadine (**3**) (entry 6, IC_{50} 0.28 μM).

Whilst the precise molecular attributes necessary for biological activity are yet to be elucidated, it is clear that substitution of the methyl amine side chain (C-5) does not adversely affect activity. Thereby, providing a possible opportunity for proteomic studies. This will be the focus of future work and will be reported in due course.

Conclusion

In conclusion, we have reported a second-generation synthesis which allowed for the rapid, enantiospecific preparation of spiroleucettadine and analogues. With larger quantities of both (-)- and (+)-spiroleucettadine we have revised our original optical rotation data and performed ORD-CD on both enantiomers of spiroleucettadine. Our results suggest that spiroleucettadine does not possess significant antibacterial properties, however, it does possess potent (nanomolar) anti-proliferative activity against H522 (lung) cancer cells. Through the synthesis of analogues, we have established that modification of the methyl amine side chain is possible without significant loss of biological activity. This information will facilitate further studies centred around determining the mode of action.

Experimental Section

Thin layer chromatography (TLC) was performed on ALUGRAM® aluminium-backed UV254 silica gel 60 (0.20 mm) plates. Compounds were initially detected using UV light, and developed with either *p*-anisaldehyde, basic permanganate, vanillin, or phosphomolybdic acid with heating. Column chromatography was performed using silica gel 60. Infrared spectra were recorded on a Bruker Optics Alpha ATR FT-IR spectrometer. No sample preparation was required. High resolution mass-spectra were recorded on a Bruker microTOFQ mass spectrometer or Shimadzu LCMS-9030 mass spectrometer using an electrospray ionisation (ESI) source in either the positive or negative modes. ^{1}H NMR spectra were recorded at either 400 MHz on a Varian 400-MR NMR system or at 500 MHz on a Varian 500 MHz AR premium shielded spectrometer. Spectra were recorded from samples in the designated deuterated solvent at 25 °C in 5 mm NMR tubes. ^{13}C NMR spectra were recorded at either 101 MHz on a Varian 400-MR NMR system or at 125 MHz on a Varian 500 MHz AR premium shielded spectrometer under the same conditions as the ^{1}H NMR spectra. Chemical shifts were reported in parts per million and referenced to the appropriate solvent peak. Melting points were measured on a DigiMelt MPA 161 apparatus. The optical rotation of chiral compounds were recorded on a Rudolph Research Analytical AUTOPOL® IV automatic polarimeter. Dichloromethane (CH_2Cl_2), diethyl ether (Et_2O), tetrahydrofuran (THF), methanol (MeOH), and acetonitrile (MeCN) were dried using the PURE SOLV MD-6 solvent purification system. All other solvents and reagents were used as received. Analytical HPLC was carried out on a Shimadzu Prominence HLPC system using a Shimadzu ELSD-LT II detector and a Diacel CHIRALPAK IC-3 chiral column, eluting with acetonitrile.

Biological assay: H522 cells (10 x 10^3cells/well) were seeded in 96-well plates for 24 h at 37 °C in RPMI growth medium supplemented with 5% FBS and 1% penicillin/streptomycin. Cells were treated with the respective compound for 72 h, using DMSO as the vehicle control. To determine cell viability, the sulforhodamine B (SRB) assay was used. Briefly cells were fixed in 10% trichloroacetic acid (TCA) for 30 min at 4 °C. To remove the TCA, plates were washed twice in distilled water and dried for 30 min. SRB (0.4% in 1% acetic acid) was added to the wells for 10 min, followed by three 1% acetic acid washes to remove unbound dye. The dye was solubilized in 10 mM Tris/HCl (pH 10.5) and the absorbance read at 510 nm on a BioRad Microplate Spectrophotometer. Cell viability was calculated as a percentage of the DMSO control and EC50values were determined using non-linear regression (equation: log(inhibitor) vs. response – variable slope (four parameters)) using GraphPad Prism 8. **Antimicrobial Susceptibility Testing:** To determine the minimum inhibitory concentration (MIC) of spiroleucettadine (**3**) or (**11**) against S. aureus ATCC 6538 or E. coli ATCC 10536, cells were grown in cation adjusted Mueller Hinton (CAMHB) broth at 37 °C with shaking (200 rpm). E. faecalis JH2-2 was grown under the same conditions without shaking. The MIC assay was performed by microbroth dilution following the CLSI guidelines.[7] Whereby a polystyrene 96-well plate was set up such that 100 μL of CAHMB media was added to column 1 (A-H) and 50 μL of media was added to the remaining wells. Spiroleucettadine (**3**) or (**11**) was added to column 1, to yield a final concentration of 512 μg mL^{-1}, and then serially diluted 2-fold (50 μL transfer) into the neighbouring wells, resulting in a serial dilution of each compound from 512 μg mL^{-1} to 0.25 μg mL^{-1}. Overnight cultures of bacteria were diluted in fresh CAMHB before adding 50 μL of culture to each well of the plate to achieve a uniform CFU mL -1 (final) of approximately 5 × 105 in the MIC plate. The plates were incubated at 37°C with shaking as appropriate for each bacteria for 16-24 h before determining the MIC. MICs were determined as the lowest concentration at which growth did not occur. Ampicillin, penicillin G, and gentamycin were used as positive controls.

5-{[4-(benzyloxy)phenyl]methyl}-3-methylimidazolidine-2,4-dione (8). Et_3N (2.32 mL, 16.7 mmol) was added to a suspension of HCl salt of **7** (4.40 g, 13.9 mmol) in THF (50 mL), followed by N-methyl carbamoylimidazole (2.10 g, 16.7 mmol), and the reaction was heated to reflux for 16 h. The solution was cooled to room temperature and partitioned between water (100 mL) and EtOAc (100 mL). The aqueous layer was extracted with EtOAc (3 × 50 mL), and the combined organic

phases were washed with brine (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude residue was then dissolved in EtOH (70 mL); Na (384 mg, 16.7 mmol) was added to the solution, and a white precipitate appeared. The reaction was then stirred at room temperature for a further 30 h then concentrated *in vacuo* to half volume. The mixture was then diluted with water (200 mL) and extracted with EtOAc/isopropanol (4:1) (3 × 100 mL). The combined organic phases were washed with brine (50 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The crude product was then triturated with cold Et_2O to yield the title compound as a white crystalline solid (3.34 g, 77% over two steps). m.p: 166−167 °C. ^{1}H NMR (400 MHz, DMSO) δ 8.18 (s, 1H), 7.49 – 7.28 (m, 5H), 7.07 (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 8.7 Hz, 2H), 5.05 (s, 2H), 4.31 (t, *J* = 5.1 Hz, 1H), 2.91 (dd, *J* = 14.1, 4.8 Hz, 1H), 2.86 (dd, *J* = 14.1, 5.3 Hz, 1H), 2.65 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ 174.1, 157.6, 157.2, 137.6, 131.1, 128.8, 128.2, 128.1, 128.0, 114.8, 69.5, 57.9, 36.1, 24.2. HRMS-ESI (*m/z*): calcd for $C_{18}H_{18}N_2NaO_3$ [M + Na$^+$], 333.1210; found, 333.1180. ν_{max} (ATR-IR), cm^{-1}: 3316, 2924, 1774, 1708, 1600, 1511. $[\alpha]_D^{22}$ = -51.0 (c = 0.32, pyridine).

4-{[4-(benzyloxy)phenyl]methyl}-5-hydroxy-5-[(4-methoxyphenyl)methyl]-1-methylimidazolidin-2-one (9). To a solution of the hydantoin **8** (1.50 g, 4.83 mmol) in THF (10 mL) was added a solution of *para*-methoxybenzyl magnesium chloride[12] in THF (56.8 mL, 10.63 mmol) at 0 °C. The reaction was then allowed to warm to room temperature and stirred overnight. The reaction mixture was quenched with saturated aqueous $NaHCO_3$ (10 mL) and partitioned between water (50 mL) and EtOAc (50 mL). The aqueous layer was washed with EtOAc (3 x 50 mL) and the combined organic layers were washed with brine (100 mL), dried over $MgSO_4$ and concentrated *in vacuo* to afford the crude compound as a yellow oil. This was triturated with Et_2O to afford the title compound (1.41 g, 68%) as a white solid. m.p: 157–158 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.47 – 7.41 (m, 2H), 7.41 – 7.34 (m, 2H), 7.35 – 7.28 (m, 1H), 7.07 – 6.99 (m, 2H), 6.95 – 6.86 (m, 4H), 6.83 – 6.76 (m, 2H), 6.23 (d, *J* = 2.0 Hz, 1H), 5.87 (s, 1H), 5.08 (s, 2H), 3.70 (s, 3H), 3.39 (ddd, *J* = 7.8, 5.7, 1.8 Hz, 1H), 2.85 (d, *J* = 14.1 Hz, 1H), 2.67 (s, 3H), 2.62 – 2.55 (m, 2H), 2.45 (dd, *J* = 14.0, 7.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 159.9, 157.9, 156.7, 137.3, 131.3, 130.9, 130.3, 128.4, 128.1, 127.7, 127.6, 114.6, 113.4, 89.0, 69.1, 57.6, 54.9, 41.1, 34.9, 24.2. HRMS-ESI (*m/z*): calcd for $C_{26}H_{28}N_2NaO_4$ [M + Na]$^+$, 455.1941; found, 455.1927. ν_{max} (ATR-IR) cm^{-1} 3380, 3253, 2920, 2835, 1695, 1613, 1512, 1248. $[\alpha]_D^{20}$ = +1.8 (c = 0.10, MeOH).

5-hydroxy-4-[(4-hydroxyphenyl)methyl]-5-[(4-methoxyphenyl)methyl]-1-methylimidazolidin-2-one (10). The benzyl ether **9** (200 mg, 0.462 mmol) and K_2HPO_4 (80.6 mg, 0.462 mmol) were taken up in MeOH (8 mL). To this was added $Pd(OH)_2$/C (20 mg, 10% wt.) and the system was purged with H_2. The reaction mixture was stirred at room temperature for 90 min. This was then diluted with EtOAc (50 mL), filtered through a plug of celite and concentrated *in vacuo* to afford the crude residue. Given the propensity to eliminate, this was used without further purification. m.p. 135-136 °C. ^{1}H NMR (400 MHz, DMSO) δ 9.16 (s, 1H), 6.95 – 6.86 (m, 4H), 6.80 (d, *J* = 8.8 Hz, 2H), 6.66 (d, *J* = 8.6 Hz, 2H), 6.18 (d, *J* = 1.8 Hz, 1H), 5.84 (s, 1H), 3.70 (s, 3H), 3.37 (td, *J* = 5.7, 1.5 Hz, 1H), 2.85 (d, *J* = 13.8 Hz, 1H), 2.67 (s, 3H), 2.62 – 2.52 (m, 2H), 2.40 (dd, *J* = 13.6, 7.6 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 160.1, 158.0, 155.7, 131.4, 130.3, 128.9, 128.2, 115.1, 113.5, 89.2, 57.7, 55.0, 41.2, 34.9, 24.3. HRMS-ESI (*m/z*): calcd for $C_{19}H_{22}N_2NaO_4$ [M + Na]$^+$, 365.14718; found, 365.14692. ν_{max} (ATR-IR) cm^{-1} 3320, 3222, 2943, 2828, 1673, 1514, 1244. $[\alpha]_D^{22}$ = +16.7 (c = 0.25, MeOH).

5-benzyl-4-{[4-(benzyloxy)phenyl]methyl}-5-hydroxy-1-methylimidazolidin-2-one (13). To a solution of the hydantoin **8** (250 mg, 0.806 mmol) in THF (7 mL) was added benzylmagnesium chloride[12] dropwise (6.91 mL, 2.42 mmol) at 0 °C. This was warmed to room temperature and stirred overnight. The reaction mixture was quenched with aqueous $NaHCO_3$ (10 mL) and partitioned between H_2O (20mL) and EtOAc (20 mL). The aqueous layer was washed with EtOAc (3 x 20 mL) and the combined organic layers were washed with brine (50 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The crude residue was triturated with Et_2O to afford the title compound (191 mg, 59%) as a white solid. m.p. 166–167 °C. ^{1}H NMR (400 MHz, DMSO) δ 7.46 – 7.41 (m, 2H), 7.41 – 7.35 (m, 2H), 7.34 – 7.28 (m, 1H), 7.27 – 7.17 (m, 3H), 7.05 – 6.96 (m, 4H), 6.91 (d, *J* = 8.8 Hz, 2H), 6.25 (d, *J* = 2.0 Hz, 1H), 5.91 (s, 1H), 5.07 (s, 2H), 3.40 (ddd, *J* = 7.5, 5.6, 1.9 Hz, 1H), 2.92 (d, *J* = 13.7 Hz, 1H), 2.72 – 2.65 (m, 4H), 2.54 (dd, *J* = 13.0, 6.1 Hz, 1H), 2.44 (dd, *J* = 13.8, 8.2 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 159.9, 156.7, 137.2, 136.3, 130.9, 130.3, 130.3, 128.4, 128.0, 127.7, 127.6, 126.4, 114.6, 88.9, 69.1, 57.7, 42.1, 34.9, 24.2. HRMS-ESI (*m/z*): calcd for $C_{25}H_{26}N_2NaO_3$ [M + Na]$^+$, 425.1836; found, 425.1820. ν_{max} (ATR-IR) cm^{-1} 3294, 2924, 2854, 1704. $[\alpha]_D^{22}$ = -276 (c = 0.077, MeOH).

3'a-[(4-methoxyphenyl)methyl]-3'-methyl-6',6'a-dihydro-1'H-spiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-diene-2',4-dione (6). The aminol **14** (122 mg, 0.356 mmol) was taken up in 2,2,2-trifluoroethanol (2.1 mL) and cooled to 0 °C. PIDA (126 mg, 0.392 mmol) was added and the solution was stirred at 0 °C for 10 min. The reaction mixture was quenched with $Na_2S_2O_3$ (1 M, 5 mL) and partitioned between EtOAc (15 mL) and H_2O (15 mL). The aqueous layer was extracted with EtOAc (3 x 15 mL) and the combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography using EtOAc as the eluent yielded the title compound (51 mg, 42%) as a white crystalline solid. m.p. 122-123 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (d, *J* = 8.5 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.84 (dd, *J* = 10.3, 3.0 Hz, 1H), 6.34 (dd, *J* = 10.1, 3.0 Hz, 1H), 6.12 (dd, *J* = 10.1, 3 Hz, 1H), 6.09 (br s, 1H), 6.06 (dd, *J* = 10.1, 3.0 Hz, 1H), 4.24 (dd, *J* = 6.5, 1.5 Hz, 1H), 3.82 (s, 3H), 3.36 and 2.84 (abq, *J* = 14.1, 2H), 2.93 (s, 3H), 1.83 (d, *J* = 13.8 Hz, 1H), 1.45 (dd, *J* = 13.8, 6.8 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.0, 159.5, 159.1, 149.2, 148.8, 131.8, 129.0, 127.3, 126.1, 113.9, 102.7, 79.7, 58.3, 55.4, 44.8, 41.1, 25.1. HRMS-ESI (*m/z*): calcd for $C_{19}H_{20}N_2NaO_4$ [M + Na]$^+$, 363.1315; found 363.1342. ν_{max} (ATR-IR) cm^{-1} 3277, 2956, 2851, 1697, 1670, 1631, 1513, 1440, 1400, 1302, 1172, 1067, 1030, 943, 827. $[\alpha]_D^{24}$ = -37.3 (c = 0.53, MeOH).

5-benzyl-5-hydroxy-4-[(4-hydroxyphenyl)methyl]-1-methylimidazolidin-2-one (14). The benzyl ether **13** (191 mg, 0.475 mmol) and K_2HPO_4 (82.7 mg, 0.475 mmol) were suspended in MeOH (8 mL). To this was added $Pd(OH)_2$/C (19 mg, 10% wt.) and the system was purged with H_2. The reaction mixture was stirred at room temperature for 90 min. Following this, it was diluted with EtOAc (50 mL), filtered through a plug of celite and concentrated *in vacuo* to yield the title compound solid (136 mg, 92%) as a white solid. m.p. 164–165 °C. ^{1}H NMR (400 MHz, DMSO) δ 9.17 (s, 1H), 7.33 – 7.13 (m, 3H), 7.00 (d, *J* = 6.8 Hz, 2H), 6.89 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 2H), 6.20 (s, 1H), 5.89 (s, 1H), 3.38 (td, *J* = 6.0, 2.9 Hz, 1H), 2.92 (d, *J* = 13.6 Hz, 1H), 2.71 – 2.64 (m, 4H), 2.54 – 2.51 (m, 1H), 2.39 (dd, *J* = 13.9, 7.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 159.9, 155.6, 136.4, 130.4, 130.2, 128.7, 127.9, 126.4, 115.0, 88.9, 57.8, 42.1, 34.9, 24.3. HRMS-ESI (*m/z*): calcd for $C_{18}H_{20}N_2NaO_3$ [M + Na]$^+$, 335.1366; found, 335.1366. ν_{max} (ATR-IR) cm^{-1} 3375, 3033, 2920, 1693, 1679, 1589, 1493, 1048. $[\alpha]_D^{20}$ = +27.5 (c = 1.01, MeOH).

3'a-benzyl-3'-methyl-6',6'a-dihydro-1'H-spiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-diene-2',4-dione (15). The aminol **14** (146 mg, 0.467 mmol) was taken up in 2,2,2-trifluoroethanol (3 mL) and cooled to 0 °C. PIDA (166 mg, 0.514 mmol) was added and the solution was stirred at 0 °C for 30 min. The reaction mixture was quenched with $Na_2S_2O_3$ (1 M, 5 mL) and partitioned between EtOAc (15 mL) and H_2O (15 mL). The aqueous layer was extracted with EtOAc (3 x 15 mL) and the combined organic extracts were washed with brine (30 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography using EtOAc as the eluent yielded the title compound (72.7 mg, 51%) as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.26 (m, 5H), 6.84 (dd, *J* = 10.3, 3.1 Hz, 1H), 6.30 (dd, *J* = 10.0, 3.0 Hz, 1H), 6.11 (dd, *J* = 10.1, 2.0 Hz, 1H), 6.07 (dd, *J* = 10.3, 2.0 Hz, 1H), 5.81 (s, 1H), 4.26 (d, *J* = 6.5 Hz, 1H), 3.43 (d, *J* = 13.7 Hz, 1H), 2.96 (s, 3H), 2.92 (d, *J* = 13.7 Hz, 1H), 1.86 (d, *J* = 13.8 Hz, 1H), 1.41 (dd, *J* = 13.8, 6.5 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.0, 159.3, 149.1, 148.7, 134.2, 130.8, 129.0, 128.6, 127.8, 127.4, 102.6, 79.7, 58.3,

44.8, 42.1, 25.1. HRMS-ESI (*m/z*): calcd for $C_{18}H_{18}N_2NaO_3$ [M + Na]$^+$, 333.1210; found, 333.1192. ν_{max} (ATR-IR) cm^{-1} 2925, 2853, 1698, 1667, 1627, 1435, 1397, 1242. $[\alpha]_D^{20}$ = +6.2 (c = 0.11, MeOH).

3'a-benzyl-3'-methyl-2',4-dioxo-1',6'-dihydrospiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-dien-6'a-yl acetate (16). To a solution of spirocycle **15** (56 mg, 0.181 mmol) in fluorobenzene (3.5 mL) was added Dess-Martin periodinane (192 mg, 0.454 mmol) at room temperature. The reaction was then heated to 60 °C for 75 min. The reaction mixture was then cooled to room temperature and quenched with solid $NaHCO_3$, filtered through a plug of silica, and concentrated *in vacuo*. Column chromatography eluting with 1:1 EtOAc to PE afforded the title compound (54 mg, 81%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.28 (m, 4H), 6.85 (dd, *J* = 10.2, 3.0 Hz, 1H), 6.30 (s, 1H), 6.08 (dd, *J* = 10.2, 2.0 Hz, 1H), 6.04 (dd, *J* = 10.0, 2.0 Hz, 1H), 5.94 (dd, *J* = 10.0, 3.0 Hz, 1H), 3.40 (d, *J* = 14.4 Hz, 1H), 3.21 (d, *J* = 14.4 Hz, 1H), 2.93 (s, 3H), 2.81 (d, *J* = 13.7 Hz, 1H), 2.20 (s, 3H), 1.86 (d, *J* = 13.7 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.6, 170.2, 156.7, 148.0, 147.4, 134.5, 132.0, 129.2, 128.1, 127.8, 127.5, 101.9, 95.1, 78.0, 47.8, 39.0, 25.5, 21.6. HRMS-ESI (*m/z*): calcd for $C_{20}H_{20}N_2NaO_5$ [M + Na]$^+$, 391.1264; found, 391.1226. ν_{max} (ATR-IR) cm^{-1} 2930, 1714, 1670, 1632, 1435. 1396, 1370, 1236. $[\alpha]_D^{20}$ = +30.4 (c = 0.32, MeOH).

3'a-benzyl-3'-methyl-6'a-(methylamino)-1',6'-dihydrospiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-diene-2',4-dione (17). To a solution of acetate **16** (10 mg, 0.027 mmol) in THF (300 µL) at room temperature was added methylamine (2 M, 27 µL, 0.054 mmol). The reaction was stirred at room temperature for 3 h before being quenched with water (5 mL). The aqueous layer was then extracted with EtOAc (3 x 5 mL) and the combined organic layers washed with brine (10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified using column chromatography, eluting with EtOAc to yield the title compound (5.2 mg, 57%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 – 7.45 (m, 2H), 7.39 – 7.29 (m, 3H), 7.00 (dd, *J* = 10.3, 3.0 Hz, 1H), 6.05 (dd, *J* = 10.3, 2.0 Hz, 1H), 5.99 (dd, *J* = 10.1, 2.0 Hz, 1H), 5.82 (dd, *J* = 10.1, 3.1 Hz, 1H), 5.80 (br s, 1H), 3.23 (ABq, *J* = 14.6 Hz, 2H), 2.90 (s, 3H), 2.49 (s, 3H), 2.15 (d, *J* = 13.2 Hz, 1H), 1.96 (d, *J* = 13.2 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.0, 158.6, 149.4, 148.9, 135.3, 131.8, 128.6, 128.3, 127.4, 127.3, 102.3, 82.9, 77.3, 48.6, 38.8, 29.2, 25.9. HRMS-ESI (*m/z*): calcd for $C_{19}H_{21}N_3NaO_3$ [M + Na]$^+$, 362.1475; found, 362.1476. ν_{max} (ATR-IR) cm^{-1} 3062, 2929, 1696, 1668, 1629, 1438, 1395, 1091. $[\alpha]_D^{20}$ = +6.7 (c = 0.23, MeOH).

3'a-benzyl-3'-methyl-6'a-(piperidin-1-yl)-1',6'-dihydrospiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-diene-2',4-dione (18). To a solution of acetate **16** (10 mg, 0.027 mmol) in THF (300 µL) at room temperature was added piperidine (5.4 µL, 0.054 mmol). The reaction was stirred at room temperature for 3 h before being quenched with water (5 mL). The aqueous layer was then extracted with EtOAc (3 x 5 mL) and the combined organic layers washed with brine (10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified using gradient eluted column chromatography, eluting with 1:1 EtOAc to PE to 3:2 EtOAc to PE to yield the title compound (10 mg, 96%) as an orange wax. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 – 7.60 (m, 2H), 7.39 – 7.30 (m, 3H), 7.05 (dd, *J* = 10.3, 3.0 Hz, 1H), 6.16 (s, 1H), 5.98 (dd, *J* = 10.3, 2.1 Hz, 1H), 5.84 (dd, *J* = 10.1, 2.0 Hz, 1H), 5.15 (dd, *J* = 10.1, 3.0 Hz, 1H), 3.21 (ABq, *J* = 14.0 Hz, 2H), 2.99 (br s, 1H), 2.94 (s, 3H), 2.84 (br s, 1H), 2.48 – 2.22 (br m, 1H), 2.07 (d, *J* = 12.9 Hz, 1H), 1.97 (d, *J* = 12.9 Hz, 1H), 1.84 (br s, 2H), 1.82 – 1.69 (br m, 4H), 1.41 – 1.21 (br s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.0, 158.6, 149.4, 148.9, 135.3, 131.8, 128.6, 128.3, 127.4, 127.3, 102.3, 82.9, 77.3, 48.6, 38.8, 29.2, 25.9. HRMS-ESI (*m/z*): calcd for $C_{23}H_{28}N_3O_3$ [M + H]$^+$, 394.21252; found, 394.21470. ν_{max} (ATR-IR) cm^{-1} 2932, 2852, 1762, 1691, 1631, 1452, 1444, 1395, 1240. $[\alpha]_D^{20}$ = +17.8 (c = 0.27, MeOH).

3'a-benzyl-3'-methyl-6'a-(morpholin-4-yl)-1',6'-dihydrospiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-diene-2',4-dione (19). To a solution of acetate **16** (10 mg, 0.027 mmol) in THF (300 µL) at room temperature was added morpholine (4.7 µL, 0.054 mmol). The reaction was stirred at room temperature for 3.5 h before an additional aliquot of morpholine (2.4 µL, 0.027 mmol) was added. The reaction was stirred for 1.5 h before a final aliquot of morpholine (2.4 µL, 0.027 mmol) was added. The reaction was then stirred a further 1.5 h before being quenched with water (5 mL). The aqueous layer was then extracted with EtOAc (3 x 5 mL) and the combined organic layers washed with brine (10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified using gradient eluted column chromatography, eluting with 3:2 EtOAc to PE to neat EtOAc to yield the title compound (8.5 mg, 79%) as a yellow-orange waxy solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 – 7.60 (m, 2H), 7.41 – 7.32 (m, 3H), 7.04 (dd, *J* = 10.4, 3.1 Hz, 1H), 6.85 (s, 1H), 6.00 (dd, *J* = 10.3, 2.0 Hz, 1H), 5.87 (dd, *J* = 10.2, 2.0 Hz, 1H), 5.19 (dd, *J* = 10.1, 3.0 Hz, 1H), 3.88 (br s, 4H), 3.22 (s, 2H), 2.93 (s, 3H), 2.78 (br s, 2H), 2.66 (br s, 2H), 2.09 (d, *J* = 12.9 Hz, 1H), 1.99 (d, *J* = 12.9 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.0, 160.0, 149.5, 135.5, 132.7, 128.3, 128.1, 127.5, 127.1, 103.0, 84.9, 77.4, 67.4, 66.4, 48.8, 46.8, 46.5, 37.9, 29.8, 26.0. HRMS-ESI (*m/z*): calcd for $C_{22}H_{25}N_3NaO_4$ [M + Na]$^+$, 418.1737; found, 418.1709. ν_{max} (ATR-IR) cm^{-1} 2951, 2858, 1719, 1695, 1666, 1618, 1512, 1425, 1249, 1091. $[\alpha]_D^{20}$ = +36.6 (c = 0.16, MeOH).

3'a-benzyl-6'a-(butylamino)-3'-methyl-1',6'-dihydrospiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-diene-2',4-dione (21). To a solution of acetate **16** (10 mg, 0.027 mmol) in THF (300 µL) at room temperature was added butylamine (5.4 µL, 0.054 mmol). The reaction was stirred at room temperature for 3.5 h before being quenched with water (5 mL). The aqueous layer was then extracted with EtOAc (3 x 5 mL) and the combined organic layers washed with brine (10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified using column chromatography, eluting with 3:2 EtOAc to PE to yield the title compound (8.1 mg, 78%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.47 (m, 2H), 7.39 – 7.29 (m, 3H), 6.99 (dd, *J* = 10.3, 3.1 Hz, 1H), 6.04 (dd, *J* = 10.3, 2.1 Hz, 1H), 5.98 (dd, *J* = 10.1, 2.0 Hz, 1H), 5.81 – 5.72 (m, 2H), 3.22 (ABq, *J* = 14.4 Hz, 2H), 2.91 (s, 3H), 2.74 – 2.65 (m, 1H), 2.65 – 2.56 (m, 1H), 2.16 (d, *J* = 13.1 Hz, 1H), 2.01 (d, *J* = 13.1 Hz, 1H), 1.57 – 1.47 (m, 2H), 1.44 – 1.34 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 185.1, 158.6, 149.5, 149.1, 135.4, 131.9, 128.4, 128.2, 127.4, 127.3, 102.6, 82.5, 77.5, 48.8, 42.6, 38.7, 32.9, 25.9, 20.6, 14.1. HRMS-ESI (*m/z*): calcd for $C_{22}H_{27}N_3NaO_3$ [M + Na]$^+$, 404.1945; found, 404.1928. ν_{max} (ATR-IR) cm^{-1} 2958, 2928, 2858, 1699, 1670, 1435, 1394, 1258. $[\alpha]_D^{20}$ = +22.6 (c = 0.22, MeOH).

6'a-(butylamino)-3'a-[(4-methoxyphenyl)methyl]-3'-methyl-1',6'-dihydrospiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-diene-2',4-dione (22). To a solution of the acetate derivative **20** (30.2 mg, 0.0758 mmol) in THF (3 mL) was added butylamine (25.0 µL, 0.252 mmol). This was stirred at room temperature for 2 h, then concentrated *in vacuo*. The crude residue was purified using column chromatography with EtOAc to afford the title compound (14.6 mg, 47%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 – 7.37 (m, 2H), 6.98 (dd, *J* = 10.3, 3.1 Hz, 1H), 6.90 – 6.84 (m, 2H), 6.06 – 5.99 (m, 2H), 5.93 (s, 1H), 5.90 (dd, *J* = 10.0, 3.1 Hz, 1H), 3.82 (s, 3H), 3.14 (ABq, J = 14.5 Hz, 2H), 2.89 (s, 3H), 2.72 – 2.65 (m, 1H), 2.64 – 2.56 (m, 1H), 2.17 (d, *J* = 13.2 Hz, 1H), 2.00 (d, *J* = 13.1 Hz, 1H), 1.55 – 1.47 (m, 2H), 1.44 – 1.33 (m, 2H), 0.94 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.1, 158.9, 158.8, 149.7, 149.1, 132.9, 128.4, 127.3, 127.2, 113.5, 102.6, 82.6, 77.4, 55.4, 48.8, 42.7, 37.9, 32.9, 25.9, 20.6, 14.1. HRMS-ESI (*m/z*): calcd for $C_{23}H_{29}N_3NaO_4$ [M + Na]$^+$, 434.2050; found, 434.2045. ν_{max} (ATR-IR) cm^{-1} 2957, 2929, 2857, 1702, 1665, 1511, 1438, 1392, 1245, 1084. $[\alpha]_D^{20}$ = +21.3 (c = 0.22, MeOH).

3'a-benzyl-3'-methyl-6'a-(prop-2-yn-1-ylamino)-1',6'-dihydrospiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-diene-2',4-dione (23). To a solution of acetate **16** (6.0 mg, 0.016 mmol) in THF (2 mL) was added propargyl amine (17 µL, 0.265 mmol). The reaction was stirred at room temperature for 3 h before being quenched with water (3 mL). The aqueous layer was extracted with EtOAc (3 x 3 mL) and the combined organic extracts were washed with brine (3 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude

residue was purified using column chromatography, eluting with EtOAc to yield the title compound as a pale orange solid (4.8 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (m, 1H), 7.38 – 7.29 (m, 3H), 6.99 (dd, *J* = 10.3, 3.1 Hz, 1H), 6.06 (dd, *J* = 10.4, 2.0 Hz, 1H), 6.00 (dd, *J* = 10.1, 2.0 Hz, 1H), 5.83 (s, 1H), 5.79 (dd, *J* = 10.0, 3.1 Hz, 1H), 3.66 (dd, *J* = 17.8, 2.5 Hz, 1H), 3.47 (dd, *J* = 17.8, 2.3 Hz, 1H), 3.22 (ABq, *J* = 14.4 2H), 2.91 (s, 3H), 2.37 (t, *J* = 2.4 Hz, 1H), 2.29 (d, *J* = 13.2 Hz, 1H), 2.00 (d, *J* = 13.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.8, 157.8, 149.1, 148.6, 135.0, 131.8, 128.5, 128.1, 127.3, 127.2, 103.0, 82.3, 77.4, 73.3, 47.3, 38.7, 32.8, 29.7, 25.8. HRMS-ESI (*m/z*): calcd for $C_{21}H_{21}N_3NaO_3$ $[M+Na]^+$, 386.14751; found 386.14534. ν_{max} (ATR-IR) cm^{-1} 3304, 3297, 2954, 2923, 2852, 1703, 1671, 1453, 1395, 1257. $[\alpha]_D^{23}$ = +27.9 (c = 0.14, MeOH).

3'a-benzyl-6'a-(benzylamino)-3'-methyl-1',6'-dihydrospiro[cyclohexane-1,5'-furo[2,3-d]imidazole]-2,5-diene-2',4-dione (24). To a solution of acetate **16** (6.0 mg, 0.016 mmol) in THF (200 µL) at room temperature was added benzylamine (3.6 µL, 0.033 mmol). The reaction was stirred at room temperature for 1.5 h before being quenched with water (5 mL). The aqueous layer was then extracted with EtOAc (3 x 5 mL) and the combined organic layers washed with brine (10 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified using column chromatography, eluting with 3:2 EtOAc to PE to yield the title compound (4.6 mg, 68%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 – 7.46 (m, 2H), 7.42 – 7.27 (m, 8H), 7.00 (dd, *J* = 10.3, 3.1 Hz, 1H), 6.05 (dd, *J* = 10.3, 2.0 Hz, 1H), 6.00 (dd, *J* = 10.1, 2.1 Hz, 1H), 5.81 (dd, *J* = 10.1, 3.1 Hz, 1H), 5.40 (s, 1H), 3.87 (br s, 2H), 3.28 (ABq, *J* = 14.4 Hz, 2H), 2.93 (s, 3H), 2.22 (d, *J* = 13.4 Hz, 1H), 2.08 (d, *J* = 13.2 Hz, 1H), 1.89 (br s, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 185.0, 158.3, 149.4, 148.9, 139.1, 135.3, 131.9, 129.1, 128.6, 128.2, 128.1, 127.9, 127.5, 127.4, 102.7, 82.5, 77.3, 48.8, 47.5, 38.9, 26.0. HRMS-ESI (*m/z*): calcd for $C_{25}H_{25}N_3NaO_3$ $[M + Na]^+$, 438.17881; found, 438.17697. ν_{max} (ATR-IR) cm^{-1} 3375, 3253, 2955, 2856, 1700, 1248, 697. $[\alpha]_D^{22}$ = +14.3 (c = 0.14, MeOH).

Acknowledgements

We acknowledge the University of Otago for a Research grant (UORG). MPB and APC gratefully acknowledge the University of Otago for the provision of PhD scholarships. We thank the National Cancer Institute (NCI) for the preliminary testing of spiroleucettadine against the NCI-60 panel of cancer cells.

Keywords: total synthesis • alkaloids • oxidative spirocyclization • hypervalent iodine • medicinal chemistry

[1] a) S. Carmelya, M. Ilanb, Y. Kashmana, *Tetrahedron* **1989**, *45*, 2193-2200; b) P. B. Koswatta, C. J. Lovely, *Nat. Prod. Rep.* **2011**, *28*, 511-528.

[2] a) B. R. Copp, C. R. Fairchild, L. Cornell, A. M. Casazza, S. Robinson, C. M. Ireland, *J. Med. Chem.* **1998**, *41*, 3909-3911; b) D. V. LaBarbera, K. Modzelewska, A. I. Glazar, P. D. Gray, M. Kaur, T. Liu, D. Grossman, M. K. Harper, S. K. Kuwada, N. Moghal, C. M. Ireland, *Anti-Cancer Drugs* **2009**, *20*, 425-436.

[3] R. Singh, C. Lovely, **2019**, pp. 43-79.

[4] a) P. Ralifo, P. Crews, *J. Org. Chem.* **2004**, *69*, 9025-9029; b) K. N. White, T. Amagata, A. G. Oliver, K. Tenney, P. J. Wenzel, P. Crews, *J. Org. Chem.* **2008**, *73*, 8719-8722; c) Y. Nagasawa, H. Kato, H. Rotinsulu, R. E. P. Mangindaan, N. J. de Voogd, S. Tsukamoto, *Tetrahedron Lett.* **2011**, *52*, 5342-5344.

[5] a) R. A. Lamb, N. S. Aberle, N. T. Lucas, G. Lessene, B. C. Hawkins, *Angew. Chem. Int. Ed.* **2017**, *56*, 14663-14666; b) R. A. Lamb, G. Lessene, B. C. Hawkins, *Synlett* **2018**, *29*, 1125-1130.

[6] *See Supporting Information for details*.

[7] P. Wayne, *M100 Performance Standards for Antimicrobial Susceptibility Testing*, 27th ed., CLSI, **2017**.

[8] R. A. Lamb, N. T. Lucas, G. Lessene, B. C. Hawkins, *J. Org. Chem.* **2018**, *83*, 10120-10133.

[9] Y. Tamura, T. Yakura, J. Haruta, Y. Kita, *J. Org. Chem.* **1987**, *52*, 3927-3930.

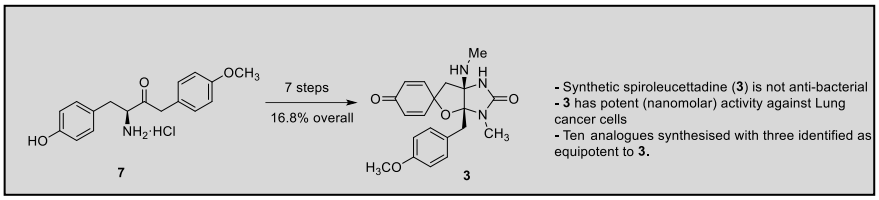
[10] L. Verna, J. Whysner, G. M. Williams, *Pharmacol. Ther.* **1996**, *71*, 83-105.

[11] a) E. E. Meyer, K. J. Rosenberg, J. Israelachvili, *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 15739-15746; b) A. Sarkar, G. E. Kellogg, *Curr. Top. Med. Chem.* **2010**, *10*, 67-83.

[12] W. Liu, H. Li, P.-J. Cai, Z. Wang, Z.-X. Yu, X. Lei, *Angew. Chem. Int. Ed.* **2016**, *55*, 3112-3116.

10.1002/cmdc.202000954

Entry for the Table of Contents



The second-generation synthesis of spiroleucettadine is described along with its unexpected anti-proliferative properties. This improved synthesis enabled access to large quantities of both enantiomers of spiroleucettadine and structural analogues. Three spiroleucettadine analogues were found to possess similar potency as spirolecuettadine against lung cancer cells.

Institute and/or researcher Twitter usernames: @HawkinsLab4