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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 6179–6185
Bioorganic & Medicinal Chemistry

Anti-*Helicobacter pylori* activity of derivatives of the phthalide-containing antibacterial agents spirolaxine methyl ether, CJ-12,954, CJ-13,013, CJ-13,102, CJ-13,104, CJ-13,108 and CJ-13,015

Fiona J. Radcliff,[a,c] John D. Fraser,[a,c,*] Zoe E. Wilson,[b,c] Amanda M. Heapy,[b,c] James E. Robinson,[b,c] Christina J. Bryant,[b,c] Christopher L. Flowers[b,c] and Margaret A. Brimble[b,c,*]

[a]*Department of Molecular Medicine and Pathology, University of Auckland, 85 Park Road, Grafton, Auckland 1142, New Zealand*
[b]*Department of Chemistry, University of Auckland, 23 Symonds Street, Auckland 1142, New Zealand*
[c]*Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand*

Received 13 February 2008; revised 12 April 2008; accepted 16 April 2008
Available online 18 April 2008

Abstract—The naturally occurring phthalide-containing antibiotics spirolaxine methyl ether, CJ-12,954, CJ-13,013, CJ-13,015, CJ-13,102, CJ-13,103, CJ-13,104 and CJ-13,108, have been reported to exhibit anti-*H. pylori* activity. However, the exact stereochemistry of spirolaxine methyl ether, CJ-12,954 or CJ-13,013, contributing to this observed activity has not been confirmed. The anti-*H. pylori* activity of several analogues of spirolaxine methyl ether, CJ-12,954 and CJ-13,013 of defined stereochemistry together with the anti-*H. pylori* activity of several indole analogues of the simpler phthalide-containing antibiotics CJ-13,102, CJ-13,104, CJ-13,108 and CJ-13,015 is reported herein. A 1:1 mixture of spiroacetals **5b** and **6b** in which the phthalide substituent exhibited (3*R*)-stereochemistry was sixty times more active than the corresponding 1:1 mixture of spiroacetals with (3*S*)-stereochemistry. Notably, the unnatural (2″*S*)-diastereomer of spirolaxine methyl ether exhibited more potent anti-*H. pylori* activity than the natural product spirolaxine methyl ether. The 4,6-dimethoxyindoles **9**, **10**, **11** and **13** were all found to be less active than their parent compounds **1**, **2**, **3** and **4**, respectively. Chain-shortened 4,6-dimethoxyindole analogue **12** of CJ-13,108 **3** and 4,6-dimethoxyindole-spiroacetal **13** exhibited weak anti-*H. pylori* activity thus providing future opportunity for drug discovery programs.

1. Introduction

Helicobacter pylori are microaerophilic Gram-negative bacteria[1] that infect over 50% of the human population.[2] Infection has been associated with gastric and duodenal ulcers, distal gastric cancer and mucosal-associated lymphoid tissue (MALT) lymphoma (cancer of the B cell lymphocytes).[3] As a result of the latter, the International Agency for Research in Cancer classified *H. pylori* as a class I carcinogen in 1994.[2,3] In most cases infection will persist for the lifetime of an individual without medical intervention.[4]

The current available treatments for *H. pylori* infections are complex, involving multiple broad-spectrum antibiotics in combination with proton pump inhibitors and/or bismuth salts. Less than 80% of patients are sucessfully treated by first line therapy and as a result, second-line or rescue treatments are often required.[5] Treatment failure is associated with the emergence of *H. pylori* strains that are resistant to the commonly used broad-spectrum antibiotics clarithromycin and metronidazole.[5] Consequently, there is considerable need for the development of a novel, specific antibiotic against *H. pylori*, preferably one that can be used effectively as a monotherapy, allowing widespread eradication of the bacteria and a reduction in the incidence of its associated diseases.[6]

In a screening program designed to discover such compounds, Dekker et al.[7] isolated several new phthalide antibiotics with specific anti-*H. pylori*

Keywords: Anti-*Helicobacter pylori* agents; Phthalide; Indole.
* Corresponding authors. Tel.: +64 9 373 7599x88259; fax: +64 9 373 7422 (M.A.B.); e-mail: m.brimble@auckland.ac.nz

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doi:10.1016/j.bmc.2008.04.037

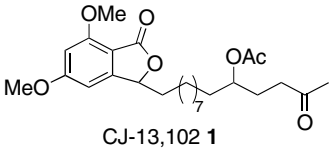
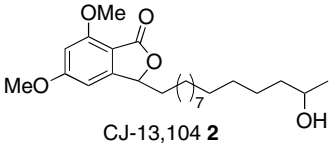
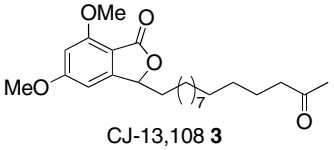
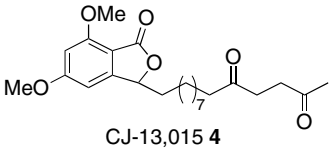
activity (**1**–**6**) from the basidiomycete *Phanerochaete velutina* CL6387. The absolute stereochemistry of these natural products was not confirmed thereby limiting the ability to construct any detailed structure–activity relationship based on these naturally occurring compounds. Two structurally related compounds, spirolaxine **7**[8a] and its methyl ether **8**[8b] have also been isolated from *Sporotrichum laxum*, *S. pruinosum* and *Phanerochaete chrysosporium*. Whilst the anti-*H. pylori* activity of spirolaxine was reported, any anti-*H. pylori* activity of spirolaxine methyl ether **8** was not mentioned.[8] Moreover, the stereochemistry of the natural spirolaxine used for the biological evaluation was not determined. Thus, whilst these phthalide-containing compounds provide promising new leads for the treatment of *H. pylori*-related diseases, any further development hinges on the establishment of detailed correlation of the observed anti-*H. pylori* activity with the structures of defined stereochemistry. We therefore herein report the anti-*H. pylori* activity of synthetic analogues of the spiroacetal-containing phthalides **5**, **6** and **8** that were prepared with defined absolute chemistry. We also report the anti-*H. pylori* activity of several 4,6-dimethoxyindole isosteres of the simpler phthalide-containing natural products CJ-13,102, CJ-13,104, CJ-13,108 and CJ-13,015.

2. Results and discussion

Initial attention focused on the anti-*H. pylori* activity of the simple phthalides CJ-13,102 **1**, CJ-13,104 **2**, CJ-13,108 **3** and CJ-13,015 **4** (Table 1). Samples of these compounds as racemic mixtures have recently been prepared by our group by Wittig coupling of a phthalide-containing aldehyde fragment with an appropriate phosphorus ylide.[9] Ready availability of these compounds allowed the comparison of our anti-*H. pylori* activity with the reported activity of the naturally occurring compounds without the consideration of more complex relative and absolute stereochemical issues associated with the spiroacetal-containing phthalides CJ-12,954 **5**, CJ-13,014 **6**, spirolaxine **7** and spirolaxine methyl ether **8**.

The methods used for determining anti-*H. pylori* activity differ from those originally employed for characterizing the activity of these phthalides,[7] but have the advantage of enabling the determination of the MIC and MBC for each compound. The MIC and MBC values for the anti-*H. pylori* activity of phthalides **1**–**4** using *H. pylori* strain 11637 are summarized in Table 1. The synthesized forms of CJ-13,102 **1**, CJ-13,108 **3** and CJ-13,015 **4** are active against *H. pylori* with a similar hierarchy of activity as the published data.[7] Unexpectedly, CJ-13,104 **2** had a comparable MIC to phthalides **1**, **3** and **4**, whereas it

Table 1. Anti-*H. pylori* activity of phthalides **1**–**4**

Compound	Anti-*H. pylori* activity (μg/mL)[a]		Published anti-*H. pylori* activity (μg)[b]
	MIC	MBC	
 CJ-13,102 **1**	1.25	2.5	0.5
 CJ-13,104 **2**	12.5	50	500
 CJ-13,108 **3**	10	10–20[c]	10
 CJ-13,015 **4**	2.5	5	2

[a] Helicobactericidal activity (*H. pylori* strain 11637) of phthalides after a 24-h culture period in liquid medium to obtain a minimum inhibitory concentration (MIC), followed by serial dilutions and culture on solid media to obtain a minimum bactericidal concentration (MBC).
[b] μg/disk that gives a 15 mm zone of inhibition on solid media, see Ref. 7.
[c] Obtained variable results with CJ-13,108 **3** due to its limited solubility in DMSO.

was determined to be 50-fold less active in the original publication.

Having demonstrated the anti-*H. pylori* activity of the simple phthalides **1**–**4**, we next investigated the anti-*H. pylori* activity of the more potent complex phthalides CJ-12,954 **5** and its C-5″ epimer CJ-13,014 **6** that contain a 5,5-spiroacetal ring joined through a polymethylene chain. The exact stereochemistry of the stereogenic centres on the phthalide ring and the 5,5-spiroacetal ring was not reported in the original isolation paper; however, the two epimeric spiroacetals were separable, and the same anti-*H. pylori* activity (0.02 μg) was reported for the individual epimers (albeit of ill-defined stereochemistry). Thus, the anti-helicobactericidal activity reported for these natural products indicated that stereochemistry associated with the spirocentre C-5″ had little effect on the activity whereas the dihydroxy ketone formed by ring opening, namely CJ-13,015 **4**, exhibited a decreased potency of 100-fold.[7]

In order to probe the detailed effect of the stereochemistry of the 5,5-spiroacetal ring and the phthalide substituent on the observed anti-*H. pylori* activity, we recently completed an enantioselective total synthesis of *ent*-CJ-12,954 **5a** and *ent*-CJ-13,014 **6a** based on the union of a heterocycle-activated spiroacetal-containing sulfone fragment with a phthalide-containing aldehyde fragment. Comparison of the ^{1}H and ^{13}C NMR data, optical rotations and HPLC retention times of the synthetic compounds (3*S*, 2″*S*, 5″*S*, 7″*S*)-**5a** and (3*S*, 2″*S*, 5″*R*, 7″*S*)-**6a** and the 3*R*-diastereomers (3*R*, 2″*S*, 5″*S*, 7″*S*)-**5b** and (3*R*, 2″*S*, 5″*R*, 7″*S*)-**6b** with the naturally occurring compounds confirmed that the synthetic isomers **5a** and **6a** were in fact enantiomeric to the natural products CJ-12,954 **5** and CJ-13,014 **6**.[10]

During the course of this synthetic investigation it was found that the separation of the individual C-5″ epimers was not possible due to rapid equilibration of the 5,5-spiroacetal spirocentre. Thus, only a 1:1 mixture of spiroacetals **5a** and **6a** and a 1:1 mixture of the corresponding spiroacetals **5b** and **6b** that were diastereomeric about the phthalide sterogenic centre were available for the anti-*H. pylori* assays (Table 2). These 1:1 mixtures of spiroacetals nevertheless revealed interesting structure–activity relationships. Notably, the 1:1 mixture of spiroacetals **5b** and **6b** in which the phthalide substituent exhibited (3*R*)-stereochemistry was sixty times more active than the corresponding 1:1 mixture of spiroacetals with (3*S*)-stereochemistry. Interestingly, the activity of the more potent 1:1 mixture of spiroacetals **5b** and **6b** was only approximately 10-fold less active than the values obtained for the individual spiroacetals **5** and **6** of ill-defined relative and absolute stereochemistry.

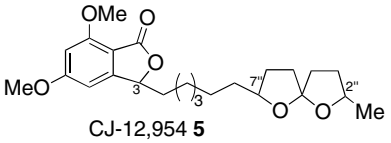
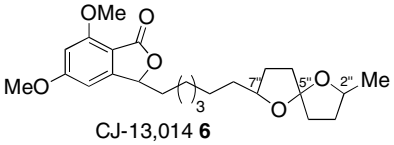
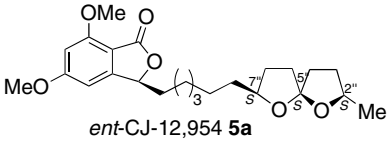
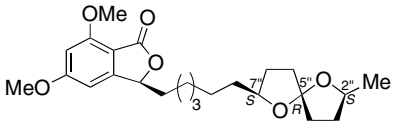
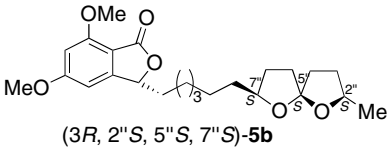
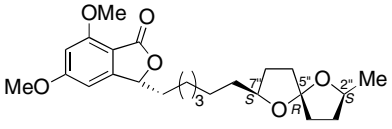
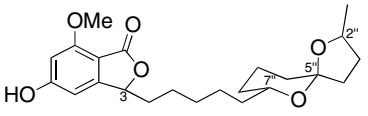
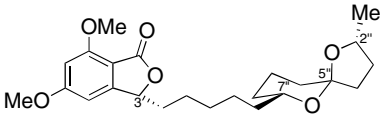
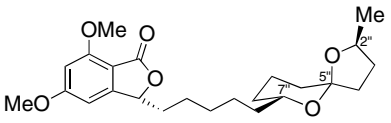
Our attention next focused on the anti-*H. pylori* activity of the 6,5-spiroacetal-containing phthalides spirolaxine **7** and spirolaxine methyl ether **8**. In contrast to the 5,5-spiroacetals 12,954 **5** and CJ-13,104 **6**, the structure–activity profiles of these 6,5-spiroacetals are not complicated by the inability to separate the individual isomers of the spiroacetal rings as the anomeric effect is operative in this case such that the 6,5-spiroacetal ring adopts the anomerically stabilized bis-axial conformation with the polymethylene chain at C-7″ occupying an equatorial position. Our research group completed the first enantioselective synthesis of the (+)-spirolaxine methyl ether **8a** establishing that the absolute stereochemistry of the natural products is in fact (3*R*, 2″*R*, 5″*R*, 7″*R*).[11] Our synthetic program also resulted in the synthesis of the unnatural (2″*S*)-diastereomer **8b** that was available for biological evaluation.

The anti-*H. pylori* activity of an authentic sample of spirolaxine **7** (of ill-defined stereochemistry) obtained from Pfizer had an MIC of 0.2 μg/mL in our assays (Table 2). In the absence of literature data available for spirolaxine methyl ether, the MIC value for our synthetic (3*R*, 2″*R*, 5″*R*, 7″*R*)-spirolaxine methyl ether **8a** (0.5 μg/mL) compared well with the value for naturally occurring spirolaxine **7**. More importantly, it was also found that the synthetic (2″*S*)-diastereomer of spirolaxine methyl ether **8b** exhibited 4-fold more anti-*H. pylori* activity in our assays than the natural compound **8a**. This result suggests that the exact stereochemistry of the 6,5-spiroacetal ring system is an important structural feature for the observed anti-*H. pylori* activity and that the conversion of the methyl ether to a free phenol is not necessary for the activity.

It was envisaged that the 4,6-dimethoxyindole would be a good bioisosteric replacement for the 5,7-dimethoxyphthalide unit. Thus, as an extension to our work on the synthesis of the above natural products we have also recently prepared[12] 4,6-dimethoxyindole analogues **9, 10, 11** and **13** of the simpler phthalides **1**–**4**. Additionally, the chain-shortened indole analogue **12** of CJ-13,108 **3** was synthesized for the present investigation via the N-alkylation of 4,6-dimethoxyindole with 10-bromodec-1-ene followed by the Wacker oxidation of the resultant alkene to a methyl ketone (Scheme 1). Indole analogue **9** of CJ-13,102 **1**, indole analogue **10** of CJ-13,104 **2**, indole analogue **11** of CJ-13,108 **3**, chain-shortened indole analogue **12** of CJ-13,108 **3** and indole analogue **13** of CJ-13,015 **4** were tested for anti-*H. pylori* activity (Table 3). The indole analogues **9**, **10**, **11** and **13** were all found to be less active (or inactive at the concentrations tested) than their parent compounds **1**, **2**, **3** and **4**, respectively. The notable exception was the chain-shortened indole analogue **12** of CJ-13,108 **3** that was twice as potent as the parent phthalide. This latter observation suggests that examination of the chain length in conjunction with bioisosteric replacement of the phthalide provides an opportunity for future drug discovery programs.

This latter result suggested that the incorporation of a spiroacetal ring system onto a chain-shortened 4,6-dimethoxyindole unit might be an exciting possibility to explore. The synthesis of indole-spiroacetal **14** was therefore undertaken due to the ready availability of a suitable spiroacetal moiety to append to a 4,6-dimethoxyindole unit. Indole-spiroacetal **14** was prepared via N-alkylation of 4,6-dimethoxyindole with bromo-spiroacetal **15** (Scheme 2). Unfortunately, indole-spiroacetal **14** exhibited weak anti-*H. pylori* activity (MIC 20 μg/mL).

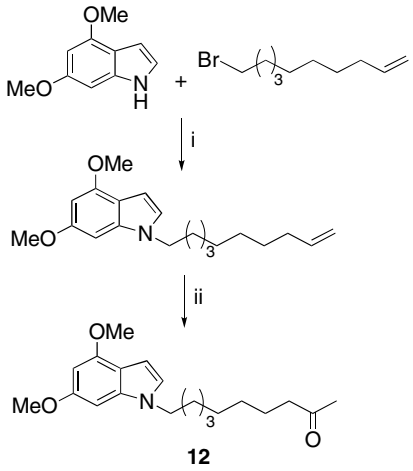
Table 2. Anti-*H. pylori* activity of spiroacetal-phthalides **5–8**

Compound	Anti-*H. pylori* activity (μg/mL)[a]		Published anti-*H. pylori* activity (μg)[b]
	MIC	MBC	
 CJ-12,954 **5** (natural product)	0.02[c]	0.02–0.2[c]	0.02
 CJ-13,014 **6** (natural product)	0.02[c]	0.2[c]	0.02
 *ent*-CJ-12,954 **5a** (3*S*, 2″*S*, 5″*S*, 7″*S*)  *ent*-CJ-13,014 **6a** (3*S*, 2″*S*, 5″*R*, 7″*S*)	20	100	—
 (3*R*, 2″*S*, 5″*S*, 7″*S*)-**5b**  (3*R*, 2″*S*, 5″*R*, 7″*S*)-**6b**	0.3	0.3	—
 spirolaxine **7** (natural product)	0.2	>2	0.01
 (3*R*, 2″*R*, 5″*R*, 7″*R*)-spirolaxine methyl ether **8a**	0.5	1	—
 (3*R*, 2″*S*, 5″*R*, 7″*R*)-**8b**	0.125	0.125	—

[a] Helicobactericidal activity (*H. pylori* strain 11637) of phthalides after a 24-h culture period in liquid medium to obtain a minimum inhibitory concentration (MIC), followed by serial dilutions and culture on solid media to obtain a minimum bactericidal concentration (MBC).
[b] μg/disk gives a 15 mm zone of inhibition on solid media, see Refs. 7 and 8.
[c] Sample obtained from Pfizer R&D, Groton, USA.

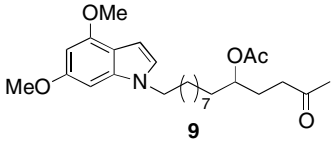
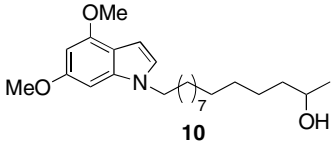
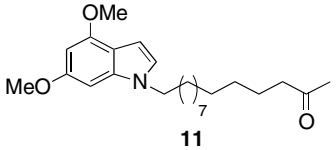
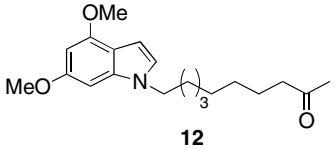
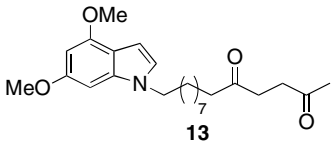
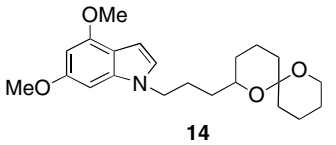
However, our assays may underestimate the potency of these compounds relative to the published values. Dekker et al.[7] determined that the MIC and MBC for spiroacetal **6** was 0.005 μg/mL using the methodology that our assays were based on. In contrast, we determined these values to be 0.02 and 0.2 μg/mL, respectively. These differences could be related to the use of different strains of *H. pylori* as our data are based on testing with the NCTC11637 type strain, rather than *H. pylori* 41. Our assays were validated with ampicillin, which was determined to have an MIC of 0.03 μg/mL and matches the expected MIC for this antibiotic.[13]

In summary, the anti-*H. pylori* activity of several analogues of the phthalide-containing natural products spirolaxine methyl ether, CJ-12,954, CJ-13,013, CJ-13,015,

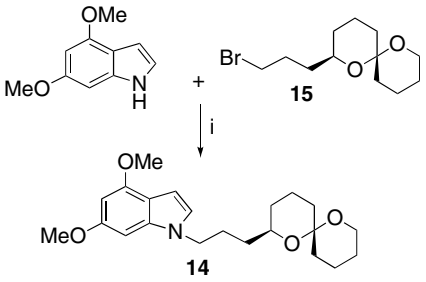


Scheme 1. Reagents and conditions: (i) KOH, DMSO, rt, 1.75 h, 82%; (ii) $PdCl_2$, CuCl, DMF, H_2O, O_2, rt, 0.75 h, 69%.

Table 3. Anti-*H. pylori* activity of indole analogues **9**–**12** of phthalides **1**–**4**

Compound	Anti-*H. pylori* activity (μg/mL)[a]	
	MIC	MBC
 **9**	>100	>100
 **10**	>100	>100
 **11**	50	50
 **12**	5	10
 **13**	10	20
 **14**	20	40

[a] Helicobactericidal activity (*H. pylori* strain 11637) of phthalides after a 24-h culture period in liquid medium to obtain a minimum inhibitory concentration (MIC), followed by serial dilutions and culture on solid media to obtain a minimum bactericidal concentration (MBC).



Scheme 2. Reagents and conditions: (i) KOH, DMSO, rt, 1.75 h, 75%.

CJ-13,102, CJ-13,103, CJ-13,104 and CJ-13,108 are reported herein. Notably, the effect of the stereochemistry of the spiroacetal ring systems present in spirolaxine methyl ether, CJ-12,954 and CJ-13,013, was probed with the observation that subtle changes to the stereochemistry of the methyl group at C-2″ do affect the observed anti-*H. pylori* activity. An examination of the effect of bioisosteric replacement of the phthalide unit with a 4,6-dimethoxyindole ring resulted in weak retention of anti-*H. pylori* activity with additional incorporation of a spiroacetal ring system in conjunction with varying the length of the polymethylene chain providing an avenue for future exploration.

3. Experimental

3.1. General methods (chemistry)

Analytical thin layer chromatography (TLC) was carried out on 0.20 mm pre-coated silica gel plates (ALUGRAM® SIL G/UV_{254}) and products were visualized by UV fluorescence. Flash chromatography was performed using Scharlau 60 (40–60 μm mesh) silica gel. Melting points in degrees Celsius (°C) were measured on an Electrothermal® melting point apparatus and are uncorrected. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AVANCE DRX400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) or a Bruker AVANCE 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer at 298 K. For ^{1}H NMR data, chemical shifts are described in parts per million (ppm) relative to tetramethylsilane (δ 0.00) and are reported consecutively as position (δ_H), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, qd = quartet of doublets, m = multiplet), coupling constant (*J*/Hz) and assignment. For ^{13}C NMR data, chemical shifts (ppm) are referenced internally to $CDCl_3$ (δ 77.0) and are reported consecutively as position (δ_C) and degree of hybridization. Assignments were aided by DEPT135, HSQC and HMBC experiments. Mass spectra were recorded on a VG-70SE mass spectrometer (EI, CI and FAB). High-resolution mass spectra were recorded at a nominal resolution of 5000.

Phthalides **1**–**4** were prepared following the previously reported methods.[9] Spiroacetals **5a/6a**, **5b/6b**, **8a** and

8b were prepared as reported in the appropriate total synthesis papers.[10,11] 4,6-Dimethoxyindoles **9, 10, 11** and **13** were prepared following our previously reported methods.[12]

(2*R*,6*R*)-2-(3-Bromopropyl)-1,7-dioxaspiro[5.5]undecane **15** was prepared via the treatment of the mesylate intermediate of the corresponding alcohol with lithium bromide following our literature procedure.[14] The corresponding alcohol was prepared via the addition of the acetylide derived from (4*S*)-4-benzyloxy-7-*tert*-butyldiphenylsilyloxyhep-1-yne to δ-valerolactone followed by the treatment with hydrogen and palladium on charcoal which effected hydrogenation of the alkyne, hydrogenolysis of the benzyl ether and subsequent spiroacetal formation.

3.1.1. 10-(4′,6′-Dimethoxy-1′H-indol-1′-yl)decan-2-one 12. Ground potassium hydroxide (0.51 g, 9.03 mmol) was mixed with dimethylsulfoxide (7.0 mL). 4,6-Dimethoxyindole (0.40 g, 2.26 mmol) was then added and the mixture stirred for 45 min at room temperature. 10-Bromodec-1-ene[14] (0.99 g, 4.52 mmol) was added dropwise to the solution, and the resultant mixture stirred for 45 min. Water (10 mL) was added and the reaction mixture extracted with diethyl ether (2× 20 mL). The aqueous layer was further extracted with ethyl acetate (2× 20 mL). The organic extracts were combined and washed with saturated $NaHCO_3$ (20 mL), brine (20 mL) and dried over $MgSO_4$. The solvent was removed in vacuo and the resultant yellow liquid purified by flash chromatography using 6:4 hexane-ethyl acetate as an eluent to afford 1-(9′-decen-1′-yl)-4,6-dimethoxy-1*H*-indole (0.58 g, 82%) as a yellow liquid. υ_{max} (film) 2995, 2927, 2853, 1622, 1588, 1499, 1455, 1251, 1209, 1147, 1069, 1049, 908, 805, 757, 707 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$), 1.28–1.47 (10H, m, 3′-H, 4′-H, 5′-H, 6′-H, 7′-H), 1.82–1.90 (2H, m, 2′-H), 2.05–2.10 (2H, m, 8′-H), 3.88 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 4.02 (2H, t, J = 7.1 Hz, NCH_2), 4.95–5.05 (2H, m, 10′-CH_2), 5.80–5.87 (1H, m, 9′-H), 6.24 (1H, s, 5-H), 6.42 (1H, s, 7-H), 6.52 (1H, d, J = 3.2 Hz, 3-H), 6.89 (1H, d, J = 3.2 Hz, 2-H); ^{13}C NMR (100 MHz, $CDCl_3$), 26.8, 28.0, 28.6, 28.8, 28.9, 29.1 (CH_2, C-2′, C-3′, C-4′, C-5′, C-6′, C-7′, C-8′), 46.3 (NCH_2), 55.1 (OCH_3), 55.5 (OCH_3), 85.3 (CH, C-7), 90.9 (CH, C-5), 98.0 (CH, C-3), 113.4 (quat., C-3a), 114.1 (CH_2, C-10′), 124.8 (CH, C-2), 137.1 (quat., C-7a), 138.9 (C–H, C-9′), 153.63 (quat., C-4) 157.1 (quat., C-6); *m*/*z* (EI^+, %) 328 (3), 316 (50), 315 (100), 314 (7), 190 (8), 176 (4), 120 (3), 91 (3), 90 (4), 89 (5). Found: M^+, 315.2194. $C_{20}H_{29}NO_2$ requires 315.2198.

The above alkene (50 mg, 0.16 mmol) was dissolved in dimethylformamide (1.5 mL), and this solution added to a mixture of $PdCl_2$ (15 mg, 0.079 mmol) and CuCl (20 mg, 0.194 mmol) in dimethylformamide (3 mL) and water (1 mL). Oxygen gas was bubbled through the solution for 1.75 h. The reaction mixture was filtered through silica and washed with ethyl acetate (100 mL) and hexane (50 mL). The volatile solvents were removed *in vacuo*, and dimethylformamide was removed under high vacuum at 40 °C to afford the title compound **12** (34 mg, 69%) as a grey solid. Mp 52–54 °C. υ_{max} (film) 2932, 2855, 1713 (C=O), 1621, 1586, 1499, 1456, 1265, 1211, 1148, 1068, 935; ^1H NMR (400 MHz, $CDCl_3$), 1.25–1.29 (8H, m, 8-H, 7-H, 6-H, 5-H), 1.49–1.62 (2H, m, 4-H), 1.79 (2H, t, J = 6.8 Hz, 9-H), 2.11 (3H, s, CH_3C=O), 2.38 (2H, t, J = 7.4 Hz, CH_2C=O), 3.85 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.00 (2H, t, J = 7.1 Hz, NCH_2), 6.21 (1H, s, 5′-H), 6.39 (1H, s, 7′-H), 6.48 (1H, d, J = 3.2 Hz, 3′-H), 6.87 (1H, d, J = 3.2 Hz, 2′-H); ^{13}C NMR (100 MHz, $CDCl_3$), 23.7, 26.8, 29.0, 29.1, 29.2, 30.0 (CH_2, C-9, C-8, C-7, C-6, C-5, C-4), 29.8 (CH_3, C-1), 43.6 (CH_2, C-3), 46.4 (CH_2, C-10), 55.3 (OCH_3), 55.7 (OCH_3), 85.5 (CH, C-7′), 91.0 (CH, C-5′), 98.0 (CH, C-3′), 113.5 (quat., C-3a′), 125.0 (CH, C-2′), 137.2 (quat., C-7a′), 153.7 (quat., C-4′), 157.2 (quat., C-6′), 209.1 (quat., C=O); *m*/*z* (EI^+, %) 332 (22), 331 (100), 316 (6), 191 (9), 190 (20), 176 (8), 69 (5), 55 (6), 43 (13), 41 (8). Found: M^+, 331.2144. $C_{20}H_{29}NO_3$ requires 331.2147.

3.1.2. 2-(3′-(4,6-Dimethoxy-(1*H*)indol-1-yl)prop-1-yl)-1,7-dioxaspiro[5.5]undecane 14. Ground potassium hydroxide (0.03 g, 0.43 mmol) was mixed with dimethylsulfoxide (1.5 mL) for 5 min. 4,6-Dimethoxyindole (0.04 g, 0.22 mmol) dissolved in dimethylsulfoxide (1.5 mL) was then added and the mixture stirred for 45 min at room temperature. 2-(3-(Bromo)-prop-1-yl)-1,7-dioxaspiro[5.5]undecane **15** (0.03 g, 0.11 mmol) also dissolved in dimethylsulfoxide (1.0 mL) was added dropwise to the solution, and the resultant mixture stirred for 45 min. Water (5 mL) was added and the reaction mixture extracted with diethyl ether (2× 15 mL). The aqueous layer was further extracted with ethyl acetate (2× 15 mL). The organic extracts were combined and washed with $NaHCO_3$ (15 mL), brine (15 mL) and dried over $MgSO_4$. The solvent was removed in vacuo and the resultant yellow oil purified by flash chromatography using 3:2 hexane-ethyl acetate as an eluent to afford the title compound **14** (0.03 g, 75%) as a yellow oil. υ_{max} (film) 2941, 2870, 2082, 1625, 1588, 1499, 1455, 1374, 1252, 1212, 1147 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$); 1.10–1.62 (10H, m, 2′-H, 1′-H, 3a-H, 4a-H, 5a-H, 9a-H, 10a-H, 11a-H), 1.76–1.94 (4H, m, 3b-H, 4b-H, 9b-H, 10b-H), 2.04–2.12 (2H, m, 5b-H, 11b-H), 3.57–3.60 (3H, m, 2-H, 8-H), 3.86 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.04–4.11 (2H, m, 3′-H), 6.22 (1H, d, J = 1.7 Hz, 5″-H), 6.42 (1H, d, J = 1.7 Hz, 7″-H), 6.50 (1H, d, J = 3.1 Hz, 3″-H), 6.90 (1H, d, J = 3.1 Hz, 2″-H); ^{13}C NMR (100 MHz, $CDCl_3$) 18.6, 18.7, 25.3, 26.7, 31.3, 33.6, 35.4, 35.9 (CH_2, C-2′, C-3′, C-3, C-4, C-5, C-9, C-10, C-11), 46.7 (CH_2, C-1′), 55.3 (OCH_3), 55.8 (OCH_3), 60.8 (CH_2, C-8), 68.8 (CH, C-2), 85.5 (CH, C-7′), 91.1 (CH, C-5″), 95.5 (quat., C-6), 98.1 (CH, C-3″), 113.5 (quat., C-3a″), 125.0 (CH, C-2″), 137.3 (quat., C-7a″), 153.8 (quat., C-4″), 157.3 (quat., C-6″); *m*/*z* (EI^+, %) 373 (100), 289 (3), 261 (6), 230 (5), 190 (13), 177 (5), 111 (4), 97 (5), 55 (5), 41 (6). Found: M^+, 373.2249. $C_{22}H_{31}NO_4$ requires 373.2253.

3.2. *Helicobacter pylori* culture and the determination of anti-*H. pylori* activity

The *H. pylori* type strain NCTC11637 was used to determine minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). Culture conditions were similar to previously published methods[7,15] except the MIC values which were based on A_{570} readings taken from small volume liquid cultures. *H. pylori* stocks were thawed and grown in brucella broth (Difco) supplemented with 2.5% v/v heat-inactivated foetal bovine serum (FBS; Invitrogen) and subcultured once before antimicrobial testing. Cultures were maintained at 37 °C in a microaerobic atmosphere using the CampyPak system (Oxoid). To achieve good gas dispersion, *H. pylori* were maintained on a gyratory shaker at 70 rpm in sterile petri dishes. All the test compounds were weighed then solubilized at 10 mg/mL in DMSO on the day of testing, followed by serial 2-fold dilution in broth. Ampicillin (Sigma) was dissolved in distilled water and then diluted in broth for testing. Cultures of *H. pylori* were grown for 16 h in standard 100 mm petri dishes with a maximum culture volume of 10 mL. Cultures were transferred to 35 mm petri dishes (2 mL/dish) with 0.5 mL of broth containing diluted compounds; all cultures had an A_{570} of $\leqslant 0.05$ at this time point ($t = 0$), which corresponded to $2–4 \times 10^7$ colony-forming units per mL. Cultures were incubated for a further 24 h, and the MIC determined for each compound by identifying the lowest concentration to give an A_{570} value within 0.02 of the A_{570} at $t = 0$. Cultures were serially diluted to 10^{-7} in broth and spread onto duplicate brucella agar plates supplemented with 2.5% v/v FBS and cultured at 37 °C for 5 days in a microaerobic atmosphere. Plated dilutions containing single colonies were counted and the average CFU/mL from duplicate plates calculated to determine the MBC for each compound and controls. The MBC was defined as the lowest concentration of each compound able to kill 99.9% of bacteria. The MIC and MBC were determined for each compound in two or more independent experiments.

Acknowledgment

The authors wish to thank the Maurice Wilkins Centre for Molecular Biodiscovery for financial support and assistance.

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