Total Synthesis of Tumor Inhibiting Didemnenone Analogues

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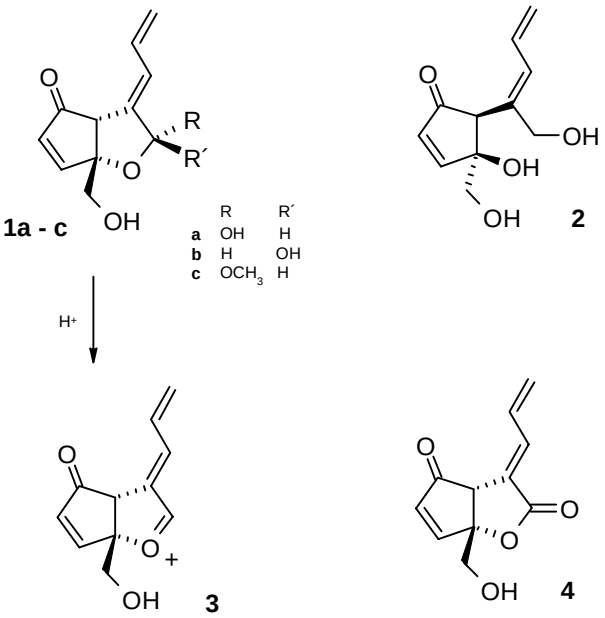
Abstract. We have previously described an enantioselective total synthesis of the tumor inhibiting didemnenones **1a**,**b** and **2**. Our investigations reported here shed light on the structure–activity relationships of these natural products. The significantly lower activity found for (3a*S**, 6a*S**)-3 [(*E*)-allyliden]-2-oxo-6a-(-hydroxymethyl)-2,3,3a,6a-tetrahydro-4*H*-cyclo-penta[*b*]furan]2(3*H*)-one (**4**) supported the hypothesis that the oxonium intermediate **3** is the active species. The strategy of the synthesis of the natural products was used to prepare acceptor substituted analogues (3a*S**, 6a*S**) [(*E*)-[3-(4-oxo-pent-2-(*E*)-enylidene]-6a-(4-hydroxymethyl)-2-methoxy-2,3,3a, 6a-tetrahydro[4*H*-cyclopenta[*b*]furan]-4-one (**18**) and (3a*S**, 6a*S**) [(*E*)-[3-(4-oxo-pent-2-(*E*)-enylidene]-6a-(4-hydroxymethyl)-2,3,3a,6a-tetrahydro-[4*H*-cyclopenta[*b*]furan]-2-(3*H*)-one (**20**). Although there were only moderate structural changes some of the key transformations differed remarkably in yield and general performance from those employed in the former synthesis. Optimization of the synthesis rewardingly led to compounds with increased biological activity against human gastric carcinoma cell-lines.

The Didemnenones A (**1a**), B (**1b**) and C (**2**), isolated by Lindquist *et al*. [1], from the Carribean tunicates *Didemnum voeltzkowi* and *Tridemnum cyanophorum*, show a broad range of biological activities, including toxicity against leukemia as well as antimicrobial and antifungal activity. The structure of these marine natural products is based on X-ray investigations with the corresponding α-methylacetal **1c** and on synthetic results [2–5].



Scheme 1 Didemnenones A (**1a**), B (**1b**) and C (**2**) and the derivatives **1c** and **4**.

Since cytotoxity is often linked to an acceptor group able to react with nucleophiles [6–8], the structure–activity considerations for the didemnenones are based on the alkylating substructures as shown in Scheme 1.

A cyclopentenone as contained in all test compounds (**1**, **2** and **4**) is well known as a potent alkylating substructure [9]. One may also argue that the lactol in the second ring (see **1** and **4**) represents a hidden Michael-acceptor. An oxidation gives rise to a allylidene-lactone which has been described as a tumor-inhibiting functionality [10]. Alternatively, acid-catalyzed dehydration could give rise to oxonium salt **3** operating as a strong electrophile.

To obtain experimental support for or against the structure activity relationship (SAR) considerations of the didemnenones as discussed above lactone **4** was to be made available *en route* to the natural products.

Results and Discussion

Scheme 2, which summarises the key steps of our synthesis, indicates the protected lactone **9** to be the intermediate of choice. While a diisobutyl aluminium hydride (DIBAH) reduction [11] was the first step in a straightforward conversion to the natural products **1a**, **1b**, and *epi*-**2**, deprotection provided lactone **4** directly.

Test runs with the tumor cell-line HM02 indicate that the monocyclic didemnenone C (**2**) is of lower activity than bicyclic compounds **1** and **4**. This argues in favour either of the *exo*-allylidene-lactol alone or a combina-

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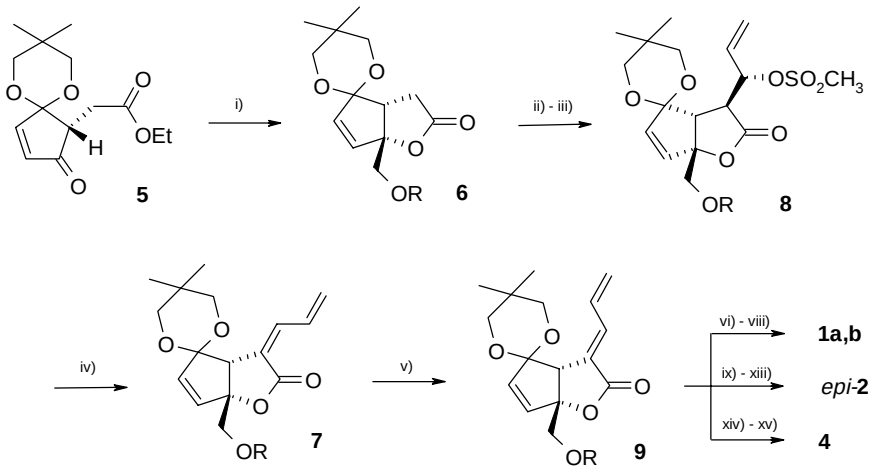
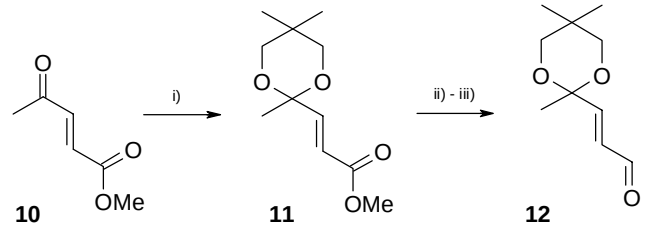


Scheme 2 i) *n*-$Bu_3SnCH_2OC_6H_4OCH_3$, *n*-BuLi, THF, –78 °C; ii) LiHMDS, acrolein, THF, –78 °C; iii) mesyl chloride, CH_2Cl_2, 0 °C; iv) DBU, CH_2Cl_2, 30 min; v) DBU, CH_2Cl_2, 3d; vi) DIBAH, Et_2O, –78 °C; vii) *p*-TsOH, MeOH; viii) CAN, aq. acetonitrile, *p*-TsOH, MeOH; ix) CAN, aq. acetonitrile; x) TBDMSCl, imidazole; xi) DIBAH, Et_2O, –78 °C; xii) $NaBH_4$, KO*t*-Bu; xiii) 6N aq. HCl, acetone, 40 °C; xiv) conc. HCl, acetone, 40 °C (71%); xv) CAN, aq. acetonitrile (76%).

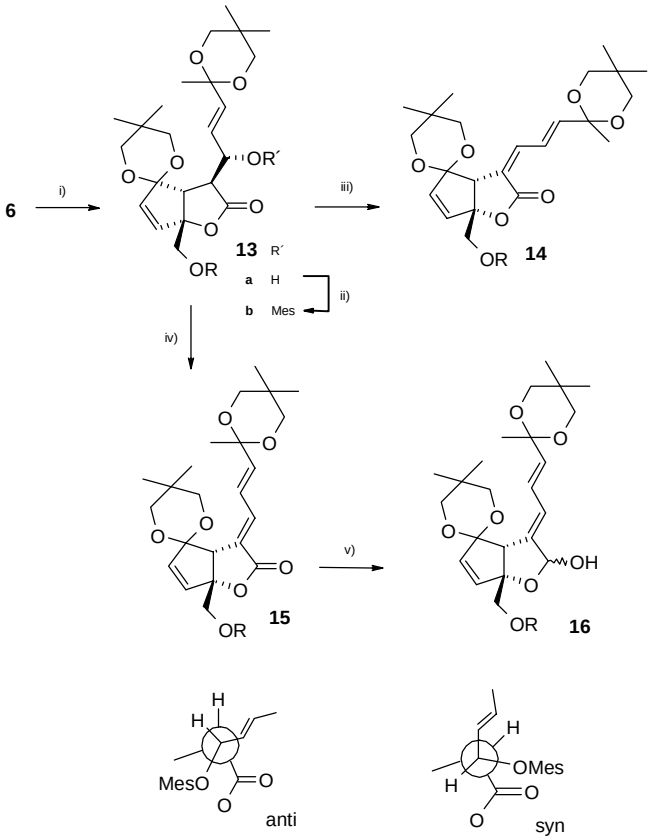
tion of both electrophilic systems, cyclopentenone and α-allylidene-lactol, as the pharmacophore of the didemnenones. In this connection it has to be mentioned that bisalkylating compounds represent highly potent lead structures against cancer (*e.g*. Mitomycin and related antitumor agents by DNA–DNA interstrand cross link [12]).

After it was found, that the lactol unit in the bicyclic compounds represents one essential part for the antitumor activity of the didemnenones, it was interesting to explore the effect of electron accepting substituents on the diene system, since they should enhance the electrophilicity of the bioactive species. Sticking to our synthetic scheme (see **6**–**8**) we decided to replace acrolein by ketal-aldehyde **12** with a hidden acceptor group on the terminal double bond (Scheme 3).



Scheme 3 i) 2,2-Dimethyl-1,3-propandiol, *p*-TsOH, toluene (99%); ii) DIBAH, CH_2Cl_2, –78 °C (92%); iii) TPAP, NMO, 4 Å sieves, CH_2Cl_2 (71%).

This aldehyde can be obtained in a few simple steps from the commercially available γ-ketoester **10** by ketal formation followed by a reduction–oxidation sequence. When **12** was added to the lithium enolate of enantiopure lactone **6** as described in our didemnenone synthesis, the *exo*-aldol was obtained with the same excellent diastereoselectivity as observed with acrolein,



Scheme 4 i) LiHMDS, **12**, THF, –78 °C (44%); ii) mesyl chloride, CH_2Cl_2, 0 °C (98%); iii) DBU, CH_2Cl_2 (86%); iv) KHMDS, THF, –78 °C (49% **15**; 6% **14**); v) DIBAH, Et_2O, –78 °C (99%).

albeit the yield was considerably lower (44%), and the obvious variations of reaction conditions led to no improvement. As determination of the biological activity was the first and most important goal no further optimization experiments were performed. Mesylate **13b** was formed in high yield, and the subsequent elimination gave rise to the *Z*-diene **14** in 86% yield (Scheme 4).

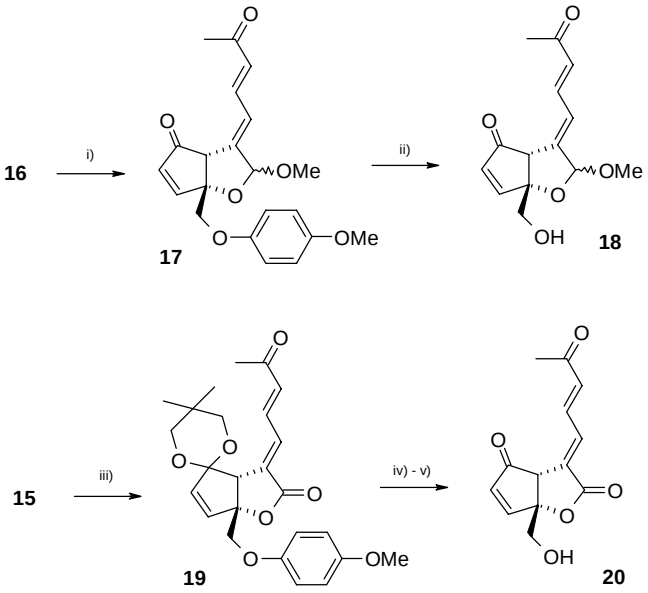
These results parallel those with the unsubstituted diene **7**, but in contrast to **7** which on further treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was completely converted into the desired *E*-diene **9**, analogue **14** did not isomerize under a whole set of reaction conditions [5, 13]. Shifting from E2 anti-elimination conditions to the E1cB syn-elimination mode was hoped to lead to the right configuration. This turned out to be the case for the main product of the reaction. However, **15** was accompanied by about 6% of the *Z*-isomer which could be removed by silicachromatography. The subsequent low-temperature DIBAH reduction of **15** finally provided the protected didemnenone analogue **16**.

Simultaneous deprotection of the two ketal groups was achieved by acid-catalyzed methanolysis, and was accompanied by a smooth acetal formation. Again, quite a difference to the didemnenone case was observed. While there the pure α-methyl acetal was obtained in a highly diastereoseletive manner, **17** was formed as a mixture of stereoisomers. Since the activity tests can be run with both epimers the hydroquinone-ether was cleaved directly with cerium(IV) ammonium nitrate (CAN) [14] to secure a 60% yield of ketolactol **18** (mixture of stereoisomers).

With lactone **15** a selective ketal hydrolysis could be achieved. Treatment with the palladium dichloride-acetonitrile complex in aqueous acetone [15] gave the monoketal **19** selectively in 90% yield, which on subsequent acid treatment provided the corresponding diketone. The final CAN-deprotection then gave the primary alcohol **20** without trans-lactonisation (Scheme 5).

At this stage in addition to glycoside **1c** and lactone **4** the corresponding derivatives **18** and **20** were available for activity tests (see Table 1) which were run according to the NCI guidelines [16] with the tumor-cell-line HM02 (human gastric carcinoma) [17]. RPMI 1640 tissue culture medium supplemented with 10% foetal calf serum served as medium in the 24 h growth-period, after which the test-material was added as 1–100 $\mu mol \cdot l^{-1}$ methanol solutions. The cultivation was then continued for another 48 h, and the subsequent cell count was done by protein-determination using sulforhodamine B [18]. This led to the following GI_{50}, TGI and LC_{50} data (Table 1).

Both lactones **4** and **20** are of inferior activity when compared with the corresponding compounds **1** and **18**. Besides, if the well known higher proton activity is taken into consideration the oxonium pathway is in line



Scheme 5 i) *p*-TsOH, MeOH (61%); ii) CAN, aq. acetonitrile, *p*-TsOH, MeOH (60%); iii) $(CH_3CN)_2PdCl_2$, aq. acetone (80%); iv) 12N aq. HCl (89%); v) CAN, aq. acetonitrile (79%).

Table 1 Antitumor activity measured towards HM02 cells (GI_{50}: drug concentration causing 50% growth inhibition; TGI: drug concentration causing 100% growth inhibition; LC_{50}: drug concentration causing 50% reduction of the cells present at time point zero, *i.e.* at 24 h).

Compound	GI_{50} ($\mu mol \cdot l^{-1}$)	TGI ($\mu mol \cdot l^{-1}$)	LC_{50} ($\mu mol \cdot l^{-1}$)
1c	1	38	>100
18	<3.8	10	>50
4	4	100	>100
20	28	60	>60

with the selectivity found out with these compounds. Particularly the TGI data prove the manipulation of the diene leads to compounds of higher activity than the simple unsubstituted systems.

Conclusion

For structure–activity relationship studies the didemnenone analogues **4**, **18** and **20** were synthesized. It was shown that the acetal **1c** is of higher activity against tumor cell line HM02 than lactone **4**. Additionally, the strategy of lowering electron density in the pharmacophore led to a compound (**18**) of increased antitumor activity compared to the corresponding natural product **1**.

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Experimental

UV spectra were measured on a Beckmann 3600 instrument and IR spectra on a Perkin Elmer 581 spectrometer. ^{1}H NMR spectra were recorded on a Bruker WP 200 and Bruker AM 400. δH Values are given relative to TMS = 0; *J* values in Hz. MS were determined with a Finnigan MAT 312 instrument and VG Autospec at 70 eV. For flash chromatography silica gel (30–60 mesh; Baker) was used at 0.3 bar. All solvents were dried by standard methods. Light petroleum refers to the fraction with *b.p.* 40–60 °C. Compounds **1a**,**b**; **3** and **5**–**9** were prepared as described [5].

(3aS,6aS*)-3[(E)-Allyliden]-2-oxo-6a-(4-methoxyphenoxymethyl)-2,3,3a,6a-tetrahydro-4H-cyclopenta[b] furan]2(3H)-one*

To a solution of **9** (175 mg, 440 μmol) in 6 mL acetone and 1 mL water a catalytic amount of 6N aqueous HCl was added at room temperature. After 8 h at 40 °C small amounts of solid sodium hydrogen carbonate were added. The mixture was evaporated and the residue partitioned between water and diethyl ether. The aqueous phase was extracted with diethyl ether, and the combined extracts were washed with brine, dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography (diethyl ether/light petroleum 1:2) yielded 97 mg (71%) of a colourless oil: λ_{max} (MeOH): 263 nm; ν/cm^{-1} ($CHCl_3$): 3040 (m), 2952 (m), 1760 (s), 1728 (s), 1508 (vs), 1232 (vs), 1036 (s). – ^{1}H NMR (200 MHz; $CDCl_3$): δ/ppm = 3.77 (3H, s), 3.95 (1H, d, 2 Hz), 4.22 (1H, d, 10 Hz), 4.28 (1H, d, 10 Hz), 5.79 (1H, dd, 10/1 Hz), 5.82 (1H, dd, 17/1 Hz), 6.44 (1H, d, 6 Hz), 6.80–6.87 (4H, m), 7.08 (1H, ddd, 17/11/10 Hz), 7.33 (1H, dd, 11/2 Hz), 7.67 (1H, d, 6 Hz). – MS (70 °C): *m/z* (%) = 312 (M^+, 2), 246 (11), 189 (3), 168 (3), 123 (100), 109 (20). – HRMS (EI) *m/z* (M^+) 312.0997 calcd. for $C_{18}H_{16}O_5$ 312.0998.

(3aS,6aS*)-3 [(E)-Allyliden]-2-oxo-6a-(4-hydroxymethyl)-2,3,3a,6a-tetrahydro-4H-cyclopenta [b]furan]2(3H)-one* (**4**)

To a cooled (0 °C) and vigorously stirred solution of (3a*S**, 6a*S**)-3 [(*E*)-allyliden]-2-oxo-6a-(4-methoxyphenoxymethyl)-2,3,3a,6a-tetrahydro-4*H*-cyclopenta[*b*] furan] 2(3*H*)-one (70 mg; 224 μmol) in 3 mL acetonitrile and 1 mL H_2O cerium(IV) ammonium nitrate (295 mg; 538 μmol; 2.4 equiv.) was added in one portion. After 15 min the mixture was poured into saturated aqueous sodium hydrogen carbonate, and the aqueous phase was extracted with dichloromethane. The combined organic phase was washed with brine, dried ($MgSO_4$) and evaporated. Purification of the residue by flash chromatography (diethyl ether/light petroleum, 1:1) yielded 35 mg (76%) of **4** as a colourless oil: λ_{max} (MeOH): 263 nm; ν/cm^{-1} ($CHCl_3$): 3688 (w_{br}), 3520 (w_{br}), 2956 (m), 1756 (s), 1724 (s), 1636 (m), 1332 (m), 1036 (s). – ^{1}H NMR (200 MHz; $CDCl_3$): δ/ppm = 3.80 – 3.90 (1H, m), 3.84 (1H, d, 12 Hz), 4.05 (1H, d, 12 Hz), 5.78 (1H, dd, 10/1 Hz), 5.81 (1H, dd, 17/1 Hz), 6.40 (1H, d, 6 Hz), 7.09 (1H, ddd, 17/12/10 Hz), 7.28 (1H, dd, 12/3 Hz), 7.64 (1H, d, 6 Hz). – MS (70 °C): *m/z* (%) = 206 (M^+, 7), 188 (2), 175 (12), 147 (25), 120 (11), 91 (26), 73 (100). – HRMS (EI) *m/z* (M^+) 206.0577 calcd. for $C_{12}H_{14}O_4$ 206.0579.

(E)-3-(2,5,5-Trimethyl-[1,3]dioxan-2-yl)-acrylic acid methyl ester (**11**)

A solution of (*E*)-4-oxo-pent-2-enoic acid methyl ester (**10**) (5.3 g, 41 mmol), 2,2-dimethyl-1,3-propandiol (21.5 g, 207 mmol; 5 equiv.) and *p*-toluenesulfonic acid (1 g, 5 mmol) was heated at reflux for 5 h with azeotropic removal of water. After cooling, the mixture was poured into saturated aqueous sodium hydrogen carbonate, then extracted with methyl *tert*-butyl ether. The combined extracts were washed with saturated aqueous sodium hydrogen carbonate, water and brine, dried ($MgSO_4$) and evaporated. Purification of the residue by flash chromatography (diethyl ether/light petroleum, 1:3) yielded 8.1 g (99%) of **11** as a colourless oil: λ_{max} (MeOH): 219 nm; ν/cm^{-1} ($CHCl_3$): 5 (m), 1720 (s), 1436 (m), 1308 (m), 1272 (m), 1176 (s), 1076 (m). – ^{1}H NMR (200 MHz; $CDCl_3$): δ/ppm = 0.71 (3H, s), 1.17 (3H, s), 1.44 (3H, s), 3.32–3.59 (4H, m), 3.77 (3H, s), 6.10 (1H, d, 16 Hz), 6.87 (1H, d, 16 Hz). – MS (RT): *m/z* (%) = 214 (M^+, 1), 199 (20), 183 (5), 155 (3), 129 (100), 113 (25), 97 (14), 69 (52). – HRMS (EI) *m/z* (M^+) 214.1205 calcd. for $C_{11}H_{18}O_4$ 214.1268.

(E)-3-(2,5,5-Trimethyl-[1,3]dioxan-2-yl)-prop-2-en-1-ol

To a cooled (–60 °C) solution of **11** (5.0 g, 25 mmol) in dry dichloromethane (25 mL) DIBAH (38 mmol; 1.5 equiv.) was slowly added. After 2 h the reaction was quenched with methanol at –60 °C. After addition of potassium carbonate paste the mixture was warmed to room temperature. The solution was decanted, and the residue was washed several times with dichloromethane. Concentration and purification of the residue by flash chromatography (diethyl ether/light petroleum, 1:1) yielded 4.3 g (92%) of a colourless oil: ν/cm^{-1} (film): 3402 (m), 2954 (m), 1372 (m), 1178 (s), 1082 (s), 1018 (m). – ^{1}H NMR (200 MHz; $CDCl_3$): δ/ppm = 0.73 (3H, s), 1.17 (3H, s), 1.43 (3H, s), 1.70 (1H, s_{br}), 3.36 (2H, dt, 12/1 Hz), 3.60 (2H, d, 12 Hz), 4.23 (2H, d, 5 Hz), 5.69 (1H, dt, 16/2 Hz), 5.98 (dt, 16/5 Hz). – MS (RT): *m/z* (%) = 186 (M^+, 2), 171 (49), 155 (23), 129 (100), 101 (62), 83 (66), 69 (82). – HRMS (EI) *m/z* (M^+) 186.1256 calcd. for $C_{10}H_{18}O_3$ 186.1257.

(E)-3-(2,5,5-Trimethyl-[1,3]dioxan-2-yl)-propenal (**12**)

To a cooled (0 °C) mixture of *N*-methyl-morpholine-*N*-oxide (NMO) (3.77 g, 32.2 mmol; 1.5 equiv.), 4 Å sieves (300 mg) and tetrapropylammonium perruthenat (TPAP) (200 mg, 570 μmol) in dry dichloromethane (25 mL) a solution of (*E*)-3-(2,5,5-trimethyl-[1,3]dioxan-2-yl)-prop-2-en-1-ol (4.0 g, 21.5 mmol) in dry dichloromethane (5 mL) was added. After 1 h a further portion of solid TPAP (177 mg, 504 μmol) was added, and the mixture was stirred for 12 h at room temperature. Evaporation and purification of the residue by flash chromatography (diethyl ether/light petroleum, 1:2) yielded 2.81 g (71%) of **12** as a colourless oil: ν/cm^{-1} ($CHCl_3$): 2960 (m), 1696 (s), 1372 (m), 1180 (s), 1076 (m). – ^{1}H NMR (200 MHz; $CDCl_3$): δ/ppm = 0.73 (3H, s), 1.18 (3H, s), 1.48 (3H, s), 3.47 (4H, m), 6.36 (1H, d, 16 Hz), 6.76 (1H, d, 16 Hz), 9.66 (1H, d, 8 Hz). – MS (RT): *m/z* (%) = 184 (M^+,

3), 169 (80), 129 (99), 99 (78), 83 (82), 69 (100). – HRMS (EI) *m/z* (M^+) 184.1099 calcd. for $C_{10}H_{16}O_3$ 184.1100.

(3R,3aS*,6aS*)-6a-(4-Methoxyphenoxymethyl)-5',5'-dimethyl-3 [(1R*)-2,5,5-trimethyl[1.3]dioxan-1-hydroxy-2-allyl)]-3a,6a-dihydrospiro[4H-cyclopenta[b]furan-4,2'-[1.3]-dioxan]-2-(3H)-one* (**13a**)

To a cooled (–78 °C) solution of lithium hexamethyldisilazide (6.72 mmol; 1.3 equiv.) in dry tetrahydrofuran (20 mL) the lactone **6** (1.86 g, 5.17 mmol), dissolved in dry tetrahydrofuran (15 mL) was added, under an atmosphere of argon. The mixture was stirred at the same temperature for 30 min after which a solution of aldehyde **12** (1.7 g, 9.3 mmol; 1.8 equiv.) in dry tetrahydrofuran (5 mL) was added. After being stirred for a further hour at –78 °C, the reaction mixture was poured into aqueous ammonium chloride and extracted with methyl *tert*-butyl ether. The combined extracts were washed with brine, dried ($MgSO_4$) and evaporated. Subsequent purification of the residue by flash chromatography (diethyl ether/light petroleum 1:3) yielded 1.24 g (44%) of a white foam: ν/cm^{-1} ($CHCl_3$): 3434 (w), 2960 (s), 2868 (m), 1772 (s), 1508 (vs), 1364 (m), 1232 (vs), 1088 (vs), 1036 (s). – ^{1}H NMR (200 MHz; $CDCl_3$): δ/ppm = 0.76 (3H, s), 0.82 (3H, s), 1.16 (3H, s), 1.22 (3H, s), 1.43 (3H, s), 3.10–3.75 (9H, m), 3.76 (3H, s), 3.98 (1H, d, 10 Hz), 4.18 (1H, d, 10 Hz), 4.75 (1H, dt_{br}, 6/4 Hz), 5.89 (1H, d, 4 Hz), 6.22 (1H, d, 6 Hz), 6.75–6.95 (6H, m). – MS (150 °C): *m/z* (%) = 544 (M^+, 2), 499 (1), 484 (2), 391 (4), 360 (25), 319 (8), 274 (11), 237 (18), 183 (25), 140 (42), 124 (90), 109 (89), 82 (88), 68 (100). – HRMS (EI) *m/z* (M^+) 544.2672 calcd. for $C_{30}H_{40}O_9$ 544.2675.

(3R,3aS*,6aS*)-6a-(4-Methoxyphenoxymethyl)-5',5'-dimethyl-3 [(1R*)-2,5,5-trimethyl [1.3] dioxan-1-methanesulfonoxy-2-allyl)]-3a,6a-dihydrospiro [4H-cyclopenta [b] furan-4,2´-[1.3]-dioxan]-2-(3H)-one* (**13b**)

To a cooled (0 °C) solution of **13a** (880 mg, 1.62 mmol) and triethylamine (449 µL, 3.24 mmol; 2 equiv.) in dry dichloromethane (20 mL) mesyl chloride (188 µL, 2.43 mmol; 1.5 equiv.) was added. After being stirred for 1 h, the reaction mixture was poured into saturated aqueous sodium hydrogen carbonate and extracted with dichloromethane. The combined extracts were washed with aqueous sodium hydrogen carbonate and brine, dried ($MgSO_4$) and concentrated to yield 987 mg (98%) of **13b** as a colourless oil: ν/cm^{-1} ($CHCl_3$): 2960 (s), 2932 (s), 1776 (s), 1508 (vs), 1364 (m), 1232 (s), 1088 (s), 1036 (s), 908 (s). – ^{1}H NMR (200 MHz; $CDCl_3$): δ/ppm = 0.69 (3H, s), 0.82 (3H, s), 1.16 (3H, s), 1.21 (3H, s), 1.43 (3H, s), 3.03–3.75 (10H, m), 3.06 (3H, s), 3.77 (3H, s), 4.00 (1H, d, 10 Hz), 4.19 (1H, d, 10 Hz), 5.55 (1H, dd, 8/5 Hz), 5.91 (1H, d, 16 Hz), 6.18 (1H, d, 6 Hz), 6.19 (1H, dd, 16/8 Hz), 6.80–6.95 (4H, m), 6.84 (1H, d, 6 Hz). – FAB-MS: *m/z* (%): 623 (M^++1, 26), 622 (M^+, 77), 583 (18), 544 (6), 527 (66), 500 (65), 124 (100).

(3aS,6aS*) [(Z)-[3-(2,5,5-Trimethyl-[1,3]dioxan-2-yl)-(E)-allylidene]-6a-(4-methoxyphenoxy-methyl)-5',5'-dimethyl-3a,6a-dihydrospiro [4H-cyclopenta[b]furan-4,2'-[1.3] dioxan]-2-(3H)-one* (**14**)

To a solution of **13b** (100 mg, 160 µmol) in dichloromethane (5 mL) DBU (50 µL, 320 µmol; 2 equiv.) was added at room temperature. After being stirred for 3 h, the reaction mixture was poured into water and extracted with dichloromethane. The combined extracts were washed with aqueous ammonium chloride and brine, dried ($MgSO_4$) and concentrated. The crude product was purified by flash chromatography (diethyl ether/light petroleum 1:2) yielded 72 mg (86%) of **14** as a colourless oil: λ_{max} (MeOH): 282 nm; ν/cm^{-1} ($CHCl_3$): 2960 (m), 2868 (m), 1748 (m), 1508 (vs), 1364 (m), 1228 (vs), 1084 (s), 1036 (s). – ^{1}H NMR (200 MHz; $CDCl_3$): δ/ppm = 0.70 (3H, s), 0.80 (3H, s), 1.16 (3H, s), 1.23 (3H, s), 1.48 (3H, s), 3.34–3.75 (9H, m), 3.76 (3H, s), 4.05 (1H, d, 10 Hz), 4.17 (1H, d, 10 Hz), 6.02 (1H, d, 16 Hz), 6.20 (1H, d, 6 Hz), 6.80–6.90 (5H, m), 6.86 (1H, d, 6 Hz), 7.81 (1H, dd, 16/12 Hz). – MS (170 °C): *m/z* (%) = 527 (M^++1, 5), 526 (M^+, 14), 440 (2), 403 (4), 389 (100), 303 (16), 275 (10), 189 (11), 129 (23), 109 (7), 69 (22). – HRMS (EI) *m/z* (M^+) 526.2559 calcd. for $C_{30}H_{33}O_8$ 526.2567.

(3aS,6aS*) [(E)-[3-(2,5,5-Trimethyl-[1,3]dioxan-2-yl)-(E)-allylidene]-6a-(4-methoxyphenoxy-methyl)-5',5'-dimethyl-3a,6a-dihydrospiro [4H-cyclopenta[b]furan-4,2'-[1.3] dioxan]-2-(3H)-one* (**15**)

To a solution of **13b** (987 mg, 1.59 mmol) in dry tetrahydrofuran (25 mL) a solution of potassium hexamethyldisilazide (2.4 mmol, 1.5 equiv.) was added at –78 °C. After being stirred for 30 min at ambient temperature, the reaction mixture was poured into aqueous saturated sodium hydrogen carbonate and extracted with methyl *tert*-butyl ether. The combined organic extracts were washed with brine, dried ($MgSO_4$) and concentrated. The crude product was purified by flash chromatography (diethyl ether/light petroleum 1:3) and yielded 411 mg (49%) of *E*-diene **15** as a white foam and 51 mg (6%) of *Z*-diene **14** as a colourless oil: λ_{max} (MeOH): 282 nm; ν/cm^{-1} ($CHCl_3$): 2960 (m), 2868 (m), 1760 (m), 1508 (vs), 1364 (m), 1228 (s), 1088 (s), 1040 (s). – ^{1}H NMR (400 MHz; $CDCl_3$): δ/ppm = 0.72 (3H, s), 0.77 (3H, s), 1.19 (3H, s), 1.23 (3H, s), 1.46 (3H, s), 3.34–3.75 (9H, m), 3.76 (3H, s), 4.05 (1H, d, 10 Hz), 4.17 (1H, d, 10 Hz), 6.21 (1H, d, 17 Hz), 6.23 (1H, d, 6 Hz), 6.80–6.90 (1H, m), 6.81 (4H, s), 6.96 (1H, d, 6 Hz), 7.36 (1H, dd, 12/2 Hz). – MS (170 °C): *m/z* (%) = 527 (M^++1, 3), 526 (M^+, 4), 496 (4), 440 (19), 390(31), 354 (12), 302 (96), 217 (18), 189 (31), 129 (50), 109 (26), 69 (100). – HRMS (EI) *m/z* (M^+) 526.2543 calcd. for $C_{30}H_{33}O_8$ 526.2567.

(3aS,6aS*) [(E)-[3-(4-Oxo-pent-2-(E)-enylidene]-6a-(4-methoxyphenoxymethyl)-5',5'-dimethyl-3a,6a-dihydrospiro[4H-cyclopenta[b]furan-4,2'-[1.3]dioxan]-2-(3H)-one* (**19**)

To a solution of **15** (90 mg, 127 µmol) in acetone (5 mL) and water (0.5 mL) $(CH_3CN)_2PdCl_2$ (5 mg, 17 µmol) was added. After 3 d at room temperature the mixture was evaporated. Purification of the residue by flash chromatography (methyl *tert*-butyl ether) yielded 60 mg (80%) of **19** as a colourless oil: λ_{max} (MeOH): 286 nm; ν/cm^{-1} ($CHCl_3$): 3012 (m), 2960 (m), 1756 (s), 1668 (m), 1508 (vs), 1232 (vs), 1092 (s), 1040 (s). – ^{1}H NMR (400 MHz; $CDCl_3$): δ/ppm = 0.78 (3H, s), 1.19 (3H, s), 2.36 (3H, s), 3.42 (1H, dd, 11.5/2.5 Hz), 3.56 (1H, dd, 11.5/2.5 Hz), 3.66 (1H, d, 11.5 Hz), 3.76 (1H, s), 3.83 (1H, d, 2 Hz), 4.04 (1H, d, 10 Hz), 4.17 (1H, d, 10 Hz),

6.23 (1H, d, 6 Hz), 6.49 (1H, d, 15.5 Hz), 6.79 (4H, s), 7.00 (1H, d, 6 Hz), 7.37 (1H, dd, 11.5/2 Hz), 7.51 (1H, dd, 15.5/11.5 Hz). – MS (100 °C): *m/z* (%) = 441 (M^++1, 8), 440 (M^+, 23), 354 (8), 302 (100), 217 (25), 175 (22), 147 (10), 123 (16), 97 (18), 77 (20), 69 (51). – HRMS (EI) *m/z* (M^+) 440.1835 calcd. for $C_{25}H_{28}O_7$ 440.1841.

(3aS,6aS*)[(E)-[3-(4-Oxo-pent-2-(E)-enylidene] -6a-(4-methoxyphenoxymethyl)-2,3,3a,6a-tetrahydro[4H-cyclopenta[b]furan]-2-(3H)-one*

To a solution of **19** (42 mg, 95 μmol) in acetone (2 mL) and water (1 mL) was added a catalytic amount of 12N aqueous HCl at room temperature. After 12 h small amounts of solid $NaHCO_3$ were added. The mixture was evaporated and the residue distributed between water and diethyl ether. The aqueous phase was extracted with diethyl ether, and the combined extracts were washed with brine, dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography (diethyl ether/light petroleum 1:2) yielded 31 mg (89%) of a colourless oil: λ_{max} (MeOH): 292 nm; ν/cm^{-1} ($CHCl_3$): 3040 (w), 2952 (w), 1764 (m), 1728 (m), 1672 (m), 1644 (m), 1596 (m), 1508 (s), 1228 (s), 1036 (s). – ^{1}H NMR (400 MHz; $CDCl_3$): δ/ppm = 2.43 (3H, s), 3.77 (3H, s), 4.08 (1H, d, 2.5 Hz), 4.24 (1H, d, 10 Hz), 4.31 (1H, d, 10 Hz), 6.47 (1H, d, 5.5 Hz), (1H, d, 15 Hz), 6.82 (4H, s), 7.37 (1H, dd, 12/2.5 Hz), 7.71 (1H, d, 5.5 Hz), 7.72 (1H, dd, 15/12 Hz). – MS (90 °C): *m/z* (%) = 354 (M^+, 2), 247 (3), 205 (2), 179 (1), 155 (3), 124 (14), 109 (8), 84 (100), 71 (18). – HRMS (EI) *m/z* (M^+) 354.1103 calcd. for $C_{20}H_{18}O_6$ 354.1103.

(3aS,6aS*)[(E)-[3-(4-Oxo-pent-2-(E)-enylidene]-6a-(4-hydroxymethyl)-2,3,3a,6a-tetrahydro-[4H-cyclopenta[b]furan]-2-(3H)-one* (**20**)

To a cooled (0 °C) and vigorously stirred solution of (3a*S**, 6a*S**)-[(*E*)-[3-(4-oxo-pent-2-(*E*)-enylidene]-6a-(4-methoxyphenoxy-methyl)-2,3,3a,6a-tetrahydro[4*H*-cyclopenta[*b*]furan]-2-(3*H*)-one (30 mg; 85 μmol) in 2 mL acetonitrile and 0.5 mL H_2O cerium(IV) ammonium nitrate (119 mg; 203 μmol; 2.4 equiv.) was added in one portion. After 15 min the mixture was poured into saturated aqueous sodium hydrogen carbonate, and the aqueous phase was extracted with dichloromethane. The combined organic phase was washed with brine, dried ($MgSO_4$) and evaporated. Purification of the residue by flash chromatography (diethyl ether/light petroleum, 1:2) yielded 17 mg (79%) of **20** as a colourless oil: λ_{max} (MeOH): 292 nm; ν/cm^{-1} ($CHCl_3$): 3604 (w), 3428 (w), 2928 (m), 1764 (vs), 1728 (vs), 1672 (s), 1644 (m), 1596 (m), 1228 (vs), 1028 (m). – ^{1}H NMR (400 MHz; $CDCl_3$): δ/ppm = 2.40 (3H, s), 3.82 (1H, d, 12 Hz), 3.97 (1H, d, 2.5 Hz), (1H, d, 12 Hz), 6.39 (1H, d, 6 Hz), 6.45 (1H, d, 16 Hz), 7.30 (1H, dd, 12/2.5 Hz), 7.64 (1H, d, 6 Hz), 7.69 (1H, dd, 16/11 Hz). – MS (110 °C): *m/z* (%) = 248 (M^+, 6), 217 (7), 205 (20), 189 (19), 175 (19), 147 (23), 125 (13), 118 (22), 109 (100), 86 (83), 71 (49). – HRMS (EI) *m/z* (M^+) 248.0685 calcd. for $C_{13}H_{12}O_5$ 248.0673.

(3aS,6aS*) [(E)-[3-(2,5,5-Trimethyl-[1,3]dioxan-2-yl)-(E)-allylidene]-6a-(4-methoxyphenoxy-methyl)-5',5'-dimethyl-3a,6a-dihydrospiro[4H-cyclopenta[b]furan-4,2'-[1.3]dioxan]-2-ol* (**16**)

To a cooled (–78 °C) solution of **15** (502 mg, 0.95 mmol) in dry diethyl ether (20 mL) DIBAH (1.4 mmol; 1.5 equiv.) was slowly added. After 1 h the reaction was quenched with methanol at –78 °C, and the mixture was poured into aqueous citric acid (2M). The mixture was extracted with chloroform, and the extracts were washed with brine, dried ($MgSO_4$) and evaporated to yield 496 mg (99%) of **16** as a colourless oil: λ_{max} (MeOH): 236 nm; ν/cm^{-1} ($CHCl_3$): 3408 (w_{br}), 3008 (m), 2956 (m), 2868 (m), 1508 (vs), 1232 (s), 1096 (s), 1036 (s). – ^{1}H NMR (400 MHz; $CDCl_3$): diastereomeres, 1:1; δ/ppm = 0.68/0.70 (3H, s_{br}), 0.76/0.78 (3H, s), 1.18/1.19 (3H, s_{br}), 1.12/1.28 (3H, s_{br}), 1.43/1.45 (3H, s_{br}), 3.29–3.74 (9H, m), 3.75 (3H, s), 3.91/3.96 (1H, d, 10 Hz), 3.97/4.18 (1H, d, 10 Hz), 4.07 (1H, s_{br}), 4.47/4.50 (1H, s), 6.17/6.34 (1H, d, 6 Hz), 6.44/6.50 (1H, d, 11 Hz), 6.77–6.90 (4H, m). – MS (250 °C): *m/z* (%) = 528 (M^+, 3), 511 (8), 425 (2), 390 (8), 375 (3), 303 (6), 269 (4), 189 (5), 175 (8), 141 (11), 129 (100), 109 (16), 81 (16), 69 (36). – HRMS (EI) *m/z* (M^+) 528.2723 calcd. for $C_{30}H_{40}O_8$ 528.2722.

(3aS,6aS*)[(E)-[3-(4-Oxo-pent-2-(E)-enylidene]-2-methoxy-6a-(4-methoxyphenoxymethyl)-2,3,3a,6a-tetrahydro[4H-cyclopenta[b]furan]-4-one* (**17**)

A solution of **16** (242 mg, 458 μmol) and a catalytic amounts of *p*-toluenesulfonic acid in methanol (5 mL) was stirred for 3 h at room temperature. After addition of small amounts of solid sodium hydrogen carbonate to the mixture was evaporated, and the residue was distributed between water and diethyl ether. The aqueous phase was extracted with diethyl ether, and the combined extracts were dried ($MgSO_4$) and evaporated. Purification of the residue by flash chromatography (diethyl ether/light petroleum 1:1) yielded 103 mg (61%) of **17** as a colourless oil: λ_{max} (MeOH): 282 nm; ν/cm^{-1} ($CHCl_3$): 3000 (w), 2928 (m), 1720 (s), 1672 (m), 1596 (m), 1508 (vs), 1360 (w), 1228 (vs), 1080 (s), 1036 (s). – ^{1}H NMR (400 MHz; $CDCl_3$): diastereomeres, 5:1; δ/ppm = 2.28/2.40 (3H, s), 3.35/3.46 (3H,s), 3.77 (3H, s), 3.81 (1H, d, 2 Hz), 4.11–4.31 (2H, m), 5.45 (1H, s), 6.16–6.29 (2H, m), 6.46 (1H, d_{br}, 11 Hz), 6.83 (4H, s), 7.61/7.72 (1H, dd, 16/11 Hz), 7.65 (1H, d, 6 Hz), 7.61/7.70 (1H, dd, 15.5/11 Hz). – MS (150 °C): *m/z* (%) = 371 (M^++1, 14), 370 (M^+, 59), 339 (6), 279 (5), 247 (9), 215 (19), 187 (48), 173 (41), 145 (44), 124 (100), 91 (26), 77 (50). – HRMS (EI) *m/z* (M^+) 370.1416 calcd. for $C_{21}H_{22}O_6$ 370.1409.

(3aS,6aS*)[(E)-[3-(4-Oxo-pent-2-(E)-enylidene]-6a-(4-hydroxymethyl)-2-methoxy-2,3,3a,6a-tetrahydro[4H-cyclopenta[b]furan]-4-one* (**18**)

To a cooled (0 °C) and vigorously stirred solution of **17** (47 mg, 127 μmol) in acetonitrile (2 mL) and water (0.5 mL) cerium(IV) ammonium nitrate (119 mg; 203 μmol; 2.4 equiv.) was added in one portion. After 15 min the mixture was poured into saturated aqueous sodium hydrogen carbonate, and the aqueous phase was extracted with dichloromethane. The combined organic phase was washed with brine, dried ($MgSO_4$) and evaporated. The residue was dissolved in dry methanol (2 mL) and, after addition of catalytic quantities of *p*-toluenesulfonic acid, the mixture was stirred at ambient temperature for 1 h. Small amounts of solid sodium hydrogen carbonate were added to the mixture which was then evaporated and the residue partitioned between water and dichlorometh-

ane. The aqueous phase was separated and extracted with dichloromethane, and the combined organic phase and extracts were washed with brine, dried ($MgSO_4$) and evaporated. Purification of the residue by flash chromatography (diethyl ether/light petroleum, 1:2) yielded 20 mg (60%) of **18** as a colourless oil: λ_{max} (MeOH): 282 nm; ν/cm^{-1} ($CHCl_3$): 3596 (w), 3432 (w_{br}), 3000 (m), 1720 (vs), 1672 (s), 1596 (m), 1364 (m), 1252 (m), 1076 (s), 1020 (s). – ^{1}H NMR (400 MHz; $CDCl_3$): diastereomeres, 3:1; δ/ppm = 2.39 (3H, s), 3.34/3.38 (3H, s), 3.68–3.76 (1H, m), 3.72 (1H, m), 3.75 (1H, d_{br}, 2 Hz), 3.91/3.93 (1H, d_{br}, 11 Hz), 5.41/5.73 (1H, s), 6.20 (1H, m), 6.21 (1H,d, 6 Hz), 6.42/6.48 (1H, dd 11/2 Hz), 7.12/7.14 (1H, dd, 15.5/12 Hz), 7.58 (1H, d, 6 Hz), 7.61/7.70 (1H, dd, 15.5/11 Hz). – MS (110 °C): *m/z* (%) = 264 (M^+, 2), 246 (4), 233 (24), 214 (18), 191 (62), 160 (100), 145 (36), 131 (46), 103 (38), 91 (35), 77 (55). – HRMS (EI) *m/z* (M^+) 264.0998 calcd. for $C_{14}H_{16}O_5$ 264.0994.

Biological Assay

The antitumor activity of the test compounds was determined in the human cancer cell line HM02 (human gastric carcinoma), according to the NCI guidelines. Cells were grown in 96-well microtitre plates (Greiner) of RPMI 1640 tissue culture medium supplemented with 10% foetal calf serum (Life Technologies) at 37 °C in a humidified atmosphere. Stock solutions of the test compounds were prepared in methanol (1 μmol·l^{-1} – 100 μmol·l^{-1}) and were added to the cells. After 48 h incubation in the presence of the test drugs the cells were fixed by addition of trichloroacetic acid and cell protein was assayed with sulforhodamine B. For each compound tested the GI_{50}, TGI and LC_{50} values were determined.

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