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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 5300–5315
Bioorganic & Medicinal Chemistry

Antiangiogenic versus cytotoxic activity in analogues of aeroplysinin-1

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Received 7 March 2007; revised 25 April 2007; accepted 2 May 2007
Available online 6 May 2007

Dedicated to Professor Miguel Ángel Yus (Universidad de Alicante) on occasion of his 60th birthday.

Abstract—A series of analogues of the potentially angiogenic inhibitor aeroplysinin-1 **1** were synthesized and their in vitro antiangiogenic and cytotoxic activities evaluated. In the case of epoxy ketone **6** and azlactone **36** the relationship *sprouting inhibition assay*/*cytotoxicity* in BAE cells was enhanced by one order and two orders of magnitude, respectively, with respect to the reference. These results imply more specific antiangiogenic properties for the synthesized derivatives.

1. Introduction

Cancer is a group of diseases characterized by the excessive and uncontrolled growth of cells that leads to the formation of tumours. In the evolutionary process of a tumour three differentiated phases can exist: (1) neoplasic transformation. (2) Tumour growth. (3) Metastasis.

Angiogenesis, the formation of new blood vessels from the pre-existing vascular bed of the guest,[1] is a necessary process induced by the tumour for its growth due to the distance limit of 100–200 μm that the original tumoral cells can stand to be from a blood vessel in order to achieve their metabolic needs.[2] Therefore, the process of angiogenesis is an important target in order to inhibit the tumour growth and, consequently, the harm of the disease.[3] The possibility to cultivate endothelial cells in vitro and the development of in vivo assays to confirm the in vitro results have led to an important increase of the angiogenesis studies in the last years.[4]

Aeroplysinin-1 **1** (Fig. 1a) is a natural product isolated, in both enantiomeric forms, as a secondary metabolite present in different marine sponges.[5,6]

Many biological properties of this compound have been described, such as antibacterial activity,[7] inhibition of the EGFR tyrosine kinase in in vitro test systems[8] and cytotoxic activity against several tumour cell lines and antileukaemic activity in both in vivo and in vitro test systems.[9] Aeroplysinin-1 **1** has also shown antiangiogenic properties[10] as it inhibits certain functions of the endothelial cells (these cells are necessary for the formation of new blood vessels) in both in vivo and in vitro assays, using less dose than the ones required for other angiogenic inhibitors previously reported.[11]

We have initiated a study on the *sprouting inhibition assay*/*cytotoxicity* relationship of aeroplysinin-1 **1** analogues in order to select structures having specific antiangiogenic properties without cytotoxic activities. For the synthesis

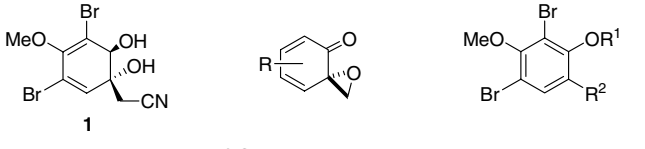

Figure 1. Structures of aeroplysinin-1 **1** and the two types of analogues.

Keywords: Antiangiogenic compounds; Cytotoxicity; Aeroplysinin-1; Spiroepoxycyclohexadienones.
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doi:10.1016/j.bmc.2007.05.011

and biological test of analogues of **1**, we have chosen two types of compounds: (1) spiroepoxycyclohexadienones as structural analogues of the non-aromatic cyclic skeleton (Fig. 1b). (2) Aromatic compounds with analogue cyclic substitution (Fig. 1c). This selection has been done mainly for three reasons: (a) the simplicity of the synthetic methods used for the preparation of compounds **1b** and **1c** (Fig. 1). That allows us for the synthesis of a significant number of compounds by means of versatile and not elaborated synthetic procedures; (b) the spiroepoxycyclohexadienone analogues can undergo ring-opening reactions of the epoxide moiety under 'physiological' conditions to yield molecules with structural similarity to aeroplysinin-1 **1**; (c) regarding the aromatic analogues, these compounds could be the final products of the aromatization process of the natural product, also under 'physiological' conditions, in the cell environment with concomitant ring-opening of the epoxide substructure. In consequence, we report in this paper the synthesis of several compounds with structure **1b** and **1c** (Fig. 1) and the biological evaluation of the *structure-specific antiangiogenic activity* relationship of these compounds.

2. Results and discussions

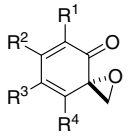
2.1. Chemistry

2.1.1. Spiroepoxycyclohexadienones. For the synthesis of epoxy ketone analogues **6**–**12** (Fig. 2) the methodology described by K. Hinterding et al.[8d,12] to obtain compound **6** was used (Scheme 1).

Compounds **6** (33% overall yield) and **7**[13] (39% overall yield) were synthesized following this procedure using, respectively, 2,4-dimethoxybenzaldehyde **2** and 2,4,6-trimethoxybenzaldehyde as starting materials. In the case of epoxy ketone **8**, the *ortho* monobromination of compound **3** using PHPB (pyridinium hydrobromide perbromide)[14,15] took place with poor regioselectivity because the formation of the brominated by-products **14** and **4** (Scheme 2). Nevertheless, compound **13** was easily isolated by column chromatography (Hex/AcOEt, 1:1). Lithium aluminium hydride reduction of **13** and Becker–Adler oxidation of the resulting product **15** afforded spiroepoxycyclohexadienone **8**.

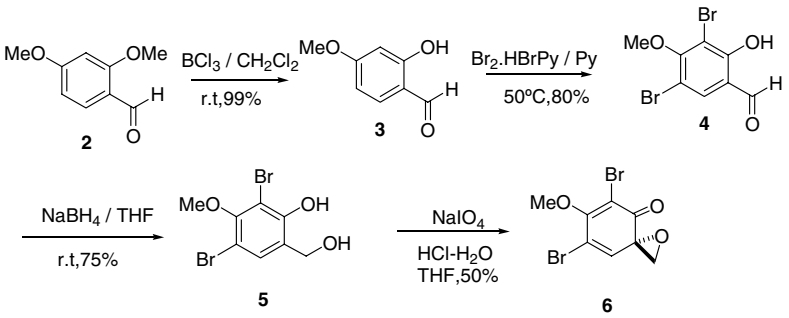
In order to achieve the synthesis of compounds **9** and **10**[16] (Scheme 3) the synthetic protocol was slightly modified due to the instability of the salicylic alcohols **17** and **18**. Thus, the reduction and oxidation processes were done in a single step without isolating those alcohols. In addition, synthesis of compound **14** was achieved by reaction of 2,4-dimethoxybenzaldehyde **2** with pyridinium tribromide and, subsequently, selective cleavage of the phenolic ether in position *ortho* respect to the aldehyde moiety. Due to the presence of the methoxy groups in *2* and *4* positions, no aromatic bromination of position *3* took place.

Spiroepoxycyclohexadienone **11**[17] was directly obtained by oxidation of commercially available salicylic alcohol **19** (Scheme 4), whereas epoxy ketone **12** was synthesized using three reaction steps with salicylic aldehyde as starting material. It is worthy of note that in the

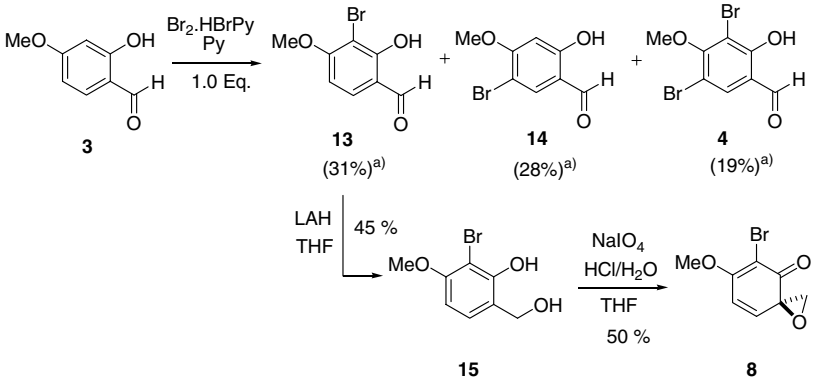


Compound number	R^1	R^2	R^3	R^4
6	Br	MeO	Br	H
7	Br	MeO	Br	MeO
8	Br	MeO	H	H
9	H	MeO	Br	H
10	H	MeO	H	H
11	H	H	Br	H
12	Br	H	Br	H

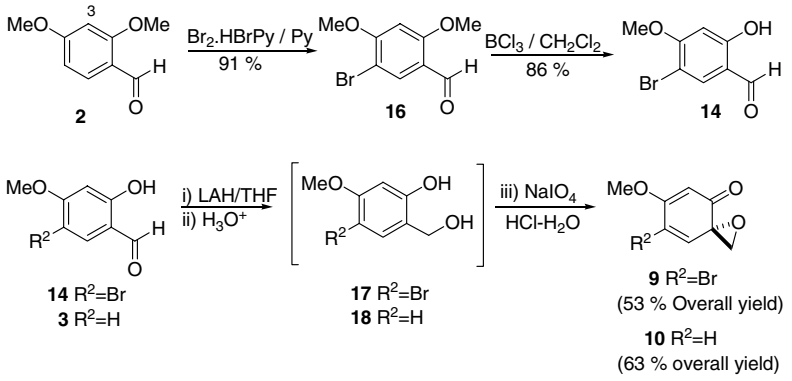
Figure 2. Spiroepoxycyclohexadienones synthesized.



Scheme 1. Previously reported synthesis of epoxy ketone **6**.

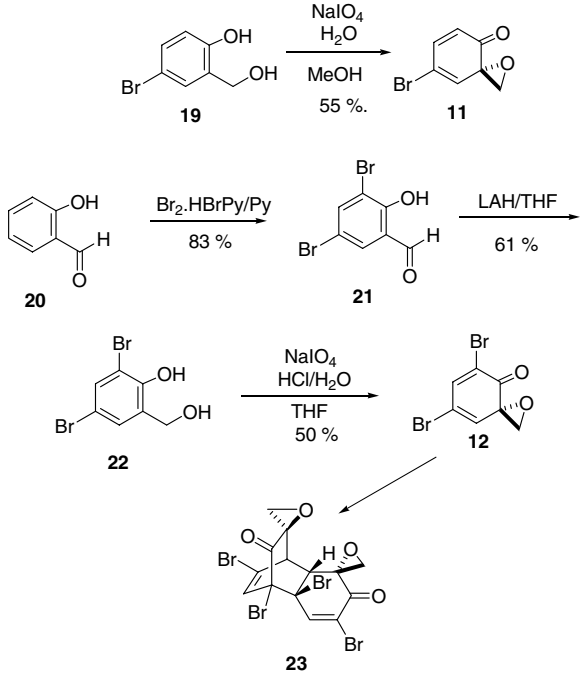


Scheme 2. Synthesis of epoxy ketone **8**. (a) Product ratio determined by ^{1}H NMR. 22% of starting material was recovered.



Scheme 3. Synthesis of epoxy ketones **9** and **10**.

treatment of alcohol **22** with sodium periodate the temperature must be controlled under 25 °C in order to avoid the dimerization process giving **23**.[18]



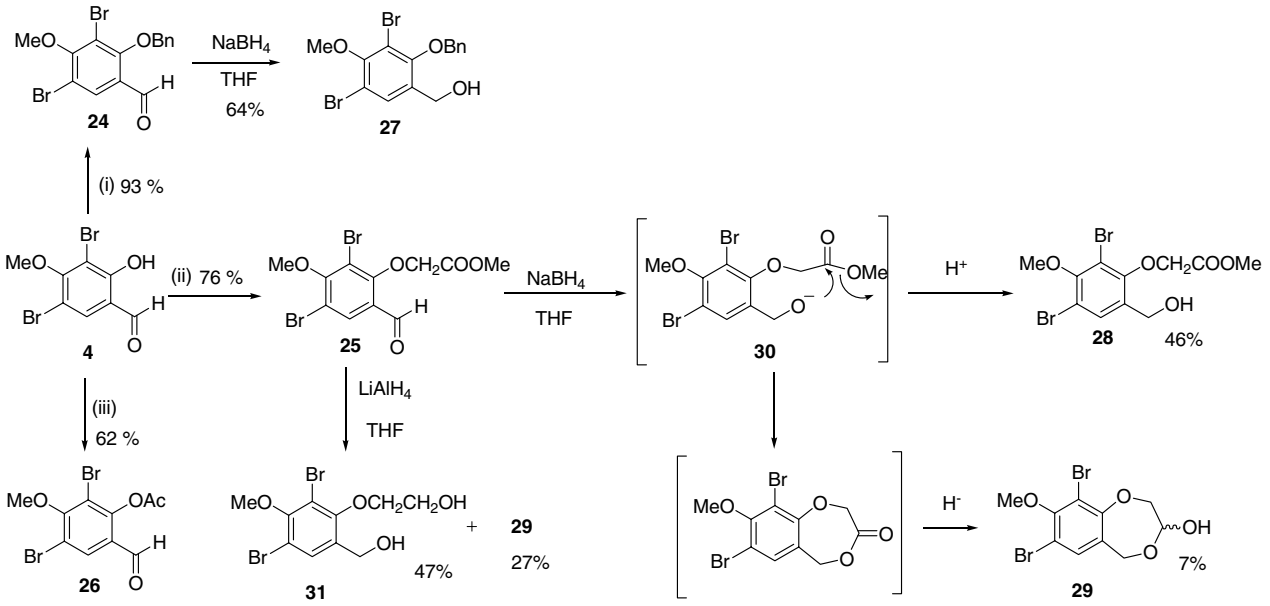
Scheme 4. Synthesis of epoxy ketones **11** and **12**.

2.1.2. Aromatic compounds. Analogues **24**, **25** and **26**, with different phenolic protection group, were obtained by simple transformations of salicylic aldehyde **4** (Scheme 5). The subsequent reduction of **24** and **25** with $NaBH_4$ led to the isolation of **27**, **28** and **29**, whereas the treatment of **25** with $LiAlH_4$ led to the isolation of **29** and **31**.

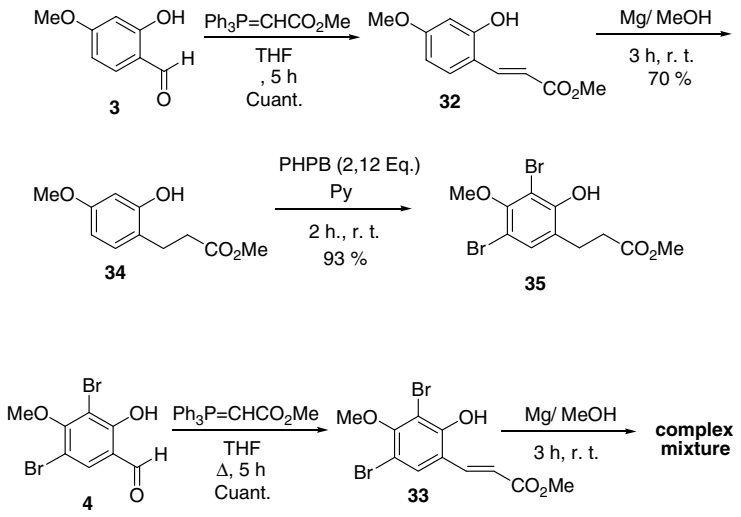
The formation of compound **29** can be explained as an intramolecular transesterification of the intermediary reaction product **30** followed by a reduction process of the carbonylic moiety.

The Wittig reaction was also used for the synthesis of aromatic analogues. Thus, the treatment of salicylic aldehydes **3** and **4** with $Ph_3P{=}CHCO_2Me$ as fosforane led to the formation of alkenes **32** and **33**, both compounds as a unique (*E*) isomer (Scheme 6). In order to attempt a selective reduction of the alkenyl moiety, these compounds were treated with magnesium in methanolic solution.[19] In the case of the non-brominated compound **32** the reaction took place with high selectivity and high yield, whereas in the case of **33** a complex mixture of debrominated products, with and without reduction of the carbon–carbon double bond, was obtained. Therefore, in order to obtain compound **35**, the non-brominated compound **34** was synthesized in first place and, subsequently, treated with 2.12 equivalents of pyridinium tribromide.

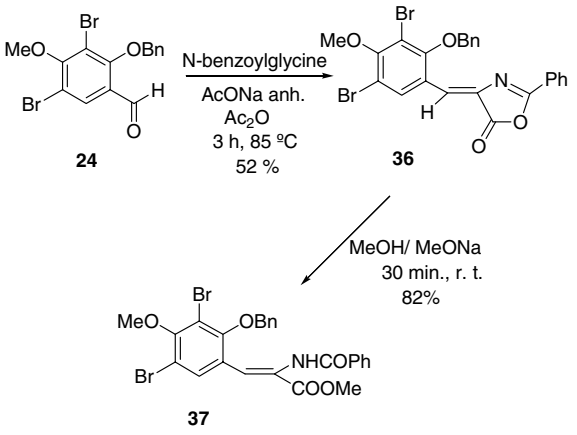
Finally, we focussed the synthesis of aromatic analogues towards the azlactone[20] **36** (Scheme 7) due to the high



Scheme 5. Synthesis of aromatic analogues. Reagents and conditions: (i) BrBn, K_2CO_3, DMF, 95 °C, 3 h; (ii) $BrCH_2CO_2Me$, K_2CO_3, 18-crown-6, acetone, rt, 3 h; (iii) AcCl, TBAH, NaOH, dioxane, rt, 32h.



Scheme 6. Synthesis of aromatic analogues.

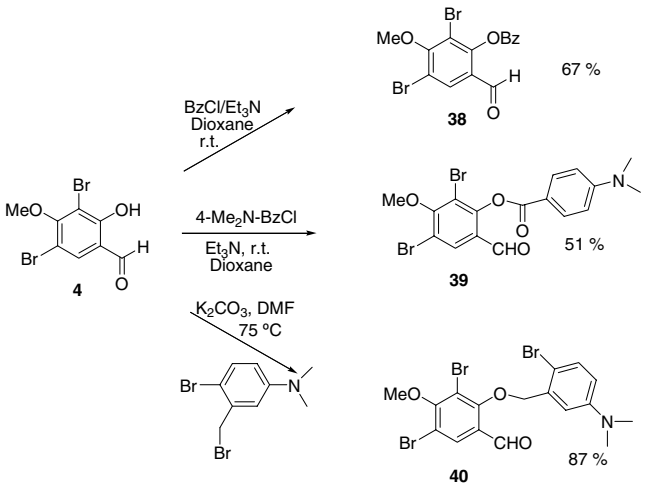


Scheme 7. Synthesis of aromatic analogues.

reactivity of aromatic aldehydes with hipuric acid (*N*-benzoylglycine) as previously described for the non-brominated compound in the Andersen and Faulkner[6a,21] synthesis of aeroplysinin-1 **1**.

Thus, the treatment of benzaldehyde **24** with hipuric acid (*N*-benzoylglycine) and AcONa in Ac_2O led to the isolation of azlactone **36** as a (*Z*) diastereomer.[22] Another analogue was isolated by methanolic treatment of azlactone **36** rendering compound **37** in an oxazolone ring opening process.

At this stage, the biological results of the aeroplysinin-1 **1** analogues synthesized were evaluated (see Section 2.2). Azlactone **36** showed, significantly, the best *sprouting inhibition assay*/*cytotoxicity* relationship.



Scheme 8. Phenolic protection for the synthesis of azlactone **36** derivatives.

Consequently, the next part of this study was focussed in the synthesis of azlactone **36** derivatives that could improve the water solubility and, therefore, the biological evaluation. For this purpose was selected in order to finally obtain a hydrochloride. In this context, the phenolic group of compound **4** was protected using benzoyl chloride, 4-*N*,*N*-dimethylbenzoyl chloride and 2-bromo-5-*N*,*N*-dimethylbenzyl bromide[23] to obtain compounds **38** (this one was synthesized in order to evaluate the effect of the *N*,*N*-dimethyl moiety in the activity), **39** and **40**, respectively (Scheme 8). The treatment of these aldehydes with hipuric acid led to the formation of azlactones **41**, **42**, **43** and **44** (Scheme 9), being **42** a partial benzoyl ester cleavage[24] by-product isolated in the synthesis of azlactone **41**. Finally, hydrochloride[25] **45** was synthesized by treatment of azlactone **44** with gaseous hydrogen chloride in dioxane.

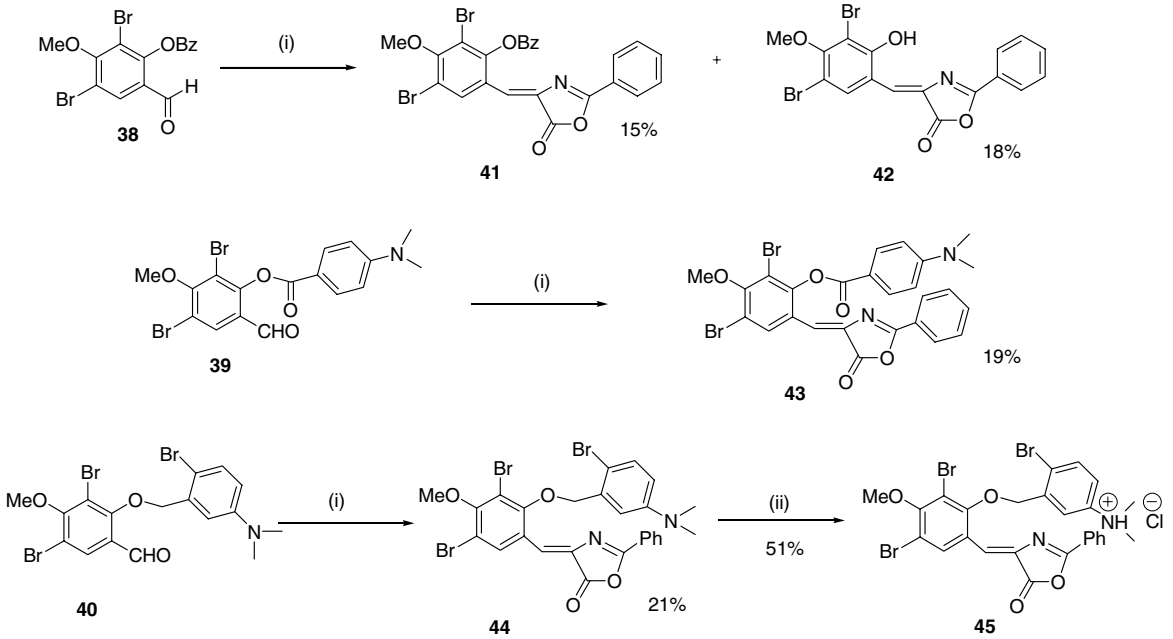
2.2. Biological results and discussion

The biological evaluation of the synthesized analogues (Table 1) has been done using bovine aortic endothelial cells (BAEC) in an in vitro assay system and taking aeroplysinin-1 **1** as a reference. Two types of tests were carried out: (a) cytotoxicity assay: IC_{50}, μM (the drug concentration inhibiting the growth value of the BAE cells by 50%); (b) sprouting inhibition assay: MIC, μM (the minimum inhibition concentration at which no tube formation is observed). In this test, the endothelial cells are supported in pearls for a 3-D angiogenic event simulation (Fig. 3).

Spiroepoxycyclohexadienones **8**, **12** and **23** showed the same magnitude values for the inhibition assay (Table 1) as the reference (aeroplysinin-1 **1**). As the reference itself, these compounds also showed cytotoxic activity against the endothelial cells, therefore, having a low ratio between angiogenesis inhibition and cytotoxicity. It is worthy of note that compounds **8** and **12** are suitable for dimerization in mild conditions, whereas **23** is a dimeric compound. The cytotoxicity of this type of dimeric spiro-epoxy compounds has been previously reported in the literature.[26]

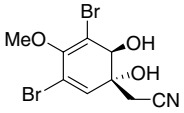
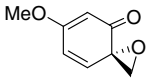
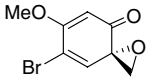
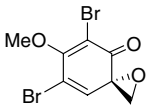
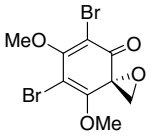
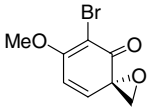
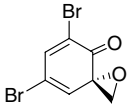
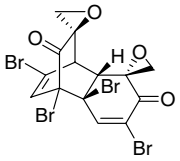
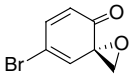
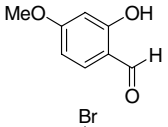
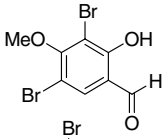
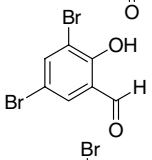
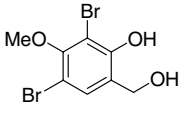
Nevertheless, spiroepoxycyclohexadienone **6**, with two bromine atoms and a methoxylic group in the dienic system substitution, showed a significant increase in the *sprouting inhibition assay*/*cytotoxicity* relationship compared with the aeroplysinin-1 **1** values.

In the case of the aromatic analogues synthesized (Table 1) only the azlactone **36** showed interesting values in the biological evaluation, exhibiting a 10^3 value for the *sprouting inhibition assay*/*cytotoxicity* ratio. This value is about 10^2-fold higher than the ratio for the



Scheme 9. Synthesis of azlactone **36** derivatives. Reagents and conditions: (i) *N*-benzoylglycine, anhydrous sodium acetate, acetic anhydride, 90 °C, 3 h; (ii) HCl (g), dioxane, rt, 30 min.

Table 1. Cytotoxicity and tube inhibition assays of bovine aortic endothelial cells for aeroplysinin-1 **1** analogues in an in vitro assay system (for the description of the assays, see Section 4)

Compound	Structure	IC_{50}[a,d]	MIC[b,d]	/[c,d]
1		29	2	**14**
10		33	33	**1**
9		>22	>22	—
6		161	3	**54**
7		>15	15	—
8		11	3	**4**
12		12	2	**6**
23		4	2	**2**
11		>25	25	—
3		>33	>33	—
4		806	>16	—
21		36	71	**0,5**
5		>16	>16	—

(*continued on next page*)

Table 1 (*continued*)

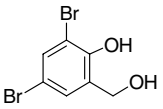
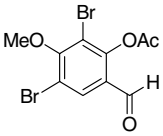
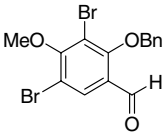
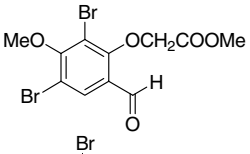
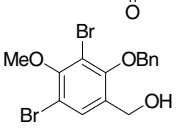
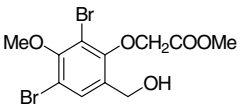
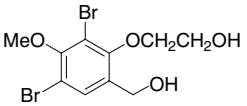
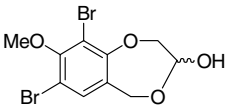
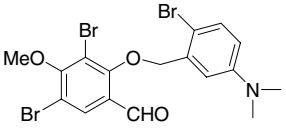
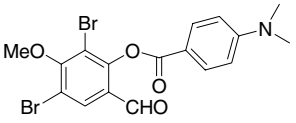
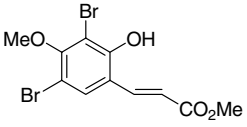
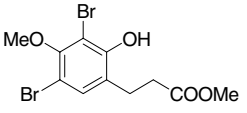
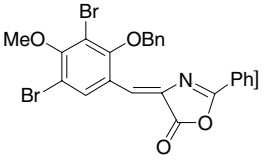
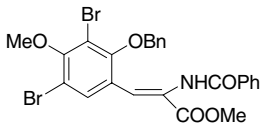
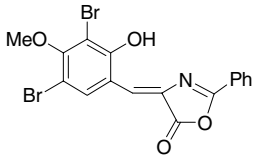
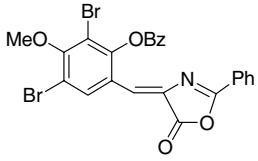
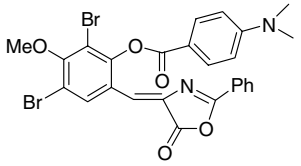
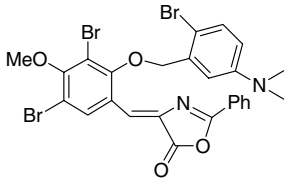
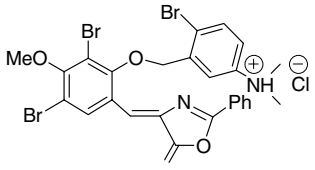
Compound	Structure	IC_{50}[a,d]	MIC[b,d]	/[c,d]
22		>35	71	—
26		23	33	**0,7**
24		>12.5	>12.5	—
25		151	52	**3**
27		>12	>12	—
28		>26	>26	—
32		>28	28	—
29		>28	>28	—
40		>19	>38	—
39		>22	>44	—
33		>14	>14	—
35		136	>27	—
36		921	1	921

Table 1 (*continued*)

Compound	Structure	IC_{50}[a,d]	MIC[b,d]	/[c,d]
37		>17	>35	—
42		>22	>22	—
41		>18	27	—
43		83	>17	—
44		>15	>30	—
45		71	>28	—

[a] Drug concentration inhibiting the growth value of the BAE cells by 50% (IC_{50}, μM).
[b] Minimum inhibition concentration at which no tube formation is observed (MIC, μM).
[c] *Sprouting inhibition assay*/*cytotoxicity* ratio.
[d] Symbol > means that IC_{50} and MIC were not found at the biggest concentration assayed. From our criteria compounds with a *sprouting inhibition tube* value <10 μM are not interesting as antiangiogenic candidates.

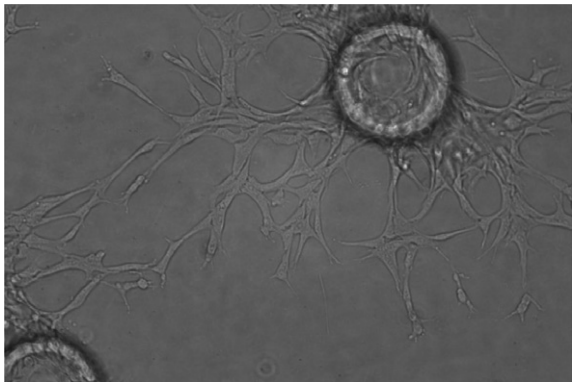


Figure 3. Tube formation between endothelial cells supported in pearls.

aeroplysinin-1 **1**. The biological activity comparison between compounds **36**, **24**, **41**, **42**, **43** and **44** shows the necessity of a benzylic group, as a protector group of the phenolic moiety, and the oxazolone ring in order to have a high *sprouting inhibition assay*/*cytotoxicity* ratio. The formation of the hydrochloride azlactone **45** did not improve the mentioned ratio.

3. Conclusions

In conclusion, two series of aeroplysinin-1 **1** analogues have been synthesized, one with the same non-aromatic cyclic skeleton and the other as aromatic derivatives with the same substitution in the ring. Their in vitro *cytotoxicity* and their tube inhibition activity have been evaluated

using in vitro assays with bovine aortic endothelial cells (BAEC). Epoxy ketone **6** and azlactone **36** have shown a relationship value of *sprouting inhibition assay*/*cytotoxicity* enhanced by one order and two orders of magnitude, respectively, with respect to the reference (aeroplysinin-1 **1**). This result implies the attribution of specific antiangiogenic properties for compounds **6** and **36**, whereas in the case of the reference the observable inhibition of tubes can be attributed either to antiangiogenic properties or to cytotoxicity. Therefore, epoxy ketone **6** and azlactone **36** seem to be attractive compounds as angiogenesis inhibitors. Further studies, including in vivo evaluations, on this interesting type of antiangiogenic compounds are underway in our laboratories.

4. Experimental

4.1. General

All reactions were carried out under argon atmosphere. Column chromatography was performed on silica gel Merck 230–400 mesh. Melting points are uncorrected and were determined using a Gallenkamp instrument. NMR spectra were recorded on Bruker 200-AM (200 MHz), Bruker AM300 (300 MHz) and on Bruker AM500 (500 MHz) instruments, using $CDCl_3$ and acetone-d_6 as solvents. Chemical shifts are in ppm relative to TMS. Mass spectra were recorded on a mass spectrometer HP-5890. Electrospray ionization (ESI) spectra were recorded on a Bruker Esquire-LC 00126 by direct method and using positive polarity.

Spiroepoxycyclohexadienone **6** was synthesized using the methodology described by Hinterding, K. et al.[8d] Compound **7** was obtained[13] following the same synthetic route and using 2,4,6-trimethoxybenzaldehyde as the starting material.

Compound **11** was obtained using the methodology described by V. Bonnarme et al.[17] Epoxy ketone **10** has been previously synthesized by Corey, E. J. et al.[16] and compounds **3**, **4** and **5** have been previously described by Hinterding, K. et al.[8d] The rest of chemicals were obtained from commercial sources and were used without further purification. Solvents were distilled and dried over molecular sieves.

4.2. Aromatic bromination: general procedure

A solution of pyridinium hydrobromide perbromide (2.12 equiv) (in the case of compound **13** only 1 equiv was used) in pyridine (1 mL/mmol) was added dropwise to a solution of the aldehyde (1 equiv) in pyridine (2 mL/mmol) at 50 °C. The reaction mixture was stirred for 2 h at 50 °C and then hydrolysed with ice-water (100 mL/g aldehyde). After filtration of the precipitate, this was purified by column chromatography (AcOEt/hexane = 1:2 v/v) (in the case of compound **13** a 1:1 ratio was used, $R_f = 0.37$).

4.2.1. 3-Bromo-2-hydroxy-4-methoxybenzal-dehyde (13). White crystals, mp 115–117 °C: 605 mg, 2.62 mmol, 25%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.95 (3H, s, OMe), 6.55 (1H, d, $^3J = 8.7$ Hz, ArH, H-5), 7.45 (1H, d, $^3J = 8.7$ Hz, ArH, H-6), 9.69 (1H, s, CHO), 11.88 (1H, s, OH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 56.90 (OMe), 99.76 (ArC—Br), 103.84 (ArCH, C-5), 116.22 (ArC—CHO), 134.60 (ArCH, C-6), 160.25 (ArC—OH), 162.85 (ArC—OMe), 194.33 (CHO) ppm. MS (70 eV, EI) *m*/*z* (%) 230/232 (82/86) ($M^{+\cdot}$), 229/231 (100/100) (M−H), 79 (22), 77 (8), 65 (24), 63 (16), 52 (25), 50 (19), 39 (24). Anal. Calcd for $C_8H_7BrO_3$: C, 41.59; H, 3.05. Found: C, 41.71; H, 3.09.

4.2.2. 5-Bromo-2,4-dimethoxybenzaldehyde (16). White crystals, mp 140–142 °C: 1.34 g, 5.47 mmol, 91%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (3H, s, OMe), 3.95 (3H, s, OMe), 6.41 (1H, s, ArH, H-3), 7.95 (1H, s, ArH, H-6), 10.20 (1H, s, CHO) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 56.0 (OMe), 56.5 (OMe), 95.6 (ArCH, C-3), 103.5 (ArC—Br), 119.4 (ArC—CHO), 132.9 (ArCH, C-6), 161.7 (ArC—OMe), 163.0 (ArC—OMe), 187.1 (CHO) ppm. MS (70 eV, EI) *m*/*z* (%) 244/246 (92/100) ($M^{+\cdot}$), 243/245 (40/50) (M−H), 227/229 (34/39) (M−OH), 226/228 (23/35) (M−H_2O), 198/200 (30/38) (M−H_2O−CO), 148 (60), 79 (19), 77 (18), 63 (34), 51 (25), 39 (10). Anal. Calcd for $C_9H_9BrO_3$: C, 44.11; H, 3.70. Found: C, 44.00; H, 3.62.

4.2.3. 3,5-Dibromo-2-hydroxybenzaldehyde (21). Yellow crystals, mp 79–81 °C: 1.90 g, 6.79 mmol, 83%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.61 (1H, d, $^4J = 2.3$ Hz, ArH, H-4), 7.85 (1H, d, $^4J = 2.3$ Hz, ArH, H-6), 9.78 (1H, s, CHO), 11.47 (1H, s, OH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 111.5 (ArC—Br), 112.5 (ArC—Br), 121.9 (ArC—CHO), 134.9 (ArCH, C-6), 142.0 (ArCH, C-4), 157.3 (ArC—OH), 194.9 (CHO) ppm. Anal. Calcd for $C_7H_4Br_2O_2$: C, 30.04; H, 1.44. Found: C, 30.19; H, 1.39.

4.2.4. 3-Methyl (3,5-dibromo-2-hydroxy-4-methoxyphenyl) propanoate (35). White crystals, mp 76–78 °C: 815 mg, 2.23 mmol, 93%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.59 (2H, t, $^3J = 7.2$ Hz, CH_2, H-2), 2.85 (2H, t, $^3J = 7.2$ Hz, CH_2, H-3), 3.66 (3H, s, COOMe), 3.81 (3H, s, OMe), 6.46 (1H, s, OH), 7.21 (1H, s, ArH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 25.4 (CH_2, C-3), 33.6 (CH_2, C-2), 51.8 (CH_3, COOMe), 60.4 (CH_3, OMe), 107.2 (2× ArC—Br), 125.0 (C, Ar*C*—CH_2), 132.5 (ArCH), 150.9 (ArC—OH), 152.7 (ArC—OMe), 173.9 (COO) ppm. Anal. Calcd for $C_{11}H_{12}Br_2O_4$: C, 35.90; H, 3.29. Found: C, 35.93; H, 3.44.

4.3. Selective cleavage of phenolic ethers

4.3.1. 5-Bromo-2-hydroxy-4-methoxybenzal-dehyde (14). A 1M solution of BCl_3 in CH_2Cl_2 (5 mL, 5 mmol) was added dropwise to a solution of the aldehyde **16** (740 mg, 3.02 mmol) in 5 mL of dry CH_2Cl_2 at 0 °C. After stirring at room temperature for 16 h the mixture was cooled to 0 °C and 5 mL of 1 N HCl followed by 10 mL of H_2O was added. The aqueous phase was extracted with CH_2Cl_2 (3× 5 mL) and the combined organic layers were washed with brine (2× 10 mL). Drying of the organic phase with $MgSO_4$ was followed by

evaporation of the solvent under vacuum. The product **14** was purified by chromatography on silica gel (AcOEt/hexane = 1:1 v/v). White crystals, mp 118–120 °C: 740 mg, 3.02 mmol, 86%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.91 (3H, s, OMe), 6.42 (1H, s, ArH-3), 7.61 (1H, s, ArH-6), 9.64 (1H, s, CHO), 11.38 (1H, s, OH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 56.6 (OMe), 100.2 (ArCH, C-3), 102.0 (ArC—Br), 115.6 (ArC—CHO), 137.1 (ArCH, C-6), 162.4 (ArC—OMe), 163.5 (ArC—OH), 193.5 (CHO) ppm. Anal. Calcd for $C_8H_7BrO_3$: C, 41.59; H, 3.05. Found: C, 41.39; H, 3.01.

4.4. Treatment of aromatic aldehydes with reductive agents

4.4.1. 2-Bromo-6-(hydroxymethyl)-3-methoxy phenol (15). A solution of compound **13** (580 mg, 2.51 mmol) in THF (2.5 mL) was added, dropwise, to a solution of $LiAlH_4$ (95 mg, 2.51 mmol) in THF (4 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min. The reaction was quenched by the dropwise addition of water, and the mixture was extracted with Et_2O (3× 30 mL). Drying of the combined organic phases with $MgSO_4$ was followed by evaporation of the solvent under vacuum. Compound **15** was purified by chromatography on silica gel (AcOEt/hexane = 1:1 v/v). White crystals, mp 106–109 °C: 263 mg, 1.13 mmol, 45%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.28 (1H, br s, *H*O—CH_2), 3.82 (3H, s, OCH_3), 4.66 (2H, s, CH_2—OH), 6.39 (1H, d, ^3J = 8.4 Hz, ArH, H-4), 6.61 (1H, s, HO—Ph), 7.02 (1H, d, ^3J = 8.4 Hz, ArH, H-5) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 56.5 (OCH_2), 62.3 (OCH_3), 100.4 (ArC—Br), 103.3 (ArCH, C-4), 120.1 (Ar*C*—CH_2OH), 127.9 (ArCH, C-5), 152.4 (ArC—OH), 156.5 (ArC—OMe) ppm. MS (70 eV, EI) *m*/*z* (%) 232/234 (20/19) ($M^{+\cdot}$), 215/217 (24/31) (M−OH), 214/216 (81/100) (M−H_2O), 186/188 (14/8) (M−H_2O—CO), 135 (39), 124 (12), 105 (17), 77 (28), 65 (14), 51 (16). Anal. Calcd for $C_8H_9BrO_3$: C, 41.23; H, 3.89. Found: C, 41.48; H, 3.98.

4.4.2. 2,4-Dibromo-6-hydroxymethylphenol (22). A solution of compound **21** (900 mg, 3.21 mmol) in THF (5 mL) was added, dropwise, to a solution of $LiAlH_4$ (126 mg, 3.21 mmol) in THF (10 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min. The reaction was quenched by the dropwise addition of water, and the mixture was extracted with Et_2O (3× 30 mL). Drying of the combined organic phases with $MgSO_4$ was followed by evaporation of the solvent under vacuum. Compound **22** was purified by chromatography on silica gel (AcOEt/hexane = 1:1 v/v). White crystals, mp 130–133 °C: 550 mg, 1.95 mmol, 61%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.40 (1H, br s, HO—CH_2), 4.82 (2H, s, CH_2—OH), 6.74 (1H, s, HO—Ph), 7.30 (1H, d, ^4J = 2.3 Hz, ArH, H-5), 7.61 (1H, d, ^4J = 2.3 Hz, ArH, H-3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 62.4 (OCH_2), 111.1 (ArC—Br), 112.4 (ArC—Br), 128.8 (ArC—CH_2OH), 130.3 (ArCH, C-3), 133.7 (ArCH, C-5), 150.4 (ArC—OH) ppm. MS (70 eV, EI) *m*/*z* (%) 280/282/284 (6/14/8) ($M^{+\cdot}$), 262/264/266 (41/100/48) (M−H_2O), 234/236/238 (3/6/2) (M−H_2O—CO), 183/185 (16/21) (M−H_2O—Br), 155/157 (9/8) (M−H_2O—Br—CO), 77 (12), 76 (19), 75 (43), 63 (24), 39 (13). Anal. Calcd for $C_7H_6Br_2O_2$: C, 29.82; H, 2.15. Found: C, 29.83; H, 2.18.

4.4.3. 2-[2,4-Dibromo-6-(hydroxymethyl)-3-methoxyphenoxy] ethanol (31) and 7,9-dibromo-8-methoxy-2,3-dihydro-5-*H*-1,4-benzodioxepin-3-ol (29). A solution of compound **25** (16 mg, 0.04 mmol) in THF (0.5 mL) was added, dropwise, to a solution of $LiAlH_4$ (3 mg, 0.08 mmol) in THF (1 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min. The reaction was quenched by the dropwise addition of water, and the mixture was extracted with Et_2O (3× 2 mL). Drying of the combined organic phases with $MgSO_4$ was followed by evaporation of the solvent under vacuum. The chromatography on silica gel (AcOEt/hexane = 1:1 v/v) yielded compound **31** (white crystals, mp 109–113 °C: 7 mg, 0.02 mmol, 47%) and compound **29** (white crystals, mp 106–107 °C: 4 mg, 0.01 mmol, 27%). Compound **31**: ^{1}H NMR (300 MHz, acetone-d_6) δ 3.88 (3H, s, OMe), 3.90 (2H, c, ^3J = 5.3 Hz, CH_2, H-1), 4.15 (2H, t, ^3J = 5.3 Hz, CH_2, H-2), 4.22 (1H, t, ^3J = 5.3 Hz, CH_2CH_2—O*H*), 4.56 (1H, t, ^3J = 6.0 Hz, ArC—CH_2—O*H*), 4.72 (2H, d, ^3J = 6.0 Hz, ArC—CH_2—OH), 7.68 (1H, s, ArH) ppm. Anal. Calcd for $C_{10}H_{12}Br_2O_4$: C, 33.74; H, 3.40. Found: C, 33.53; H, 3.40. Compound **29**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.93 (1H, d, ^3J = 5.4 Hz, OH, exchangeable with D_2O), 3.84 (3H, s, OMe), 4.02 (1H, dd, ^2J = 13.0 Hz, ^3J = 6.3 Hz, CH_2, H-2), 4.19 (1H, dd, ^2J = 13.0 Hz, ^3J = 2.8 Hz, CH_2, H-2), 4.37 (1H, d, ^2J = 14.4 Hz, CH_2, H-5), 5.09 (1H, d, ^2J = 14.4 Hz, CH_2, H-5), 5.31 (1H, m, C*H*—OH; with D_2O: dd, ^3J = 6.3 Hz, ^3J = 2.8 Hz), 7.22 (1H, s, ArH) ppm. MS (70 eV, EI) *m*/*z* (%) 352/354/356 (19/38/18) ($M^{+\cdot}$), 306/308/310 (41/50/51) (M−H_2O—CO), 291/293/295 (13/29/19) (M-61), 263/265/267 (12/24/10) (M-89), 212/214 (38/34), 148 (34), 77 (29), 75 (24).

Anal. Calcd for $C_{10}H_{10}Br_2O_4$: C, 33.93; H, 2.85. Found: C, 34.19; H, 2.97.

4.4.4. Methyl [2,4-dibromo-6-(hydroxy-methyl)-3-methoxyphenoxy] acetate (28) and 7,9-dibromo-8-methoxy-2,3-dihydro-5-*H*-1,4-benzodioxepin-3-ol (29). To a solution of compound **25** (50 mg, 0.13 mmol) in THF (1.3 mL) was added $NaBH_4$ (3.8 mg, 0.13 mmol) at room temperature. After stirring for 20 h the reaction was quenched by the addition of 1 N HCl (0.5 mL) and the aqueous phase was extracted with Et_2O (3× 1 mL). Drying of the combined organic phases with $MgSO_4$ was followed by evaporation of the solvent under vacuum. The chromatography on silica gel (AcOEt/hexane = 1:1 v/v) yielded compound **28** (white crystals: 28 mg, 0.06 mmol, 46%) and compound **29** (white crystals, mp 100–103 °C: 4 mg, 0.01 mmol, 7%). Compound **28**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.28 (1H, br s, *H*O—CH_2), 3.85 (3H, s, COOMe), 3.89 (3H, s, OMe), 4.70 (2H, s, CH_2—OH), 4.77 (2H, s, O—CH_2—COOMe), 7.54 (1H, s, ArH) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 52.4 (CH_3, COO*Me*), 60.6 (OMe), 60.6 (HO—*C*H_2), 69.9 (O—*C*H_2—COOMe), 113.2 (ArC—Br), 113.3 (ArC—Br), 132.5 (ArCH), 133.1 (Ar*C*—CH_2OH), 154.6 (Ar*C*—OCH_2), 162.6 (Ar*C*—OMe), 169.8 (COO) ppm. MS (70 eV, EI) *m*/*z* (%) 382/384/386 (3/7/3) ($M^{+\cdot}$), 350/352/354 (8/16/8) (M−MeOH), 309/311/313 (66/100/45) (M−CH_2COOMe), 308/310/313 (18/28/17) (M-74), 293/295/297 (9/17/11), 292/294/296 (25/47/26) (M-90),

204 (16), 202 (19), 75 (13), 74 (28), 45 (18). Anal. Calcd for $C_{11}H_{12}Br_2O_5$: C, 34.40; H, 3.15. Found: C, 34.29; H, 3.21.

4.4.5. [2-(Benzyloxy)-3,5-dibromo-4-methoxy-phenyl] methanol (27). To a solution of compound **24** (100 mg, 0.25 mmol) in THF (2.5 mL) was added $NaBH_4$ (9.5 mg, 0.25 mmol) at room temperature. After stirring for 90 min the reaction was quenched by the addition of 1 N HCl (1 mL) and the aqueous phase was extracted with Et_2O (3× 1.5 mL). Drying of the combined organic phases with $MgSO_4$ was followed by evaporation of the solvent under vacuum. Compound **27** was purified by chromatography on silica gel (AcOEt/hexane = 1:1 v/v). White crystals, mp 85–87 °C: 64 mg, 0.16 mmol, 64%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.85 (1H, br s, *H*O—CH_2), 3.92 (3H, s, OMe), 4.57 (2H, s, C*H*$_2$—OH), 5.09 (2H, s, CH_2—O—Ph), 7.31–7.55 (5H, m, 5× ArCH), 7.58 (1H, s, ArH, H-6) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 60.6, 60.8 (OMe, HO—CH_2), 75.9 (Ph—O—*C*H_2), 113.2 (ArC—Br), 114.5 (ArC—Br), 128.6 (2× ArCH), 128.79 (2× ArCH), 128.82 (ArCH), 131.9 (ArCH, C-6), 133.3, 136.4 (ArC—CH_2OH, ArC—CH_2OPh), 154.2, 154.8 (ArC—OBn, ArC—OMe) ppm. MS (70 eV, EI) *m*/*z* (%) 400/402/404 (1/2/1) ($M^{+\cdot}$), 292/294/296 (6/12/6) (M−PhCH$_2$OH), 92 (8), 91 (100) ($PhCH_2^+$), 65 (7). Anal. Calcd for $C_{15}H_{14}Br_2O_3$: C, 44.81; H, 3.51. Found: C, 44.69; H, 3.37.

4.5. Synthesis of (±)-spiroepoxycyclohexa-dienones[12b]

4.5.1. 5-Bromo-6-methoxy-1-oxa-spiro[2.5]octa-5,7-dien-4-one (8). To a solution of compound **15** (200 mg, 0.86 mmol) in THF (4.3 mL) and 1 N HCl (0.53 mL) was added, dropwise, a solution of $NaIO_4$ (1.48 g, 6.9 mmol) in H_2O (8.1 mL) and 1 N HCl (2.7 mL) at room temperature. After stirring for 90 min the aqueous phase was extracted with Et_2O (3× 15 mL). Drying of the combined organic phases with $MgSO_4$ was followed by evaporation of the solvent under vacuum. Compound **8** was purified by chromatography on silica gel (AcOEt/hexane = 1:5 v/v). Yellow crystals, mp 121–126 °C: 99 mg, 0.43 mmol, 50%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.19 (1H, d, ^2J = 8.2 Hz AB system, CH_2O), 3.36 (1H, d, ^2J = 8.2 Hz AB system, CH_2O), 4.02 (3H, s, OMe), 6.28 (1H, d, ^3J = 10.3 Hz, CH, H-7), 6.73 (1H, d, ^3J = 10.3 Hz, CH, H-8) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 57.0 (C—O), 57.7 (OMe), 58.9 (OCH_2), 103.6 (C—Br), 120.1 (CH, C-7), 141.2 (CH, C-8), 166.7 (*C*—OMe), 194.3 (C=O) ppm. MS (70 eV, EI) *m*/*z* (%) 230/232 (95/100) ($M^{+\cdot}$), 229/231 (96/100) (M−H), 215/217 (39/40) (M−CH_3), 214/216 (29/42) (M-16), 135 (17), 79 (24), 77 (22), 63 (19), 57 (25), 55 (29), 51 (32), 43 (26), 41 (20), 39 (19). Anal. Calcd for $C_8H_7BrO_3$: C, 41.59; H, 3.05. Found: C, 41.60; H, 3.11.

4.5.2. 7-Bromo-6-methoxy-1-oxa-spiro[2.5] octa-5,7-dien-4-one (9). A solution of compound **14** (300 mg, 1.30 mmol) in THF (2 mL) was added, dropwise, to a solution of $LiAlH_4$ (51 mg, 1.30 mmol) in THF (4 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min. The reaction was quenched by the dropwise addition of water, and the mixture was extracted with Et_2O (3× 10 mL). The combined organic phases were dried with $MgSO_4$ and filtered. To the solution obtained [**17**] a solution of $NaIO_4$ (1.11 g,) in H_2O (8.3 mL) and 1 N HCl (2 mL) at room temperature was added. After stirring for 90 min the aqueous phase was extracted with Et_2O (2× 10 mL). Drying of the combined organic phases with $MgSO_4$ was followed by evaporation of the solvent under vacuum. Compound **9** was purified by chromatography on silica gel (AcOEt/hexane = 1:1 v/v). Yellow crystals, mp 114–116 °C: 1.59 g, 0.69 mmol, 53%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.11 (1H, d, ^2J = 7.8 Hz AB system, CH_2O), 3.33 (1H, d, ^2J = 7.8 Hz AB system, CH_2O), 3.82 (3H, s, OMe), 5.76 (1H, s, H-5) 6.53 (1H, s, H-8) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 56.0 (C—O), 57.2 (OMe), 57.7 (OCH_2), 101.3 (CH, C-5), 118.5 (CBr), 139.9 (CH, C-8), 166.2 (C—OMe), 191.3 (C=O) ppm. MS (70 eV, EI) *m*/*z* 230/232 (28/28) ($M^{+\cdot}$), 215/217/218/219/220 (12/27/10/13/8), 124 (19) (M-106), 86 (49), 84 (100), 53 (22), 51 (45), 49 (94), 45 (55). Anal. Calcd for $C_8H_7BrO_3$: C, 41.59; H, 3.05. Found: C, 41.43; H, 3.20.

4.5.3. 5,7-Dibromo-1-oxa-spiro[2.5]octa-5,7-dien-4-one (12) and 1′,3′,7′,8a′-tetrabromo-2′,4′,4a′, 8′-tetrahydro-di-spiro-[oxiran-2-5′(6′H)-[1,4]ethanenaphthalen-9′,2″-oxiran]-6′,10′-dione (23). To a solution of compound **22** (250 mg, 0.89 mmol) in THF (4.5 mL) and 1 N HCl (0.6 mL) was added, dropwise, a solution of $NaIO_4$ (1.52 g, 7.1 mmol) in H_2O (8.3 mL) and 1 N HCl (2.8 mL) at room temperature. After stirring for 90 min the aqueous phase was extracted with Et_2O (3× 20 mL). Drying of the combined organic phases with $MgSO_4$ was followed by evaporation of the solvent under vacuum. Compound **12** was purified by chromatography on silica gel (AcOEt/hexane = 1:5 v/v). Yellow crystals: 124 mg, 0.44 mmol, 50%. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.21 (1H, d, ^2J = 8.0 Hz AB system, CH_2—O), 3.36 (1H, d, ^2J = 8.0 Hz AB system, CH_2—O), 6.32 (1H, d, ^4J = 2.2 Hz, CH, H-8), 7.60 (1H, d, ^4J = 2.2 Hz, CH, H-6) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) δ 58.7 (C—O), 60.3 (CH_2—O), 117.3 (C—Br, C-7), 124.2 (C—Br, C-5), 138.0 (CH, C-6), 146.8 (CH, C-8), 187.0 (C=O) ppm. MS (70 eV, EI) *m*/*z* (%) 278/280/282 (52/100/42) ($M^{+\cdot}$), 277/279/281 (29/70/32) (M−H), 262/264/266 (24/33/17) (M-16), 63 (62), 62 (38), 45 (25), 43 (23). Anal. Calcd for $C_7H_4Br_2O_2$: C, 30.04; H, 1.44. Found: C, 30.30; H, 1.21. When the treatment of the reaction was carried out at $T > 25$ °C a progressive dimerization of epoxy ketone **12** to render compound **23** was observed. The dimer **23** was purified by chromatography on silica gel (AcOEt/hexane = 1:5 v/v). White crystals, mp 160–162 °C. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.08 (1 H, dd, ^3J = 2.0, ^4J = 2.6 Hz, H-4a), 3.10 (1H, d, ^2J = 5.9 Hz AB system, CH_2—O, oxirane-5), 3.15 (1H, d, ^2J = 5.9 Hz AB system, CH_2—O, oxirane-5), 3.20 (1H, d, ^2J = 6.4 Hz AB system, CH_2—O, oxirane-9), 3.34 (1H, d, ^2J = 6.4 Hz AB system, CH_2—O, oxirane-9), 3.45 (1H, d, ^3J = 2.0 Hz, H-4), 6.48 (1H, d, ^4J = 2.6 Hz, H-8), 7.47 (1H, s, H-10) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz) δ 51.1 (CH, C-4 or C-4a), 53.4 (CH, C-4 or C-4a), 54.2 (CH_2—O), 56.2 (C—O), 56.5 (C—O), 59.3 (CH_2—O), 61.2 (CBr, C-8a), 76.8 (CBr, C-1), 124.4 (CBr, C-3 or 7), 125.1 (CBr, C-3 or 7), 132.8 (CH, C-8 or 2), 146.1 (CH, C-2 or 8), 190.3

(2× C=O) ppm. ESI *m/z* (%) 579/581/583/585/587 (15/35/62/40/11) $(M+Na)^+$. Anal. Calcd for $C_{14}H_8Br_4O_4$: C, 30.04; H, 1.44. Found: C, 30.23; H, 1.50.

4.6. Phenolic hydroxyl protection of the salicylic aldehyde (4)

4.6.1. 2-(Benzyloxy)-3,5-dibromo-4-methoxy benzaldehyde (24). To a solution of aldehyde **4** (2.05 g, 6.61 mmol) in DMF (1.55 mL) were added, successively, K_2CO_3 (823 mg, 5.95 mmol) and BnCl (0.84 mL, 7.27 mmol). The reaction mixture was heated up to 95 °C and allowed to react for 3 h. The reaction mixture was then cooled to room temperature and diluted with H_2O. After filtration of the brown precipitate formed, compound **24** was purified by chromatography on silica gel (AcOEt/hexane = 1:10 v/v). Green crystals, mp 91–93 °C: 2.45 g, 6.12 mmol, 93%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.00 (3 H, s, OMe), 5.12 (2H, s, CH_2), 7.30–7.60 (5H, m, Ph), 8.01 (1H, s, ArH), 9.94 (1H, s, CHO) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 61.1 (OMe), 78.2 (CH_2O), 114.6 (ArC—Br), 115.5 (ArC—Br), 128.5 (Ar*C*—CHO), 128.92 (2× ArCH), 128.96 (2× ArCH), 129.2 (ArCH), 131.6 (ArCH, C-6), 159.4 (ArC—O), 160.5 (ArC—O), 187.2 (CHO). MS (70 eV, EI) *m/z* (%) 398/400/402 (1/2/1) ($M^{+\cdot}$), 369/371/373 (2/4/2) (M−CHO), 307/309/311 (1/2/1) (M−CH_2Ph), 92 (7), 91 (100) ($C_7H_7^+$), 65 (8). Anal. Calcd for $C_{15}H_{12}Br_2O_3$: C, 45.03; H, 3.02. Found: C, 44.83; H, 3.12.

4.6.2. Methyl (2,4-dibromo-6-formyl-3-methoxyphenoxy) acetate (25). To a solution of aldehyde **4** (300 mg, 0.93 mmol) in acetone (2.80 mL) were added, successively, K_2CO_3 (115 mg, 0.84 mmol), ether-18-crown-6 (26 mg, 0.09 mmol) and $BrCH_2CO_2Me$ (0.10 mL, 1.02 mmol). The reaction mixture was allowed to react for 3 h at room temperature. The inorganic salt was eliminated by filtration and the resulting solution was dried with $MgSO_4$ followed by evaporation of the solvent under vacuum. Compound **25** was purified by chromatography on silica gel (AcOEt/hexane = 1:1 v/v). White crystals, mp 114–116 °C: 280 mg, 0.73 mmol, 76%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.74 (3H, s, COOMe), 3.89 (3H, s, OMe), 4.76 (2H, s, CH_2), 7.97 (1H, s, ArH), 10.35 (1H, s, CHO) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 52.5 (CH_3, COOMe), 61.1 (OMe), 70.5 (CH_2O), 114.1 (ArC—Br), 114.8 (ArC—Br), 128.4 (Ar*C*—CHO), 132.0 (ArCH), 158.5 (Ar*C*—OCH_2), 161.3 (Ar*C*—OMe), 168.6 (COO), 187.7 (CHO) ppm. MS (70 eV, EI) *m/z* (%) 380/382/384 (12/25/13) ($M^{+\cdot}$), 348/350/352 (9/18/9) (M−MeOH), 321/323/325 (16/27/13) (M−COOMe), 307/309/311 (50/100/49) (M−CH_2COOMe), 306/308/310 (18/38/28), 47 (64), 45 (29), 43 (17). Anal. Calcd for $C_{11}H_{10}Br_2O_5$: C, 34.59; H, 2.64. Found: C, 34.57; H, 2.72.

4.6.3. 2,4-Dibromo-6-formyl-3-methoxy-phenyl acetate (26). A solution of AcCl (0.03 mL, 0.38 mmol) in dioxane (0.32 mL) was added, dropwise, during 30 min to a mixture of aldehyde **4** (100 mg, 0.32 mmol) in dioxane (0.39 mL), TBAH (0.4 mg) and NaOH (32 mg, 0.81 mmol). The mixture was stirred at room temperature for 32 h. The reaction mixture was filtered washing with dioxane. Drying of the resulting solution with $MgSO_4$ was followed by evaporation of the solvent under vacuum. White solid, mp 77–81 °C: 70 mg, 0.20 mmol, 62%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.47 (3 H, s, Ac), 3.98 (3H, s, OMe), 8.05 (1H, s, ArH), 9.91 (1H, s, CHO) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 20.3 (CH_3, Ac), 60.8 (OMe), 115.31 (ArC—Br), 115.36 (ArC—Br), 126.5 (Ar*C*—CHO), 133.8 (ArCH), 149.9 (Ar*C*—OC=O), 159.8 (Ar*C*—OMe), 167.7 (*C*OOMe), 185.7 (CHO) ppm. MS (70 eV, EI) *m/z* (%) 307/309/311 (18/39/29) (M−Ac), 308/310/312 (34/74/29) (M-42), 86 (59), 84 (78), 51 (38), 49 (15), 43 (31).

4.6.4. 2,4-Dibromo-6-formyl-3-methoxy-phenyl benzoate (38). To a solution of aldehyde **4** (100 mg, 0.32 mmol) in dioxane (0.4 mL) were added, successively, Et_3N (0.11 mL, 0.81 mmol) and BzCl (0.05 mL, 0.39 mmol). The reaction mixture was stirred at room temperature for 30 min. The reaction mixture was filtered washing, several times, with Et_2O. Drying of the resulting solution with $MgSO_4$ was followed by evaporation of the solvent under vacuum. Compound **38** was purified by chromatography on silica gel (AcOEt/hexane = 1:5 v/v). White crystals, mp 104–107 °C: 90 mg, 0.22 mmol, 67%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.02 (3H, s, OMe), 7.58 (2H, t, ^3J = 7.8 Hz, 2× ArCH, H-2′ and 6′), 7.73 (1H, t, ^3J = 7.8 Hz, ArCH, H-4′), 8.14 (1H, s, ArH, H-5), 8.27 (2H, d, ^3J = 7.8 Hz, 2× ArCH, H-3′ and 5′), 9.49 (1H, s, CHO) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 61.1 (OMe), 115.5 (ArC—Br), 116.2 (ArC—Br), 127.2 (Ar*C*—C=O), 127.8 (Ar*C*—C=O), 128.9 (2× ArCH), 130.7 (2× ArCH), 133.0 (ArCH), 134.6 (ArCH), 150.9 (Ar*C*—OC=O), 160.1 (Ar*C*—OMe), 163.9 (COO), 185.8 (CHO) ppm. Anal. Calcd for $C_{15}H_{10}Br_2O_4$: C, 43.51; H, 2.43. Found: C, 43.51; H, 2.41.

4.6.5. 4-(Dimethylamino) 2,4-dibromo-6-formyl-3-methoxyfenyl benzoate (39). To a solution of aldehyde **4** (310 mg, 0.99 mmol) in dioxane (1.1 mL) were added, successively, Et_3N (0.33 mL, 2.44 mmol) and 4-(dimethylamin) benzoyl chloride (198 mg, 1.08 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered washing, several times, with Et_2O. Drying of the resulting solution with $MgSO_4$ was followed by evaporation of the solvent under vacuum. Compound **39** was purified by chromatography on silica gel (AcOEt/hexane = 1:5 v/v). White crystals, mp 140–142 °C: 230 mg, 0.50 mmol, 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.11 (6H, s, $N(CH_3)_2$), 4.00 (3H, s, OMe), 6.73 (2H, d, ^3J = 8.9 Hz, 2× ArCH, H-3′ and 5′), 8.11 (2H, d, ^3J = 8.8 Hz, 2× ArCH, H-2′ and 6′), 8.14 (1H, s, ArH, H-5), 10.02 (1H, s, CHO) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 40.1 ($N(CH_3)_2$), 61.0 (OMe), 111.2 (2× ArCH, C-3′ and 5′), 113.6 (ArC—Br), 115.6 (Ar*C*—COO), 115.8 (ArC—Br), 127.6 (Ar*C*—CHO), 131.9 (ArCH, C-5), 132.7 (2× ArCH, C-2′ and 6′), 152.0 (ArC-N), 154.4 (Ar*C*—OC=O), 159.9 (Ar*C*—OMe), 164.1 (COO), 186.2 (CHO) ppm. MS (70 eV, EI) *m/z* (%) 455/3457/459 (1/2/1) ($M^{+\cdot}$), 149 (11), 148 (100), 120 (3), 105 (3), 104 (2), 79 (3), 42 (4). Anal. Calcd for $C_{17}H_{15}Br_2NO_4$: C, 44.67; H, 3.31. Found: C, 44.50; H, 3.58.

4.6.6. 3,5-Dibromo-2-{[2-bromo-5-(dimethyl-amino)benzyl]oxy}-4- methoxybenzaldehyde (40). (a) *Synthesis of 4-bromo-3-(bromomethyl)-N,N-dimethylaniline.* A

suspension of NBS (1.72 g, 9.66 mmol) in anhydrous CH_2Cl_2 (33 mL) was cooled to 0 °C and SMe_2 (0.87 mL, 10.63 mmol) was added dropwise during 5 min. The reaction mixture was then cooled to −20 °C and a solution of 3-(hydroxymethyl)-*N*,*N*-dimethylaniline (710 mg, 4.70 mmol) in anhydrous CH_2Cl_2 (2.5 mL) was added. After the addition, the temperature was raised to 0 °C and the mixture stirred for 3 h. The reaction mixture was then diluted with pentane and 50 mL of a water/ice mixture was added. The organic phase was separated, washed with cold brine (5× 30 mL), dried with $MgSO_4$ and the solvent evaporated under vacuum. Purification by chromatography on silica gel (AcOEt/hexane = 1:5 v/v) gave yellow crystals, mp 87–90 °C: 751 mg, 2.56 mmol, 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.96 (6H, s, $N(CH_3)_2$), 4.57 (2H, s, CH_2Br), 6.55 (1H, dd, ^3J = 8.9 Hz, ^4J = 3.2 Hz, ArH-6), 6.77 (1H, d, ^4J = 3.2 Hz, ArH-2), 7.37 (1H, d, ^3J = 8.9 Hz, ArH-5) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 34.9 (CH_2Br), 40.9 ($N(CH_3)_2$), 110.5 (ArC—Br), 114.7 (ArCH), 115.0 (ArCH), 133.8 (ArCH, C-5), 137.3 (Ar*C*—CH_2), 150.4 (ArC-N) ppm. MS (70 eV, EI) *m*/*z* (%) 291/293/295 (36/78/32) ($M^{+\cdot}$), 212/214 (97/100) (M−Br), 133 (52) (M−Br_2), 132 (30), 118 (27), 91 (33). Anal. Calcd for $C_9H_{11}Br_2N$: C, 36.89; H, 3.78. Found: C, 37.05; H, 3.72.

(b) *Phenolic group protection.* To a solution of aldehyde **4** (298 mg, 0.93 mmol) in DMF (0.5 mL) were added, successively, K_2CO_3 (116 mg, 0.84 mmol) and 4-bromo-3-(bromomethyl)-*N*,*N*-dimethylaniline (300 mg, 1.03 mmol) in DMF (0.5 mL). The reaction mixture was heated up to 75 °C and allowed to react for 2 h. The reaction mixture was then cooled to room temperature and diluted with H_2O. Filtration of the yellow precipitate formed yielded compound **40**. Yellow crystals, mp 140–142 °C: 425 mg, 0.81 mmol, 87%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.95 (6H, s, $N(CH_3)_2$), 3.98 (3H, s, OMe), 5.19 (2H, s, CH_2O), 6.58 (1H, dd, ^3J = 8.9 Hz, ^4J = 3.1 Hz, ArH-3′), 6.86 (1H, d, ^4J = 3.1 Hz, ArH-6′), 7.38 (1H, d, ^3J = 8.9 Hz, ArH-4′), 8.01 (1H, s, ArH-6), 10.00 (1H, s, CHO) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 40.5 ($N(CH_3)_2$), 61.3 (OMe), 77.5 (CH_2O), 109.1 (ArC—Br), 114.2 (ArCH, C-4′ or 6′), 114.4 (ArCH, C-4′ or 6′), 114.4 (ArC—Br), 115.3 (ArC—Br), 128.7 (ArC—CHO), 131.4 (ArCH, C-3′ó 6), 133.2 (ArCH, C-3′ or 6), 134.5 (Ar*C*—CH_2), 150.1 (ArC-N), 159.5 (Ar*C*—OCH_2), 160.2 (Ar*C*—OMe), 187.4 (CHO) ppm. MS (70 eV, EI) *m*/*z* (%) 519/521/523/525 (8/23/22/7) ($M^{+\cdot}$), 212/214 (100/96), 133 (30), 91 (21).

4.7. Wittig reactions

4.7.1. Methyl (2*E*)-3-(2-hydroxy-4-methoxy-phenyl) propionate (32). To a solution of aldehyde **3** (1.00 g, 6.58 mmol) in THF (65 mL) was added $Ph_3P{=}CH_2$ COOMe (2.20 g, 6.60 mmol). The reaction mixture was refluxed at 95 °C for 5 h. After evaporation of the solvent under vacuum, compound **32** was purified by chromatography on silica gel (AcOEt/hexane = 1:1 v/v). White crystals, mp 142–145 °C: 1.38 g, 6.58 mmol, quantitative. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.71 (3H, s, COOMe), 3.74 (3H, s, OMe), 6.36 (1H, s, ArH, H-3′), 6.40 (1H, d, ^3J = 8.3 Hz, ArH, H-5′), 6.97 (1H, d, $^3J_{trans}$ = 15.9 Hz, CH, H-2), 7.31 (1H, d, ^3J = 8.3 Hz, ArH, H-6′), 7.92 (1H, d, $^3J_{trans}$ = 15.9 Hz, CH, H-3) ppm. ^{13}C NMR: (50 MHz, $CDCl_3$) δ 51.7 (*Me*OOC), 55.4 (OMe), 101.9 (ArCH, C-3′ or 5′), 107.0 (ArCH, C-3′ or 5′), 115.0 (Ar*C*—CH), 115.1 (CH, C-2), 130.6 (ArCH, C-6′), 140.9 (CH, C-3), 157.3 (ArC—OH), 162.6 (Ar*C*—OMe), 169.3 (*C*OOMe) ppm. Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81. Found: C, 63.33; H, 5.84.

4.7.2. Methyl (2*E*)-3-(3,5-dibromo-2-hydroxy-4-methoxyphenyl) acrylate (33). To a solution of aldehyde **4** (5.08 g, 16.40 mmol) in THF (165 mL) was added $Ph_3P{=}CH_2$ COOMe (5.49 g, 16.44 mmol). The reaction mixture was refluxed at 95 °C for 5 h. After evaporation of the solvent under vacuum, compound **33** was purified by chromatography on silica gel (AcOEt/hexane = 1:1 v/v). White crystals, mp 177–179 °C: 6.02 g, 16.40 mmol, quantitative. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.71 (3H, s, COOMe), 3.85 (3H, s, OMe), 5.98 (1H, s, OH), 6.45 (1H, d, $^3J_{trans}$ = 16.1 Hz, CH, C-2), 7.58 (1H, s, ArH), 7.73 (1H, d, $^3J_{trans}$ = 16.1 Hz, CH, C-3) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 51.9 (*Me*OOC), 60.9 (OMe), 107.9 (ArC—Br), 108.2 (ArC—Br), 119.8 (Ar*C*—CH), 119.9 (CH, C-2), 131.8 (ArCH), 137.9 (CH, C-3), 151.7 (ArC—OH), 155.4 (Ar*C*—OMe), 167.2 (*C*OOMe) ppm. MS (70 eV, EI) *m*/*z* (%) 364/366/368 (13/27/14) ($M^{+\cdot}$), 332/334/336 (50/100/46) (M−MeOH), 304/306/308 (15/32/15) (M−MeOH—CO), 289/291/293 (21/40/23) (M-75), 75 (16). Anal. Calcd for $C_{11}H_{10}Br_2O_4$: C, 36.10; H, 2.75. Found: C, 37.25; H, 2.64.

4.8. Selective reduction of the alkenyl moiety

4.8.1. Methyl 3-(2-hydroxy-4-methoxyphenyl) propionate (34). To a solution of ester **32** (3.69 g, 17.74 mmol) in dry MeOH (83 mL) was added metallic Mg (4.31 g, 177.40 mmol). The reaction mixture was stirred at room temperature for 3 h. The reaction was hydrolysed by the dropwise addition of HCl (0.5 N) and the mixture was extracted with Et_2O (3× 25 mL). Drying of the resulting solution with $MgSO_4$ was followed by evaporation of the solvent under vacuum. Compound **34** was purified by chromatography on silica gel (AcOEt/hexane=1:1 v/v). White crystals, mp 55–57 °C: 2.61 g, 12.42 mmol, 70%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.62 (2H, t, ^3J = 7.0 Hz, CH_2, H-2), 2.80 (2H, t, ^3J = 7.0 Hz, CH_2, H-3), 3.62 (3H, s, COOMe), 3.70 (3H, s, OMe), 6.35 (1H, d, ^3J = 7.9 Hz, ArH, H-5′), 6.38 (1H, s, ArH, H-3′), 6.91 (1H, d, ^3J = 7.9 Hz, ArH, H-6′), 7.33 (1H, s, OH) ppm. Anal. Calcd for $C_{11}H_{14}O_4$: C, 62.85; H, 6.71. Found: C, 62.86; H, 6.84.

4.9. Methanolysis of the oxazolone ring

4.9.1. Methyl (2*Z*)-2-(benzoylamino)-3-[2-(benzyloxy)-3,5-dibromo-4-methoxyphenyl]acrylate (37). A solution of azlactone **36** (38 mg, 0.07 mmol) in dry MeOH (1.2 mL) was stirred at room temperature for 90 min. The reaction mixture was filtered followed by evaporation of the solvent under vacuum. Finally, cold water was added until the formation of a yellow precipitate. Compound **37** was purified by chromatography on silica

gel (AcOEt/hexane = 1:5 v/v). White crystals, mp 109–112 °C: 33 mg, 0.06 mmol, 82%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.90 (3H, s, OMe), 3.91 (3H, s, OMe), 4.88 (2H, s, CH_2O), 7.05 (1H, s, Ar*C*—C*H*=C), 7.10–7.60 (10H, m, 10× ArH, 2× Ph), 8.54 (1H, s, ArH, H-6′) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 52.95 (COO*Me*), 60.81 (OMe), 76.81 (CH_2—O), 113.70 (ArC—Br), 115.32 (ArC—Br), 121.03 (CH, Ar*C*—*C*H=C), 127.05 (ArC), 127.43 (2× ArCH), 128.23 (ArCH), 128.68 (2× ArCH), 128.81 (2× ArCH), 129.15 (ArCH), 129.60 (2× ArCH), 132.15 (ArCH), 132.80 (ArCH), 133.32 (CH=*C*—N), 135.26 (ArC), 153.22, 155.62 (Ar*C*—OCH_2, Ar*C*—OMe), 165.25, 165.46 (O—*C*=N, O—*C*=O) ppm. MS (70 eV, EI) *m*/*z* (%) 573/575/577 (1/2/1) ($M^{+\cdot}$), 541/543/545 (1/2/1) (M−MeOH), 494/496 (1/1) (M−Br), 452/454/456 (7/12/6) (M−H_2NCOPh), 436/438/440 (3/6/3), 420/422/424 (2/4/2), 106 (7), 105 (100) ($PhCO^+$), 91 (6), 77 (28). Anal. Calcd for $C_{25}H_{21}Br_2NO_5$: C, 52.20; H, 3.68. Found: C, 22.43; H, 654.

4.10. Synthesis of azlactones: general procedure

To a solution of the corresponding aldehydes (1 equiv) in Ac_2O (2.3 mL/mmol) were added, successively, anhydrous AcONa (2.94 equiv) and BzGly (2 equiv). The reaction mixture was stirred at 85–90 °C for 3 h. The reaction mixture was filtered washing, several times, with Ac_2O. The corresponding azlactones were purified by chromatography on silica gel (AcOEt/hexane = 1:5 v/v).

4.10.1. (4*Z*)-4-[2-(benzyloxy)-3,5-dibromo-4-methoxybenzyliden]-2-phenyl-1,3-oxazol-5(4*H*)-one (36). Yellow crystals, mp 191–192 °C: 200 mg, 0.37 mmol, 52%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (3H, s, OMe), 5.05 (2H, s, CH_2O), 7.38 (1H, s, Ar*C*—C*H*=C), 7.30–7.80 (8H, m, 8× ArH, H-3′,4′,5′,2″,3″,4″,5″ and 6″), 8.19 (2H, dd, ^3J = 6.8 Hz, ^4J = 1.6 Hz, 2× ArH, H-2′ and 6′), 9.10 (1H, s, ArH, 6‴) ppm. ^{13}C RMN (50 MHz, $CDCl_3$) δ 60.92 (OMe), 77.33 (CH_2—O), 113.92 (ArC—Br), 114.78 (ArC—Br), 123.11 (CH, *ArC*—*C*H=C), 125.28 (C, (Ar*C*) C-1′ or Ar*C*—CH=C), 126.76 (C, (Ar*C*) C-1′ or Ar*C*—CH=C), 128.55 (2× ArCH), 128.79 (2× ArCH), 128.82 (ArCH, C-4′), 129.00 (2× ArCH), 129.04 (2× ArCH), 133.72 (ArCH, C-4″ or C-6‴), 134.03 (C, (ArC) C-1‴ or CH=*C*—N), 135.19 (ArCH, C-4″ or C-6‴), 135.24 (C, (ArC) C-1‴ or CH=*C*—N), 156.63 (Ar*C*—OCH_2 or Ar*C*—OMe), 157.24 (Ar*C*—OCH_2 or Ar*C*—OMe), 164.37 (O—C=N), 166.73 (O—C=O) ppm. MS (70 eV, EI) *m*/*z* (%) 541/543/545 (1/2/1) ($M^{+\cdot}$), 436/438/440 (7/13/16) (M−Ph—CO), 308/310/312 (3/6/3), 106 (6), 105 (68), 92 (18), 91 (100) ($PhCH_2^+$), 77 (31), 65 (6), 47 (8), 43 (8). Anal. Calcd for $C_{24}H_{17}Br_2NO_4$: C, 53.07; H, 3.15; N, 2.58. Found: C, 52.81; H, 3.21; N, 2.57.

4.10.2. Phenyl 2,4-dibromo-3-methoxy-6-[(*Z*)-(5-oxo-2-phenyl-1,3-oxazol-4(5*H*)-yliden)-methyl]benzoate (41) and (4*Z*)-4-(3,5-dibromo-2-hydroxy-4-methoxybenzyliden)2-phenyl-1,3-oxazol-4(5*H*)-one (42). Compound **41**: Yellow crystals, mp >200 °C: 40 mg, 0.07 mmol, 15%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.99 (3H, s, OMe), 7.16 (1H, s, Ar*C*—C*H*=C), 7.45–7.85 (6H, m, 8× ArH, H-3′,4′,5′,3″,4″ and 5″), 8.16 (2H, d, ^3J = 8.3 Hz, 2× ArH, H-2′ and 6′ or H-2″and 6″), 8.28 (2H, d, ^3J = 8.6 Hz, 2× ArH, H-2′ and 6′ or H-2″ and 6″), 9.29 (1H, s, ArH, H-5) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 61.2 (OMe), 115.0 (ArC—Br), 116.0 (ArC—Br), 121.5 (CH, Ar*C*—*C*H=C), 125.2, 126.2, 128.1 (3× ArC, C-1″ or Ar*C*—CH=C or Ar*C*—C=O), 128.8 (2× ArCH), 129.1 (2× ArCH), 129.2 (2× ArCH), 130.9 (ArCH), 134.7 (ArCH), 135.4 (ArCH), 135.4 (CH=*C*—N), 149.5 (Ar*C*—O—C=O), 156.5 (Ar*C*—OMe), 163.9 (O—C=N), 165.2 (COO), 167.2 (COO) ppm. MS (70 eV, EI) *m*/*z* (%) 555/557/559 (3/6/3) ($M^{+\cdot}$), 106 (8), 105 (100) ($PhCO^+$), 77 (24). Anal. Calcd for $C_{24}H_{15}Br_2NO_5$: C, 51.73; H, 2.71; N, 2.51. Found: C, 51.87; H, 2.61; N, 2.60. Compound **42**: Yellow crystals, mp 184–187 °C: 40 mg, 0.09 mmol, 18%. ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.92 (3H, s, OMe), 7.35–7.75 (3H, m, 3× ArH, H-3′,4′ and 5′), 7.78 (1H, s, Ar*C*—C*H*=C), 7.86 (2H, d, ^3J = 8.1 Hz, ^4J = 1.5 Hz, 2× ArH, H-2′ and 6′), 8.72 (1H, s, ArH, H-6″), 8.76 (1H, s ancho, ArH, HO) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ 61.1 (OMe), 106.9 (ArC—Br), 114.2 (ArC—Br), 118.4 (Ar*C*—CH=C), 121.3 (CH, Ar*C*—*C*H=C), 124.2 (ArC, C-1′), 127.2 (2× ArCH), 129.0 (2× ArCH), 130.0 (ArCH), 132.7 (ArCH), 133.2 (CH=*C*—N), 147.3 (ArC—OH), 155.6 (Ar*C*—OMe), 157.9 (O—C=N), 166.1 (COO) ppm. MS (70 eV, EI) *m*/*z* (%) 451/453/455 (8/17/9) ($M^{+\cdot}$), 106 (8), 105 (100) ($PhCO^+$), 77 (29). Anal. Calcd for $C_{17}H_{11}Br_2NO_4$: C, 45.07; H, 2.45; N, 3.09. Found: C, 45.29; H, 2.65; N, 3.09.

4.10.3. Phenyl 2,4-Dibromo-3-methoxy-6-[(*Z*)-(5-oxo-2-phenyl-1,3-oxazol-4(5*H*)-yliden)methyl]4-(dimethylamino) benzoate (43). Yellow crystals, mp >200 °C: 20 mg, 0.03 mmol, 19%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.13 (6H, s, $N(CH_3)_2$), 4.01 (3H, s, OMe), 6.77 (2H, d, ^3J = 9.1 Hz, 2× ArCH, H-3′ and 5′), 7.23 (1H, s, Ar*C*—C*H*=C), 7.58 (2H, t, ^3J = 7.5 Hz, 2× ArCH, H-3″ and 5″), 7.67 (1H, t, ^3J = 7.5 Hz, 2× ArCH, H-4″), 8.13 (2H, d, ^3J = 9.1 Hz, 2× ArH, H-2′ and 6′), 8.19 (2H, d, ^3J = 7.5 Hz, 2× ArCH, H-2″ and 6″), 9.28 (1H, s, ArH, H-5) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 40.51 ($N(CH_3)_2$), 61.41 (OMe), 111.37 (2× ArCH, C-3 and 5), 114.24 (ArC—Br), 115.24, 115.77 (ArC—Br, Ar*C*—CH=C), 122.60 (Ar*C*—*CH*=C), 125.26, 126.79 (Ar*C*—COO, Ar*C*—C=N), 133.07 (2× ArCH, C-2′ and 6′), 134.26, 135.26, 135.54 (ArCH, ArCH, CH=*C*—N), 150.32 (ArC—N), 154.67 (Ar*C*—O—C=O), 157.31 (Ar*C*—OMe), 164.38 (O—C=N), 165.03 (COO), 167.16 (COO) ppm. MS (70 eV, EI) *m*/*z* (%) 598/600/602 (1/2/1) ($M^{+\cdot}$), 149 (11), 148 (100) ($(Me)_2N$—$PhCO^+$), 120 (2), 106 (2), 105 (21) ($PhCO^+$), 79 (2), 77 (11). Anal. Calcd for $C_{26}H_{20}Br_2N_2O_5$: C, 52.02; H, 3.36; N, 4.67. Found: C, 52.18; H, 3.56; N, 4.85.

4.10.4. (4*Z*)-4-(3,5-Dibromo-2-{[2-bromo-5-(dimethylamino)benzyl]oxy}-4-methoxy-benzyliden)-2-phenyl-1,3-oxazol-5(4*H*)-one (44). Orange crystals, mp >200 °C: 105 mg, 0.16 mmol, 21%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.95 (6H, s, $N(CH_3)_2$), 3.99 (3H, s, OMe), 5.11 (2H, s, CH_2O), 6.56 (1H, dd, ^3J = 8.9 Hz, ^4J = 2.9 Hz, ArH, H-4″), 6.95 (1H, d, ^4J = 2.9 Hz, ArH, H-6″), 7.37 (1H, d, ^3J = 8.9 Hz, ArH, H-3″), 7.46 (1H, s, Ar*C*—C*H*=C), 7.56 (2H, t, ^3J = 6.7 Hz, 2× ArH, H-3′ and 5′), 7.67 (1H, t, ^3J = 6.7 Hz, ArH, H-4′), 8.18 (2H, d,

^3J = 6.7 Hz, 2× ArH, H-2′ and 6′), 9.10 (1H, s, ArH, H-6‴) ppm. MS (70 eV, EI) *m/z* (%) 662/664/666/668 (2/8/8/3) ($M^{+\cdot}$), 582/584/586/588 (2/5/5/2), 583/585/587 (5/11/6) (M−Br), 555/557/559 (3/5/3) (M−Br—CO), 504/506 (3/3) ($M-Br_2$), 451/453/455 (4/7/3), 212/214 (64/63) ((Br)($(Me)_2N$)—$C_6H_3(CH_2)^+$), 133 (21), 132 (11), 118 (11), 106 (10), 105 (100) ($PhCO^+$), 77 (41). Anal. Calcd for $C_{26}H_{20}Br_2N_2O_5$: C, 52.02; H, 3.36; N, 4.67. Found: C, 52.18; H, 3.56; N, 4.85. Anal. Calcd for $C_{26}H_{21}Br_3N_2O_4$: C, 46.95; H, 3.18; N, 4.21. Found: C, 47.12; H, 3.08; N, 4.25.

4.11. Synthesis of hydrochlorides

4.11.1. (4*Z*)-4-(3,5-dibromo-2-{[2-bromo-5-(dimethylamonium chloride) benzyl]oxy}4-methoxybenzyliden)-2-phenyl-1,3-oxazol-5(4*H*)-one (45). A solution of the azlactone **44** (70 mg, 0.11 mmol) in dioxane (30 mL) was treated with gaseous HCl at room temperature for 30 min. Filtration of the yellow precipitate washing with dioxane yielded hydrochloride **45**. Yellow crystals, mp >200 °C: 40 mg, 0.06 mmol, 51%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.22 (6H, s, $N(CH_3)_2$), 4.00 (3H, s, OMe), 5.12 (2H, s, CH_2O), 7.42 (1H, s, Ar*C*—C*H*=C), 7.57 (2H, t, ^3J = 7.4 Hz, 2× ArH, H-3′ and 5′), 7.69 (1H, t, ^3J = 7.4 Hz, ArH, H-4′), 7.73 (1H, br d, ^3J = 8.7 Hz, ArH, H-3″), 7.83 (1H, dd, ^3J = 8.7 Hz, ArH, H-4″), 7.98 (1H, br s, ArH, H-6″), 8.20 (2H, d, ^3J = 7.4 Hz, 2× ArH, H-2′ and 6′), 9.17 (1H, s, ArH, H-6‴) ppm. ESI *m/z* (%) 663/665/667/669 (30/90/75/25) (M− Cl)$^+$.

4.12. Biological assays

4.12.1. Endothelial cell isolation and culture. The protocol for bovine aortic endothelial cell (BAEC) isolation has been published before (Ariane Scoumanne, Tahereh Kalamati, Jill Moss, Janet T. Powell, Martin Gosling and Nessa Carey *Atherosclerosis* **2002**, *160*, 59). In summary, aortic cords were isolated from calves younger than a year. The endothelium was isolated by digestion with 1 mg/mL Type I collagenase (Sigma) in Phosphate-Buffered Saline (PBS; Sigma) for 20 min at 37 °C. The endothelial cells were collected by centrifugation. BAE cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented with 20% foetal bovine serum (FBS; Sigma) and endothelial cell growth supplement (ECGS; Sigma) at 37 °C and 5% CO_2.

4.12.2. Cytotoxicity assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT; Sigma Chemical Co., St. Louis, MO) dye reduction assay in 96-well microplates was used, essentially as described (Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55). The assay is dependent on the reduction of MTT by mitochondrial dehydrogenases of viable cell to a blue formazan product which is measured spectrophotometrically. BAE cells (8×10^3 cells in a total volume of 200 mL of DMEM/20% FBS) were incubated in each well with serial dilutions of the tested compounds. After 2 days of incubation (37 °C, 5% CO_2 in a humid atmosphere) 50 mL of MTT (5 mg/mL in PBS) was added to each well and the plate was incubated for a further 2 h (37 °C). The resulting formazan was dissolved in 100 mL DMSO and read at 490 nm. All determinations were carried out in triplicate. IC_{50} value was calculated as the concentration of drug yielding a 50% of cell survival.

4.12.3. Sprouting inhibition assay. BAE cells were mixed with Cytodex 3 microcarriers beads (Amersham Pharmacia Bioech) and the mixture was incubated for 48 h at 37 °C and 5% CO_2. Following incubation cell-coated beads were washed and resuspended in fresh medium at a concentration of 250 beads per millilitre of media. 96-Well tissue culture plates with the compound to be tested were coated with 40 μL Matrigel Basement Membrane Matrix (BD Biosciences) and cell-coated beads (10 μL) were seeded on. Plates were incubated in 37 °C and 5% CO_2 incubator and 30 min later 50 μL of culture medium was added. After 48 h the extent and the length of the sprouting formed is assessed by observation at TMS-Nikon microscope and compared with non-treated well cells.

Acknowledgments

We thank Dr. Jesús Ángel de la Fuente (Instituto Biomar S.A., León, Spain) for valuable suggestions. This work has been supported by Instituto Biomar S.A. and Ministerio de Educación y Ciencia (Spain, Grant No. PTR1995-0651-OP).

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