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ARTICLE

Synthesis and biological evaluation of cyclic derivatives of combretastatin A-4 containing group 14 elements

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Several tricyclic compounds inspired in the structure of combretastatin A-4 and bearing group 14 elements have been synthesized by homocoupling of lithiated aryl fragments followed by ring-closing metathesis. These tricyclic compounds and their diolefin precursors were evaluated for their antiproliferative action on the tumor cell lines HT-29, MCF-7, HeLa and A-549 and on the non-tumor cell line HEK-293. In addition, their effects on the cell cycle were also measured. The tricyclic compounds show antiproliferative activity similar to combretastatin A-4, even though they are not so active in arresting cell cycle. However, some diolefin precursors are able to cause accumulation of cells in the G2/M phase in a higher percentage than combretastatin A-4 itself. Inhibition of endothelial tube formation and VEGFR-2 phosphorylation of some selected compounds are comparable to that of combretastatin A-4, particularly those of tin-containing compounds **23c** and **26c**, whose actions exceed those of sorafenib, a clinically used VEGFR-2 inhibitor.

Introduction

Evolution of life on Earth has originated a myriad of organic compounds from plants and microorganisms. These natural products display a wide variety of structures, many of them endowed with a plethora of organic functions resulting from countless biosynthetic pathways. Since ancient times humankind has used these compounds, particularly those extracted from plants, as drugs to cure various diseases.[1] Furthermore, natural products have an ample range of binding groups that allow them to interact within various biological targets in an optimal way.[2] The vast chemical diversity and novel mechanisms of action of natural products explain the pivotal role they have been playing in many drug development programmes.[3]

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. Cancer is caused by both external and internal factors that may act together or in sequence to initiate or promote carcinogenesis. Chemotherapy with cytotoxic drugs is the main treatment for certain types of cancer. Among the anti-cancer drugs approved in the period from 1940-2002, approximately 54% were derived from natural products or derivatives thereof.[4] Developments in synthetic chemistry have allowed the modification of natural products and the synthesis of new chemical structures endowed with new and better pharmacological properties. The incorporation of various types of metal atoms into synthetic molecules, for instance, has allowed the preparation of new pharmacologically valuable drugs, with cis-platinum and its derivatives being archetypal examples.[5] Our interest on natural product analogues[6] has led us to prepare several of such analogues and to investigate their activity as antiproliferative agents.[7] Combretastatin A-4 (hereafter CA-4, see structure **I** in Fig. 1), for instance, is a compound that belongs, like the well-known resveratrol, to the stilbene class of natural products.[8] Several compounds of the combretastatin family have acquired an outstanding status in the last years and found utility in various pharmacological applications, most particularly as anticancer agents.[9] Preclinical and clinical developments over the last decade have been rapidly accelerating for drugs such as **I**, most particularly in the form of its more water-soluble phosphate prodrug CA-4P (structure **II**, Fig. 1).[10] These encouraging developments have stimulated a variety of efforts devoted to the synthesis and biological evaluation of numerous structurally modified combretastatin derivatives and analogues.[11]

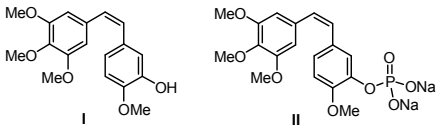

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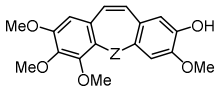
Fig. 1 Structures of combretastatin A-4 (**I**) and its phosphate prodrug (**II**)

Very recently, we have published several reports on the biological properties of several CA-4 analogues with cytotoxic activity.[12] One of the potential drawbacks of **I** and derivatives is the fact that they are prone to *cis-trans* isomerization induced by light or traces of acid impurities. This is undesirable because the *trans* isomers are practically devoid of biological activity.[13] We thus conceived the synthesis and biological evaluation of a family of CA-4 analogues such as **III** (Fig. 2) in which the presence of an additional cycle prevents the possibility of *cis-trans* isomerization.[14] The atom Z may belong in principle to any element of groups 14-16 in the periodic system but we have selected for the initial phase several elements of group 14 (Si, Ge, Sn).[15] It is expected that the differences in C–Z bond lengths, according to the nature of Z, will be reflected in differences in the dihedral angle between the two benzene rings, a feature which has been found to have an influence on the biological activity.[16]



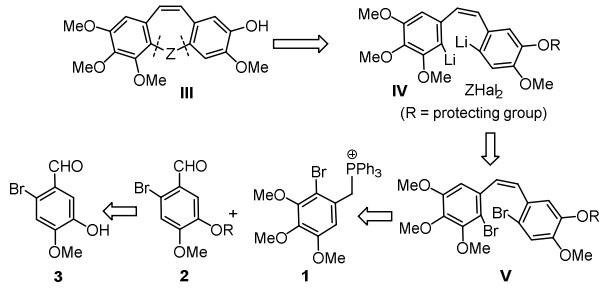
III

Fig. 2 General structure of tricyclic derivatives of CA-4

Results and discussion

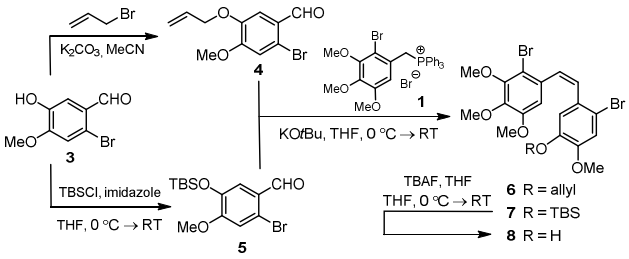
Chemistry

The initial synthetic concept towards compounds of type **III** is that depicted in Scheme 1. Thus, cyclic compounds such as **III** would be obtained by reaction of the electrophilic reagent $ZHal_2$ (Z = atom from group 14, Hal = halogen) with the dilithium derivative **IV** to be prepared in turn from dibromo derivative **V**. For the synthesis of the latter compound we envisaged a *Z*-selective Wittig olefination, for which there is a close precedent.[17] The precursors would be the ylide generated from phosphonium salt **1** and the protected aldehyde **2** generated from the commercially available **3**.



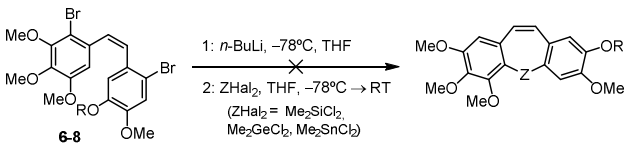
Scheme 1 Retrosynthetic analysis

Scheme 2 shows the results of the synthetic work. Aldehyde **3** was protected as its allyl and *tert*-butyldimethylsilyl (TBS) derivatives, **4** (R = allyl) and **5** (R = TBS), respectively. These were then allowed to react with the known phosphonium salt **1** in the presence of potassium *tert*-butoxide to yield the *cis* stilbene derivatives **6** and **7**, respectively, with excellent stereoselectivity (see Experimental for details on reaction conditions and yields).



Scheme 2 Synthesis of dibromo derivatives **6-8**. Abbreviations: TBS, *tert*-butyldimethylsilyl; TBAF, tetra-*n*-butylammonium fluoride; DIPEA, *N*,*N*-diisopropyl ethylamine.

The following step in the sequence proved disappointing. All attempts to cyclize compounds **6** and **7** by means of bromine-lithium interchange followed by treatment with the three electrophilic reagents indicated below met with failure (Scheme 3). In the case of compound **6** extensive decomposition took place whereas in the case of **7**, the product of reductive dehalogenation was isolated in low yield. Its structure was confirmed by the fact that desilylation yielded CA-4. A second side product isolated in low yield seemed to be the result of lithiation followed by a retro-Brook-type silyl migration.[18] Conjecturing that the nature or else the presence of the protecting group R might be the cause of the failures, we also attempted the cyclization with the unprotected compound **8**, prepared as shown in Scheme 2. However, this approach was not successful, as only CA-4 was isolated as a product of reductive dehalogenation in low yield. While disappointing, these results confirmed that at least bromine-lithium interchange had actually taken place.



Scheme 3 Attempts to prepare tricyclic derivatives

After this lack of success, we reasoned that the failure in the cyclization was possibly due to a too slow reaction of the electrophile $ZHal_2$ with the C–Li bonds in the lithiated intermediate **IV** (see Scheme 1). Since structurally close organolithium compounds with a lower substitution degree in the rings reacted in the expected way,[17] these negative results may be due to both C–Li bonds in **IV** being sterically hindered (*ortho* disubstitution). In view of this, we decided to revert the order of formation of bonds of the cyclic system. The two C–Z bonds would now be formed first by means of an intermolecular process, followed by subsequent creation of the C=C bond through an intramolecular olefin metathesis. This new type of strategy, which has precedent,[19] is depicted in Scheme 4. Thus, **III** should be obtained by means of ring-closing metathesis (RCM) of diolefin **VI**, to be in turn prepared through sequential coupling of the electrophile $ZHal_2$ with the two

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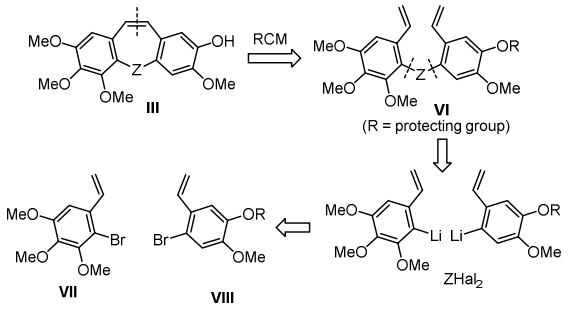
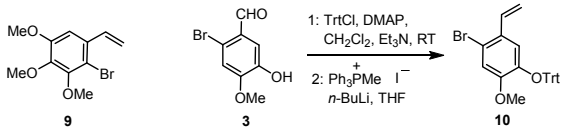
indicated organolithium reagents. The latter would be synthesized by means of bromine-lithium interchange in bromoarenes **VII** and **VIII**.



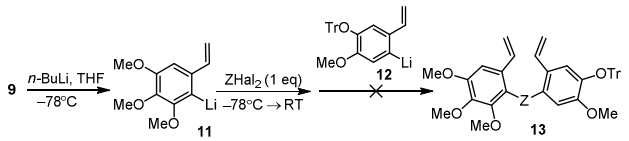
Scheme 4 Retrosynthetic analysis based on RCM

Compound **9** was synthesized by means of Wittig methylenation of the commercially available 2-bromo-3,4,5-trimethoxy-benzaldehyde. Likewise, **10** was prepared from aldehyde **3** through sequential tritylation (Trt = trityl) and Wittig methylenation (Scheme 5).



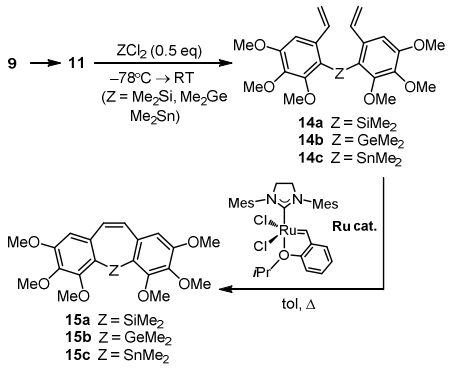
Scheme 5. Structure of **9** and synthesis of **10**

Bromoarene **9** was then converted into the corresponding organolithium derivative **11** (*n*-BuLi, THF, −78°C), and the latter was treated with one equivalent of the appropriate electrophilic reagent $ZHal_2$ and stirred for 15 min. at the same temperature (Scheme 6). Then the bath was allowed to reach room temperature and further stirred for 12 h. Subsequently, the reaction mixture was treated with one equivalent of the organolithium derivative **12** (generated from **10**) and further stirred for 12 h at room temperature.



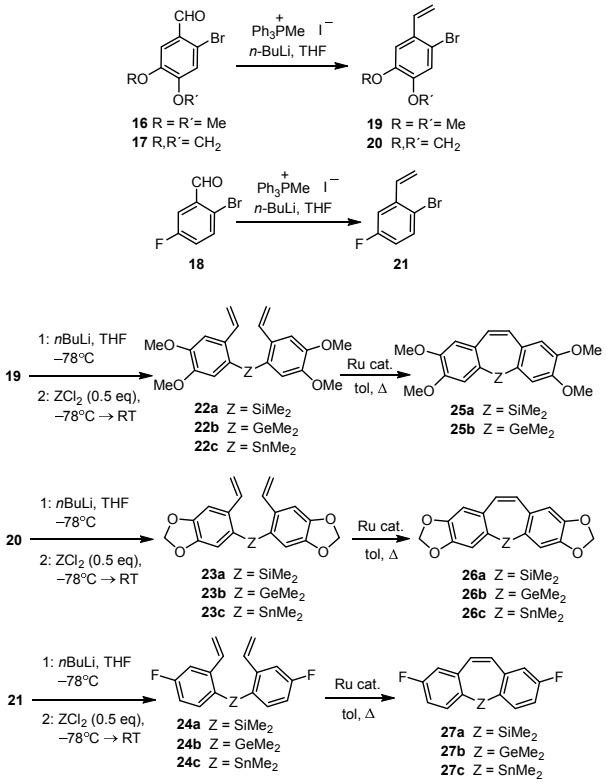
Scheme 6 Attempts to prepare **13**

Unfortunately, none of the desired heterocoupling compound **13** was obtained. Products of reductive dehalogenation were isolated in low yield, together with products of homocoupling (see below). We then tried an inversion of the order of the steps: organolithium **12** was allowed to react with the electrophile $ZHal_2$ followed by addition of **11**. The same lack of success was observed. As commented, the reaction mixtures above (Scheme 6) were shown to contain products of homocoupling. In view of this, we considered the possibility of preparing symmetric compounds such as **14** (Scheme 7), which still display the pharmacologically important *vic*-trimethoxyphenyl fragment. Indeed, compounds **14** (Z = $SiMe_2$, $GeMe_2$, $SnMe_2$) were obtained in two steps from **9** under the aforementioned reaction conditions.[20] Subsequently, ring-closing olefin metathesis to combretastatin-type compounds **15** (Z = $SiMe_2$, $GeMe_2$, $SnMe_2$) was performed by means of heating at reflux a solution of **14** and a second-generation Hoveyda-Grubbs ruthenium catalyst[21] in dry, deoxygenated toluene. The RCM step took place with good yields for Z = $SiMe_2$ and $GeMe_2$ but with a low yield for Z = $SnMe_2$ (see Experimental for details on reaction conditions and yields).



Scheme 7 Synthesis of tricyclic derivatives

In order to expand the range of cyclic combretastatin-like structures for biological investigation, we further performed the same reaction sequence starting with three other aromatic, commercially available aldehydes **16**-**18** (Scheme 8).



Scheme 8 Synthesis of tricyclic derivatives **25**-**27**

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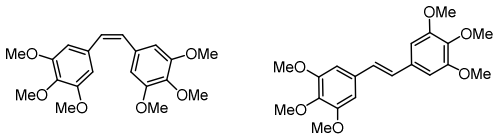
Thus, the cyclic, combretastatin-like compounds **25**-**27** were obtained (see Experimental for details on reaction conditions and yields). As in the previous case, the RCM step gave good yields when Z = $SiMe_2$, $GeMe_2$ but low yields when Z = $SnMe_2$ (indeed, 0% for **25c**). In these cases, extrusion of the tin fragment took place with formation of phenantrene derivatives. This behavior has precedent in similar systems with group 15 elements.[19c]

Biological results

Effect on the inhibition of cell proliferation

The inhibition of cell proliferation of CA-4 and its tricyclic derivatives was measured by means of their IC_{50} values towards the tumor cell lines HT-29 (human colon adenocarcinoma), MCF-7 (breast adenocarcinoma), HeLa (human epithelioid cervix carcinoma), A-549 (human lung adenocarcinoma) and in non-tumor cell line HEK-293 (human embryonic kidney). In addition to the compounds indicated in schemes 7 and 8, the acyclic geometrical isomers **28** and **29**[22] drawn in Scheme 9 were also evaluated for comparison purposes.



28 **29**

Scheme 9 Structures of (*Z*) and (*E*)-1,2-bis(3,4,5-trimethoxyphenyl) ethylene (**28** and **29**, respectively)

IC_{50} values are presented in Table 1 along with the calculated selectivity indexes (SI) obtained by dividing the IC_{50} values of the non-tumor cell line (HEK-293) by those of the corresponding tumor cell line. The higher the SI index of the compound, the higher its therapeutic safety margin.

Most of the cyclic derivatives show antiproliferative activity in the low micromolar range although they are not as active as CA-4. The most active compounds are **15a-c**, **25a** and **26a-c**, particularly on the HT-29 and MCF-7 lines. Fluorinated compounds **27a-c** exhibited much lower antiproliferative actions. As regards selectivity indexes, the best compounds are **15b**, **26c** and **27b** with SI values well above 1. Furthermore, when the IC_{50} values of **28** and **29** are compared, it is observed that the *trans* **29** isomer is much less active than the *cis* isomer **28**. This confirms previous observations that a *cis* configuration is needed in order to achieve a noticeable antiproliferative acivity.[14] Besides, IC_{50} values of the acyclic compound **28** are quite similar to those of tricyclic analogues **15a-c**, which means that the presence of the bridging atom does not perturb the antiproliferative action.

The acyclic precursor compounds **22a-c**, **23a-c** and **24a-c** were also evaluated. Their IC_{50} values were also in the μM range, although they were slightly higher than those of their tricyclic analogues and their SI values were lower than their tricyclic derivatives. In this sense, it is worth mentioning that compound **23c** is the one with the highest selectivity index. The higher antiproliferative activity of the tricyclic derivatives could be due to a more favourable relative spatial orientation of the benzene rings in these rigid compounds, although deeper studies would be necessary to confirm this hypothesis.

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Table 1. IC_{50} (μM) values and SI indexes for combretastatin A-4, acyclic and cyclic derivatives.[a]

Comp.	HT-29	MCF-7	HeLa	A-549	HEK-293	SI_A[b]	SI_B[c]	SI_C[d]	SI_D[e]
CA-4	4.2 ± 0.5	1.0 ± 0.2	2.5 ± 7	0.43 ± 0.004	25 ± 3	6	25	10	58
14a	7 ± 2	6.2 ± 1	3.9 ±0.7	7.4 ± 0.9	1.4 ± 0.2	0.2	0.2	0.3	0.2
14b	11 ± 2	6.0 ± 0.8	4.7 ± 0.9	8 ± 2	0.42 ± 0.05	0.04	0.07	0.09	0.09
14c	3.2 ± 0.8	7.0 ± 0.3	5.6 ± 0.4	8.6 ± 0.8	0.26 ± 0.03	0.08	0.04	0.06	0.03
15a	8.3 ± 0.7	11.3 ± 0.2	6.6 ± 0.8	7.2 ± 0.3	14 ± 2	4.2	5.7	0.6	2.3
15b	12.5 ± 0.8	9.15 ± 0.05	80 ± 15	22.7 ± 0.5	52 ± 9	2.5	1.2	2.8	1.2
15c	2.2 ± 0.4	4.75 ± 0.02	2.0 ± 0.9	4.49 ± 0.02	5.6 ± 0.3	0.8	2	1.3	0.2
22a	13 ± 2	43 ± 2	29 ± 8	57 ± 2	64 ± 2	5	1.5	2.2	1.1
22b	23 ± 5	50 ± 8	5.5 ± 0.9	76 ± 2	3.8 ± 0.3	0.2	0.07	0.7	0.05
22c	37 ± 2	35 ± 3	20 ± 4	10 ± 2	26 ± 4	0.7	0.7	1.3	2.6
23a	5.6 ± 0.9	36 ± 2	5.5 ± 0.8	19 ± 2	2.5 ± 0.3	0.4	0.07	0.4	0.1
23b	8 ± 1.5	7.0 ± 1.2	7.6 ± 0.7	>100	6.5 ± 1.0	0.8	0.8	0.8	0.07
23c	27 ± 2	68 ± 5	48 ± 4	>100	>100	2.7	6.8	48	1
24a	>100	44 ± 3	52 ± 6	74 ± 5	>100	1	2.3	2	1.3
24b	45 ± 2	82 ± 2	70 ± 9	68 ± 4	85 ± 9	2	1	1.2	1.2
24c	57 ± 5	18 ± 1	>100	21 ± 1	36 ± 5	0.6	2	0.4	1.7
25a	7.5 ± 0.7	3.2 ± 0.6	5.0 ± 0.8	26 ± 3	6.4 ± 0.2	0.9	0.3	0.1	0.4
25b	12 ± 2	32 ± 5	89 ± 2	25 ± 6	11 ± 2	0.4	0.8	0.8	0.4
26a	8.4 ± 0.3	32 ± 10	6.6 ± 0.8	>100	6.4 ± 0.5	0.8	0.2	1	0.06
26b	8.5 ± 0.6	6 ± 2	80 ± 15	54 ± 3	4.2 ± 0.3	0.5	0.7	0.05	0.08
26c	15 ± 3	2.5 ± 0.2	2.0 ± 0.9	5.1 ± 0.2	11 ± 2	0.7	4.4	5.5	2.1
27a	>100	57 ± 3	55 ± 10	>100	45 ± 2	0.4	0.8	0.8	0.4
27b	54 ± 9	45 ± 4	62 ± 8	>100	92 ± 7	1.7	2	1.5	0.9
27c	35 ± 2	53 ± 2	66 ± 4	76 ± 7	32 ± 5	0.9	0.6	0.5	0.4
28	8.0 ± 0.9	2.2 ± 0.2	1.82 ± 0,04	5.6 ± 0.5	2.4 ± 0.3	0.3	1.1	1.3	0.43
29	>100	>100	>100	21.5 ± 0.8	>100	-	-	-	-

[a]IC_{50} values are expressed as the compound concentration (μM) that inhibits the cell growth by 50%. Data are the average (±SD) of three experiments. [b]SI_A = IC_{50}(HEK-293)/IC_{50}(HT-29). [c]SI_B = IC_{50}(HEK-293)/IC_{50}(MCF-7). [d]SI_C= IC_{50} (HEK-293)/IC_{50} (HeLa). [e]SI_D= IC_{50} (HEK-293)/IC_{50} (A-549).

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Effect on the cell cycle

The effects of compounds on the cell cycle distribution were evaluated in A-549 cells. Thus, cells were incubated for 20 h in the presence of each compound at concentrations half of their IC_{50} values. Then the ADN content was measured by flow cytometry (see experimental section). Along with the tricyclic compounds and compounds **28** and **29**, diolefin compounds **23a-c** were also evaluated. These compounds were specifically selected for evaluation because they caused the formation of rounded cells.

Table 2. Cell cycle distribution.[a]

Comp.	Conc.	SubG0	G0	G1/S	G2/M
Control	---	5	69	12	13
CA-4	50 nM	15	21	22	43
14a	5 μM	2	87	5	4
14b	5 μM	2	86	6	6
14c	5 μM	6	70	10	12
15a	5 μM	2	88	6	4
15b	5 μM	2	89	6	3
15c	5 μM	10	80	7	3
22a	50 μM	13	68	10	7
22b	50 μM	10	71	8	9
22c	5 μM	54	44	5	3
23a	5 μM	5	24	10	61
23b	50 μM	5	23	11	60
23c	50 μM	2	20	12	65
24a	50 μM	3	84	2	4
24b	50 μM	8	74	2	12
24c	5 μM	9	76	4	10
25a	25 μM	5	80	9	6
25b	25 μM	13	71	7	8
26a	50 μM	9	35	10	46
26b	50 μM	11	47	12	30
26c	5 μM	11	26	7	49
27a	50 μM	3	65	12	19
27b	50 μM	19	72	4	5
27c	50 μM	7	80	6	7
28	5 μM	32	39	14	14
29	5 μM	15	72	8	4

[a]At least three measurements were performed in each case.

The results shown in Table 2 indicate that the tricyclic analogues are unable to accumulate cells on G2/M phase to a significant extent. In other words, these compounds are practically devoid of antimitotic action. However, it is worth noting that the diolefinic precursors **23a-c** show a noticeable percentage of cells in G2/M phase, with larger values than CA-4. Even though this effect is exerted at concentrations >100 times greater than that of CA-4, this suggests a certain degree of antimitotic activity of these compounds.

Effect on microtubules

CA-4 is able to arrest the cell cycle because it interferes with the formation of the microtubule cytoskeleton. In order to check whether compounds **23a**-**c** and **26a**-**c** exhibited the same mode of action as CA4, an immunofluorescence assay on A-549 cell line was performed. Thus, A549 cells were incubated for 16 h in the presence of CA-4 and compounds **23a**-**c** and **26a**-**c**. Figure 3 depicts the images of the effect caused by these compounds at their minimum concentration. As a control assay, in which A-549 cells were treated with DMSO (Fig. 3a), a normal filamentous microtubule network was observed, with microtubules extending from the central regions of the cell to the periphery. It can be appreciated that in the presence of a 50 nM dosis of CA-4 (Fig. 3b), tubulin appears aggregated and nuclei are compressed and fragmented, which is characteristic of cell division disruption.[23]

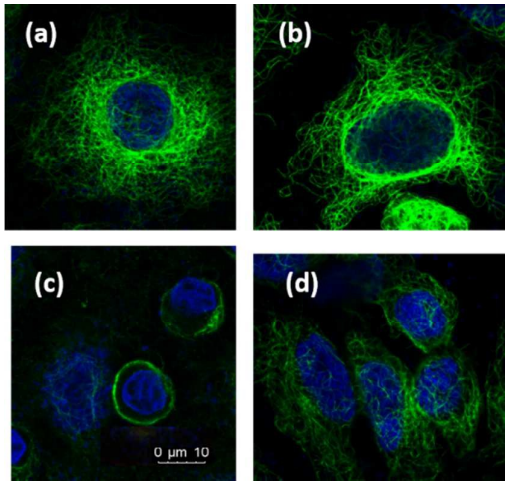

Fig. 3. Effects on the microtubule network. A-549 cells were treated for 16 hours and processed for immunofluorescence microscopy: (a) DMSO; (b) 50 nM CA-4; (c) 25 μM of **23c**; (d) 5 μM of **26c**.

Compound **23c** caused depolymerization and solubilization of the microtubule cytoskeleton at concentrations around 25 μM (Fig. 3c). Furthermore, cells treated with a 5 μM concentration of compound **26c** (Fig. 3d) showed disorganized interphase microtubules. These morphological changes of microtubules indicate that compounds of this structural type are able to disrupt the microtubule morphology in a similar manner to CA-4.

Effect on endothelial cells

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It has recently been shown that CA-4 might exhibit anti-angiogenic properties by interfering the VEGF/VEGFR-2 signalling pathway in endothelial cells.[24] Thus, we studied the effect of our synthetic derivatives on the proliferation of human microvascular endothelial (HMEC-1) cells by MTT assay. For this study we selected derivatives **23a**-**c** and **26a**-**c**, which were the ones that accumulated more cells in the G2/M phase. CA-4 and Sorafenib,[25] a clinically used VEGFR-2 inhibitor,[26] were employed as positive controls. Table 3 shows the IC_{50} values obtained for the tested compounds.

Table 3. IC_{50} values on HMEC-1 cells and minimum active concentration.[a]

Compound	IC_{50} ± SD HMEC-1 (μM)	Minimum active concentration (μM)
CA-4	3.4 ± 0.4	0.003
Sorafenib	34 ± 3	10
23a	35 ± 2	5
23b	32 ± 9	5
23c	12 ± 7	0.025
26a	39 ± 9	5
26b	27 ± 8	1
26c	15 ± 4	0.25

[a]At least three measurements were performed in each case.

The data of Table 3 indicate a decrease of IC_{50} values for HMEC-1 cells with the atomic number of the heteroatom present in the compound. Thus, the tin compounds **23c** and **26c** exhibit half the IC_{50} value of the silicon derivatives **23a** and **26a**.

Table 3 also shows the Minimum Active Concentration (MAC) at which the selected compounds begin to inhibit VEGF-induced endothelial tube formation. In order to measure this effect, HMEC-1 cells were seeded on Matrigel and the selected derivatives **23a**-**c** and **26a-c**, along with CA-4 and sorafenib, were immediately added at seriate concentrations ranging from 50 μM to 10 nM. The effect of the compounds on the endothelial tube formation was evaluated after 24 h of treatment. As an example, the photos of the inhibitory action of compound **23c** (MAC 0.025 μM) are presented in Fig. 3. The most active compound is CA-4 which starts to inhibit endothelial tube formation at 0.003 μM. In this biological action all synthetic compounds are more active than sorafenib itself. As above, the tin derivatives **17c** and **24c** proved the most active.

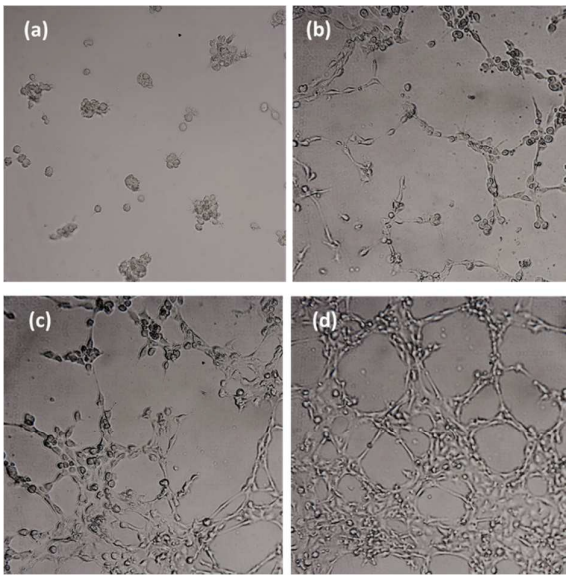

Fig. 3. Effect of compound **23c** at 5 μM (a), 0.5 μM (b), 0.05 μM (c) and 0.025 μM (d) concentrations.

Inhibition of VEGFR-2 kinase activity

The effect of the selected compounds exerted over VEGFR-2, a kinase receptor that plays an important role in the tumour angiogenic process, was also evaluated. In this assay, HMEC-1 cells were treated with the selected compounds for 2 hours, then VEGF (50 ng/mL) was added to the cell media and 30 minutes later, cells were lysed. Finally, phospho-VEGFR-2 was quantified from the lysates by ELISA analysis. The percentage of phospho-VEGFR-2 is expressed for each compound referred to control, which corresponds to 100 %.

Table 4. Detection of p-VEGFR-2 by ELISA assay.

Compound	Conc. (μM)	% p-VEGFR-2
CA-4	5	74 ± 8
Sorafenib	25	60 ± 6
23a	25	64 ± 3
23b	25	61 ± 3
23c	25	52 ± 4
26a	25	84 ± 6
26b	25	82 ± 2
26c	25	70 ± 4

It can be deduced from Table 4 that acyclic compounds **23a-c** are more active than cyclic derivatives **26a-c** in causing the inhibition of the kinase action of VEGFR-2 in endothelial cells. Moreover, compounds **23a-c** exert an inhibitory effect similar to that of sorafenib or the lead compound, CA-4. Again, the action of the compounds depends on the heteroatom they contain, the tin-

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containing compounds being a little more active than the rest of the synthetic compounds.

Conclusions

A range of 23 compounds containing silicon, germanium and tin have been synthesized and biologically evaluated as regards their antiproliferative action, their effect on cell cycle, and their effects on endothelial tube formation and inhibition of VEGFR-2 kinase activity. As compounds with structures inspired by that of combretastatin A-4, their biological actions have been compared with those of the natural product.

In the case of the tricyclic analogues **15**, **25**, **26** and **27**, where the third ring was expected to rigidify the structure, it has been found that their antiproliferative action and their effect on the cell cycle are weaker than that of CA-4. Thus, and in contrast with our expectations, the introduction of the third ring containing the heteroelement (Si, Ge, Sn) does not cause the desired increase of the antiproliferative action. However, their inhibition of endothelial tube formation and their inhibition of VEGFR-2 phosphorylation are comparable to that of CA-4, particularly in the case of the tin-containing compounds **23c** and **26c**, whose actions exceed those of sorafenib, a clinically used VEGFR-2 inhibitor.

The presence of compounds containing elements such as tin immediately raises questions related to their potential toxicity. While we have not performed toxicity studies on these tricyclic analogues of CA-4, it is worth mentioning that among the non-transition metal derivatives, more organotin(IV) compounds have undergone testing as potential anticancer agents than any other single group of compounds.[27] The design of improved organotin(IV) antitumor agents therefore still occupies a significant place in cancer chemotherapy.[27a]

Experimental

General features

NMR spectra (300 MHz for ^{1}H, 75 MHz for ^{13}C and 282 MHz for ^{19}F) were measured at 25°C. The signals of the deuterated solvent ($CDCl_3$) were taken as the reference for ^{1}H and ^{13}C spectra. For ^{19}F spectra (measured under ^{1}H decoupling), δ values were referenced to $CFCl_3$ (δ = 0). ^{13}C NMR signal multiplicities were determined with the DEPT pulse sequence. Mass spectra were run in the electrospray (ESMS) mode. Experiments which required an inert atmosphere were carried out under dry Ar in a flame-dried glassware. Commercially available reagents were used as received. Acronyms are defined in the synthetic schemes.

5-(Allyloxy)-2-bromo-4-methoxybenzaldehyde (**4**). Aldehyde **3** (231 mg, 1 mmol) was dissolved under Ar in dry acetonitrile (8 mL) and treated with anhydrous K_2CO_3 (207 mg, 1.5 mmol). Then allyl bromide (105 μL, 1.2 mmol) was added dropwise. The mixture was then stirred for 24 h at room temperature. After this time, water (10 mL) was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with brine and then dried over anhydrous $MgSO_4$. Removal of volatiles under reduced pressure gave **4**, sufficiently pure for further use: oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.16 (1H, s), 7.41 (1H, s), 7.05 (1H, s), 6.05 (1H, ddt, J = 17.3, 10.5, 5.5 Hz), 5.43 (1H, ddt, J = 17.3, 1.5, 1.5 Hz), 5.32 (1H, ddt, J = 10.5, 1.5, 1.5 Hz), 4.63 (2H, dt, J = 5.5, 1.5 Hz), 3.95 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.0, 147.9, 126.6, 120.6 (C), 190.9, 132.3, 115.8, 112.2 (CH), 119.0, 70.0 (CH_2), 56.6 (CH_3).

2-Bromo-5-(*tert*-butyldimethylsilyloxy)-4-methoxybenzaldehyde (**5**). Aldehyde **3** (231 mg, 1 mmol) was dissolved under Ar in dry THF (6 mL), cooled to 0°C and treated with TBSCl (226 mg, 1.5 mmol) and imidazole (204 mg, 3 mmol). The mixture was then stirred for 16 h at room temperature. After this time, water (10 mL) was added and the reaction mixture was extracted with EtOAc. The organic layer was washed with satd $NaHCO_3$, then with brine and finally dried over anhydrous $MgSO_4$. Removal of volatiles under reduced pressure gave an oily residue which was chromatographed on silica gel (elution with hexane-EtOAc 4:1). This gave **5**, sufficiently pure for further use (259 mg, 75%): oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.14 (1H, s), 7.39 (1H, s), 7.03 (1H, s), 3.88 (3H, s), 0.98 (9H, s), 0.16 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 145.1, 126.9, 120.5, 18.5 (C), 190.9, 120.6, 116.1 (CH), 56.1, 25.7 (x 3), −4.5 (x 2) (CH_3).

(*Z*)-1-(5-Allyloxy-2-bromo-4-methoxystyryl)-2-bromo-3,4,5-trimethoxybenzene (**6**). A solution of triphenyl (2-bromo-3,4,5-trimethoxybenzyl) phosphonium bromide **1** (300 mg, 0.5 mmol) in dry THF (8 mL) was cooled to 0°C and treated under Ar with KO*t*Bu (62 mg, 0.55 mmol). The mixture is then stirred for 30 min. at 0 °C. A solution of the appropriate aldehyde **4** (0.4 mmol) in dry THF (8 mL) is then added dropwise, and the mixture is stirred at room temperature for 16 h. After this time, water (30 mL) was added and the reaction mixture was extracted with EtOAc. The organic layer was washed with brine and finally dried over anhydrous $MgSO_4$. Removal of volatiles under reduced pressure gave an oily residue which was chromatographed on silica gel (elution with hexane-EtOAc 9:1). The desired olefination product was obtained as an off-white solid in 93% yield: oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (1H, s), 6.68 (1H, d, *J* = 11.8 Hz), 6.59 (1H, d, *J* = 11.8 Hz), 6.53 (1H, s), 6.40 (1H, s), 5.76 (1H, ddt, *J* = 17.3, 10.5, 5.5 Hz), 5.16 (1H, ddt, *J* = 17.3, 1.5, 1.5 Hz), 5.11 (1H, ddt, *J* = 10.5, 1.5, 1.5 Hz), 4.18 (2H, dt, *J* = 5.5, 1.5 Hz), 3.89 (3H, s), 3.84 (3H, s), 3.83 (3H, s), 3.49 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.5, 151.1, 149.4, 146.9, 142.4, 132.9, 128.9, 114.8, 110.3 (C), 132.5, 130.3, 130.1, 115.3, 115.2, 109.8 (CH), 118.4, 69.8 (CH_2), 61.2, 61.1, 56.2, 56.0 (CH_3).

(*Z*)-[4-Bromo-5-(2-bromo-3,4,5-trimethoxystyryl)-2-methoxy-phenoxy](*tert*-butyl)dimethylsilane (**7**). Prepared as above in 82% yield from phosphonium salt **1** and aldehyde **5**: off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (1H, s), 6.65 (1H, d, *J* = 11.8 Hz), 6.62 (1H, d, *J* = 11.8 Hz), 6.52 (1H, s), 6.39 (1H, s), 3.89 (3H, s), 3.85 (3H, s), 3.78 (3H, s), 3.51 (3H, s), 0.84 (9H, s), −0.08 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.4, 151.3, 151.1,

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144.2, 142.4, 133.0, 129.4, 115.2, 110.3, 18.4 (C), 130.3 (x 2), 122.7, 115.8, 109.7 (CH), 61.2, 61.1, 56.0, 55.9, 25.6 (x 3), −4.8 (x 2) (CH_3).

(*Z*)-4-Bromo-5-(2-bromo-3,4,5-trimethoxystyryl)-2-methoxy-phenol (**8**). Compound **7** (118 mg, 0.2 mmol) was dissolved under Ar in dry THF (6 mL) and cooled to 0°C. A solution of 1M TBAF in THF (0.3 mL, 0.3 mmol) was slowly added dropwise. The mixture is then stirred at room temperature for 1 h. After this time, satd aq $NaHCO_3$ (10 mL) was added and the reaction mixture was extracted with EtOAc. The organic layer was washed three times with brine and finally dried over anhydrous $MgSO_4$. Compound **8** was obtained in almost quantitative yield, sufficiently pure for further use: off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.02 (1H, s), 6.68 (1H, d, *J* = 11.8 Hz), 6.67 (1H, s), 6.62 (1H, d, *J* = 11.8 Hz), 6.44 (1H, s), 3.90 (3H, s), 3.86 (6H, s), 3.48 (3H, s) (OH proton not detected); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.2, 151.0, 146.6, 144.7, 142.6, 132.3, 130.1, 113.4, 110.7 (C), 130.2, 130.0, 116.4, 114.6, 109.7 (CH), 61.3, 61.1, 56.3, 56.0 (CH_3).

2-Bromo-3,4,5-trimethoxy-1-vinylbenzene (**9**). A solution of methyl triphenylphosphonium iodide (1 mmol, 404 mg) in dry THF (4 mL) was treated under an inert atmosphere with 2.5M *n*-BuLi (1 mmol, 0.4 mL). The mixture was then stirred for 20 min. at room temperature. A solution of 2-bromo-3,4,5-trimethoxy-benzaldehyde (1 mmol) in dry THF (3 mL) is then added dropwise, and the mixture is stirred at room temperature for 2 h. After this time, satd aq NH_4Cl (5 mL) was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed three times with brine and finally dried over anhydrous $MgSO_4$. Removal of volatiles under reduced pressure gave an oily residue which was chromatographed on silica gel (elution with hexane-EtOAc 9:1) to yield **9** (74%): oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (1H, dd, *J* = 17.3, 10.8 Hz), 6.89 (1H, s), 5.60 (1H, dd, *J* = 17.3, 1 Hz), 5.31 (1H, dd, *J* = 10.8, 1 Hz), 3.88 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.8, 150.9, 143.1, 133.3, 110.6 (C), 136.0, 105.3 (CH), 116.0 (CH_2), 61.3, 61.0, 56.2 (CH_3).

2-Bromo-3,4-dimethoxy-5-(triphenylmethyl)oxy-1-vinylbenzene (**10**). Aldehyde **3** (2.16 mmol, 500 mg) was dissolved in dry CH_2Cl_2 (12 mL), cooled to 0°C and treated with triethylamine (5.40 mmol, 752 μL) and triphenylmethyl chloride (4.30 mmol, 1.2 g). The mixture was then stirred for 14 h at room temperature. After this time, satd aq NH_4Cl (5 mL) was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed three times with brine and finally dried over anhydrous MgSO4. Removal of volatiles under reduced pressure gave an oily residue of the tritylated aldehyde, which was then subjected to Wittig methylenation under the conditions described above to yield **10** (60%): yellowish gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53-7.48 (6H, m), 7.35-7.25 (9H, m), 6.88 (1H, s), 6.85 (1H, s), 6.78 (1H, dd, *J* = 17.3, 10.8 Hz), 5.03 (1H, dd, *J* = 10.8, 1 Hz), 4.98 (1H, dd, *J* = 17.3, 1 Hz), 3.62 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.8, 145.2, 144.0 (x 3), 128.7, 116.0, 91.6 (C), 135.2, 129.3 (x 6), 127.7 (x 6), 127.5 (x 3), 119.9, 115.8 (CH), 114.4 (CH_2), 56.1 (CH_3).

Dimethyl bis(2,3,4-trimethoxy-6-vinylphenyl)silane (**14a**). Compound **9** (2.1 mmol) was dissolved under Ar in dry THF (7 mL) and cooled to −78°C. A 2.5M solution of *n*-BuLi in hexane (0.84 mL, 2.1 mmol) was added dropwise and the mixture was stirred at the same temperature for 30 min. Then, Me_2SiCl_2 (1 mmol) dissolved in dry THF (2 mL) was added dropwise with stirring, and the mixture was allowed to reach room temperature. The stirring was maintained for 12 h. After this time, satd aq NH_4Cl (5 mL) was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed three times with brine and finally dried over anhydrous $MgSO_4$. Removal of volatiles under reduced pressure gave an oily residue which was chromatographed on silica gel (elution with hexane-Et_2O 95:5) to yield **14a** (40%): off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (2H, dd, *J* = 17.3, 10.8 Hz), 6.78 (2H, s), 5.40 (2H, dd, *J* = 17.3, 1.5 Hz), 5.08 (2H, dd, *J* = 10.8, 1.5 Hz), 3.87 (6H, s), 3.81 (6H, s), 3.50 (6H, s), 0.60 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.0 (x 2), 154.3 (x 2), 141.1 (x 2), 139.9 (x 2), 124.6 (x 2) (C), 139.2 (x 2), 106.0 (x 2) (CH), 114.2 (x 2) (CH_2), 60.6 (x 2), 60.1 (x 2), 55.9 (x 2), 5.0 (x 2) (CH_3); HR ESMS *m/z* 467.1873 ($M+Na^+$). Calcd. for $C_{24}H_{32}NaO_6Si$, 467.1866.

Dimethyl bis(2,3,4-trimethoxy-6-vinylphenyl)germane (**14b**). Compound **9** and Me_2GeCl_2 (1 mmol) were allowed to react under the same reaction conditions as above for **14a**. Work-up gave an oily residue which was chromatographed on silica gel (elution with hexane-Et_2O 95:5) to yield **14b** (43%): off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86 (2H, dd, *J* = 17.3, 10.8 Hz), 6.73 (2H, s), 5.33 (2H, dd, *J* = 17.3, 1.5 Hz), 5.00 (2H, dd, *J* = 10.8, 1.5 Hz), 3.80 (6H, s), 3.75 (6H, s), 3.52 (6H, s), 0.67 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.0 (x 2), 154.0 (x 2), 141.2 (x 2), 139.2 (x 2), 127.1 (x 2) (C), 138.6 (x 2), 105.6 (x 2) (CH), 114.1 (x 2) (CH_2), 60.7 (x 2), 60.5 (x 2), 55.9 (x 2), 5.1 (x 2) (CH_3); HR ESMS *m/z* 513.1315 ($M+Na^+$). Calcd. for $C_{24}H_{32}{}^{74}GeNaO_6$, 513.1308.

Dimethyl bis(2,3,4-trimethoxy-6-vinylphenyl)stannane (**14c**). Compound **9** and Me_2SnCl_2 (1 mmol) were allowed to react under the same reaction conditions as above for **14a**. Work-up gave an oily residue which was chromatographed on silica gel (elution with hexane-Et_2O 95:5) to yield **14c** (57%): off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (2H, s), 6.84 (2H, dd, *J* = 17.3, 10.8 Hz), 5.48 (2H, dd, *J* = 17.3, 1.5 Hz), 5.10 (2H, dd, *J* = 10.8, 1.5 Hz), 3.88 (6H, s), 3.82 (6H, s), 3.65 (6H, s), 0.55 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.2 (x 2), 154.4 (x 2), 140.7 (x 2), 140.5 (x 2), 128.8 (x 2) (C), 139.4 (x 2), 105.0 (x 2) (CH), 114.0 (x 2) (CH_2), 60.7 (x 2), 60.6 (x 2), 56.0 (x 2), −2.6 (x 2) (CH_3); HR ESMS *m/z* 559.1124 ($M+Na^+$). Calcd. for $C_{24}H_{32}NaO_6{}^{120}Sn$, 559.1118.

2,3,4,6,7,8-Hexamethoxy-5,5-dimethyl-5*H*-dibenzo[b,f]silepine (**15a**). The second-generation Hoveyda-Grubbs ruthenium catalyst (0.01 mmol, 6.3 mg) was disolved under Ar in dry, deoxygenated toluene (40 mL). A solution of diolefin **14a** (0.1 mmol) in dry, deoxygenated toluene (27 mL) was then added dropwise. The mixture was then heated at reflux until

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consumption of the starting material (TLC monitoring). After the required time, the volatiles were removed under reduced pressure and the oily residue was chromatographed on silica gel (elution with hexane-Et_2O 95:5) to yield **15a** (86%): off-white solid, mp 200-202°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.84 (2H, s), 6.67 (2H, s), 3.87 (6H, s), 3.85 (6H, s), 3.84 (6H, s), 0.58 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.6 (x 2), 153.8 (x 2), 142.3 (x 2), 137.6 (x 2), 123.5 (x 2) (C), 133.0 (x 2), 109.4 (x 2) (CH), 61.4 (x 2), 60.8 (x 2), 56.0 (x 2), 1.0 (x 2) (CH_3); HR ESMS *m/z* 439.1554 ($M+Na^+$). Calcd. for $C_{22}H_{28}NaO_6Si$, 439.1553.

2,3,4,6,7,8-Hexamethoxy-5,5-dimethyl-5*H*-dibenzo[b,f]germepine (**15b**). Prepared from diolefin **14b** under the same reaction conditions as for **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **15b** (90%): off-white solid, mp 202-204°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (2H, s), 6.65 (2H, s), 3.86 (6H, s), 3.85 (6H, s), 3.84 (6H, s), 0.74 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.7 (x 2), 153.6 (x 2), 142.1 (x 2), 137.1 (x 2), 126.0 (x 2) (C), 133.1 (x 2), 109.4 (x 2) (CH), 61.3 (x 2), 60.8 (x 2), 56.0 (x 2), 1.3 (x 2) (CH_3); HR ESMS m/z 485.0976 ($M+Na^+$). Calcd. for $C_{22}H_{28}{}^{74}GeNaO_6$, 485.0995.

2,3,4,6,7,8-Hexamethoxy-5,5-dimethyl-5*H*-dibenzo[b,f]stannepine (**15c**). Prepared from diolefin **14c** under the same reaction conditions as for **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **15c** (21%): off-white solid, mp 173-175 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.66 (2H, s), 6.65 (2H, s), 3.86 (6H, s), 3.85 (6H, s), 3.84 (6H, s), 0.57 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9 (x 2), 154.0 (x 2), 141.2 (x 2), 139.0 (x 2), 127.7 (x 2) (C), 133.4 (x 2), 110.3 (x 2) (CH), 61.1 (x 2), 60.8 (x 2), 56.1 (x 2), −6.4 (x 2) (CH_3); HR ESMS m/z 531.0818 ($M+Na^+$). Calcd. for $C_{22}H_{28}NaO_6{}^{120}Sn$, 531.0805.

1-Bromo-4,5-dimethoxy-2-vinylbenzene (**19**). Prepared in 85% yield by means of Wittig methylenation of aldehyde **16** as described for the synthesis of **9**: oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (1H, s), 6.99 (1H, s), 6.97 (1H, dd, *J* = 17.3, 10.8 Hz), 5.58 (1H, dd, *J* = 17.3, 1 Hz), 5.26 (1H, dd, *J* = 10.8, 1 Hz), 3.89 (3H, s), 3.86 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.5, 148.7, 129.6, 114.4 (C), 135.5, 115.4, 108.8 (CH), 114.7 (CH_2), 56.3, 56.1 (CH_3).

5-Bromo-6-vinylbenzo[d][1,3]dioxole (**20**). Prepared in 79% yield by means of Wittig methylenation of aldehyde **17** as described for the synthesis of **9**: oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (1H, s), 6.99 (1H, s), 6.98 (1H, dd, *J* = 17.3, 10.8 Hz), 5.97 (2H, s), 5.55 (1H, dd, *J* = 17.3, 1 Hz), 5.26 (1H, dd, *J* = 10.8, 1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.2, 147.8, 131.0, 114.8 (C), 135.6, 112.7, 106.1 (CH), 115.1, 101.9 (CH_2).

1-Bromo-4-fluoro-2-vinylbenzene (**21**). Although this compound is commercial, we prepared it as an oil in 47% yield by means of Wittig methylenation of aldehyde **18** as described for the synthesis of **9**. Its spectral data are in good agreement with those described in the literature.[28]

Bis(4,5-dimethoxy-2-vinylphenyl)dimethylsilane (**22a**). Prepared in 36% yield from **19** under the same reaction conditions as in the synthesis of **14a**: off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (2H, s), 6.98 (2H, s), 6.80 (2H, dd, *J* = 17.3, 10.8 Hz), 5.47 (2H, dd, *J* = 17.3, 1.2 Hz), 5.04 (2H, dd, *J* = 10.8, 1.2 Hz), 3.92 (6H, s), 3.83 (6H, s), 0.60 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.2 (x 2), 148.2 (x 2), 137.5 (x 2), 128.7 (x 2) (C), 137.7 (x 2), 117.5 (x 2), 108.4 (x 2) (CH), 113.1 (x 2) (CH_2), 56.0 (x 2), 55.8 (x 2), 0.4 (x 2) (CH_3).

Bis(4,5-dimethoxy-2-vinylphenyl)dimethylgermane (**22b**). Prepared in 34% yield from **19** under the same reaction conditions as in the synthesis of **14b**: off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (2H, s), 6.88 (2H, s), 6.77 (2H, dd, *J* = 17.3, 10.8 Hz), 5.52 (2H, dd, *J* = 17.3, 1.2 Hz), 5.08 (2H, dd, *J* = 10.8, 1.2 Hz), 3.92 (6H, s), 3.80 (6H, s), 0.72 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.9 (x 2), 148.5 (x 2), 136.4 (x 2), 131.0 (x 2) (C), 137.6 (x 2), 116.7 (x 2), 108.2 (x 2) (CH), 113.0 (x 2) (CH_2), 55.9 (x 2), 55.8 (x 2), 0.3 (x 2) (CH_3).

Bis(4,5-dimethoxy-2-vinylphenyl)dimethylstannane (**22c**). Prepared in 50% yield from **19** under the same reaction conditions as in the synthesis of **14c**: off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (2H, s), 6.88 (2H, s), 6.71 (2H, dd, *J* = 17.3, 10.8 Hz), 5.55 (2H, dd, *J* = 17.3, 1.2 Hz), 5.14 (2H, dd, *J* = 10.8, 1.2 Hz), 3.92 (6H, s), 3.79 (6H, s), 0.57 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.0 (x 2), 148.6 (x 2), 137.9 (x 2), 132.5 (x 2) (C), 139.2 (x 2), 118.7 (x 2), 106.0 (x 2) (CH), 113.3 (x 2) (CH_2), 55.9 (x 2), 55.8 (x 2), −6.9 (x 2) (CH_3).

Dimethyl bis(6-vinylbenzo[d][1,3]dioxol-5-yl)silane (**23a**). Prepared in 36% yield from **20** under the same reaction conditions as in the synthesis of **14a**: off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (2H, s), 6.97 (2H, s), 6.77 (2H, dd, *J* = 17.3, 10.8 Hz), 5.96 (4H, s), 5.43 (2H, dd, *J* = 17.3, 1.2 Hz), 5.02 (2H, dd, *J* = 10.8, 1.2 Hz), 0.55 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.2 (x 2), 147.2 (x 2), 138.9 (x 2), 130.4 (x 2) (C), 137.3 (x 2), 113.9 (x 2), 106.0 (x 2) (CH), 113.5, 101.0 (x 2) (CH_2), 0.3 (x 2) (CH_3).

Dimethyl bis(6-vinylbenzo[d][1,3]dioxol-5-yl)germane (**23b**). Prepared in 41% yield from **20** under the same reaction conditions as in the synthesis of **14b**: off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (2H, s), 6.88 (2H, s), 6.73 (2H, dd, *J* = 17.3, 10.8 Hz), 5.96 (4H, s), 5.48 (2H, dd, *J* = 17.3, 1.2 Hz), 5.07 (2H, dd, *J* = 10.8, 1.2 Hz), 0.68 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.0 (x 2), 147.3 (x 2), 137.7 (x 2), 132.6 (x 2) (C), 137.3 (x 2), 113.3 (x 2), 105.8 (x 2) (CH), 113.5, 101.0 (x 2) (CH_2), 0.2 (x 2) (CH_3).

Dimethyl bis(6-vinylbenzo[d][1,3]dioxol-5-yl)stannane (**23c**). Prepared in 47% yield from **20** under the same reaction conditions as in the synthesis of **14c**: off-white gum; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (2H, s), 6.87 (2H, s), 6.67 (2H, dd, *J* = 17.3, 10.8 Hz), 5.95 (4H, s), 5.53 (2H, dd, *J* = 17.3, 1 Hz), 5.13 (2H, dd, *J* = 10.8, 1 Hz), 0.55 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.2 (x 2), 147.4 (x 2), 139.1 (x 2), 134.1 (x 2) (C), 138.8 (x 2), 113.8 (x 2), 105.8 (x 2) (CH), 115.3, 101.0 (x 2) (CH_2), −6.8 (x 2) (CH_3).

Bis(4-fluoro-2-vinylphenyl)dimethylsilane (**24a**). Prepared in 20% yield from **21** under the same reaction conditions as in the synthesis of **14a**: oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (2H, dd, *J* = 8.3, 6.5 Hz), 7.22 (2H, dd, *J* = 10.7, 2.5 Hz), 6.97 (2H, td, *J* = 8.3, 2.5 Hz), 6.75 (2H, ddd, *J* = 17.3, 10.8, 1.7 Hz), 5.55 (2H,

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dd, *J* = 17.3, 1 Hz), 5.13 (2H, dd, *J* = 10.8, 1 Hz), 0.58 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.6 (x 2, center point of doublet, $^1J_{C-F}$ = 246.5 Hz), 146.6 (x 2, doublet, $^3J_{C-F}$ = 7 Hz), 132.2 (x 2, doublet, $^4J_{C-F}$ = 3 Hz) (C), 137.0 (x 2, doublet, $^3J_{C-F}$ = 7.5 Hz), 136.9 (x 2, doublet, $^4J_{C-F}$ = 2 Hz), 114.2 (x 2, doublet, $^2J_{C-F}$ = 19.5 Hz), 112.4 (x 2, doublet, $^2J_{C-F}$ = 20.5 Hz) (CH), 116.3 (x 2) (CH_2), 0.0 (x 2) (CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −112.4.

Bis(4-fluoro-2-vinylphenyl)dimethylgermane (**24b**). Prepared in 22% yield from **21** under the same reaction conditions as in the synthesis of **14b**: oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (2H, dd, *J* = 8.3, 6.5 Hz), 7.27 (2H, dd, *J* = 10.7, 2.5 Hz), 6.95 (2H, td, *J* = 8.3, 2.5 Hz), 6.73 (2H, ddd, *J* = 17.2, 10.8, 1.7 Hz), 5.59 (2H, dd, *J* = 17.2, 1 Hz), 5.18 (2H, dd, *J* = 10.8, 1 Hz), 0.70 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.1 (x 2, center point of doublet, $^1J_{C-F}$ = 246 Hz), 145.7 (x 2, doublet, $^3J_{C-F}$ = 6.7 Hz), 134.3 (x 2, doublet, $^4J_{C-F}$ = 3.3 Hz) (C), 136.8 (x 2, doublet, $^4J_{C-F}$ = 2.7 Hz), 136.0 (x 2, doublet, $^3J_{C-F}$ = 7.7 Hz), 114.4 (x 2, doublet, $^2J_{C-F}$ = 20 Hz), 112.3 (x 2, doublet, $^2J_{C-F}$ = 20.5 Hz) (CH), 116.4 (x 2) (CH_2), −0.2 (x 2) (CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −113.4.

Bis(4-fluoro-2-vinylphenyl)dimethylstannane (**24c**). Prepared in 25% yield from **21** under the same reaction conditions as in the synthesis of **14c**: oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (2H, dd, *J* = 8, 6.5 Hz), 7.30 (2H, dd, *J* = 10.7, 2.5 Hz), 6.95 (2H, td, *J* = 8, 2.5 Hz), 6.70 (2H, ddd, *J* = 17.2, 10.8, 1.7 Hz), 5.63 (2H, dd, *J* = 17.2, 1 Hz), 5.26 (2H, dd, *J* = 10.8, 1 Hz), 0.57 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.2 (x 2, center point of doublet, $^1J_{C-F}$ = 245 Hz), 147.2 (x 2, doublet, $^3J_{C-F}$ = 6.7 Hz), 135.8 (x 2, doublet, $^4J_{C-F}$ = 3.5 Hz) (C), 138.3 (x 2, doublet, $^4J_{C-F}$ = 2.6 Hz), 138.2 (x 2, doublet, $^3J_{C-F}$ = 7 Hz), 114.6 (x 2, doublet, $^2J_{C-F}$ = 19.5 Hz), 112.2 (x 2, doublet, $^2J_{C-F}$ = 20.5 Hz) (CH), 116.7 (x 2) (CH_2), −7.2 (x 2) (CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −113.4.

2,3,7,8-Tetramethoxy-5,5-dimethyl-5*H*-dibenzo[b,f]silepine (**25a**). Prepared from diolefin **22a** under the same reaction conditions as in the synthesis of **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **25a** (90%): off-white solid, mp 200-202 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (2H, s), 6.89 (2H, s), 6.85 (2H, s), 3.92 (6H, s), 3.88 (6H, s), 0.48 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.6 (x 2), 149.0 (x 2), 135.3 (x 2), 129.0 (x 2) (C), 131.7 (x 2), 114.6 (x 2), 112.8 (x 2) (CH), 56.1 (x 2), 55.9 (x 2), −4.1 (x 2) (CH_3); HR ESMS *m/z* 379.1347 ($M+Na^+$). Calcd. for $C_{20}H_{24}NaO_4Si$, 379.1342.

2,3,7,8-Tetramethoxy-5,5-dimethyl-5*H*-dibenzo[b,f]germepine (**25b**). Prepared from diolefin **22b** under the same reaction conditions as in the synthesis of **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **25b** (78%): off-white solid, mp 203-205 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (2H, s), 6.88 (2H, s), 6.78 (2H, s), 3.91 (6H, s), 3.87 (6H, s), 0.60 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.2 (x 2), 149.0 (x 2), 134.7 (x 2), 131.3 (x 2) (C), 132.0 (x 2), 114.2 (x 2), 112.7 (x 2) (CH), 56.1 (x 2), 56.0 (x 2), −4.7 (x 2) (CH_3); HR ESMS *m/z* 403.0969 ($M+H^+$). Calcd. for $C_{20}H_{25}{}^{74}GeO_4$, 403.0965.

Silepine 26a. Prepared from diolefin **23a** under the same reaction conditions as in the synthesis of **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **26a** (86%): oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (2H, s), 6.85 (2H, s), 6.78 (2H, s), 5.93 (4H, s), 0.43 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.6 (x 2), 147.8 (x 2), 136.4 (x 2), 130.5 (x 2) (C), 131.7 (x 2), 111.4 (x 2), 110.0 (x 2) (CH), 101.0 (CH_2), −4.2 (x 2) (CH_3); HR ESMS *m/z* 325.0886 ($M+H^+$). Calcd. for $C_{18}H_{17}O_4Si$, 325.0896.

Germepine 26b. Prepared from diolefin **23b** under the same reaction conditions as in the synthesis of **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **26b** (80%): oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (2H, s), 6.83 (2H, s), 6.71 (2H, s), 5.92 (4H, s), 0.56 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.3 (x 2), 147.8 (x 2), 135.6 (x 2), 132.8 (x 2) (C), 131.9 (x 2), 111.0 (x 2), 109.8 (x 2) (CH), 101.0 (CH_2), −4.8 (x 2) (CH_3); HR ESMS *m/z* 371.0358 ($M+H^+$). Calcd. for $C_{18}H_{17}{}^{74}GeO_4$, 371.0340.

Stannepine 26c. Prepared from diolefin **23c** under the same reaction conditions as in the synthesis of **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **26c** (23%): oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (2H, s), 6.83 (2H, s), 6.71 (2H, s), 5.92 (4H, s), 0.56 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.4 (x 2), 147.5 (x 2), 137.5 (x 2), 134.3 (x 2) (C), 133.1 (x 2), 113.2 (x 2), 109.7 (x 2) (CH), 100.9 (CH_2), −11.2 (x 2) (CH_3); HR ESMS *m/z* 417.0110 ($M+H^+$). Calcd. for $C_{18}H_{17}O_4{}^{120}Sn$, 417.0149.

2,8-Difluoro-5,5-dimethyl-5*H*-dibenzo[b,f]silepine (**27a**). Prepared from diolefin **24a** under the same reaction conditions as in the synthesis of **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **27a** (80%): off-white solid, mp 102-104°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55-7.50 (2H, m), 7.10-7.00 (4H, m), 6.92 (2H, s), 0.48 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.7 (x 2, center point of doublet, $^1J_{C-F}$ = 246 Hz), 143.4 (x 2, doublet, $^3J_{C-F}$ = 7 Hz), 133.2 (x 2, doublet, $^4J_{C-F}$ = 3 Hz) (C), 134.6 (x 2, doublet, $^3J_{C-F}$ = 7.5 Hz), 133.1 (x 2, doublet, $^4J_{C-F}$ = 2 Hz), 116.2 (x 2, doublet, $^2J_{C-F}$ = 20 Hz), 114.9 (x 2, doublet, $^2J_{C-F}$ = 20 Hz) (CH), −4.2 (x 2) (CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −113.3.

2,8-Difluoro-5,5-dimethyl-5*H*-dibenzo[b,f]germepine (**27b**). Prepared from diolefin **24b** under the same reaction conditions as in the synthesis of **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **27b** (70%): off-white solid, mp 98-100°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45-7.40 (2H, m), 7.10-7.00 (4H, m), 6.86 (2H, s), 0.60 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.4 (x 2, center point of doublet, $^1J_{C-F}$ = 245 Hz), 143.0 (x 2, doublet, $^3J_{C-F}$ = 6.7 Hz), 135.3 (x 2, doublet, $^4J_{C-F}$ = 3.5 Hz) (C), 133.8 (x 2, doublet, $^3J_{C-F}$ = 7.5 Hz), 133.2 (x 2, doublet, $^4J_{C-F}$ = 2.5 Hz), 116.2 (x 2, doublet, $^2J_{C-F}$ = 20 Hz), 114.9 (x 2, doublet, $^2J_{C-F}$ = 19.5 Hz) (CH), −4.8 (x 2) (CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −114.2.

2,8-Difluoro-5,5-dimethyl-5*H*-dibenzo[b,f]stannepine (**27c**). Prepared from diolefin **24c** under the same reaction conditions as in the synthesis of **15a**. Chromatography on silica gel (elution with hexane-Et_2O 95:5) gave **27c** (28%): off-white solid, mp 96-98°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (2H, dd, *J* = 8, 6.5 Hz), 7.03 (2H, dd, *J* = 10.8, 2.5 Hz), 6.98 (2H, td, *J* = 8.5, 2.5 Hz), 6.86 (2H, s), 0.45 (6H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5 (x 2, center point of doublet, $^1J_{C-F}$ = 245 Hz), 145.1 (x 2,

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doublet, $^{3}J_{C-F}$ = 6.5 Hz), 136.9 (x 2, doublet, $^{4}J_{C-F}$ = 3.5 Hz) (C), 136.3 (x 2, doublet, $^{3}J_{C-F}$ = 7.5 Hz), 134.0 (x 2, doublet, $^{4}J_{C-F}$ = 2.7 Hz), 116.0 (x 2, doublet, $^{2}J_{C-F}$ = 20 Hz), 114.7 (x 2, doublet, $^{2}J_{C-F}$ = 19 Hz) (CH), −11.2 (x 2) (CH_3); ^{19}F NMR (282 MHz, $CDCl_3$) δ −114.1.

Biological studies. Materials and methods

Cell culture

Cell culture media were purchased from Gibco (Grand Island, NY). Fetal bovine serum (FBS) was a product of Harlan-Seralab (Belton, U.K.). Supplements and other chemicals not listed in this section were obtained from Sigma Chemical Co. (St. Louis, MO). Plastics for cell culture were supplied by Thermo Scientific BioLite. All tested compounds were dissolved in DMSO at a concentration of 20 μM and stored at -20°C until use.

HT-29, MCF-7, HeLa and HEK-293 cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) containing glucose (1g/L), glutamine (2 mM), penicillin (50 IU/mL), streptomycin (50 μg/mL), and amphotericin B (1.25 μg/mL), supplemented with 10% FBS. HMEC-1 cell line was maintained in Dulbecco's modified Eagle's medium (DMEM) low glucose, glutamine (2 mM), penicillin (50 IU/mL), streptomycin (50 μg/mL), and amphotericin B (1.25 μg/mL), supplemented with 10% FBS.

MTT assay

A total of 5 × 10^3 HT-29, MCF-7, HeLa or HEK-293 cells in a total volume of 100 μL of their respective growth media were incubated with serial dilutions of the tested compounds. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co.) dye reduction assay in 96-well microplates was used, as previously described [26]. After 2 days of incubation (37 °C, 5% CO_2 in a humid atmosphere), 10 μL of MTT (5 mg/mL in phosphate-buffered saline, PBS) was added to each well, and the plate was incubated for a further 3 h (37 °C). The supernatant was discarded and replaced by 100 μL of DMSO to dissolve formazan crystals. The absorbance was then read at 490 nm by spectrophotometry. For all concentrations of compound, cell viability was expressed as the percentage of the ratio between the mean absorbance of treated cells and the mean absorbance of untreated cells. Three independent experiments were performed, and the IC_{50} values (i.e., concentration half inhibiting cell proliferation) were graphically determined.

Cell cycle analysis

Progression of the cell cycle was analysed by means of flow cytometry with propidium iodide. After incubation with compounds for 24 h, A549 cells were fixed, treated with RNase and stained with propidium iodide following instructions of BD Cycletest™ DNA Kit. Analysis was performed with a BD Accuri™ C6 flow cytometer.

Microtubule network study by immunofluorescence

Immunofluorescent analysis of the microtubule network was performed on the A-549 cell line. In this assay, 1,5x105 cells were plated on a coverglass and incubated with the different concentrations of selected compounds for 16 h. Cells were then washed with PEMP, permeabilized with PEM-Triton X-100 0.5% for 90 seconds at room temperature and fixed in 3.7% formaldehyde (in PEM pH 7.4) for 30 min at rt. Direct immunostaining was carried out for 2.5 h at 37°C in darkness with primary FITC-conjugated anti-□-tubulin antibody (dilution 1:400 in PBS-BSA 1% from a 1 mg/mL solution; monoclonal antibody, clone DM1A, Sigma-Aldrich). Next, cells were washed with PBS and incubated for 30 min at room temperature in darkness with Hoechst 2 mM in water. Then, cells were washed in PBS and coverglasses were mounted with 10 μL of Glycine/Glycerol buffer. The cytoskeleton was imaged by a confocal laser scanning microscope (CLSM) Leica SP5 with a Leica inverted microscope, equipped with a Plan-Apochromat 63□ oil immersion objective (NA=1.4). Each image was recorded with the CLSM's spectral mode selecting specific domains of the emission spectrum. The FITC fluorophore was excited at 488 nm with an argon laser and its fluorescence emission was collected between 496 nm and 535 nm.

Tube formation inhibition assay

Wells of a 96-well μ-plate for angiogenesis were coated with 12 μL of Matrigel (10 mg/mL, BD Biosciences) at 4ºC. After gelatinization at 37ºC for 30 min, HMEC-1 cells were seeded at 2 x 10^4 cells/well in 25 μL of culture medium on top of the Matrigel and were incubated 30 min at 37ºC while are attached. Then, compounds were added dissolved in 25 μL of culture medium and after 20 h of incubation at 37ºC, tube destruction was evaluated and photographed.

Phospho-VEGFR2 quantification by ELISA

HMEC-1 cells were seeded at 5·10^5 cells/well in 6-well plates and once they were at 80% of their confluency, they were starved with medium containing 0.1% of FBS for 24 h. Then, cells were incubated with the corresponding compounds for 2 hours and next cells were stimulated with 50 ng/ml of Recombinant VEGF-165 for 30 minutes at 37ºC. After that, lysates were collected, protein quantification was carried out by Bradford test and, then, and phospho-VEGFR2 was quantified using PathScan® Phospho-VEGFR2(Tyr1175) Sandwich ELISA Kit according to the manufacturer's instructions.

Conflicts of interest

There are no conflicts to declare.

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