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Synthesis of Honokiol Analogues and Evaluation of their Modulating Action on VEGF Protein Secretion and Telomerase-Related Gene Expressions

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Abstract: A group of 36 biphenyl derivatives structurally related to honokiol were synthesised by means of Suzuki coupling reactions. Their cytotoxicities were evaluated and compared to that of honokiol. Some of the compounds were then evaluated for their ability to downregulate the secretion of the VEGF protein and the expression of the *VEGF*, *hTERT* and *c-Myc* genes, the two latter involved in the activation of telomerase in tumoral cells. Some of the synthetized derivatives showed promising pharmacological features as they exhibited IC_{50} values in low micromolar range, good therapeutic margins and a multiple mode of action on tumor cells based on the inhibition of VEGF and, at the same time, of the expression of genes related to the activation of telomerase.

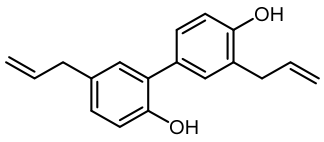
Keywords: honokiol analogues, angiogenesis, gene regulation, VEGF, telomerase, *hTERT*, *c-Myc*

Molecules containing biaryl moieties are relatively common within natural products. For their preparation, Nature has developed an ample array of biosynthetic strategies (1). A number of these biaryl natural products belong to the biogenetic class of lignans (2). Honokiol (Figure 1), has aroused a great degree of interest because of their various pharmacological properties. This product and many derivatives thereof, both of natural and synthetic origin, have been reported to display anti-oxidative, anti-inflammatory, anti-tumor, anti-diabetic, anti-microbial, anti-neurodegenerative, anti-depressant, pain control, gastrointestinal, cardiovascular and liver protective properties, among others (3,4).

We have been investigating a range of analogues of natural products (5) for their cytotoxicity and potential value in anticancer therapy (6). The latter feature may be related to the ability of the compounds to disrupt microtubule dynamics (7), to inhibit the angiogenesis process (8) or to inhibit the expression of genes related to telomerase activation (9) among other alternative mechanisms.

Thanks to the intense studies on genes that mediate cancer progression and therapeutic resistance, many gene targets that regulate apoptosis, proliferation and cell signaling have been identified.

Multiple cumulative genetic and/or epigenetic changes are needed to cause cancer. Molecules that can inhibit expression of such genes are powerful tools in cancer research (10). In this sense, methodologies focused on sequence-specific gene suppression strategies involving antisense oligonucleotides and ribozymes or else involving gene silencing using RNA interference (RNAi) have been developed (11). However, their adaptation as broadly applicable functional genomic and therapeutic tools has proven difficult because of problems regarding stability and poor efficiency of delivery.



Honokiol

Figure 1. Structure of honokiol

Many experiments have shown that targeting a single gene can inhibit the growth and proliferation of tumor cells (12). However, interference targeting a single gene has limitations in the prevention and treatment of cancer as it is known that tumorigenesis results in many cases from multiple gene mutation. Therefore, therapies targeting multiple genes may have better effects on malignant tumors.

In most solid tumors, angiogenesis is an important process for tumor growth and metastasis (13). Many different mediators are involved in this process, including VEGF, which has been shown to play a critical role in pathological angiogenesis (14). VEGF levels in serum are tightly associated to a more aggressive disease state and may serve as a marker to evaluate diagnosis. Blocking VEGF expression can inhibit tumor growth and prevent metastasis (15).

Most cancer cells also exhibit telomerase activity. The latter mantains the length of the telomeres, thus preserving genomic stability (16). Telomerase is a ribonucleoprotein composed of two main subunits which, in the case of human beings, are called human telomerase RNA (hTR) and human telomerase protein (hTERT). Many studies have demonstrated that interference in the expression of the *hTERT* gene can efficiently inhibit the growth and tumorigenicity of cancer cells (17), as the *hTERT* gene is a rate-limiting factor in telomerase synthesis and activity. Equally important is the *c-Myc* gene, which has been found to be amplified in various types of human cancers such as, for example, lung carcinoma (18), breast carcinoma (19) and colon carcinoma (20). The result of the expression of this gene, the c-Myc protein, is a transcriptional factor with an important role in cell proliferation, differentiation, invasion and adhesion of tumor cells (21). It is also involved in the activation of *hTERT* gene transcription (17).

Since on one hand tumoral cell secretion of VEGF is an important factor in metastasis and, on the other hand, telomerase is responsible for the immortality of tumoral cells, we consider that the potential multiple ability (22) of some compounds to perturb microtubule dynamics and, at the same time, to inhibit VEGF secretion by tumoral cells and the expression of the *VEGF*, *hTERT* and *c-Myc* genes is a goal worth pursuing.

As the natural biphenyl derivative honokiol displays valuable anticancer activity, we decided to prepare a number of synthetic biphenyl derivatives, such as **1-36** (Figure 2), and to investigate their behavior in each of the aforementioned three types of biological activities. In fact, many of these compounds can be viewed as analogues of honokiol that differ in the relative positions of the *C*-allyl and OH groups. In some cases, hydroxyl functions have been replaced by OMe groups. The *C*-allyl groups were introduced by means of Claisen rearrangement (23) in precursors having *O*-allyl groups. The latter have also been used in the biological evaluations. It is worth noting that the biphenyl moiety present in all compounds can be considered a privileged structure (24) that may prove useful in the development of lead compounds. Indeed, small molecules have always aroused interest in cancer therapy (25).

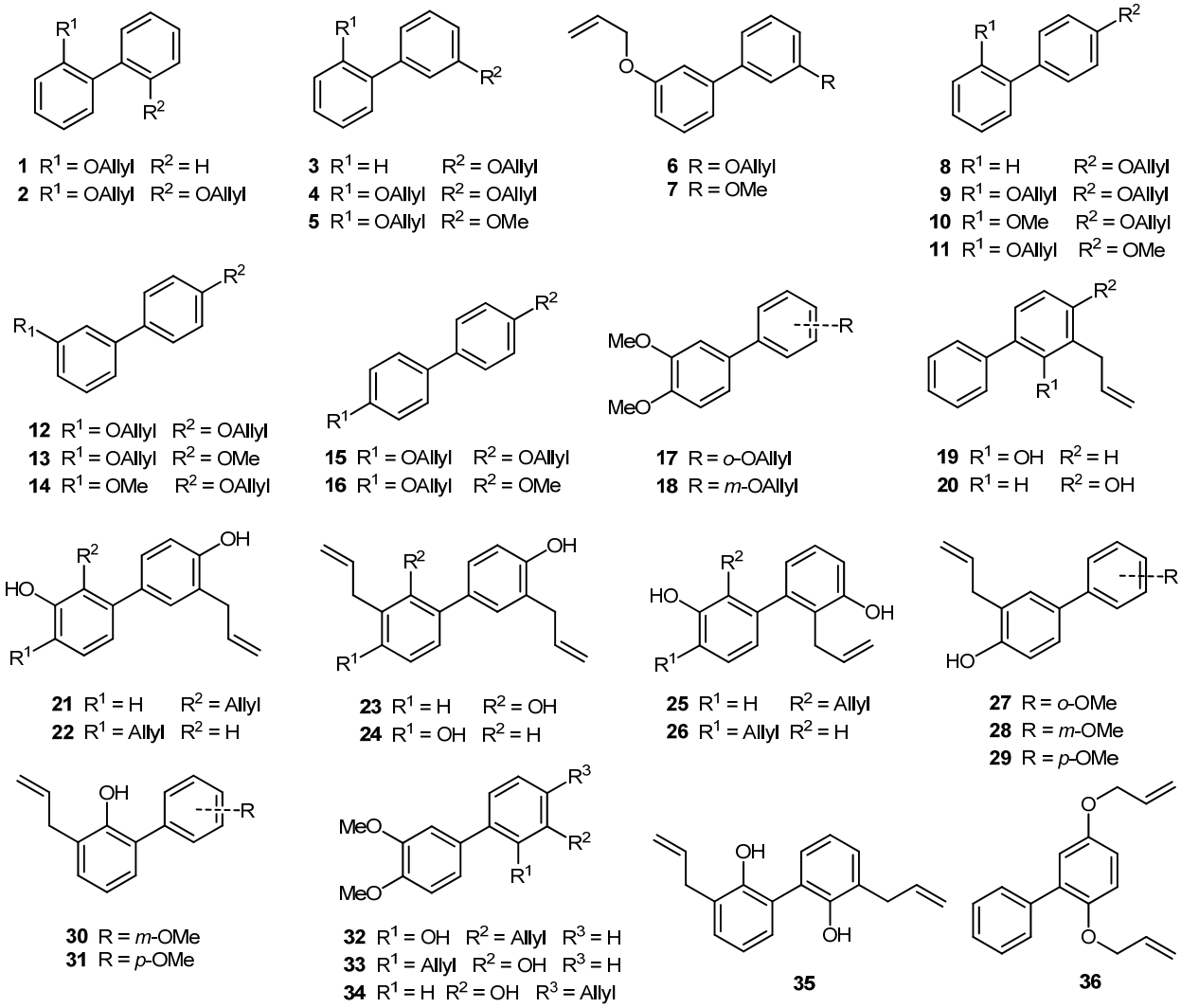
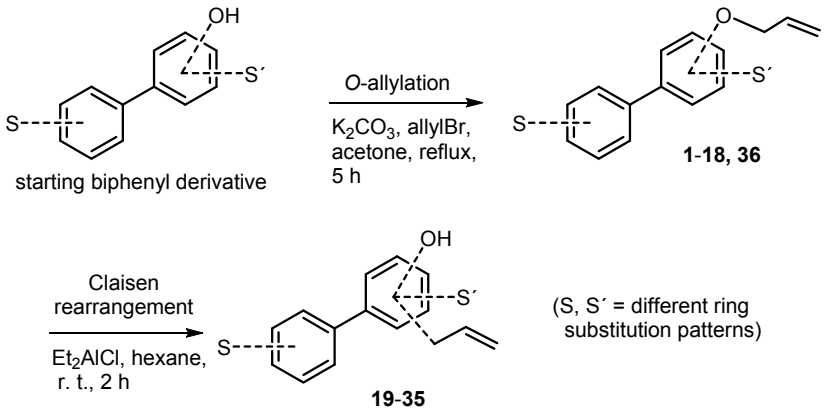


Figure 2. Structure of biphenyl derivatives investigated in this study.

Chemistry

The starting materials for the synthesis of compounds **1**-**36** were hydroxylated biphenyl derivatives prepared by means of palladium-catalyzed Suzuki coupling (26). The phenolic functions were then *O*-allylated and the resulting *O*-allyl derivatives subjected to Claisen rearrangement (Scheme 1) under Lewis acid catalysis (23). Compounds with *O*-allyl groups *ortho* or *para* to the other benzene ring (**1**, **2**, **5**, **8**-**11**, **14**-**17**) gave single compounds in the Claisen rearrangements. Those having *O*-allyl groups *meta* to the other benzene ring (**3**, **4**, **6**, **7**, **12**, **13**, **18** and **36**) gave mixtures of regioisomeric rearrangement products. In the case of compounds **6**, **12** and **18**, the mixtures could be chromatographically resolved into their individual components. These were then subjected to biological evaluation. However, compounds **3**, **4**, **7**, **13** and **36** gave mixtures which could not be separated into pure components. No biological evaluation of such mixtures was carried out.



Scheme 1. Synthesis and structures of biphenyl derivatives.

Methods and Materials

Chemistry

Conditions for O-allylations (27)

A mixture of the appropriate phenol (10 mmol) and K_2CO_3 (2.07 g, 15 mmol) was dissolved in acetone (15 mL). Allyl bromide (1.1 mL, ca. 12.5 mmol) was then added dropwise and the reaction mixture was stirred at reflux for 5 h. The mixture was then cooled, and the volatiles removed under reduced pressure. Addition of a 10% aqueous NaOH solution (10 mL) was followed by extraction with Et_2O (3 x 15 mL). The organic layers were then washed with brine and dried on anhydrous $MgSO_4$, followed by removal of all volatiles under reduced pressure. This afforded an oily material which was subjected to column chromatography on silica gel (hexane-EtOAc mixtures) to yield the desired *O*-allylated derivative. Yields were in the range between 50 and 85%.

Conditions for Claisen rearrangements (28)

A mixture of the appropriate *O*-allylated derivative (4 mmol) in dry hexane (40 mL) was treated dropwise under N_2 with a 1M solution of Et_2AlCl in dry hexane (8 mL for compounds having one *O*-allyl group and 16 mL for compounds having two *O*-allyl groups). The mixture was then stirred at

room temperature until consumption of the starting materials (ca. 2 h, TLC monitoring). The mixture was then cooled in an ice bath, followed by slow dropwise addition of a 2M aqueous HCl solution (40 mL). The mixture was poured into a separation funnel, and the organic phase was separated from the aqueous layer, which then was additionally extracted with EtOAc (4 x 15 mL). The combined organic layers were washed with brine, desiccated over anhydrous Na_2SO_4, followed by removal of all volatiles under reduced pressure. This afforded an oily material which was subjected to column chromatography on silica gel (hexane-EtOAc mixtures) to yield the desired *C*-allyl derivative. Yields were in the range between 30 and 60%. Crystallization of the solid products purified by means of column chromatography was performed using methanol.

Physical data of individual compounds are given in the Supporting Information section.

Biology

Cell culture

Cell culture media were purchased from Gibco (Grand Island, NY, USA). 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 Chemicals Co. (St. Louis, Mo., USA). Plastics for cell culture were supplied by Thermo Scientific™ BioLite. All tested compounds were dissolved in DMSO at a concentration of 10 mg/mL and stored at –20ºC until use.

Cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) containing glucose (1 g/L), glutamine (2 mM), penicillin (50 IU/mL), streptomycin (50 µg/mL) and amphotericin B (1.25 µg/mL), supplemented with 10% FBS.

Cytotoxicity assays

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO) dye reduction assay in 96-well microplates was used, as previously described (29). Some 5 x 10^3 cells of HT-29, MCF-7 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. After 3 days of incubation (37 °C, 5% CO_2 in a humid atmosphere), 10 µl of MTT (5 mg/ml in PBS) were added to each well and the plate was incubated for further 4 h (37 °C). The resulting formazan was dissolved in 150 µL of 0.04 N HCl/2-propanol and read at 550 nm. All determinations were carried out in triplicate.

ELISA analysis

HT-29 cells at 70–80% confluence were collected after serum starvation for 24 h. Cells were incubated at the concentrations showed in Table 2 of the corresponding drugs in DMSO for 72 h (Fig. 3). Culture supernatants were collected and VEGF secreted by HT-29 cells was determined using Invitrogen Human Vascular Endothelial Growth Factor ELISA Kit according to the manufacturer's instructions.

RT-qPCR analysis

HT-29 cells at 70–80% confluence were collected after serum starvation for 24 h. Cells were incubated at the concentrations showed in Table 2 of the corresponding drugs in DMSO for 48 h. Cells were collected and the total cellular RNA from HT-29 cells was isolated using Ambion RNA extraction Kit according to the manufacturer's instructions. The cDNA was synthesized by MMLV-RT with 1-21 μg of extracted RNA and oligo(dT)15 according to the manufacturer's instructions.

Amplification of the genes was performed by use of a StepOnePlus™ thermalcycler. Fast TaqMan Gene Expression Master Mix containing the appropriate buffer for the amplification conditions, dNTPs, thermostable DNA polymerase enzyme and a passive reference probe was used. Each of the genes were amplified using predesigned primers by Life Technologies TaqMan® Gene Expression Assays, Hs99999903-m1 (β-actin), Hs00900055-m1 (VEGF), Hs00972646-m1 (hTERT) and Hs00153408-m1 (c-Myc).

Statistical analysis

Data are expressed as the mean ± SEM. Statistical analyses were done using Microsoft Excel and GraphPad Prism®. Differences between means were determined using one-way ANOVA with Dunnett's Multiple Comparison Test, and considered to be statistically significant at $\alpha \leq 0.05$.

Results and Discussion

Cytotoxicity of the biphenyl derivatives

The cytotoxic ability of compounds **1-36** was measured by means of their IC_{50} values towards the tumoral cell lines HT-29 and MCF-7 and towards the non-tumoral cell line HEK-293 (see Table 1). We have also evaluated the therapeutic safety margin of each compound as expressed by means of the α and β coefficients. These are obtained by dividing the IC_{50} value of each compound for the nontumoral HEK-293 line by those for the HT-29 (α) and the MCF-7 (β) tumoral cell line, respectively. The higher the value of either coefficient, the higher the therapeutic safety margin for the corresponding compound.

The observed IC_{50} values are in the low to medium micromolar range. In general terms, these biphenyl derivatives exhibit the same cytotoxicity range in the HT-29 line as in the MCF-7 line. Compounds **3**, **6** and **36** showed comparable or better IC_{50} values than honokiol towards the HT-29 cell line and, in addition, exhibited a markedly higher therapeutic margin than honokiol ($\alpha > 7$). Compounds **4**, **9**, **15** and **16** exhibited better IC_{50} values than honokiol towards the MCF-7 cell line even though only **4** and **15** exhibited a higher therapeutic margin than honokiol ($\beta > 3$).

Thirteen compounds (**3**, **6**, **10**, **12**, **13**, **15**, **16**, **17**, **20**, **22**, **25**, **28** and **36**) were selected for further biological evaluations. The selection was made on the basis of products showing either comparatively low IC_{50} values (high cytotoxicity) or else $\alpha > 1$ (good safety margin).

Table 1. IC_{50} values (μM) and selectivity coefficients for biphenyls **1-36**.[a]

Compound	HEK-293	HT-29	MCF-7	α*	β**
Honokiol	35 ± 7	25 ± 3	18.2 ± 0.5	1.5	1.9
1	320 ± 30	227 ± 24	247 ± 24	1.4	1.3
2	67 ± 6	101 ± 13	80.0 ± 0.9	0.7	0.8
3	258 ± 5	27 ± 12	242 ± 4	9.7	1.1
4	49 ± 5	208 ± 14	14 ± 4	0.2	3.6
5	70 ± 30	210 ± 30	109 ± 15	0.3	0.7
6	>100	11 ± 3	110 ± 14	>9	1
7	104 ± 12	232 ± 22	230 ± 30	0.4	0.5
8	140 ± 30	>100	230 ± 50	>1.4	0.6
9	21 ± 5	69.5 ± 0.7	18 ± 4	0.3	1.2
10	110 ± 9	56.0 ± 0.7	228 ± 13	2.0	0.5
11	31 ± 6	180 ± 10	214 ± 10	0.2	0.1
12	>100	70 ± 40	97 ± 5	>1.5	>1
13	120 ± 40	70 ± 30	210 ± 3	1.6	0.6
14	60 ± 30	219 ± 13	52.9 ± 2.1	0.3	1.2
15	26 ± 5	38 ± 12	2.9 ± 0.14	0.7	8.7
16	4.5 ± 2.3	32 ± 10	6.5 ± 2.2	0.1	1.1
17	53 ± 13	44 ± 6	130 ± 40	1.2	0.4
18	48 ± 3	>100	187 ± 14	0.5	0.3
19	240 ± 11	290 ± 40	230 ± 50	0.8	1.0
20	138 ± 14	120 ± 30	123 ± 5	1.2	1.1
21	85 ± 10	105 ± 11	66 ± 6	0.8	1.3
22	56 ± 18	60 ± 30	46.4 ± 0.6	0.9	1.2
23	131 ± 13	175 ± 18	129 ± 13	0.7	1.0
24	81 ± 3	>100	176 ± 23	0.8	0.5
25	93 ± 4	79 ± 23	56 ± 13	1.2	1.7
26	189 ± 9	>100	182 ± 7	1	1.1
27	320 ± 70	23 ± 3	350 ± 30	0.6	0.7
28	121 ± 19	69 ± 8	107 ± 12	1.7	1.1
29	57 ± 3	50 ± 10	88 ± 14	0.3	0.3
30	170 ± 40	147.7 ± 2.1	282 ± 20	0.4	0.7
31	271 ± 8	290 ± 30	530 ± 40	0.5	0.7
32	260 ± 30	270 ± 30	150 ± 40	0.9	0.6
33	270 ± 30	280 ± 40	239 ± 6	0.1	0.2
34	>100	209 ± 7	235 ± 3	0.2	0.2
35	292 ± 10	240 ± 50	303 ± 9	0.1	0.5
36	130 ± 30	19 ± 9	190 ± 30	7.1	0.7

[a]Values are the average (± s.d.) of three different measurements performed as described in the Experimental Section. [b]$\alpha = IC_{50}$ (HEK-293) / IC_{50} (HT-29). [c]$\beta = IC_{50}$ (HEK-293) / IC_{50} (MCF-7). Values of α and β have been rounded off to a decimal figure.

Table 2. Concentrations for testing selected compounds.

Concentration	Biphenyl derivatives
10 μM	**6**, **36**
20 μM	Honokiol, **3**, **16**, **17**
40 μM	**10**, **12**, **13**, **15**, **22**, **25**
60 μM	**28**
90 μM	**20**

Effect of biphenyl derivatives on VEGFA protein secretion and VEGF gene inhibition into HT-29

The influence of the synthetic biphenyl derivatives on the secretion of the VEGFA protein and the expression of the VEGF gene was performed on the HT-29 line. The thirteen aforementioned compounds were tested at concentrations that were close to their respective IC_{50} values in the HT-29 line (see Table 2).

The amount of VEGFA protein was first determined by means of the ELISA procedure (30), as described in the Methods and Materials Section. Figure 3 shows the percentage of VEGFA secreted to the culture medium after 72 h of incubation in the presence of each of the selected compounds at concentrations showed in Table 2. The values observed when cells were treated only with DMSO were used as the control (standardized to 100%).

Most of the studied derivatives showed an ability to diminish the secretion of VEGFA (Fig. 3) (31). Compound **16** showed an activity (59%) similar to that of honokiol at the same concentration whereas **36** showed a 61% decrease in VEGFA secretion at half the concentration of honokiol. The strongest effect was observed with compounds **25** and **28**, which were able to lower VEGFA protein secretion to 40% and 47% of the control value, respectively, an effect stronger than that observed for honokiol (55%). However, it should also be noted that **25** and **28** were acting at a concentration twice and three times, respectively, higher than that of the natural product.

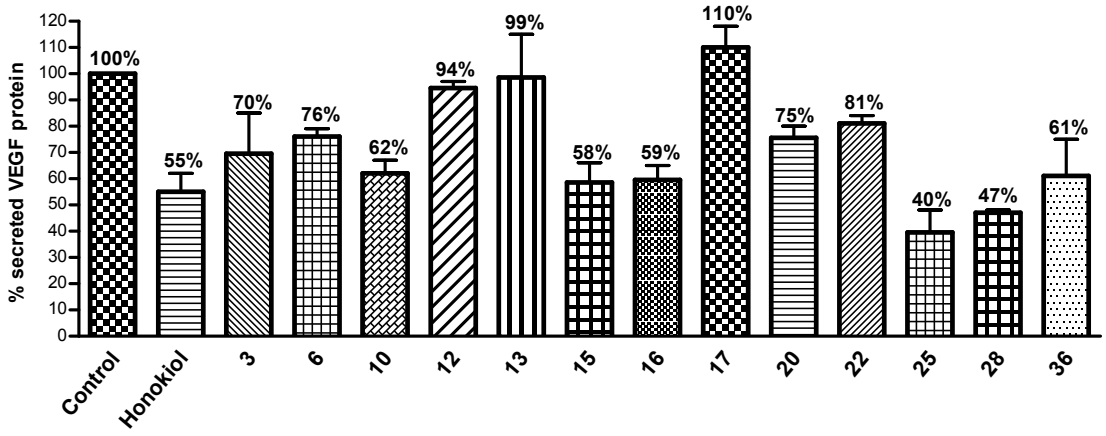


Figure 3. VEGFA protein secretion from HT-29 cells determined by means of the ELISA procedure. At least three measurements were performed in each case. Bars represent mean values of VEGFA secretion (percentage values related to control) and error bars indicate standard errors of the mean. The statistical analysis was evaluated using one-way ANOVA with Dunnett's Multiple Comparison Test ($P < 0.001$).

In order to find out whether the selected biphenyl derivatives were able to downregulate the secretion of VEGFA protein by means of a mechanism based on interference at the transcriptional level, we tested the ability of the compounds to inhibit the expression of the *VEGFA* gene. In this case, HT-29 cells were incubated for 48 h with the selected derivatives at the concentrations shown in Table 2, as well as with DMSO as the control test. The real time quantitative PCR (RT-qPCR) methodology (32) was then used as described in the Experimental Section to determine the percentage of *VEGFA* gene expression related to the control value. The results are shown in Figure 4.

Seven of the thirteen studied synthetic derivatives showed an ability to diminish the expression of VEGFA gene to a noticeable degree (< 50% of the control value, with honokiol showing a 38% value). The most active compound was **6**, which inhibited gene expression to 16% of the control value and at the lowest concentration of all of the tested compounds (half the concentration of honokiol). At the same concentration as the natural product, biphenyl derivatives **16** and **17** were able to decrease the expression of the *VEGFA* gene to less than 40% of the control value. Finally, compounds **12**, **15** and **22** lowered the expression of the *VEGFA* to 23, 24 % and 35%, respectively, of the control value, even though at a concentration twice higher than for honokiol. We can observe that most of these compounds display one allyloxy group at one at least of the two aromatic rings and the *ortho* position is usually not occupied. Furthermore, the most frequent substitution patterns for the oxygen atoms in these structures are *meta-meta, meta-para* and *para-para*.

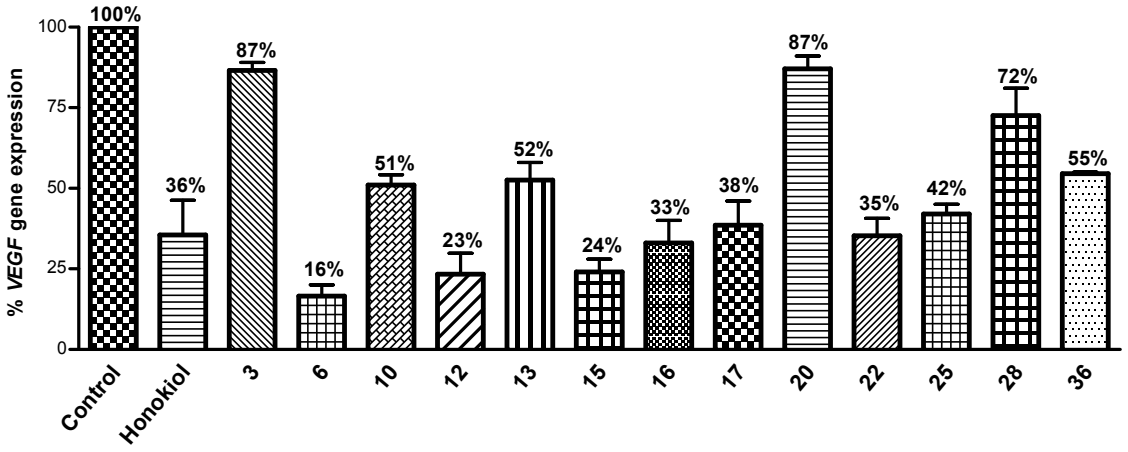


Figure 4. Expression percentage of the *VEGFA* gene after 48 h of incubation of HT-29 cells determined by means of the RT-qPCR methodology. At least three measurements were performed in each case. Error bars indicate standard errors of the mean. The statistical analysis was evaluated using one-way ANOVA with Dunnett's Multiple Comparison Test ($P < 0.001$).

It is worth mentioning here that the results discussed above do not show a good correlation between the VEGFA amount excreted to the medium and the degree of gene expression. This suggests that these compounds exert the control of VEGFA production at a phase different from that of gene transcription (33).

Effect of biphenyl derivatives on the inhibition of the hTERT and c-Myc genes

In order to determine whether the studied compounds were able to downregulate the expression of the *hTERT* and *c-Myc* genes, we have performed an RT-qPCR analysis on HT-29 tumoral cells. The cells were incubated for 48 h in the presence of DMSO (control) and the selected compounds (see Table 2). Figures 5 and 6 show the results observed for the expression of the *hTERT* and *c-Myc* genes, respectively.

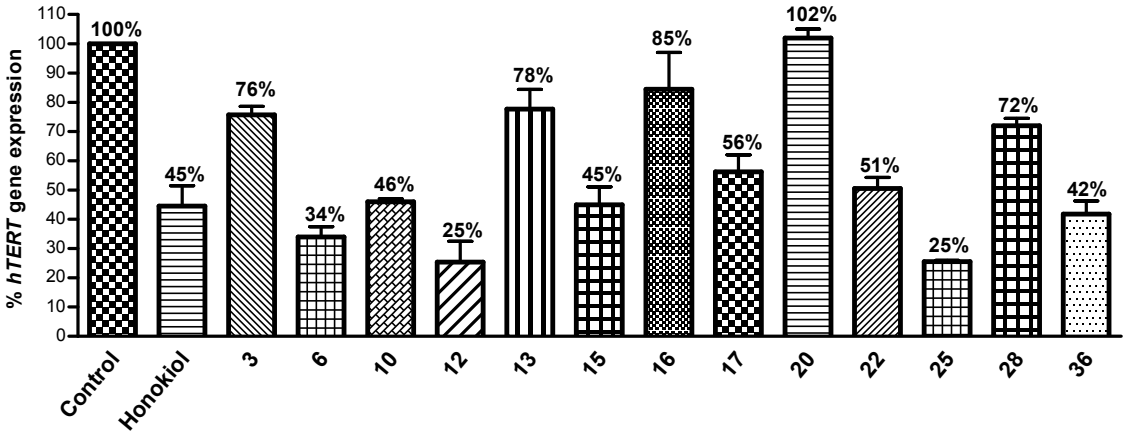


Figure 5. Expression percentage of the *hTERT* gene after 48 h of incubation. At least three measurements were performed in each case. Error bars indicate standard errors of the mean. The statistical analysis was evaluated using one-way ANOVA with Dunnett's Multiple Comparison Test ($P < 0.001$).

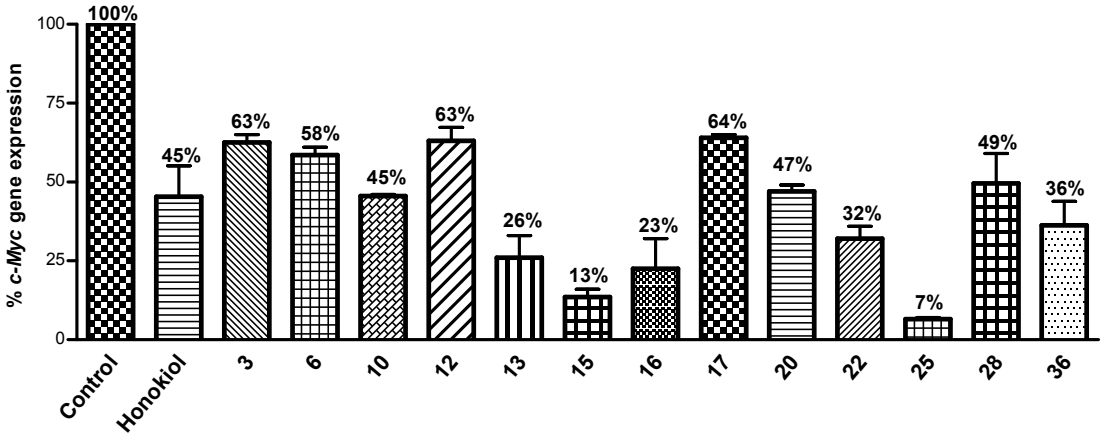


Figure 6. Expression percentage of the *c-Myc* gene after 48 h of incubation. At least three measurements were performed in each case. Error bars indicate standard errors of the mean. The statistical analysis was evaluated using one-way ANOVA with Dunnett's Multiple Comparison Test ($P < 0.001$).

Honokiol and several of the synthetic biphenyl derivatives were able to inhibiting *hTERT* gene expression to levels below 50% of the control (45% for honokiol). Again, compound **6** and **36** deserve mention as they were able to lower *hTERT* gene expression to 34 % and 42%, respectively, of the control and at concentration one half that of the natural product (Fig. 5). Compounds **12** and **25** also diminished the expression of the gene to about 25% of the control value although at a concentration twice higher than that of honokiol. In this case, the most frequent substitution patterns for the oxygen atoms in the most active derivatives are *meta-meta* or *meta-para*.

As regards the downregulation of the expression of the *c-Myc* gene (Fig. 6), compounds **13**, **15**, **16**, **22**, **25** and **36** were able to diminish the expression of this gene to levels below 45% (the value for honokiol) of the control value. As above, compound **36** is also noteworthy as it caused a decrease of gene expression to 36 % of the control at half the concentration of honokiol. Compounds **15** and **25** were also quite active, with gene expression reduced to 13% and 7%, respectively, of the control value, but at a concentration twice than that of honokiol. Another compound that showed a noticeable activity was **16**, which lowered gene expression to 23% of the control at the same concentration as the natural product. Thus, the most frequent oxygen substitution patterns in this case are *para-para* or *meta-para.*

Conclusions

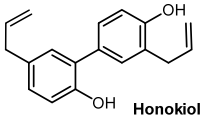
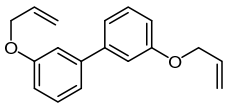
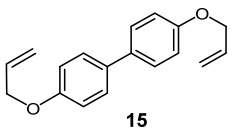
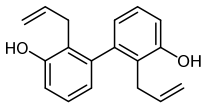
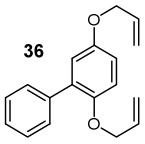
Thirty-six biphenyl derivatives structurally related to honokiol have been synthesized and biologically evaluated. All of them bear either *O*- or *C*-allyl groups.

IC_{50} values were first determined for all these biphenyl derivatives towards the HT-29, MCF-7 and HEK-293 cell lines. Some of these derivatives exhibited comparable or lower IC_{50} values than honokiol towards the HT-29 cell line or else higher therapeutic margin (α values) than the natural product.

Thirteen of the derivatives (**3**, **6**, **10**, **12**, **13**, **15**, **16**, **17**, **20**, **22**, **25**, **28** and **36**) were then selected for further biological evaluation on the basis of showing either comparatively low IC_{50} values or else $\alpha > 1$ (good safety margin). These derivatives were specifically evaluated for their ability to inhibit the secretion of the VEGFA protein and to inhibit the expression of the *VEGFA*, *hTERT* and *c-Myc* genes. We observed that, in general, the most active derivatives in these particular types of biological properties exhibited in many cases substitution patterns *meta-meta* or *meta-para* in their oxygen atoms.

Table 3 summarizes the results obtained for compounds that showed the highest activity in simultaneously inhibiting the VEGFA protein secretion and the expression of the *VEGFA*, *hTERT* and *c-Myc* genes. All these derivatives exhibit two oxygen functions (OH or OR) and also two allyl units, either of the *C*-allyl or the *O*-allyl type.

Table 3. Compounds with the highest activity in simultaneously inhibiting the VEGFA protein secretion and the expression of the *VEGFA*, *hTERT* and *c-Myc* genes.

Biological activity	 Honokiol	 **6**	 **15**	 **25**	 **36**
IC_{50} (μM) HT-29	25	11	38	79	19
α	1.5	>9	0.7	1.2	7.1
% VEGFA secretion	55	76	58	40	61
% *VEGFA* gene expr.	36	16	24	42	55
% *hTERT* gene expr.	45	34	45	25	42
% *c-Myc* gene expr.	45	58	13	7	36

Compound **6** turns out to be a very promising derivative as a potential anticancer agent because it is able to simultaneously diminish the expression of all three targeted genes at a concentration in the low micromolar range (half the concentration used for honokiol). In addition, **6** exhibits the highest therapeutic margin of all the tested derivatives, honokiol included, and has no free hydroxyl groups.

This feature could be advantageous because, as it is not able to form glucuronide conjugates, it may exhibit a lower metabolization rate. Compounds of this type therefore deserve to be the object of further pharmacological investigation.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Physical data. Physical and spectral data of compounds **1-36**.