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Selective synthesis of (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-bromo-2(5*H*)-furanones

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Abstract—4-Aryl-3-bromo-2(5*H*)-furanones have been selectively synthesized in satisfactory yields by treatment of easily available 3,4-dibromo-2(5*H*)-furanone either with arylboronic acids in the presence of Ag_2O and a catalytic amount of $PdCl_2(MeCN)_2$ or with aryl(trialkyl)stannanes in the presence of a catalyst precursor consisting of $AsPh_3$ and a Pd(II) or a Pd(0) compound. These monobromo derivatives have been then used as precursors to a variety of (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-bromo-2(5*H*)-furanones including the compound with the structure corresponding to that reported for naturally occurring rubrolide N. The structure and stereochemistry of these synthetic compounds have been unambiguously established by NMR techniques. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

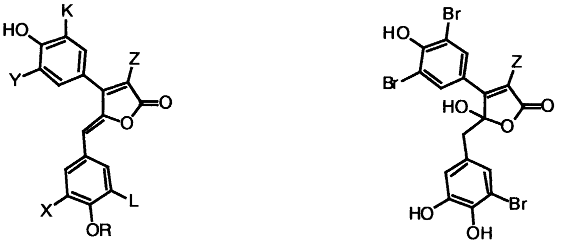
Rubrolides are a family of biologically active marine ascidian (tunicate) metabolites which have been isolated from *Ritterella rubra*[1] and *Synoicum blochmanni* (Fig. 1).[2]

Rubrolides A (**1a**) to H (**2b**) are potent antibiotics and show moderate but selective inhibition of protein phosphatases 1 and 2A.[1] On the other hand, rubrolides K (**1i**), I (**1g**), M (**1k**) and L (**1j**) show significant cytotoxicity against P-388 suspension culture of mouse limphoid neoplasm, the monolayer cultures of human lung carcinoma (A-549), human colon carcinoma (HT-29) and human melanoma (MEL-28).[2] Efficient and selective procedures for the synthesis of rubrolides, which are unsubstituted at the 3-position of their 2(5*H*)-furanone ring, have been reported.[3–5] Nevertheless, no synthetic route toward rubrolides which contain an halogen atom in their 3-position have been reported to date.

Recently, in connection with an ongoing project directed towards the development of concise and efficient procedures for the selective synthesis of natural products and their analogs which are potentially cytotoxic against human tumor cell lines, we decided to develop a general procedure for the synthesis of rubrolide N (**1l**) and some related substances of general formula **3** (Fig. 2) and to evaluate the cytotoxic activities of these compounds.

We speculated that readily available 3,4-dibromo-2(5*H*)-furanone (**4**) might be an ideal starting material to access either **1l** or the related compounds **3a–h** if it was possible to achieve a regioselective arylation of **4** at the bromine-bearing carbon atom 4 of this dibromo derivative. On the other hand, **4** represents the cyclic analog of methyl (*Z*)-2,3-dibromopropenoate (**5**) (Fig. 3) and similarly to this last compound it might exhibit regioselectivity in Pd-catalyzed cross-coupling reactions with organometallic reagents.[6,7]

Thus, we anticipated that **4** would be able to react with an equimolar amount of an arylmetal derivative in the presence of a suitable Pd-catalyst to give selectively the corresponding 4-aryl-3-bromo-2(5*H*)-furanone **6**. The synthesis of



1a : R = Z = H; K = L = X = Y = Br
1b : R = H; K = L = X = Y = Br; Z = Cl
1c : R = K = Y = Z = H; L = X = Br
1d : R = L = X = Z = H; K = Y = Br
1e : R = L = K = X = Y = Z = H
1f : R = Me; L = K = X = Y = Z = H
1g : R = K = H; L = X = Y = Br; Z = Cl
1h : R = K = Z = H; L = X = Y = Br
1i : R = K = L = H; X = Y = Br; Z = Cl
1j : R = K = Y = H; L = X = Br; Z = Cl
1k : R = K = L = Y = H; X = Br; Z = Cl
1l : R = K = L = H; Y = Cl; X = Z = Br

2a : Z = H
2b : Z = Cl

Figure 1. Chemical structures of rubrolides.

Keywords: furanones; coupling reactions; boron and compounds; tin and compounds; biologically active compounds.

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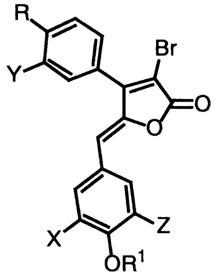
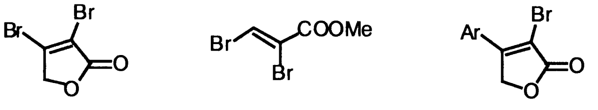


3a : R = OMe; R^1 = Me; X = Br; Y = Cl; Z = H
3b : R = OH; R^1 = Me; X = Br; Y = Cl; Z = H
3c : R = OMe; R^1 = Me; Y = Cl; X = Z = H
3d : R = OH; R^1 = X = Z = H; Y = Cl
3e : R = SMe; R^1 = Me; X = Br; Y = Z = H
3f : R = OMe; R^1 = Me; X = Y = Z = H
3g : R = OH; R^1 = X = Y = Z = H
3h : R = OMe; R^1 = Me; Y = H; X = Z = Br

Figure 2. Chemical structures of compounds **3a**–**h**.



4 **5** **6**

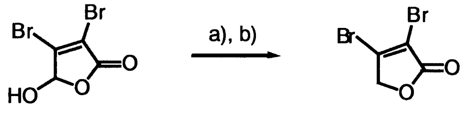
Figure 3. Chemical structures of compounds **4**, **5** and **6**.

rubrolide N (**1l**) and the structural analogs **3a**–**h** might then be completed by subsequent appendage of a 1-(aryl)methylidene substituent at C-5 of compounds **6**, which we envisaged to perform by a furanolate chemistry similar to that previously employed to synthesize rubrolides C and E starting from 4-(4-methoxyphenyl)-2(5*H*)-furanone.[4]

The results obtained in the study of the synthesis of 4-aryl-3-bromo-2(5*H*)-furanones **6** starting from **4**[8] and the use of these monobromo derivatives as precursors to (*Z*)-4-aryl-5-[1-(aryl)methylidene]-2(5*H*)-furanones such as compound **1l** and the related substances **3a**–**h** form the subject of this paper. We will also report that the NMR parameters of **1l** prepared from **4** did not match those described in the literature for the naturally occurring compound with the surmised structure of rubrolide N.[2] Finally, we will report the results of tests to evaluate the cytotoxic activities exhibited by some (*Z*)-4-aryl-5-[1-(aryl)methylidene]-2(5*H*)-furanones prepared from **4** against the National Cancer Institute (NCI) 3-cell and 60-cell line panels.

2. Results and discussion

3,4-Dibromo-2(5*H*)-furanone (**4**), which we used as a starting material for the synthesis of compounds **6**, was prepared from commercially available mucobromic acid (**7**) by a procedure similar to that previously used for the conversion of mucochloric acid to 3,4-dichloro-2(5*H*)-furanone.[9] Thus, a methanol solution of **7** was treated at 0°C with 1.5 equiv. of $NaBH_4$, added in portionwise manner, and then with 1.0 equiv. of concentrated sulfuric acid in methanol (Scheme 1). The crude reaction product was recrystallized from hexane and Et_2O to give dibromide **4** in 75% yield.



7 **4**

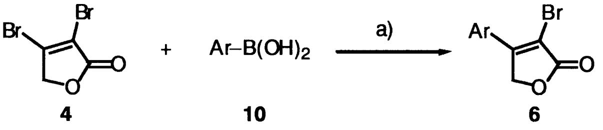
Scheme 1. (a) $NaBH_4$ (1.5 equiv.), MeOH, 0°C; (b) conc. H_2SO_4, 0°C.

Based on our success with $Pd(PPh_3)_4$ as catalyst in Negishi-type reactions of compound **5** or the corresponding ethyl ester,[6,10] we then investigated a similar protocol for the synthesis of a typical 4-aryl-3-bromo-2(5*H*)-furanone **6** starting from **4**. However, we observed that reaction of this dibromide with 1.2 equiv. of phenylzinc bromide (**8**) in THF or in a 1:1 mixture of THF and DMF at 20°C, in the presence of 5 mol% $Pd(PPh_3)_4$, did not produce the required compound **6a**. It should be noted that compound **4** was completely consumed in this reaction and that the crude reaction mixture proved to contain a significant amount of biphenyl (**9**).

On the other hand, no better result was obtained when **4** was reacted with 3.0 equiv. of **8** at 65°C in a mixture of THF and DMF, in the presence of a catalytic amount of $Pd(PPh_3)_4$.

An initial attempt to prepare selectively **6a** by a Suzuki-type reaction proved also to be unsuccessful.[11] In fact, treatment of **4** with 1.2 equiv. of phenylboronic acid (**10a**) in a mixture of toluene and EtOH at 20°C for 3 h, in the presence of 2.1 equiv. of Na_2CO_3 and 5 mol% $Pd(PPh_3)_4$ provided a reaction mixture which did not contain either **6a** or unreacted **4**. Instead, this mixture contained a small amount of 3,4-diphenyl-2(5*H*)-furanone (**11**) together with unreacted **10a**. On the other hand, the product of the reaction of **4** with 1.3 equiv. of **10a**, which was performed in dioxane for 21 h at 80°C, in the presence of 3.0 equiv. of K_3PO_4 and 3 mol% $Pd(PPh_3)_4$, proved to be constituted of a complex mixture in which **9** was one of the major components and compounds **6a** and **11** were in a ca. 1:1 molar ratio. Nevertheless, we were gratified to find that compounds **6** could be regioselectively prepared using a procedure similar to that we recently developed for the synthesis of 4-alkyl-3-bromo-2(5*H*)-furanones from **4**.[7a] In fact, when **4** was reacted with 1.1 equiv. of an arylboronic acid **10** in THF under reflux for 18–26 h, in the presence of 3.0 equiv. of Ag_2O, 5 mol% $PdCl_2(MeCN)_2$ and 20 mol% $AsPh_3$, the required cross-coupled product **6** was obtained in yields ranging from 66 to 79% (Scheme 2, Table 1).

Interestingly, in all of the cases examined (entries 1–3,



4 **10** **6**

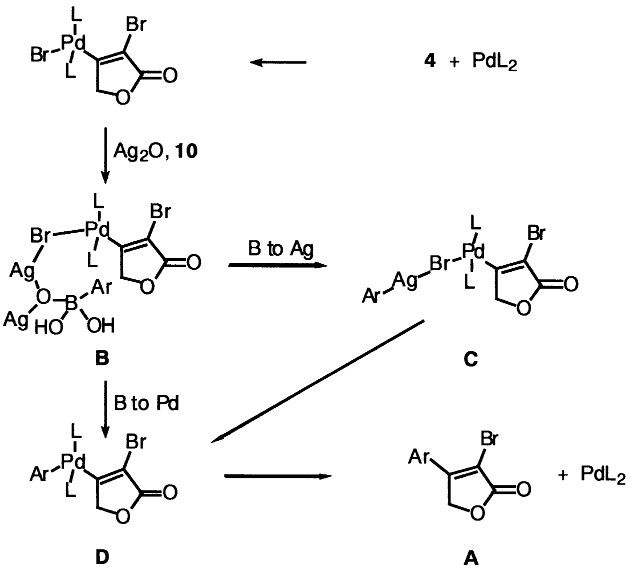
Scheme 2. (a) **10** (1.1 equiv.), $PdCl_2(MeCN)_2$ (5 mol%), $AsPh_3$ (20 mol%), Ag_2O (3.0 equiv.), THF, 65°C, 18–26 h.

Table 1. Palladium-catalyzed cross-coupling reaction of **4** with arylboronic acids **10**

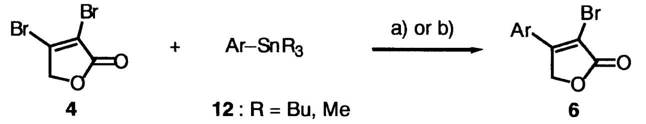
Entry	Arylboronic acid Ar	Reaction time (h)	Product **6**	Isolated yield (%)
1	C_6H_5	18	**6a**	66
2	3-Cl, 4-$MeOC_6H_3$	21	**6b**	79
3	4-ClC_6H_4	26	**6c**	61

These reactions were performed in THF at 65°C using 1.1 equiv. of **10**, 5 mol% $PdCl_2(MeCN)_2$, 20 mol% $AsPh_3$ and 3.0 equiv. of Ag_2O.

Table 1) this Ag_2O-mediated Pd-catalyzed reaction did not provide the 3,4-diaryl-2(5*H*)-furanones derived from the cross-coupling at the bromine-bearing carbon atoms C-3 and C-4 of **4**. Instead, significant amounts of biaryls **9**, which derived from homocoupling of compounds **10**, were found to be present in the crude reaction mixtures.



Scheme 3. Plausible mechanism for the Ag_2O-mediated Pd-catalyzed reaction between **4** and **10**.



Scheme 4. (a) **12** (1.1 equiv.), $PdCl_2(PhCN)_2$ (5 mol%), $AsPh_3$ (10 mol%), NMP, 20°C; (b) **12** (1.0 equiv.), $Pd_2(dba)_3$ (2.5 mol%), $AsPh_3$ (10 mol%), NMP, 20°C.

A plausibile mechanism of this process is presented in Scheme 3. In particular, it is proposed that the catalytic cycle of the cross-coupling reaction involves oxidative addition of the C-4 carbon–bromine bond of **4** to a palladium(0) species and that the resulting organopalladium(II) bromide **A** interacts with Ag_2O and **10** to give the intermediate **B**. Transmetalation of this intermediate with **10** followed by reductive elimination would then produce the required derivative **6**. The transmetalation from boron to palladium, which produces **D**, could occur by prior boron to silver(I) transmetalation, i.e. via **C** or directly from complex **B**.

Similar mechanisms have been recently suggested either for the Ag_2O-mediated, Pd-catalyzed coupling of silanols with aryl and alkenyl iodides[12] or for the Pd-catalyzed coupling of aryl and alkenyl iodides with boronic acids mediated by copper(I) thiophene-2-carboxylate.[13]

We were also pleased to observe that compounds **6** could also be regioselectively obtained in yields ranging from 58 to 76% by reaction of **4** with 1.0–1.1 equiv. of an aryl-(trialkyl)stannane **12** in NMP at 20°C in the presence of the catalyst precursor consisting of 5 mol% $PdCl_2(PhCN)_2$ and 10 mol% $AsPh_3$ (*catalyst A*)[14] or that obtained by treatment of 2.5 mol% $Pd_2(dba)_3$ with 10 mol% $AsPh_3$ (*catalyst B*) (Scheme 4, Table 2).

Similarly to the above mentioned Ag_2O-mediated Pd-catalyzed reactions of **4** with **10**, these cross-coupling reactions did not afford any trace of the diarylated compounds **11**, but provided significant amounts of biaryls **9** derived from homocoupling of **12** when catalyst *A* or *B* was used.

Another significant feature of some of these reactions was that, unexpectedly, the cross-coupling reactions of **4** with aryl(trimethyl)stannanes **12b** and **12c** (entries 2 and 4, Table

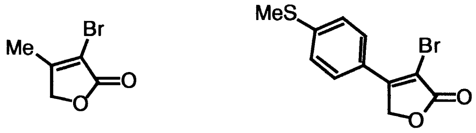
Table 2. Palladium-catalyzed cross-coupling reactions of **4** with aryl(trialkyl)stannanes **12**

Entry	Organotin			Catalyst precursor	Reaction time (h)	**6**/**9** molar ratio	Cross-coupled product	Isolated yield (%)
	12	Ar	R					
1	**12a**	C_6H_5	Bu	Cat A	93	92/8	**6a**	76
2	**12b**	C_6H_5	Me	Cat A	39	90/10[a]	**6a**	70
3	**12a**	C_6H_5	Bu	Cat B	94	92/8	**6a**	67
4	**12c**	4-$MeSC_6H_4$	Me	Cat B	22	91/9[a]	**6d**	59
5	**12d**	4-$MeOC_6H_4$	Bu	Cat B	89	92/8	**6e**	68
6	**12e**	3-FC_6H_4	Bu	Cat B	89	96/4[b]	**6f**	58
7	**12f**	4-MeC_6H_4	Bu	Cat B	112	93/7	**6g**	73
8	**12g**	3-Cl, 4-$MeOC_6H_3$	Bu	Cat B	250	n.d.	**6c**	62

All reactions were performed at 20°C in the presence of 5 mol% $PdCl_2(PhCN)_2$ and 10 mol% $AsPh_3$ (Cat A) or in the presence of 2.5 mol% $Pd_2(dba)_3$ and 10 mol% $AsPh_3$ (Cat B).

[a] The crude reaction product was also contaminated by 3-bromo-4-methyl-2(5*H*)-furanone (**13**). The desired monoarylated compound and **13** were in a ca. 84:16 molar ratio, respectively.

[b] The crude reaction product was also contaminated by ca. 10% of **4**.



13 **6d**

Figure 4. Chemical structures of compounds **13** and **6d**.

2) furnished 3-bromo-4-methyl-2(5*H*)-furanone (**13**) in addition to the desired compounds **6a** and **6d**, respectively (Fig. 4).

The required monobromo derivatives and compound **13** were found to be present in the crude reaction products of these reactions in a ca. 84:16, molar ratio, respectively. Purification of these crude products by MPLC on silica gel allowed us to isolate pure **6a** and **6d** in 70 and 59% yield, respectively.

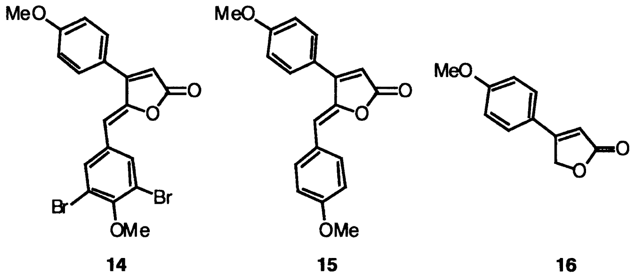
It should also be noted that preliminary tests indicated that the regioselectivity of the cross-coupling reactions between **4** and organostannanes **12**, which were carried out using the catalyst precursor A, was higher than that obtained when these reactions were performed using catalyst A and 10 mol% CuI. In fact, significant amounts of symmetrically 3,4-diaryl substituted 2(5*H*)-furanones **11** were formed under these last conditions.

Having set up two different protocols for the regioselective synthesis of compounds **6**, we examined the conversion of these heterocyclic derivatives into a precursor to rubrolide N, i.e. compound **3a**, and some of its structural analogs of general formula **3** which are characterized by methoxyaryl moieties, by a procedure similar to that recently used to prepare the dimethyl ethers **14** and **15** corresponding to rubrolide C (**1c**) and rubrolide E (**1e**), respectively, starting from 4-(4-methoxyphenyl)-2(5*H*)-furanone (**16**) (Fig. 5).[4]

Thus, a CH_2Cl_2 solution of a compound **6** was sequentially treated with 1.2 equiv. of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf), 3.0 equiv. of diisopropylethylamine and 1.0 equiv. of an arylaldehyde **17**.

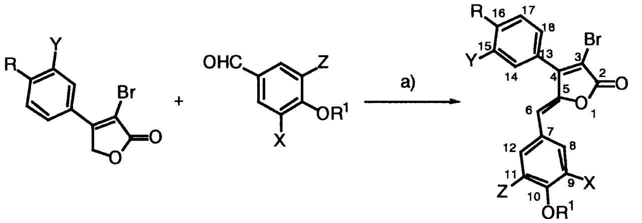
After stirring for 2 h at room temperature, the reaction mixture was treated with 2.0 equiv. of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at 20°C for 4.5 h to give stereoselectively the required compound **3** (Scheme 5).

This one-pot procedure was used to prepare compounds **3a** and **3c** in 87 and 95% yield starting from aldehydes **6b** and **17a** and **17b**, respectively (entries 1 and 2, Table 3), compound **3e** in 86% yield starting from **6d** and **17a** (entry 3, Table 3), and compounds **3f** and **3h** in 99 and 24% yield starting from **6e** and **17b** and **17c**, respectively (entries 4 and 5, Table 3).



14 **15** **16**

Figure 5. Chemical structures of compounds **14**, **15** and **16**.



6 **17** **3**

Scheme 5. (a) TBDMSOTf (1.2 equiv.), *i*-Pr_2EtN (3.0 equiv.), CH_2Cl_2, 20°C, 2 h, then DBU (2.0 equiv.), 20°C, 4 h.

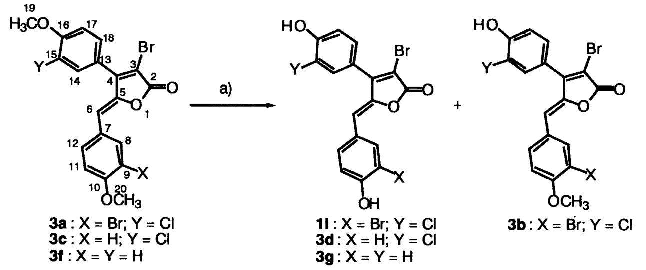
All these compounds were characterized by MS, IR and NMR analyses as well as by elemental analysis. Moreover, the structure and stereochemistry of **3a**, **3c**, **3f** and **3h** were unambiguously assigned on the basis of their ^1H and ^{13}C NMR spectra at 600 and 150 MHz, respectively, and by a combination of NMR techniques, which included homonuclear shift correlation (^1H–^1H COSY), nuclear Overhauser enhancement spectroscopy (NOESY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC). Unfortunately, in the case of compound **3e** the structural and stereochemical assignments by NMR spectroscopy could not be performed owing to the very low solubility of this substance in solvents such as acetone-d_6, $CDCl_3$, C_6D_6, CD_3OD and DMSO-d_6.

Finally, we investigated the conversion of the methyl aryl ether subunits of compounds **3a**, **3c** and **3f** so prepared into the corresponding phenol moieties and, first of all, we tried to prepare compound **1l** from **3a**. Thus, 5.0 equiv. of BBr_3 in CH_2Cl_2 were added to a CH_2Cl_2 solution of **3a** maintained at −78°C and the resulting mixture was allowed to warm up to 20°C, stirred at this temperature for 48 h and then hydrolyzed and extracted with $CHCl_3$. A TLC analysis of the dried organic extract showed the presence of two new compounds. This extract was concentrated and the residue was purified by MPLC on silica gel. Concentration of the first eluted chromatographic fractions followed by a purification of the resulting product by a second MPLC on silica

Table 3. Synthesis of (*Z*)-4-aryl-5-[1-(aryl)methylidene]-2(5*H*)-furanones **3** by introduction of a1-(aryl)methylidene unit at the C-5 position of compounds **6**

Entry	Reagents					Product	Isolated yield (%)
	Compound **6**	**17**	Aldehyde				
			X	Z	R^1		
1	**6b**	**17a**	Br	H	Me	**3a**	87
2	**6b**	**17b**	H	H	Me	**3c**	95
3	**6d**	**17a**	Br	H	Me	**3e**	86
4	**6e**	**17b**	H	H	Me	**3f**	99
5	**6e**	**17c**	Br	Br	Me	**3h**	24

These syntheses were performed using the experimental conditions indicated in Scheme 5.



Scheme 6. (a) BBr_3 (5.0 equiv.), CH_2Cl_2, 20°C, 15–48 h, then H_2O.

gel provided 3-bromo-4-(3-chloro-4-hydroxyphenyl)-5-[1-(3-bromo-4-methoxy-phenyl)methylidene]-2(5*H*)-furanone (**3b**) in 51% yield (Scheme 6).

On the other hand, concentration of the last eluted chromatographic fractions of the first MPLC on silica gel followed by purification of the resulting residue by a second MPLC on silica gel allowed us to obtain **1l** in 47% yield (Scheme 6). The structure and stereochemistry of **3b** and **1l** were confirmed by their ^1H and ^{13}C NMR spectra and by a combination of NMR techniques which included NOESY, ^1H–^1H COSY, HMQC and HMBC.

However, it was observed that several NMR parameters of **1l** did not match those reported for the natural product with the structure of rubrolide N.[2] In fact, the chemical shift of C-3 in synthetic **1l** (δ=107.9 ppm) was significantly different from that of the corresponding carbon atom in the natural compound (δ=119.0 ppm).[2] On the other hand, it must be taken into account that: (i) the chemical shift for C-3 either in a compound similar to **1l**, i.e. **3a**, or in 3-bromo-4-aryl-2(5*H*)-furanones such as **6a** and **6f** is usually included between 107 and 110 ppm; (ii) unexpectedly, the chemical shift value reported for C-3 in rubrolide N is very similar to the chemical shift values for C-3 in rubrolides which are characterized by a chlorine atom in their position 3 and are included in the range δ 116.9–118.5 ppm;[2] (iii) C-3 of 3-chloro-4-phenyl-2(5*H*)-furanone, which recently we selectively synthesized from 3,4-dichloro-2(5*H*)-furanone,[15] absorbs at 117.1 ppm. It should also be noted that the assignments, which have been reported in the literature for C-17 and C-15 of the naturally occurring compound with the surmised structure of rubrolide N (δ=123.4 and 117.0 ppm, respectively),[2] ought to be interchanged. In fact, for compounds characterized by a similarly substituted aromatic moiety, the chemical shift of C-17 is expected upfield compared with that of C-15. On the other hand, this interchange would lead to a better agreement between the literature data and our assignments even though not negligible differences should be still present among the chemical shift values of the protons ($\Delta\delta\sim$0.8 ppm) or of the carbon atoms ($\Delta\delta\sim$4 ppm) of the aryl moiety linked to C-4 of synthetic **1l** and of the natural product. Thus, all these observations indicate that some NMR assignments made in the literature for the natural product with the surmised structure of rubrolide N[2] are incorrect and that probably the substituent at C-3 of the naturally occurring compound is a chlorine atom instead of a bromine atom.

It is worth mentioning that the procedure, which was used to prepare **1l** from **3a**, proved to be suitable for the preparation of compounds **3d** and **3g** from **3c** and **3f**, respectively, in almost quantitative yield (Scheme 5). On the contrary, attempts to prepare **1l** from **3a** in high selectivity were unsuccessful. In fact, demethylation of **3a** either by treatment with 7.0 equiv. of BBr_3 in CH_2Cl_2 at 20°C for 60 h and at 35°C for 6 h, followed by hydrolysis, or by reaction with 10.0 equiv. of $AlCl_3$ and 12.0 equiv. of ethanethiol at room temperature for 1 h, followed by hydrolysis with dilute aqueous HCl,[16] provided **1l** contaminated by significant amounts of **3b**.

Finally, compounds **1l**, **3a**, **3d**, **3e**, **3f** and **3g** were evaluated in the NCI 3-cell line, one dose primary anticancer assay at a 1.00×10^{-4} M concentration. This 3-cell line panel consisted of MCF7 (breast), NCI-H460 (lung) and SF-268 (CNS). The results for each test agent are reported in Table 4 as the percent of growth of the treated cells when compared to the untreated control cells. The data summarized in Table 4 indicate that compounds **1l** and **3d** were highly cytotoxic (entries 1 and 3). In fact, their values for the growth reduction of any one of the cell lines were negative numbers, which indicate cell kill. On the contrary, compounds **3a**, **3e**, **3f** and **3g** were found to be inactive (entries 2, 4–6). Thus, these data seem to suggest that the presence either of two phenol subunits or a chlorine atom at C-15 are necessary for cytotoxicity of (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-bromo-2(5*H*)-furanones. It should also be noted that compounds **1l** and **3d** were further evaluated against the NCI 60 cell line panel but showed limited cytotoxicity, displaying average GI_{50} values 1.70×10^{-5} and 1.29×10^{-5} M, respectively.

Table 4. Results of the cytotoxicity tests for compounds **1l**, **3a**, **3d**, **3e**, **3f** and **3g** in the NCI 3-cell line, one dose primary anticancer assay

Entry	Compound	Growth percentages			Activity
		NCI-H460 (lung)	MCF-7 (breast)	SF-268 (CNS)	
1	**1l**	−64	−79	−75	Active
2	**3a**	155	41	41	Inactive
3	**3d**	−97	−73	−88	Active
4	**3e**	99	141	121	Inactive
5	**3f**	100	145	130	Inactive
6	**3g**	82	104	97	Inactive

In the protocol used, each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a 1.00×10^{-4} M concentration in DMSO and the culture incubated for 48 h. End-point determinations were made with alamar blue. Results for each test are reported as the percent of growth of the treated cells when compared to the untreated control cells.

3. Conclusions

The results summarized earlier show that, when suitable reaction conditions are employed, 3,4-dibromo-2(5*H*)-furanone (**4**) is able to undergo highly regioselective cross-coupling reactions with arylboronic acids or aryl(trialkyl)-stannanes to give 4-aryl-3-bromo-2(5*H*)-furanones **6** in moderate to good yields. We have also shown that compounds **6** represent useful precursors to (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-bromo-2(5*H*)-furanones such as compounds **1l** and **3a–g**, which represent a not previously synthesized class of compounds with potential cytotoxic activities. The structure and the stereochemistry of these substances were unambiguously assigned by NMR techniques. These assignments allowed us to conclude that the structure reported in the literature for rubrolide N[2] is incorrect. In fact, several NMR parameters of compound **1l**, which has the structure of rubrolide N, did not match those reported for this natural product.[2]

Finally, it should be noted that two (*Z*)-4-aryl-5-[1-(aryl)-methylidene]-3-bromo-2(5*H*)-furanones so prepared, which are characterized by two phenol subunits and a chlorine atom at C-15, were found to be significantly active in the NCI 3-cell line, one dose primary anticancer assay. Nevertheless, these compounds showed limited cytotoxicity against the NCI 60 cell line panel.

We are currently investigating a protocol similar to that used to prepare compounds **1l** and **3a–g** for the synthesis of cytotoxic rubrolides, which are characterized by a chlorine atom linked at C-3 of their 2(5*H*)-furanone ring.

4. Experimental

4.1. General

Melting points and boiling points are uncorrected. Precoated aluminum silica gel sheets Merck 60 F_{254} were used for TLC analyses. GLC analyses were performed on a Dani GC 1000 instrument with a PTV injector, which was equipped with a Dani data station 86.01. Two types of capillary columns were used: an Alltech AT-1 bonded FSOT column (30 m×0.25 mm i.d.) and an Alltech AT-35 bonded FSOT column (30 m×0.25 mm i.d.). Purifications by MPLC on silica gel (Merck silica gel 60, particle size 0.015–0.040 mm) were performed on a Büchi B-680 system using a Knauer K-2400 differential refractometer as detector. GLC/MS analyses were performed using a Q-mass 910 spectrometer interfaced with a Perkin–Elmer gas-chromatograph. The ionspray mass spectra of compound **1l** and **3b** were acquired using a Perkin–Elmer Sciex API III$^+$ triple quadrupole mass spectrometer (Sciex, Concord, Ont., Canada) equipped with a source for atmospheric pressure ionization and an articulated ionspray interface. The ESI spectra, in negative-ion mode, were obtained under the following conditions: ionspray voltage, −4.0 kV; orifice voltage, −60 V; scan range, *m*/*z*=400–600; scan time, 4.73 s; no interscan delay; unit-mass resolution. The APCI-MS spectra of compounds **3d–h** were registered on a quadrupole mass spectrometer equipped with the Sciex API source and a heated nebulizer probe for APCI ionization. The experimental conditions used included: discharge current, 3 mA (ionspray voltage read-back, 5012 V); orifice voltage, 60 V (−60 V in negative-ion mode); scan range, *m*/*z*=400–600. IR spectra were recorded on a Perkin–Elmer 1725 FT-IR spectrophotometer. NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer or on a Bruker AMX 600 spectrometer using TMS and $CDCl_3$ as an internal standard, respectively. Nevertheless, the ^1H and ^{13}C NMR spectra of **3b** and **3d** were referenced to DMSO-d_6 at δ 2.50 ppm (downfield to TMS) and at δ 40.40 ppm, respectively. On the other hand, the ^1H NMR spectra of **1l** and **3g** were referenced to the residual CHD_2OD proton of CD_3OD at 3.37 ppm downfield to TMS, whereas the ^{13}C NMR spectra of these compounds were referenced to CD_3OD at 49.30 ppm. The structure of compounds **6a**, **6f**, **1l**, **3a–d** and **3f–h** was assigned on the basis of the ^1H and ^{13}C NMR spectra of these compounds at 600 and 150 MHz, respectively, and by a combination of NMR techniques which included ^1H–^1H COSY, NOESY (mixing time: 400 ms), HMQC and HMBC. All reactions of air and water sensitive materials were performed in flame-dried glassware under an atmosphere of nitrogen or argon using standard syringe, cannula and septa techniques. Solvents were dried and distilled before use. The following compounds were prepared by published procedures: $PdCl_2(PhCN)_2$,[17] $PdCl_2(MeCN)_2$,[18] 3-chloro-4-methoxyphenylboronic acid (**10b**),[19] 4-methylthiophenyl-trimethylstannane (**12c**),[20] 3-fluorophenyltributylstannane (**12e**),[21] 4-methoxyphenyltributylstannane (**12d**)[22] and 4-tolyltributylstannane (**12f**).[23]

4.1.1. 3,4-Dibromo-2(5*H*)-furanone (4). A stirred solution of mucobromic acid (**7**) (28.67 g, 111.2 mmol) in methanol (160 ml), which was cooled to 0°C, was treated with $NaBH_4$ (6.31 g, 166.7 mmol), added in a portionwise manner, and the resulting mixture was stirred for 15 min at 0°C. A solution of concentrated sulfuric acid (10.89 g, 111.2 mmol) in methanol (55 ml), which was cooled to 0°C, was then added and the resulting mixture was stirred for 15 min. It was then diluted with Et_2O (1.5 l) and washed with brine (4×200 ml). The organic phase was dried and concentrated under reduced pressure and the solid residue was recrystallized from a mixture of hexane and Et_2O (1:1) to give **4** (20.20 g, 75% yield) as a colorless solid. Mp 90–91°C. MS, *m*/*z* (%): 244 (19) [M^+], 242 (39) [M^+], 240 (19) [M^+], 163 (86), 161 (100), 119 (19), 117 (17). ^1H NMR (200 MHz, $CDCl_3$): δ 4.90 (2H, s, H-5). Anal. Calcd for $C_4H_2Br_2O_2$: C, 19.86; H, 0.84; Br, 66.07. Found: C, 19.67; H, 1.02; Br, 66.18.

4.2. Palladium-catalyzed reaction between 4 and arylboronic acids 10

A mixture of compound **4** (2.42 g, 10.0 mmol), an arylboronic acid **10** (11.0 mmol), $PdCl_2(CH_3CN)_2$ (0.13 g, 0.5 mmol), $AsPh_3$ (0.61 g, 2.0 mmol) and Ag_2O (6.95 g, 30.0 mmol) was placed in a reaction vessel under argon atmosphere and then deaereated. Deaereated THF (40 ml) was added and the reaction mixture, which was periodically monitored by GLC and TLC, was stirred under reflux. On completion of the reaction the mixture was cooled to room temperature, diluted with EtOAc (250 ml) and filtered over Celite. The filtrate was concentrated under reduced pressure and the residue was purified by MPLC on silica gel and/or by recrystallization. This procedure was used to prepare compounds **10a**, **10b** and **10c**. The reaction time of these preparations and the isolated yields of compounds **10** so prepared are reported in Table 1.

4.2.1. 3-Bromo-4-phenyl-2(5*H*)-furanone (6a). The crude reaction product, which was obtained from the reaction of phenylboronic acid (**10a**) with **4** according to Section 4.2 (entry 1, Table 1), was purified by MPLC on silica gel, using a mixture of benzene and EtOAc (99:1) as eluant, to give compound **6a** in 66% yield as a colorless solid. Mp 105–107°C. MS, *m/z* (%): 240 (20) [M$^+$], 238 (21) [M$^+$], 211 (41), 209 (46), 105 (46), 102 (100). IR (KBr): ν 1752, 1602, 1338, 1190, 1029, 984, 766, 749, 692 cm^{-1}. ^{1}H NMR (600 MHz, CDCl$_3$): δ 7.84 (2H, dd, J=8.3, 1.5 Hz, H-7 and H-11), 7.56 (1H, m, H-9), 7.53 (2H, m, H-8 and H-10), 5.20 (2H, s, H-5). Anal. Calcd for $C_{10}H_7BrO_2$: C, 50.24; H, 2.95. Found: C, 50.44; H, 3.11. GLC analysis showed that **6a** had chemical purity higher than 98%.

4.2.2. 3-Bromo-4-(3-chloro-4-methoxyphenyl)-2(5*H*)-furanone (6b). The crude reaction product, which was obtained from the reaction of 3-chloro-4-methoxyphenylboronic acid (**10b**) with **4** according to Section 4.2 (entry 2, Table 1), was recrystallized from a mixture of CH_2Cl_2 and hexane to give **6b** in 78% yield as a colorless solid. Mp 162–164°C. MS, *m/z* (%): 307 (3) [M$^+$], 306 (20) [M$^+$], 305 (22) [M$^+$], 304 (100) [M$^+$], 302 (74) [M$^+$], 275 (57), 166 (78). IR (KBr): ν 1759, 1598, 1510, 1260, 1193, 1035, 988, 831, 702 cm^{-1}. ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.90 (1H, d, J=2.2 Hz, H-7), 7.82 (1H, dd, J=8.8, 2.2 Hz, H-11), 7.05 (1H, d, J=8.8 Hz, H-10), 5.16 (2H, s, H-5), 3.99 (3H, s, OCH$_3$). Anal. Calcd for $C_{11}H_8BrClO_3$: C, 43.53; H, 2.66. Found: C, 43.23; H, 2.48. GLC analysis showed that **6b** had chemical purity higher than 98%.

4.2.3. 3-Bromo-4-chlorophenyl-2(5*H*)-furanone (6c). The crude reaction product, which was obtained from the reaction of 4-chlorophenylboronic acid (**10c**) with **4** according to Section 4.2 (entry 3, Table 1), was recrystallized from a mixture of $CHCl_3$ and hexane to give **6c** as a beige solid. Mp 173–175°C. Purification of the mother liquor of this recrystallization by MPLC on silica gel, using a mixture of $CHCl_3$ and hexane (50:50) as eluant, allowed to obtain a further amount of **6c** in 61% overall yield. MS, *m/z* (%): 194 (61), 167 (32), 165 (100), 138 (22), 137 (37), 136 (52), 75 (27). IR (KBr): ν 1744, 1611, 1491, 1443, 1192, 1061, 1036, 990, 824 cm^{-1}. ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.80 (2H, m, H$_{arom}$), 7.50 (2H, m, H$_{arom}$), 5.18 (2H, s, H-5). Anal. Calcd for $C_{10}H_6BrClO_2$: C, 43.91; H, 2.21. Found: C, 44.22; H, 2.45. ^{1}H NMR analysis showed that **6c** had chemical purity higher than 96%.

4.2.4. 3-Chloro-4-methoxyphenyltributylstannane (12g). Chlorotributylstannane (16.33 g, 50.2 mmol) was added dropwise to a 0.81 M THF solution of 3-chloro-4-methoxyphenylmagnesium bromide (71 ml, 57.5 mmol) and the resulting mixture was refluxed for 19 h. It was then cooled to room temperature, poured into water (300 ml) and extracted with Et_2O (4×60 ml). The dried organic extract was concentrated under reduced pressure and the residue was fractionally distilled to give **12g** (18.84 g, 87% yield) as a colorless liquid. Bp 148°C/0.1 mbar. MS, *m/z* (%): 375 (75), 373 (60), 319 (41), 317 (35), 261 (75), 260 (100), 154 (37). IR (film): ν 1577, 1488, 1462, 1288, 1252, 1067, 1027, 804, 701 cm^{-1}. ^{1}H NMR (200 MHz, CDCl$_3$): δ 7.39 (1H, d, J=1.5 Hz, H-2), 7.28 (1H, dd, J=7.7, 1.5 Hz, H-6), 6.91 (1H, d, J=7.7 Hz, H-5), 1.61–1.25 (12H, m, Sn–C–CH$_2$–CH$_2$–), 1.04 (6H, t, J=7.9 Hz, Sn–CH$_2$–), 0.89 (9H, t, J=7.2 Hz, Sn–C–C–C–CH$_3$). Anal. Calcd for $C_{19}H_{30}ClOSn$: C, 52.87; H, 7.71. Found: C, 53.14; H, 7.45.

4.3. Palladium-catalyzed reaction between 4 and aryl(trialkyl)stannanes 12

The preparation of compounds **6** by the title reaction was performed using one of the following Pd-catalyst precursors: that consisting of a mixture of $PdCl_2(PhCN)_2$ (0.192 g, 0.50 mmol) and $AsPh_3$ (0.307 g, 1.00 mmol) (catalyst A); or that consisting of a mixture of $Pd_2(dba)_3$ (0.229 g, 0.25 mmol) and $AsPh_3$ (0.307 g, 1.00 mmol) (catalyst B). One of these catalyst precursors was sequentially treated with a deaereated solution of **4** (10.0 mmol) in NMP (15 ml) and a deaereated solution of an aryl(trialkyl)stannane **12** (10.0–11.0 mmol) in NMP (10 ml). The resulting mixture, which was periodically monitored by GLC and/or TLC analysis, was stirred at room temperature for the period of time reported in Table 2. After completion of the reaction the mixture was poured into a saturated aqueous NH_4Cl solution (150 ml) and extracted with EtOAc (4×80 ml). The organic extract, which was obtained from reactions involving aryl(tributyl)stannanes, was then stirred for 4.5 h at 20°C with a 8 M aqueous KF solution (200 ml), filtered over Celite and the filtrate was extracted with EtOAc (3×60 ml). The organic extract was washed with water (80 ml), dried, filtered, concentrated under reduced pressure and the residue was analyzed by GLC/MS. In the case of the residues obtained from reactions involving aryl(tributyl)stannanes, this analysis showed the presence of three major products, i.e. the required cross-coupled products **6**, the homocoupling compounds **9** derived from the organostannanes, and $AsPh_3$. On the other hand, in the case of the residues obtained from reactions involving aryl(trimethyl)stannanes **12b** and **12c** (entries 2 and 4, Table 2), GLC/MS analysis also showed the presence of significant amounts of a compound having a MS spectrum corresponding to that of 3-bromo-4-methyl-2(5*H*)-furanone (**13**). The residues were then purified by MPLC on silica gel and/or by recrystallization. This general procedure was used to prepare compounds **6a**, **6c**, **6d**, **6e**, **6f** and **6g**. Table 2 summarizes the reaction time and the catalyst precursor used for these preparations, the molar ratios between the required compounds **6** and the homocoupling products derived from organostannes **12**, and the isolated yields of compounds **6** so obtained. It should be noted that the physical and spectral properties of so prepared **6a** and **6c** proved to be in agreement with those of the same compounds obtained by Pd-catalyzed reaction of **4** with **10a** and **10c**, respectively.

4.3.1. 3-Bromo-4-methylthiophenyl-2(5*H*)-furanone (6d). The crude reaction product, which was obtained by Pd-catalyzed reaction between **4** and 4-methylthiophenyltrimethylstannane (**12c**) (entry 4, Table 2), was purified by MPLC on silica gel, using a mixture of $CHCl_3$, petroleum ether and EtOAc (70:30:1) as eluant. The chromatographic fractions containing the required compound **6d** were concentrated under reduced pressure band the solid residue was recrystallized from a mixture of CH_2Cl_2 and hexane to give pure **6d** in 59% yield as a colorless solid. Mp 144–146°C. MS, *m/z* (%): 207 (16), 206 (100), 177 (49), 148 (81), 134 (49), 133 (49), 102 (45). IR (KBr): ν 1768, 1605, 1593, 1192, 1060, 1041, 990, 820 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.78 (2H, m, H_{arom}), 7.32 (2H, m, H_{arom}), 5.18 (2H, s, H-5), 2.54 (3H, s, SCH_3). Anal. Calcd for $C_{11}H_9BrO_2S$: C, 46.33; H, 3.18. Found: C, 46.56; H, 3.17.

4.3.2. 3-Bromo-4-(4-methoxyphenyl)-2(5*H*)-furanone (6e). The crude reaction product, which was obtained by Pd-catalyzed reaction between **4** and 4-methoxyphenyltributylstannane (**12d**) (entry 5, Table 2), was purified by MPLC on silica gel, using a mixture of $CHCl_3$, petroleum ether and EtOAc (70:30:1) as eluant. The chromatographic fractions containing the required compound **6e** were concentrated under reduced pressure and the solid residue was recrystallized from a mixture of CH_2Cl_2 and hexane to give pure **6e** in 68% yield as a colorless solid. Mp 133–134°C. MS, *m/z* (%): 270 (6) [M^+], 268 (7) [M^+], 159 (11), 152 (100), 132 (40), 102 (12), 77 (14). IR (KBr): ν 1752, 1605, 1513, 1276, 1262, 1182, 1060, 989, 826 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.85 (2H, m, H_{arom}), 7.01 (2H, m, H_{arom}), 5.18 (2H, s, H-5), 3.89 (3H, s, OCH_3). Anal. Calcd for $C_{11}H_9BrO_3$: C, 49.10; H, 3.37. Found: C, 49.28; H, 3.52.

4.3.3. 3-Bromo-3(fluorophenyl)-2(5*H*)-furanone (6f). The crude reaction product, which was obtained by Pd-catalyzed reaction between **4** and 3-fluorophenyltributylstanne (**12e**) (entry 6, Table 2), was purified by MPLC on silica gel, using a mixture of toluene and EtOAc (99:1) as eluant, to give pure **6f** in 58% yield as a pale yellow solid. Mp 109–111°C. MS, *m/z* (%): 258 (3) [M^+], 256 (3) [M^+], 229 (8), 227 (10), 123 (27), 120 (100), 99 (30). IR (KBr): ν 1751, 1608, 1582, 1439, 1157, 1071, 1033, 986, 801 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.61 (1H, ddd, J=9.1, 2.7, 1.8 Hz, H-7), 7.58 (1H, dd, J=8.2, 1.8 Hz, H-11), 7.51 (1H, ddd, J=8.2, 8.2, 5.5 Hz, H-10), 7.26 (1H, ddd, J=8.2, 8.2, 2.7 Hz, H-9), 5.17 (2H, s, H-5). Anal. Calcd for $C_{10}H_6BrFO_2$: C, 46.72; H, 2.35. Found: C, 46.93; H, 2.41.

4.3.4. 3-Bromo-4-(4-tolyl)-2(5*H*)-furanone (6g). The crude reaction product, which was obtained by Pd-catalyzed reaction between **4** and 4-tolyltributylstannane (**12f**) (entry 7, Table 2), was recrystallized from a mixture of $CHCl_3$ and petroleum ether to give pure **6g** as a pale yellow solid. Mp 169–171°C. Purification of the mother liquor of this recrystallization by MPLC on silica gel, using a mixture of toluene and EtOAc (98:2), as eluant, allowed to obtain a further amount of pure **6g** in 73% overall yield. MS, *m/z* (%): 254 (10) [M^+], 252 (8) [M^+], 225 (14), 223 (11), 143 (39), 119 (29), 116 (100). IR (KBr): ν 1747, 1607, 1445, 1191, 1061, 1032, 986, 813, 752 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 7.75 (2H, m, H_{arom}), 7.30 (2H, m, H_{arom}), 5.17 (2H, s, H-5), 2.42 (3H, s, CH_3). Anal. Calcd for $C_{11}H_9BrO_2$: C, 52.22; H, 3.58. Found: C, 52.47; H, 3.84.

4.3.5. 3,5-Dibromo-4-methoxybenzaldeyde (17c). A stirred mixture of 3,5-dibromo-4-hydroxybenzaldehyde (15.03 g, 53.5 mmol), anhydrous K_2CO_3 (powder, 325 mesh, 37.03 g, 267.9 mmol) and methyl iodide (76.61 g, 539.9 mmol) in acetone (225 ml) was stirred under reflux for 6 h. After cooling, the mixture was filtered and the filtrate was concentrated under reduced pressure. The solid residue was recrystallized from hexane to give **17c** (13.38 g, 85% yield) as a colorless crystalline solid. Mp 88–89°C (lit[24] mp 92°C). ^1H NMR (200 MHz, $CDCl_3$): δ 9.85 (1H, s, CHO), 8.03 (2H, s, H_{arom}), 3.97 (3H, s, OCH_3). The spectral properties of this compound were in agreement with those previously reported.[24]

4.4. General procedure for the synthesis of (*Z*)-4-aryl-5-[1-(aryl)methylidene]-3-bromo-2(5*H*)-furanones 3 starting from compounds 6 and arylaldehydes 17

A solution of a compound **6** (3.3 mmol) in CH_2Cl_2 (20 ml) was sequentially treated with *tert*-butyldimethylsilyl trifluoromethanesulfonate (1.04 g, 3.9 mmol), diisopropylethylamine (1.72 ml, 9.9 mmol) and an arylaldehyde **17** (3.9 mmol) and the resulting mixture was stirred for 2 h at room temperature under argon. 1,8-Diazabicyclo[5.4.0]-undec-7-ene (1.0 g, 6.6 mmol) was then added and the mixture, which was periodically monitored by TLC analysis, was stirred at room temperature for 4.5 h. It was then diluted with CH_2Cl_2 (100 ml) and washed with 3 M HCl (2×50 ml). The aqueous phase was extracted with CH_2Cl_2 (3×50 ml) and the collected organic extracts were washed with brine (5×50 ml) until neutrality, dried and concentrated under reduced pressure. The solid residue was purified by recrystallization or by MPLC on silica gel. This procedure was employed to prepare compounds **3a**, **3c**, **3e**, **3f** and **3h**. The results of the preparation of these compounds are summarized in Table 3.

4.4.1. (*Z*)-3-Bromo-4-(3-chloro-4-methoxyphenyl)-5-[1-(3-bromo-4-methoxyphenyl)methylidene]-2-(5*H*)-furanone (3a). The crude reaction product, which was obtained from the reaction of **6b** with 3-bromo-4-methoxybenzaldehyde (**17a**) according to Section 4.4 (entry 1, Table 3), was recrystallized from a mixture of CH_2Cl_2 and hexane to give pure **3a** in 87% yield as a yellow solid. Mp 225–227°C. MS, *m/z* (%): 502 (43) [M^+], 501 (51) [M^+], 500 (89) [M^+], 364 (19), 226 (37), 193 (68), 178 (35), 156 (39), 119 (100). IR (KBr): ν 1778, 1606, 1499, 1291, 1272, 1003, 822, 750, 711 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.93 (1H, d, J=2.1 Hz, H-8), 7.79 (1H, dd, J=8.7, 2.1 Hz, H-12), 7.75 (1H, d, J=2.1 Hz, H-14), 7.42 (1H, dd, J=8.5, 2.1 Hz, H-18), 7.10 (1H, d, J=8.5 Hz, H-17), 6.91 (1H, d, J=8.7 Hz, H-11), 6.01 (1H, s, H-6), 4.01 (3H, s, H-19), 3.94 (3H, s, H-20). ^{13}C NMR (150 MHz, $CDCl_3$): δ 164.96 (C-2), 157.12 (C-10), 156.92 (C-16), 152.21 (C-4), 146.84 (C-5), 135.81 (C-8), 131.66 (C-12), 130.91 (C-14), 129.07 (C-18), 126.79 (C-7), 123.57 (C-13), 121.97 (C-15), 113.00 (C-6), 112.43 (C-9), 112.41 (C-17), 112.17 (C-11), 108.11 (C-3), 56.58 (C-20), 56.57 (C-19). Anal. Calcd for $C_{19}H_{13}Br_2ClO_4$: C, 45.59; H, 2.62. Found: C, 45.27; H, 2.66.

4.4.2. (*Z*)-3-Bromo-4-(3-chloro-4-methoxyphenyl)-5-[1-(4-methoxyphenyl)methylidene]-2(5*H*)-furanone (3c). The crude reaction product, which was obtained by reaction of **6b** with 4-methoxybenzaldehyde (**17b**) according to Section 4.4 (entry 2, Table 3), was purified by recrystallization from a mixture of CH_2Cl_2 and hexane to give **3c** in 95% yield as a yellow solid. Mp 184–186°C. MS, *m/z*(%): 424 (28) $[M^+]$, 422 (100) $[M^+]$, 420 (81) $[M^+]$, 326 (5), 285 (35), 178 (5), 148 (15). IR (KBr): ν 1763, 1600, 1498, 1297, 1273, 1174, 1019, 872 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.75 (2H, m, H-8 and H-12), 7.56 (1H, d, *J*=2.2 Hz, H-14), 7.43 (1H, dd, *J*=8.5, 2.2 Hz, H-18), 7.10 (1H, d, *J*=8.5 Hz, H-17), 6.92 (2H, m, H-9 and H-11), 6.08 (1H, s, H-6), 4.01 (3H, s, H-19), 3.85 (3H, s, H-20). ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.11 (C-2), 160.93 (C-10), 156.63 (C-16), 152.15 (C-4), 145.91 (C-5), 132.77 (C-8 and C-12), 130.78 (C-14), 128.92 (C-18), 125.37 (C-7), 123.29 (C-15 or C-13), 122.07 (C-13 or C-15), 114.59 (C-6), 114.53 (C-9 and C-11), 112.18 (C-17), 107.11 (C-3), 56.37 (C-19), 55.41 (C-20). Anal. Calcd for $C_{19}H_{14}BrClO_4$: C, 54.12; H, 3.35. Found: C, 53.87; H, 3.16. GLC analysis showed that **3c** had chemical purity higher than 97%.

4.4.3. (*Z*)-3-Bromo-4-(4-methylthiophenyl)-5-[1-(3-bromo-4-methoxyphenyl)methylidene]-2(5*H*)-furanone (3e). The crude reaction product, which was obtained by reaction of **6d** with 3-bromo-4-methoxybenzaldehyde (**17a**) according to Section 4.4 (entry 3, Table 3), was recrystallized from a mixture of benzene and hexane to give **3e** in 86% yield as a yellow crystalline solid. Mp 245–246°C. APCI-MS, *m/z*: $[M+H]^+$=481. IR (KBr): ν 1765, 1595, 1487, 1266, 1224, 1093, 1054, 995, 818 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 8.09 (1H, d, *J*=2.0 Hz, H-8), 7.83 (1H, dd, *J*=8.8, 2.0 Hz, H-12), 7.50 (4H, m, H-13, H-14, H-17 and H-18), 7.21 (1H, d, *J*=8.8 Hz, H-11), 6.31 (1H, s, H-6), 3.90 (3H, s, H-20), 2.56 (3H, s, H-19). It should be noted that a complete structural and stereochemical assignment for **3e** by NMR spectroscopy could not be performed owing to the very low solubility of this substance in solvents such as acetone-d^6, $CDCl_3$, C_6D_6, CD_3OD and DMSO-d^6. Anal. Calcd for $C_{19}H_{14}Br_2O_3S$: C, 47.33; H, 2.93. Found: C, 47.78; H, 2.73. TLC analysis showed that **3e** was chemically pure.

4.4.4. (*Z*)-3-Bromo-4-(4-methoxyphenyl)-5-[1-(4-methoxyphenyl)methylidene]-2(5*H*)-furanone (3f). The crude reaction product, which was obtained by reaction of **6e** with **17b** according to Section 4.4 (entry 4, Table 3), was recrystallized from a mixture of CH_2Cl_2 and hexane to give pure **3f** in 99% yield as a yellow amorphous solid. Mp 148–150°C. APCI-MS, *m/z*: $[M+H]^+$=387. IR (KBr): ν 1771, 1604, 1514, 1504, 1258, 1176, 1022, 989, 828 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.74 (2H, m, H-8 and H-12), 7.49 (2H, m, H-14 and H-18), 7.07 (2H, m, H-15 and H-17), 6.91 (2H, m, H-9 and H-11), 6.11 (1H, s, H-6), 3.90 (3H, s, H-19), 3.85 (3H, s, H-20). ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.45 (C-2), 161.26 (C-16), 160.77 (C-10), 153.49 (C-4), 146.24 (C-5), 132.67 (C-8 and C-12), 130.66 (C-14 and C-18), 125.55 (C-7), 121.29 (C-13), 114.51 (C-6), 114.47 (C-15 and C-17), 114.41 (C-9 and C-11), 106.32 (C-3), 55.47 (C-19), 55.39 (C-20). Anal. Calcd for $C_{19}H_{15}BrO_4$: C, 58.93; H, 3.91. Found: C, 58.59; H, 4.18.

4.4.5. (*Z*)-3-Bromo-5-[1-(3,5-dibromo-4-methoxyphenyl)-methylidene]-4-(4-methoxyphenyl)-2(5*H*)-furanone (3h). The crude reaction product, which was obtained by reaction of **6e** with **17c** according to Section 4.4 (entry 5, Table 3), was purified by MPLC on silica gel, using a mixture of $CHCl_3$, hexane and EtOAc (60:40:1) as eluant, to give compound **3e** in 24% yield as a yellow amorphous solid. Mp 175–178°C. APCI-MS, *m/z*: $[M+H]^+$=543. IR (KBr): ν 1780, 1608, 1508, 1471, 1259, 1180, 1026, 982, 831 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 7.91 (2H, m, H-8 and H-12), 7.47 (2H, m, H-14 and H-18), 7.07 (2H, m, H-15 and H-17), 5.97 (1H, s, H-6), 3.91 (3H, s, H-20), 3.90 (3H, s, H-19). ^{13}C NMR (150 MHz, $CDCl_3$): δ 164.58 (C-2), 161.53 (C-16), 154.78 (C-10), 153.17 (C-4), 148.52 (C-5), 134.41 (C-8 and C-12), 131.32 (C-7), 130.59 (C-14 and C-18), 120.61 (C-13), 118.65 (C-9 and C-11), 114.59 (C-15 and C-17), 110.56 (C-6), 108.53 (C-3), 60.80 (C-20), 55.51 (C-19). Anal. Calcd for $C_{19}H_{13}Br_3O_4$: C, 41.87; H, 2.40. Found: C, 41.80; H, 2.25. ^1H NMR analysis showed that compound **3h** had chemical purity higher than 97%.

4.4.6. Demethylation of 3a: synthesis of (*Z*)-3-bromo-4-(3-chloro-4-hydroxyphenyl)-5-[1-(3-bromo-4-methoxyphenyl)methylidene]-2(5*H*)-furanone (3b) and (*Z*)-3-bromo-4-(3-chloro-4-hydroxyphenyl)-5-[1-(3-bromo-4-hydroxyphenyl)methylidene]-2(5*H*)-furanone (1l). To a solution of **3a** (1.00 g, 2.0 mmol) in dry CH_2Cl_2 (40 ml), which was stirred at −78°C, was added a 1 M solution of BBr_3 in CH_2Cl_2 (10 ml, 10.0 mmol) over 15 min. The reaction mixture was allowed to warm up to room temperature and stirred at this temperature for 48 h. Water (20 ml) and $CHCl_3$ (100 ml) were sequentially added and the organic phase was separated. The aqueous phase was extracted with $CHCl_3$ (5×20 ml) and Et_2O (40 ml). The collected organic extracts were washed with brine (2×25 ml), dried and concentrated under reduced pressure. The solid residue was purified by MPLC on silica gel using a mixture of $CHCl_3$ and methanol (96:4) as eluant. Concentration of the first eluted chromatographic fractions allowed to **3b** (0.50 g, 51% yield) as a yellow solid. Mp 243–245°C. ESI-MS, *m/z*: $[M-H]^-$=483. IR (KBr): ν 3321, 1724, 1592, 1493, 1300, 1257, 1057, 1034, 1018 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 10.94 (1H, s, H-19), 8.09 (1H, d, *J*=2.1 Hz, H-8), 7.84 (1H, dd, *J*=8.7, 2.1 Hz, H-12), 7.58 (1H, d, *J*=2.2 Hz, H-14), 7.39 (1H, dd, *J*=8.3, 2.2 Hz, H-18), 7.21 (1H, d, *J*=8.7 Hz, H-11), 7.17 (1H, d, *J*=8.3 Hz, H-17), 6.32 (1H, s, H-6), 3.90 (3H, S, H-20). ^{13}C NMR (150 MHz, DMSO-d_6): δ 165.33 (C-2), 157.06 (C-10), 156.00 (C-16), 153.12 (C-4), 147.12 (C-5), 135.67 (C-8), 132.74 (C-12), 131.45 (C-14), 130.26 (C-18), 127.60 (C-7), 121.04 (C-15), 120.82 (C-13), 117.78 (C-17), 113.84 (C-11), 113.16 (C-6), 111.90 (C-9), 107.85 (C-3), 57.36 (C-20). Anal. Calcd for $C_{18}H_{11}Br_2ClO_4$: C, 44.44; H, 2.28. Found: C, 44.15; H, 1.99.

Concentration of the last eluted chromatographic fractions allowed to obtain compound **1l** (0.45 g, 47% yield) as a yellow solid. Mp 219–223°C. ESI-MS, *m/z*: $[M-H]^-$= 469. IR (KBr): ν 3360, 1746, 1603, 1497, 1410, 1280, 1193, 1005, 753 cm^{-1}. ^1H NMR (600 MHz, CD_3OD): δ 8.04 (1H d, *J*=2.2 Hz, H-8), 7.66 (1H, dd, *J*=8.4, 2.2 Hz, H-12), 7.61 (1H, d, *J*=2.2 Hz, H-14), 7.41 (1H, dd, *J*=8.4, 2.2 Hz, H-18), 7.16 (1H, d, *J*=8.4 Hz, H-17), 6.97 (1H, d, *J*=8.5 Hz, H-11), 6.18 (1H, s, H-6). ^{13}C NMR (150 MHz, CD_3OD): δ 166.80 (C-2), 157.45 (C-10), 157.03 (C-16), 154.45 (C-4), 147.89 (C-5), 136.96 (C-8), 133.15 (C-12), 132.20 (C-14), 130.57 (C-18), 127.45 (C-7), 122.73 (C-15 or C-13), 122.36 (C-13 or C-15), 118.28 (C-17), 117.76

(C-11), 114.78 (C-6), 111.81 (C-9), 107.86 (C-3). Anal. Calcd for $C_{17}H_9Br_2ClO_4$: C, 43.21; H, 1.92. Found: C, 42.97; H, 1.88.

4.4.7. Demethylation of 3c: synthesis of (*Z*)-3-bromo-4-(3-chloro-4-hydroxyphenyl)-5-[1-(4-hydroxyphenyl)-methylidene]-2(5*H*)-furanone (3d). A solution of compound **3c** (0.50 g, 1.2 mmol) in CH_2Cl_2 (15 ml) was reacted with a 1 M CH_2Cl_2 solution of BBr_3 (6.0 ml, 6.0 mmol) at room temperature for 17 h according to the same procedure used to prepare **1l** from **3a**. The crude reaction product, which was obtained after usual work up, was purified by MPLC on silica gel, using a mixture of $CHCl_3$ and methanol (96:4) as eluant, to give **3d** (0.45 g, 99% yield) as a yellow solid. Mp 284–285°C. APCI-MS, *m/z*: $[M+1]^+=393$; $[M-H]^-=391$. IR (KBr): ν 3462, 3235, 1733, 1601, 1277, 1172, 1031, 840 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 10.93 (1H, s, H-19), 10.11 (1H, s, H-20), 7.68 (2H, m, H-8 and H-12), 7.58 (1H, d, J= 2.1 Hz, H-14), 7.38 (1H, dd, J=8.4, 2.1 Hz, H-18), 7.17 (1H, d, J=8.4 Hz, H-17), 6.84 (2H, m, H-9 and H-11), 6.22 (1H, s, H-6). ^{13}C NMR (150 MHz, DMSO-d_6): δ 165.58 (C-2), 160.06 (C-10), 155.91 (C-16), 153.31 (C-4), 145.76 (C-5), 133.76 (C-8 and C-12), 131.42 (C-14), 130.20 (C-18), 124.61 (C-7), 121.10 (C-13), 121.02 (C-15), 117.78 (C-17), 116.91 (C-9 and C-11), 115.48 (C-6), 106.47 (C-3). Anal. Calcd for $C_{17}H_{10}BrClO_4$: C, 51.87; H, 2.56. Found: C, 51.65; H, 2.28.

4.4.8. Demethylation of 3f: synthesis of (*Z*)-3-bromo-4-(4-hydroxyphenyl)-5-[1-(4-hydroxyphenyl)-methylidene]-2(5*H*)-furanone (3g). A solution of compound **3f** (0.50 g, 1.3 mmol) in CH_2Cl_2 (15 ml) was reacted with a 1 M CH_2Cl_2 solution of BBr_3 (6.5 ml, 6.5 mmol) at room temperature for 15 h according to the same procedure used to prepare **1l** from **3a**. The crude reaction product, which was obtained after usual work up, was purified by MPLC on silica gel, using a mixture of $CHCl_3$ and methanol (96:4) as eluant, to give **3g** (0.45 g, 99% yield) as a yellow solid. Mp 264–266°C. APCI-MS, *m/z*: $[M+1]^+=359$; $[M-H]^-=357$. IR (KBr): ν 3291, 1737, 1715, 1606, 1506, 1265, 1013, 841 cm^{-1}. ^1H NMR (600 MHz, CD_3OD): δ 7.72 (2H, m, H-8 and H-12), 7.49 (2H, m, H-14 and H-18), 7.03 (2H, m, H-15 and H-17), 6.88 (2H, m, H-9 and H-11), 6.24 (1H, s, H-6). ^{13}C NMR (150 MHz, CD_3OD): δ 167.44 (C-2), 161.32 (C-16), 160.89 (C-10), 156.05 (C-4), 147.38 (C-5), 134.31 (C-8 and C-12), 132.27 (C-14 and C-18), 126.08 (C-7), 121.51 (C-13), 117.23 (C-9 and C-11), 117.08 (C-15 and C-17), 116.68 (C-6), 106.05 (C-3). Anal. Calcd for $C_{17}H_{11}BrO_4$: C, 56.85; H, 3.09. Found: C, 56.69; H, 2.97.

4.5. Cytoxicity assays

The compounds were evaluated at the National Cancer Institute (Bethesda, Maryland). in the NCI 3-cell line, one dose primary anticancer assay. The 3-cell line panel consisted of MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS). The following protocol was used. Each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a 1.00×10^{-4} M concentration in DMSO and the culture was incubated for 48 h. End-point determinations were made with sulforhodamine B, a protein-binding dye. Results of each test agent were reported as the percent of growth of the treated cells when compared to the untreated control cells. Table 4 summarizes the results of the assays performed. Compounds **1l** and **3d** were also evaluated against the NCI 60 cell line panel[25] at five concentrations at 10-fold dilutions. A 48 h continuous drug exposure protocol was used, and a sulforhodamine B protein assay was used to evaluate cell viability or growth.

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