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Synthesis of 3-arylisocoumarins, including thunberginols A and B, unsymmetrical 3,4-disubstituted isocoumarins, and 3-ylidenephthalides via iodolactonization of methyl 2-ynylbenzoates or the corresponding carboxylic acids

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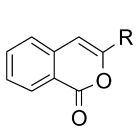
Abstract—3-Aryl-4-iodoisocoumarins, which were readily and efficiently prepared by regioselective iodolactonization of methyl 2-ynylbenzoates or the corresponding carboxylic acids, were used as precursors either to 3-arylisocoumarins, including naturally-occurring thunberginols A and B, or to unsymmetrical 3,4-disubstituted isocoumarins. On the other hand, (*Z*)- and (*E*)-3-iodomethylidenephthalides, which were regioselectively prepared by iodolactonization of methyl 2-ethynylbenzoate, were employed as starting materials for the stereospecific synthesis of (*Z*)- and (*E*)-3-ylidenephthalides, respectively. Some 3-arylisocoumarins and unsymmetrical 3,4-disubstituted isocoumarins showed certain cytotoxic activity against human cancer cell lines in vitro.

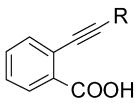
1. Introduction

Isocoumarin structures are important components in many natural products that exhibit a broad range of biological activitities including antiallergic and antimicrobial,[1,2] immunomodulatory,[3] cytotoxic,[4] antifungal,[5] antiinflammatory,[6] antiangiogenic,[7] anticalmodulin-sensitive cyclic guanosine 3′,5′-monophosphate phosphodiesterease[8] effects, and differentiation inducing activity against leukemic cells.[9] Isocoumarins[10] are also useful intermediates in the synthesis of a variety of important compounds including some isoquinoline alkaloids.[11] Thus, a number of methods have been reported in the literature for the synthesis of isocoumarins.[12] For example, in the last 10 years 3-substituted isocoumarins with no substituent at the 4-position have been synthesized either by a variety of traditional approaches[13] or by utilizing transition metal-catalyzed reactions.[14] The synthesis of 3,4-disubstituted isocoumarins has received considerable attention too[6,15] and, recently, a number of these compounds have been prepared in good yields via palladium-catalyzed annulation of internal alkynes.[15d] However, this last catalytic method is somewhat limited in synthetic scope since it is highly regioselective only for symmetrical disubstituted acetylenes containing a highly sterically demanding group in the position adjacent to the carbon–carbon triple bond.

During the course of our earlier studies on the synthesis of biologically active naturally-occurring heterocycles and their analogues,[16] we investigated the synthesis of 3-alkyl- and 3-(1-alkenyl)isocoumarins **1** and we found that these substances, including naturally-occurring 3-propylisocoumarin (**1a**) and artemidin (**1b**), can be selectively prepared by $AgNO_3$-catalyzed cyclization of 2-(1-ynyl)-benzoic acids **2**.[14e]



1a : R = *n*-C_3H_7
1b : R = (*E*)-C_2H_5-CH=CH



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More recently, we decided to investigate new general procedures for the efficient and selective synthesis of 3-arylisocoumarins **3**, including thunberginols A (**3a**)[17] and B (**3b**)[18] and derivatives of these natural products, and of unsymmetrical 3,4-disubstituted isocoumarins **4**, and to perform a preliminary evaluation of the cytotoxic activity of these substances against human cancer cell lines.

We were led to prepare compounds **3a** and **3b** by the impracticality of their previously developed syntheses[17,18] and by the interesting biological activities of these isocoumarins. In fact, compounds **3a** and **3b**, which have been isolated from Hydrangea Dulcis Folium, the fermented and dried leaves of *Hydrangea macrophylla* SERINGE var.

Keywords: isocoumarins; phthalides; iodine; palladium catalysis; cytotoxicity; thunberginols.

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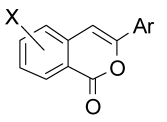

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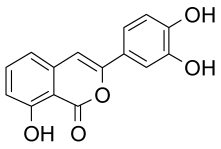
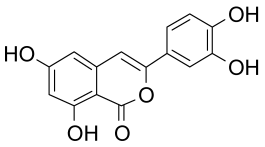
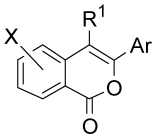


3a



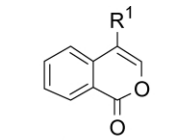
3b



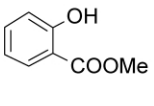
4 : R^1 = Me, aryl, alkenyl

thunbergii MAKINO, possess a variety of biological properties,[2,3] including activity against mouse myeloid leukemia cells[9] and, thus, are of potential interest for treatment of cancers. As shown in Scheme 1, we planned to synthesize compounds **3** and **4** from 3-aryl-4-iodoisocoumarins **5** by highly chemoselective palladium-catalyzed hydrodehalogenation of these halides[19] and by Stille-type reactions,[20] respectively. In fact, we recently found that a variety of iodides **5** can be selectively prepared in moderate to excellent yields by iodolactonization of 2-(arylethynyl)-benzoic acids **7** or the corresponding methyl esters **6**.[21]

The results obtained in the study of the synthesis of compounds **3**, including thunberginols A (**3a**) and B (**3b**) and their precursors, and of unsymmetrical 3,4-disubstituted isocoumarins **4** form the subject of this paper.[22] Herein, we will also report that in an attempt to synthesize 4-substituted isocoumarins **9** with no substituent at the 3-position[23] starting from methyl salicylate (**10**) by a methodology similar to that used to prepare compounds **4**, i.e. by conversion of **10** into methyl 2-ethynylbenzoate (**11**) and regioselective iodolactonization of this compound followed by Stille-type reactions, we developed a convenient new procedure for the preparation of 3-ylidenephthalides (**12**). Finally, we will describe the results of tests to evaluate the cytotoxic activity exhibited by several compounds **3** and **4** against human cancer cell lines.



9 : R^1 = Me, aryl, I



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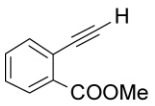

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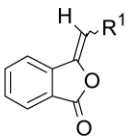
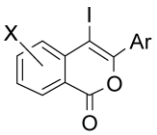


12 : R^1 = Me, aryl, I

2. Results and discussion

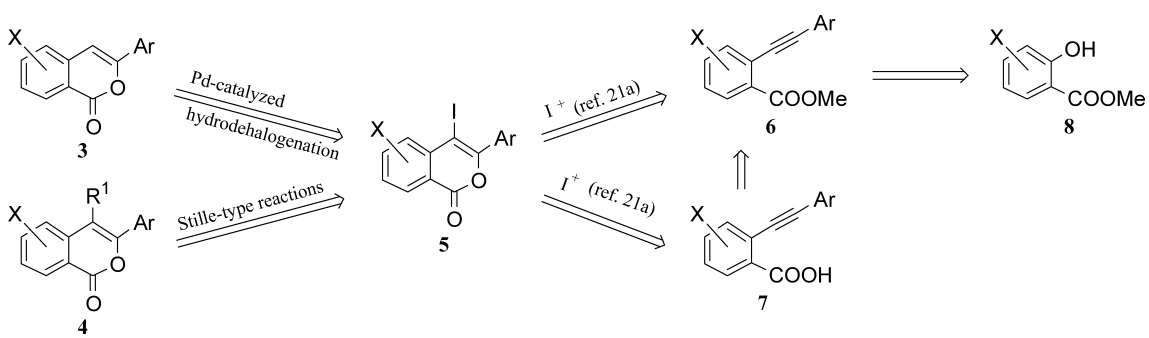
2.1. Synthesis of starting materials

The 3-aryl-4-iodoisocoumarins used in this study were compounds **5a**–**e**.

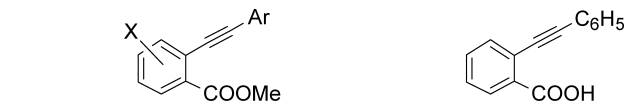


5a : X = 8-MeO; Ar = 3,4-$(MeO)_2C_6H_3$
5b : X = 8-MeO; Ar = C_6H_5
5c : X = 6-MeO; Ar = 4-$MeOC_6H_4$
5d : X = 6,8-$(MeO)_2$; Ar = 3,4-$(MeO)_2C_6H_3$
5e : X = H; Ar = C_6H_5

Recently, we synthesized four of these iodo derivatives, i.e. compounds **5a**–**5c**, and **5e**, by iodolactonization of the corresponding methyl 2-(arylethynyl)benzoates **6** or 2-(arylethynyl)benzoic acids **7**.[21a] In particular, compounds **5a** and **5c** were selectively prepared in 89 and 98% yield, respectively, by reaction of methyl 2-(3,4-dimethoxyphenyl)ethynyl-6-methoxybenzoate (**6a**) and methyl 4-methoxy-2-(4-methoxyphenyl)ethynylbenzoate (**6c**) with 3.0 equiv. of iodine in MeCN at room temperature for 3 h.[21a] Compound **5b** was prepared in 47% yield by treatment of methyl 6-methoxy-2-phenylethynylbenzoate (**6b**) with 1.0 equiv. of ICl in CH_2Cl_2 at room temperature for 3.5 h and iodide **5e** was selectively obtained in 88% yield by reaction of 2-(phenylethynyl)benzoic acid (**7a**) with 3.0 equiv. of iodine and 3.0 equiv. of $NaHCO_3$ in MeCN at room temperature for 1.5 h.[21a,24] The substrates of these iodolactonization reactions, i.e. esters **6a**–**6c** and the carboxylic acid **7a**, were prepared in 80–86% overall yield starting from methyl 2-hydroxybenzoates **8a**, **8b** and **10** via palladium-catalyzed reaction between 2-(arylethynyl)zinc chlorides and nonaflates (perfluorobutane sulfonates) **13** derived from these *o*-hydroxyesters.[21a]

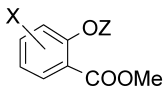


Scheme 1. Retrosynthesis of compounds **3** and **4**.



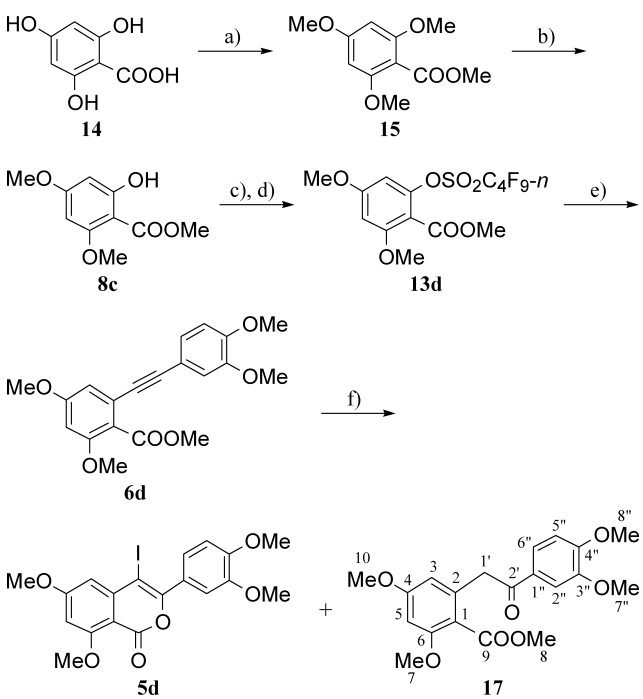
6a : X = 6-MeO; Ar = 3,4-$(MeO)_2C_6H_3$
6b : X = 6-MeO; Ar = C_6H_5
6c : X = 4-MeO; Ar = 4-$MeOC_6H_4$

7a



8a : X = 6-MeO; Z = H
8b : X = 4-MeO; Z = H
10 : X = H; Z = H
13a : X = 6-MeO; Z = *n*-$C_4F_9SO_2$
13b : X = 4-MeO; Z = *n*-$C_4F_9SO_2$
13c : X = H; Z = *n*-$C_4F_9SO_2$

On the other hand, the previously unknown 4-aryl-3-iodoisocoumarin **5d** was selectively prepared in five steps and in 35% overall yield starting from commercially available 2,4,6-trihydroxybenzoic acid monohydrate (**14**) by the reaction sequence illustrated in Scheme 2, which was similar to that previously employed in our laboratory to synthesize **5a** and **5c**.[21a] Thus, methyl ester **15**, which was prepared in 82% yield from **14**, was converted in 80% yield into the *o*-hydroxyester **8c** by treatment with 1.1 equiv. of BCl_3 in CH_2Cl_2. Reaction of **8c** with NaH in DMF followed by treatment with perfluoro-1-butanesulfonyl fluoride provided **13d** in 94% yield. This nonaflate was then reacted



Scheme 2. (a) K_2CO_3 (6.0 equiv.), Me_2SO_4 (6.0 equiv.), acetone, 26 h, 42–45°C, 82% yield. (b) BCl_3 (1.1 equiv.), CH_2Cl_2, −70 to +20°C, 40 min, 80% yield. (c) NaH (1.5 equiv.), DMF, 0.5 h at 0°C, then 1 h at rt. (d) *n*-$C_4F_9SO_2F$ (1.15 equiv.), 2 h, rt, 94% yield based on **8c**. (e) 3,4-$(MeO)_2C_6H_3C{\equiv}CZnCl$ (**16**) (1.20 equiv.), $Pd_2(dba)_3$ (1 mol%), dppf (2 mol%), THF, 60°C, 17 h, 85% yield. (f) I_2 (3.0 equiv.), MeCN, rt, 2 h then aq. $Na_2S_2O_3$, 67% yield for **5d** and 9% yield for **17**.

with a molar excess of 3,4-dimethoxyphenylethynylzinc chloride (**16**) in THF at 60°C in the presence of 1 mol% $Pd_2(dba)_3$ and 2 mol% 1,1′-bis(diphenylphosphino)ferrocene (dppf) to give compound **6d** in 85% yield. Finally, reaction of this ester with 3.0 equiv. of iodine in MeCN at room temperature followed by treatment with an aqueous $Na_2S_2O_3$ solution and purification of the resulting reaction mixture by MPLC on silica gel provided regioselectively iodide **5d** in 67% yield. Concentration of the first eluted fractions of this chromatographic purification also allowed isolation of methyl 4,6-dimethoxy-2-(3,4-dimethoxybenzoyl)methylbenzoate (**17**) in ca. 9% yield. The structure of this unexpected compound was established on the basis of its IR, ^1H and ^{13}C NMR spectra and by a combination of NMR techniques which included ^1H–^1H COSY, heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC).

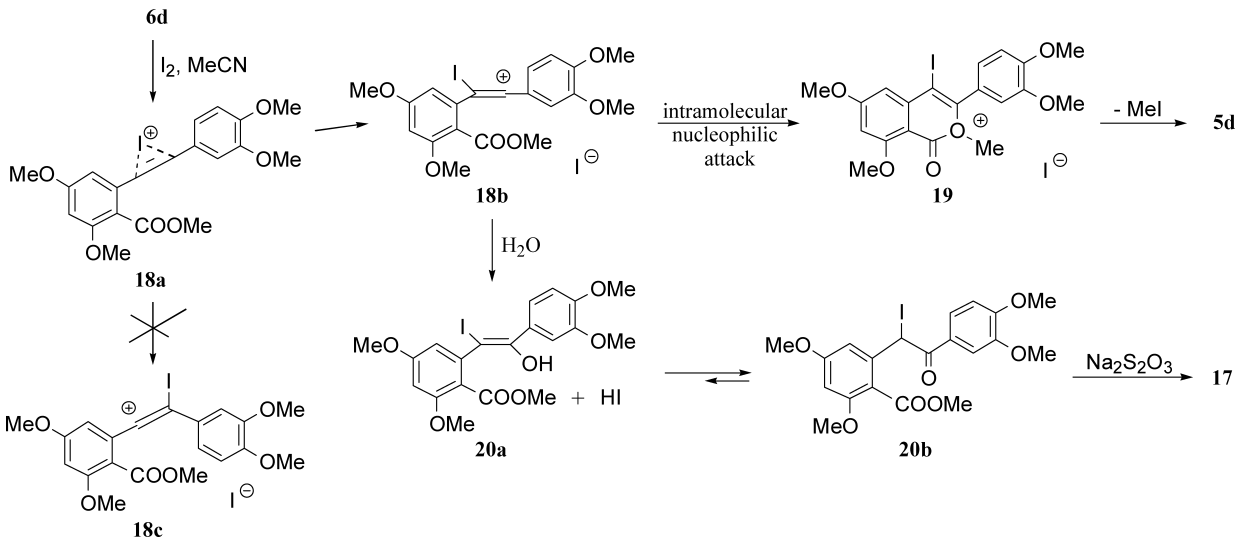
Possible processes for the formation of compounds **5d** and **17** are shown in Scheme 3. The desired reaction to form **5d** should involve the electrophilic attack of iodine on the carbon–carbon triple bond of **6d** to form regioselectively vinyl cation **18b** in which the positive charge is stabilized by the electron-rich 3,4-dimethoxyphenyl group. Subsequent intramolecular nucleophilic attack of the methoxycarbonyl group followed by elimination of methyl iodide from methyloxonium iodide **19** so obtained should then lead regioselectively to **5d**. On the other hand, nucleophilic attack of water on **18** during the workup of the reaction mixture should provide α-iodoketone **20b** and hydrogen iodide. Protodeiodination of **20b** in the presence of $Na_2S_2O_3$ as a scavenger of iodine should then produce ketone **17**.

2.2. Synthesis of 3-arylisocoumarins from 3-aryl-4-iodoisocoumarins

With 3-aryl-4-iodoisocoumarins **5** in hand we then turned our attention to the conversion of these iodo derivatives into 3-arylisocoumarins **3** and we found that the palladium-catalyzed triethylammonium formate reduction[19] of iodides **5a**–**5d** provided selectively compounds **3c**, **3d**, **3f**, and **3j** in 93, 90, 93, and 98% yield, respectively (Scheme 4). Compound **3c** was then demethylated by treatment with 5.2 equiv. of BBr_3 in CH_2Cl_2 at room temperature followed by hydrolysis to give thunberginol A (**3a**) in 85% yield[17] (Scheme 4). The spectral data of this compound were in agreement with those of the natural product isolated from the fermented and dried leaves of *H. macrophylla*.[2]

On the other hand, reaction of **3d** and **3j** with 4.5 and 10.0 equiv. of BBr_3, respectively, in CH_2Cl_2 at room temperature followed by hydrolysis furnished compound **3e** and thunberginol B (**3b**) in 98 and 81% yield, respectively (Scheme 4). The spectral properties of **3b** proved to be in agreement with those previously reported.[2,25]

We next attempted to perform the complete O-demethylation of compound **3f** by treatment with 5.2 equiv. of BBr_3 in CH_2Cl_2 at room temperature for 1.42 h followed by hydrolysis. However, we found that this reaction provided selectively the monohydroxy derivative **3h** in 72% yield. The structure of this compound was unambiguously established on the basis of its ^1H and ^{13}C NMR spectra at



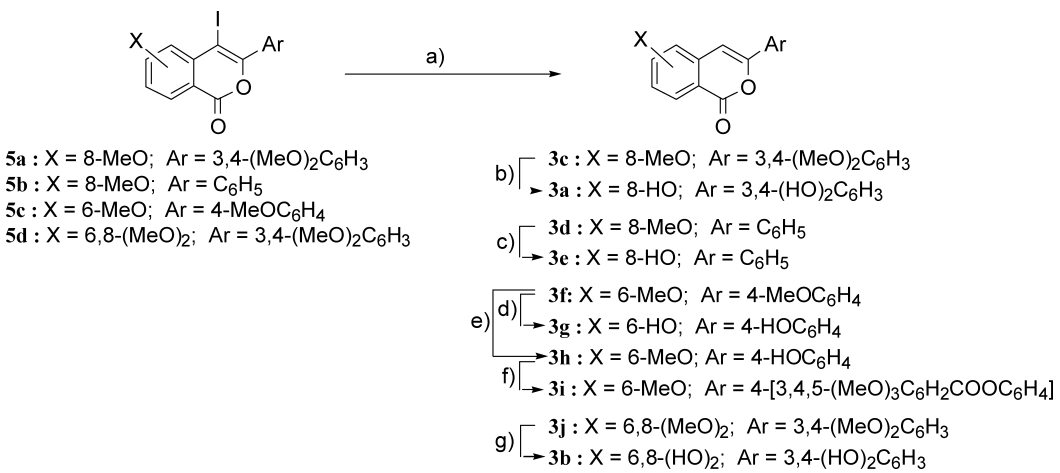
Scheme 3. Proposed processes for the reaction between **6d** and iodine in MeCN followed by treatment with aqueous $Na_2S_2O_3$.

600 and 150 MHz, respectively, and by a combination of NMR techniques which included $^1H-^1H$ COSY, nuclear Overhauser enhancement spectroscopy (NOESY), HMQC, and HMBC. We then prepared a derivative of **3h**, i.e. compound **3i**, in 74% yield by reaction with 3,4,5-trimethoxybenzoyl chloride (**21**) in the presence of pyridine (Scheme 4). Finally, we succeeded to obtain the dihydroxy derivative **3g** in 87% yield by reaction of **3f** with 8.3 equiv. of BBr_3 in CH_2Cl_2 at 40°C for 27.5 h followed by hydrolysis (Scheme 4).

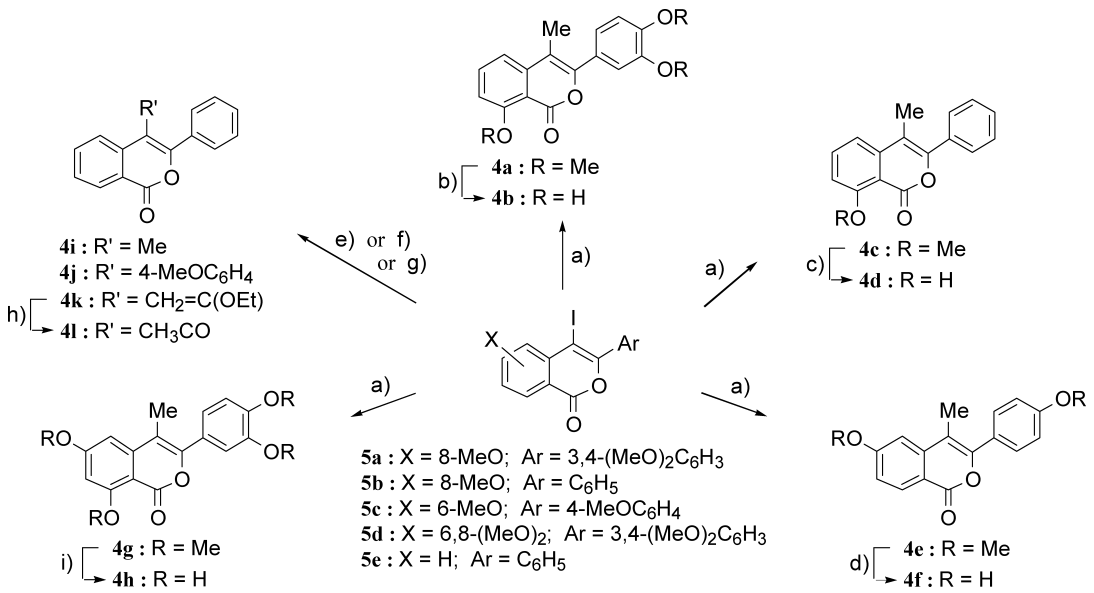
2.3. Palladium-catalyzed reactions between 3-aryl-4-iodoisocoumarins and organotin derivatives

We next examined the use of 4-aryl-3-iodoisocoumarins **5a–e** as starting materials for the synthesis of unsymmetrical 3,4-disubstituted isocoumarins of general formula **4** and, first of all, we investigated the preparation of 3-aryl-4-methylisocoumarins **4a**, **4c**, **4e**, **4g**, and **4i** by Stille-type reactions of these iodo derivatives with tetramethyltin (**22**) (Scheme 5).

Specifically, compounds **4a**, **4c**, **4e**, and **4g** were synthesized in 86, 78, 72 and 89% yield, respectively, by treatment of **5a–5d** with 3.0 equiv. of **22** in 1-methyl-2-pyrrolidinone (NMP) at 76–82°C for 4.5–10 h in the presence of a catalyst system consisting of 5 mol% $PdCl_2[P(o\text{-Tolyl})_3]_2$ and 10 mol% CuI.[26,27] On the other hand, **4i** was prepared in 66% yield by reaction of **5e** with 3.0 equiv. of **22** in NMP at 60°C in the presence of 5 mol% $PdCl_2(PhCN)_2$, 10 mol% $AsPh_3$ and 10 mol% CuI.[27] Compounds **4a**, **4c**, **4e** and **4g** were then O-demethylated by treatment with BBr_3 in CH_2Cl_2 at 20–32°C to yield compounds **4b**, **4d**, **4f** and **4h** in 89, 96, 89 and 58% yield, respectively (Scheme 5). It should be noted that we successfully used the catalyst system consisting of 5 mol% $PdCl_2(PhCN)_2$, 10 mol% $AsPh_3$ and 10 mol% CuI also for the Stille-type reaction of iodide **5e** with an aryltributyltin derivative and with tributyl-(1-ethoxyethenyl)tin (**24**). In fact, treatment of **5e** with 1.2 equiv. of tributyl(4-methoxyphenyl)tin (**23**) in NMP at 60°C for 54 h in the presence of this catalyst system gave compound **4j** in 58% yield. On the other hand, reaction of **5e** with 1.35 equiv. of **24** in NMP at 81–83°C for 47 h in the



Scheme 4. (a) $Pd(OAc)_2$ (2 mol%), PPh_3 (4 mol%), Et_3N (3.0 equiv.), HCOOH (2.0 equiv.), DMF, 60–63°C, 3.5–4.5 h, 93% yield for **3c**, 90% yield for **3d**, 93% yield for **3f**, 98% yield for **3j**. (b) BBr_3 (5.2 equiv.), CH_2Cl_2, rt, 2 h, 85% yield. (c) BBr_3 (4.5 equiv.), CH_2Cl_2, rt, 1.75 h, 98% yield. (d) BBr_3 (8.3 equiv.), CH_2Cl_2, 40°C, 27 h, 87% yield. (e) BBr_3 (5.2 equiv.), CH_2Cl_2, rt, 1.42 h, 72% yield. (f) 3,4,5-$(MeO)_3C_6H_2COCl$ (**21**) (1.5 equiv.), pyridine, 48°C, 48 h, 74% yield. (g) BBr_3 (10.0 equiv.), CH_2Cl_2, rt, 72 h, 81% yield.



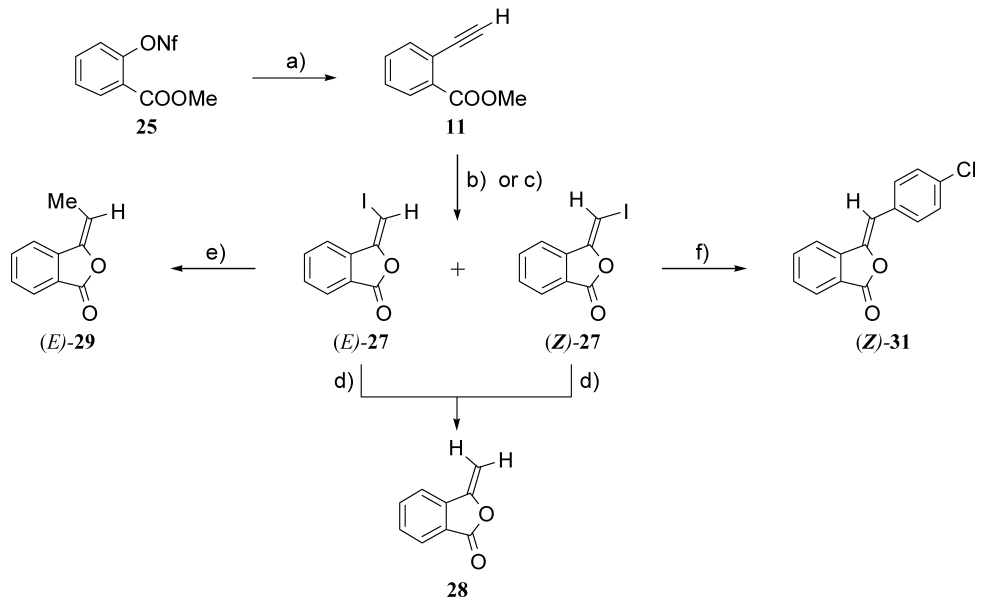
Scheme 5. (a) Me_4Sn (**22**) (3.0 equiv.), $PdCl_2[P(o\text{-Tolyl})_3]_2$ (5 mol%), CuI (10 mol%), NMP, 76–82°C, 4.5–10 h, 86% yield for **5a**, 78% yield for **5b**, 72% yield for **5c**, 89% yield for **5d**. (b) BBr_3 (5.2 equiv.), CH_2Cl_2, rt, 2 h, 89% yield. (c) BBr_3 (4.5 equiv.), CH_2Cl_2, rt, 2.5 h, 96% yield. (d) BBr_3 (8.3 equiv.), CH_2Cl_2, 30–32°C, 48 h, 89% yield. (e) Me_4Sn (**22**) (3.0 equiv.), $PdCl_2(PhCN)_2$ (5 mol%), $AsPh_3$ (10 mol%), CuI (10 mol%), NMP, 60°C, 117 h, 66% yield. (f) 4-$MeOC_6H_4SnBu_3$ (**23**), (1.2 equiv.), $PdCl_2(PhCN)_2$ (5 mol%), $AsPh_3$ (10 mol%), CuI (10 mol%), NMP, 60°C, 54 h, 58% yield. (g) $CH_2{=}C(OEt)SnBu_3$ (**24**), (1.35 equiv.), $PdCl_2(PhCN)_2$ (5 mol%), $AsPh_3$ (10 mol%), CuI (10 mol%), NMP, 81–83°C, 47 h, 70% yield. (h) 2N HCl, THF, rt, 2.25 h, 93% yield. (i) BBr_3 (10.0 equiv.), CH_2Cl_2, rt, 96 h, 58% yield.

presence of this same catalyst system furnished compound **4k** in 70% yield. Acidic hydrolysis of this vinyl ether allowed then compound **4l** to be obtained in 93% yield (Scheme 5).

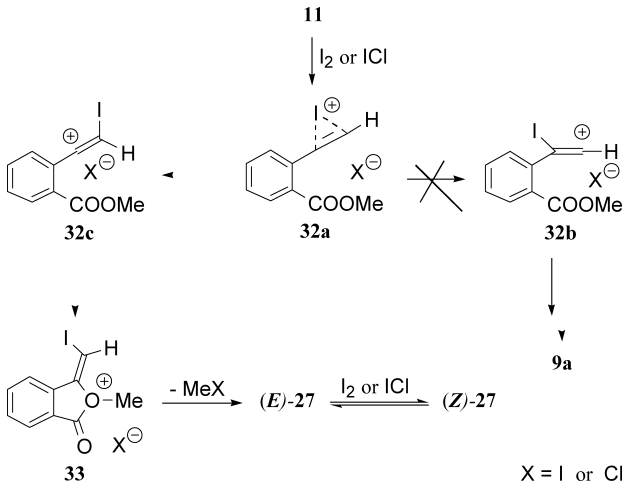
2.4. Synthesis of 3-ylidenephthalides from methyl salicylate

Only a few methods have been reported in the literature for the synthesis of 4-substituted isocoumarins with no substituent at the 3-position.[23] Therefore, we deemed it interesting to establish if these compounds might be prepared starting from methyl salicylate (**10**) by a reaction sequence similar to that used for the preparation of 3,4-disubstituted isocoumarins **4**. To this end, nonaflate **25**, which derived from **10**, was reacted with ethynylzinc chloride (**26**) in THF at 62–66°C in the presence of 1 mol% $Pd_2(dba)_3$ and 2 mol% dppf to give compound **11** in 60% yield (Scheme 6).

However, iodolactonization of **11** by a procedure very



Scheme 6. (a) HC≡C–ZnCl (**26**), THF, 62–66°C, 13 h, $Pd_2(dba)_3$ (1 mol%), dppf (2 mol%), 60% yield. (b) I_2 (3 equiv.), MeCN, 3 h, then MPLC on silica gel, 38% yield for (*E*)-**27** and 56% yield for (*Z*)-**27**. (c) ICl, CH_2Cl_2, rt, 6 h (*E*)- and (*Z*)-**27** in a 70:30 molar ratio). (d) $Pd(OAc)_2$ (2 mol%), PPh_3 (4 mol%), Et_3N (3.0 equiv.), HCOOH (2.0 equiv.), DMF, 60–63°C, 30 h, 45% yield from (*E*)-**27** and 51% yield from (*Z*)-**27**. (e) (for (*E*)-**27**): Me_4Sn (**22**) (3.0 equiv.), $PdCl_2[P(o\text{-Tolyl})_3]_2$ (5 mol%), CuI (10 mol%), NMP, 80°C, 5 h, 80% yield. (f) (for (*Z*)-27): 4-Cl-$C_6H_4SnBu_3$ (**30**), (1.35 equiv.), $PdCl_2(PhCN)_2$ (5 mol%), $AsPh_3$ (10 mol%), CuI (10 mol%), NMP, 80°C, 24 h, 59% yield.



Scheme 7. Proposed mechanism for the regioselective iodolactonization reaction of **11**.

similar to that employed for the synthesis of **5a–d**, i.e. by treatment with 3.0 equiv. of iodine in MeCN at room temperature, furnished a mixture of (*E*)- and (*Z*)-3-iodomethylenephthalide, (*E*)- and (*Z*)-**27**, in a ca. 37:63 ratio, respectively, as the only reaction product. These iodides were separated chromatographically to give 97% pure (*E*)-**27** and stereoisomerically pure (*Z*)-**27** in 38 and 56% yield, respectively. We then attempted to modify the regio- and/or stereoselectivity of the iodolactonization reaction of **11** and we found that treatment of this compound with 1.0 equiv. of ICl in CH_2Cl_2 at room temperature furnished a mixture of (*E*)- and (*Z*)-**27**, in a 70:30 ratio, respectively. Moreover, we observed that the reaction between a solution of ICl in CH_2Cl_2 and a molar excess of **11** provided a mixture of (*E*)- and (*Z*)-**27**, in a 97:3 ratio, respectively. The structural assignment of these iodides was performed taking into account that the palladium-catalyzed triethylammonium formate reduction of each of these compounds provided 3-methylenephthalide (**28**) (Scheme 6). Moreover, the presence of absorption at 1776–1780 cm^{-1} in the IR spectra of these iodides indicated a γ-lactone ring. On the other hand, the stereochemistry of (*E*)-**27** was assigned on the basis of the known stereochemistry of (*E*)-**29**,[28,29] which we prepared in 80% yield by reaction of (*E*)-**27** with a molar excess of tetramethyltin (**22**) in NMP at 80°C in the presence of catalytic amounts of $PdCl_2[P(o\text{-Tolyl})_3]_2$ and CuI. Furthermore, we found that reaction of stereoisomerically pure (*Z*)-**27** with tributyl(4-chlorophenyl)tin (**30**) in NMP at 80°C in the presence of 5 mol% $PdCl_2(PhCN)_2$, 10 mol% $AsPh_3$ and 10 mol% CuI provided the known compound (*Z*)-**31**[30] in 59% yield (Scheme 6).[31]

Finally, it is worth mentioning that no further attempts were performed to synthesize 4-substituted isocoumarins of general formula **9** via iodolactonization of **11**. In fact, the results obtained for the reaction of **11** with iodine in MeCN or with ICl in CH_2Cl_2, indicated that this electrophilic ring closure does not involve intermediate **32b**, which might be the precursor to 4-iodoisocoumarin (**9a**) (Scheme 7). We rationalized these results assuming that the cyclization process selectively proceeds via the benzyl cationic intermediate **32c** in which the positive charge is better stabilized by the aromatic ring, although this ring bears an electron-withdrawing group in the *ortho*-position. Subsequent *endo*-5-*trig* cyclization should then produce iodide (*E*)-**27**, which easily undergoes partial stereomutation in the presence of iodine or ICl (Scheme 7).

Table 1. Primary anticancer assay of 3-arylisocoumarins and unsymmetrical 3,4-disubstituted isocoumarins

Compound	Percentage of growth inhibition		
	NCI-H460 (lung)	MCF-7 (breast)	SF-268 (CNS)
3a	4	9	51
3b	30	4	54
3c	35	84	92
3d	115	109	118
3e	45	71	89
3f	112	107	120
3g	21	45	76
3h	114	106	130
3i	76	90	81
3j	85	104	112
4a	107	107	115
4b	0	9	21
4c	57	75	73
4d	77	105	79
4e	78	100	104
4f	23	6	31
4g	96	100	106
4h	29	9	50
4i	−48	−39	−57
4k	78	59	84
4l	88	72	80

2.5. Biological results

The cytotoxic activity of compounds **3a–j** and **4a–i**,**k**,**l** was evaluated in vitro against the National Cancer Institute (NCI) 3-cell lines panel consisting of MCF-7 (breast), SF-268 (CNS), and NCI-H460 (lung). In this protocol, each cell line was inoculated and preincubated on a microtiter plate. Test agents were then added at a single concentration (1.00×10^{-4} M) and the culture incubated for 48 h. End-point determinations were made with sulforhodamine B, a protein-binding dye. Results for each test (Table 1) are reported as the percent of growth of the treated cells when compared to the untreated control cells.

Compounds which reduced the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) were considered to be active and some of them were passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range. Results from Table 1 indicate that compounds **3a**, **3b**, **3g**, **4b**, **4f**, **4h**, and **4i** passed the 3-cell lines primary screening. The structural features of all these bioactive compounds save **4i** include the presence of a hydroxy group at the 6-position and/or a 3,4-dihydroxyphenyl or a 4-hydroxyphenyl group at the 3-position of the isocoumarin ring. However, **4i**, which was shown to be highly active in this primary screening, does not contain hydroxy groups. Analysis of the data reported in Table 1 seems also to link the cytotoxicity of the tested compounds to the presence of a methyl group at the 4-position of their isocoumarin ring. In fact, compounds **4b**, **4f** and **4h**, which are characterized by the presence of a methyl group at their 4-position, exhibited higher cytotoxicity than the

Table 2. Cytotoxicity of compounds **3a**, **3g**, **4b** and **4i**

Compound	log molar drug concentration required for 50% growth inhibition (log GI_{50})									
	Leukemia MOLT-4	Lung HOP-92	Colon HCT-15	CNS SF-268	Melanoma SK-MEL-5	Ovarian IGROV1	Renal UO31	Prostate PC-3	Breast T-470	MGM[a]
3a	−5.82	−6.52	−5.28	−4.84	−5.17	−5.77	−4.93	−4.84	−5.80	−4.88
3g	−4.73	−4.66	−4.67	−4.58	−4.95	−4.70	−4.88	−4.60	−4.70	−4.58
4b	−5.52	−4.97	−5.40	−5.04	−4.58	−5.24	−4.95	−4.89	−5.81	−5.05
4i	−4.96	−4.66	−4.70	−4.76	−4.73	−4.69	−4.72	−4.61	−4.51	−4.70

[a] Mean graph midpoint for all human cancer cell lines tested.

corresponding substances, i.e. **3a**, **3b** and **3g**, respectively, which do not contain this methyl group.

Compounds **3a**, **3g**, **4b**, and **4i** were then tested in US NCI's human tumor cell line screen.[32] This assay involves determination of the effect of a test agent on growth parameters against a panel of approximately 60 human tumor cell lines, mostly derived from solid tumors. For each compound, dose–response curves for each cell line were measured with five different drug concentrations. The log GI_{50} values (GI_{50} being the molar drug concentration required for half growth inhibition) obtained with selected cell lines, along with the mean graph mid-point (MGM) values, are summarized in Table 2. The MGM is based on a calculation of the average log GI_{50} for all of the cell lines tested in which GI_{50} values below and above the test range (10^{-4}–10^{-8} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentration used in the screening test. The data summarized in Table 2 indicate an indisputable cytotoxicyty of compound **4b**, which showed a MGM log GI_{50} below −5. Moreover, the complete set of data obtained in this screening panel qualitatively shows that either the leukemia cell lines or the breast cell line were relatively more sensitive to compound **4b** than were other cell lines.

3. Conclusions

In summary, we have explored a useful and versatile approach to 3-aryliscoumarins **3**, including two natural products, and unsymmetrical 3,4-disubstituted isocoumarins **4** characterized by an aryl group at the 3-position. A key step of this approach involves the regioselective iodolactonization of methyl 2-(arylethynyl)benzoates **6** or the corresponding carboxylic acids **7**. The examples we presented demonstrate that the strategy is general and should be applicable to the regioselective synthesis of a large variety of isocoumarin derivatives save 4-substituted isocoumarins **9** with no substituent at the 3-position. In fact, we have shown that an attempt to prepare compounds **9** from methyl 2-ethynylbenzoate (**11**) according to the same strategy used for the synthesis of compounds **3** and **4** provided 3-ylidenephthalides **12** with complete regioselectivity. Finally, it should be noted that seven isocoumarin derivatives so prepared, including naturally-occurring thunberginols A (**3a**) and B (**3b**), were found to be among those significantly active in the NCI 3-cell line, one dose primary anticancer assay. Four compounds which passed the 3-cell lines primary screening were then tested in US NCI's human tumor cell line screen. However, among these substances only **4b** exhibited an indisputable cytotoxicity.

4. Experimental

4.1. General

Melting points are uncorrected. Merck precoated 60 F_{254} aluminum silica gel sheets 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 DDS 1000 data station. 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. Electron impact mass spectra were measured at 70 eV by GLC/MS. GLC/MS analyses were performed using a Q-mass 910 spectrometer interfaced with a Perkin–Elmer 8500 gas-chromatograph. The MS spectrum of compounds **3b** and **4f** was recorded on a Perkin–Elmer SCIEX API III triple quadrupole mass spectrometer using the atmospheric pressure photoionization (APPI) technique in a tandem mass spectrometry manner. NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer and a Varian Gemini 300 MHz spectrometer with TMS as the internal reference or with a Bruker AMX 600 spectrometer with solvent ($CDCl_3$ or DMSO-d_6) as the internal reference, respectively. The structures of compounds **3h**, **4i**, **4j** and **17** were assigned on the basis of their ^1H NMR and ^{13}C NMR spectra 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. IR spectra were recorded on a Perkin–Elmer 1725-X FT-IR spectrophotometer. All reactions of air and water sensitive materials were performed in flame dried glassware under a positive 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: $PdCl_2(PPh_3)_2$,[33] $Pd(PPh_3)_4$,[34] tributyl(4-methoxyphenyl)tin (**23**),[35] tributyl(4-chlorophenyl)tin (**30**),[27e] (3,4-dimethoxyphenyl)ethynylzinc chloride (**16**).[21a]

4.1.1. Methyl 2,4,6-trimethoxybenzoate (15). K_2CO_3 (92.9 g, 672 mmol) and dimethyl sulfate (84.80 g, 672 mmol)

were added to a solution of 90% pure 2,4,6-trihydroxybenzoic acid monohydrate (**14**) (21.00 g, 112 mmol) in dry acetone (420 ml) and the resulting mixture was stirred for 26 h at 42–45°C. It was then filtered and the filtrate was concentrated under reduced pressure. The solid residue was recrystallized from hexane to give **15** (14.60 g, 58% yield) as a colorless solid. Concentration of the mother liquors followed by recrystallization from methanol of the resulting solid residue provided a further amount of pure **15** (6.22 g, 24% yield). Mp 70°C (lit.[36] mp 69–70°C). MS, *m/z* (%): 226 (23), 196 (11), 195 (100), 180 (10), 165 (4), 152 (6), 137 (10), 109 (3), 77 (3). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.79 (6H, s, OMe), 3.81 (3H, s, OMe), 3.87 (3H, s, OMe), 6.09 ppm (2H, s, H-3 and H-5).

4.1.2. Methyl 2-hydroxy-4,6-dimethoxybenzoate (8c). A 1 M solution of BCl_3 in CH_2Cl_2 (66.1 ml, 66.1 mmol) was added during 20 min to a solution of compound **15** (13.59 g, 60.07 mmol) in dry CH_2Cl_2 (74 ml), which was stirred at −70°C. The reaction mixture was then allowed to warm up to 20°C and stirred at this temperature for 40 min. It was then poured into 10% aqueous HCl (115 ml) and extracted with CH_2Cl_2 (5×100 ml). The organic extract was washed with water (3×70 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by MPLC on silica gel with CH_2Cl_2 as eluent to give compound **8c** (10.20 g, 80% yield) as a colorless solid. Mp 107–109°C (lit.[37] mp 107°C). IR (KBr): ν 1642, 1614, 1444, 1314, 1276, 1221, 1162, 1112, 821 cm^{-1}. MS, *m/z* (%): 212 (32), 181 (24), 180 (100), 152 (35), 138 (7), 137 (46), 109 (10), 95 (13), 81 (9). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.80 (3H, s, OMe), 3.82 (3H, s, OMe), 3.91 (3H, s, OMe), 5.96 (1H, d, *J*=2.6 Hz, H-5 or H-3), 6.10 (1H, d, *J*=2.6 Hz, H-3 or H-5), 12.03 ppm (1H, s, OH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 52.1, 55.4, 56.0, 91.4, 93.3, 96.4, 162.0, 165.2, 165.8, 171.5 ppm. The spectral properties of this compound were in agreement with those previously reported.[37]

4.1.3. Methyl 4,6-dimethoxy-2-(perfluoro-1-butanesulfonyloxy)benzoate (13d). A 60% dispersion of NaH in mineral oil (2.26 g, 56.6 mmol), which was maintained under an atmosphere of nitrogen, was washed with pentane (4×20 ml). Dry DMF (75 ml) was added to the residue and the mixture was stirred at 0°C. A solution of **8c** (8.00 g, 37.7 mmol) in dry DMF (22 ml) was then added dropwise and the mixture was stirred at 0°C for 0.5 h and at rt for 1 h. Perfluoro-1-butanesulfonyl fluoride (7.79 ml, 43.4 mmol) was added and the resulting mixture was stirred at rt for 2 h. It was then poured into a saturated aqueous NH_4Cl solution (300 ml) and extracted with Et_2O (5×150 ml). The organic extract was washed with water (3×50 ml), dried over Na_2SO_4 and concentrated under reduced pressure to give compound **13d** (17.53 g, 94% yield) as a pale yellow solid. Mp 44–47.5°C. MS, *m/z* (%): 494 (58), 463 (94), 183 (100), 181 (15), 180 (88), 179 (20), 152 (17), 137 (36), 69 (36). ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.85 (3H, s, OMe), 3.86 (3H, s, OMe), 3.90 (3H, s, OMe), 6.43 (1H, d, *J*=2.2 Hz, H-5 or H-3), 6.48 ppm (1H, d, *J*=2.2 Hz, H-3 or H-5). GLC analysis showed that **13d** had chemical purity higher than 97%. This product was used in the next step without any further purification and characterization.

4.1.4. Methyl 4,6-dimethoxy-2-[(3,4-dimethoxyphenyl)-ethynyl]benzoate (6d). A slurry of (3,4-dimethoxyphenyl)-ethynylzinc chloride (**16**) in THF was prepared by addition of a THF solution of the corresponding Grignard reagent (0.505 M, 76.0 ml, 38.4 mmol) to a slurry of dry $ZnCl_2$ (6.80 g, 49.9 mmol) in THF (52 ml), which was stirred at 0°C. After stirring for additional 20 min at this temperature, a solution of **13d** (15.81 g, 31.99 mmol), $Pd_2(dba)_3$ (0.293 g, 0.320 mmol), and dppf (0.355 g, 0.640 mmol) in THF (128 ml) was added. The resulting mixture was allowed to warm up to rt and then heated to 60°C for 17 h. After usual workup the crude reaction product was diluted with a mixture of benzene and AcOEt (9:1) (150 ml) and filtered. The filtrate was concentrated and the residue, which was diluted with CH_2Cl_2 (35 ml), was purified by MPLC on silica gel with a mixture of benzene and AcOEt (9:1) as eluent, to give **6d** (9.69 g, 85% yield) as a colorless solid. Mp 58–61°C. IR (KBr): ν 2205, 1731, 1598, 1578, 1514, 1266, 1245, 1157, 1134 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.82 (3H, s, OMe), 3.83 (3H, s, OMe), 3.89 (3H, s, OMe), 3.90 (3H, s, OMe), 3.94 (3H, s, OMe), 6.46 (1H, d, *J*=2.1 Hz, H-3 or H-5), 6.66 (1H, d, *J*=2.1 Hz, H-5 or H-3), 6.83 (1H, d, *J*=8.1 Hz, H-5′), 6.99 (1H, d, *J*=2.0 Hz, H-2′), 7.10 ppm (1H, dd, *J*=8.1, 2.0 Hz, H-6′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 52.3, 55.6, 55.9 (2C), 56.0, 85.4, 89.5, 92.9, 107.8, 111.0, 114.3, 115.0, 118.8, 123.6, 125.2, 148.7, 148.9, 158.0, 161.4, 167.4 ppm. Anal. calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.11; H, 5.61.

4.1.5. 4-Iodo-6,8-dimethoxy-3-(3,4-dimethoxyphenyl)isocoumarin (5d) and methyl 4,6-dimethoxy-2-(3,4-dimethoxybenzoyl)methylbenzoate (17). To a solution of **6d** (6.67 g, 18.7 mmol) in MeCN (214 ml) was added iodine (14.28 g, 56.28 mmol) and the mixture was stirred under nitrogen in the dark at rt for 2 h. It was then poured into a 10% aqueous $Na_2S_2O_3$ solution (300 ml) and extracted with $CHCl_3$ (6×250 ml). The organic extract was washed with water (75 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The solid residue was recrystallized from THF to give chemically pure **5d** (4.63 g) as a yellow solid. Evaporation of the mother liquors furnished a residue, which was purified by MPLC on silica gel with a mixture of CH_2Cl_2 and AcOEt (8:2) as eluent to give a further amount of chemically pure **5d** (1.22 g). The overall yield was 67%. Mp 237.5–238.5°C. IR (KBr): ν 1739, 1609, 1514, 1465, 1342, 1257, 1231, 1132, 1000 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.95 (6H, s, OMe), 3.97 (3H, s, OMe), 4.01 (3H, s, OMe), 6.57 (1H, d, *J*=2.2 Hz, H-7 or H-5), 6.95 (1H, d, *J*=8.0 Hz, H-5′), 6.99 (1H, d, *J*=2.2 Hz, H-5 or H-7), 7.21 (1H, d, *J*=1.8 Hz, H-2′), 7.31 ppm (1H, dd, *J*=8.0, 1.8 Hz, H-6′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 55.8, 55.9, 56.1, 56.6, 75.8, 98.9, 102.6, 107.6, 110.1, 112.8, 123.4, 127.9, 142.5, 148.1, 150.3, 156.0, 157.9, 163.5, 165.5 ppm. Anal. calcd for $C_{19}H_{17}IO_6$: C, 48.74; H, 3.66. Found: C, 48.68; H, 3.60. Concentration of the first eluted fractions of the above mentioned chromatographic purification allowed isolation of compound **17** (0.60 g, 9% yield) as a pale yellow solid. Mp 190–193°C. IR (KBr): ν 2984, 1702, 1680, 1586, 1514, 1311, 1256, 1153, 1024 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): 3.76 (3H, s, COOMe), 3.78 (3H, s, H-10), 3.82 (3H, s, H-7), 3.92 (3H, s, H-7″), 3.94 (3H, s, H-8″), 4.26 (2H, s, H-1′), 6.36 (1H, d, *J*=2.1 Hz, H-3), 6.40 (1H, d, *J*=2.1 Hz, H-5), 6.89 (1H, d, *J*=8.3 Hz, H-5″), 7.56 (1H, d, *J*=1.9 Hz, H-2″), 7.65 ppm (1H, dd, *J*=8.3, 1.9 Hz, H-6″). ^{13}C NMR

(150 MHz, $CDCl_3$): δ 43.3, 52.1, 55.4, 56.0, 56.1 (2C), 97.7, 107.2, 110.1, 110.7, 116.1, 123.2, 129.8, 136.3, 149.0, 153.4, 159.1, 161.8, 168.3, 195.5 ppm.

4.2. General procedure for the synthesis of 3-arylisocoumarins by palladium-catalyzed triethylammonium formate reduction of 3-aryl-4-iodoisocoumarins

Formic acid (99%, 393 mg, 8.54 mmol) was added to a degassed mixture of a 3-aryl-4-iodoisocoumarin **5** (4.27 mmol), Et_3N (1.79 ml, 12.8 mmol), $Pd(OAc)_2$ (19.2 mg, 0.0854 mmol), and triphenylphosphine (44.8 mg, 0.171 mmol) in dry DMF (120 ml) and the mixture was stirred at 60–63°C for 3.5–4.5 h under argon. After this period of time GLC analysis showed that compound **5** had been completely consumed. The mixture was then cooled to rt, diluted with water (300 ml), and extracted with AcOEt (5×120 ml). The organic extract was dried over Na_2SO_4, concentrated under reduced pressure and the residue was purified by MPLC on silica gel. This procedure was used to prepare compounds **3c**, **3d**, **3f** and **3j** from **5a**–**5d**, respectively. It should be noted that the crude reaction mixture which was obtained by palladium-catalyzed triethylammonium formate reduction of **5c** was extracted with $CHCl_3$ (5×120 ml) instead of being extracted with AcOEt.

4.2.1. 3-(3,4-Dimethoxyphenyl)-8-methoxyisocoumarin (3c). The crude reaction product, which was obtained by palladium-catalyzed triethylammonium formate reduction of **5a**, was purified by MPLC on silica gel with a mixture of benzene and AcOEt (65:35) as eluent, to give compound **3c** as a pale yellow solid in 93% yield. Mp 148–150°C (lit.[13b] 152.8–154.4°C) MS, *m/z* (%): 312 (100), 284 (46), 269 (22), 165 (12), 142 (10), 119 (15), 76 (10). IR (KBr): ν 1729, 1698, 1566, 1515, 1254, 1077, 1021, 991, 805 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 3.93 (3H, s, OMe), 3.98 (3H, s, OMe), 4.01 (3H, s, OMe), 6.74 (1H, s, H-4), 6.90 (1H, br d, *J*=8.1 Hz, H-7), 6.92 (1H, d, *J*=8.4 Hz, H-5′), 6.99 (1H, dd, *J*=8.1, 1.0 Hz, H-5), 7.35 (1H, d, *J*=2.2 Hz, H-2′), 7.46 (1H, dd, *J*=8.4, 2.2 Hz, H-6′), 7.59 ppm (1H, pseudo-t, *J*=8.1 Hz, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 56.0, 56.1, 56.3, 100.5, 108.2, 108.8, 109.4, 111.0, 117.7, 118.5, 124.6, 135.6, 140.6, 149.0, 150.6, 153.8, 159.0, 161.5 ppm. The spectral properties of this compound were in agreement with those previously reported.[2,21a]

4.2.2. 8-Methoxy-3-phenylisocoumarin (3d). The crude reaction product, which was obtained by palladium-catalyzed triethylammonium formate reduction of **5b**, was purified by MPLC on silica gel with a mixture of benzene and AcOEt (87.5:12.5) as eluent, to give **3d** as a pale yellow solid in 90% yield. Mp 144–146°C (lit.[38] mp 143–145°C). MS, *m/z* (%): 252 (100), 223 (57), 205 (27), 165 (27), 152 (32), 119 (23), 105 (88), 91 (28), 77 (80). IR (KBr): ν 1725, 1568, 1477, 1274, 1219, 1108, 992, 767, 689 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 4.03 (3H, s, OMe), 6.86 (1H, s, H-4), 6.94 (1H, d, *J*=8.1 Hz, H-7 or H-5), 7.03 (1H, d, *J*=8.1 Hz, H-5 or H-7), 7.39–7.51 (3H, m, H-arom), 7.62 (1H, pseudo-t, *J*=8.1 Hz, H-6), 7.83–7.93 ppm (2H, m, H-arom). ^{13}C NMR (50 MHz, $CDCl_3$): δ 56.3, 101.7, 109.2, 109.8, 118.0, 125.2 (2C), 128.6 (2C), 129.8, 131.7, 135.7, 140.4, 153.8, 158.9, 161.5 ppm. The spectral properties of this compound, which had chemical purity higher than 98%, were in agreement with those previously reported.[38]

4.2.3. 6-Methoxy-3-(4-methoxyphenyl)isocoumarin (3f). The crude reaction product, which was obtained by palladium-catalyzed triethylammonium formate reduction of **5c**, was purified by MPLC on silica gel with a mixture of benzene and AcOEt (95:5) as eluent, to give **3f** as a colorless solid in 93% yield. Mp 145–147°C. MS, *m/z* (%): 282 (91), 254 (100), 239 (18), 211 (50), 195 (22), 139 (18), 127 (20), 119 (17), 77 (21). IR (KBr): ν 1736, 1606, 1515, 1493, 1266, 1175, 1065, 1023, 831 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 3.86 (3H, s, OMe), 3.92 (3H, s, OMe), 6.76 (1H, s, H-4), 6.83 (1H, d, *J*=2.6 Hz, H-5), 6.91–6.99 (2H, m, H-arom), 6.99 (1H, dd, *J*=8.8, 2.6 Hz, H-7), 7.75–7.86 (2H, m, H-arom), 8.20 ppm (1H, d, *J*=8.8 Hz, H-8). ^{13}C NMR (50 MHz, $CDCl_3$): 55.4, 55.6, 100.2, 107.5, 113.3, 114.1 (2C), 116.0, 124.5, 126.7 (2C), 131.7, 140.1, 154.1, 160.9, 162.1, 164.6 ppm. Anal. calcd for $C_{17}H_{14}O_4$: C, 72.33; H, 5.00. Found: C, 72.25; H, 4.67.

4.2.4. 6,8-Dimethoxy-3-(3,4-dimethoxyphenyl)isocoumarin (3j). The crude reaction product, which was obtained by palladium-catalyzed triethylammonium formate reduction of **5d**, was purified by MPLC on silica gel with a mixture of CH_2Cl_2 and AcOEt (80:20) as eluent, to give **3j** as a colorless solid in 98% yield. Mp 144.5–146°C (lit.[13b] mp 155.9–157.2°C). IR (KBr): ν 1721, 1640, 1598, 1565, 1516, 1254, 1209, 1151, 991 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 3.89 (3H, s, OMe), 3.92 (3H, s, OMe), 3.95 (3H, s, OMe), 3.97 (3H, s, OMe), 6.39 (1H, d, *J*=2.2 Hz, H-7 or H-5), 6.41 (1H, d, *J*=2.2 Hz, H-5 or H-7), 6.64 (1H, s, H-4), 6.88 (1H, d, *J*=8.4 Hz, H-5′), 7.31 (1H, d, *J*=2.1 Hz, H-2′), 7.41 ppm (1H, dd, *J*=8.4, 2.1 Hz, H-6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.5, 55.9, 56.0, 56.2, 98.3, 100.0, 100.6, 102.9, 108.1, 110.9, 118.5, 124.6, 142.4, 149.0, 150.5, 154.1, 158.8, 163.1, 165.2 ppm. Anal. calcd for $C_{19}H_{18}O_6$: C, 66.66; H, 5.30. Found: C, 66.56; H, 5.27.

4.2.5. 8-Hydroxy-3-(3,4-dihydroxyphenyl)isocoumarin (thunberginol A) (3a). A mixture of **3c** (0.781 g, 2.50 mmol) and a 1 M CH_2Cl_2 solution of BBr_3 (5.2 equiv., 13.0 ml, 13.0 mmol) was stirred at rt under nitrogen for 2 h. The solution was then poured into ice water (100 ml) and the mixture was extracted with AcOEt (6×150 ml). The organic extract was washed with brine (2×70 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by MPLC on silica gel with a mixture of $CHCl_3$, MeOH and water (10:1:1, lower phase) as eluent to give **3a** (0.57 g, 85% yield) as a colorless solid. Mp 249–252°C (lit.[2] mp 240°C). IR (KBr): ν 3428, 1666, 1611, 1566, 1527, 1454, 1217, 1171, 820 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 6.87 (1H, br d, *J*=8.1 Hz, H-5′), 6.93 (1H, br d, *J*=8.0 Hz, H-7), 7.10 (1H, br d, *J*=8.0 Hz, H-5), 7.23 (1H, s, H-4), 7.24 (1H, br d, *J*=8.1 Hz, H-6′), 7.29 (1H, br s, H-2′), 7.69 (1H, dd, *J*=8.1, 8.0 Hz, H-6), 10.85 ppm (3H, br s, OH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 100.5, 105.0, 112.0, 113.9, 115.9, 116.4, 116.8, 122.1, 137.4, 138.4, 145.5 147.6, 152.6, 160.2, 165.0 ppm. These NMR data were in satisfactory agreement with those of **3a**, which was isolated from the fermented and dried leaves of *H. macrophylla*.[2]

4.2.6. 8-Hydroxy-3-phenylisocoumarin (3e). A mixture of **3d** (0.500 g, 1.98 mmol) and a 1 M CH_2Cl_2 solution of BBr_3 (4.54 equiv., 8.98 ml, 8.98 mmol) was stirred at rt under nitrogen for 1.75 h. The crude reaction product, which was obtained after usual workup, was purified by MPLC on silica gel with toluene as eluent to give **3e** (0.47 g, 98% yield) as a colorless solid. Mp 128–130°C (lit.[39] mp 142°C). MS, *m/z* (%): 239 (16), 238 (100), 210 (64), 181 (36), 153 (10), 152 (15), 105 (63), 92 (10), 77 (52). IR (KBr): ν 3445, 1677, 1628, 1452, 1223, 1168, 1094, 759, 683 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 7.01 (1H, d, J=8.1 Hz, H-7 or H-5), 7.16 (1H, d, J=8.1 Hz, H-5 or H-7), 7.48–7.60 (3H, m, H-arom), 7.74 (1H, pseudo-t, J=8.1 Hz, H-6), 7.55 (1H, s, H-4), 7.84–7.94 (2H, m, H-arom), 10.85 ppm (1H, s, OH). ^{13}C NMR (75 MHz, DMSO-d_6): δ 103.1, 105.7, 114.9, 117.0, 124.8 (2C), 129.0 (2C), 130.1, 131.1, 137.7, 137.9, 151.9, 160.4, 164.8 ppm. Anal. calcd for $C_{15}H_{10}O_3$: C, 75.62; H, 4.23. Found: C, 75.45; H, 4.36.

4.2.7. 6-Hydroxy-3-(4-hydroxyphenyl)isocoumarin (3g). A mixture of **3f** (1.00 g, 3.54 mmol) and a 1 M CH_2Cl_2 solution of BBr_3 (8.33 equiv., 29.5 ml, 29.5 mmol) was stirred at 40°C under nitrogen for 27 h. The mixture was then cooled to rt, poured into ice water (80 ml) and concentrated at rt at 20 Torr. The resulting aqueous suspension was cooled to 0°C and filtered. The solid was washed with cold water (5×5 ml), dissolved in THF (10 ml) and purified by MPLC on silica gel with a mixture of $CHCl_3$, MeOH and water (10:2:1, lower phase) as eluent to give **3g** (0.79 g, 87% yield) as a pale yellow solid. Mp 281–284°C. IR (KBr): ν 3260, 1679, 1636, 1595, 1463, 1360, 1256, 1174, 844 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 6.85–6.96 (2H, m, H-3′ and H-5′), 6.94 (1H, d, J=2.2 Hz, H-5), 6.97 (1H, dd, J=8.4, 2.2 Hz, H-7), 7.16 (1H, s, H-4), 7.49–7.61 (2H, m, H-2′ and H-6′), 8.02 (1H, d, J=8.4 Hz, H-8), 10.10 (1H, br s, OH), 10.70 ppm (1H, br s, OH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 99.5, 109.9, 110.9, 115.6 (2C), 116.6, 122.4, 128.5 (2C), 131.3, 140.0, 153.1, 159.0, 161.0, 163.3 ppm. Anal. calcd for $C_{15}H_{10}O_4$: C, 70.86; H, 3.96. Found: C, 70.78; H, 3.99.

4.2.8. 3-(4-Hydroxyphenyl)-6-methoxyisocoumarin (3h). A mixture of **3f** (1.09 g, 3.86 mmol) and a 1 M CH_2Cl_2 solution of BBr_3 (5.20 equiv., 20.1 ml, 20.1 mmol) was stirred at rt under nitrogen for 1.42 h. The crude reaction product, which was obtained after usual workup, was purified by MPLC on silica gel with a mixture of benzene and AcOEt (80:20) as eluent to give **3h** (0.75 g, 72% yield) as a pale yellow solid. Mp 251–254°C. IR (KBr): ν 3446, 1691, 1636, 1610, 1272, 1259, 1245, 1077, 831 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): δ 3.91 (3H, s, OMe), 6.90 (2H, m, H-3′ and H-5′), 7.10 (1H, dd, J=8.7, 2.6 Hz, H-7), 7.13 (1H, d, J=2.6 Hz, H.5), 7.21 (1H, s, H-4), 7.72 (2H, m, H-2′ and H-6′), 8.05 (1H, d, J=8.7 Hz, H-8), 10.02 ppm (1H, s, OH). ^{13}C NMR (150 MHz, DMSO-d_6): δ 56.5, 100.5, 108.9, 113.2, 116.6 (2C), 117.2, 123.3, 127.5 (2C), 131.8, 141.0, 154.4, 160.0, 161.8, 165.3 ppm. Anal. calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.48; H, 4.48.

4.2.9. 6-Methoxy-3-[4-(3,4,5-trimethoxybenzoyloxy)-phenyl]isocoumarin (3i). A solution of **3h** (0.349 g, 1.30 mmol) in dry pyridine (18 ml) was added to a solution of 3,4,5-trimethoxybenzoyl chloride (**17**) (0.450 g, 1.95 mmol) in dry pyridine (3 ml) and the mixture was stirred at 48°C for 48 h. It was then cooled to rt, poured into ice water (60 ml), acidified with cold 2 M HCl and extracted with AcOEt (5×50 ml). The organic extract was washed with water (2×30 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The solid residue was recrystallized from a mixture of benzene and AcOEt (6:1) to give **3i** (0.44 g, 74% yield) as a pale yellow solid. Mp 135–137°C. IR (KBr): ν 1726, 1606, 1463, 1417, 1336, 1203, 1167, 1120, 1058 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 3.92 (3H, s, OMe), 3.94 (6H, s, OMe), 3.95 (3H, s, OMe), 6.87 (1H, d, J=2.4 Hz, H-5), 6.87 (1H, s, H-4), 7.03 (1H, dd, J=8.8, 2.4 Hz, H-7), 7.26–7.36 (2H, m, H-3′ and H-5′), 7.46 (2H, s, H-2″ and H-6″), 7.89–7.99 (2H, m, H-2′ and H-6′), 8.22 ppm (1H, d, J=8.8 Hz, H-8). ^{13}C NMR (50 MHz, $CDCl_3$): δ 55.6, 56.3 (2C), 60.9, 101.9, 107.4 (2C), 107.9, 113.6, 116.5, 122.2 (2C), 123.9, 126.5 (2C), 129.7, 131.8, 139.6, 142.9, 152.1, 153.0 (2C), 153.3, 161.8, 164.5, 164.7 ppm. Anal. calcd for $C_{26}H_{22}O_7$: C, 69.95; h, 4.97. Found: C, 70.00; H, 4.68.

4.2.10. 6,8-Dihydroxy-3-(3,4-dihydroxyphenyl)isocoumarin (thunberginol B) (3b). A mixture of **3j** (0.750 g, 2.19 mmol) and a 1 M CH_2Cl_2 solution of BBr_3 (10.0 equiv., 21.9 ml, 21.9 mmol) was stirred at rt under nitrogen for 72 h. The mixture was then poured into ice water (60 ml) and concentrated at rt at 20 Torr. The resulting aqueous suspension was cooled to 0°C and filtered. The solid was washed with cold water (5×4 ml) and purified by MPLC on silica gel with a mixture of $CHCl_3$, MeOH and water (10:3:1, lower phase) as eluent to give compound **3b** (0.51 g, 81% yield) as a pale yellow solid. Mp 281–285°C (lit.[2] mp 244°C). Tandem mass spectrometry on the 287 (M+H) ion, *m/z* (%): 287 (52), 269 (100), 259 (4), 241 (13), 213 (11), 177 (23), 149 (3), 137 (4), 121 (7). IR (KBr): ν 3176, 1676, 1530, 1620, 1384, 1246, 1191, 1168, 1094 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 6.33 (1H, d, J=2.1 Hz, H-5 or H-7), 6.50 (1H, d, J=2.1 Hz, H-7 or H-5), 6.87 (1H, d, J=8.4 Hz, H-5′), 7.12 (1H, s, H-4), 7.23 (1H, dd, J=8.4, 2.2 Hz, H-6′), 7.28 (1H, d, J=2.2 Hz, H-2′), 9.30 (1H, s, OH), 9.57 (1H, s, OH), 10.96 ppm (2H, s, OH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 97.8, 100.4, 101.3, 103.0, 112.1, 115.2, 116.8, 122.3, 139.9, 145.4, 147.5, 152.6, 162.4, 164.8, 165.4 ppm. Anal. calcd for $C_{15}H_{10}O_6$: C, 62.94; H, 3.52. Found: C, 62.90; H, 3.49. These NMR data were in satisfactory agreement with those of **3j**, which was isolated from the fermented and dried leaves of *H. macrophylla*.[2]

4.2.11. 3-(3,4-Dimethoxyphenyl)-8-methoxy-4-methylisocoumarin (4a). A deaerated solution of tetramethyltin (**22**) (1.84 g, 10.3 mmol) in dry NMP (12 ml) was added to a degassed mixture of **5a** (1.50 g, 3.42 mmol), $PdCl_2[P(o\text{-Tolyl})_3]_2$ (134 mg, 0.171 mmol), and CuI (65.2 mg, 0.342 mmol) in dry NMP (38 ml) and the mixture was stirred under nitrogen at 76–79°C for 4.5 h. It was then cooled to rt, poured into a saturated aqueous NH_4Cl solution (150 ml) and extracted with $CHCl_3$ (6×100 ml). The organic extract was washed with brine (2×50 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The resulting solid was dissolved in $CHCl_3$ (15 ml) and purified by MPLC on silica gel with a mixture of benzene and AcOEt (75:25) as eluent to give **4a** (0.96 g, 86% yield) as a

pale yellow solid. Mp 195.5–198.5°C. MS, *m/z* (%): 327 (15), 326 (70), 299 (21), 298 (100), 283 (38), 252 (15), 165 (13), 149 (11), 77 (8). IR (KBr): ν 1728, 1516, 1480, 1254, 1215, 1173, 1048, 1024, 806 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 2.27 (3H, s, C–Me), 3.94 (6H, s, OMe), 4.03 (3H, s, OMe), 6.92 (1H, d, J=8.9 Hz, H-5′ or H-6′), 7.00 (1H, d, J=8.1 Hz, H-5 or H-7), 7.08–7.20 (3H, m, H-arom), 7.70 ppm (1H, pseudo-t, J=8.1 Hz, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.3, 55.9, 56.0, 56.3, 107.9, 109.4, 109.6, 110.3, 112.3, 115.1, 122.7, 125.8, 135.4, 141.8, 148.5, 149.7, 151.6, 159.2, 161.7 ppm. Anal. calcd for $C_{19}H_{18}O_5$: C, 69.93; H, 5.56. Found: C, 69.85; H, 5.43.

4.2.12. 8-Hydroxy-3-(3,4-dihydroxyphenyl)-4-methylisocoumarin (4b). A solution of **4a** (1.13 g, 3.46 mmol) in a 1 M CH_2Cl_2 solution of BBr_3 (5.20 equiv., 18.0 ml, 18.0 mmol) was stirred for 2 h at rt under nitrogen. The mixture was then poured into water (150 ml) and extracted with AcOEt (6×200 ml). The organic extract was washed with water (1×50 ml) and concentrated under reduced pressure. The resulting solid residue was dissolved in THF (10 ml) and purified by MPLC on silica gel with a mixture of $CHCl_3$, MeOH and water (10:1:1, lower phase) as eluent to give **4b** (0.88 g, 89% yield) as a pale yellow solid. Mp 242–244°C. IR (KBr): ν 3297, 1662, 1606, 1513, 1292, 1240, 1215, 1127, 691 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 2.21 (3H, s, C–Me), 6.91 (2H, s, H-5′ or H-6′), 6.99 (1H, d, J=8.4 Hz, H-7 or H-5), 7.04 (1H, s, H-2′), 7.11 (1H, d, J=8.2 Hz, H-5 or H-7), 7.75 (1H, pseudo-t, J=8.2 Hz, H-6), 9.35 (1H, s, OH), 9.52 (1H, s, OH), 11.22 ppm (1H, s, OH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 13.6, 105.1, 108.8, 114.0, 114.3, 115.2, 116.3, 121.0, 123.1, 137.3, 139.0, 144.9, 146.8, 150.3, 160.6, 165.4 ppm. Anal. calcd for $C_{16}H_{12}O_5$: C, 67.60; H, 4.26. Found: C, 67.91; H, 4.41.

4.2.13. 8-Methoxy-4-methyl-3-phenylisocoumarin (4c). Reaction of **5b** (0.700 g, 1.85 mmol) with tetramethyltin (**22**) (0.993 g, 5.55 mmol) in NMP (20 ml) at 82°C for 4.5 h in the presence of 5 mol% $PdCl_2[P(o\text{-Tolyl})_3]_2$ and 10 mol% CuI according to the same procedure used for the synthesis of **4a** provided 98% chemically pure **4c** (0.38 g, 78% yield) as a pale yellow solid. Mp 161–162°C. MS, *m/z* (%): 266 (100), 238 (39), 237 (28), 223 (26), 219 (17), 195 (19), 165 (17), 105 (60), 77 (65). IR (KBr): ν 1725, 1594, 1566, 1479, 1267, 1230, 1095, 1049, 812 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.25 (3H, s, C–Me), 4.02 (3H, s, OMe), 7.01 (1H, d, J=8.3 Hz, H-5 or H-7), 7.17 (1H, d, J=8.3 Hz, H-7 or H-5), 7.39–7.48 (3H, m, H-arom), 7.54–7.61 (2H, m, H-arom), 7.70 ppm (1H, pseudo-t, J=8.3 Hz, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 56.4, 108.4, 109.7, 109.9, 115.3, 128.1 (2C), 129.2, 129.5 (2C), 133.3, 135.5, 141.7, 151.8, 159.1, 161.8 ppm. Anal. calcd for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30. Found: C, 76.50; H, 5.28.

4.2.14. 8-Hydroxy-4-methyl-3-phenylisocoumarin (4d). A mixture of **4c** (0.312 g, 1.17 mmol) and a 1 M CH_2Cl_2 solution of BBr_3 (4.54 equiv., 5.31 ml, 5.31 mmol) was stirred for 2.5 h at rt under nitrogen. The mixture was then poured into ice-water (140 ml) and the whole was extracted with AcOEt (5×70 ml). The organic extract was washed with water (2×30 ml) and concentrated under reduced pressure. The solid residue was purified by MPLC on silica gel with toluene as eluent to give chemically pure **4d** (0.28 g, 96% yield) as a pale yellow solid. Mp 161–162.5°C. IR (KBr): ν 3434, 1678, 1634, 1457, 1240, 1186, 813, 749, 702 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 2.22 (3H, s, C–Me), 7.07 (1H, d, J=8.1 Hz, H-5 or H-7), 7.22 (1H, d, J=8.1 Hz, H-7 or H-5), 7.46–7.68 (5H, m, H-2′, H-3′, H-4′, H-5′, and H-6′), 7.82 (1H, pseudo-t, J=8.2 Hz, H-6), 11.20 ppm (1H, s, OH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 13.4, 105.4, 110.2, 114.3, 114.9, 128.3 (2C), 129.1 (2C), 129.5, 132.2, 137.6, 138.6, 149.7, 160.6, 165.1 ppm. Anal. calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.11; H, 4.70.

4.2.15. 6-Methoxy-3-(4-methoxyphenyl)-4-methylisocoumarin (4e). Reaction of **5c** (600 mg, 1.47 mmol) with tetramethyltin (**22**) (789 mg, 4.41 mmol) in NMP (16 ml) at 80°C for 10 h in the presence of 5 mol% $PdCl_2[P(o\text{-Tolyl})_3]_2$ and 10 mol% CuI according to the same procedure used for the synthesis of **4a** provided 98% chemically pure **4e** (0.31 g, 72% yield) as a colorless solid. Mp 69–71°C. MS, *m/z* (%): 296 (40), 269 (18), 268 (100), 253 (66), 225 (15), 182 (9), 135 (17), 92 (18), 77 (22). IR (KBr): ν 1703, 1601, 1493, 1441, 1254, 1231, 1182, 1023, 850 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.27 (3H, s, C–Me), 3.87 (3H, s, OMe), 3.95 (3H, s, OMe), 6.97 (1H, d, J=2.2 Hz, H-5), 6.93–7.02 (2H, m, H-3′ and H-5′ or H-2′ and H-6′), 7.06 (1H, dd, J=8.7, 2.2 Hz, H-7), 7.49–7.56 (2H, m, H-2′ and H-6′ or H-3′ and H-5′), 8.29 ppm (1H, d, J=8.7 Hz, H-8). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.7, 55.4, 55.6, 106.5, 108.2, 113.7 (2C), 114.0, 115.1, 125.9, 130.9 (2C), 132.7, 141.4, 151.8, 160.3, 162.4, 164.8 ppm. Anal. calcd for $C_{18}H_{16}O_4$: C, 72.96; H, 5.44. Found: C, 72.85; H, 5.47.

4.2.16. 6-Hydroxy-3-(4-hydroxyphenyl)-4-methylisocoumarin (4f). A mixture of **4e** (237 mg, 0.800 mmol) and a 1 M CH_2Cl_2 solution of BBr_3 (8.33 equiv., 6.67 ml, 6.67 mmol) was stirred at 30–32°C for 48 h. After workup according to the procedure used in the synthesis of **3g**, the crude reaction product was dissolved in THF (35 ml) and purified by MPLC on silica gel with a mixture of toluene and AcOEt (50:50) as eluent to give chemically pure **4f** (191 mg, 89% yield) as a colorless solid. Mp 309–313°C (decomposition). Tandem mass spectrometry on the 287 (M+H) ion, *m/z* (%): 269 (100), 251 (33), 235 (26), 226 (95), 223 (40), 195 (22), 175 (17), 147 (39), 121 (19). IR (KBr): ν 3382, 1687, 1625, 1595, 1495, 1462, 1275, 1109, 830 cm^{-1}. ^1H NMR (200 MHz, DMSO-d_6): δ 2.17 (3H, s, C–Me), 6.83–6.95 (2H, m, H-3′ and H-5′ or H-2′ and H-6′), 6.97 (1H, d, J=2.2 Hz, H-5), 7.02 (1H, dd, J=8.8, 2.2 Hz, H-7), 7.36–7.47 (2H, m, H-2′ and H-6′ or H-3′ and H-5′), 8.06 (1H, d, J=8.8 Hz, H-8), 9.94 (1H, br s, OH), 10.80 ppm (1H, br s, OH). ^{13}C NMR (50 MHz, DMSO-d_6): δ 13.3, 107.4, 108.2, 111.5, 115.0 (2C), 116.6, 123.7, 130.7 (2C), 131.5, 140.9, 151.0, 158.3, 161.1, 163.4 ppm. Anal. calcd for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51. Found: C, 71.53; H, 4.48.

4.2.17. 4-Methyl-3-phenylisocoumarin (4i). A degassed solution of tetramethyltin (**22**) (3.54 g, 19.8 mmol) in dry NMP (3 ml) was added to a deaerated mixture of **5e** (2.30 g, 6.61 mmol), $PdCl_2(PhCN)_2$ (0.13 g, 0.33 mmol), $AsPh_3$ (0.20 g, 0.66 mmol), and CuI (0.13 g, 0.66 mmol) in dry NMP (10 ml) and the mixture was stirred under nitrogen for 117 h at 60°C. The crude reaction product, which was obtained after usual workup, was purified by MPLC on

silica gel with a mixture of hexane, CH_2Cl_2 and AcOEt (80:15:5) as eluent to give 98% chemically pure **4i** (1.03 g, 66% yield) as an orange solid. Mp 109–113°C. MS, *m/z* (%): 236 (92), 208 (91), 178 (19), 131 (10), 105 (84), 102 (16), 77 (100). IR (KBr): ν 1718, 1637, 1486, 1244, 1098, 1057, 767, 705, 694 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 2.32 (3H, s, C–Me), 7.44 (1H, m, H-4′), 7.46 (2H, m, H-3′ and H-5′), 7.55 (1H, ddd, *J*=7.8, 7.2, 1.2 Hz, H-7), 7.59 (2H, dd, *J*=8.0, 1.2 Hz, H-2′ and H-6′), 7.65 (1H, d, *J*=8.0 Hz, H-5), 7.81 (1H, ddd, *J*=8.0, 7.2, 1.2 Hz, H-6), 8.38 ppm (1H, dd, *J*=7.8, 1.2 Hz, H-8). ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.6, 109.1, 120.9, 123.4, 127.9, 128.3 (2C), 129.4, 129.5 (2C), 129.8, 133.3, 134.8, 138.8, 151.2, 162.5 ppm. Anal. calcd for $C_{16}H_{12}O_2$: C, 81.34; H, 5.12. Found: C, 80.97; H, 4.93.

4.2.18. 3-Phenyl-4-(4-methoxyphenyl)isocoumarin (4j). The crude reaction mixture, which was obtained by reaction of **5c** (1.57 g, 4.51 mmol) with 4-methoxyphenyltributyltin (**23**) (2.14 g, 5.39 mmol) in NMP at 60°C for 54 h in the presence of $PdCl_2(PhCN)_2$ (90 mg, 0.23 mmol), $AsPh_3$ (0138 mg, 0.45 mmol) and CuI (85.6 mg, 0.45 mmol), was poured into a saturated aqueous NH_4Cl solution (150 ml) and extracted with AcOEt (5×50 ml). The organic extract was then stirred for 4.5 h at rt with a 8 M aqueous KF solution (200 ml), filtered over Celite®, and the filtrate was extracted with AcOEt (3×50 ml). The organic extract was washed with water (50 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by MPLC on silica gel with toluene as eluent to give chemically pure **4j** (0.86 g, 58% yield) as a colorless solid. Mp 173–174°C. MS, *m/z*(%): 328 (100), 251 (49), 223 (31), 207 (18), 152 (31), 105 (83), 77 (62). IR (KBr): ν 1732, 1604, 1511, 1480, 1290, 1252, 1171, 766, 695 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ 3.86 (3H, s, C–Me), 6.95 (2H, d, *J*=8.8 Hz, H-3″ and H-5″), 7.16 (2H, d, *J*=8.8 Hz, H-2″ and H-6″), 7.22 (2H, m, H-3′ and H-5′), 7.23 (1H, ddd, *J*=8.0, 1.2, 0.6 Hz, H-5), 7.24 (1H, m, H-4′), 7.36 (2H, d, *J*=6.7 Hz, H-2′ and H-6′), 7.52 (1H, ddd, *J*=7.9, 7.2, 1.2 Hz, H-7), 7.64 (1H, ddd, *J*=8.0, 7.2, 1.4 Hz, H-6), 8.40 ppm (1H, ddd, *J*=7.9, 1.4, 0.6 Hz, H-8). ^{13}C NMR (150 MHz, $CDCl_3$): δ 55.3, 114.5 (2C), 116.5, 120.5, 125.4, 127.9 (2C), 128.0, 128.3, 128.8, 129.2 (2C), 129.5, 132.3 (2C), 133.1, 134.6, 139.2, 151.0, 159.4, 162.3 ppm. Anal. calcd for $C_{22}H_{16}O_3$: C, 80.47; H, 4.91. Found: C, 80:68; H, 4.95.

4.2.19. 4-(1-Ethoxyethenyl)-3-phenylisocoumarin (4k). Iodide **5e** (1.50 g, 4.31 mmol) was reacted with tributyl (1-ethoxyethenyl)tin (**24**) (2.10 g, 5.81 mmol) in dry NMP (16 ml) for 47 h at 81–83°C in the presence of $PdCl_2(PhCN)_2$ (82.7 mg, 0.216 mmol), $AsPh_3$ (13.2 mg, 0.431 mmol) and CuI (82.1 mg, 0.431 mmol). The reaction mixture was then cooled to rt and worked up according to the same procedure employed in the preparation of **4j**. The crude reaction product was purified by MPLC on silica gel with benzene as eluent to give 97% chemically pure **4k** (0.88 g, 70% yield) as a yellow solid. Mp 100–101°C. MS, *m/z*(%): 292 (69), 291 (60), 263 (100), 247 (30), 207 (26), 178 (36), 173 (27), 105 (69), 77 (89). IR (KBr): ν 1733, 1621, 1316, 1223, 1084, 1060, 1018, 759, 698 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 1.38 (3H, t, *J*=7.2 Hz, Me), 3.94 (2H, q, *J*=7.2 Hz, OCH_2), 4.19 (1H, d, *J*=2.6 Hz, C=CH), 4.43 (1H, d, *J*=2.6 Hz, C=CH), 7.33–7.42 (3H, m, H-arom), 7.51 (1H, pseudo-dt, *J*=7.6, 1.6 Hz, H-6 or H-7), 7.62 (1H, dd, *J*=8.0, 1.6 Hz, H-5), 7.68–7.81 (3H, m, H-arom), 8.33 ppm (1H, dd, *J*=7.4, 1.6 Hz, H-8). ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.4, 63.4, 90.1, 113.9, 120.1, 124.7, 127.9, (2C), 128.0, 128.3 (2C), 129.2, 129.5, 132.8, 134.7, 137.6, 152.9, 155.0, 161.8 ppm. Anal. calcd for $C_{19}H_{16}O_3$: C, 78.06; H, 5.52. Found: C, 78.21; H, 5.40.

4.2.20. 4-Acetyl-3-phenylisocoumarin (4l). A solution of **4k** (0.400 g, 1.37 mmol) in THF (20 ml) was treated at 0°C with 2 M HCl (10.0 ml, 20.0 mmol) and the resulting mixture was stirred at rt for 2.25 h. It was then poured into brine (8 ml) and extracted with benzene (4×50 ml). The organic extract was washed with a saturated aqueous $NaHCO_3$ solution (3×30 ml) and water (30 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in benzene (4 ml) and purified by MPLC on silica gel with a mixture of petroleum ether and AcOEt (85:15) as eluent to give **4l** (0.34 g, 93% yield) as a colorless solid. Mp 126.5–129.5°C (lit.[40] mp 72–73°C). MS, *m/z*(%): 265, (15), 264 (94), 263 (100), 249 (50), 205 (12), 187 (14), 165 (20), 105 (71), 77 (55). IR (KBr): ν 1746, 1694, 1619, 1358, 1240, 1096, 1076, 765, 696 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 2.14 (3H, s, Me), 7.45–7.59 (4H, m, H-arom), 7.59–7.70 (3H, m, H-arom), 7.78 (1H, dt, *J*=7.7, 1.4 Hz, H-6 or H-7), 8.38 ppm (1H, dd, *J*=8.0, 1.8 Hz, H-5 or H-8). ^{13}C NMR (50 MHz, $CDCl_3$): δ 32.4, 118.2, 120.0, 123.8, 128.6 (3C), 128.9 (2C), 129.9, 131.0, 131.9, 134.2, 135.2, 153.2, 161.0, 201.9 ppm. Anal. calcd for $C_{17}H_{12}O_3$: C, 77.26; H, 4.58. Found: C, 77.17; H, 4.53.

4.2.21. 6,8-Dimethoxy-3-(3,4-dimethoxyphenyl)-4-methylisocoumarin (4g). Compound **5d** (1.00 g, 2.14 mmol) was reacted with tetramethyltin (**22**) (1.15 g, 6.41 mmol) at 81–82°C for 5.7 h according to the same procedure used to prepare **4a**. The crude reaction product was dissolved in CH_2Cl_2 (35 ml) and purified by MPLC on silica gel with a mixture of CH_2Cl_2 and AcOEt (80:20) as eluent to give **4g** (0.67 g, 89% yield) as a pale yellow solid. Mp 188–191°C. IR (KBr): ν 1720, 1602, 1518, 1249, 1208, 1173, 1139, 1078, 1017 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 2.24 (3H, s, C–Me), 3.94 (6H, br s, OMe), 3.95 (3H, s, OMe), 4.00 (3H, s, OMe), 6.50–6.60 (2H, m, H-5 and H-7), 6.92 (1H, d, *J*=8.5 Hz, H-5′), 7.08–7.18 ppm (2H, m, H-2′ and H-6′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 55.6, 56.0, 56.1, 56.4, 97.9, 98.6, 103.6, 107.9, 110.4, 112.5, 122.8, 126.1, 143.7, 148.6, 149.9, 152.2, 159.1, 163.7, 165.3 ppm. Anal. calcd for $C_{20}H_{20}O_6$: C, 67.41; H, 5.66. Found: C, 67.38; H, 5.60.

4.2.22. 6,8-Dihydroxy-3-(3,4-dihydroxyphenyl)-4-methylisocoumarin (4h). A mixture of **4g** (0.450 g, 1.26 mmol) and a 1 M CH_2Cl_2 solution of BBr_3 (10.0 equiv., 12.6 ml, 12.6 mmol) was stirred at rt for 96 h. The reaction mixture was worked up according to the procedure used for the synthesis of **3g**. The resulting crude reaction product was dissolved in THF (4 ml) and purified by MPLC on silica gel with a mixture of benzene and THF (60:40) as eluent to give **4h** (0.22 g, 58% yield) as a pale yellow solid. This compound was recrystallized from a mixture of AcOEt and hexane. Mp 274–276°C (decomposition). IR (KBr): ν 3211,

1655, 1612, 1509, 1451, 1376, 1296, 1256, 1169 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 2.14 (3H, s, C–Me), 6.40 (1H, s, H-arom), 6.49 (1H, s, H-arom), 6.87 (2H, s, H-arom), 6.98 (1H, s, H-arom), 9.38 (2H, br s, OH), 10.85 (1H, br s, OH), 11.33 ppm (1H, br s, OH). ^{13}C NMRv (50 MHz, $CDCl_3$): δ 13.5, 97.9, 101.2 (2C), 108.4, 115.1, 116.2, 120.9, 123.2, 140.7, 144.8, 146.7, 150.4, 162.8, 165.0, 165.5 ppm. Anal. calcd for $C_{16}H_{12}O_6$: C, 64.00; H, 4.03. Found: C, 63.95; H, 4.17.

4.2.23. Methyl 2-ethynylbenzoate (11). A slurry of ethynylzinc chloride (**26**) was prepared by addition of a THF solution of ethynylmagnesium bromide (0.50 M, 91.9 ml, 45.9 mmol) to a slurry of dry $ZnCl_2$ (8.14 g, 59.7 mmol) in THF (62 ml), which was stirred at 0°C. After stirring for additional 20 min at this temperature, a solution of methyl 2-(perfluoro-1-butanesulfonyloxy)benzoate (**25**) (16.62 g, 38.27 mmol), $Pd_2(dba)_3$ (351 mg, 0.383 mmol) and dppf (424 mg, 0.766 mmol) in THF (153 ml) was added and the resulting mixture was stirred at 62–66°C for 13 h. The crude reaction product, which was obtained after usual workup, was purified by MPLC on silica gel with a mixture of petroleum ether and Et_2O (90:10) as eluent to give **11** (3.68 g, 60% yield) as a pale yellow liquid. MS, *m/z*(%): 160 (70), 159 (44), 131 (13), 130 (16), 129 (87), 102 (77), 101 (100), 75 (45), 74 (27). IR (film): ν 2952, 1729, 1434, 1298, 1275, 1258, 1132, 1080, 758 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 3.42 (1H, s, $C_{sp}H$), 3.92 (3H, s, OMe), 7.38 (1H, pseudo-dt, *J*=7.7, 1.7 Hz, H-4 or H-5), 7.46 (1H, pseudo-dt, *J*=7.7, 1.7 Hz, H-4 or H-5), 7.62 (1H, dd, *J*=7.7, 1.7 Hz, H-3 or H-6), 7.93 ppm (1H, dd, *J*=7.7, 1.7 Hz, H-6 or H-3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 52.0, 81.8, 82.2, 122.4, 128.2, 130.1, 131.5, 132.2, 134.7, 166.1 ppm. Anal. calcd for $C_{10}H_8O_2$: C, 74.99; H, 5.03. Found: C, 74.89; H, 4.98.

4.2.24. (*E*)- and (*Z*)-3-(Iodomethylidene)phthalide [(*E*)- and (*Z*)-27]. To a deaerated solution of **11** (0.500 g, 3.12 mmol) in MeCN (17 ml) was added iodine (2.38 g, 9.36 mmol) and the mixture was stirred in the dark under nitrogen for 3 h. After this period of time a GLC analysis showed the presence of two compounds in a ca. 37:63 molar ratio, which were subsequently identified as (*E*)- and (*Z*)-**27**, respectively. The reaction mixture was then poured into a 10% aqueous $Na_2S_2O_3$ solution (30 ml) and extracted with AcOEt (4×30 ml). The organic extract was washed with water (10 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by MPLC on silica gel using a mixture of petroleum ether and AcOEt (90:10) as eluent. Concentration of the first eluted chromatographic fractions furnished 97% pure (*E*)-**27** (0.33 g, 38% yield) as a light brown solid. Mp 77.5–80°C. MS, *m/z*(%): 272 (100), 231 (12), 127 (14), 117 (10), 104 (17), 89 (84), 76 (25), 74 (15), 63 (16). IR (KBr): ν 1776, 1627, 1467, 1227, 1096, 1018, 785, 766, 686 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 6.54 (1H, s, =CH), 7.66 (1H, pseudo-t, *J*=7.5 Hz, H-6 or H-5), 7.79 (1H, pseudo-t, *J*=7.5 Hz, H-5 or H-6), 7.93 (1H, d, *J*=7.5 Hz, H-4 or H-7), 8.68 ppm (1H, d, *J*=7.5 Hz, H-7 or H-4). ^{13}C NMR (75 MHz, $CDCl_3$): δ 57.7, 124.1, 125.7, 126.4, 131.2, 131.4, 137.7, 148.9, 165.4 ppm. Anal. calcd for $C_9H_5IO_2$: C, 39.74; H, 1.85. Found: C, 39.61; H, 1.72. Concentration of the last eluted chromatographic fractions furnished pure (*Z*)-**27** (0.47 g, 56% yield) as a pale yellow solid. Mp 120–121.5°C. MS, *m/z* (%): 273 (10), 272 (100), 231 (10), 127 (16), 117 (13), 104 (15), 89 (74), 76 (24), 63 (15). IR (KBr): ν 1780, 1643, 1474, 1273, 1066, 983, 741, 713, 685 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 6.42 (1H, s, =CH), 7.60 (1H, pseudo-t, *J*=7.5 Hz, H-6 or H-5), 7.66 (1H, d, *J*=7.5 Hz, H-4 or H-7), 7.74 (1H, pseudo-t, *J*=7.5 Hz, H-5 or H-6), 7.88 ppm (1H, d, *J*=7.5 Hz, H-7 or H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 55.2, 120.3, 125.1, 125.7, 130.5, 134.7, 137.5, 152.9, 165.2 ppm. Anal. calcd for $C_9H_5IO_2$: C, 39.74; H, 1.85. Found: C, 39.69; H, 1.80. On the other hand, a crude mixture of (*E*)- and (*Z*)-**27** in a 70:30 molar ratio, respectively, was obtained by reaction of **11** with 1.0 equiv. of ICl in CH_2Cl_2 solution in the dark at rt for 6 h. Purification of this mixture by MPLC on silica gel allowed isolation of compounds (*E*)- and (*Z*)-**27** in 44 and 38% yield, respectively. It should also be noted that a crude mixture of (*E*)- and (*Z*)-**27** in a ca. 97:3 molar ratio, respectively, was obtained by reaction of a CH_2Cl_2 solution of ICl with a molar excess of **11** at rt.

4.2.25. 3-Methylidenephthalide (28). The crude reaction product of the palladium-catalyzed triethylammonium formate reduction of (*E*)-**27** (225 mg, 0.827 mmol), which was performed according to a procedure very similar to that used to prepare compounds **3c**, **3d**, **3f**, and **3j**, was purified by MPLC on silica gel with a mixture of petroleum ether and AcOEt (90:10) as eluent to give **28** (54.7 mg, 45% yield) as a colorless solid. Mp 54–56°C (lit.[41] mp 54–55°C). MS, *m/z* (%): 146 (100), 118 (18), 105 (13), 104 (70), 90 (40), 89 (16), 76 (55), 74 (12), 63 (8). IR (KBr): ν 1767, 1661, 1473, 1273, 1013, 950, 763, 686 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 5.23 (1H, d, *J*=3.0 Hz, =CH), 5.25 (1H, d, *J*=3.0 Hz, =CH), 7.65–7.70 (1H, m, H-arom), 7.69–7.76 (2H, m, H-arom), 7.92 ppm (1H, d, *J*=7.2 Hz, H-arom). ^{13}C NMR (50 MHz, $CDCl_3$): δ 91.2, 120.5, 125.0, 125.3, 130.4, 134.4, 138.9, 151.8, 166.8 ppm. These NMR data were in agreement with those previously reported.[42] It should be noted that compound **28** was also synthesized in 51% yield by palladium-catalyzed triethylammonium formate reduction of (*Z*)-**27**.

4.2.26. (*E*)-3-Ethylidenephthalide [(*E*)-29]. Compound (*E*)-**27** (0.800 g, 2.94 mmol) was reacted with tetramethyltin (**22**) (1.22 ml, 8.82 mmol) in NMP (11 ml) at 80°C for 5 h in the presence of $PdCl_2[P(o\text{-Tolyl})_3]_2$ (116 mg, 0.147 mmol) and CuI (56.0 mg, 0.294 mmol). The crude reaction product, which was obtained after usual workup, was purified by MPLC on silica gel with a mixture of petroleum ether and AcOEt (90:10) as eluent to give 97% pure (*E*)-**29** (0.38 g, 80% yield) as a colorless solid. Mp 57–59°C (lit.[43] mp 59–60°C). MS, *m/z*(%): 160 (82), 132 (13), 131 (24), 105 (25), 104 (100), 103 (20), 77 (20), 76 (41), 74 (12). IR (KBr): ν 2923, 1773, 1713, 1673, 1403, 1261, 1071, 908, 714 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 2.15 (3H, d, *J*=7.8 Hz, Me), 5.91 (1H, q, *J*=7.8 Hz, =CH), 7.56 (1H, pseudo-dt, *J*=7.5, 1.2 Hz, H-5 or H-6), 7.73 (1H, pseudo-dt, *J*=7.5, 1.2 Hz, H-5 or H-6), 7.86 (1H, d, *J*=7.5 Hz, H-4 or H-7), 7.93 ppm (1H, d, *J*=7.5 Hz, H-7 or H-4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 11.5, 108.0, 123.0, 125.4, 125.6, 129.4, 134.2, 138.2, 146.2, 166.9 ppm. The ^1H NMR data of this compound were in agreement with those previously reported.[44]

4.2.27. (*Z*)-3-(4-Chlorophenyl)methylidenephthalide [(*Z*)-31]. The crude reaction mixture, which was obtained by reaction of (*Z*)-**27** (424 mg, 1.56 mmol) with tributyl-4-chlorophenyltin (**30**) (847 mg, 2.11 mmol) in NMP (11 ml) at 80°C for 24 h in the presence of $PdCl_2(PhCN)_2$ 830 mg, 0.0780 mmol), $AsPh_3$ (47.8 mg, 0.156 mmol) and CuI (29.7 mg, 0.156 mmol), was poured into a saturated aqueous NH_4Cl solution (60 ml) and extracted with Et_2O (7×50 ml). The organic extract was partially concentrated and the residue (50 ml) was stirred for 18 h with a 8 M aqueous KF solution (50 ml) and filtered over Celite®. The solid residue was washed with AcOEt (5×40 ml). The collected filtrates were extracted with AcOEt (4×40 ml) and the organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by MPLC on silica gel with a mixture of hexane and benzene (50:50) as eluent to give compound (*Z*)-**31** (234 mg, 59% yield) as a pale yellow solid. Mp 148–150°C (lit.[30] mp 150°C). MS, *m*/*z*(%): 258 (31), 257 (16), 256 (100), 221 (12), 193 (27), 165 (41), 163 (11), 89 (16), 76 (16). IR (KBr): ν 1795, 1355, 1268, 1077, 971, 850, 758, 686, 516 cm^{-1} ^1H NMR (200 MHz, $CDCl_3$): δ 6.34 (1H, s, =CH), 7.31–7.38 (2H, br d, *J*=8.4 Hz, H-arom), 7.50–7.58 (1H, m, H-4), 7.69–7.80 (4H, m, H-arom), 7.91 ppm (1H, br d, *J*=7.6 Hz, H-7). ^{13}C NMR (50 MHz, $CDCl_3$): δ 105.6, 119.3, 123.2, 125.5, 128.8 (2C), 129.9, 131.1 (2C), 131.5, 134.0, 134.5, 140.2, 144.8, 166.6 ppm. The ^1H NMR data of this compound were in agreement with those previously reported.[30]

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