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Journal of Fluorine Chemistry 123 (2003) 101–108
JOURNAL OF FLUORINE CHEMISTRY
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Synthesis and anticancer activity of fluorinated analogues of combretastatin A-4

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Received 6 March 2003; received in revised form 8 April 2003; accepted 11 April 2003

Dedicated to Professor Eric Banks on the occasion of his 70th birthday

Abstract

The synthesis of a series of fluorinated benzaldehydes and their use in the Wittig synthesis of fluoro-substituted stilbenes is described. 3,5-Difluoro-4-hydroxybenzaldehyde (**6**) and 3-fluoro-4-methoxybenzaldehyde (**11**) are prepared by Duff formylation of 3,5-difluorophenol and 2-fluoroanisole, respectively. 2-Methoxy-3,4-difluorobenzaldehyde was obtained by Friedel–Crafts formylation of 2,3-difluoroanisole with α,α-dichloromethyl methyl ether. The aldehydes were used to make a series of fluorinated analogues of the anticancer combretastatins A-1, A-2 and A-4. The in vitro anticancer properties of the fluoro combretastatins are reported. The most active fluoro analogue 3-deoxy-3-fluoro-combretastatin A-4 (*Z*-**2**) retains the potent cell growth inhibitory properties of CA-4.

Keywords: Combretastatin A-4; *Combretum caffrum*; Stilbenes; Wittig; Benzaldehydes

1. Introduction

The combretastatins are a class of stilbenoid natural product isolated from the genus *Combretum* that comprises numerous shrubs and trees. Whilst being structurally simple these materials are exceptionally potent inhibitors of cell division. Combretastatin A-4, isolated from the bark of the African bush willow *Combretum caffrum*, is a promising anticancer drug [1]. Combretastatin A-4 (Scheme 1) has been found to strongly inhibit the polymerisation of tubulin by binding to the colchicine site [2]. Combretastatin A-4 is also able to elicit selective [3] and irreversible vascular shutdown within solid tumours [4], leaving normal vasculature intact. The cancer cells within the tumour are starved of nutrients and die. The antivascular effect of these agents derives from the role that tubulin and microtubules play in determining the elongated shape of vascular endothelial cells. The cellular microtubule network—a principal part of the cytoskeleton—plays a major role in maintaining cell shape, particularly in the case of neovasculature. The drugs cause the microtubules to rapidly depolymerize and the endothelial cells round up and very quickly block blood flow through the tumour vascular network. A pro-drug of combretastatin A-4, the water-soluble phosphate derivative CA-4P is now in phase II clinical trials. Related compounds also isolated from *C. caffrum* such as CA-1 [5] and CA-2 [6,7] (Scheme 1) share many of the interesting biological effects of CA-4.

We are actively engaged in a programme developing novel antimitotic agents [8] that target tubulin [9–12]. As part of this programme we needed to introduce fluorine into the combretastatins to investigate their ability to induce tumour vasculature damage via ^{18}F positron emission tomography (PET) and study their interaction with tubulin by ^{19}F NMR spectroscopy. We describe herein the synthesis of a series of ^{19}F-containing analogues of combretastatins (**1**–**4**, Scheme 1) along with some preliminary biological data. The replacement of a hydrogen or hydroxyl group by a fluorine atom is a strategy widely used in drug development to alter biological function. The substitution of the hydroxyl group with a fluorine atom would also clearly block any metabolic

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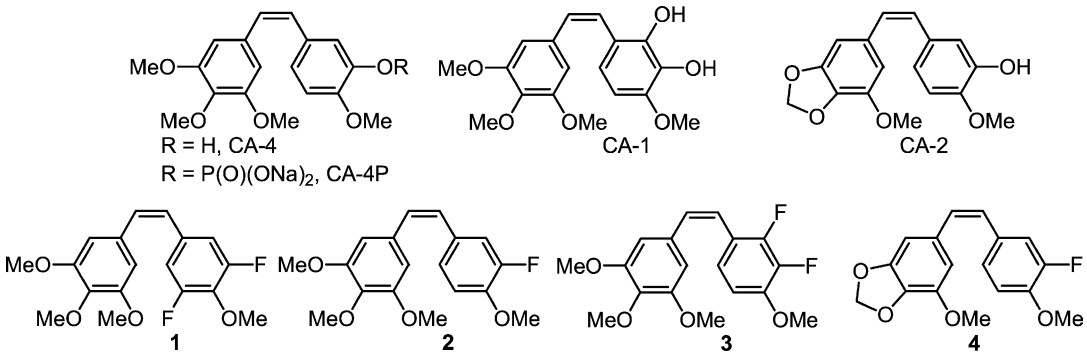
doi:10.1016/S0022-1139(03)00117-9



Scheme 1. Combretastatins A-1, A-2, A-4 and the target fluoro analogues **1**–**4**.

clearance via glucuronidation. Fluorine substitution can alter the chemical properties, cellular and systemic distribution and biological activity of drugs [13]. Fluorine substitution has been used widely to extend the biological half-life of many synthetic compounds and thereby produce drugs with a longer duration of action. With a series of fluoro-substituted combretastatins we would be able to study the effect fluorine substitution has upon their in vitro activity and in vivo drug distribution, clearance and the rate, route and extent of drug metabolism [14].

2. Results and discussion

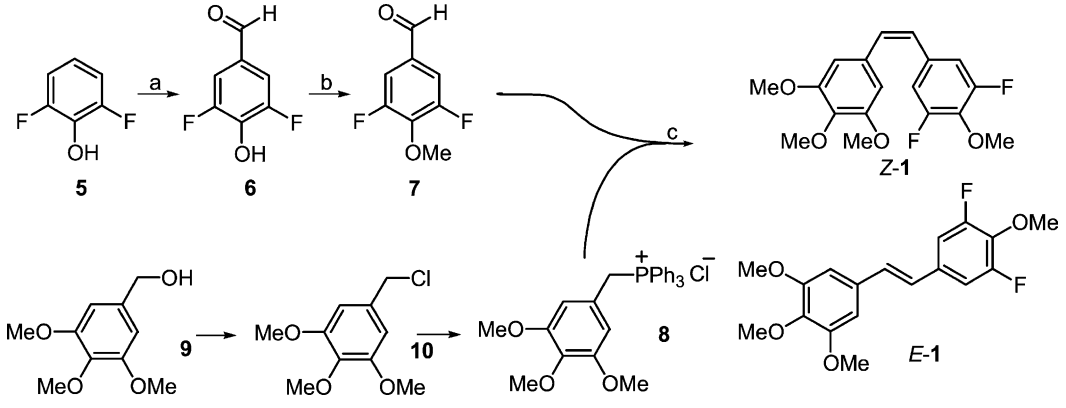
2.1. Synthesis of the fluorinated combretastatin analogues

We used a Wittig strategy to synthesise the fluorostilbenes **1**–**4**. The poor stereoselectivity observed in the usual reaction of a semi-stabilised yield derived from a benzyl phosphonium salt, provides convenient access to both *E*- and *Z*-stereoisomers, each of which can be assessed separately for biological activity. The cost of this convenience is, of course, met in the separation of the isomers.

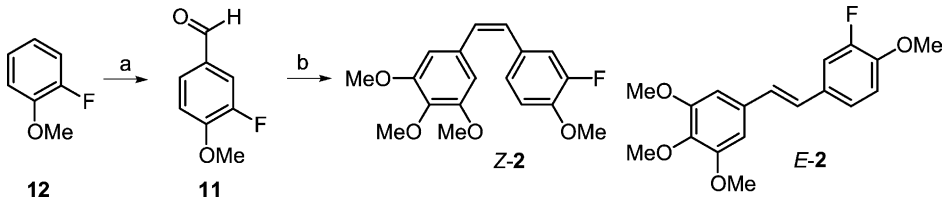
The synthesis of the 3,5-difluoro CA-4 derivative **1**, illustrated in Scheme 2, first required us to make 3,5-difluoro-4-methoxybenzaldehyde (**7**). This was achieved by means of the Duff reaction [15–17]. 3,5-Difluoro-4-hydroxybenzaldehyde (**6**) was thereby obtained by reaction of 2,6-difluorophenol (**5**) with hexamethylenetetramine and trifluoroacetic acid. Subsequent methylation of the intermediate aldehyde **6** gave 3,5-difluoro-4-methoxybenzaldehyde (**7**) (Scheme 2).

The triphenyl(3,4,5-trimethoxybenzyl)phosphonium chloride (**8**) was prepared by reaction of 3,4,5-trimethoxybenzyl chloride (**10**) (itself prepared [18] from the benzyl alcohol **9**) with triphenylphosphine. We strongly recommend that contact and exposure to the benzyl chloride **10** be avoided. Even taking the usual suitable precautions two members of our laboratory developed extreme allergic reactions when handling this material. Thus, the benzyl chloride **10** was obtained by treating the alcohol **9** with thionyl chloride. Removal of the solvent and excess thionyl chloride gave the benzyl chloride, which was immediately quaternized with triphenylphosphine in the same reaction flask.

The aldehyde **7** was reacted with triphenyl(3,4,5-trimethoxybenzyl)phosphonium chloride (**8**) and potassium *tert*-butoxide (*tert*-BuOK) to furnish the desired product **1** as a 1:1 mixture of the *cis*- and *trans*-isomers. The two isomers were then carefully separated by slow column chromatography to give *E*-**1** (35%; *E*:*Z* > 95:5) and *Z*-**1**



Scheme 2. Reagents and conditions: (a) hexamethylenetetramine, CF_3CO_2H, reflux, overnight, 67%; (b) MeI, K_2CO_3, DMF, RT, overnight, 73%; (c) *tert*-BuOK, MeOH, −78 °C.



Scheme 3. Reagents and conditions: (a) hexamethylenetetramine, CF_3CO_2H, reflux, overnight, 54%; (b) **8**, *tert*-BuOK, MeOH, −78 °C, 68%.

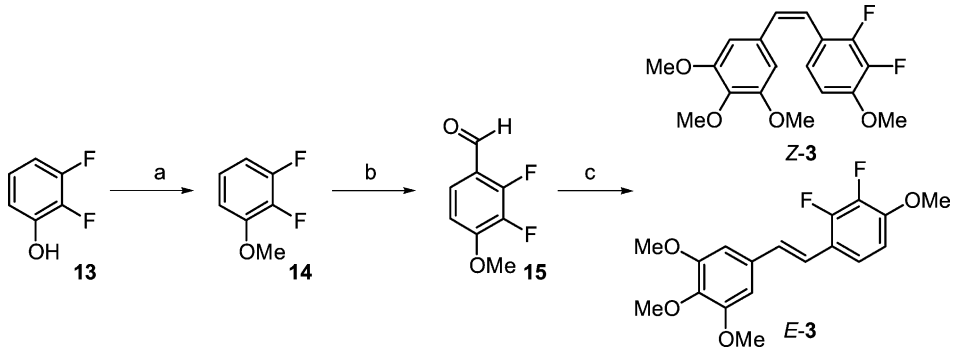
(35%; *Z*:*E* > 95:5). The stilbene components of the reaction exhibited R_f values of 0.52 and 0.49 (SiO_2, hexane:EtOAc, 7:1 (v/v)). The component showing the lowest R_f value appeared as a bright blue fluorescent spot, indicative of a *trans*-stilbene. The component possessing an R_f of 0.52 was identified by ^{1}H NMR as the *Z*-isomer, since a coupling constant of 12.4 Hz was observed for the olefinic protons (δ 6.44 and 6.52 ppm). The lower running spot was identified as the *E*-isomer, again from the olefinic proton coupling constant which was found to be 16.2 Hz (δ 6.85 and 6.95 ppm), a characteristic value for a *trans*-alkene.

The synthesis of the mono-fluorinated CA-4 analogue **2** is illustrated in Scheme 3. Although 3-fluoro-4-methoxybenzaldehyde (**11**) is commercially available it is expensive (Aldrich, 1 g, £27.50), so we sought to prepare it ourselves. Duff formylation of 2-fluoroanisole (**12**) (Aldrich, 28 g, £17.20) conveniently and exclusively afforded benzaldehyde **11** in acceptable yield (54%), with recovery of unreacted 2-fluoroanisole (∼35%).

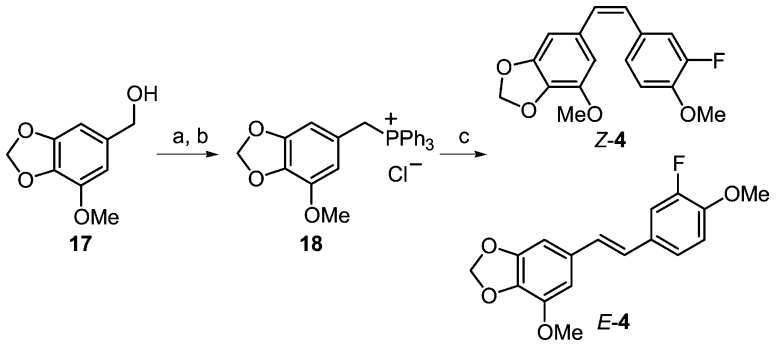
The aldehyde **11** underwent the Wittig reaction with triphenyl(3,4,5-trimethoxybenzyl)phosphonium chloride (**8**) using the conditions developed for the synthesis of **1**. The required alkene was obtained as an equal mixture of *E*- and *Z*-alkenes in 68% yield. The isomers were separated by column chromatography to give *E*-**2** (34%; *E*:*Z* > 95:5) and *Z*-**2** (34%; *Z*:*E* > 95:5). Once again the assignment of the geometries was determined by examining the ^{1}H NMR coupling constants of the olefinic proton signals. The signal for the olefinic protons of the *Z*-isomer appeared at δ 6.42 and 6.46 ppm with a coupling constant of 12.4 Hz whilst the signals for the *E*-isomer appeared within a multiplet (3H, m, δ 6.90–6.97 ppm). This downfield shift of the *E*-alkene olefin signals relative to those of the *Z*-alkene is characteristic for stilbenes.

The 2,3-dideoxy-2,3-difluoro CA-1 derivative **3** was synthesised from commercially available 2,3-difluorophenol (**13**) (Scheme 4). Methylation of this phenol with MeI and K_2CO_3 furnished 2,3-difluoroanisole (**14**) in 78% yield. Attempts to formylate this ether using the Duff reaction resulted in the destruction of the starting material and the formation of a sticky black tar. However, treatment of **14** with $TiCl_4$ and α,α-dichloromethyl methyl ether efficiently gave the aldehyde **15**. A small amount (3%) of 2-methoxy-3,4-difluorobenzaldehyde was also obtained, but the two aldehydes were easily separated by column chromatography. The Wittig reaction between the phosphonium salt **8** and the aldehyde **15** gave the 2,3-difluoro-3-deoxy CA-4 derivative **3** as a near equal mixture of *E*- and *Z*-isomers. The *cis*-isomer, isolated in 36% yield eluted first upon purification by column chromatography as the faster running material and was assigned as Z-**3** from the alkene coupling constant (doublets at δ 6.52 and 6.73 ppm, *J* 12.4 Hz). The *trans*-alkene *E*-**3**, obtained in 34% yield, again exhibited the characteristic downfield shifted alkene signal (2H, s, δ 7.23 ppm).

The phosphonium salt **18** for the synthesis of the 3-deoxy-3-fluoro CA-2 derivative **4** (Scheme 5) was prepared by sequential treatment of the benzyl alcohol **17** [19] with thionyl chloride and triphenylphosphine. The phosphonium salt **18** was then treated with 3-fluoro-4-methoxybenzaldehyde (**11**) to provide a 1:1 mixture of the *E*- and *Z*-isomers of the fluorinated combretastatin analogue **4**. The *E*- and



Scheme 4. Reagents and conditions: (a) MeI, K_2CO_3, DMSO, RT, 30 min, 78%; (b) $TiCl_4$, Cl_2CHOMe, RT, 30 min, 71%; (c) **8**, *tert*-BuOK, MeOH, −78 °C, 72%.



Scheme 5. Reagents and conditions: (a) $SOCl_2$, RT, 1 h; (b) PPh_3, 1,2-Cl_2–C_6H_4, 180 °C, 30 min, 79%; (c) **11**, *tert*-BuOK, MeOH, −78 °C, 37%.

Z-isomers were isolated pure by column chromatography although not very efficiently since the R_f of the *E*- and *Z*-isomers were very similar. As a result the yields of the two isomers were significantly less than those seen previously for similar compounds [*Z*-**4** (6%; *Z*:*E* > 95:5) and *E*-**4** (31%; *E*:*Z* > 95:5)]. Their geometries were assigned by examining the ^{1}H NMR spectra. For the case of the *Z*-isomer, the coupling constant of the olefinic hydrogens (δ 6.40 and 6.44 ppm) was found to be 12.0 Hz, characteristic of a *Z*-double bond. However, the coupling constant could not be determined for what was thought to be the *E*-isomer since the signal for the two olefinic protons appeared as a singlet at δ 6.84 ppm. Nevertheless there is little doubt that it is indeed the *E*-isomer. It also appeared under ultra violet light as a bright blue fluorescent spot on a TLC plate and was the lower running spot, both features of the *E*-stilbenes in the other examples described above.

2.2. Biological activity of the analogues

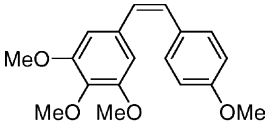
The in vitro cell cytotoxicity of the fluoro-substituted analogues was determined using the MTT assay [20]. This important assay is very widely used to determine the cytotoxicity of anticancer drugs. Cultured cancer cells are grown in the presence of the putative anticancer agent. The amount of viable cells remaining is then determined spectrophotometrically. This is based on the reduction of the yellow coloured MTT [3-(4′,5′-dimethylthiazol-2′-yl)-2,5-diphenyltetrazolium bromide] by mitochondrial dehydrogenases of metabolically active cancer cells to a purple–blue formazan precipitate. The activity is most often reported as its IC_{50} concentration—the concentration that results in a 50% decrease in cell growth relative to an untreated control. In these studies the growth inhibitory activity of the stilbenes was determined using the K562 human chronic myelogenous leukaemia cell-line. Our results are shown in Table 1.

It is clear that the presence of a fluorine atom in the monofluorinated analogues does not seriously compromise biological activity. All the analogues are cytotoxic. The fluoro CA-4 derivative *Z*-**2** retains all the activity of CA-4. This is significant and bodes well for the use of *Z*-**2** as a probe of combretastatin-like drug mechanism. The activity of *Z*-**4** is significantly less than that of CA-2, although both are much less potent than CA-4. Though in this case some caution is required when making this comparison as the data is derived from different cell-lines. The activity of *Z*-**1** is significantly reduced by the presence of two fluorine atoms flanking the 4-methoxy group. The position of the fluorine atoms clearly effects activity. The 2,3-difluoro derivative *Z*-**3** is a little more active than the 3,5-difluoro isomer *Z*-**1**. Nevertheless *Z*-**3** is remarkably more active than CA-1 itself. The stilbene **19** (3-deoxy CA-4) is as active as CA-4 itself in most cell-lines [21], although data for the K562 cell-line is not available. It is therefore noteworthy that fluorine can also be considered a good replacement for hydrogen on the combretastatin B-ring.

In conclusion we have developed efficient syntheses of 3,5-difluoro-4-methoxybenzaldehyde (**7**), 3-fluoro-4-methoxybenzaldehyde (**11**) and 3-fluoro-3-deoxy CA-4 (*Z*-**2**). The most active fluoro analogue *Z*-**2** retains the potent cell growth inhibitory properties of CA-4. Further biological studies of *Z*-**2**, including in vivo data, will be described in due course.

Table 1

Compound	Cell growth inhibition[a], IC_{50} (nM)
CA-4	3
Z-**2**	4
Z-**1**	50
CA-1	332
Z-**3**	20
CA-2	90[b]
Z-**4**	750
19	0.3[c]



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[a] Against the K562 human myelogenous leukaemia cell-line.
[b] Reported using the P388 murine leukaemia cell-line.
[c] Reported using the SKMEL-5 human melanoma cell-line (CA-4 has an IC_{50} of 0.1 nM with SKMEL-5).

3. Experimental details

3.1. General

200 MHz ^{1}H NMR spectra were recorded using a Bruker AC 200 NMR spectrometer whilst all 300 MHz ^{1}H and 75 MHz ^{13}C NMR spectra were recorded using a Bruker AC 300 Spectrometer. ^{13}C NMR spectra were recorded using distortionless enhancement by polarisation transfer. Both ^{1}H and ^{13}C spectra were recorded using $CHCl_3$ as an internal standard. Chemical ionisation (CI) mass spectra were recorded using a Kratos MS25 mass spectrometer; fast atom bombardment (FAB) mass spectra were recorded with a Kratos MS50 with a *meta*-nitrobenzyl alcohol matrix. Accurate mass determinations were carried out on a Kratos Concept IS spectrometer. Elemental analyses were performed using a Carlo-Erba 1106 elemental analyser. Infra red spectra were recorded using a Perkin-Elmer 783 spectrometer equipped with a PE 600 data station. Melting points were determined using an electrothermal melting point apparatus and were uncorrected. Column chromatography was conducted using silica gel 60 230–400 mesh (Merck). Silica gel TLC was conducted on precoated aluminum sheets (60 F_{254}) with a 0.2 mm thickness (Aldrich). Anhydrous methanol was obtained from Aldrich and used as supplied.

3.2. Preparation of 3,5-difluoro-4-hydroxybenzaldehyde (*6*)

Using the method developed by Diana et al. [22], a stirring solution of 2,6-difluorophenol (4.50 g, 34.6 mmol) and hexamethylenetetramine (4.85 g, 34.6 mmol) in TFA (35 cm^3) was heated at reflux under argon overnight. On cooling to room temperature the solvent was evaporated in vacuo and the crude residue taken up in DCM (75 cm^3). The mixture was washed with an aqueous solution (saturated) of $NaHCO_3$ (2×50 cm^3) and the separated aqueous layer acidified to pH 1 with concentrated HCl. The aqueous layer was extracted with DCM (2×50 cm^3), the combined organic fractions dried ($MgSO_4$) and evaporated in vacuo to afford **6** as a cream solid (3.65 g, 67%), mp 116–118 °C; Analysis: Found C, 53.5; H, 2.4%. Calculated for $C_7H_4O_2F_2$: C, 53.2; H, 2.6%. IR (KBr disc) ν 3200, 1690, 1600, 1540, 1340 cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ_H (ppm) 6.29 (1H, s, OH), 7.52 (2H, d, *J* 9.0 Hz, H-2 and H-6), 9.84 (1H, s, CHO); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C (ppm) 113.4 (CH, dd, J_{CF} 20.0 and 10.0 Hz), 128.6 (C, t, J_{CF} 10.0 Hz), 139.3 (C, t, J_{CF} 20.0 Hz), 152.7 (C, dd, J_{CF} 240.0 and 10.0 Hz), 189.5 (CH); ^{19}F NMR (188 MHz, $CDCl_3$) δ_F (ppm) –53.2 (d); FABMS *m/z* (%) 159 [(MH$^+$), 80%], 137 (100).

3.3. Preparation of 3,5-difluoro-4-methoxybenzaldehyde (*7*)

Iodomethane (0.70 cm^3, 11.5 mmol) was added to a stirring solution of 3,5-difluoro-4-hydroxybenzaldehyde (**6**) (1.52 g, 9.6 mmol) and K_2CO_3 (1.99 g, 14.4 mmol) in DMF (7.5 cm^3). The mixture was stirred at room temperature under argon overnight, diluted with DCM (50 cm^3) and washed with an aqueous solution (saturated) of $NaHCO_3$ (2×25 cm^3). The organic fraction was dried ($MgSO_4$) and evaporated in vacuo to afford **7** as a white solid (1.20 g, 73%), mp 37–38 °C [23] [mp 37–38 °C]; Analysis: Found C, 55.7; H, 3.5; F, 21.8%. Calculated for $C_8H_6O_2F_2$: C, 55.8; H, 3.5; F, 22.1%. ^{1}H NMR (300 MHz; $CDCl_3$) δ_H (ppm) 4.12 (3H, s, OCH_3), 7.43 (2H, d, *J* 9.0 Hz, H-2 and H-6), 9.82 (1H, s, CHO).

3.4. Preparation of triphenyl(3,4,5-trimethoxybenzyl)phosphonium chloride (*8*)

Thionyl chloride (15 cm^3, 205 mmol) was added to a solution of 3,4,5-trimethoxybenzyl alcohol (**9**) (Aldrich, 10.1 g, 51 mmol) in $CHCl_3$ (100 cm^3). The mixture was then stirred at RT for 1 h. The $CHCl_3$ and excess $SOCl_2$ were evaporated in vacuo. *CAUTION*: Avoid contact with the crude benzyl chloride. Several workers have developed severe allergic reactions with this material. 1,2-Dichlorobenzene (50 cm^3) was immediately added to the crude substituted benzyl chloride (in the same flask fitted with a reflux condenser) followed by PPh_3 (13.4 g, 51 mmol). The mixture was heated to reflux for 5 min and left to cool overnight. The brown crystals that formed were filtered off and washed with distilled hexane (3×100 cm^3). The product was recrystallised from $CHCl_3$/hexane to furnish the phosphonium salt **8** (17.49 g, 72%), mp 232–242 °C [18] [mp 234–236 °C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm) 3.47 (6H, s, 3 and 5-OCH_3), 3.72 (3H, s, 4-OCH_3), 5.45 (2H, d, J_{PH} 19 Hz, CH_2), 6.43 (2H, d, J_{PH} 4.0 Hz, H-2 and H-6), 7.55–7.79 (15H, m).

3.5. Preparation of (Z)-1-(3′,4′,5′-trimethoxyphenyl)-2-(3″,5″-difluoro-4″-methoxyphenyl)ethene (Z-*1*) and (E)-1-(3′,4′,5′-trimethoxyphenyl)-2-(3″,5″-difluoro-4″-methoxyphenyl)ethene (E-*1*)

Potassium *tert*-butoxide (0.75 g, 6.68 mmol) in MeOH (4 cm^3) was added to a stirred solution of phosphonium chloride **8** (1.6 g, 3.34 mmol) and 3,5-difluoro-*p*-anisaldehyde (**7**) (0.30 g, 1.86 mmol) in anhydrous MeOH (8 cm^3), at −78 °C. The resulting clear milky solution was stirred for a further 20 min at −78 °C before being allowed to slowly warm to room temperature. A dense off-white precipitate formed and the mixture was stirred at room temperature overnight. Water was added to the mixture and the resulting solution was extracted with DCM (3×30 cm^3). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to furnish a white solid. Column chromatography (SiO_2, hexane:EtOAc, 7:1 (v/v)) provided *Z*-**1** as a white crystalline solid (0.22 g, 35%), mp 60–62 °C; Found C, 64.2; H, 5.6; F, 10.9%. Calculated for $C_{18}H_{18}F_2O_4$: C, 64.3; H, 5.4; F, 11.3%; R_f 0.52 (SiO_2, hexane:EtOAc, 7:1 (v/v)); IR

(KBr disc) ν 3000–2800 (s), 1580, 1140 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ_H (ppm) 3.70 [6H, s, $(OCH_3)_2$], 3.83 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 6.36 (1H, d, J 12.4 Hz, C=CH), 6.44 (2H, s, H-2′ and H-6′), 6.52 (1H, d, J 12.4 Hz, CH=C), 6.85 (2H, d, J 9.0 Hz, H-2″ and H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C (ppm) 56.0, 61.0, 61.9, 103.7, 105.9, 112.7 (2C, dd, J_{CF} 10 Hz and J_{CF} 20 Hz), 127.3, 131.5, 132.2, 135.3, 137.6, 153.1, 155.3 (2C, dd, J_{CF} 250 Hz and J_{CF} 10 Hz); FABMS m/z (%) 336 [(M^+), 100%].

Further elution provided *E*-**1** as a white crystalline solid (0.22 g, 35%), mp 160–163 °C; Analysis: Found C, 64.5; H, 5.3%. Calculated for $C_{18}H_{18}F_2O_4$: C, 64.3; H, 5.3%; R_f 0.49 (SiO_2, hexane:EtOAc, 7:1 (v/v)); IR (KBr disc) ν 3000–2900 (m), 2860–2840, 1580, 1520–1510, 1130 (s), 960 (s) cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ_H (ppm) 3.85 (3H, s, OCH_3), 3.89 [6H, s, $(OCH_3)_2$], 3.99 (3H, t, J_{HF} 1.0 Hz, 4″-OCH_3), 6.68 (2H, s, H-2′ and H-6′), 6.85 (1H, d, J 16.2 Hz, C=CH), 6.95 (1H, d, J 16.2 Hz, CH=C), 7.05 (2H, d, J 9.8 Hz, H-2″ and H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C (ppm) 56.1 (2 × OCH_3), 61.0 (OCH_3), 61.9 (OCH_3), 103.6, 109.9 (2C, d, J_{CF} 20 Hz), 125.7, 129.9, 132.2, 132.6 (1C, t, J_{CF} 10 Hz), 135.7 (1C, t, J_{CF} 10 Hz), 138.3, 153.4, 155.8 (2C, dd, J_{CF} 250 Hz and J_{CF} 10 Hz); FABMS m/z (%) 336 [(M^+), 100%].

3.6. Preparation of 3-fluoro-4-methoxybenzaldehyde (*11*)

A stirring solution of 2-fluoroanisole (**12**) (4.46 cm^3, 39.7 mmol) and hexamethylenetetramine (5.57 g, 39.7 mmol) in TFA (35 cm^3) was heated at reflux under argon overnight. On cooling to room temperature the solvent was evaporated in vacuo and the crude residue dissolved in DCM (75 cm^3). The mixture was washed with an aqueous solution (saturated) of $NaHCO_3$ (2 × 30 cm^3), dried ($MgSO_4$) and evaporated in vacuo to afford the aldehyde **11** as a pale yellow solid (3.32 g, 54%), mp 30–31 °C [24] [mp 29–30 °C]; Analysis: Found C, 62.3; H, 4.6%. Calculated for $C_8H_7O_2F$: C, 62.0; H, 4.5%; ^1H NMR (300 MHz, $CDCl_3$) δ_H (ppm) 3.98 (3H, s, OCH_3), 7.08 (1H, t, $J_{HF,HH}$ 8.0 Hz, H-5), 7.55–7.65 (2H, m, H-2 and H-6), 9.87 (1H, d, J_{HF} 5.0 Hz, CHO).

3.7. Preparation of (Z)-1-(3′,4′,5′-trimethoxyphenyl)-2-(3″-fluoro-4″-methoxyphenyl)ethene (*Z*-**2**) and (E)-1-(3′,4′,5′-trimethoxyphenyl)-2-(3″-fluoro-4″-methoxyphenyl)ethene (*E*-**2**)

Potassium *tert*-butoxide (175 mg, 1.56 mmol) in EtOH (4 cm^3) was added to the phosphonium chloride **8** (622 mg, 1.3 mmol) and 3-fluoro-*p*-anisaldehyde (**11**) (100 mg, 0.65 mmol) in anhydrous EtOH (5 cm^3) at −78 °C. The resulting clear pink solution was stirred for a further 20 min at −78 °C before being allowed to slowly warm to room temperature. A pink precipitate quickly formed and the mixture was stirred overnight. Water (10 cm^3) was added causing the precipitate to redissolve and the resulting mixture was extracted with DCM (3 × 30 cm^3). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo yielding a pale pink solid. Column chromatography (SiO_2, hexane:EtOAc, 4:1 (v/v)) provided *Z*-**2** as a white powder (71 mg, 34%), mp 75–78 °C; Analysis: Found C, 68.0; H, 6.3; F, 5.9%. Calculated for $C_{18}H_{19}FO_4$: C, 67.9; H, 6.0; F, 6.0%; R_f 0.27 (SiO_2, hexane:EtOAc, 4:1 (v/v)); IR (KBr disc) ν 3000–2860, 2840–2800, 1620 (*cis*-C=C), 1140–1110 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ_H (ppm) 3.68 [6H, s, $(OCH_3)_2$], 3.83 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 6.42 (1H, d, J 12.5 Hz, C=CH), 6.46 (1H, d, J 12.5 Hz, CH=C), 6.47 (2H, s, H-2′ and H-6′), 6.82 (1H, t, J 8.8 Hz, H-5″), 6.98 (1H, d, J 8.8 Hz, H-6″), 7.03 (1H, dd, J 12.5 Hz and 2.0 Hz, H-2″); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C (ppm) 55.8, 56.0, 60.9, 105.8, 112.8, 116.3 (1C, d, J_{CF} 20 Hz), 125.1, 128.3, 129.9, 130.2, 132.2, 137.2, 146.4 (1C, d, J_{CF} 10 Hz), 151.8 (d, J_{CF} 240 Hz), 152.9; FABMS m/z (%) 318 [(M^+), 100%].

Further elution provided *E*-**2** as a white powder (71 mg, 34%), mp 173–177 °C; Analysis: Found C, 68.2; H, 5.7; F, 6.4. Calculated for $C_{18}H_{19}FO_4$: C, 67.9; H, 6.0; F, 6.0%; R_f 0.48 (SiO_2, hexane:EtOAc, 4:1 (v/v)); IR (KBr disc) ν 3000–2900 (s), 2840–2820, 1580, 1140, 960 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ_H (ppm) 3.86 (3H, s, OCH_3), 3.92 [9H, s, $(OCH_3)_3$], 6.71 (2H, s, H-2′ and H-6′), 6.90–6.97 (3H, m), 7.18 (1H, d, J 8.3 Hz, H-5″), 7.30 (1H, d, J 2.3 Hz, H-2″); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C (ppm) 56.0 (2 × OCH_3), 56.2 (OCH_3), 60.9 (OCH_3), 103.3 (CH), 113.1 (2 × CH), 122.8 (CH), 126.4 (CH), 127.8 (CH), 130.7, 132.8, 137.8, 147.1 (1C, d, J_{CF} 20 Hz), 152.5 (1C, d, J_{CF} 250 Hz), 153.3; FABMS m/z (%) 318 [(M^+), 100%].

3.8. Preparation of 1,2-difluoro-3-methoxybenzene (*14*)

Freshly ground K_2CO_3 (4.04 g, 28.8 mmol) was added to a solution of 2,3-difluorophenol (**13**) (2.5 g, 19.23 mmol) in DMSO (5 cm^3), followed by MeI (2.39 cm^3, 38.5 mmol). The resulting pale green solution was stirred for 30 min at RT after which time the solution appeared yellow in colour. Water (25 cm^3) was added and the mixture extracted with DCM (3 × 30 cm^3). The combined organic extracts were thoroughly washed with water (5 × 50 cm^3), dried ($MgSO_4$) and concentrated in vacuo to yield a pale yellow oil. Kugelrohr distillation (bp 50 °C at 2 mmHg) gave the ether **14** [25] as an oil (2.17 g, 78%), R_f 0.81 (SiO_2, hexane:EtOAc, 3:1 (v/v)); IR (Neat) ν 3020–2900, 1240 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ_H (ppm) 3.93 (3H, s, OMe), 6.75–6.82 (2H, m, H-1 and H-5), 6.99–7.05 (1H, m, H-6); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C (ppm) 56.4 (OMe), 108.4 (d, J_{CF} 2 Hz), 108.8 (d, J_{CF} 8 Hz), 123.1 (dd, J_{CF} 9 Hz and 5 Hz), 141.1 (dd, J_{CF} 247 and 15 Hz), 149.2 (dd, J_{CF} 8 Hz and 4 Hz), 151.3 (dd, J_{CF} 245 and 9 Hz); EIMS m/z (%) 129 [$(M-Me)^+$, 90%], 101 (100%).

3.9. Preparation of 2,3-difluoro-4-methoxybenzaldehyde (*15*)

Using the method developed by Scarpati et al. [26] $TiCl_4$ (2.74 cm^3, 25 mmol) in DCM (5 cm^3) was added dropwise

to 2,3-difluoroanisole (**14**) (1.5 g, 10.42 mmol) in DCM (5 cm^3) at 0 °C. The resulting orange solution was stirred at 0 °C for 5 min before a solution of dichloromethyl methyl ether (1.13 cm^3, 12.5 mmol) in DCM (5 cm^3) was added. The mixture was warmed to RT and stirred for 30 min. The now deep red reaction mixture was poured onto ice, and to this was added concentrated HCl (1 cm^3). Ether (30 cm^3) was added and the subsequent mixture was stirred for 30 min. The aqueous layer was separated and extracted with ether (2 × 30 cm^3). The combined organic extracts were repeatedly washed with water, dried ($MgSO_4$) and concentrated in vacuo furnishing an off-white solid. Column chromatography (SiO_2, hexane:EtOAc, 3:1 (v/v)) afforded the aldehyde **15** as a white solid (1.27 g, 71%), mp 65–66 °C; Analysis: Found C, 56.1; H, 3.2; F, 21.8%. Calculated for $C_8H_6F_2O_2$: C, 55.8; H, 3.5; F, 22.1%; R_f 0.43 (SiO_2, hexane:EtOAc, 3:1 (v/v)); IR (KBr disc) ν 2900–2700, 1590 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ_H (ppm) 4.01 (3H, s, OCH_3), 6.86–6.90 (1H, m, H-5), 7.62–7.67 (1H, m, H-6), 10.20 (1H, s, CHO); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C (ppm) 56.8 (OCH_3), 108.2 (d, J 10 Hz, Ar–<u>C</u>H), 118.7 (d, J_{CF} 10 Hz), 123.8 (d, J_{CF} 10 Hz, Ar–<u>C</u>H), 140.4 (dd, J_{CF} 248 and J_{CF} 14 Hz), 153.2 (dd, J_{CF} 237 and J_{CF} 12 Hz), 154.5 (d, J_{CF} 8 Hz), 185.4 (m, <u>C</u>HO); CIMS m/z (%) 190 [$(M$H + $NH_4)^+$, 80%], 35 (100%).

3.10. Preparation of (Z)-1-(3′,4′,5′-trimethoxyphenyl)-2-(2″,3″-difluoro-4″-methoxyphenyl)ethene (Z-3) and (E)-1-(3′,4′,5′-Trimethoxyphenyl)-2-(2″,3″-difluoro-4″-methoxyphenyl)ethene (E-3)

A solution of *tert*-BuOK (391 mg, 3.49 mmol) in MeOH (5 cm^3) was added to a mixture of 2,3-difluoro-*p*-anisaldehyde (**15**) (500 mg, 2.91 mmol) and the phosphonium chloride **8** (2.5 g, 5.23 mmol) in MeOH (10 cm^3) at −78 °C. The resulting brown solution was stirred at −78 °C for a further 30 min, after which time the mixture was allowed to spontaneously warm to RT. After stirring overnight a pale brown precipitate was observed. Water (25 cm^3) was added to the precipitate and the mixture was extracted with DCM (3 × 30 cm^3). The combined organics extracts were dried ($MgSO_4$) and concentrated in vacuo to yield a brown solid. Chromatography (SiO_2, hexane:EtOAc, 3:1 (v/v)) furnished *Z*-**3** as a white crystalline solid (348 mg, 36%), mp 103–105 °C; Analysis: Found C, 64.4; H, 5.3; F, 11.7%. Calculated for $C_{18}H_{18}F_2O_4$: C, 64.3; H, 5.4; F, 11.3%; R_f 0.46 (SiO_2, hexane:EtOAc, 3:1 (v/v)); IR (KBr disc) ν 3020–2900, 1580, 1520 cm^{-1}; ^{1}H NMR (400 MHz; CD_3COCD_3) δ_H (ppm) 3.70 [6H, s, $(OCH_3)_2$], 3.75 (3H, s, OCH_3), 3.96 (3H, s, OCH_3), 6.52 (1H, d, J 12.4 Hz, C=CH), 6.61 (2H, s, H-2′, 6′), 6.73 (1H, d, J 12.4 Hz, CH=C), 6.93–6.99 (1H, m, H-5″), 7.08–7.14 (1H, m, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C (ppm) 55.6, 55.9, 60.9, 105.7, 107.6 (d, J_{CF} 4 Hz), 118.9, 119.0, 120.8 (t, J_{CF} 3 Hz), 123.9 (t, J_{CF} 5 Hz), 131.9 (d, J_{CF} 2 Hz), 132.1, 137.4, 141.2 (dd, J_{CF} 246 and 15 Hz), 149.3 (dd, J_{CF} 225 and 11 Hz), 152.9; EIMS m/z (%) 336 [(M^+), 90 %], 49 (100%).

Further elution provided *E*-**3** as a white solid (330 mg, 34%), mp 159–161 °C; Analysis: Found C, 64.1; H, 5.3; F, 11.0%. Calculated for $C_{18}H_{18}F_2O_4$: C, 64.3; H, 5.4; F, 11.3%; R_f 0.39 (SiO_2, hexane:EtOAc, 3:1 (v/v)); IR (KBr disc) ν 3000–2800, 1590, 1520, 1100 cm^{-1}; ^{1}H NMR (400 MHz; CD_3COCD_3) δ_H (ppm) 3.79 (3H, s, OCH_3), 3.93 [6H, s, $(OCH_3)_2$], 3.99 (3H, s, OCH_3), 6.98 (2H, s, H-2′ and H-6′), 7.03–7.09 (1H, m, H-5″), 7.23 (2H, s, CH=CH), 7.47–7.53 (1H, m, H-6″); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C (ppm) 56.1 (OCH_3), 56.7 (OCH_3), 61.0 (OCH_3), 103.5, 108.4, 119.5 (d, J_{CF} 10 Hz), 120.6 (2C), 130.1, 132.9, 138.1, 141.4 (dd, J_{CF} 260 and 20 Hz), 149.7 (dd, J_{CF} 250 and 20 Hz), 148.2, 153.4; EIMS m/z (%) 336 [(M^+), 100%].

3.11. Preparation of (7-methoxy-1,3-benzodioxol-5-yl)methyltriphenylphosphonium chloride (18)

Thionyl chloride (1.5 cm^3, 20.5 mmol) was added dropwise to a solution of the alcohol **17** [19] (1 g, 5.49 mmol) in a 25 cm^3 round bottomed flask under nitrogen. The bubbling solution was stirred at room temperature for 1 h. The excess $SOCl_2$ was removed in vacuo. 1,2-Dichlorobenzene (5 cm^3) was added to the crude substituted benzyl chloride (in the same flask now fitted with a reflux condenser) followed by PPh_3 (1.44 g, 5.49 mmol). The mixture was heated to reflux for 30 min and left to cool overnight. The brown crystals that formed were filtered off and washed with distilled hexane (3 × 25 cm^3). The crystals were dissolved in hot $CHCl_3$ (15 cm^3). Hexane (10 cm^3) was added to the hot solution, which was then cooled in an ice-bath. After a white precipitate had formed further hexane (15 cm^3) was added. The product was filtered, washed with hexane (2 × 20 cm^3) and dried in vacuo to give the phosphonium salt **18** (2.2 g, 79%); ^{1}H NMR (300 MHz; $CDCl_3$) δ_H (ppm) 3.58 (3H, s, OCH_3), 5.44 (2H, d J_{PH} 19.0 Hz, CH_2), 5.88 (2H, s, 4-CH_2), 6.16 (1H, s, Ar–H), 6.66 (1H, s, Ar–H), 7.60–7.79 (15H, m).

3.12. Preparation of (Z)-1-(6′-methoxy-3′,5′-benzodioxol)-2-(3″-fluoro-4″-methoxy)ethene (Z-4) and (E)-1-(6′-Methoxy-3′,5′-benzodioxol)-2-(3″-fluoro-4″-methoxy)ethene (E-4)

A solution of K_2CO_3 (140 mg, 1.25 mmol) in MeOH was added to a mixture of 3-fluoroanisaldehyde (**11**) (100 mg, 0.65 mmol) and the phosphonium chloride **18** (478 mg, 1.04 mmol) in MeOH (4 cm^3) at −78 °C. The resulting brown solution was stirred at −78 °C for a further 30 min, after which time the mixture was allowed to spontaneously warm to RT. After stirring overnight a pale brown precipitate was observed. Water (25 cm^3) was added to the precipitate and the mixture was extracted with DCM (3 × 30 cm^3). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to yield a brown solid. Purification was achieved by chromatography (SiO_2, hexane:EtOAc, 5:1 (v/v)) furnishing *Z*-**4** as a white

crystalline solid (12 mg, 6%), mp 86–88 °C; Analysis: Found C, 67.5; H, 5.0; F, 6.6%. Calculated for $C_{17}H_{15}FO_4$: C, 67.5; H, 5.0; F, 6.3%; R_f 0.54 (SiO_2, hexane:EtOAc, 3:1 (v/v)); IR (KBr disc) ν 3000–2800, 1620, 1520, 1090 cm^{-1}; ^1H NMR (200 MHz; $CDCl_3$) δ_H (ppm) 3.78 (3H, s, OMe), 3.89 (3H, s, OMe), 5.97 (2H, s, CH_2), 6.40 (1H, d, J 12.0 Hz, CH=C), 6.44 (1H, d, J 1.8 Hz, H-7′), 6.47 (1H, d, J 1.8 Hz, H-2′), 6.49 (1H, d, J 12.0 Hz, C=CH), 6.85 (1H, t, J 8.8 Hz, H-5″), 7.01 (2H, m, H-2″ and H-6″); CIMS m/z (%) 302 (100%).

Further elution provided *E*-**4** as a brown powder (60 mg, 31%), mp 112–114 °C; Analysis: Found C, 67.5; H, 4.7; F, 6.0%. Calculated for $C_{17}H_{15}FO_4$: C, 67.5; H, 5.0; F, 6.3%; R_f 0.43 (SiO_2, hexane:EtOAc, 3:1 (v/v)); IR (KBr disc) ν 3200–2900, 1520, 1110 cm^{-1}; ^1H NMR (300 MHz; $CDCl_3$) δ_H (ppm) 3.91 (3H, s, OMe), 3.94 (3H, s, OMe), 5.98 (2H, s, CH_2), 6.63 (1H, d, J 2.2 Hz, H-7′), 6.72 (1H, d, J 2.2 Hz, H-2′), 6.84 (2H, s, olefinic), 6.93 (1H, t, J 8.5 Hz, H-5″), 7.13–7.16 (1H, dm, J 8.5 H-6″), 7.24 (1H, dd, J 12.6 and 2.1 Hz, H-2″); ^{13}C NMR (75 MHz; $CDCl_3$) δ_C (ppm) 56.3 (OCH_3), 56.6 (OCH_3), 99.8, 101.5 (CH_2), 107.0, 113.1, 113.5 (d, J_{CF} 3 Hz), 122.7 (d, J_{CF} 3 Hz), 126.1, 127.7, 131.4 (d, J_{CF} 8 Hz), 132.2, 135.1, 143.7, 147.2 (d, J_{CF} 8 Hz), 149.2, 151.0–154.3 (d, J_{CF} 244 Hz); FABMS m/z (%) 302 [(M^+), 100%].

3.13. Assessment of cytotoxicity

This was performed as previously described [27].

Acknowledgements

We thank the Cancer Research Campaign, EPSRC (Total Technology Studentship to L.A.H.) and the Chemistry Department of UMIST for support of this work and Sally Haran, Dee Whittacker and Dr Tim Ward of the PICR Cell Culture Unit for providing invaluable assistance during the cell growth inhibition experiments. Finally, we would like to thank Professor Eric Banks for all his generous support and encouragement over the years and for showing us so enthusiastically the power and beauty of fluorine chemistry. To an inspirational teacher, valued colleague and friend, we say a great thank you.

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