Structural Studies on Bioactive Compounds. 32.[1] Oxidation of Tyrphostin Protein Tyrosine Kinase Inhibitors with Hypervalent Iodine Reagents

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Hydroxylated styrenes (tyrphostins) undergo oxidation by hypervalent iodine oxidants such as [(diacetoxy)iodo]benzene (DAIB) to give a range of products depending on the structure of the phenolic substrate, the solvent, the oxidant stoichiometry, and the purification strategy. Conditions have been developed to modify the phenolic component of the tyrphostin without affecting the appended substituted-vinyl moiety. Novel products include: unstable 2-acyloxy-2-methoxy-4-(substituted-vinyl)cyclohexadienones and their rearrangement products 2-acyloxy-4-hydroxy-3-methoxy-1-(substituted-vinyl)benzenes; phenyliodoniophenolates and their rearrangement products iodophenoxytyrphostins; and 3,3′-dialkoxy-2,2′-dihydroxy-5,5′-di(substituted-vinyl)biphenyls. None of these oxidation products displayed enhanced activity in vitro in the NCI 60-cell line panel or in a panel of human breast cancer cell lines, compared to their tyrphostin precursors. The inhibitory activity of three representative tyrphostins (**3e**,**n**, **28**) was not modulated by aerobic/anaerobic conditions in MCF-7 and MDA 468 cells and was independent of EGFR status in clones of ZR75B cells transfected with this receptor. Basal growth of MCF-7 cells was unaffected by co-administration of the growth factors EGF, TGF-α, IGF-I, and IGF-II, and the new agents did not inhibit EGFR and c-erbB2 autophosphorylation in cell lysates from MDA 468 or SkBr3 cells, respectively, suggesting that receptor tyrosine kinases are not targets for these compounds. Growth stimulation by the tyrphostin **3n** in the ER^+ breast cell lines MCF-7, T47D, and ZR75-1 was abolished by 1 μM tamoxifen, suggesting that this compound has estrogen agonist activity.

Introduction

Protein tyrosine kinases (PTKs) occupy critical loci in cell signaling pathways. They catalyze the transfer of the γ-phosphate group of ATP to the phenolic group of a specific tyrosine residue in the substrate.[2] Phosphotyrosine-containing proteins then trigger a cascade of signaling events downstream of the kinase. Although less than 0.01% of intracellular phosphorylation involves tyrosine residues,[3] the involvement of PTKs and their regulatory phosphatases in human cancer is demonstrated by the fact that a large proportion of viral oncogene products have been identified as constitutively active PTKs, which often have human proto-oncogenic counterparts.[4]

Certain peptidic growth factors, e.g. epidermal growth factor (EGF), exert their stimulatory effects via tyrosine kinase enzymes, with aberrant expression of the growth factor or kinase being evident in many cancers. The epidermal growth factor receptor (EGFR) is overexpressed in a range of tumor types, notably breast, prostatic, hepatic, renal, bladder, esophageal, laryngeal, stomach, lung, and ovarian cancers. For example, 57% of primary breast tumor biopsies were shown to be EGFR +ve; moreover, the receptor is overexpressed in estrogen receptor (ER) −ve tumors[5] which respond poorly to hormonal therapy.[6] Also, in ovarian cancer the presence of EGFR mRNA correlates with poor prognosis and reduced survival.[7] Not surprisingly, there has been great interest in therapeutic strategies to antagonize the functions of receptor PTKs: these include the development and marketing of an antibody (herceptin) raised against the extracellular domain of EGFR and the clinical evaluation of a family of orally active 4-anilinoquinazolines which compete with ATP and inhibit EGFR autophosphorylation with IC_{50} values in the nanomolar range.[8]

After the identification of the flavone quercetin **(1)** (Chart 1) as a PTK inhibitor,[9] a diverse array of structures ranging from complex natural product macrocyclic quinones such as herbimycin A and geldanamycin to low molecular weight heterocycles to simple phenols has been studied.[3,10] In the phenolic class the lead structure erbstatin **(2)** inhibits EGFR autophosphorylation in membrane fractions and intact cells by partially competing with ATP and the substrate at the enzyme active site.[11] Structurally related hydroxylated styrenes of general structure **3** (the 'tyrphostins' where group R is often a carboxylic acid-derived moiety and R^1, R^2, and R^3 comprise at least one phenolic group) developed by Gazit, Levitzky, and co-workers[12] can differentiate between the highly homologous EGFR and c-erbB2 receptor in some cases.[13]

Our interest in phenolic PTK inhibitors has focused on a characteristic property of the phenolic group − its instability, especially under oxidizing conditions. Erbstatin has a short half-life (<30 min) in fetal calf serum,[14] and the lack of correlation between the activity of tyrphostins against isolated enzymes and their effects in vitro and in vivo could be attributed to oxidative decomposition or bioconversion of the inhibi-

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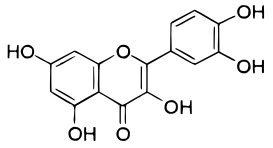
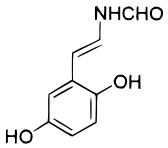
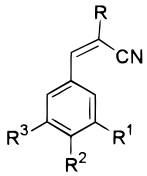
Chart 1



1 quercetin



2 erbstatin



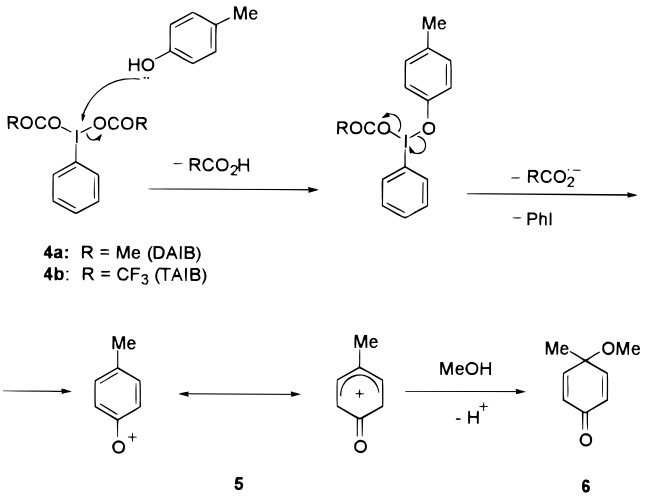
3 tyrphostins

	R	R^1	R^2	R^3
a:	CN	OMe	OH	H
b:	$CONH_2$	OMe	OH	H
c:	CO_2Me	OMe	OH	H
d:	CN	OEt	OH	H
e:	pyridin-2-yl	OMe	OH	H
f:	CN	OH	OMe	H
g:	$CONH_2$	OH	OMe	H
h:	CN	H	OH	H
i:	$CONH_2$	H	OH	H
j:	CO_2Me	H	OH	H
k:	$CONH(CH_2)_3Ph$	H	OH	H
l:	$CSNH_2$	H	OH	H
m:	$C(NH_2):C(CN)_2$	H	OH	H
n:	pyridin-2-yl	H	OH	H
o:	CN	NO_2	OH	H
p:	CN	OH	OH	H
q:	$CSNH_2$	OH	OH	H
r:	pyridin-3-yl	OMe	OMe	H
s:	CN	OMe	OAc	H
t:	CN	OAc	OAc	H
u:	CN	OH	OH	OH
v:	$CONH_2$	OMe	OH	(benzothiazol-2-yl)-methylthio

tors to other moieties more/or less bioactive. Perhaps significantly, the di- and triphenolic tyrphostins **3p,u** decompose in solution to chemical species which are more active PTK inhibitors.[15] The slow onset of action of tyrphostin **3q**[16] may be explained by the agent requiring prior oxidative activation: in contrast, tyrphostins devoid of hydroxy groups (e.g. **3r**) have a rapid onset of activity in cellular systems.[17] This raises the intriguing possibility that the unstable phenolic substrates might undergo redox interconversions between the phenolic state and more bioactive quinone (or other) oxidized states.

In this paper we describe our results using the hypervalent iodine reagents [(diacetoxy)iodo]benzene (DAIB, **4a**) and [bis(trifluoroacetoxy)iodo]benzene (TAIB, **4b**) to oxidize a range of tyrphostins and some simple model phenols. These oxidants have been used to mimic key steps in opioid biosynthesis as well as in the construction of natural product skeletons and thus may be regarded as 'biomimetic' in certain cases.[18] The biological properties of certain of the oxidized products have been compared with the starting tyrphostins to ascertain whether the oxidative activation hypothesis is sustainable.

Scheme 1



4a: R = Me (DAIB)
4b: R = CF_3 (TAIB)

5 **6**

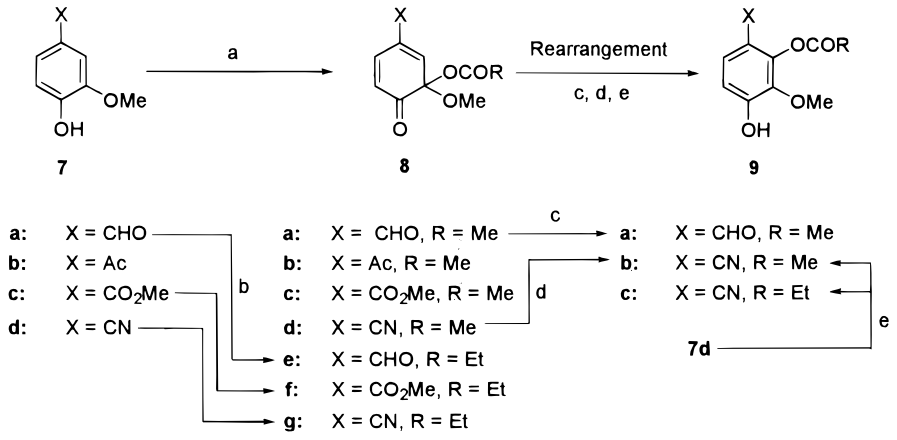
Chemistry

At its simplest oxidation of a phenol (e.g. 4-methylphenol) by DAIB **(4a)** in methanol generates a phenoxenium cation ↔ π-carbocation reactive intermediate **5** which is quenched by methanol to yield the cyclohexadienone **6** (Scheme 1).[19] A wide range of nucleophiles can participate as trapping partners (including intramolecular trapping leading to spirocyclic products), and the reactive dienone products can subsequently participate in a range of intermolecular processes, such as dimerizations, rearrangements, and Diels–Alder reactions. These reactions are exquisitely sensitive to the nature of the phenolic substrate, solvent, participating nucleophile, reaction conditions, and isolation procedures but, notwithstanding these variables, have great synthetic utility.[20]

Representative tyrphostins **3a–u** required as starting materials for this study were prepared by Knoevenagel condensations between substituted benzaldehydes and acetonitriles in ethanol–piperidine in near-quantitative yields.

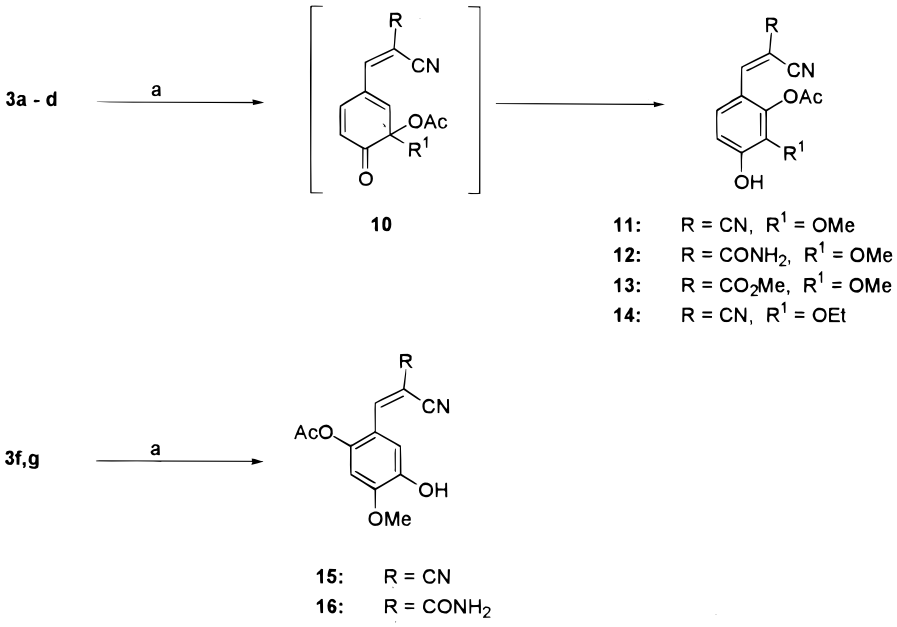
Oxidation of Model Phenols and Tyrphostins with DAIB in Acetic Acid: Formation and Rearrangement of Cyclohexadienones. Oxidations of some model disubstituted phenols with DAIB gave clues to the complexities of these reactions. Thus when a dilute solution of vanillin **(7a)** in an acetic acid–nitromethane mixture was oxidized with 1.1 equiv of DAIB at 25 °C the product was an unstable 2-acetoxy-4-formyl-2-methoxycyclohexadienone **(8a)** (72%). Nitromethane is a solvent which facilitates oxidations of phenols bearing EWGs,[21] and high concentrations of acetic acid ensure that the reactive intermediate is efficiently quenched. In a consistent pattern, the reaction can be extended to other phenols bearing EWGs **7b–d** to yield cyclohexadienones **8b–d** in >60% isolated yields and also to the use of propionic acid as solvent, but yields of propionyloxycyclohexadienones **8e–g** were lower (38–54%) (Scheme 2). The poorer yields are probably due to a combination of steric effects and also to the competitive intervention of a stoichiometric amount of acetic acid liberated from the DAIB which

Scheme 2[a]



[a] Reagents and conditions: (a) DAIB, AcOH−$MeNO_2$, 25 °C; (b) DAIB, $EtCO_2H$−$MeNO_2$, 25 °C; (c) $BF_3{\cdot}Et_2O$, Et_2O; (d) AcOH, reflux; (e) DAIB, $EtCl_2H$−$MeNO_2$, 25 °C, then reflux in AcOH.

Scheme 3[a]



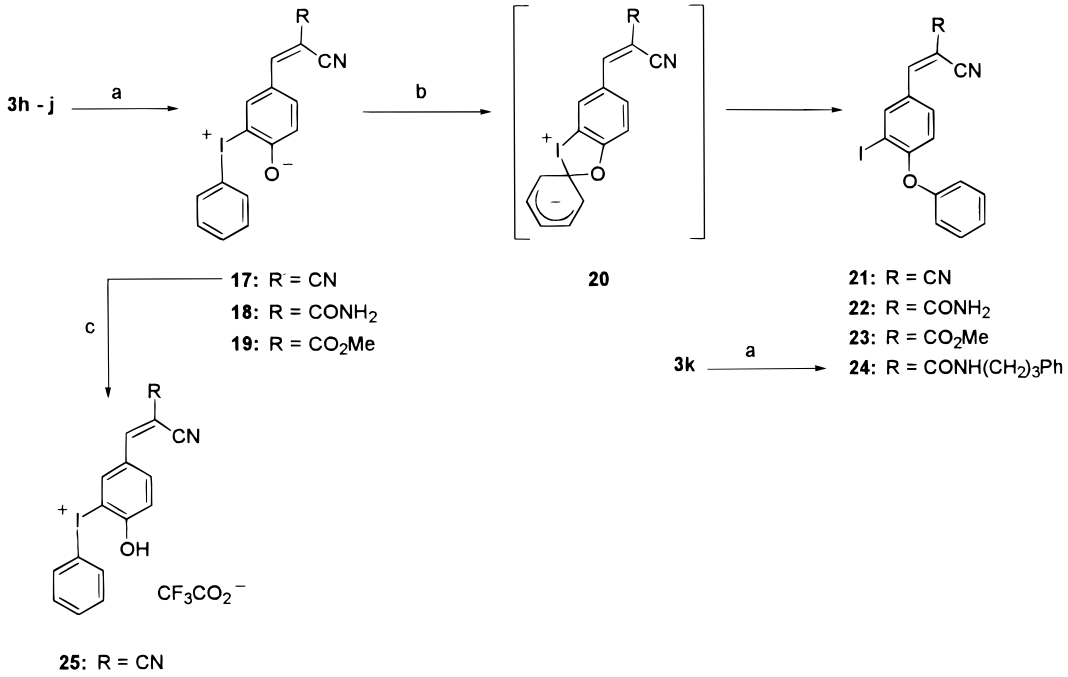
[a] Reagents and conditions: (a) DAIB, AcOH, 50 °C, 2 h.

competes with propionic acid for the reactive intermediate and complicates chromatographic purification. Possibly use of TAIB as oxidant, which liberates a weaker nucleophile trifluoroacetate, would widen the scope of this reaction to participation of a range of acids.

The cyclohexadienones are unstable yellow oils, which solidify below 0 °C. They decompose readily on silica gel, and isolated yields vary according to the speed with which chromatography columns are eluted: faster elution gives higher yields. The ^{13}C NMR spectra of **8a**−**g** show the dienone carbonyl at δ 189.2−191.0 and the tetrahedral carbon C-2 at δ 91.8−93.1, characteristic of analogous monoketal structures,[22] as are the IR absorptions at 1726−1744 and 1692−1701 cm^{-1} for the acyloxy and dienone carbonyl groups, respectively.[23] Further evidence that the compounds are cyclohexadienones comes from their acid-catalyzed rearrangement to 3-acyloxy-2-methoxy-4-(substituted)phenols **9** (Scheme 2). Thus, when vanillin **(7a)** was oxidized with DAIB at 25 °C in acetic acid alone the expected cyclohexadienone **8a** (49%) was accompanied by the acetoxyphenol **9a** (16%); from similar oxidation of **7d** cyclohexadienone **8d** (59%) and acetoxyphenol **9b** (14%) were isolated. A pure sample of **8a** was rearranged to the phenol **9a** by boron tribromide etherate in ether, suggestive of an intramolecular rearrangement. However, when **7d** was oxidized in propionic acid and then the product rapidly isolated and washed with sodium bicarbonate to remove residual acid, followed by refluxing in acetic acid, both the acetoxyphenol **9b** and the corresponding propionyloxyphenol **9c** were isolated in low yields, implying a competitive intermolecular aromatization.

Tyrphostins **3a**−**d** bearing a 3-alkoxy-4-hydroxy substitution pattern do not oxidize to form isolable cyclohexadienones, but the presence of these unstable intermediates **10** can be inferred by the isolation of phenols **11**−**14** albeit in poor yields (27−50%) (Scheme 3). The isomeric 4-alkoxy-3-hydroxytyrphostins **3f**,**g** also gave the 2-acetoxy-4-methoxy-5-hydroxytyrphostins **15** and **16** (30−40%). The poor isolated yields in these oxidations reflect the fact that products **11**−**16** are still phenolic and possible substrates for further oxidation,

Scheme 4[a]



[a] Reagents and conditions: (a) DAIB, AcOH, 25 °C, 3−9 days; (b) AcOH, reflux; (c) CF_3CO_2H.

as well as chromatographic difficulties in isolating the main components of the reactions.

Oxidation of Tyrphostins with DAIB in Acetic Acid: Formation and Rearrangement of Phenyliodoniophenolates. When the monohydroxytyrphostins **3h**−**j** were oxidized by DAIB in acetic acid over 3−9 days at 25 °C the reaction took a different path and the products were yellow phenyliodoniophenolates **17**−**19** (61−80%) (Scheme 4). Similar products have been isolated from phenols bearing conjugated EWGs[24,25] where there is a balance between the nucleophilicity of the phenol and the electron deficiency of the ring. Although relatively stable when stored below 25 °C, these zwitterions readily decompose when exposed to light or heat or when stored in solution. Compound **17** rearranged to the iodophenoxytyrphostin **21** in hot acetic acid. The mechanism may involve a 1,4-sigmatropic process[24] or an intramolecular nucleophilic substitution via a spiro intermediate **20** (R = CN). A 'one-pot' process is a more efficient approach to form these iodophenoxytyrphostins: thus phenols **3h**−**k** could be converted to iodoniophenolates with DAIB in acetic acid at 25 °C; refluxing acetic acid then completed the rearrangement to iodophenoxy compounds **21**−**24**. Although isolated yields were only moderate (33−70%) these products could be separated readily from starting phenols and iodoniophenolates by column chromatography, the polar zwitterions remaining at the origin. These novel stable tyrphostins could be chromatographed to analytical purity and have further synthetic potential for conversion to dibenzofuran derivatives by photolytic, radical, or palladium(0)-mediated cyclizations.

The structure of the unstable phenyliodoniophenolate **17** was confirmed by the compound showing the correct molecular ion (by HRMS) and by the ^{1}H NMR spectrum (in DMSO-d_6) which showed an upfield resonance for the H-6 proton adjacent to the phenolate oxygen at δ 6.47−6.49 (Figure 1A). Protons in the *ortho* position of the pendant phenyl ring absorb downfield at δ 8.03−8.06 due to the effect of the positively charged iodine. If the spectrum of **17** is run in TFA, the phenolate is protonated (Scheme 4) which results in a downfield shift of the H-6 proton from δ 6.49 to 7.14 for the trifluoroacetate salt **25** (Figure 1B). The *ortho* protons in the phenoxy substituent of the iodophenoxytyrphostin **21** (Figure 1C) show a marked upfield shift to δ 7.14 in DMSO-d_6.

Although other monohydroxytyrphostins **3l**−**o** did not afford iodophenoxytyrphostins under similar DAIB/acetic acid oxidations (no reaction with **3l**,**o**; complex mixture with **3m**,**n**), an alternative route to such compounds is feasible. For example, oxidation of 4-hydroxybenzaldehyde **(26a)** in the 'one-pot' process gave the iodophenoxybenzaldehyde **27a** (32%); similarly methyl 4-hydroxybenzoate **(26b)** afforded methyl 3-iodo-4-phenoxybenzoate **(27b)** (76%) with DAIB/acetic acid. Knoevenagel condensation of **27a** with 2-pyridylacetonitrile furnished the iodophenoxytyrphostin **28** which could not be formed directly from **3n**.

Oxidation of the dihydroxytyrphostin **3p** with DAIB resulted in black resinous material. The choice of oxidant (DAIB, TAIB, DDQ, Fe^{3+}, AcO_2H), solvent (MeOH, EtOH, EtOAc, AcMe, Et_2O, DCM, THF, TFE, 1,1,1,3,3,3-hexafluoro-2-propanol), and reaction temperature (0−50 °C) had no effect on the observed result. The dihydroxy functionality is responsible for this negative outcome, because tyrphostins **3r**−**t** in which both hydroxyl groups are protected are unreactive under these conditions. Attempts to trap the presumed *o*-quinone intermediates with nucleophiles[26] or as Diels−Alder adducts[27] proved unsuccessful with these very electron-deficient catechols.

Oxidation of Tyrphostins with DAIB in Acetonitrile: Formation of 2,2′-Dihydroxybiphenyls. Oxidative phenolic coupling can be accomplished by a

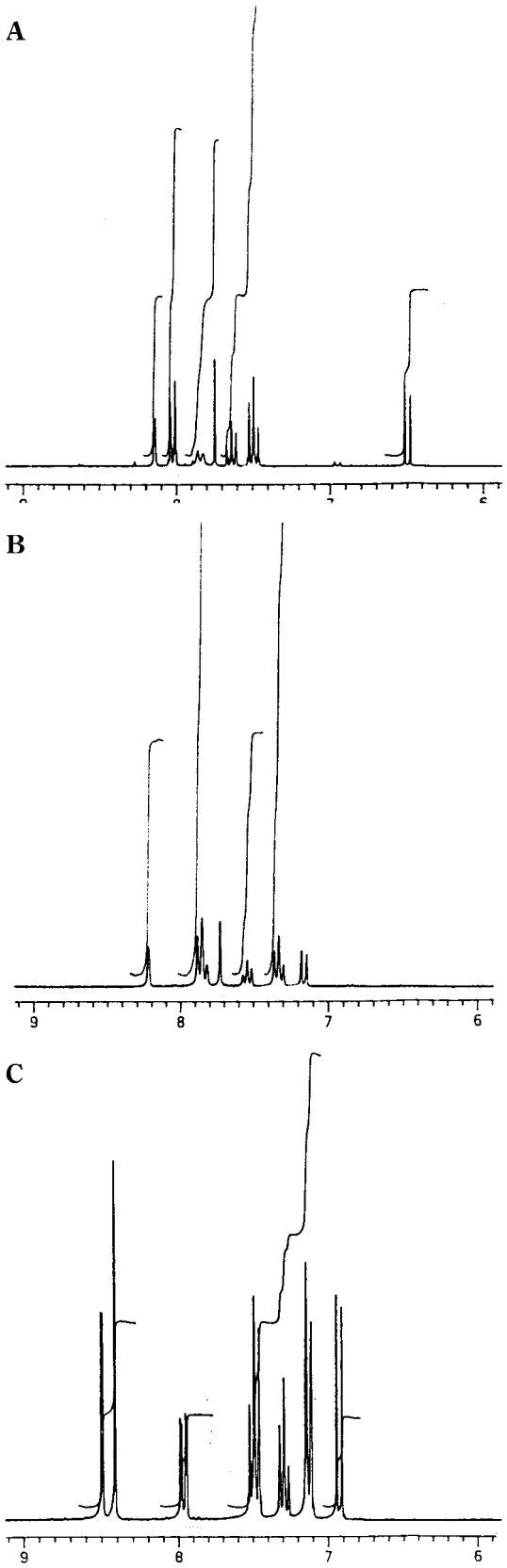
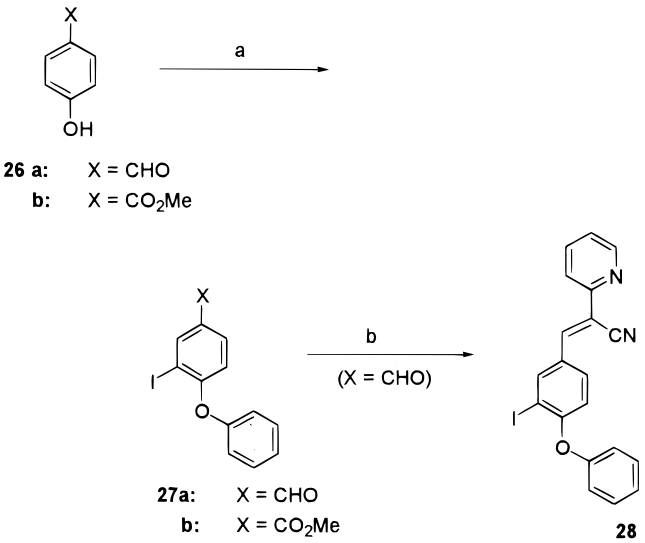


Figure 1. A, ^{1}H NMR spectrum of **17** in DMSO-d_6; B, ^{1}H NMR spectrum of **25** in TFA; C, ^{1}H NMR spectrum of **21** in DMSO-d_6.

range of oxidants, in aqueous or organic solvents or in the solid state. Most recently 2-naphthols have been converted to 1,1′-binaphthols (>90%) using atmospheric oxygen catalyzed by solid Lewis acids.[28] When DAIB (0.5 mol equiv) was added to a solution of vanillin **(7a)** in acetonitrile the color changed from green to yellow and the biphenyl **29a** precipitated (52%) (Scheme 6). Similarly, phenols **7b**,**c** afforded biphenyls **29b**,**c**. The sparingly soluble biphenyl **29a** could be converted to a more soluble monotosyl derivative **30** with tosyl chloride in pyridine. Surprisingly, **30** underwent a double Knoevenagel condensation with methyl 2-cyanoacetate in piperidine−EtOH to form the unusual bis(tyrphostin) **31** (52%) which, potentially, can exist as a pair of separable enantiomers (atropisomers) noninterconvertible by restricted rotation around the pivotal phenyl−phenyl bond. Oxidation of the tyrphostins **3a**−**e** similarly gave bis(tyrphostins) **32**−**36** in yields ranging from 45% to 69%: in these cases the more insoluble products precipitated in a pure state from the reaction medium. This oxidative coupling is limited to 2-alkoxy-4-(substituted)phenols, but the reaction fails with 2-methoxy-4-nitrophenol. Coupling, when it occurs, is exclusively *ortho*−*ortho* to yield 2,2′-dihydroxybiphenyls: no *para* coupling was observed when *o*-vanillin (2-hydroxy-3-methoxybenzaldehyde) **(37)** was oxidized with DAIB in acetonitrile. Instead, a small yield (10%) of 5-acetoxy-2-hydroxy-3-methoxybenzaldehyde **(38)** was isolated (Scheme 6), the source of the acetoxy group being the oxidant DAIB.

Scheme 5[a]

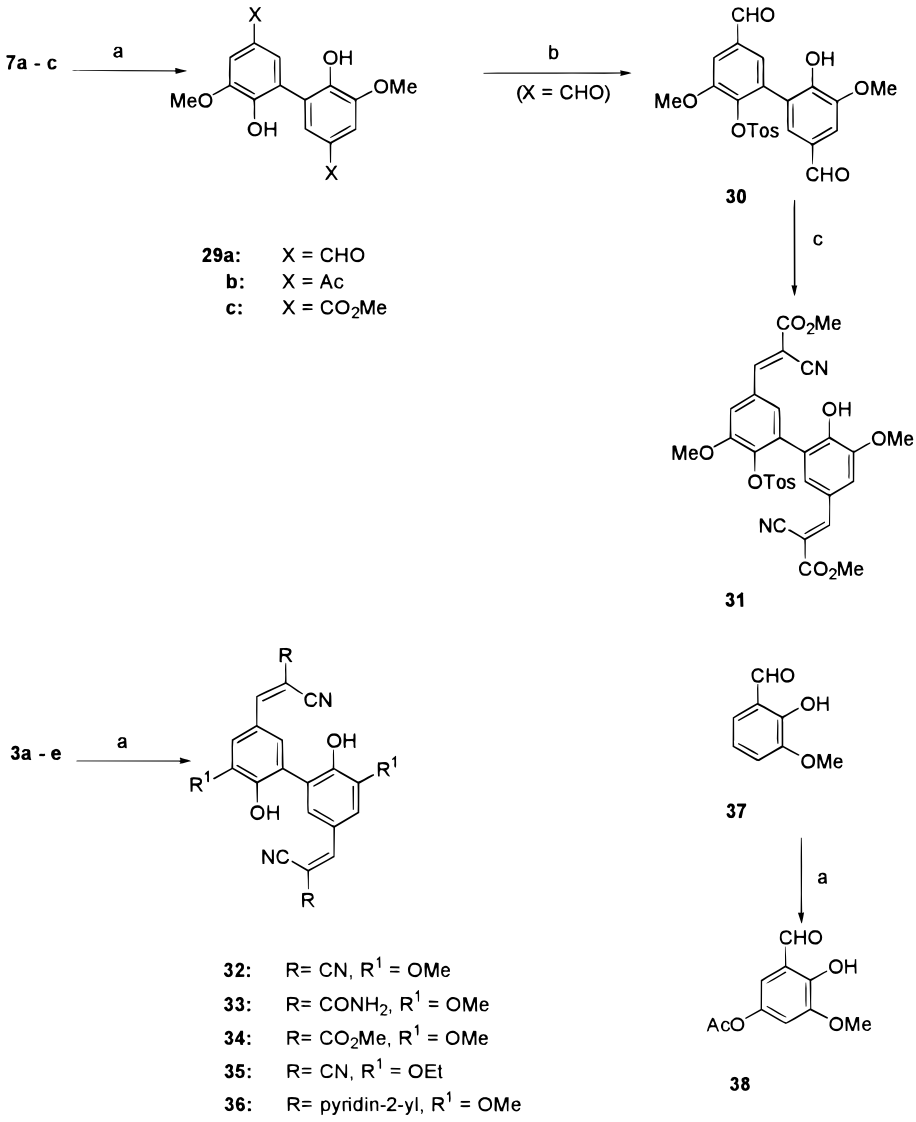


[a] Reagents and conditions: (a) DAIB, AcOH, reflux; (b) 2-pyridylacetonitrile, piperidine, EtOH, reflux.

Biological Results and Discussion

In Vitro Activity. Representative novel acetoxytyrphostins **11**−**16**, iodoniophenolates **17**−**19**, iodophenoxytyrphostins **21**−**24** and **28**, and biphenyls **29a**−**c** and **32**−**36** were tested in the NCI panel of 60 human cell lines.[29] Mean GI_{50} values were in the range 20−80 μM with no notable pattern of selectivity in cells of a particular tumor type. These values were comparable to mean GI_{50} values for quercetin, erbstatin, and representative 4-anilinoquinazolines extracted from the NCI Developmental Therapeutics Program Internet Site.[30] The quinones herbimycin A and geldanamycin gave mean GI_{50} values of 2.32 and 0.327 μM, respectively. The poor activity of the tyrphostin type of PTK inhibitors (GI_{50} generally > 100 μM)[30] in this 48-h drug

Scheme 6[a]



[a] Reagents and conditions: (a) DAIB, MeCN, 25 °C, 24 h; (b) tosyl chloride, pyridine, 25 °C; (c) methyl cyanoacetate, piperidine, EtOH, reflux.

exposure assay may reflect the fact that these compounds require a prolonged drug exposure to exert inhibitory effects on their target enzymes and thence cell growth. Also, their profile of activity does not appear to correlate with levels of EGFR and erbB-2 mRNA in the cells lines.[31] Evidently the new tyrphostins tested in the present work, which are all variants of the prototypic agents, albeit at a higher oxidation level, do not have enhanced activity in this assay.

Three tyrphostins **(3e**,**n** and **28)** were evaluated by MTT assay in a panel of human breast cell lines with varying receptor characteristics[32] in a 7-day exposure assay (Table 1). All three compounds were less active against MCF-7/adr than MCF-7 wt suggesting they can act as substrates for P-glycoprotein-mediated efflux. The most potent agent was tyrphostin **3e,** and the most sensitive cell line to the compounds was the ER^-,$EGFR^+$ MDA 468. Efficacy of **3e** against MDA 468 was directly related to drug exposure time: after a 24-h exposure to drug the IC_{50} value was 100 μM; 48 h, 2 μM; 72 h, 0.7 μM; and 96 h, 0.5 μM. This corroborates our conclusion that short-term assays (e.g. 48-h drug exposure) are inappropriate as a primary screen for this class of compound.

Table 1. Activity of Tyrphostins Against Human Breast Tumor Cell Lines in Vitro

	IC_{50} (μM)[a]						
compd	MCF-7	MCF-7/adr	MDA 468	MDA 231	T47D	SkBr3	ZR75-1
3e	0.7	52.8	0.06	51.0	6.0	0.5	2.6
3n	15.0	51.2	0.45	40.3	36.3	7.8	48.5
28	20.0	25.2	0.3	39.8	48.7	6.5	24.7

[a] All IC_{50} values were the mean of at least three experiments.

The activities of **3e**,**n** and **28** were assessed in MTT assays against MCF-7 and MDA 468 cells under aerobic and anaerobic conditions where any observed differential cytotoxicity might indicate a requirement for oxidative or reductive activation, respectively.[33] However, no differential activity was observed (data not shown).

Effects of Growth Factors on the Activity of Tyrphostins. As the activity of the tyrphostins against the MDA 468 line may be related to its high EGFR content, compounds **3e**,**n** and **28** were evaluated against clones of human breast ZR75B cells transfected with the

Table 2. Comparisons of Basal Cell Growth of MCF-7 Human Breast Tumor Cells with Growth Factor Stimulation in the Presence of Tyrphostins

	IC_{50} (μM)[a]				
compd	basal growth	+EGF	+TGF-α	+IGF-I	+IGF-II
3e	2.7	2.1	2.3	2.9	3.2
3n	73.8	71.7	67.7	72.8	77.1
28	41.8	45.8	41.0	39.1	46.5

[a] All IC_{50} values are the mean of at least three experiments.

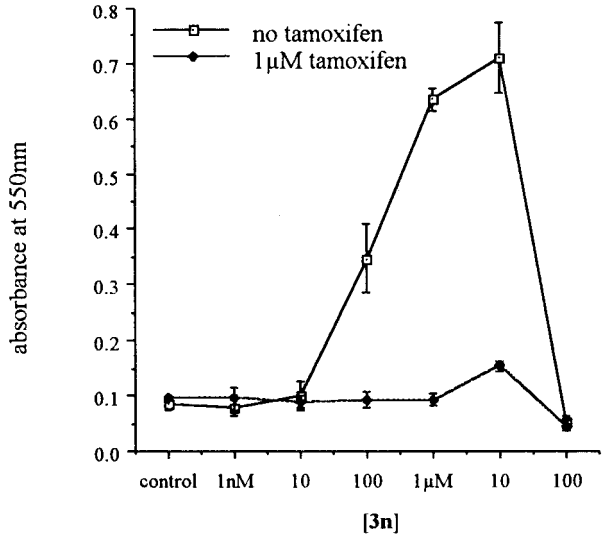


Figure 2. Effect of tamoxifen on the growth stimulation caused by **3n** in MCF-7 wt cells.

human EGF receptor and the pSV2-*neo* selectable marker.[34] The ZR75B parental line had 2×10^4 receptor sites/cell; clone 2, 4.3×10^4 sites (marker transfect only); clone 13, 3.1×10^5 sites; and clone 11, $>1 \times 10^6$ sites. The overexpressing clones have been reported to be more resistant to doxorubicin, vinblastine, and 5-FU[34] but had not previously been used to evaluate potential EGFR inhibitors to which they should be more sensitive. However, clones 13 and 11 do not show hypersensitivity to any of the compounds or reference tyrphostin **3u** (data not shown).

Because the growth of MCF-7 cells can be sustained in media deprived of growth factors (phenol red-free medium supplemented with charcoal-stripped FCS) the effects of individual exogenously applied RTK ligands (e.g. EGF, TGF-α, IGF-I, IGF-II) on tyrphostin inhibition of basal cell growth can be evaluated. Stimulated MCF-7 cell growth was compared with basal growth. No modulation of the activity of compounds **3e**,**n** and **28** was observed (Table 2), suggesting that the compounds exhibit no selectivity toward any of these phosphorylation pathways.

Of the new compounds **3n** elicited growth stimulation in three of the panel of breast cells lines when cultured in phenol red-free medium supplemented with charcoal-stripped FCS. Significantly, the mitogenic effect was restricted to the ER^+ cell lines MCF-7, T47D, and ZR75-1. For example, growth stimulation of MCF-7 cells was completely abolished in the presence of 1 μM tamoxifen, suggesting that this monophenolic compound has estrogen agonist activity (Figure 2).

Tyrosine Kinase Inhibitory Effects of Novel Oxidized Tyrphostins. Certain tyrphostins have been shown to be selective tyrosine kinase inhibitors able to distinguish between the highly homologous EGFR and c-erbB2.[12,13] Novel compounds **3e**,**n** and **28** were assessed (Western blotting followed by densitometry) for their ability to inhibit EGFR and c-erbB2 autophosphorylation in cell lysates from MDA 468 (overexpress EGFR) or SkBr3 cells (overexpress c-erbB2). Whereas the known EGFR inhibitor **3u** (IC_{50} 3 μM)[12] in this study partially inhibited EGFR autophosphorylation (70% of control at 100 μM) none of the test compounds were inhibitory at this concentration over a 24-h drug exposure time (Figure 3A).

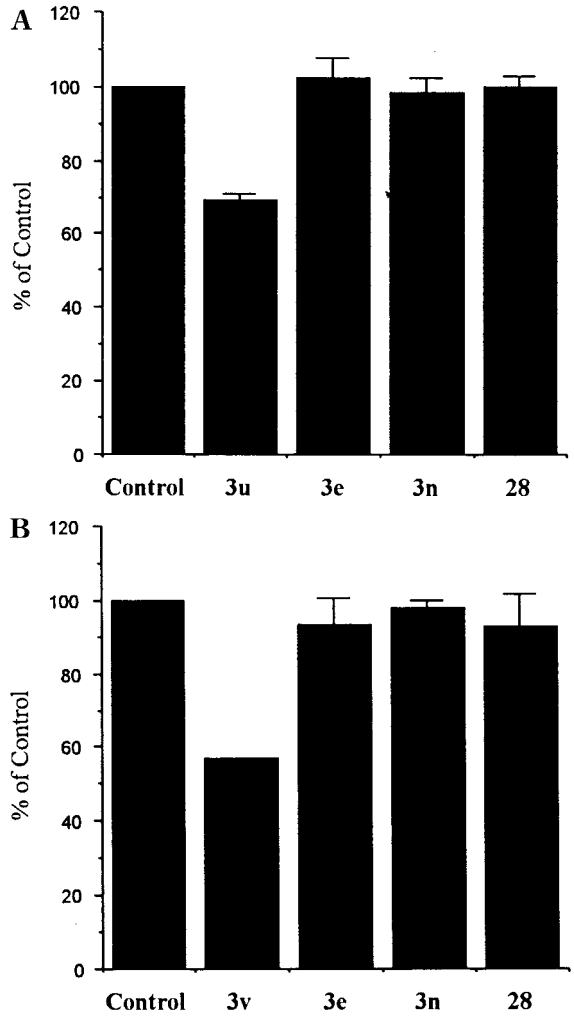


Figure 3. (A) Inhibitory effect of tyrphostins on EGFR autophosphorylation. Results are expressed as % band intensity relative to the control and are the mean of at least three experiments (EGFR band ca. 170 kDa). (B) Inhibitory effect of tyrphostins on c-erbB2 autophosphorylation. Results are expressed as % band intensity relative to the control and are the mean of at least three experiments (c-erbB2 band ca. 185 kDa).

The tyrphostin AG825 **(3v)** is known to be a selective inhibitor of c-erbB2, exhibiting an IC_{50} value of 0.35 μM (cf. 19 μM against EGFR).[13] In the present work **3v** only partially inhibited c-erbB2 autophosphorylation (69% of control at 100 μM) (Figure 3B). In contrast, compounds **3e**,**n** and **28** had no effects at the same concentration. These results suggests that EGF-stimulated EGFR and c-erbB2 are not targets for these agents.

Conclusions

The DAIB oxidation chemistry serves to demonstrate both the diversity of products that can be synthesized from tyrphostin type bioactive phenols and the marked dependency of the reactions on phenol substitution, solvent, and oxidant stoichiometry. Phenyliodoniophenolates can be formed from 4-hydroxytyrphostins and 2-acyloxy-2-alkoxycyclohexadienones or 3,3′-dialkoxy-2,2′-dihydroxybiphenyls from 3-alkoxy-4-hydroxytyrphostins. The initial products of oxidation may undergo subsequent rearrangement. Thus phenyliodoniophenolates rearrange to iodophenoxytyrphostins when heated, and 2-acyloxy-2-alkoxycyclohexadienones aromatize to 2-acyloxy-4-hydroxy-3-methoxy-1-(substituted) benzenes in acid. It is therefore apparent that, depending on the choice of phenol and conditions, a range of oxidation products can be synthesized.

The oxidation products generally proved to be poor inhibitors of cell growth in the NCI screen. This result is not entirely unsurprising in view of the relatively poor performance of other known PTK inhibitors and tyrphostin type compounds in the panel and may reflect the relatively slow onset of PTK inhibition with these types of compound. Compounds **3e**,**n** and **28** showed some selective toxicity in a panel of breast cancer cell lines, being especially active in MDA468 cells ($EGFR^+$). However the compounds did not affect EGFR or erbB-2 receptor autophosphorylation or modulate MCF-7 cell growth induced by EGF, TGF-α, IGF-I, or IGF-II, suggesting that they are not receptor tyrosine kinase inhibitors. The growth inducing effects of **3n** in MCF-7 cells grown in phenol red-free medium could be reversed by the antiestrogen tamoxifen, suggesting **3n** can behave as an estrogenic mitogen.

Experimental Section

General. Melting points were obtained using a Gallenkamp melting point apparatus and are uncorrected. IR spectra were measured using a Mattson 2020 Galaxy Series FT-IR spectrometer as KBr disks. ^1H and ^{13}C NMR spectra were acquired using a Bruker ARX250 spectrometer at 250.13 and 62.9 MHz, respectively. Coupling constants are in hertz (Hz). Mass spectra were recorded using a Micromass platform spectrometer or an AEI MS-902 spectrometer using electron impact (EI), electrospray (ES), chemical ionization (CI), or atmospheric pressure CI (AP) techniques. Nominal mass spectra were obtained using an AP+ ionization technique and accurate mass spectra using an EI+ technique unless otherwise stated. Flash column chromatography refers to medium-pressure silica gel (C60 (40−60 μm)) preparative column chromatography, unless otherwise stated. Petrol ether refers to the fraction which boils between 60 and 80 °C.

General Method A for Synthesis of Tyrphostins. To the substituted benzaldehyde (2.0 g) dissolved in ethanol (20 mL) were added the 1-substituted acetonitrile (1.1 mol equiv) and piperidine (5−10 drops). The mixture was refluxed for 0.5−18 h, then allowed to cool. Addition of water precipitated the desired tyrphostin which was collected on a filter, washed with water and dried in vacuo.

The following known tyrphostins were synthesized and had melting points and spectroscopic properties consistent with literature values: 4-hydroxy-3-methoxybenzylidenemalononitrile, **3a**; 3-ethoxy-4-hydroxybenzylidenemalononitrile, **3b**; 2-cyano-3-(4-hydroxy-3-methoxyphenyl)propenamide, **3c**; methyl 2-cyano-3-(4-hydroxy-3-methoxyphenyl)propenoate, **3d**; 3-hydroxy-4-methoxybenzylidenemalononitrile, **3f**; 2-cyano-3-(3-hydroxy-4-methoxyphenyl)propenamide, **3g**; 4-hydroxybenzyl-denemalononitrile, **3h**; 2-cyano-3-(4-hydroxyphenyl)propenamide, **3i**; methyl 2-cyano-3-(4-hydroxyphenyl)propenoate, **3j**; 2-cyano-3-(4-hydroxyphenyl)propenethioamide, **3l**; 2-amino-1,1,3-tricyano-4-(4-hydroxyphenyl)-1,3-butadiene, **3m**; 3-(4-hydroxyphenyl)-2-(2-pyridyl)propenenitrile, **3n**; 4-hydroxy-3-nitrobenzylidenemalonitrile, **3o**; 3,4-dihydroxybenzylidenemalonitrile, **3p**; 4-acetoxy-3-methoxybenzylidenemalonitrile, **3q**; 3,4-diacetoxybenzylidenemalonitrile, **3r**.

3-(4-Hydroxy-3-methoxyphenyl)-2-(2-pyridyl)propenitrile, 3e. A mixture of vanillin (1.0 g, 6.6 mmol) and 2-pyridylacetonitrile (0.82 g, 6.9 mmol) in ethanol (15 mL) was reacted for 18 h according to general method A to give a white solid (1.53 g, 92%): mp 121 °C; IR 3520, 2211 (CN), 1586, 1518, 1439, 1298, 1169, 779 cm^{-1}; δ_H (DMSO-d_6) 10.08 (brs, 1H, OH), 8.62 (m, 1H, pyridine H-6), 8.34 (s, 1H, vinylic H), 7.90 (m, 1H, pyridine H-4), 7.74 (m, 2H, pyridine H-3 or 5, H-2), 7.53 (dd, 1H, J = 1.9, 8.3, H-6), 7.37 (m, 1H, pyridine H-3 or 5), 6.94 (d, 1H, J = 8.3, H-5), 3.84 (s, 3H, OCH_3); δ_C (DMSO-d_6) 152.4 (C), 151.2 (C), 150.4 (CH), 148.5 (C), 146.0 (CH), 138.5 (CH), 125.8 (CH), 125.4 (C), 124.1 (CH), 120.9 (CH), 119.2 (C), 116.7 (CH), 113.9 (CH), 106.7 (C), 56.4 (CH_3); m/z 253 (M^+ + 1), 221 ($M^+ - OCH_3$). Anal. ($C_{15}H_{12}N_2O_2 \cdot 0.5H_2O$) C, H, N.

2-Cyano-3-(4-hydroxyphenyl)-*N*-(3-phenylpropyl)propenamide, 3k. A mixture of 4-hydroxybenzaldehyde (0.40 g, 3.3 mmol) and *N*-(3-phenylpropyl)-2-cyanoethanamide (0.66 g, 3.3 mmol) in ethanol (40 mL) was reacted for 18 h according to the general method A to give a yellow solid (0.61 g, 60%): mp 158−60 °C; IR 3235, 2214 (CN), 1655, 1545, 1510, 1289, 1173, 837 cm^{-1}; δ_H (DMSO-d_6) 10.51 (brs, 1H, OH), 8.32 (brs, 1H, NH), 8.02 (s, 1H, vinylic-H), 7.87 (d, 2H, J = 10.0, H-2,6), 7.23 (m, 5H, Ph-H), 6.92 (d, 2H, J = 10.0, H-3,5), 3.23 (q, 2H, J = 7.5, CH_2), 2.60 (t, 2H, J = 7.5, CH_2), 1.80 (q, 2H, J = 7.5 Hz, CH_2); δ_C (DMSO-d_6) 162.6 (C), 162.4 (C), 151.0 (CH), 142.5 (C), 133.7 (CH), 129.1 (CH), 126.6 (CH), 123.8 (C), 118.1 (C), 117.1 (CH), 102.2 (C), 40.2 (CH_2), 33.4 (CH_2), 31.5 (CH_2); m/z 307 (M^+ + 1). Anal. ($C_{19}H_{18}N_2O_2$) C, H, N.

General Method B for Synthesis of 2-Acyloxy-2-methoxy-4-(substituted)cyclohexa-3,5-dienones. To a solution of 2-methoxy-4-(substituted)phenol (0.50 g) in nitromethane (15 mL) and a carboxylic acid (5 mL) was added solid DAIB (1.1 mol equiv). After 10 min the solvent was removed by vacuum evaporation and the resultant yellow oil was purified by rapid flash column chromatography (EtOAc−hexane, 1:1) to give the product.

2-Acetoxy-4-formyl-2-methoxy-3,5-cyclohexadienone, 8a. A mixture of vanillin **(7a)** (0.50 g, 3.3 mmol), DAIB (1.17 g, 3.6 mmol) and acetic acid (5 mL) was reacted according to general method B to give a yellow oil (yellow needles below 0 °C) (0.50 g, 72%): IR 3379, 2861, 1720, 1691, 1251, 1103, 950, 824 cm^{-1}; δ_H ($CDCl_3$) 9.63 (s, 1H, CHO), 7.41 (dd, 1H, J = 2.1, 10.1, H-5), 6.92 (d, 1H, J = 2.1, H-3), 6.28 (d, 1H, J = 10.1, H-6), 3.51 (s, 3H, OCH_3), 2.16 (s, 3H, s, CH_3CO_2); δ_C ($CDCl_3$) 190.9 (C), 189.4 (C), 170.3 (C), 147.9 (CH), 136.5 (C), 134.0 (CH), 127.8 (CH), 93.1 (C), 52.2 (CH_3), 20.8 (CH_3); m/z (EI^+) 168 ($M^+ - C_2H_2O$), 151 (−OH), 136 (−CH_3), 108 (−CO), 79 (−CHO).

2-Acetoxy-4-acetyl-2-methoxy-3,5-cyclohexadienone, 8b. A mixture of acetovanillinone **(7b)** (0.50 g, 3.3 mmol), DAIB (1.07 g, 3.6 mmol) and acetic acid (5 mL) was reacted according to general method B to give a yellow oil (0.45 g, 66%): IR 3065, 1730, 1696, 1682, 1360, 1253, 1113, 960 cm^{-1}; δ_H ($CDCl_3$) 7.56 (dd, 1H, J = 2.2, 10.2, H-5), 6.95 (d, 1H, J = 2.2, H-3), 6.24 (d, 1H, J = 10.2, H-6), 3.51(s, 3H, OCH_3), 2.46 (s, 3H, s, CH_3CO), 2.18 (s, 3H, CH_3CO_2); δ_C ($CDCl_3$) 195.5 (C), 190.9 (C), 170.2 (C), 141.1 (CH), 136.6 (C), 135.8 (CH), 126.7 (CH), 93.0 (C), 52.0 (CH_3), 25.8 (CH_3), 20.9 (CH_3); m/z (EI^+) 182 ($M^+ - C_2H_2O$), 165 (−OH), 151 (−CH_3), 122 (−CO), 107 (−CH_3), 79 (−CO).

2-Acetoxy-2-methoxy-4-methoxycarbonyl-3,5-cyclohexadienone, 8c. A mixture of methyl vanillate **(7c)** (0.50 g, 2.7 mmol), DAIB (0.97 g, 3.0 mmol) and acetic acid (5 mL) was reacted according to general method B to give a yellow oil (0.40 g, 61%): IR 3435, 1730, 1692, 1439, 1240, 1072, 1017, 766 cm^{-1}; δ_H ($CDCl_3$) 7.45 (dd, 1H, J = 2.1, 10.2, H-5), 7.17 (d, 1H, J = 2.1, H-3), 6.23 (d, 1H, J = 10.2, H-6), 3.86 (s, 3H, OCH_3), 3.51 (s, 3H, OCH_3), 2.15 (s, 3H, CH_3CO_2); δ_C ($CDCl_3$) 191.0

(C), 170.2 (C), 164.6 (C), 141.8 (CH), 137.5 (CH), 129.7 (C), 126.7 (CH), 92.7 (C), 53.1 (CH_3), 52.2 (CH_3), 20.8 (CH_3); *m/z* (EI^+) 198 ($M^+ - C_2H_2O$), 181 ($-CH_3CO_2$), 166 ($-CH_3$), 138 ($-CO$), 123 ($-CH_3$).

2-Acetoxy-4-cyano-2-methoxy-3,5-cyclohexadienone, 8d. A mixture of 4-hydroxy-3-methoxybenzonitrile **(7d)** (0.50 g, 3.4 mmol), DAIB (1.19 g, 3.7 mmol) and acetic acid (5 mL) was reacted according to general method B to give a yellow oil (yellow needles below 0 °C) (0.50 g, 73%): IR 2953, 2232 (CN), 1744, 1690, 1242, 1181, 1080, 960, 831 cm^{-1}; δ_H (DMSO-d_6) 7.65 (d, 1H, $J = 2.0$, H-3), 7.24 (dd, 1H, $J = 2.0$, 10.1, H-5), 6.36 (d, 1H, $J = 10.1$, H-6), 3.43 (s, 3H, OCH_3), 2.11 (s, 3H, s, CH_3CO_2); δ_C ($CDCl_3$) 190.2 (C), 170.8 (C), 149.5 (CH), 137.5 (CH), 127.7 (CH), 117.1 (C), 112.9 (C), 92.5 (C), 53.1 (CH_3), 20.9 (CH_3); *m/z* (EI^+) 165 ($M^+ - C_2H_2O$), 148 ($-OH$), 133 ($-CH_3$), 106 ($-HCN$).

4-Formyl-2-methoxy-2-propionyloxy-3,5-cyclohexadienone, 8e. A mixture of vanillin **(7a)** (0.50 g, 3.3 mmol), DAIB (1.17 g, 3.6 mmol) and propionic acid (5 mL) was reacted according to general method B to give a yellow oil (0.40 g, 54%): IR 2947, 1730, 1697, 1460, 1181, 1074, 1003, 824 cm^{-1}; δ_H ($CDCl_3$) 9.65 (s, 1H, CHO), 7.44 (dd, 1H, $J = 2.0$, 10.1, H-5), 6.93 (d, 1H, $J = 2.0$, H-3), 6.31 (d, 1H, $J = 10.1$, H-6), 3.53 (s, 3H, OCH_3), 2.41 (qd, 2H, $J = 7.5$, CH_2CH_3), 1.16 (t, 3H, $J = 7.5$, CH_2CH_3); δ_C ($CDCl_3$) 191.0 (C), 189.5 (CH), 173.9 (C), 148.0 (CH), 136.4 (C), 134.0 (CH), 127.8 (CH), 92.9 (C), 52.2 (CH_3), 27.4 (CH_2), 9.0 (CH_3). EI (M^+) $C_{11}H_{12}O_5$ Requires: 224.0685. Found: 224.0678.

2-Methoxy-4-methoxycarbonyl-2-propionyloxy-3,5-cyclohexadienone, **8f.** A mixture of methyl vanillate **(7c)** (0.50 g, 2.7 mmol), DAIB (0.97 g, 3.0 mmol) and propionic acid (5 mL) was reacted according to general method B to give a yellow oil (0.39 g, 38%): IR 2953, 1720, 1692, 1439, 1248, 1071, 1005, 974 cm^{-1}; δ_H ($CDCl_3$) 7.47 (dd, 1H, $J = 2.1$, 10.2, H-5), 7.18 (d, 1H, $J = 2.1$, H-3), 6.25 (d, 1H, $J = 10.2$, H-6), 3.88 (s, 3H, OCH_3), 3.52 (s, 3H, OCH_3), 2.46 (qd, 2H, $J = 7.6$, CH_2CH_3), 1.16 (t, 3H, $J = 7.6$, CH_2CH_3); δ_C ($CDCl_3$) 190.9 (C), 180.5 (C), 170.8 (C), 164.2 (C), 142.0 (CH), 129.9 (C), 126.7 (CH), 93.1 (C), 53.1 (CH_3), 27.6 (CH_2), 9.0 (CH_3); *m/z* (EI^+) 198 ([$M^+ + 1$] − EtCO).

4-Cyano-2-methoxy-2-propionyloxy-3,5-cyclohexadienone, 8g. A mixture of 4-hydroxy-3-methoxybenzonitrile **(7d)** (0.50 g, 3.4 mmol), DAIB (1.19 g, 3.7 mmol) and propionic acid (5 mL) was reacted according to general method B to give a yellow oil (0.33 g, 44%): IR 2949, 2232 (CN), 1730, 1701, 1460, 1269, 1177, 1074, 824 cm^{-1}; δ_H ($CDCl_3$) 6.89 (dd, 1H, $J = 2.1$, 10.6, H-5), 6.88 (d, 1H, $J = 2.3$, H-3), 6.31 (d, 1H, $J = 10.6$, H-6), 3.51 (s, 3H, OCH_3), 2.46 (qd, 2H, $J = 7.5$, CH_2CH_3), 1.21 (t, 3H, $J = 7.5$, CH_2CH_3); δ_C ($CDCl_3$) 189.2 (C), 180.6 (C), 173.9 (C), 146.9 (CH), 135.7 (C), 128.4 (CH), 116.2 (C), 113.5 (C), 91.8 (C), 52.1 (CH_3), 27.3 (CH_2), 9.0 (CH_3). EI (M^+) $C_{11}H_{11}NO_4$ Requires: 221.0688. Found: 221.0685.

Synthesis of 3-Acyloxy-2-methoxy-4-(substituted)phenols: 2-Acetoxy-4-hydroxy-3-methoxybenzaldehyde, 9a. (i) To vanillin (0.50 g, 2.3 mmol) in acetic acid (30 mL) was added DAIB (1.17 g, 2.6 mmol) in acetic acid (20 mL). After mixing the reaction was maintained at 25 °C for 3 days, solvent was removed by vacuum evaporation and the products were purified by flash column chromatography (eluted with ethyl acetate−hexane, 3:7) to give **8a** as a yellow oil (0.34 g, 49%) and **9a** as white needles (0.11 g, 16%): mp 115 °C; IR 3079, 1775, 1667, 1570, 1319, 1180, 1086, 822 cm^{-1}; δ_H (DMSO-d_6) 10.96 (brs, 1H, OH), 9.83 (s, 1H, CHO), 7.50 (d, 1H, $J = 8.6$, H-6), 6.94 (d, 1H, $J = 8.6$, H-5), 3.72 (s, 3H, OCH_3), 2.36 (s, 3H, CH_3CO_2); δ_C (DMSO-d_6) 189.2 (CH), 169.6 (C), 158.3 (C), 146.5 (C), 140.7 (C), 127.9 (CH), 121.9 (C), 115.2 (CH), 61.1 (CH_3), 21.2 (CH_3); *m/z* 211 ($M^+ + 1$), 169 ([$M^+ + 1$] − C_2H_4O). Anal. ($C_{10}H_{10}O_5$) C, H, N.

(ii) Compound **9a** can also be prepared in situ after the oxidation of vanillin in the presence of acetic acid. To vanillin (0.50 g, 3.3 mmol) dissolved in nitromethane (15 mL) and acetic acid (5 mL) was added DAIB (1.17 g, 3.6 mmol) with stirring at 25 °C. After 15 min the reaction was cooled to 0 °C and boron trifluoride etherate (1 mL) was added, followed after 5 min with water (50 mL). The mixture was extracted with EtOAc (2 × 50 mL) and the organic layer was washed with water (2 × 50 mL), then dried ($MgSO_4$). Removal of the solvent followed by flash column chromatography (eluted with EtOAc−hexane, 1:4) gave **9a** as a white crystalline solid (0.31 g, 45%), the product being contaminated with a small amount of a compound tentatively identified as 2,4-dihydroxy-3-methoxybenzaldehyde.

(iii) To compound **8a** (0.10 g, 0.5 mmol) dissolved in dry ether (2 mL) was added boron trifluoride etherate (0.4 mL) under nitrogen. The reaction was stirred at 25 °C for 3 h, diluted with ether (20 mL) and washed with water (2 × 20 mL). The organic layer was dried ($MgSO_4$) and the solvent removed to give a white solid identified as primarily **9a** (^1H NMR).

2-Acetoxy-4-cyano-2-methoxyphenol, **9b.** DAIB (1.19 g, 3.7 mmol) in acetic acid (10 mL) was added to 4-hydroxy-3-methoxybenzaldehyde (0.50 g, 3.4 mmol) in acetic acid (20 mL) at 25 °C. The reaction was left to stand for 3 days, the solvent was removed and the products were purified by flash column chromatography (eluted with EtOAc−hexane, 1:4) to give **8d** as a yellow oil (0.41 g, 59%) and **9b** as a white solid (97 mg, 14%): mp 67 °C; IR 3239, 2249 (CN), 1777, 1601, 1317, 1168, 1059, 808 cm^{-1}; δ_H (DMSO-d_6) 11.06 (brs, 1H, OH), 7.45 (d, 1H, $J = 8.6$, H-5), 6.91 (d, 1H, $J = 8.6$, H-6), 3.73 (s, 3H, OCH_3), 2.39 (s, 3H, CH_3CO_2); δ_C (DMSO-d_6) 169.0 (C), 157.4 (C), 147.1 (C), 141.1 (C), 129.2 (CH), 116.5 (C), 116.2 (CH), 97.6 (C), 61.1 (CH_3), 21.0 (CH_3); *m/z* 208 ($M^+ + 1$), 166 ([$M^+ + 1$] − C_2H_4O). Anal. ($C_{10}H_9NO_4$) C, H, N.

2-Cyano-5-hydroxy-6-methoxyphenyl Propionate, **9c.** To TAIB (0.88 g, 2.0 mmol) in acetonitrile (10 mL) was added propionic acid (1 mL) with stirring at 25 °C. A solution of 4-hydroxy-3-methoxybenzonitrile (0.298 g, 2.0 mmol) dissolved in acetonitrile (10 mL) was then added dropwise over 10 min. After a further 10 min the reaction was diluted with ether (50 mL) and extracted with saturated sodium hydrogen carbonate solution (50 mL), then washed with water (2 × 50 mL) and dried ($MgSO_4$). Removal of the solvent gave a yellow oil which was mixed with acetic acid (50 mL) and heated at reflux for 2 h. Purification of the products by flash column chromatography (eluted with EtOAc−hexane, 3:7) gave two products, **9c** as a white crystalline solid (42 mg, 9%): mp 121 °C; IR 3252, 2241 (CN), 1771, 1599, 1462, 1319, 1117, 810 cm^{-1}; δ_H (DMSO-d_6) 11.09 (brs, 1H, OH), 7.46 (d, 1H, $J = 8.6$, H-5), 6.92 (d, 1H, $J = 8.9$, H-6), 3.74 (s, 3H, OCH_3), 2.71 (q, 2H, $J = 7.5$, CH_2CH_3), 1.21 (t, 3H, $J = 7.5$, CH_2CH_3); δ_C (DMSO-d_6) 171.8 (C), 154.8 (C), 146.0 (C), 140.4 (C), 129.1 (CH), 115.7 (C), 114.5 (CH), 100.2 (C), 61.9 (CH_3), 27.9 (CH_2), 9.4 (CH_3); *m/z* 222 ($M^+ + 1$), 196 ([$M^+ + 1$] − CN). Anal. ($C_{11}H_{11}NO_4$) C, H, N.

Also isolated was **9b** (53 mg, 13%), identical (^1H NMR) to the sample characterized (above).

General Method C for Synthesis of 2-Acetoxy-3-alkoxy-4-hydroxytyrphostins. To the phenol in acetic acid was added DAIB (1.1 mol equiv) and the mixture was stirred at 50 °C for 2 h. After removal of solvent the product was purified by flash column chromatography (EtOAc−hexane) to give the acetoxytyrphostin as a white or yellow solid.

2-Acetoxy-4-hydroxy-3-methoxybenzylidenemalonitrile, 11. Tyrphostin **3a** (1.00 g, 5.0 mmol) and DAIB (1.62 g, 5.0 mmol) in acetic acid (100 mL) were oxidized according to general method C to give a yellow solid (0.35 g, 27%): mp 138−139 °C; IR 3351, 2221 (CN), 1757, 1572, 1501, 1202, 895, 826 cm^{-1}; δ_H ($CDCl_3$) 8.12 (d, 1H, $J = 9$, H-6), 7.69 (s, 1H, vinylic-H), 7.01 (d, 1H, $J = 9.0$, H-5), 3.88 (s, 3H, OCH_3), 2.46 (s, 3H, CH_3CO_2); δ_C ($CDCl_3$) 168.0 (C), 156.3 (C), 152.2 (CH), 145.0 (C), 140.0 (C), 125.5 (CH), 118.3 (C), 115.2 (CH), 114.5 (C), 112.9 (C), 81.3 (C), 61.9 (CH_3), 21.0 (CH_3); *m/z* (AP−) 257 ($M^- - 1$). Anal. ($C_{13}H_{10}N_2O_4$) C, H, N.

2-Cyano-3-(2-acetoxy-4-hydroxy-3-methoxyphenyl)propenamide, 12. Compound **3b** (0.50 g, 2.3 mmol) and DAIB (0.81 g, 2.5 mmol) in acetic acid (100 mL) were reacted according to general method C to give a white solid (0.25 g, 40%): mp 175−176 °C; IR 3374, 3163, 2212 (CN), 1777, 1691, 1497, 1171, 822 cm^{-1}; δ_H (DMSO-d_6) 10.88 (brs, 1H, OH), 8.07

(brs, 1H, NH), 7.97 (s, 1H, vinylic-H), 7.89 (d, 1H, J = 8.9, H-6), 7.77 (brs, 1H, NH), 6.98 (d, 1H, J = 8.9, H-5), 3.79 (s, 3H, s, OCH_3), 2.43 (s, 3H, s, CH_3CO_2); δ_C (DMSO-d_6) 169.3 (C), 163.7 (C), 156.4 (C), 145.7 (C), 144.0 (CH), 140.7 (C), 124.2 (CH), 117.6 (C), 117.5 (C), 115.7 (CH), 105.9 (C), 60.8 (CH_3), 21.2 (CH_3); m/z (AP−) 275 (M^- − 1). Anal. ($C_{13}H_{12}N_2O_5$) C, H, N.

Methyl 2-Cyano-3-(2-acetoxy-4-hydroxy-3-methoxyphenyl)propenoate, 13. Compound **3c** (0.50 g, 2.1 mmol) and DAIB (0.76 g, 2.4 mmol) in acetic acid (100 mL) were reacted according to general method C to give a white solid (0.31 g, 50%): mp 131 °C; IR 3269, 2228 (CN), 1782, 1698, 1586, 1238, 1061, 806 cm^{-1}; δ_H (DMSO-d_6) 11.10 (brs, 1H, OH), 8.11 (s, 1H, vinylic-H), 7.98 (d, 1H, J = 8.9, H-6), 7.01 (d, 1H, J = 8.9, H-5), 3.83 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 2.39 (s, 3H, CH_3CO_2); δ_C (DMSO-d_6) 169.3 (C), 163.5 (C), 157.9 (C), 147.8 (CH), 146.5 (C), 140.8 (C), 124.8 (CH), 116.9 (C), 116.7 (C), 116.1 (CH), 100.6 (C), 60.9 (CH_3), 54.1 (CH_3), 21.0 (CH_3); m/z 292 (M^+ + 1), 250 ([M^+ + 1] − C_2H_4O). Anal. ($C_{14}H_{13}NO_6$) C, H, N.

2-Acetoxy-3-ethoxy-4-hydroxybenzylidenemalonitrile, 14. Tyrphostin **3d** (0.50 g, 2.3 mmol) and DAIB (0.79 g, 2.5 mmol) in acetic acid (100 mL) were reacted according to general method C to give a yellow solid (0.32 g, 50%): mp 120−121 °C; IR 3283, 2239 (CN), 1740, 1572, 1321, 1190, 1061, 824 cm^{-1}; δ_H (DMSO-d_6) 11.33 (brs, 1H, OH), 8.34 (s, 1H, vinylic-H), 7.93 (d, 1H, J = 9.0, H-6), 7.02 (d, 1H, J = 9.0, H-5), 3.99 (q, 2H, J = 7.0, CH_2), 2.39 (s, 3H, CH_3CO_2), 1.25 (t, 3H, J = 7.0, CH_3); δ_C (DMSO-d_6) 169.3 (C), 158.9 (C), 154.7 (CH), 146.8 (C), 139.8 (C), 124.6 (CH), 117.4 (C), 116.1 (CH), 115.6 (C), 114.8 (C), 79.0 (C), 68.9 (CH_2), 21.5 (CH_3), 16.3 (CH_3); m/z 231 ([M^+ + 1] − C_2H_4O), 185 (−EtOH). Anal. ($C_{14}H_{12}N_2O_4$) C, H, N.

Synthesis of 2-Acetoxy-5-hydroxy-4-methoxytyrphostins: 2-Acetoxy-5-hydroxy-4-methoxybenzylidenemalonitrile, 15. Oxidation of tyrphostin **3f** (0.50 g, 2.5 mmol) and DAIB (0.89 g, 2.8 mmol) in acetic acid (100 mL) according to general method C gave a yellow solid (0.24 g, 38%): mp 161 °C; IR 3441, 2226 (CN), 1750, 1588, 1512, 1312, 1229, 918 cm^{-1}; δ_H (DMSO-d_6) 9.96 (brs, 1H, OH), 8.26 (s, 1H, vinylic-H), 7.67 (s, 1H, H-6), 7.00 (s, 1H, H-3), 3.85 (s, 3H, OCH_3), 2.34 (s, 3H, OCH_3); δ_C (DMSO-d_6) 170.3 (C), 155.4 (C), 154.3 (CH), 146.4 (C), 145.6 (C), 117.1 (C), 115.6 (C), 114.6 (C), 112.6 (CH), 108.4 (CH), 79.2 (C), 57.3 (CH_3), 21.9 (CH_3); m/z 259 (M^+ + 1), 217 ([M^+ + 1] − C_2H_4O). Anal. ($C_{13}H_{10}N_2O_4$) C, H, N.

2-Cyano-3-(2-acetoxy-5-hydroxy-4-methoxyphenyl)propenamide, 16. Compound **3g** (0.50 g, 2.3 mmol) and DAIB (0.78 g, 2.4 mmol) in acetic acid (10 mL) were reacted according to general method C to give **16** as pale yellow needles (0.19 g, 30%): mp 171 °C; IR 3435, 2214 (CN), 1757, 1670, 1512, 1219, 1181, 922 cm^{-1}; δ_H (DMSO-d_6) 9.70 (brs, 1H, OH), 8.00 (brs, 1H, NH), 7.94 (s, 1H, vinylic-H), 7.72 (brs, 1H, NH), 7.68 (s, 1H, H-6), 6.96 (s, 1H, H-3), 3.84 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3CO_2); δ_C (DMSO-d_6) 170.2 (C), 163.7 (C), 153.1 (C), 145.4 (C), 145.2 (C), 143.9 (CH), 117.4 (C), 117.3 (C), 113.4 (CH), 108.1 (CH), 105.7 (C), 57.0 (CH_3), 21.6 (CH_3); m/z 277 (M^+ + 1), 235 ([M^+ + 1] − C_2H_4O). Anal. ($C_{13}H_{12}N_2O_5 \cdot 0.5H_2O$) C, H, N.

General Method D for Synthesis of 4-(Substituted)-phenyliodoniophenolates. To the substituted phenol dissolved in acetic acid was added DAIB (1 mol equiv) in acetic acid for 3−9 days in darkness at 25 °C. Removal of solvent gave a yellow oil which solidified on addition of EtOAc or petrol ether. The solid was collected, washed with petrol ether and dried in vacuo over KOH.

4-(2,2-Dicyanovinyl)-2-phenyliodoniophenolate, 17. Compound **3h** (0.50 g, 2.9 mmol) and DAIB (0.95 g, 2.9 mmol) in acetic acid (65 mL) were reacted for 9 days according to general method D to give a mustard yellow solid (0.88 g, 80%): mp 104−105 °C; IR 2226 (CN), 1574, 1470, 1402, 1263, 1210, 1190, 745 cm^{-1}; δ_H (DMSO-d_6) 8.16 (d, 1H, J = 2, H-3), 8.03 (m, 2H, Ph-H), 7.82 (m, 1H, H-5), 7.76 (s, 1H, s, vinylic-H), 7.64 (m, 1H, Ph-H), 7.50 (m, 2H, Ph-H), 6.49 (d, 1H, J = 10, H-6); δ_C (DMSO-d_6) 157.0 (CH), 136.1(CH), 132.4 (CH), 121.2 (C), 118.0 (C), 117.1 (C), 117.0 (C), 113.7 (C), 64.1 (C). EI (M^+) $C_{16}H_9IN_2O$ Requires: 371.9760. Found: 371.9769.

4-(2-Carbamoyl-2-cyanovinyl)-2-phenyliodoniophenolate, 18. Compound **3i** (1.0 g, 5.3 mmol) and DAIB (1.71 g, 5.3 mmol) in acetic acid (10 mL) were reacted for 3 days according to general method D to give a mustard yellow solid (1.37 g, 65%): mp 105−106 °C; IR 3391, 2211 (CN), 1688, 1576, 1470, 1388, 1254, 1200 cm^{-1}; δ_H (DMSO-d_6) 8.06 (m, 2H, Ph-H), 8.00 (d, 1H, J = 2.3, H-3), 7.86 (dd, 1H, J = 2.3, H-5), 7.83 (s, 1H, s, vinylic-H), 7.66 (m, 1H, Ph-H), 7.51 (m, 2H, Ph-H), 7.33 (brs, 2H, NH_2), 6.47 (d, 1H, J = 9.0, H-6); δ_C (DMSO-d_6) 156.1 (CH), 136.3(CH), 132.4 (CH), 120.1 (CH), 119.6 (C), 118.8 (C), 117.3 (C), 113.1 (C), 93.6 (C). EI (M^+) $C_{16}H_{11}IN_2O_2$ Requires: 390.9944. Found: 390.9950.

4-(Cyano-2-methoxycarbonylvinyl)-2-phenyliodoniophenolate, 19. Compound **3j** (0.50 g, 2.5 mmol) and DAIB (0.83 g, 2.6 mmol) in acetic acid (100 mL) were reacted for 3 days according to general method D to give a yellow solid (0.61 g, 61%): mp 105−106 °C; IR 3440, 2214 (CN), 1719, (CO_2Me), 1562, 1491, 1280, 1190, 829 cm^{-1}; δ_H (DMSO-d_6) 8.31 (d, 1H, J = 1.75, H-3), 8.04 (m, 3H, Ph-H, H-5), 7.91 (s, 1H, s, vinylic-H), 7.64 (m, 1H, Ph-H), 7.50 (m, 2H, Ph-H), 6.49 (d, 1H, J = 9.0, H-6); δ_C (DMSO-d_6) 165.5 (C), 153.3 (CH), 136.0(CH), 132.4 (CH), 120.9 (C), 119.1 (C), 116.8 (C), 116.2 (C), 113.8 (C), 87.6 (C), 53.2 (CH_3). EI (M^+) $C_{17}H_{12}INO_3$ Requires: 405.9940. Found: 405.9928.

General Method E for Synthesis of 3-Iodo-4-phenoxy-1-(vinyl-substituted)benzenes. To the 4-(substituted)phenol dissolved in acetic acid was added DAIB (1.1 mol equiv) in acetic acid. The mixture was stirred at 25 °C in the dark for 24 h, then refluxed (24 h). Removal of solvent in vacuo was followed by isolation of the product by column chromatography (elution with DCM−hexane).

3-Iodo-4-phenoxybenzylidenemalonitrile, 21. (i) Compound **3h** (1.00 g, 5.9 mmol) and DAIB (1.89 g, 5.9 mmol) in acetic acid (200 mL) were reacted according to general method E to give a white solid (1.53 g, 70%): mp 110−112 °C; IR 2226 (CN), 1574, 1478, 1402, 1265, 1196, 745, 691 cm^{-1}; δ_H (d_6-DMSO) 8.49 (d, 1H, J = 2.2, H-2), 8.41 (s, 1H, vinylic-H), 7.96 (dd, 1H, J = 2.2, 8.7, H-5), 7.49 (m, 2H, Ph-H), 7.30 (m, 1H, Ph-H), 7.14 (m, 2H, Ph-H), 6.93 (d, 1H, J = 8.7, H-6); δ_H ($CDCl_3$) 8.32 (d, 1H, J = 2.0, H-2), 7.92 (dd, 1H, J = 2.0, 10.0, H-5), 7.62 (s, 1H, vinylic-H), 7.45 (m, 2H, Ph-H), 7.31 (m, 1H, Ph-H), 7.10 (m, 2H, Ph-H), 6.77 (d, 1H, J = 10.0, H-6); δ_C ($CDCl_3$) 162.9 (C), 157.4 (CH), 154.8 (C), 143.6 (CH), 132.2 (CH), 130.8 (CH), 127.4 (C), 126.3 (C), 120.9 (CH), 116.6 (CH), 114.1 (C), 113.0 (C), 87.9 (C), 81.8 (C); m/z 372 (M^+). Anal. ($C_{16}H_9IN_2O$) C, H, N.

Compound **21** was also prepared (50%) by rearrangement of the phenyliodoniophenolate **17** (0.1 g, 0.4 mmol) in refluxing acetic acid (50 mL) for 18 h.

2-Cyano-3-(3-iodo-4-phenoxyphenyl)propenamide, 22. Compound **3i** (1.00 g, 5.4 mmol) and DAIB (1.71 g, 5.4 mmol) in acetic acid (200 mL) were reacted according to general method E to give a white solid (0.71 g, 33%): mp 145−147 °C; IR 3412, 2211 (CN), 1690, 1591, 1576, 1371m 1246, 752 cm^{-1}; δ_H ($CDCl_3$) 8.40 (d, 1H, J = 2.0, H-2), 8.20 (s, 1H, vinylic-H), 7.92 (dd, 1H, J = 2.0, 10.0, H-5), 7.43 (m, 2H, Ph-H), 7.28 (m, 1H, Ph-H), 7.08 (m, 2H, Ph-H), 6.80 (d, 1H, J = 7.5, H-6), 6.33 (brs, 1H, NH), 5.92 (brs, 1H, NH); δ_C ($CDCl_3$) 162.1 (C), 161.5 (C), 155.4 (C), 151.8 (CH), 143.3 (CH), 132.3 (CH), 130.7 (CH), 128.3 (C), 125.8 (CH), 120.6 (CH), 117.4 (C), 117.1 (CH), 102.5 (C), 87.9 (C), 88.1 (C); m/z 391 (M^+ + 1). Anal. ($C_{16}H_{11}IN_2O_2$) C, H, N.

Methyl 2-Cyano-3-(3-iodo-4-phenoxyphenyl)propenoate, 23. Prepared from **3j** (0.5 g) and DAIB (0.83 g) g) in acetic acid (50 mL) according to general method E, the propenoate **23** was isolated as a white solid (0.60 g, 60%): mp 121−123 °C; IR 2218 (CN), 1721 (CO_2Me), 1576, 1481, 1258, 1208, 976, 764 cm^{-1}; δ_H ($CDCl_3$) 8.38 (d, 1H, J = 2.2, H-2), 8.11 (s, 1H, vinylic-H), 7.99 (dd, 1H, J = 2.2, 8.7, H-5), 7.41 (m, 2H, Ph-H), 7.25 (m, 1H, Ph-H), 7.07 (m, 2H, Ph-H), 6.80 (d, 1H, J = 7.5, H-6), 3.92 (s, 3H, OCH_3); δ_C ($CDCl_3$) 163.4 (C), 161.8 (C), 155.3 (C), 153.0 (CH), 143.8 (CH), 132.5 (CH), 130.7 (CH),

128.3 (C), 125.8 (CH), 120.7 (CH), 117.0 (C), 115.8 (C), 101.0 (C), 87.9 (C), 53.9 CH_3); *m/z* 405 (M^+). Anal. ($C_{17}H_{12}INO_3$) C, H, N.

2-Cyano-3-(3-iodo-4-phenoxyphenyl)-*N*-(3-phenylpropyl)propenamide, 24. Compound **3k** (0.20 g, 0.7 mmol) and DAIB (0.21 g, 0.7 mmol) in acetic acid (100 mL) were reacted according to general method E to give a white solid (0.11 g, 34%): mp 116−118 °C; IR 3376, 2214 (CN), 1680, 1528, 1470, 1252, 1198, 692 cm^{-1}; δ_H ($CDCl_3$) 8.36 (d, 1H, J = 2.3, H-2), 8.17 (s, 1H, vinylic-H), 7.88 (dd, 1H, J = 2.3, 9.0, H-6), 7.40 (m, 2H, Ph-H), 7.23 (m, 6H, Ph-H), 7.07 (m, 2H, Ph-H), 6.78 (d, 1H, J = 8.8, H-5), 6.32 (brt, 1H, NH), 3.46 (q, 2H, J = 6.0, $NHCH_2$), 2.70 (t, 2H, J = 7.3, $CH_2CH_2CH_3$), 1.96 (q, 2H, J = 7.8, $CH_2CH_2CH_3$); δ_C ($CDCl_3$) 161.2 (C), 160.4 (C), 155.5 (C), 150.6 (CH), 143.0 (CH), 141.4 (C), 132.1 (CH), 130.6 (CH), 129.0 (CH), 128.8 (CH), 128.6 (C), 126.6 (CH), 125.6 (CH), 120.6 (CH), 117.3 (C), 117.2 (CH), 103.5 (C), 88.1 (C), 77.6 (C), 40.6 (CH_2), 33.5 (CH_2), 31.3 (CH_3); *m/z* 509 ($M^+ + 1$).

3-Iodo-4-phenoxybenzaldehyde, 27a. A mixture of 4-hydroxybenzaldehyde **(26a)** (2.00 g, 16.4 mmol) and DAIB (5.54 g, 17.2 mmol) in acetic acid (100 mL) was reacted according to general method E to give a white solid (1.68 g, 32%): mp 58−59 °C; IR 2851, 1670, 1580, 1479, 1252, 1192, 891, 750 cm^{-1}; δ_H ($CDCl_3$) 9.86 (s, 1H, CHO), 8.39 (d, 1H, J = 2.0, H-2), 7.75 (dd, 1H, J = 2.0, 7.5, H-5), 7.44 (m, 1H, Ph-H), 7.28 (m, 2H, Ph-H), 7.09 (m, 2H, Ph-H), 6.82 (d, 1H, J = 7.5, H-6); δ_C ($CDCl_3$) 189.8 (CH), 162.6 (C), 155.5 (C), 142.1 (C), 133.2 (C), 131.6 (C), 130.7 (CH), 125.7 (CH), 120.6 (CH), 116.9 (CH), 88.0 (C); *m/z* 325 ($M^+ + 1$). Anal. ($C_{13}H_9IO_2$) C, H, N.

Methyl 3-Iodo-4-phenoxybenzoate, 27b. Similarly prepared, from methyl 4-hydroxybenzoate **(26b)** (2.00 g, 13.2 mmol) and DAIB (4.66 g, 14.5 mmol) in acetic acid (100 mL) according to general method E, the white crystalline benzoate **27b** (3.57 g, 76%) had: mp 57 °C (lit. mp 63 °C);[35] IR 2945, 1709, 1584, 1479, 1260, 1192, 1119, 756 cm^{-1}; δ_H ($CDCl_3$) 8.40 (d, 1H, J = 2.0, H-2), 7.91 (dd, 1H, J = 2.1, 8.6, H-6), 7.47 (m, 2H, Ph-H), 7.29 (m, 1H, Ph-H), 7.09 (m, 2H, Ph-H), 6.86 (d, 1H, J = 8.6, H-5), 3.85 (s, 3H, OCH_3); δ_C ($CDCl_3$) 165.3 (C), 161.2 (C), 156.0 (C), 141.4 (CH), 132.1 (CH), 131.3 (CH), 130.8 (CH), 126.9 (C), 125.7 (CH), 120.2 (CH), 118.1 (CH), 116.1 (CH), 89.1 (C), 53.2 (CH3); *m/z* 355 ($M^+ + 1$).

3-(3-Iodo-4-phenoxyphenyl)-2-(pyridin-2-yl)propenenitrile, 28. Compound **27a** (0.32 g, 1.0 mmol) and 2-pyridylacetonitrile (0.12 g, 1.0 mmol) in ethanol (10 mL) were reacted at room temperature for 3 days according to general method A to give a white solid (0.17 g, 40%): mp 139−140 °C; IR 2209 (CN), 1578, 1470, 1256, 1200, 1034, 887, 777 cm^{-1}; δ_H ($CDCl_3$) 8.70 (m, 1H, pyridine H-6), 8.62 (d, 1H, J = 2.2, H-2), 8.43 (s, 1H, vinylic-H), 8.06 (dd, 1H, J = 2.2, 8.8, H-6), 7.97 (dd, 1H, J = 1.8, 7.5, pyridine H-4), 7.87 (m, 1H, pyridine H-3 or H-5), 7.48 (m, 3H, pyridine H-5 or H-3, Ph-H), 7.25 (m, 1H, Ph-H), 7.12 (m, 2H, Ph-H), 7.01 (d, 1H, J = 8.6, H-5); δ_C ($CDCl_3$) 159.1 (C), 156.4 (C), 151.9 (C), 150.5 (CH), 143.4 (CH), 141.8 (CH), 138.7 (CH), 132.4 (CH), 131.2 (CH), 125.3 (CH), 124.9 (CH), 121.4 (CH), 119.8 (CH), 119.2 (CH), 118.2 (C), 111.2 (C), 90.2 (C); *m/z* 425 ($M^+ + 1$), 297 ($M^+ - HI$). Anal. ($C_{20}H_{13}IN_2O$) C, H, N.

General Method F for Synthesis of 2,2′-Dihydroxybiphenyls. To the 2-alkoxy-4-(substituted)phenol in acetonitrile was added DAIB (0.505 mol equiv) in acetonitrile. The mixture was stirred at 25 °C in the dark for 24 h. The precipitated biphenyl was collected, washed with more acetonitrile and dried in vacuo.

The following known biphenyls were prepared: (from **7a**) 5,5′-diformyl-2,2′-dihydroxy- 3,3′-dimethoxybiphenyl **(29a)**, mp 296 °C (56%); and (from **7b**) 5,5′-diacetyl-2,2′-dihydroxy-3,3′-dimethoxybiphenyl **(29b)**, mp >300 °C (43%).

Dimethyl 2,2′-Dihydroxy-3,3′-dimethoxybiphenyl-5,5′-dicarboxylate, 29c. A mixture of methyl vanillate **(7c)** (0.50 g, 2.7 mmol) and DAIB (0.45 g, 1.4 mmol) in acetonitrile (40 mL) was reacted according to general method F to give a white solid (0.20 g, 40%): mp 230−231 °C; IR 3420, 2959, 1711, 1591, 1425, 1231, 1047, 762 cm^{-1}; δ_H (DMSO-d_6) 9.54 (brs, 2H, OH), 7.46 (d, 2H, J = 2.0, H-6,6′), 7.44 (d, 2H, J = 2.0, H-4,4′), 3.90 (s, 6H, CH_3), 3.79 (s, 6H, CH_3); δ_C (DMSO-d_6) 167.0 (C), 149.7 (C), 148.3 (C), 126.2 (CH), 125.2 (C), 120.3 (C), 111.7 (CH), 56.9 (CH_3), 52.7 (CH_3); *m/z* 363 ($M^+ + 1$). Anal. ($C_{18}H_{18}O_8$) C, H, N.

5,5′-Diformyl-2-hydroxy-3,3′-dimethoxy-2′-tosyloxybiphenyl, 30. To compound **29a** (0.25 g, 0.8 mmol) suspended in pyridine (20 mL) was added tosyl chloride (0.17 g, 0.8 mmol). After 10 min the resulting clear solution was concentrated in vacuo and triturated with water and the white precipitate was collected, washed with water and dried to give **30** as a white solid (0.32 g, 83%): mp 151−152 °C; IR (KBr disk) 3412, 1688, 1593, 1341, 1132, 1090, 855, 721 cm^{-1}; δ_H (DMSO-d_6) 10.00 (brs, 1H, OH), 9.98 (s, 1H, CHO), 9.71 (s, 1H, CHO), 7.65 (d, 1H, J = 1.8, H-6 or 6′), 7.51 (d, 1H, J = 1.8, H-6 or 6′), 7.34 (d, 2H, J = 8.3, tosyl-H-2,6), 7.27 (d, 1H, J = 1.8, H-4 or 4′), 7.20 (d, 1H, J = 1.7, H-4 or 4′), 7.15 (d, 2H, J = 8.5, tosyl-H-3,5), 3.88 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 2.33 (s, 3H, tosyl CH_3); δ_C (DMSO-d_6) 192.9 (CH), 191.8 (CH), 154.2 (C), 150.7 (C), 148.7 (C), 145.5 (C), 141.6 (C), 135.8 (C), 134.4 (C), 134.1 (C), 130.4 (CH), 128.8 (C), 128.7 (CH), 127.8 (CH), 126.5 (CH), 123.6 (C), 112.3 (CH), 110.1 (CH), 57.3 (CH_3), 56.8 (CH_3), 21.9 (CH_3); *m/z* 457 ($M^+ + 1$), 303 ($M^+ - C_7H_7SO_2$). Anal. ($C_{23}H_{20}O_8S$) C, H, N.

5,5′-Di(2-cyano-2-methoxycarbonylvinyl)-2-hydroxy-3,3′-dimethoxy-2′-tosyloxybiphenyl, 31. Compound **30** (0.10 g, 0.2 mmol) and methyl cyanoacetate (28 mg, 0.3 mmol) in EtOH (40 mL) were reacted for 4 h according to general method A to give a yellow solid (46 mg, 52%): mp 224−225 °C; IR (KBr disk) 3434, 2218 (CN), 1736, 1584, 1418, 1260, 1094, 856 cm^{-1}; δ_H (DMSO-d_6) 10.31 (brs, 1H, OH), 8.45 (s, 1H, vinylic-H), 8.17 (s, 1H, vinylic-H), 7.94 (d, 1H, J = 1.8, H-6 or 6′, or H-4,4′), 7.66 (d, 2H, H-6 or 6′, or H-4,4′), 7.35 (d, 2H, J = 8.6, tosyl-H-2,6), 7.16 (d, 2H, J = 8.4, tosyl-H-3,5), 3.88 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 2.3 (s, 3H, tosyl-CH_3); δ_C (DMSO-d_6) 164.0 (C), 163.0 (C), 162.5 (C), 155.6 (CH), 154.7 (CH), 153.8 (C), 150.5 (C), 148.2 (C), 145.4 (C), 140.5 (C), 134.5 (C), 133.7 (C), 131.3 (C), 130.3 (CH), 129.9 (CH), 127.7 (CH), 126.7 (C), 124.1 (C), 123.1 (C), 117.4 (C), 116.3 (C), 115.6 (CH), 113.4 (CH), 104.4 (C), 104.1 (C), 98.0 (C), 57.3 (CH_3), 56.7 (CH_3), 54.3 (CH_3), 54.0 (CH_3), 22.0 (CH_3); *m/z* 619 ($M^+ + 1$), 447 ($M^+ - C_7H_7SO_2$). Anal. ($C_{31}H_{26}N_2O_{10}S$) C, H, N.

5,5′-Di(2,2-dicyanovinyl)-2,2′-dihydroxy-3,3′-dimethoxybiphenyl, 32. Compound **3a** (0.50 g, 2.5 mmol) and DAIB (0.40 g, 1.3 mmol) in acetonitrile (20 mL) were reacted according to general method F to give a yellow solid (0.30 g, 59%): mp >300 °C; IR 3389, 2226 (CN), 1595, 1566, 1411, 1298, 1188, 629 cm^{-1}; δ_H (DMSO-d_6) 8.32 (s, 2H, vinylic-H), 7.72 (d, 2H, J = 2.0, H-6,6′), 7.46 (d, 2H, J = 2.0, H-4,4′), 3.90 (s, 6H, CH_3); δ_C (DMSO-d_6) 161.3 (CH), 152.3 (C), 148.7 (C), 130.1 (CH), 125.7 (C), 123.1 (C), 115.9 (C), 115.2 (C), 112.6 (CH), 76.3 (C), 56.8 (CH_3); *m/z* 398 (M^+), 381 ($M^+ - OH$). Anal. ($C_{22}H_{14}N_4O_4 \cdot 0.5H_2O$) C, H, N.

5,5′-Di(2-carbamoyl-2-cyanovinyl)-2,2′-dihydroxy-3,3′-dimethoxybiphenyl, 33. Compound **3b** (0.25 g, 1.1 mmol) and DAIB (0.19 g, 0.6 mmol) in acetonitrile (10 mL) were reacted according to general method F to give a yellow solid (0.17 g, 69%); mp >300 °C; IR (KBr disk) 3368, 2216 (CN), 1690, 1574, 1279, 1180, 1042, 631 cm^{-1}; δ_H (DMSO-d_6) 9.67 (brs, 2H, OH), 8.07 (s, 2H, vinylic-H), 7.71 (d, 2H, J = 2.0, H-6,6′), 7.55 (brs, 4H, NH_2), 7.45 (d, 2H, J = 2.0, H-4,4′), 3.91 (s, 6H, CH_3); δ_C (DMSO-d_6) 164.1 (C), 151.6 (CH), 150.0 (C), 148.6 (C), 128.5 (CH), 126.0 (C), 123.2 (C), 118.3 (C), 112.8 (CH), 102.5 (C), 56.8 (CH_3); *m/z* (AP−) 433 ($M^- - 1$). Anal. ($C_{22}H_{18}N_4O_6 \cdot 0.5H_2O$) C, H, N.

2,2′-Dihydroxy-3,3′-dimethoxy-5,5′-(2-cyano-2-methoxycarbonylvinyl)biphenyl, 34. Compound **3c** (0.50 g, 2.1 mmol) and DAIB (0.35 g, 1.1 mmol) in acetonitrile (30 mL) were reacted according to general method F to give a yellow solid (0.34 g, 69%): mp 244−245 °C; IR 3391, 2220 (CN), 1729, 1582, 1415, 1252, 1098, 1047 cm^{-1}; δ_H (DMSO-d_6) 10.20 (brs, 2H, OH), 8.29 (s, 2H, vinylic-H), 7.83 (d, 2H, J = 2.0, H-6,6′), 7.64 (d, 2H, J = 2.0, H-4,4′), 3.91 (s, 6H, CH_3), 3.83 (s, 6H, CH_3); δ_C (DMSO-d_6) 164.0 (C), 155.9 (CH), 151.2 (C), 148.6 (C),

129.6 (CH), 125.9 (C), 122.9 (C), 117.5 (C), 113.8 (CH), 97.8 (C), 56.8 (CH_3), 53.9 (CH_3); *m/z* 465 ($M^+ + 1$). Anal. ($C_{24}H_{20}N_2O_8 \cdot H_2O$) C, H, N.

5,5′-Di(2,2-dicyanovinyl)-3,3′-diethoxy-2,2′-dihydroxybiphenyl, **35.** Compound **3d** (0.50 g, 2.3 mmol) and DAIB (0.38 g, 1.2 mmol) in acetonitrile (25 mL) were reacted according to general method E to give a yellow solid (0.23 g, 45%): mp 294−295 °C; IR 3293, 2231 (CN), 1570, 1431, 1290, 1186, 1074, 625 cm^{-1}; δ_H (DMSO-d_6) 10.28 (brs, 2H, OH), 8.32 (s, 2H, vinylic-H), 7.73 (d, 2H, $J = 1.8$, H-6,6′), 7.45 (d, 2H, $J = 1.9$, H-4,4′), 4.18 (q, 4H, $J = 7.0$, CH_2), 1.44 (t, 6H, $J = 7.0$, CH_3); δ_C (DMSO-d_6) 161.3 (CH), 152.4 (C), 147.8 (C), 130.0 (CH), 125.8 (C), 123.2 (C), 115.9 (C), 115.1 (C), 113.3 (CH), 76.3 (C), 65.3 (CH_2), 15.3 (CH_3); *m/z* 426 (M^+). Anal. ($C_{24}H_{18}N_4O_4$) C, H, N.

5,5′-Di[2-cyano-2-(pyridin-2-yl)vinyl]-2,2′-dihydroxy-3,3′-dimethoxybiphenyl, 36. Compound **3e** (0.50 g, 2.0 mmol) and DAIB (0.32 g, 1.0 mmol) in acetonitrile (50 mL) were reacted according to general method F to give a yellow solid (0.27 g, 55%): mp 267−268 °C; IR 3516, 2209 (CN), 1581, 1408, 1181, 1148, 1045, 777 cm^{-1}; δ_H (DMSO-d_6) 9.67 (brs, 2H, OH), 8.63 (m, 2H), 8.36 (s, 2H, vinylic-H), 7.92 (m, 2H), 7.81 (m, 4H), 7.58 (d, 2H), 7.39 (m, 2H), 3.94 (s, 6H, CH_3); δ_C (DMSO-d_6) 152.5 (C), 150.4 (CH), 148.7 (C), 148.5 (C), 145.9 (CH), 138.6 (CH), 127.7 (CH), 126.2 (C), 124.5 (C), 124.1 (CH), 120.9 (CH), 119.2 (C), 112.6 (CH), 106.9 (C), 56.8 (CH_3); *m/z* (AP−) 501 ($M^- - 1$). Anal. ($C_{30}H_{22}N_4O_4 \cdot 0.5H_2O$) C, H, N.

Oxidation of 2-Hydroxy-3-methoxybenzaldehyde, 37. *o*-Vanillin (0.50 g, 3.3 mmol) in acetonitrile (20 mL) was added to DAIB (0.54 g, 1.7 mmol) in acetonitrile (20 mL). The reaction was left to stand overnight, after which the solvent was removed in vacuo and the product purified by flash column chromatography (EtOAc−hexane, 1:4), to give (probably) 5-acetoxy-2-hydroxy-3-methoxybenzaldehyde **(38)** as a yellow oil (69 mg, 10%): IR 2851, 1748, 1669, 1470, 1221, 1021, 910, 743 cm^{-1}; δ_H ($CDCl_3$) 11.02 (brs, 1H, OH), 9.89 (s, 1H, CHO), 6.98 (d, 1H, $J = 2.6$, H-6), 6.89 (d, 1H, $J = 2.6$, H-4), 3.94 (s, 3H, s, OCH_3), 2.34 (s, 3H, CH_3CO_2); δ_C ($CDCl_3$) 196.2 (C), 170.0 (C), 150.0 (C), 149.4 (C), 143.3 (C), 120.0 (C), 115.9 (CH), 112.8 (CH), 56.9 (CH_3), 21.4 (CH_3); *m/z* (EI+) 211 ($M^+ + 1$).

NCI in Vitro Cytotoxicity Assays. The cytotoxicity of test agents was assessed in a panel of 60 cell lines using a sulforhodamine B assay.[29] Briefly, cell lines were inoculated into a series of 96-well microtiter plates, with varied seeding densities depending on the growth characteristics of the particular cell lines. Following a 24-h drug-free incubation, test agents were added routinely at five 10-fold dilutions with a maximum concentration of 10^{-4} M. After 2 days of drug exposure, the change in protein stain optical density allowed the inhibition of cell growth to be analyzed.

MTT Colorimetric Assays. Cells were seeded in 96-well microtiter plates at densities of 250−300 cells/well for 7-day assays in 10% FCS/1% penicillin/streptomycin solution supplemented RPMI 1640 medium (volume/well 180 μL). Cells were allowed to adhere during a 24-h drug-free incubation period at 37 °C/5% CO_2. Drug dilutions (20 μL) were added to the wells to give a concentration range of 1 nM−100 μM. After drug exposures of 7 days, MTT was added to each well at a final concentration of 400 μg/mL. After a 4-h incubation, allowing metabolism of MTT by mitochondrial dehydrogenase to an insoluble formazan product, medium was aspirated and formazan solubilized by the addition of 125 μL of DMSO−glycine buffer (4:1). Cell viability was determined as absorbance at 550 nm, read on an Anthos Labtec systems plate reader.

Test agents were stored as 10 mM stock solutions in DMSO at 4 °C protected from light. Serial dilutions were prepared in media prior to each assay, with final DMSO concentration < 0.25%.

Differential Hypoxic Cytotoxicity. The method of Stratford and Stephens[33] was used. Cells were seeded into glass wells at an initial seeding density of 5×10^3 cells (MCF-7) or 6×10^3 cells (MDA 468) in 500 μL of medium and incubated at 37 °C/5% CO_2 for 3 h to allow them to adhere. Medium was removed and replaced with 250 μL of medium containing the test drug. Plates were placed in gastight Perspex boxes and gassed with either air or nitrogen with 5% CO_2 for 3 h at 37 °C. The drug-containing medium was removed by aspiration and 500 μL of fresh medium was added to each well. The plates were returned to the incubator for a further 3 days, after which time an MTT assay was used (see above) to assess viable cell numbers.

Effects of Growth Factors on the Activity of Tyrphostins. MCF-7 cells (5×10^3) were seeded into sterile flat-bottomed 96-well plates (volume/well 160 μL) in phenol red-free RPMI 1640 medium supplemented with 5% charcoal-stripped FCS, 1% penicillin/streptomycin solution and 300 μg/mL L-glutamine. Cells were allowed to attach overnight, then 20 μL of the required growth factor solution (in RPMI 1640 medium) and 20 μL of the appropriate drug solution were added. Control wells received medium and all wells contained a total volume of 200 μL. Plates were returned to the incubator for a further 7 days and numbers of viable cells were determined by MTT assays (see above).

Western Blot Assays To Determine Tyrosine Kinase Inhibitory Activity of Tyrphostins. Lysates from MDA 468 cells were used as a source of EGF receptors[36] and SkBr3 cells as a source of c-erbB2 tyrosine kinase.[37] In both cases cells were stimulated with EGF.[37] Cells were grown to +70% confluency in 75-cm^2 flasks in RPMI 1640 medium supplemented with 10% FCS and 1% penicillin/streptomycin solution. Medium was replaced with fresh RPMI 1640 medium supplemented with 0.1% FCS and 1% penicillin/streptomycin solution and cells were incubated for 24 h before addition of test drug (100 μM). Following drug addition, cells were incubated overnight before stimulation with EGF (20 ng mL^{-1}) for 10 min at 37 °C. Cells were washed twice with ice-cold PBS, lysed at 4 °C for 20 min, transferred to eppendorfs, sonicated to ensure complete lysis, then finally centrifuged at 13000 rpm for 10 min. The cell lysates were mixed with sample buffer (0.125 M Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 0.2 M dithiothreitol, 0.25 mg/mL bromophenol blue) and boiled for 5 min to denature the proteins.

Protein content of lysates was measured using the Bio-Rad protein assay;[38] 1:200 protein dilutions were prepared in cuvettes containing 0.8 mL of distilled water and 0.2 mL of Bradford reagent. Absorbance at 595 nm was read on a Pharmacia Biotech Ultrospec 2000 UV/vis spectrophotometer. Protein content was calculated from a BSA protein standard curve (1−25 μg/mL).

For the detection of proteins containing phosphorylated tyrosine residues, 50 μg of cellular proteins was separated on denaturing polyacrylamide gels (10% for EGFR, 7.5% for c-erbB2). Phosphorylated A431 protein preparation was included as a positive control. Electrophoresis was performed at 0.06 A for 1 h. Separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes (100 V, 1 h) then probed for phosphotyrosine-containing proteins according to manufacturer's protocol (Upstate biotechnology catalog). Protein bands were subjected to densitometry using a Sharp JX330P scanner and Phoretix ID Advanced V.400 software.

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