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1,1-Diarylalkenes as anticancer agents: Dual inhibitors of tubulin polymerization and phosphodiesterase 4

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ABSTRACT

A series of 1,1-diarylalkene derivatives were prepared to optimize the properties of CC-5079 (**1**), a dual inhibitor of tubulin polymerization and phosphodiesterase 4 (PDE4). By using the 3-ethoxy-4-methoxyphenyl PDE4 pharmacophore as one of the aromatic rings, a significant improvement in PDE4 inhibition was achieved. Compound **28** was identified as a dual inhibitor with potent PDE4 (IC_{50} = 54 nM) and antitubulin activity (HCT-116 IC_{50} = 34 nM and tubulin polymerization IC_{50} ~1 μM). While the nitrile group at the alkene terminus was generally required for potent antiproliferative activity, its replacement was tolerated if there was a hydroxyl or amino group on one of the aryl rings. Conveniently, this group could also serve as a handle for amino acid derivatization to improve the compounds' solubility. The glycinamide analog **45** showed significant efficacy in the HCT-116 xenograft model, with 64% inhibition of tumor growth upon dosing at 20 mg/kg qd.

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1. Introduction

Microtubules are a crucial component of the intracellular machinery responsible for mitosis and cell division. They consist of polymerized chains of α-tubulin and β-tubulin heterodimers; compounds that perturb the polymerization dynamics of α,β-tubulin dimers cause mitotic arrest and can trigger cell death.[1,2] For this reason, microtubules are an important target in cancer therapy.[3–5] Moreover, recent literature reports indicate that by disruption of the endothelial cytoskeleton, certain tubulin-binding agents can block the blood supply to tumors.[6–9] Combretastatin A-4 (CA-4) (Fig. 1) is a *cis* stilbene natural product that binds at the colchicine site of tubulin and exhibits potent antineoplastic and antivascular activity by virtue of its microtubule destabilizing activity. Its phosphate derivative is currently undergoing clinical trials.[10–12] Since the initial reports describing CA-4's isolation[13–15] and biological activity,[16,17] extensive structure–activity studies have investigated related stilbenes as well as modifications of the double bond.[18–27] For example, phenstatin is an analog wherein the double bond of CA-4 is replaced by a carbonyl.[28,29] Previously, we have disclosed the structure and biological activity of CC-5079 (**1**, Fig. 1).[30,31] The diarylacrylonitrile **1** has significant cytotoxic effects on fast-proliferating cells but not on quiescent cells, and was shown to inhibit tubulin polymerization by binding to the colchicine-binding site. Unlike colchicine, vinblastine, and paclitaxel, **1** was equipotent in parental cancer cells and their multi-drug resistant variants, indicating that this novel analog can evade P-gp-mediated drug resistance seen with other chemotherapeutic agents in tested cell lines.

In addition to its activity as a tubulin inhibitor, **1** also potently inhibits phosphodiesterase 4 (PDE4) enzymatic activity. PDE4 inhibitors are commonly known for their general anti-inflammatory and bronchodilatory properties.[32–34] Inhibition of PDE4 results in accumulation of the second messenger cAMP, which attenuates multiple pro-inflammatory processes in neutrophils, eosinophils, mast cells, basophils, lymphocytes, monocytes, and macrophages.[35] Notably, inhibition of PDE4 results in decreased production of the inflammatory cytokine tumor necrosis factor-α (TNF-α) in monocytes.[36] Inhibitors of PDE4 have most frequently been investigated within the context of respiratory and inflammatory diseases such as asthma and chronic obstructive pulmonary disease, but there is evidence indicating that it might also be an useful target in oncology. PDE4 is the principal enzyme responsible for cAMP hydrolysis in 41 of 60 human tumor cell lines evaluated, and lymphoid tumors such as chronic lymphocytic leukemia cells appear to be susceptible to PDE4 inhibitor-induced apoptosis.[37–39] PDE4 has also been identified as a target for brain tumor therapy.[40,41] In addition, there have been reports of PDE4 inhibitors having anti-angiogenic activity.[42] PDE4 inhibitors have direct apoptotic effects on CLL, ALL, and some NHL cells, and while the direct antiproliferative properties of PDE4 inhibitors against solid tumors is very modest, they could have

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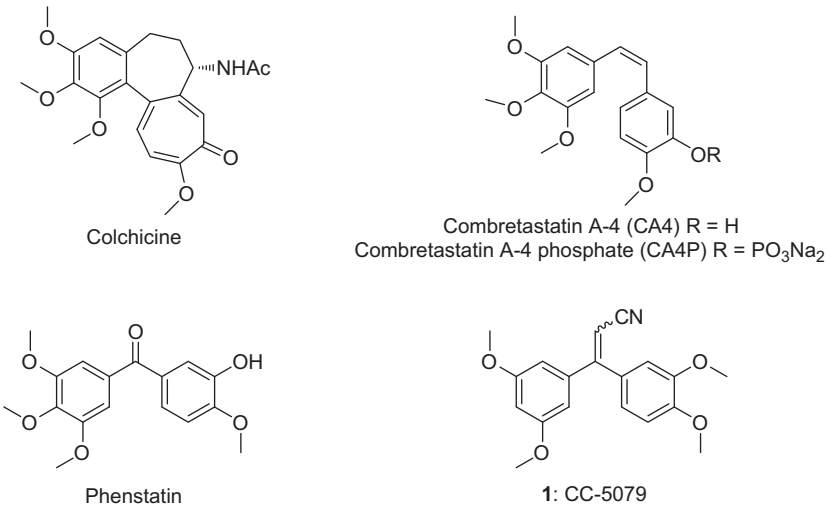


Figure 1. Structures of colchicine, combretastatin A-4 and its phosphate prodrug, phenstatin, and **1**.

antitumor effects by virtue of their antiinflammatory activity. For these reasons, we hypothesize that dual inhibitors of PDE4 and tubulin polymerization might have superior therapeutic value in the treatment of some forms of cancer. The combination of these two independent pharmacological activities into a single small molecule is a new concept in anticancer therapy. The additional biological target also serves to differentiate **1** from other known tubulin interactive agents, such as CA-4 and paclitaxel.

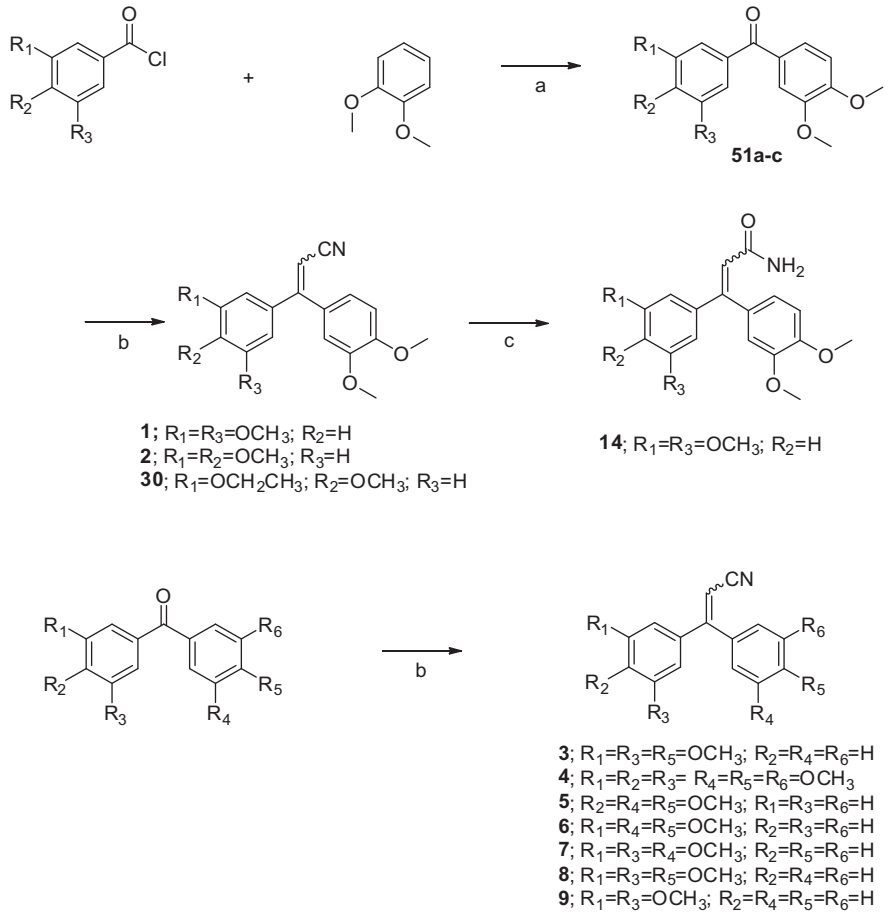
In common with CA-4, the diarylalkene **1** has a simple structure and impressive antineoplastic potency. Unfortunately, **1** also possesses poor aqueous solubility and metabolic stability, detriments also observed for CA-4.[43] In order to both optimize PDE4 and tubulin inhibitory activities as well as improve upon physicochemical properties, we have undertaken a detailed study of the structure–activity relationships (SAR). In this report, we describe results of an exploratory project to study the SAR of this novel series of anti-tumor agents, which were discovered as an offshoot of our Apremilast PDE4 inhibitor program.[44] A full description of chemical synthesis and SAR is provided. The results of xenograft studies for selected analogs are also presented.

2. Results and discussion

2.1. Chemistry

The general method for the synthesis of **1** and related 3,3-diarylacrylonitrile analogs, as well as the 3,3-diarylacrylamide **14** is outlined in Scheme 1. Friedel–Crafts reaction of commercially available acid chlorides with veratrole provided benzophenone intermediates, which were then subjected to Horner–Wadsworth–Emmons (HWE) alkenylation with diethyl cyanomethylphosphonate. The products were typically obtained as a ~1:1 mixture of geometric isomers. The *E*- and *Z*-isomers of **1**, compounds **1*E*** and **1*Z***, were separated by preparative HPLC. The stereochemical assignment was made based on NOE, as shown in Figure 2. Compound 1***E***, with the nitrile trans to the 3,5-dimethoxyphenyl ring, displays NOE between the alkene proton and the ortho protons of the 3,5-dimethoxyphenyl ring, while compound 1***Z***, which has the nitrile cis to the 3,5-dimethoxyphenyl ring, shows NOE between the alkene proton and the ortho protons on the 3,4-dimethoxyphenyl ring. Compound **1** could be converted to acrylamide **14** upon treatment with potassium trimethylsilanolate in toluene. Commercially available benzophenones were also converted to diarylacrylonitriles **3**–**9** using HWE conditions. Related procedures were used to prepare analogs possessing a 3-ethoxy-4-methoxyphenyl ring, depicted in Scheme 2. 4-Bromo-2-ethoxy-1-methoxybenzene was converted to the corresponding aryllithium and then allowed to react with various benzaldehydes. The resultant alcohol intermediates **52a**–**d** were oxidized (MnO_2) to benzophenones and then HWE olefination provided the targeted analogs. The benzophenone intermediates could be accessed directly by reaction of 2-ethoxy-4-lithio-1-methoxybenzene with the appropriate Weinreb amide.[45]

The synthesis of analogs bearing other alkene terminus substitutions was performed as outlined in Scheme 3. Methyl acrylate **13** was prepared by HWE olefination of the benzophenone **49a** with methyl diethylphosphonoacetate, and saponification provided acrylic acid **14**. Methyl ketone **15** was obtained by a two-step sequence involving Wittig olefination of 3,5-dimethoxybenzaldehyde to provide the benzalacetone **55**, followed by Heck coupling under Jeffery's ligand-free conditions.[46,47] Intermediate **51a** reacted with phosphorus ylides to provide alkyl-, aminoalkyl-, and methoxy-substituted alkenes **18**–**21**, as well the unsubstituted analog **13**. Intermediate **51a** was also converted to enyne **22** using the Peterson olefination reaction with 1,3-bis(trimethylsilyl)propyne.[48,49] The synthesis of phenol-containing analogs started with TBS protection of 4-bromo-2-hydroxyphenol and by way of the analogous synthetic route, the targeted analogs were obtained upon TBS deprotection (Scheme 4). For the synthesis of anilines, 4-methoxy-3-nitrobenzaldehyde was added to a solution of the appropriate Grignard reagent (Scheme 5). Subsequent synthetic manipulation provided nitro intermediates, which were then reduced using either stannous chloride or catalytic hydrogenation. Aniline **35** was treated with 2,5-dimethoxytetrahydrofuran in refluxing THF to provide the corresponding pyrrole **39**. For the synthesis of dimethylamino analog **38**, 4-dimethylaminobenzaldehyde was added to a solution of 3-ethoxy-4-methoxyphenyllithium. Surprisingly, treating the resultant benzyl alcohol **63** with excess manganese dioxide resulted in partial demethylation of the aniline; a separable mixture of mono- and dimethylamino benzophenones was obtained in 23% and 17% yields, respectively, after flash column chromatographic purification. The individual products were subsequently carried forward to diarylacrylonitrile analogs **37** and **38**. The anilines **11** and **36** were converted to amide derivatives as shown in Scheme 6. Treatment of **11** with acetyl chloride in refluxing THF provided **44**. Aniline **36** was coupled with Boc-protected glycine and alanine (DCC, HOBt) prior to acidic deprotection. Scheme 7 depicts the synthesis of carbamate- and ester-containing analogs. Reaction of **35** with *N*,*N*-dimethylcarbamyl chloride provided dimethyl carbamate **47**. The phenol **35** was also converted to amino acid esters by DCC coupling with Boc-protected glycine, alanine, and valine, followed by deprotection of the Boc group.



Scheme 1. Synthesis of 3,3-diarylacrylonitriles and 3,3-diarylacrylamide **14**. Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2; (b) $(EtO)_2P(O)CH_2CN$, LiHMDS or NaH, THF; (c) $KOSi(CH_3)_3$, $PhCH_3$.

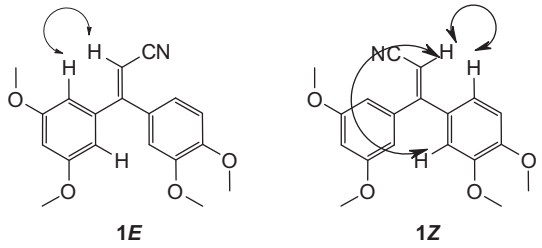
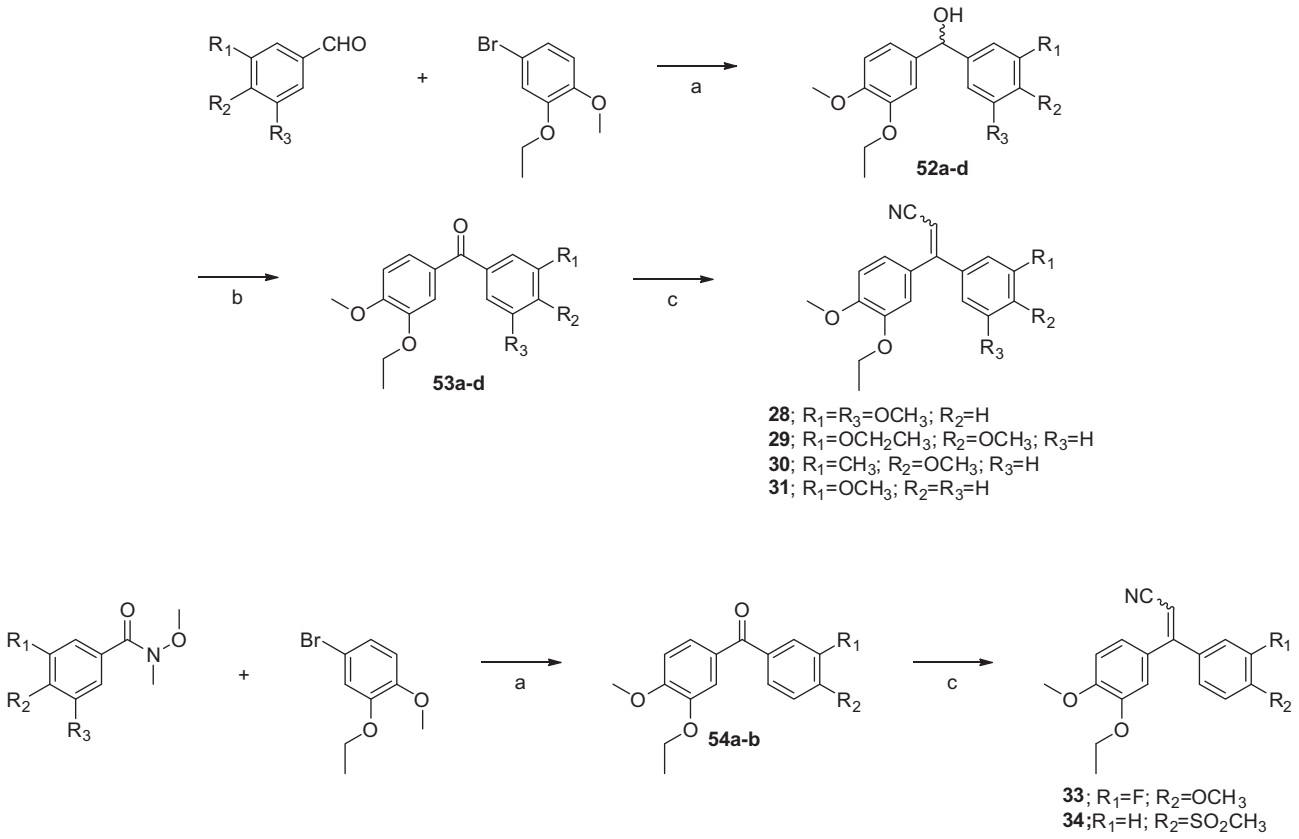


Figure 2. Key NOE correlations for **1*E*** and **1*Z***.

2.2. Biological evaluation

All analogs were evaluated for in vitro cell growth inhibition against the human colon carcinoma cell line HCT-116 to evaluate antitumor activity. Additionally, PDE4 enzyme inhibition was measured in a cell-free cAMP hydrolysis assay.[50] To quantify the downstream effect of PDE4 inhibition, TNF-α inhibition was measured in lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cells (hPBMC).[51] Since this is a phenotypic assay using human cells, it may have greater clinical relevance than inhibition of purified PDE4. However, care should be taken in interpreting the results of the TNF-α data, as other cellular phenomena can also result in the inhibition of TNF-α release. For example, a compound which is toxic to hPBMC would probably interfere with the measurable physiological response (release of TNF-α), without inhibiting the PDE4 enzyme.

Table 1 summarizes the biological data for inhibition of HCT-116 cell growth and inhibition of PDE4 and TNF-α. Data obtained with CA-4 are also presented for reference. The lead analog **1** potently inhibited the growth of HCT-116 cells, with an IC_{50} of 18 nM. It also inhibited PDE4 and TNF-α, albeit with ~10-fold decrease in potency (IC_{50} values for PDE4 and TNF-α were 350 and 270 nM, respectively). When the individual isomers were tested, **1*Z*** was about 10-fold more potent in the HCT-116 assay, and about five-fold more potent in the PDE4 and TNF-α assays. The greater activity of the **1*Z*** isomer is in accord with results reported for a previous series of analogs.[52] To facilitate rapid synthesis and screening, subsequent analogs were tested as the ~1:1 *E*/*Z* mixtures obtained in the HWE reactions. The 3,5-dimethoxyphenyl moiety was found to be important for potent antiproliferative activity; its replacement with a 3,4-dimethoxyphenyl group (i.e., **2**) resulted in a nearly 10-fold loss of antiproliferative potency in the HCT-116 assay, but had no effect on PDE4 inhibitory activity. On the other hand, if this ring was replaced with the 3,4,5-trimethoxyphenyl ring found in CA-4 (i.e., **3**), then good antiproliferative potency was maintained, but PDE4 inhibitory potency dropped six-fold. Substitution of both phenyl rings with 3,4,5-trimethoxyl (**4**) resulted in reduced activity in all assays. Analogs **5**–**8** are trimethoxy analogs, and among them **6–8** result from deletion of a single methoxyl from the structure of **1**. Data from this series indicates that the 3,5-dimethoxyphenyl moiety is required for potent antiproliferative activity and so is at least one methoxyl on the other ring. The 3,4-dialkoxyphenyl motif, a well-known PDE4 pharmacophore,[53,54] was essential for PDE4 inhibitory activity.

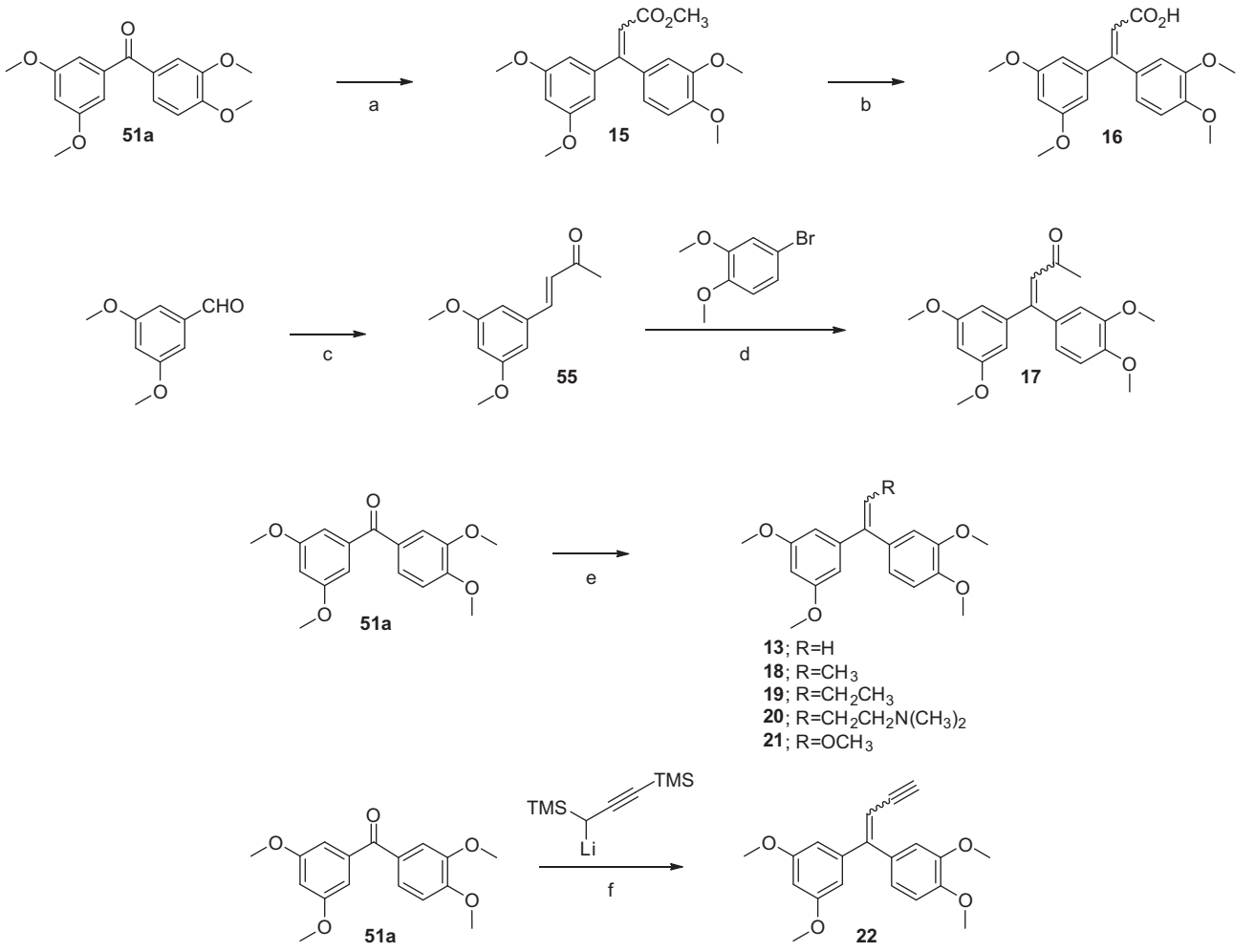


Scheme 2. Synthesis of diarylacrylonitriles with a 4-ethoxy-3-methoxyphenyl ring. Reagents and conditions: (a) *n*-BuLi or Mg, THF; (b) MnO_2, CH_2Cl_2; (c) $(EtO)_2P(O)CH_2CN$, LiHMDS, THF.

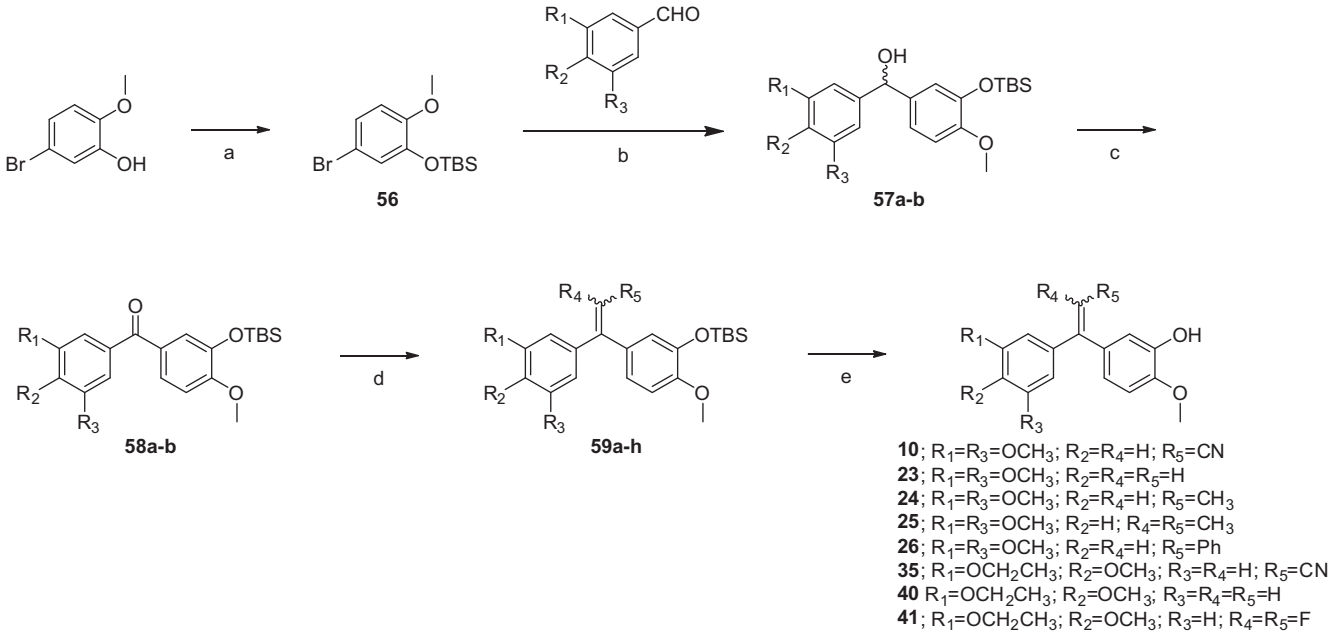
Compound **10**, which possesses the 3-hydroxy-4-methoxyphenyl ring found in CA-4, was very potent in the HCT-116 assay (IC_{50} ~2 nM), as were the amino-substituted analogs **11** and **12**. These analogs suffered substantial loss of PDE4 inhibitory activity; nevertheless, they did inhibit the release of TNF-α from hPBMC. This may be a result of toxicity to hPBMC or it may be due to effects on another signaling pathway. Combretastatin A-4 was extremely potent in the HCT-116 assay (IC_{50} of 0.7 nM), but was not active as a PDE4 inhibitor. Although CA-4 did inhibit the release of TNF-α from hPBMC, it evidently did so by a mechanism other than inhibition of PDE4.

The role of the group at the alkene terminus was explored in a series of analogs shown in Table 2. In general, the nitrile proved to be critical for potent antiproliferative and PDE4 inhibitory activity. Its removal provided **13**, which showed a 5-fold and 20-fold drop in potency in the PDE4 and TNF-α assays, and was entirely inactive against HCT-116 cells. Replacement of the nitrile with amide (**14**), methyl ester (**15**), or carboxylic acid (**16**) resulted in a loss of both antiproliferative activity (IC_{50} >10 μM) and PDE4 inhibition (IC_{50} >1 μM). Compound **17**, which possesses a methyl ketone at this site, retained activity in the PDE4 and TNF-α assays, but was not active against HCT-116 cells. Analogs **18–22**, variously substituted with alkyl, aminoalkyl, methoxyl, and ethyne groups, all showed reduced activity relative to **1**. The only one of these analogs to display any activity against HCT-116 was the methyl analog **18**, with an IC_{50} of 480 nM. Interestingly, when the 3-hydroxy-4-methoxyphenyl ring found in CA-4 was used instead of 3,4-dimethoxyphenyl, antiproliferative activity was restored. Compound **23**, which is unsubstituted at the alkene terminus, and compound **24**, which possesses a methyl group at this site, both displayed similar activity against HCT-116 as **1**. These analogs are structurally reminiscent of the recently reported isocombretastatins.[55,56] Like the other hydroxyl-substituted diarylacrylonitrile **10** without a 3,4-alkoxyphenyl moiety, these analogs were not active against PDE4. When the alkene was disubstituted (**25**) or substituted with a phenyl ring (**26**), activity dropped substantially. Use of an amino group in lieu of the hydroxyl (e.g., **27**) was also found to restore activity in the HCT-116 assay. It is unclear why the presence of polar functionality on the aromatic ring seems to compensate for the loss of the nitrile group, but its requirement is in accord with previously disclosed SAR for the structurally related phenstatins[28,29] and isocombretastatins.[55,56]

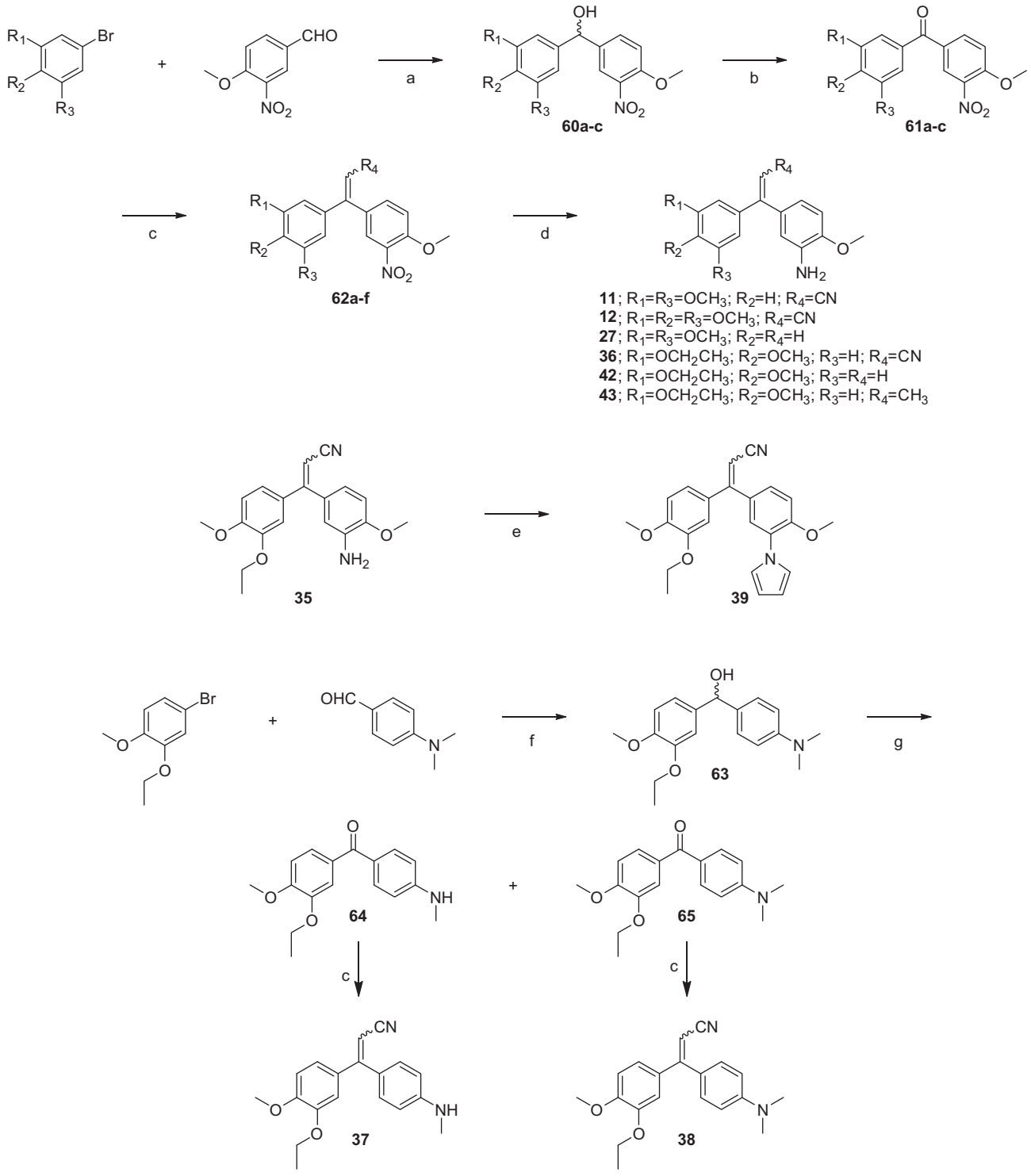
The 3,4-dialkoxyphenyl moiety is a key known pharmacophore for PDE4 inhibitors. Generally, a methoxy group at the 4-position is optimal, and a bulky group such as cyclopentoxy at the 3-position leads to more potent analogs in a majority of reported series of PDE4 inhibitors which contain the 3,4-dialkoxyphenyl group.[53,54] Previously, we have shown that an ethoxy group is sufficient to confer the same potency boost as a cyclopentoxy at the 3-position in some series of PDE4 inhibitors.[44] In order to enhance potency for PDE4 inhibition, therefore, diarylacrylonitrile analogs bearing a 3-ethoxy-4-methoxyphenyl ring were prepared and evaluated for activity (Table 3). Use of this ring system in conjunction with a 3,5-dimethyoxyphenyl ring provided **28**, and resulted in potent activity in all three assays, with all three IC_{50} values ~50 nM. In this case, substitution of ethoxy for methoxy (i.e., **28** vs **1**) provided a sevenfold improvement in PDE4 activity, with no loss of HCT-116 antiproliferative potency. Use of the 3-ethoxy-4-methoxy substitution motif on both phenyl rings (**29**) resulted in a 10-fold drop-off in HCT-116 antiproliferative activity while PDE4 activity remained unchanged relative to **28**. Analogs **29**–**34**, with various mono- or 3,4-disubstitutions, displayed reduced antiproliferative activity



Scheme 3. Synthesis of analogs featuring nitrile replacements. Reagents and conditions: (a) $(EtO)_2P(O)CH_2CO_2CH_3$, LiHMDS, THF; (b) KOH, H_2O, CH_3OH;(c) $Ph_3P{=}CHCOCH_3$, CH_3CN; (d) $Pd(OAc)_2$, NaOAc, Bu_4NBr, DMF; (e) $Ph_3P{=}CHR$, THF; (f) THF.

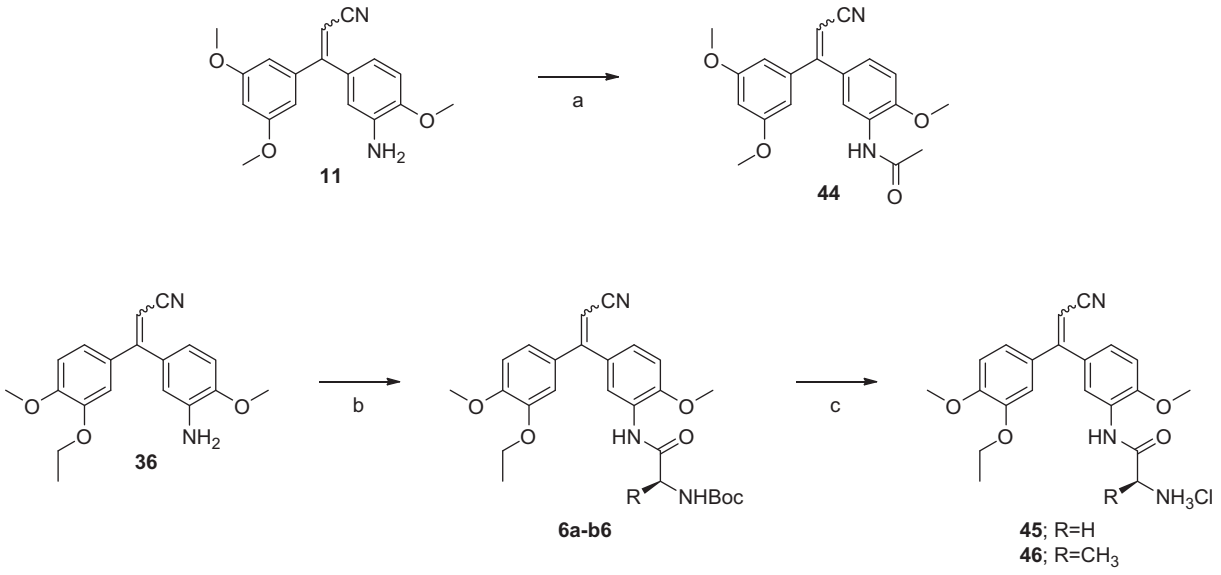


Scheme 4. Synthesis of hydroxy-substituted 3,3-diarylacrylonitriles. Reagents and conditions: (a) TBSCl, DIEA, DMF; (b) *n*-BuLi, THF; (c) MnO_2, CH_2Cl_2; (d) $(EtO)_2P(O)$ CHR_4R_5 or $XPh_3PCHR_4R_5$, LiHMDS, THF (X = Br or I); (e) TBAF, THF.

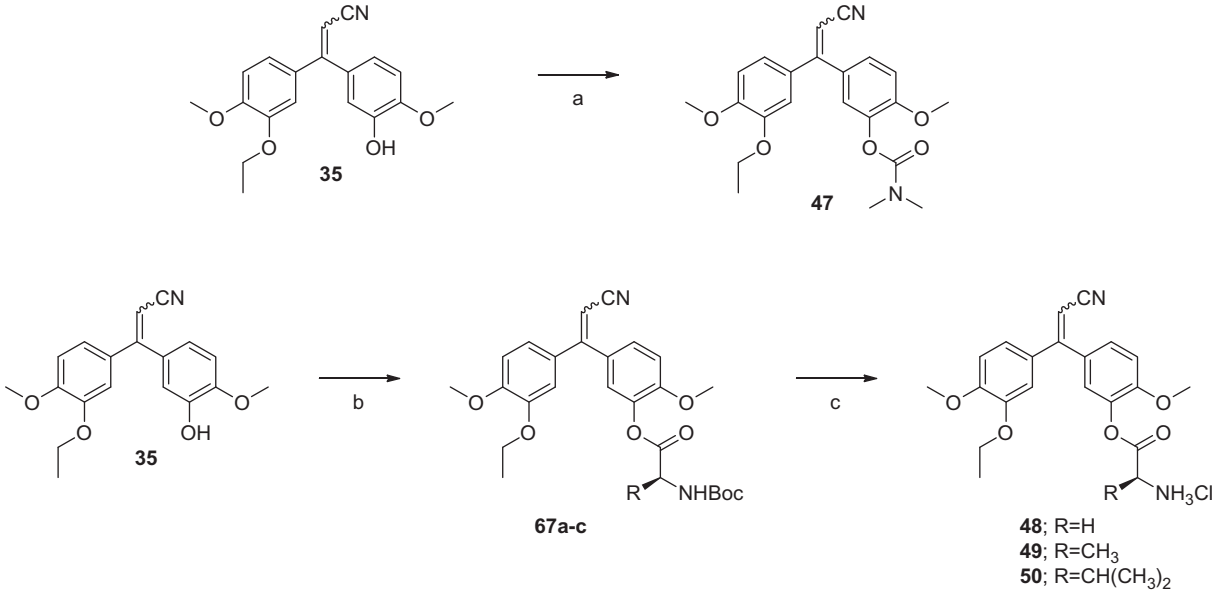


Scheme 5. Synthesis of pyrrole- and amino-substituted 3,3-diarylacrylonitriles. Reagents and conditions: (a) Mg, cat. I_2, THF; (b) MnO_2, CH_2Cl_2 or PCC, CH_2Cl_2; (c) $(EtO)_2P(O)CH_2CN$ or $BrPh_3PCH_2R_4$, LiHMDS, THF; (d) $SnCl_2$, EtOH, EtOAc or H_2, Pd/C, EtOAc; (e) 2,5-dimethoxytetrahydrofuran, AcOH; (f) *n*-BuLi, THF; (g) MnO_2, CH_2Cl_2

but similar PDE4 activity to **28**. However, the inclusion of a small polar group such as hydroxyl or amino was again found to have a beneficial effect on antiproliferative potency. Use of the 3-hydroxy-4-methoxyphenyl ring found in CA-4 resulted in **35**, which had similar activity as **28**. The corresponding amino analog **26** exhibited comparable activity, with IC_{50} values in the range of 10–50 nM across the three different assays. Use of a mono- or dimethylamino group at the *para*-position provided compounds **37**–**38**, which also had IC_{50} values ~50 nM in all three assays. Incorporation of the amino group into a pyrrole provided **39**, and had no significant impact upon activity. Since these data indicate that the 3-ethoxy-4-methoxyphenyl group is compatible with the 3-hydroxy-4-methoxyphenyl, compounds des-nitrile analogs **40**–**43** were prepared. Unlike des-nitrile analogs **23**–**25** and **27**, which retained antiproliferative activity but not PDE4 inhibitory activity, these des-nitrile analogs inhibited PDE4 as well as HCT-116, although



Scheme 6. Synthesis of amide-containing 3,3-diarylacrylonitriles. Reagents and conditions: (a) AcCl, THF; (b) BocHNCH(R)CO_2H, DCC, HOBt, DMF; (c) HCl, CH_2Cl_2.

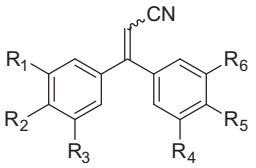


Scheme 7. Synthesis of carbamate- and ester-containing 3,3-diarylacrylonitriles. Reagents and conditions: (a) ClCON$(Me)_2$, THF; (b) BocHNCH(R)CO_2H, DCC, 4-pyrrolidin-1-yl-pyridine, CH_2Cl_2; (c) HCl, CH_2Cl_2.

they still were ~10-fold more potent in the HCT-116 assay. Removal of the nitrile from **35** provided **40**, which had similar activity to the lead compound **1**. Difluorination at the alkene terminus provided **41**, and had no effect on activity. The des-nitrile aniline **42** had very similar activity to phenol analog **40**, while addition of a methyl to the alkene terminus of **42** provided **43**, which had similar activity against HCT-116 and PDE4.

In order to improve on physicochemical properties, some of the more potent agents were derivatized as amides and esters (Table 4). The effect of acylation upon activity was first examined by acetylation of **11**. Disappointingly, acetamide **44** was ~50-fold less active against HCT-116, though the IC_{50} was still a reasonable 92 nM. Neither **11** nor **44** inhibited PDE4 at submicromolar concentrations, although both inhibited TNF-α release. For the preparation of more water-soluble analogs, **36** was derivatized with small amino acids. These analogs **45**–**46** displayed a modest decrease in HCT-116 and PDE4 activity, with a 2–3-fold decline in activity for alanine derivative **46**, and a slightly greater dropoff for glycine analog **45**. Among derivatives of phenol **35,** the carbamate **47** was inactive against HCT-116 but retained good inhibitory activity against PDE4, while the amino acid esters (**48**–**50**) had no noticeable loss of activity, with very similar potency to the parent compound. Considering that many of the analogs in Table 4 exhibit similar activity to their respective parent compounds, it is possible that some hydrolysis of these analogs may occur during the assays to release the parent active agents **35** or **36**; no efforts were made to detect **35** or **36** in the assay media. It is therefore not possible to say whether the activity observed in these assays is intrinsic to the analogs themselves or if it is due to hydrolysis to active agents. However, the stability of **45–50** was tested in human plasma to determine whether they can function as prodrugs of **35** and **36**. The ester analogs **48–50** were highly unstable in human plasma,

Table 1
Inhibitory effect of **1** and related 3,3-diarylacrylonitrile analogs on the growth of HCT-116 cells, PDE4 activity, and TNF-α release from hPBMC



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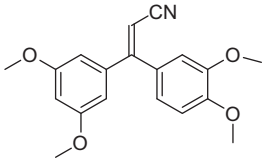
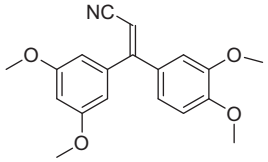


1*E*

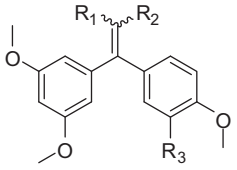


1*Z*

Compd	R_1	R_2	R_3	R_4	R_5	R_6	HCT-116[a] (μM)	PDE4[a] (μM)	TNF-α[a] (μM)
1[30]	OCH_3	H	OCH_3	OCH_3	OCH_3	H	0.018 ± 0.001	0.35 ± 0.20	0.27 ± 0.036
1*E*							0.11 ± 0.012	0.73 ± 0.24	0.65 ± 0.23
1*Z*							0.011 ± 0.0003	0.15 ± 0.032	0.093 ± 0.0006
2	H	OCH_3	OCH_3	OCH_3	OCH_3	H	0.14 ± 0.0085	0.31 ± 0.0088	0.6
3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	H	0.041 ± 0.0044	1.8 ± 0.26	0.54 ± 0.15
4	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	>10	2.4 ± 0.61	0.87 ± 0.093
5	H	OCH_3	H	OCH_3	OCH_3	H	0.024 ± 0.0016	0.19 ± 0.042	0.35 ± 0.010
6	OCH_3	H	H	OCH_3	OCH_3	H	0.31 ± 0.080	0.16 ± 0.037	0.66 ± 0.13
7	OCH_3	H	OCH_3	OCH_3	H	H	0.015 ± 0.0005	3.4 ± 0.55	0.29 ± 0.14
8	OCH_3	H	OCH_3	H	OCH_3	H	0.0012 ± 0.0005	3.8 ± 1.2	0.44 ± 0.11
9	OCH_3	H	OCH_3	H	H	H	0.97 ± 0.14	1.9 ± 0.56	2.4 ± 0.81
10	OCH_3	H	OCH_3	OH	OCH_3	H	0.0025 ± 0.0013	2.4 ± 0.87	0.0012 ± 0.001
11	OCH_3	H	OCH_3	NH_2	OCH_3	H	0.0014 ± 0.0005	9.8 ± 1.73	0.011 ± 0.024
12	OCH_3	OCH_3	OCH_3	NH_2	OCH_3	H	0.0018 ± 0.0003	>10	0.2 ± 0.007
CA-4							0.00074 ± 0.0002	>10	0.011 ± 0.004

[a] IC_{50} values are the means of at least three independent determinations.

Table 2
Inhibitory effect of analogs bearing nitrile replacements on the growth of HCT-116 cells, PDE4 activity, and TNF-α release from hPBMC



Compd	R_1	R_2	R_3	HCT-116[a] (μM)	PDE4[a] (μM)	TNF-α[a] (μM)
1[30]	CN	H	OCH_3	0.018 ± 0.001	0.35 ± 0.20	0.27 ± 0.036
13	H	H	OCH_3	>10	2.2 ± 0.55	4.8 ± 0.63
14	$CONH_2$	H	OCH_3	>10	6.7 ± 0.67	5.5 ± 2.2
15	CO_2CH_3	H	OCH_3	>10	1.3 ± 0.22	5.4 ± 0.62
16	CO_2H	H	OCH_3	>10	>10	>10
17	$COCH_3$	H	OCH_3	>10	0.14 ± 0.025	0.75 ± 0.16
18	CH_3	H	OCH_3	0.48 ± 0.12	1.5 ± 0.34	2.6 ± 0.29
19	CH_2CH_3	H	OCH_3	>10	2.0 ± 0.30	>10
20	$CH_2CH_2N(CH_3)_2$	H	OCH_3	>10	>10	>10
21	OCH_3	H	OCH_3	>10	3.6 ± 3.1	6.3 ± 1.8
22	C≡CH	H	OCH_3	>10	3.5	7.2 ± 3.1
23	H	H	OH	0.021	>10	3.0 ± 1.2
24	CH_3	H	OH	0.030 ± 0.002	>10	0.15 ± 0.15
25	CH_3	CH_3	OH	0.97 ± 0.15	>10	7.0 ± 1.6
26	Ph	H	OH	>10	>10	>10
27	H	H	NH_2	0.013 ± 0.0002	>10	0.33 ± 0.046

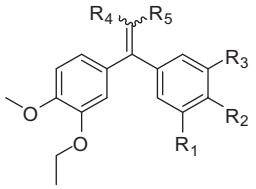
[a] IC_{50} values are the means of at least three independent determinations.

completely falling apart to the parent phenol **35** in under 20 min. On the other hand, amide analogs **45** and **46** were quite stable in human plasma, with half-lives of 56 h and 27 h, respectively, and the formation of aniline **36** was not observed. The modifications did result in improved solubility. For example, the solubility of **36** (<3 μg/mL at both pH 5.0 and 7.4) was greatly improved by its conversion to **45** (356 μg/mL at pH 5.0 and 205 μg /mL at pH 7.4) or **46** (1,045 μg/mL at pH 5.0 and 405 μg/mL at pH 7.4).

It has been shown previously that the cytotoxicity of **1** is based on binding to tubulin at the colchicine binding site, thereby inhibiting its polymerization.[33] To confirm that the same mechanism underlies the cytotoxic activity of the new analogs described herein, selected compounds were assayed at 5 and 10 μM for inhibition of tubulin polymerization. As shown in Table 5, the new analogs inhibited tubulin polymerization as expected. In the tubulin inhibition assay, CA-4 was somewhat more potent than the diarylalkene analogs, in accord with its greater potency against HCT-116. These findings strongly suggest that the compounds' cytotoxicity is based on the same mechanism as that of **1**, namely inhibition of tubulin polymerization.

We have docked compound **28** into the colchicine binding site of the tubulin crystal structure (PDB code 1SA0)[57] using MOE software.[58] Considering the greater activity of **1*Z*** compared to **1*E***, the molecule was docked in the *Z*-configuration. Figure 3 shows the docking results for compound **28** overlaid upon the crystal structure of colchicine. Due to the relatively large binding pocket, two

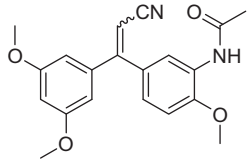
Table 3
Inhibitory effect of analogs with a 3-ethoxy-4-methoxyphenyl ring on the growth of HCT-116 cells, PDE4 activity, and TNF-α release from hPBMC



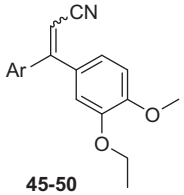
Compd	R_1	R_2	R_3	R_4	R_5	HCT-116[a] (μM)	PDE4[a] (μM)	TNF-α[a] (μM)
28	OCH_3	H	OCH_3	CN	H	0.034 ± 0.0029	0.054 ± 0.011	0.043 ± 0.014
29	OCH_2CH_3	OCH_3	H	CN	H	0.44 ± 0.0077	0.070 ± 0.015	0.048 ± 0.010
30	OCH_3	OCH_3	H	CN	H	0.20 ± 0.083	0.083 ± 0.020	0.18 ± 0.019
31	CH_3	OCH_3	H	CN	H	0.087 ± 0.019	0.082 ± 0.0005	0.10 ± 0.032
32	OCH_3	H	H	CN	H	0.45 ± 0.014	0.11 ± 0.0099	0.23 ± 0.058
33	F	OCH_3	H	CN	H	0.13 ± 0.0011	0.07 ± 0.009	0.031 ± 0.040
34	H	SO_2CH_3	H	CN	H	>10	0.20 ± 0.0064	0.13 ± 0.057
35	OH	OCH_3	H	CN	H	0.015 ± 0.0018	0.091 ± 0.030	0.044 ± 0.0056
36	NH_2	OCH_3	H	CN	H	0.019 ± 0.0048	0.047 ± 0.0099	0.031 ± 0.0066
37	H	$NHCH_3$	H	CN	H	0.055 ± 0.011	0.041 ± 0.005	0.14 ± 0.026
38	H	$N(CH_3)_2$	H	CN	H	0.031 ± 0.0063	0.052 ± 0.0095	0.13 ± 0.057
39	Pyrrol-1-yl	OCH_3	H	CN	H	0.049 ± 0.0054	0.070 ± 0.014	0.067 ± 0.033
40	OH	OCH_3	H	H	H	0.036 ± 0.0014	0.38 ± 0.048	0.029 ± 0.0017
41	OH	OCH_3	H	F	F	0.074 ± 0.012	0.36 ± 0.058	0.054 ± 0.046
42	NH_2	OCH_3	H	H	H	0.038 ± 0.0075	0.68 ± 0.10	0.38 ± 0.24
43	NH_2	OCH_3	H	CH_3	H	0.11 ± 0.026	0.59 ± 0.090	2.8 ± 0.43

[a] IC_{50} values are the means of at least three independent determinations.

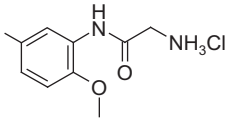
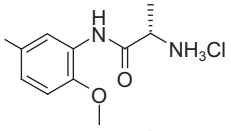
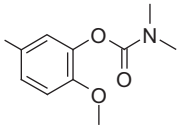
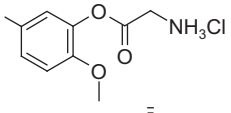
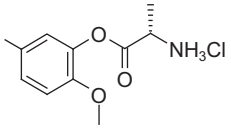
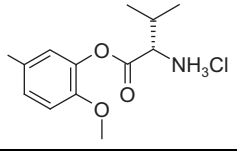
Table 4
Inhibitory effect of ester- and amide-containing analogs on the growth of HCT-116 cells, PDE4 activity, and TNF-α release from hPBMC



44

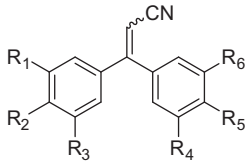


45-50

Compd	Ar	HCT-116[a] (μM)	PDE4[a] (μM)	TNF-α[a] (μM)
44		0.092 ± 0.021	>10	0.066 ± 0.026
45		0.093 ± 0.0018	0.4 ± 0.072	0.031 ± 0.020
46		0.04 ± 0.0014	0.15 ± 0.016	0.027 ± 0.014
47		>10	0.12 ± 0.01	0.24 ± 0.062
48		0.019 ± 0.001	0.049 ± 0.007	0.058 ± 0.014
49		0.017 ± 0.0017	0.069	0.057 ± 0.0054
50		0.022 ± 0.0033	0.12 ± 0.010	0.067 ± 0.0030

[a] IC_{50} values are the means of at least three independent determinations.

Table 5
Tubulin polymerization inhibition data and inhibition of HCT-116



Compd	R_1	R_2	R_3	R_4	R_5	R_6	Tubulin inhib% at 5 μM	Tubulin inhib% at 10 μM	HCT-116[a] (μM)
1	OCH_3	H	OCH_3	OCH_3	OCH_3	H	65	84	0.018
7	OCH_3	H	OCH_3	OCH_3	H	H	67	88	0.015
10	OCH_3	H	OCH_3	OH	OCH_3	H	77	N/D	0.0025
11	OCH_3	H	OCH_3	NH_2	OCH_3	H	76	N/D	0.0014
28	OCH_3	H	OCH_3	OCH_2CH_3	OCH_3	H	48	59	0.034
36	OCH_2CH_3	OCH_3	H	NH_2	OCH_3	H	65	76	0.019
44	OCH_3	H	OCH_3	$NHC(O)CH_3$	OCH_3	H	46	74	0.092
45	OCH_2CH_3	OCH_3	H	$NHC(O)CH_2NH_3Cl$	OCH_3	H	59	70	0.023
CA-4							88	N/D	0.00074

[a] % inhibition values are the means of at least two independent determinations; N/D: not determined.

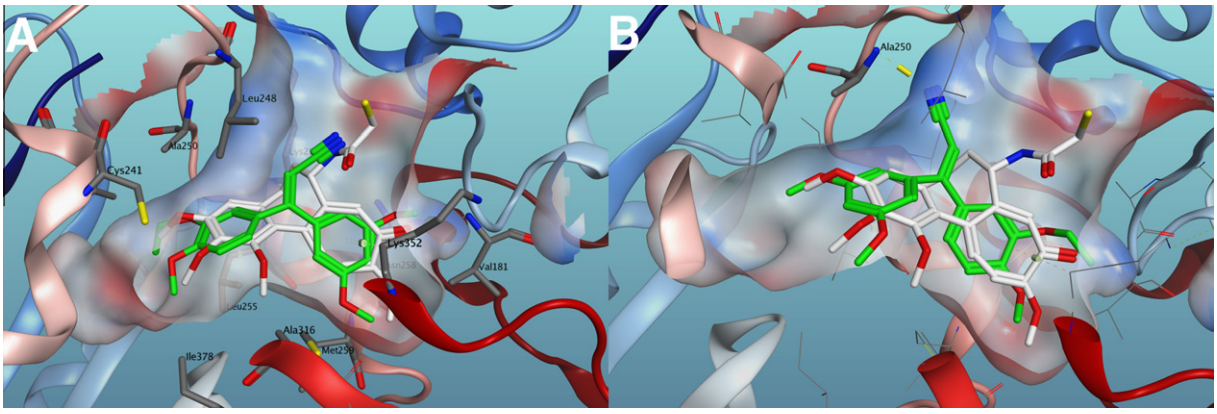


Figure 3. Docked models of **28** (green) overlaid on the crystal structure of colchicine (white). A and B display two different binding modes.

different binding modes were possible and these are shown in Figure 3A and B. The first binding mode, shown in Figure 3A, shows good overlay of the aromatic rings of compound **28** with colchicine. The nitrile moiety resides in a similar region as the acetamide of colchicine. The decision to lock the ligand in the *Z*-configuration resulted in a reversal of the 3,4- and 3,5-dialkoxy rings relative to the corresponding rings in colchicine. This is reasonable considering that results from the SAR study have shown that the 3,4,5-dimethoxy substitution is not critical in this series; for example, replacement of the 3,4,5-trimethoxyphenyl with a 4-methoxyphenyl ring (as in compounds **3** and **5**) resulted in comparable activity against HCT-116. In this binding mode, all of the contacts are nonpolar, forming a fairly large lipophilic pocket. The other binding mode, shown in Figure 3B, has the 3,4- and 3,5-dialkoxy rings in the analogous positions to the corresponding rings in colchicine. Although the nitrile has no overlay with colchicine, it does engage in an H-bonding interaction with Ala250 in the tubulin protein backbone.

2.3. HCT-116 xenograft study of selected analogs

Compounds **28** and **45** were selected for further evaluation in the HCT-116 xenograft model. The compounds were administered intraperitoneally (ip) to mice bearing subcutaneous tumors at 20 mg/kg once daily (qd). Figure 4 shows the compounds' effect on HCT-116 tumor mouse xenografts, and Figure 5 shows the compounds' effect on the body weights of treated groups. The positive control in the study was Camptosar (irinotecan), a topoisomerase I inhibitor with good efficacy in this model.[59,60] As anticipated, Cam-

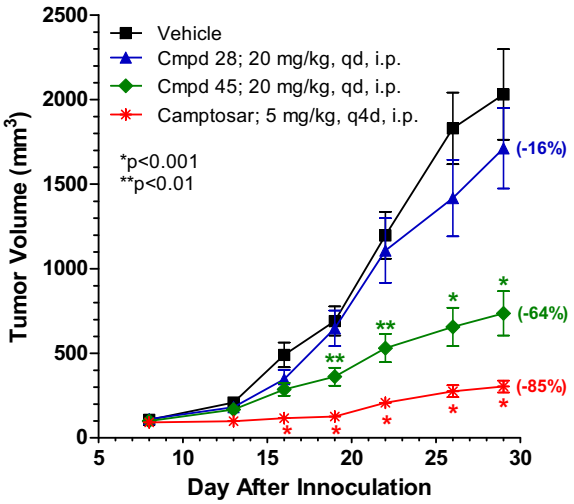


Figure 4. Effect of **28** and **45** on the growth of human colorectal cancer (HCT-116) mouse xenograft. HCT-116 cells (2×10^6) were injected s.c. into SCID mice. When the tumors reached ~100 mm^3, the mice were treated ip with vehicle control, **28** (20 mg/kg once daily), **45** (20 mg/kg once daily), or positive control Camptosar[57,58] (5 mg/kg once every four days). *Data Points*, mean tumor volumes plotted against time; *bars*, SE.*, p <0.001; **, p <0.01.

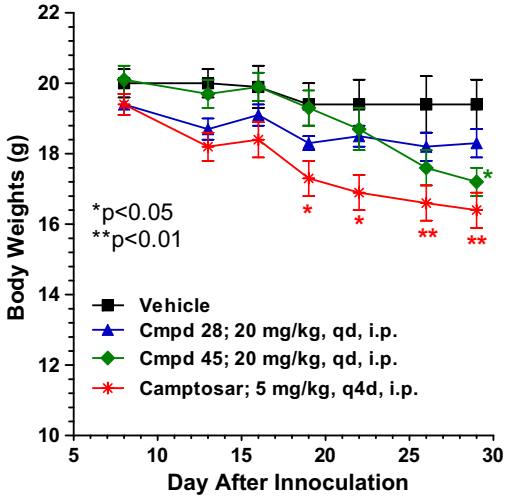


Figure 5. Effect of **28** and **45** on body weights of mice from HCT-116 colorectal cancer mouse xenograft study. Mice were treated ip with vehicle control, **28** (20 mg/kg once daily), **45** (20 mg/kg once daily), or positive control Camptosar[57,58] (5 mg/kg once every four days).*Data points*, mean body weights plotted against time; *bars*, SE.*, p <0.01; **, p <0.05.

ptosar administration resulted in efficacious anti-cancer effect, with 85% inhibition of tumor growth compared to the vehicle group. Compound **45** demonstrated significant efficacy, with a 64% tumor growth inhibition over the vehicle group. Overall, the compound was well tolerated. The mice in this group showed a 15% decrease in body weight, with a majority of the body weight loss in this group occurring after day 19 of the study. Tumor volume in mice treated with compound **28** was reduced by only 16% with respect to the vehicle-treated mice. For this compound, it is likely that poor solubility was partly responsible for the lack of efficacy observed in the study.

3. Conclusion

The novel analogs reported herein, dual inhibitors of tubulin polymerization and PDE4, were designed to elicit an antineoplastic effect by means of two independent molecular mechanisms. The SAR for antitubulin activity was similar to that of other combretastatin analogs, with the additional requirement of the nitrile group at the alkene terminus. No other alkene substitution was found to restore antitubulin activity in the absence of polar functionality on the ring. However, the nitrile could be replaced or eliminated in the presence of an appropriate hydroxyl or amino phenyl substitution without loss of antiproliferative activity. The presence of a 3,4-dialkoxy motif in these analogs did provide PDE4 inhibition. The use of 3-ethoxy-4-methoxyphenyl PDE4 pharmacophore resulted in a boost in PDE4 inhibition relative to the 3,4-dimethoxy analogs such as **1**. The critical structural motifs were combined in compounds **35** and **36**, which possess the 3-ethoxy-4-methoxyphenyl PDE4 pharmacophore and another ring bearing a hydroxyl or amino respectively. These analogs inhibited HCT-116 with IC_{50} ~20 nM and PDE4 with IC_{50} ~50 nM. Their respective amino acid ester or amide derivatives, prepared for improved solubility, showed comparable in vitro activity. One such analog, the glycinamide analog **45** also displayed significant in vivo efficacy in the HCT-116 xenograft study, and merits further study to identify the active isomer and develop a stereoselective synthesis.

In recent years, a multitude of tubulin interactive anti-cancer agents have been reported in the literature, with scattered examples in preclinical and clinical development. The diarylalkenes described herein are distinguished by the introduction of PDE4 inhibitory activity, and it is to be hoped that this additional mechanism of action will result in enhanced clinical efficacy. The success of this strategy will be determined in future studies.

4. Experimental section

Commercial reagents and solvents were used as received without further purification. Reactions were run under nitrogen atmosphere, unless noted otherwise. Column chromatography refers to flash chromatography using Isco brand prepacked silica gel cartridges. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Brucker 250 spectrometer. NMR spectra (250 MHz ^{1}H and 62.5 MHz ^{13}C) were recorded in the deuterated solvent indicated with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in hertz (Hz). Elemental analyses and were obtained from QTI Intertek, Whitehouse, NJ. Elemental analyses were within 0.4% of theory. Purity of compounds was also analyzed by reverse phase HPLC using the solvent systems indicated. All compounds submitted for pharmacological testing possessed purity $\geqslant$95% as evidenced by the results of elemental analysis and HPLC analysis. Melting points were obtained from QTI Intertek, Whitehouse, NJ. CA-4 was prepared according to the literature procedure.[15]

4.1. Preparation of 3,3-diarylacrylonitriles by Horner–Wadsworth–Emmons reaction. General procedure A. (*E*/*Z*)-3-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)acrylonitrile (1)[30,31]

To a stirred solution of diethylcyanomethylphosphonate (0.42 mL, 2.5 mmol) in THF (15 mL) was added a 1.3 M solution of LiHMDS in THF (1.9 mL, 2.5 mmol) at 0 °C. The solution was allowed to warm to room temperature and was stirred for 30 min, and then a mixture of **51a** (0.7 g, 2.3 mmol) in THF (20 mL) was added. The reaction mixture was refluxed overnight and was then cooled and quenched with water (100 mL). The resulting mixture was extracted with methylene chloride (2 × 50 mL). The combined extracts were washed with water (100 mL), dried ($MgSO_4$), and concentrated under vacuum. The residue was chromatographed in 99:1 CH_2Cl_2/EtOAc to provide 0.66 g (81% yield) of a mixture of the *E* and *Z* isomers as a white solid: mp 88–90 °C. ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3H), 3.80 (s, 3H), 3.84–3.87 (m, 3H), 3.91–3.94 (m, 3H), 5.61–5.66 (m, 1H), 6.40–6.61 (m, 3H), 6.80–7.10 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 55.4, 55.5, 55.9, 56.0, 93.1, 93.4, 102.0, 102.1, 107.0, 107.6, 110.7, 110.7, 110.9, 112.6, 118.0, 118.2, 122.1, 123.2, 129.2, 131.1, 138.9, 141.3, 148.5, 148.8, 150.6, 151.1, 160.6, 160.7, 162.5, 162.7. Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 70.33; H, 5.89; N, 4.03.

4.2. (*E*)-3-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-acrylonitrile (1*E*)

The geometric isomers of compound **1** were separated on a Waters Prep LC 4000 System, eluting with a 2:3 mixture of acetonitrile–water, providing the product as a white solid: mp 114–116 °C. ^{1}H NMR (DMSO-d_6) δ 3.74–3.83 (m, 12H), 6.21 (s, 1H), 6.49 (d, J = 2.2 Hz, 2H), 6.63 (t, J = 2.1 Hz, 1H), 6.93 (dd, J = 8.1 Hz, J = 1.8 Hz, 1H), 6.99 (d, J = 1.8 Hz, 1H), 7.08 (d, J = 8.3 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ 55.4, 55.5, 55.6, 94.0, 95.2, 100.5, 102.0, 106.7, 111.4, 112.6, 118.4, 122.5, 129.0, 140.4, 148.2, 150.1, 160.3, 161.4. Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 69.88; H, 5.88; N, 4.40.

4.3. (*Z*)-3-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-acrylonitrile (1*Z*)

Compound **1*Z*** was also obtained from the separation that provided **1*E***: mp 129–131 °C. ^{1}H NMR (DMSO-d_6) δ 3.76–3.78 (m, 12H), 6.32 (s, 1H), 6.47 (d, J = 1.8 Hz, 2H), 6.65 (t, J = 2.1 Hz, 1H), 6.74 (dd, J = 8.5 Hz, J = 1.7 Hz, 1H), 6.96 (d, J = 8.5 Hz, 1H), 7.14 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 55.4, 55.6, 55.6, 93.9, 101.0, 107.2, 110.6, 111.2, 118.3, 122.3, 129.7, 139.2, 148.7, 151.0, 160.3, 161.1. Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.30. Found: C, 69.81; H, 5.87; N, 4.19.

4.4. 3,3-Bis(3,4-dimethoxyphenyl)acrylonitrile (2)

Compound **2** was prepared from **51b**[61,62] (1.51 g, 5.0 mmol) using general procedure A. Chromatographic purification using 95:5 CH_2Cl_2/EtOAc gave 1.0 g (61% yield), as a tan glass: ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 3H), 3.87 (s, 3H), 3.92 (s, 3H), 3.94 (s, 3H), 5.57 (s, 1H), 7.95 (br, 6H). ^{13}C NMR (DMSO-d_6) δ 55.9, 55.9, 56.0, 91.9, 110.7, 110.7, 111.4, 112.7, 118,6, 122.2, 123.2, 129.5, 131.8, 148.5, 148.8, 150.5, 151.0, 162.4. Anal. Calcd for $C_{19}H_{19}NO_4{\cdot}0.325H_2O$: C, 68.90; H, 5.97; N, 4.23. Found: C, 68.99; H, 5.84; N, 4.10.

4.5. (*E*/*Z*)-3-(3,4-dimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-acrylonitrile (3)

Compound **3** was prepared from (3,4-dimethoxyphenyl) (3′,4′,5′-trimethoxyphenyl)methanone (3.3 g, 10 mmol) using general procedure A. Chromatographic purification using 95:5 CH_2Cl_2/EtOAc gave 2.7 g (77% yield) as a white solid: mp 119–121 °C. ^{1}H NMR (DMSO-d_6) δ 3.84–3.71 (m, 15H), 6.19–6.25 (m, 1H), 6.66 (d, J = 3 Hz, 2H), 7.00–6.77 (m, 2H), 7.14–7.07 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 55.5, 55.5, 55.6, 56.0, 56.0, 60.1, 93.5, 94.2, 106.3, 106.9, 111.0, 111.2, 111.3, 112.7, 118.6, 118.7, 122.4, 122.7, 128.9, 130.0, 132.5, 133.7, 138.4, 139.4, 148.2, 148.6, 150.1, 151.0, 152.6, 161.3, 161.4. Anal. Calcd for $C_{20}H_{21}NO_5$: C, 67.59; H, 5.96; N, 3.94. Found: C, 67.66; H, 5.98; N, 3.88.

4.6. 3,3-Bis(3,4,5-trimethoxyphenyl)acrylonitrile (4)

Compound **4** was prepared from bis(3,4,5-trimethoxyphenyl)methanone (0.32 g, 0.88 mmol) using general procedure A. Chromatographic purification using 95:5 CH_2Cl_2/EtOAc gave 0.25 g (74% yield) as a white solid: mp 146–148 °C. ^{1}H-NMR (DMSO-d_6) δ 3.77–3.72 (m, 18H), 6.32 (s, 1H), 6.69 (d, J = 5 Hz, 4H). ^{13}C NMR (DMSO-d_6) δ 56.1, 56.1, 60.1, 60.1, 95.2, 100.4, 106.2, 107.1, 118.4, 131.9, 133.1, 138.7, 139.6, 152.6, 152.7, 161.3. Anal. Calcd for $C_{21}H_{24}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.08; H, 5.99; N, 3.45.

4.7. (*E*/*Z*)-3-(3,4-Dimethoxyphenyl)-3-(4-methoxyphenyl)-acrylonitrile (5)

Compound **5** was prepared from (3,4-dimethoxyphenyl)(4-methoxyphenyl)methanone (2.00 g, 7.3 mmol) using general procedure A. Chromatographic purification using a hexanes/EtOAc gradient gave 2.08 g (96% yield) as a pink oil: ^{1}H NMR ($CDCl_3$) δ 3.83–3.94 (m, 9H), 5.56 (s, 1H), 6.79–7.42 (m, 7H). ^{13}C NMR ($CDCl_3$) δ 55.5, 55.5, 56.1, 56.1, 56.29, 91.8, 91.9, 110.9, 111.5, 112.9, 114.0, 114.1, 118.8, 118.8, 122.3, 123.2, 129.5, 129.8, 130.3, 131.5, 131.7, 132.1, 148.8, 149.0, 150.7, 151.2, 161.2, 161.6, 162.5, 162.6. Anal. Calcd for $C_{18}H_{17}NO_3{\cdot}0.3H_2O$: C, 71.89; H, 5.90; N, 4.66. Found: C, 71.95; H, 6.04; N, 4.54.

4.8. (*E*/*Z*)-3-(3,4-Dimethoxyphenyl)-3-(3-methoxyphenyl)-acrylonitrile (6)

Compound **6** was prepared from (3,4-dimethoxyphenyl)(3-methoxyphenyl)methanone (2.0 g, 7.3 mmol) using general procedure A. Chromatographic purification using a hexanes/ethyl acetate gradient gave 1.99 g (92% yield) as a pink oil: ^{1}H NMR ($CDCl_3$) δ 3.79–3.94 (m, 9H), 5.61–5.68 (m, 1H), 6.82–7.38 (m, 7H, Ar). ^{13}C NMR ($CDCl_3$) δ 55.5, 56.1, 56.1, 56.2, 93.3, 93.8, 110.9, 110.9, 111.2, 112.8, 114.5, 115.0, 115.9, 116.0, 118.2, 118.4, 121.3, 122.2, 122.3, 123.4, 129.5, 129.7, 129.7, 131.4, 138.6, 140.9, 148.8, 149.0, 150.8, 151.3, 159.6, 159.7, 162.7, 162.8. Anal. Calcd for $C_{18}H_{17}NO_3{\cdot}0.25H_2O$: C, 72.10; H, 5.88; N, 4.67. Found: C, 72.33; H, 6.10; N, 4.60.

4.9. (*E*/*Z*)-3-(3,5-Dimethoxyphenyl)-3-(3-methoxyphenyl)-acrylonitrile (7)

Compound **7** was prepared from (3,5-dimethoxyphenyl)(3-methoxyphenyl)methanone (1.02 g, 3.8 mmol) using general procedure A. Chromatographic purification using a hexanes/ethyl acetate gradient gave 1.06 g (96% yield) as an off-white solid: mp 81–83 °C. ^{1}H NMR ($CDCl_3$) δ 3.76–3.79 (m, 6H), 3.82 (s, 3H), 5.72 (s, 1H), 6.42–7.35 (m, 7H). ^{13}C NMR ($CDCl_3$) δ 55.5, 55.6, 55.6, 95.4, 95.4, 102.2, 102.34, 107.0, 107.8, 114.3, 115.0, 115.9, 116.1, 117.8, 117.8, 121.0, 122.1, 129.7, 129.8, 138.2, 138.8, 140.1, 141.0, 159.6, 159.8, 160.8, 160.9, 163.0, 163.0. Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.10; H, 5.72; N, 4.68.

4.10. (*E*/*Z*)-3-(3,5-Dimethoxyphenyl)-3-(4-methoxyphenyl)-acrylonitrile (8)

Compound **8** was prepared from (3,5-dimethoxyphenyl)(4-methoxyphenyl)methanone (1.86 g, 6.8 mmol) using general procedure A. Chromatographic purification using a hexanes/ethyl acetate gradient gave 1.97 g (98% yield) as an off-white solid: mp 79–81 °C. ^{1}H NMR ($CDCl_3$) δ 3.76–3.86 (m, 9H), 5.60–5.65 (m, 1H), 6.42–7.43 (m, 7H). ^{13}C NMR ($CDCl_3$) δ 55.5, 55.6, 55.6, 93.0, 93.8, 102.1, 102.2, 107.2, 107.8, 114.0, 114.2, 118.2, 118.4, 129.3, 130.1, 131.0, 131.5, 139.2, 141.7, 160.8, 160.9, 161.2, 161.7, 162.6, 162.8. Anal. Calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 72.90; H, 5.52; N, 4.67.

4.11. (*E*/*Z*)-3-(3,5-Dimethoxyphenyl)-3-phenylacrylonitrile (9)

Compound **9** was prepared from (3,5-dimethoxy-phenyl) (phenyl)methanone (0.34 g, 1.4 mmol) using general procedure A. Chromatographic purification using a hexanes/ethyl acetate gradient gave 0.33 g (89% yield) as a light beige oil: ^{1}H NMR ($CDCl_3$) δ 3.76 (s, 3H), 3.79 (s, 3H), 5.73 (s, 1H), 6.41–7.44 (m, 8H). ^{13}C NMR ($CDCl_3$) δ 55.6, 55.7, 55.6, 95.1, 95.3, 102.2, 102.3, 107.0, 107.8, 107.8, 117.9, 128.5, 128.6, 128.8, 129.7, 130.2, 130.57, 137.0, 138.7, 139.0, 141.1, 160.9, 160.9, 163.1, 163.2. Anal. Calcd for $C_{18}H_{17}NO_3{\cdot}0.25H_2O$: C, 76.44; H, 5.74; N, 5.24. Found: C, 76.41; H, 5.40; N, 5.18.

4.12. Deprotection of *tert*-butyldimethylsilyl ethers. General procedure B. (*E*/*Z*)-3-(3,5-Dimethoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)acrylonitrile (10)

A 1.0 M solution of tetrabutylammonium fluoride (1.5 mL, 1.5 mmol) was added to a stirred solution of **59a** (0.53 g, 1.3 mmol) in THF (15 mL) at room temperature. After 1 h, ice water (10 mL)

was poured into the reddish solution, followed by addition of ether (50 mL). The mixture was washed with water (2 × 80 mL), dried over $MgSO_4$, filtered and evaporated under vacuum, providing the product as a foamy solid (0.35 g, 90% yield): mp 43–45 °C. ^{1}H NMR (DMSO-d_6) δ 3.78–3.81 (m, 6H), 3.84–3.87 (m, 3H), 6.17–6.19 (m, 1H), 6.49 (t, J = 2.0 Hz, 2H), 6.65–6.69 (m, 1H), 6.82–6.91 (m, 2H), 7.00–7.07 (m, 1H), 9.28–9.36 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 55.6, 56.1, 56.2, 93.5, 94.2, 102.1, 102.2, 107.1, 107.8, 110.4, 110.4, 114.4, 116.0, 118.1, 118.2, 121.3, 122.3, 130.2, 131.9, 139.1, 141.4, 145.5, 145.7, 148.2, 148.7, 160.8, 160.8, 162.6, 162.8. Anal. Calcd for $C_{18}H_{17}NO_4 \cdot 0.08H_2O$: C, 69.12; H, 5.53; N, 4.48. Found: C, 68.76; H, 5.69; N, 4.22.

4.13. Tin (II) chloride reduction of nitro compounds. General procedure C. (*E*/*Z*)-3-(3-Amino-4-methoxyphenyl)-3-(3,5-dimethoxyphenyl)acrylonitrile (11)

A suspension of **62a** (1.5 g, 4.4 mmol) and tin chloride dihydrate (5.4 g, 23.0 mmol) in ethanol (25 mL) was heated at 70 °C for 1 h. The mixture was cooled and poured onto ice (200 mL). The pH was made strongly alkaline by the addition of 10 N NaOH. The mixture was extracted with EtOAc (5x50 mL) and the combined organic extracts were washed with water (40 mL), brine (40 mL), and dried ($MgSO_4$). The solvent was removed and the residue was purified by column chromatography eluting with 75:25 hexane/EtOAc, to afford the product (0.8 g, 54% yield) as a yellow solid: mp 93–95 °C. ^{1}H NMR ($CDCl_3$) δ 3.73 -3.79 (m, 6H), 3.70–3.87 (br, 2H), 3.87–3.89 (m, 3H), 5.54 (s, 1H), 6.42–6.82 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 55.5, 55.6, 92.7, 93.4, 101.9, 102.0, 107.0, 107.6, 109.8, 109.9, 114.2, 115.8, 119.4, 120.7, 129.5, 131.2, 136.0, 136.2, 139.3, 141.7, 148.8, 149.2, 160.6, 160.6, 162.9, 163.1. Anal. Calcd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.60; H, 5.58; N, 8.88.

4.14. Catalytic hydrogenation of nitro compounds. General procedure D. (*E*/*Z*)-(3-Amino-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)acrylonitrile (12)

A mixture of **62b** (0.9 g, 2.4 mmol) and 10% Pd/C (0.1 g) in EtOAc (100 mL) was hydrogenated under 50 psi of hydrogen for 16 h. The mixture was filtered through Celite and the filtrate was concentrated. The crude product was purified by flash chromatography (CH_2Cl_2–EtOAc 85:15) to afford the product (0.3 g, 41% yield) as a yellow solid: mp 97–99 °C. ^{1}H NMR ($CDCl_3$) δ 3.80–3.91 (m, 14H), 5.51–5.75 (s, 1H), 6.51–6.87 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 55.5, 55.6, 56.2, 60.9, 92.1, 92.6, 106.2, 107.2, 109.8, 109.8, 114.4, 115.9, 119.5, 120.8, 129.5, 131.5, 132.6, 135.1, 136.0, 136.2, 139.3, 139.9, 148.8, 149.2, 152.9, 153.0, 163.0, 163.1. Anal. Calcd for $C_{19}H_{20}N_2O_4$: C, 67.05; H, 5.92; N, 8.23. Found: C, 67.08; H, 6.08; N, 7.94.

4.15. Wittig reaction of benzophenones. General procedure E. 4-(1-(3,5-Dimethoxyphenyl)vinyl)-1,2-dimethoxybenzene (13)

To a stirred suspension of methyltriphenylphosphonium bromide (2.4 g, 6.6 mmol) in THF (20 mL) was added a 1.0 M solution of LiHMDS in THF (6.6 mL, 6.6 mmol) at 0 °C. The solution was allowed to warm to room temperature and was stirred for 30 min, and then a mixture of **51a** (1.0 g, 3.3 mmol) in THF (20 mL) was added. The reaction mixture was heated to reflux for 16 h and was then quenched with water (100 mL). The resulting mixture was extracted with methylene chloride (2 × 50 mL). The combined extracts were washed with water, dried ($MgSO_4$), and concentrated under vacuum. The residue was chromatographed using a hexanes/ethyl acetate gradient, to provide 0.76 g (74% yield) of the product as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 6H), 3.84 (s, 3H), 3.90 (s, 3H), 5.39 (s, 1H), 5.40 (s, 1H), 6.44 (t, J = 2.1 Hz, 1H), 6.51 (d, J = 2.4 Hz, 2H), 6.81–6.93 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 55.5, 56.1, 100.1, 106.8, 110.9, 111.6, 113.4, 121.1, 134.2, 143.9, 148.7, 149.0, 149.8, 160.6. Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71; N, 0.00. Found: C, 71.65; H, 7.08; N, <0.05.

4.16. (*E*/*Z*)-3-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-acrylamide (14)

$KOSi(CH_3)_3$ (0.39 g, 3.1 mmol) was added to a stirred solution of compound **1** (0.50 g, 1.5 mmol) in toluene (10 mL), and the resulting mixture was heated to reflux under nitrogen for 20 h. The mixture was cooled and evaporated under vacuum. The residue was partitioned between water (100 mL) and EtOAc (75 mL), and the aqueous phase was extracted with EtOAc (2 × 75 mL). The combined organic phases were washed with water (100 mL) and brine (100 mL), and were dried ($MgSO_4$) and evaporated, to afford 0.45 g of the product, in 85% yield: mp 119–121 °C. ^{1}H NMR ($CDCl_3$) δ 3.50–4.00 (m, 12H), 6.32–7.24 (m, 9H). ^{13}C NMR ($CDCl_3$) δ 55.2, 55.4, 55.4, 55.5, 55.5, 99.2, 100.0, 106.2, 107.5, 110.5, 111.0, 111.3, 113.4, 121.0, 121.3, 122.1, 122.8, 131.0, 133.1, 141.2, 143.8, 147.6, 147.8, 148.5, 149.4, 159.9, 160.3, 167.4, 167.6. Anal. Calcd for $C_{19}H_{21}NO_5$: C, 66.46; H, 6.16; N, 4.08. Found: C, 66.09; H, 6.32; N, 3.92.

4.17. (*E*/*Z*)-Methyl 3-(3,4-dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-acrylate (15)

To a stirred suspension of methyl diethylphosphonoacetate (1.1 g, 5.4 mmol) in THF (10 mL) was added a 1.0 M solution of LiHMDS in THF (5.4 mL, 5.4 mmol) at 0 °C. The solution was allowed to warm to room temperature and was stirred for 30 min, and then a mixture of **51a** (1.5 g, 4.9 mmol) in THF (20 mL) was added, and the mixture was stirred at rt for 4 h. Then, methyl diethylphosphonoacetate (1.1 g, 5.4 mmol) and 1.0 M LiHMDS in THF (5.4 mL, 5.4 mmol) were added, and the mixture was heated to reflux for 6 days. The reaction mixture was cooled to room temperature and quenched with water (100 mL). The resulting mixture was extracted with methylene chloride (2 × 50 mL). The combined extracts were washed with water (80 mL), dried ($MgSO_4$), and concentrated under vacuum. The residue was purified using reverse phase preparative HPLC, running a water–acetonitrile gradient. The product, a mixture of geometric isomers, was obtained as a colorless oil, 0.45 g (25% yield): ^{1}H NMR ($CDCl_3$) δ 3.62–3.64 (m, 3H), 3.75–3.77 (m, 6H), 3.82–3.84 (m, 3H), 3.89–3.92 (m, 3H), 5.39 (s, 1H), 6.28–6.29 (m, 1H), 6.29–6.49 (m, 3H), 6.72–6.91 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 51.3, 51.3, 55.4, 55.4, 55.8, 56.0, 100.2, 101.4, 106.9, 107.2, 110.4, 110.7, 112.8, 115.0, 116.7, 122.1, 122.4, 131.0, 132.9, 140.9, 143.4, 148.3, 148.8, 149.2, 150.5, 156.4, 156.5, 160.3, 160.6, 166.4, 166.6. Anal. Calcd for $C_{20}H_{22}O_6 \cdot 0.3CH_2Cl_2$: C, 63.52; H, 5.93; N, 0.00. Found: C, 66.02; H, 5.81; N, <0.05.

4.18. (*E*/*Z*)-3-(3,4-Dimethoxyphenyl)-3-(3,5-dimethoxyphenyl)-acrylate (16)

A mixture of **15** (0.76 g, 2.1 mmol), and KOH (2.3 g, 40 mmol) in 4:1 MeOH–water (10 mL) was stirred at room temperature under nitrogen for 4 h. The mixture was evaporated under vacuum to remove the methanol, and the remaining solution was diluted with water (10 mL). This mixture was washed with ethyl ether (2 × 60 mL) and then acidified to pH 2–3 (conc. HCl). The resulting mixture was extracted with CH_2Cl_2 (2 × 100 mL), and the combined extracts were dried ($MgSO_4$) and evaporated, providing the product as an off-white foam: mp 90–92 °C. ^{1}H NMR ($CDCl_3$) δ 3.75–3.92 (m, 12H), 6.24–6.50 (m, 6H), 6.75–6.90 (m, 2H). ^{13}C

NMR ($CDCl_3$) δ 55.5, 55.6, 56.0, 56.1, 100.7, 101.9, 106.1, 107.2, 107.5, 110.6, 110.8, 111.0, 113.2, 114.7, 116.3, 122.5, 122.9, 130.6, 132.9, 140.5, 143.5, 148.4, 148.9, 149.7, 150.9, 158.4, 158.7, 160.5, 160.7, 170.6, 170.9. Anal. Calcd for $C_{19}H_{20}O_6$: C, 66.27; H, 5.85; N, 0.00. Found: C, 66.02; H, 5.81; N, <0.05.

4.19. (*E*/*Z*)-4-(3,4-Dimethoxyphenyl)-4-(3,5-dimethoxyphenyl)-but-3-en-2-one (17)

A mixture of $Pd(OAc)_2$ (67 mg, 0.30 mmol) in DMF (1 mL) was added to a mixture of **55**[63,64] (2.1 g, 10 mmol), 4-bromoveratrole (3.2 g, 14.9 mmol), NaOAc (1.4 g, 17 mmol), and Bu_4NBr (3.5 g, 11 mmol) in DMF (20 mL). The mixture was heated to 60 °C for 48 h, and then cooled to room temperature. The mixture was partitioned between water (150 mL) and EtOAc (50 mL), and the aqueous phase was extracted with EtOAc (2 × 50 mL), brine (50 mL), dried ($MgSO_4$) and evaporated. The residue was chromatographed using a hexanes/ethyl acetate gradient. Further purification using reverse phase preparative HPLC in 38–62 acetonitrile–water provided 200 mg of the product as an oil, in 6% yield: ^{1}H NMR ($CDCl_3$) δ 1.91 (s, 3H), 3.79 (s, 6H), 3.85 (s, 3H), 3.89 (s, 3H), 6.38 (d, J = 2.3 Hz, 2H), 6.51–6.53 (m, 2H), 6.77–6.82 (m, 2H), 6.92–6.93 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 30.0, 55.4, 55.6, 55.8, 56.1, 100.9, 110.8, 113.2, 122.4, 126.5, 132.9, 141.1, 148.9, 150.7, 153.8, 160.9. Anal. Calcd for $C_{20}H_{22}O_5$: C, 70.16; H, 6.48; N, 0.00. Found: C, 70.26; H, 6.37; N, <0.05.

4.20. (*E*/*Z*)-4-(1-(3,5-Dimethoxyphenyl)prop-1-en-1-yl)-1,2-dimethoxybenzene (18)

Compound **18** was prepared from **51a** (0.96 g, 3.2 mmol) and ethyltriphenylphosphonium bromide (2.4 g, 6.4 mmol) using general procedure E. Chromatographic purification using a hexanes/EtOAc gradient provided 0.97 g (97% yield) of the product as a pale yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.78–1.74 (m, 3H), 3.74 (s, 3H), 3.78 (s, 3H), 3.83 (s, 3H), 3.86–3.91 (m, 3H), 6.20–6.03 (m, 1H), 6.34–6.43 (m, 3H), 6.68–6.76 (m, 2H), 6.83–6.89 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 15.8, 15.9, 55.4, 55.5, 56.0, 56.0, 99.0, 99.2, 105.8, 108.2, 110.3, 110.9, 111.0, 113.3, 119.9, 122.6, 122.8, 124.4, 132.5, 135.6, 142.2, 142.3, 142.3, 145.4, 148.0, 148.3, 148.7, 148.7, 160.6, 160.7. Anal. Calcd for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05; N, 0.00. Found: C, 72.43; H, 6.96; N, <0.05.

4.21. (*E*/*Z*)-4-(1-(3,5-Dimethoxyphenyl)but-1-en-1-yl)-1,2-dimethoxybenzene (19)

Compound **19** was prepared from **51a** (2.0 g, 6.5 mmol) and propyltriphenylphosphonium bromide (5.0 g, 13 mmol) using general procedure E. Chromatographic purification using a hexanes/EtOAc gradient provided 2.1 g (98% yield) of the product as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.04 (t, J = 7.40 Hz, 3H), 2.04–2.16 (m, 2H), 3.74–3.91 (m, 12H), 6.08–5.94 (m, 1H), 6.33–6.43 (m, 3H), 6.67–6.76 (m, 2H), 6.84–6.88 (1H). ^{13}C NMR ($CDCl_3$) δ 14.7, 14.8, 23.4, 23.4, 55.4, 26.0, 99.0, 99.2, 105.8, 108.0, 110.3, 110.9, 110.9, 113.2, 120.0, 122.4, 130.3, 132.0, 132.8, 135.5, 140.7, 140.8, 142.5, 145.3, 148.1, 148.4, 148.7, 160.6, 160.7. Anal. Calcd for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37; N, 0.00. Found: C, 72.98; H, 6.25; N, <0.05.

4.22. (*E*/*Z*)-4-(3,4-Dimethoxyphenyl)-4-(3,5-dimethoxyphenyl)-*N*,*N*-dimethylbut-3-en-1-aminium chloride (20)

Compound **20** was prepared from **51a** (0.88 g, 2.9 mmol) and (3-(dimethylamino)propyl)triphenylphosphonium bromide (2.5 g, 5.8 mmol) using general procedure E. Chromatographic purification (CH_2Cl_2–MeOH–concd NH_4OH 98.5:1:0.5) provided 0.37 g as an oil. This oil was dissolved in 4.0 M HCl in 1,4-dioxane (8 mL), and then evaporated. The residue was triturated in ether, filtered, and dried under vacuum, providing 0.40 g (37% yield), as an off-white solid: mp 123–125 °C. ^{1}H NMR ($CDCl_3$) δ 2.42–2.46 (m, 2H), 2.68 (s, 6H), 3.16 (m, 2H), 3.70–3.79 (m, 12H), 6.11 (m, 1H), 6.28 (d, J = 2.3 Hz, 1H), 6.38–6.42 (m, 1H), 6.50–6.57 (m, 1H), 6.67–6.70 (m, 1H), 6.85–7.07 (m, 2H), 10.56 (br, 1H). ^{13}C NMR ($CDCl_3$) δ 24.7, 24.7, 41.9, 55.2, 55.4, 55.5, 55.6, 55.7, 55.8, 98.9, 99.1, 105.5, 107.2, 110.1, 111.3, 111.6, 112.9, 120.0, 121.7, 123.7, 131.1, 133.6, 141.2, 142.8, 143.0, 143.8, 148.0, 148.4, 148.5, 160.2, 160.5. Anal. Calcd for $C_{22}H_{30}NO_4Cl$: C, 64.78; H, 7.41; N, 3.43. Found: C, 64.43; H, 7.40; N, 3.36.

4.23. (*E*/*Z*)-4-(1-(3,5-Dimethoxyphenyl)-2-methoxyvinyl)-1,2-dimethoxybenzene (21)

Compound **21** was prepared from **51a** (1.0 g, 3.3 mmol) and (methoxymethyl)triphenylphosphonium chloride (2.3 g, 6.6 mmol) using general procedure E. Chromatographic purification using a hexanes/ethyl acetate gradient provided 0.77 g as a colorless oil, in 77% yield: ^{1}H NMR ($CDCl_3$) δ 3.75 (m, 9H), 3.82 (s, 3H), 3.88 (s, 3H), 6.38 (m, 3H), 6.66 (m, 1H), 6.86 (m, 2H), 6.97 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 55.3, 55.3, 55.8, 55.8, 55.9, 60.6, 98.6, 98.9, 106.4, 108.0, 110.7, 111.0, 111.8, 113.1, 120.1, 120.2, 120.7, 122.5, 130.0, 133.0, 139.6, 142.6, 145.9, 146.0, 147.7, 147.9, 148.3, 148.6, 160.3, 160.6. Anal. Calcd for $C_{19}H_{22}O_5$: C, 69.07; H, 6.71; N, 0.00. Found: C, 69.10; H, 6.85; N, 0.00.

4.24. (*E*/*Z*)-4-(1-(3,5-Dimethoxyphenyl)but-1-en-3-yn-1-yl)-1,2-dimethoxybenzene (22)

A solution of prop-1-yne-1,3-diylbis(trimethylsilane) (2.2 g, 12 mmol) in THF (40 mL) was cooled to −78 °C, and then a 1.7 M solution of *t*-BuLi in pentane (7.1 mL, 12 mmol) was added dropwise, and stirring proceeded at this temperature for 1 h following completion of the addition. Intermediate **51a** (1.8 g, 6.0 mmol) was then added and after 5 min the cooling bath was removed. The reaction flask was allowed to warm to room temperature and was then heated to reflux for 1 h. The mixture was cooled to room temperature and then quenched by the addition of water (20 mL). The mixture stirred at rt for 16 h. The solvent was evaporated, and the residue was partitioned between ethyl acetate (100 mL) and water (100 mL), and the aqueous phase was extracted with ethyl acetate (100 mL). The combined organic phases were washed with water (2 × 100 mL), 1 N HCl (2 × 100 mL), and water (100 mL), were dried (Na_2SO_4) and evaporated under vacuum. The residue was chromatographed using a hexanes/ethyl acetate gradient, providing 900 mg (47% yield) of the product as a colorless oil which darkened over time: ^{1}H NMR ($CDCl_3$) δ 3.05 (d, J = 2.5 Hz, 1H), 3.73 (s, 3H), 3.75 (s, 3H), 3.81–3.89 (m, 6H), 5.92 (m, 1H), 6.43–6.47 (m, 2H), 6.61–6.62 (m, 1H), 6.79–6.85 (m, 2H), 6.97–7.16 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 55.3, 55.8, 55.9, 81.7, 81.8, 82.5, 82.8, 100.6, 100.8, 104.6, 105.3, 106.5, 108.0, 110.4, 110.7, 110.8, 113.2, 121.1, 123.0, 131.1, 131.5, 140.7, 143.5, 148.1, 148.6, 149.1, 149.6, 154.1, 154.2, 160.2, 160.5. Anal. Calcd for $C_{20}H_{20}O_4 \cdot H_2O$: C, 70.16; H, 6.48; N, 0.00. Found: C, 70.41; H, 6.14; N, 0.08.

4.25. 5-(1-(3,5-Dimethoxyphenyl)vinyl)-2-methoxyphenol (23)

Compound **23** was prepared from **59b** (0.6 g, 1.5 mmol) using general procedure B. The product was purified by column chromatography using a hexanes/ethyl acetate gradient, providing the product as an oil (0.4 g, 93% yield): ^{1}H NMR ($CDCl_3$) δ 3.78 (s, 6H), 3.88 (s, 3H), 5.36 (d, J = 1.1 Hz, 1H), 5.41 (d, J = 1.1 Hz, 1H), 5.72 (s, 1H), 6.46 (t, J = 2.1 Hz, 1H), 6.52 (d, J = 2.1 Hz, 2H),

6.77–6.83 (m, 2H), 6.99 (d, J = 2.0 Hz, 1H). ^{13}C NMR ($CDCl_3$) δ 55.3, 55.9, 99.8, 106.7, 110.2, 113.3, 114.5, 120.2, 134.6, 143.9, 145.2, 146.4, 149.5, 160.5. Anal. Calcd for $C_{17}H_{18}O_4{\cdot}0.1H_2O$: C, 70.87; H, 6.37; N, 0.00. Found: C, 70.77; H, 6.17; N, <0.05.

4.26. (*E*/*Z*)-5-(1-(3,5-Dimethoxyphenyl)prop-1-en-1-yl)-2-methoxyphenol (24)

Compound **24** was prepared from **59c** (1.0 g, 2.4 mmol) using general procedure B. The product was purified by column chromatography using a hexanes/ethyl acetate gradient, eluting the product at 25:75 ethyl acetate/hexanes as an oil (0.7 g, 97% yield): ^{1}H NMR ($CDCl_3$) δ 1.71–1.78 (m, 3H), 3.75–3.91 (m, 9H), 5.50–5.57 (m, 1H), 6.05–6.13 (m, 1H), 6.31–6.41 (m, 3H), 6.63–6.77 (m, 2H), 6.82–6.88 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 15.6, 15.7, 55.3, 55.9, 56.0, 98.7, 99.0, 105.7, 108.0, 110.2, 110.3, 113.2, 116.3, 118.8, 121.8, 122.7, 124.2, 133.1, 136.1, 141.9, 142.2, 145.1, 145.4, 145.4, 145.6, 160.4, 160.6. Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71; N, 0.00. Found: C, 71.72; H, 6.48; N, <0.05.

4.27. 5-(1-(3,5-Dimethoxyphenyl)-2-methylprop-1-en-1-yl)-2-methoxyphenol (25)

Compound **25** was prepared from **59d** (0.85 g, 2.05 mmol) using general procedure B. The product was purified by column chromatography using a hexanes/ethyl acetate gradient, eluting the product at 25:75 ethyl acetate/hexanes as an oil (0.6 g, 96% yield): ^{1}H NMR ($CDCl_3$) δ 1.77 (s, 3H), 1.80 (s, 3H), 3.75 (s, 6H), 3.86 (s, 3H), 5.53 (s, 1H), 6.28–6.32 (m, 3H), 6.63 (dd, J = 8.3, J = 2.0 Hz, 1H), 6.74–6.78 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 22.3, 22.5, 55.3, 55.9, 98.1, 107.9, 110.1, 116.0, 121.3, 130.7, 136.4, 136.5, 144.9, 145.6, 160.3. Anal. Calcd for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05; N, 0.00. Found: C, 72.59; H, 7.10; N, <0.05.

4.28. (*E*/*Z*)-5-(1-(3,5-Dimethoxyphenyl)-2-phenylvinyl)-2-methoxyphenol (26)

Compound **26** was prepared from **59e** (1.2 g, 2.6 mmol) using general procedure B. The product was purified by column chromatography using a hexanes/ethyl acetate gradient, eluting the product at 30:70 ethyl acetate/hexanes as an oil (0.9 g, 96% yield): ^{1}H NMR ($CDCl_3$) δ 3.69 (s, 3H), 3.77 (s, 3H), 3.90–3.91 (m, 3H), 5.54–5.55 (m, 1H), 6.35–6.49 (m, 3H), 6.69–6.90 (m, 4H), 6.99–7.15 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 55.4, 55.9, 56.0, 99.5, 99.9, 106.1, 108.1, 110.2, 113.6, 116.5, 119.5, 122.3, 126.6, 126.7, 126.8, 127.9, 128.1, 129.4, 129.5, 133.2, 136.6, 137.3, 137.4, 141.8, 142.1, 142.3, 145.2, 145.6, 145.9, 146.0, 160.5, 161.0. Anal. Calcd for $C_{17}H_{18}O_4{\cdot}0.1H_2O$: C, 70.87; H, 6.37; N, 0.00. Found: C, 70.77; H, 6.17; N, <0.05.

4.29. 5-(1-(3,5-Dimethoxyphenyl)vinyl)-2-methoxyaniline (27)

Compound **27** was prepared from **62c** (1.12 g, 3.55 mmol) using general procedure C. The product was purified by column chromatography using a hexanes/ethyl acetate gradient, providing the product as a solid (0.64 g, 63% yield): mp 93–95 °C. ^{1}H NMR ($CDCl_3$) δ 3.77–3.87 (m, 11H), 5.31–5.37 (m, 2H), 6.43–6.73 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 55.36, 55.53, 99.77, 106.7, 109.9, 112.8, 114.9, 118.6, 134.1, 135.6, 144.2, 147.2, 149.9, 160.4. Anal. Calcd for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.91. Found: C, 71.31; H, 6.76; N, 4.92.

4.30. (*E*/*Z*)-3-(3,5-Dimethoxyphenyl)-3-(3-ethoxy-4-methoxyphenyl)acrylonitrile (28)

Compound **28** was prepared from **53a** (0.63 g, 2.0 mmol) using general procedure A. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a white solid (0.60 g, 89% yield): mp 110–112 °C. ^{1}H NMR ($CDCl_3$) δ 1.41–1.49 (m, 3H), 3.77 (s, 3H), 3.80 (s, 3H), 3.90–3.92 (m, 3H), 3.99–4.13 (m, 2H), 5.59–5.65 (m, 1H), 6.43–6.55 (m, 3H), 6.82–7.04 (m, 3H). ^{13}C NMR ($CDCl_3$) δ 14.8, 55.7, 56.1, 56.2, 64.7, 93.2, 93.8, 102.2, 102.3, 107.2, 107.8, 111.1, 111.2, 112.7, 114.2, 118.2, 122.2, 123.3, 129.33, 131.2, 139.1, 141.6, 148.0, 148.3, 151.1, 151.7, 160.8, 160.9, 162.8, 162.9. Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.62; H, 6.25; N, 4.01.

4.31. 3,3-Bis(3-ethoxy-4-methoxyphenyl)acrylonitrile (29)

Compound **29** was prepared from **53b** (7.3 g, 22 mmol) using general procedure A. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a white solid (3.92 g, 50% yield): mp 142–144 °C. ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7.0 Hz, 6H), 3.79 (s, 3H), 3.83 (s, 3H), 3.94–4.04 (m, 4H), 6.11 (s, 1H), 6.77–7.09 (m, 6H). ^{13}C NMR (DMSO-d_6) δ 14.6, 14.6, 55.5, 55.5, 63.8, 92.6, 111.3, 111.5, 112.3, 113.8, 118.9, 122.3, 122.4, 129.3, 130.6, 147.3, 147.8, 150.1, 151.0, 161.4. Anal. Calcd for $C_{21}H_{23}NO_4$: C, 71.37; H, 6.56; N, 3.96. Found: C, 71.42; H, 6.42; N, 3.97.

4.32. (*E*/*Z*)-3-(3,4-Dimethoxyphenyl)-3-(3-ethoxy-4-methoxyphenyl) acrylonitrile (30)

Compound **30** was prepared from **51c** (1.6 g, 5.0 mmol) using general procedure A. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a white solid (0.6 g, 35% yield): mp 103–106 °C. ^{1}H NMR ($CDCl_3$) δ 1.36–1.54 (m, 3H), 3.76–4.17 (m, 11H), 5.55 (s, 1H), 6.75–7.10 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 14.6, 55.9, 56.0, 64.5, 91.8, 110.7, 110.7, 110.9, 110.9, 111.4, 112.8, 112.9, 114.2, 118.6, 122.0, 122.1, 123.1, 123.2, 129.5, 129.6, 131.7, 131.9, 147.8, 148.1, 148.6, 148.8, 150.5, 150.8, 151.0, 162.5. Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.78; H, 6.24; N, 4.13. Found: C, 70.62; H, 6.21; N, 4.07.

4.33. (*E*/*Z*)-3-(3-Ethoxy-4-methoxyphenyl)-3-(4-methoxy-3-methylphenyl)acrylonitrile (31)

Compound **31** was prepared from **53c** (1.2 g, 4.0 mmol) using general procedure A. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a white solid (0.97 g, 75% yield): mp: 86–88 °C. ^{1}H NMR (DMSO-d_6) δ 1.30 (t, J = 7.0 Hz, 3H), 2.13–2.17 (m, 3H), 3.78–3.86 (m, 3H), 3.82 (s, 3H), 3.93–4.04 (m, 2H), 6.01–6.08 (m, 1H), 6.72–7.26 (m, 6H). ^{13}C NMR (DMSO-d_6) δ 14.6, 14.6, 16.0, 63.8, 92.1, 92.4, 110.0, 110.2, 111.4, 111.5, 112.3, 113.8, 119.0, 122.3, 122.4, 125.6, 125.9, 128.1, 128.6, 128.9, 129.4, 130.1, 130.4, 130.8, 131.3, 147.4, 147.8, 150.2, 151.0, 158.4, 159.3, 161.5, 161.5. Anal. Calcd for $C_{20}H_{21}NO_3$: C, 74.28; H, 6.55; N, 4.33. Found: C, 74.16; H, 6.48; N, 4.30.

4.34. (*E*/*Z*)-3-(3-Ethoxy-4-methoxyphenyl)-3-(3-methoxyphenyl)-acrylonitrile (32)

Compound **32** was prepared from **53d** (1.6 g, 5.5 mmol) using general procedure A. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a white solid (1.6 g, 93% yield): mp 96–98 °C. ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7 Hz, 3H), 3.77–3.83 (m, 6H), 3.94–4.05 (m, 2H), 6.18–6.29 (m, 1H), 6.72–7.43 (m, 7H). ^{13}C NMR (DMSO-d_6) δ 14.5, 14.6, 55.2, 55.3, 55.5, 55.6, 63.8, 63.9, 93.9, 94.9, 111.3, 111.5, 111.8, 113.7, 114.6, 115.0, 115.9, 118.4, 118.5, 121.0,

121.3, 122.2, 122.4, 129.1, 129.7, 129.8, 129.9, 138.6, 139.8, 147.4, 147.8, 150.3, 151.1, 159.0, 161.2, 161.4. Anal. Calcd for $C_{19}H_{19}NO_3$: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.43; H, 6.13; N, 4.54.

4.35. (*E*/*Z*)-3-(3-Ethoxy-4-methoxyphenyl)-3-(3-fluoro-4-methoxyphenyl)acrylonitrile (33)

Compound **33** was prepared from **54a** (0.35 mg, 1.2 mmol) using general procedure A. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as an off-white solid (0.26 g, 69% yield): mp 122–124 °C. ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7 Hz, 3H), 3.78–3.94 (s, 6H), 4.01 (q, J = 7 Hz, 2H), 6.15–6.21 (m, 1H), 6.74 (dd, J =2, 8 Hz, 1H), 6.92–7.36 (m, 5H). ^{13}C NMR (DMSO-d_6) δ 14.5, 14.56, 55.5, 55.5, 56.1, 56.1, 63.8, 63.8, 93.6, 93.8, 111.4, 111.6, 112.1, 113.8, 115.3, 115.6, 116.7, 116.9, 118.5, 122.2, 122.3, 125.7, 125.7, 126.2, 128.8, 129.6, 129.7, 130.1, 130.8, 130.9, 147.4, 147.9, 148.1, 148.2, 148.9, 149.0, 150.2, 150.8 (d, J = 240), 151.1 (d, J = 240), 151.2, 160.0, 160.1. Anal. Calcd for $C_{19}H_{18}NO_3F$: C, 69.71; H, 5.54; N, 4.28. Found: C, 69.55; H, 5.58; N, 4.25.

4.36. (*E*/*Z*)-3-(3-Ethoxy-4-methoxyphenyl)-3-(4-(methylsulfonyl)phenyl)acrylonitrile (34)

Compound **34** was prepared from **54b** (0.8 g, 2.4 mmol) using general procedure A. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a white solid (0.53 g, 62% yield): mp, 159–161 °C. ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7 Hz, 3H), 3.26–3.37 (m, 3H), 3.78–3.84 (m, 3H), 3.94–4.07 (m, 2H), 6.30–6.48 (m, 1H), 6.61 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H), 6.88–7.14 (m, 2H), 7.60–8.08 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 14.6, 14.6, 55.6, 55.6, 63.9, 64.0, 95.2, 97.3, 111.5, 111.6, 111.7, 113.6, 118.1, 122.4, 122.5, 127.2, 128.5, 129.4, 129.4, 130.2, 141.6, 142.0, 142.4, 143.3, 147.6, 148.1, 150.5, 151.4, 159.8, 159.9. Anal. Calcd for $C_{19}H_{19}NO_4S$: C, 63.85; H, 5.36; N, 3.92. Found: C, 63.56; H, 5.21; N, 3.73.

4.37. (*E*/*Z*)-3-(3-Ethoxy-4-methoxyphenyl)-3-(3-hydroxy-4-methoxyphenyl)acrylonitrile (35)

Compound **35** was prepared from **59f** (0.61 g, 1.4 mmol) using general procedure B. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a white solid (0.4 g, 89% yield): mp 63–65 °C. ^{1}H NMR ($CDCl_3$) δ 1.40–1.47 (m, 3H), 3.89–3.94 (m, 6H), 3.98–4.12 (m, 2H), 5.53–5.55 (m, 1H), 5.68 (m, 1H), 6.80–7.09 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 14.7, 55.9, 56.0, 64.5, 92.0, 110.2, 111.0, 112.8, 114.1, 114.7, 116.0, 118.6, 118.7, 121.4, 122.1, 122.2, 123.1, 129.5, 130.4, 131.7, 132.5, 145.3, 145.5, 145.5, 147.9, 148.0, 148.1, 148.4, 150.8, 151.4, 162.4; Anal. Calcd for $C_{19}H_{19}NO_4$: C, 70.14; H, 5.89; N, 4.31. Found: C, 69.93; H, 5.84; N, 4.06.

4.38. (*E*/*Z*)-3-(3-Amino-4-methoxyphenyl)-3-(3-ethoxy-4-methoxyphenyl)acrylonitrile (37)

Compound **37** was prepared from **62d** (3.3 g, 9.7 mmol) using general procedure C. The product was purified by column chromatography eluting with 7:3 hexanes/ethyl acetate to provide the product as a yellow solid (1.1 g, 33% yield): mp 141–143°C. ^{1}H NMR ($CDCl_3$) δ 1.40–1.47 (m, 3H), 3.88–3.92 (m, 8H), 3.98–4.12 (m, 2H), 5.49 (m, 1H), 6.64–7.00 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 14.7, 55.5, 55.6, 109.8, 110.9, 113.0, 114.2, 114.7, 116.0, 118.8, 118.9, 119.6, 120.7, 122.1, 123.1, 129.8, 129.9, 132.1, 135.9, 136.1, 147.8, 148.0, 146.7, 150.7, 151.2, 163.0. Anal. Calcd for $C_{19}H_{20}N_2O_3$: C, 70.35; H, 6.21; N, 8.64. Found: C, 70.25; H, 6.21; N, 8.46.

4.39. (*E*/*Z*)-3-(3-Ethoxy-4-methoxyphenyl)-3-(4-(methylamino)-phenyl)acrylonitrile (37)

Compound **37** was prepared from **64** (0.29 g, 1.0 mmol) using general procedure A. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as an off-white solid (0.26 g, 81% yield): mp 81–83 °C. ^{1}H NMR (DMSO-d_6) δ 1.28–1.34 (m, 3H), 2.69–2.74 (m, 3H), 3.79–3.83 (m, 3H), 3.94–4.02 (m, 2H), 5.77–5.87 (m, 1H), 6.25–6.36 (m, 1H), 6.50–7.19 (m, 7H). ^{13}C NMR (DMSO-d_6) δ 14.6, 14.6, 29.2, 29.3, 55.5, 55.5, 63.7, 88.3, 89.4, 110.8, 111.1, 111.3, 111.4, 112.8, 113.9, 119.6, 122.2, 123.4, 124.5, 129.8, 129.9, 130.9, 131.6, 147.3, 147.6, 149.8, 150.7, 151.3, 151.9, 161.9, 162.1. Anal. Calcd for $C_{19}H_{20}N_2O_2$: C, 74.00; H, 6.54; N, 9.08. Found: C, 73.67; H, 6.70; N, 8.81.

4.40. (*E*/*Z*)-3-(4-(Dimethylamino)phenyl)-3-(3-ethoxy-4-methoxyphenyl)acrylonitrile (38)

Compound **38** was prepared from **65** (0.22 g, 0.74 mmol) using general procedure A. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as an off-white solid (0.21 g, 87% yield): mp 81–83 °C. ^{1}H NMR (DMSO-d_6) δ 1.28–1.34 (m, 3H), 2.96–2.99 (m, 6H), 3.79–3.83 (m, 3H), 3.94–4.03 (m, 2H), 5.84–5.93 (s, 1H), 6.68–7.26 (m, 7H). ^{13}C NMR (DMSO-d_6) δ 14.6, 14.6, 39.6, 39.7, 55.5, 55.5, 63.8, 89.0, 90.0, 111.2, 111.3, 111.4, 111.5, 112.8, 113.9, 119.5, 122.2, 123.7, 124.7, 129.6, 129.8, 130.7, 131.5, 147.3, 147.7, 149.9, 150.8, 151.2, 151.6, 161.7, 161.9. Anal. Calcd for $C_{20}H_{22}N_2O_2$: C, 74.51; H, 6.88; N, 8.69. Found: C, 74.42; H, 7.11; N, 8.61.

4.41. (*E*/*Z*)-3-(3-Ethoxy-4-methoxyphenyl)-3-(4-methoxy-3-(1H-pyrrol-1-yl)phenyl)acrylonitrile (39)

2,5-Dimethoxytetrahydrofuran (0.22 g, 1.7 mmol) was added to a stirred solution of **36** (0.50 g, 1.5 mmol) in AcOH (10 mL), and the mixture was heated to reflux for 1 h. The mixture was cooled to room temperature and diluted with ethyl acetate (100 mL). Saturated aqueous sodium bicarbonate (40 mL) was then carefully added. The organic phase was washed with water (60 mL) and brine (60 mL), dried ($MgSO_4$) and evaporated. The residue was purified using a hexanes/ethyl acetate gradient, providing the product as a white solid (0.35 g, 60% yield): mp 109–111 °C. ^{1}H NMR ($CDCl_3$) δ 1.45 (m, 3H), 3.91 (m, 6H), 4.05 (m, 2H), 5.57–5.59 (m, 1H), 6.29–6.32 (m, 2H), 6.81–6.91 (m, 3H), 6.93–7.10 (m, 3H), 7.24–7.27 (m, 1 H), 7.35–7.47 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 14.7, 56.0, 56.1, 64.6, 92.5, 109.2, 109.3, 111.1, 112.7, 112.0, 112.1, 114.0, 118.4, 118.5, 121.9, 122.1, 122.2, 123.1, 125.9, 127.1, 127.9, 129.0, 129.8, 130.1, 131.4, 132.1, 148.3, 148, 151.0, 151.6, 153.9, 154.4, 161.6, 161.7. Anal. Calcd for $C_{23}H_{22}N_2O_3$: C, 73.78; H, 5.92; N, 7.48. Found: C, 73.46; H, 5.71; N, 7.35.

4.42. 5-(1-(3-Ethoxy-4-methoxyphenyl)vinyl)-2-methoxyphenol (40)

Compound **40** was prepared from **59g** (1.1 g, 2.7 mmol) using general procedure B. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a white solid (0.65 g, 83% yield): mp 99–101 °C. ^{1}H NMR ($CDCl_3$) δ 1.42 (t, J = 7.0 Hz, 3H), 3.89 (s, 3H), 3.91 (s, 3H), 4.06 (q, J = 7.0 Hz, 2H), 5.29 (d, J = 1.2 Hz, 1H), 5.32 (d, J = 1.2 Hz, 1H), 5.60 (s, 1H), 6.78–6.98 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 14.8, 55.9, 56.0, 64.3, 110.1, 111.0, 112.1, 113.1, 114.6, 120.2, 121.0, 134.5, 135.2, 145.2, 145.3, 147.8, 149.1, 149.2. Anal. Calcd for $C_{18}H_{20}O_4$: C, 71.98; H, 6.71; N, 0.00. Found: C, 71.90; H, 6.74; N, <0.05.

4.43. 5-(1-(3-Ethoxy-4-methoxyphenyl)-2,2-difluorovinyl)-2-methoxyphenol (41)

Compound **41** was prepared from **59h** (0.80 g, 1.8 mmol) using general procedure B. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a white solid (0.5 g, 83% yield); mp 74–76 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, J = 7.0 Hz, 3H), 3.88 (s, 3H), 3.90 (s, 3H), 4.03 (q, J = 7.0 Hz, 2H), 5.57 (s, 1H), 6.74–6.86 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 14.7, 55.9, 64.4, 95.5 (t, J = 19), 110.4, 111.2, 114.3 (t, J = 3.4), 115.76 (t, J = 3.8), 121.5 (t, J = 3.0), 122.3 (t, J = 3.4 Hz), 126.8 (t, J = 2.6), 127.7 (t, J = 3.0), 145.4, 145.9, 148.0, 148.8, 153.5 (t, J = 290). Anal. Calcd for $C_{18}H_{18}F_2O_4$: C, 64.28; H, 5.39; N, 0.00. Found: C, 64.27; H, 5.46; N, <0.05.

4.44. 5-(1-(3-Ethoxy-4-methoxyphenyl)vinyl)-2-methoxyaniline (42)

Compound **42** was prepared from **62e** (0.9 g, 2.8 mmol) using general procedure C. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a solid (0.4 g, 48% yield): mp 91–93 °C. ^{1}H NMR ($CDCl_3$) δ 1.43 (t, J = 6.8 Hz, 3H), 3.75 (br, 2H), 3.86 (s, 3H), 3.88 (s, 3H), 4.06 (q, J = 7.1 Hz, 2H), 5.28 (d, J = 6.5 Hz, 2H), 6.72–6.91 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 14.8, 55.5, 56.0, 64.3, 109.9, 110.9, 111.5, 113.1, 115.0, 118.7, 120.9, 134.6, 134.7, 135.6, 147.1, 147.7, 149.0, 149.6. Anal. Calcd for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68. Found: C, 72.16; H, 7.10; N, 4.74.

4.45. (*E*/*Z*)-5-(1-(3-Ethoxy-4-methoxyphenyl)prop-1-en-1-yl)-2-methoxyaniline (43)

Compound **43** was prepared from **62f** (1.10 g, 3.2 mmol) using general procedure C. The product was purified by column chromatography using a hexanes/ethyl acetate gradient to provide the product as a solid (0.73 g, 73% yield): mp 108–110 °C. ^{1}H NMR ($CDCl_3$) δ 1.39–1.46 (m, 3H), 1.72–1.77 (m, 3H), 3.71–3.75 (br, 2H), 3.83–3.90 (m, 6H), 3.99–4.09 (m, 2H), 5.97–6.05 (m, 1H), 6.54–6.61 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 14.8, 15.7, 15.7, 55.5, 55.5, 55.9, 56.0, 64.2, 64.3, 109.9, 110.0, 111.0, 111.0, 112.1, 114.1, 114.8, 116.8, 117.5, 119.9, 120.4, 121.8, 122.0, 122.4, 132.9, 132.9, 135.5, 135.6, 136.3, 136.5, 141.9, 142.0, 146.3, 146.5, 147.8, 148.0, 148.3. Anal. Calcd for $C_{19}H_{23}NO_3$: C, 73.82; H, 7.40; N, 4.47. Found: C, 72.57; H, 7.47; N, 4.40.

4.46. (*E*/*Z*)-N-(5-(2-Cyano-1-(3,5-dimethoxyphenyl)vinyl)-2-methoxyphenyl)acetamide (44)

A mixture of **11** (0.4 g, 1.3 mmol) and acetyl chloride (0.2 mL) in THF (50 mL) was refluxed overnight, and then cooled and quenched with water (100 mL). The resulting mixture was extracted with methylene chloride (2 × 50 mL). The combined extracts were washed with water (100 mL), dried ($MgSO_4$), and concentrated under vacuum. The crude product was purified by flash chromatography (hexane-ethyl acetate 1:1) to afford the product as a white solid (0.3 g, 62% yield): mp 163–165 °C. ^{1}H NMR ($CDCl_3$) δ 2.06 (s, 3H), 3.76 (s, 6H), 3.91 (s, 3H), 6.14–6.22 (m, 1H), 6.46 (d, J = 1.8 Hz, 2H), 6.62 (s, 1H), 7.17 (m, 2H), 7.93 (s, 1H), 9.28 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 23.8, 55.4, 55.8, 95.0, 102.0, 106.7, 110.9, 118.3, 122.5, 125.5, 127.3, 128.5, 140.3, 150.5, 160.3, 161.3, 168.7. Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95. Found: C, 68.03; H, 5.54; N, 7.85.

4.47. Boc deprotection reactions. General procedure F. (*E*/*Z*)-2-Amino-*N*-(5-(2-cyano-1-(3-ethoxy-4-methoxyphenyl)vinyl)-2-methoxyphenyl)acetamide hydrochloride (45)

A solution of 4 N HCl/dioxane (3 mL) was added to a stirred solution of **66a** (1.1 g, 2.4 mmol) in CH_2Cl_2 (35 mL). The resulting solution was stirred at room temperature overnight. Ether (10 mL) was added and the mixture was stirred for 2 h. The slurry was filtered and was washed with ether to afford the product as a white solid (0.44 g, 44% yield): mp 208–210 °C. ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 6 Hz, 3H), 3.78 (s, 2H), 3.83 (s, 3H), 3.90 (s, 3H), 3.99 (q, J = 7 Hz, 2H), 6.01 (s, 1H), 7.36–6.91 (m, 5H), 7.89 (d, J = 1 Hz, 1H), 8.20 (br, 3H), 9.88 (s, 1H). ^{13}C NMR (DMSO-d_6) δ 14.6, 41.0, 55.5, 56.1, 63.8, 92.8, 111.5, 113.8, 118.7, 122.4, 125.2, 126.2, 129.2, 130.5 147.4, 150.1, 151.4, 161.4, 165.4. Anal. Calcd for $C_{21}H_{24}N_3O_4Cl{\cdot}1.4H_2O$: C, 56.92; H, 6.10; N, 9.48. Found: C, 56.58; H, 5.85; N, 9.72.

4.48. (*S*)-(*E*/*Z*)-2-Amino-*N*-(5-(2-cyano-1-(3-ethoxy-4-methoxyphenyl)vinyl)-2-methoxyphenyl)propan-amide hydrochloride (46)

Compound **46** was prepared from **66b** (2.1 g, 4.4 mmol) using general procedure F, to afford the product as a white solid (1.4 g, 74% yield): mp 207–209 °C. ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 6 Hz, 3H), 1.41 (m, 3H), 3.79–3.83 (m, 3H), 3.91–3.94 (m, 3H), 3.99 (m, 2H), 4.19 (m, 1H), 6.02–6.16 (m, 1H), 6.75–7.92 (m, 6H), 8.31 (br, 3H), 9.90–9.94 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 14.6, 14.6, 17.4, 48.6, 55.5, 55.6, 56.0, 56.1, 63.8, 63.8, 92.9, 93.0, 111.3, 111.4, 111.6, 114.5, 112.3, 113.8, 118.7, 118.8, 122.2, 122.4, 122.9, 123.1, 125.6, 126.1, 126.6, 129.1, 129.2, 130.4, 130.5, 147.4, 147.8, 151.0, 151.1, 150.2, 151.9, 161.0, 161.3, 168.8. Anal. Calcd for $C_{22}H_{26}N_3O_4Cl{\cdot}0.03H_2O$: C, 61.10; H, 6.07; N, 9.72. Found: C, 60.75; H, 5.84; N, 9.51.

4.49. (*E*/*Z*)-5-(2-Cyano-1-(3-ethoxy-4-methoxyphenyl)vinyl)-2-methoxyphenyl dimethylcarbamate (47)

A mixture of **35** (0.57 g, 1.8 mmol), dimethylcarbamyl chloride (1.5 g, 14 mmol), and DIEA (0.91 g, 7.0 mmol) was heated to reflux for 5 h. The mixture was cooled and evaporated, and the residue was purified by column chromatography using a hexanes/ethyl acetate gradient, to provide the product as an off-white solid (0.58 g, 84% yield): mp 114–116 °C. ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7.0 Hz, 3H), 2.88–3.01 (m, 6H), 3.79–3.85 (m, 6H), 3.95–4.05 (m, 2H), 6.13–6.15 (m, 1H), 6.74–7.35 (m, 6H). ^{13}C NMR (DMSO-d_6) δ 14.6, 14.6, 36.1, 36.3, 55.5, 55.6, 55.9, 56.0, 63.8, 93.1, 93.2, 111.4, 111.6, 112.3, 112.5, 112.5, 113.8, 118.7, 118.7, 122.1, 122.2, 123.2, 124.3, 127.0, 127.7, 129.0, 129.1, 130.4, 139.6, 139.9, 147.4, 147.8, 150.2, 151.1, 152.6, 153.3, 153.4, 160.3, 160.4. Anal. Calcd for $C_{22}H_{24}N_2O_5$: C, 66.65; H, 6.10; N, 7.07. Found: C, 66.47; H, 6.20; N, 6.95.

4.50. (*E*/*Z*)-5-(2-Cyano-1-(3-ethoxy-4-methoxyphenyl)vinyl)-2-methoxyphenyl 2-aminoacetate hydrochloride (48)

Compound **48** was prepared from **67a** (0.36 g, 0.76 mmol) using general procedure F, to afford the product as a white solid (0.18 g, 58% yield): mp 190–192 °C. ^{1}H NMR (DMSO-d_6) δ 1.32 (t, J = 7.0 Hz, 3H), 3.79–3.88 (m, 6H), 3.94–4.04 (m, 2H), 4.10–4.13 (m, 2H), 6.14–6.22 (m, 1H), 6.73–7.48 (m, 6H), 8.54 (br, 3H). ^{13}C NMR (DMSO-d_6) δ 14.6, 14.6, 55.5, 55.6, 56.1, 63.8, 93.5, 93.7, 111.4,

111.6, 112.2, 113.0, 113.2, 113.7, 118.5, 122.1, 122.3, 122.7, 123.6, 127.9, 128.9, 128.9, 129.5, 130.3, 130.8, 137.9, 138.0, 147.5, 147.9, 150.3, 151.1, 151.7, 152.3, 159.9, 160.1, 166.0. Anal. Calcd for $C_{20}H_{23}N_2O_5Cl{\cdot}0.5H_2O$: C, 58.95; H, 5.65; N, 6.55. Found: C, 58.57; H, 5.52; N, 6.35.

4.51. (S)-(*E*/*Z*)-5-(2-Cyano-1-(3-ethoxy-4-methoxyphenyl)vinyl)-2-methoxyphenyl 2-aminopropanoate hydrochloride (49)

Compound **49** was prepared from **67b** (0.89 g, 1.8 mmol) using general procedure F, to afford the product as a white solid (0.52 g, 67% yield): mp 173–175 °C. ^{1}H NMR (DMSO-d_6) δ 1.32 (t, J = 7.0 Hz, 3H), 1.55–1.59 (m, 3H), 3.74–3.88 (m, 6H), 3.94–4.05 (m, 2H), 4.30–4.39 (m, 1H), 6.14–6.22 (m, 1H), 6.73–7.42 (m, 6H), 8.64 (br, 3H). ^{13}C NMR (DMSO-d_6) δ 14.6, 14.6, 15.8, 24.4, 25.3, 33.3, 47.5, 47.8, 55.5, 55.6, 56.2, 56.3, 63.8, 93.6, 93.8, 111.4, 111.6, 112.2, 113.0, 113.1, 113.7, 118.5, 122.2, 122.3, 122.5, 123.4, 128.0, 128.9, 129.0, 129.5, 130.2, 130.9, 138.0, 138.2, 147.5, 147.9, 150.3, 151.2, 151.5, 152.3, 159.9, 160.1, 168.3, 168.3. Anal. Calcd for $C_{20}H_{25}N_2O_5Cl{\cdot}0.6H_2O$: C, 59.55; H, 5.95; N, 6.31. Found: C, 59.20; H, 5.83; N, 6.67.

4.52. (S)-(*E*/*Z*)-5-(2-Cyano-1-(3-ethoxy-4-methoxyphenyl)vinyl)-2-methoxyphenyl 2-amino-3-methylbut-anoate hydrochloride (50)

Compound **50** was prepared from **67c** (0.82 g, 1.6 mmol) using general procedure F, to afford the product as a white solid (0.51 g, 70% yield): mp 192–194 °C. ^{1}H NMR (DMSO-d_6) δ 1.07–1.14 (m, 6H), 1.32 (t, J = 7.0 Hz, 3H), 2.30–2.37 (m, 1H), 3.79–3.88 (m, 6H), 3.94–4.02 (m, 2H), 4.13–4.19 (m, 1H), 6.14–6.22 (m, 1H), 6.74–7.45 (m, 6H), 8.61 (br, 3H). ^{13}C NMR (DMSO-d_6) δ 14.6, 17.4, 18.0, 29.5, 55.5, 55.6, 56.0, 56.1, 57.3, 63.8, 93.6, 93.8, 111.4, 111.6, 112.2, 113.0, 113.1, 113.7, 118.5, 122.1, 122.3, 122.6, 123.5, 128.0, 128.8, 129.0, 129.5, 130.9, 137.9, 138.0, 147.5, 147.9, 150.3, 151.2, 151.5, 152.2, 159.9, 160.0, 167.0. Anal. Calcd for $C_{24}H_{29}N_2O_5Cl{\cdot}0.1H_2O$: C, 62.29; H, 6.39; N, 6.05. Found: C, 62.07; H, 6.30; N, 6.06.

4.53. HCT-116 cell proliferation assay

The human colorectal carcinoma tumor cell line HCT-116 was purchased from American Type Culture Collection (Manassas, VA). Cell proliferation was assessed by [^{3}H] thymidine incorporation assay. Briefly, cells were seeded on 96-well microtiter plates 24 h before addition of compound to allow them to adhere to plates. Each compound was tested at serial dilutions in triplicate. Following compound treatment, the cells were incubated at 37 °C for additional 72 h. [^{3}H]thymidine (1 μCi in 20 μL medium) was added to each well for the last 6 h of incubation time. The cells were then harvested for detection of tritium incorporation with a TopCount® Microplate Scintillation Counter (Packard Instrument Company, Meriden, CT). Final cumulative half-maximal inhibitory concentrations (IC_{50}s) were calculated using non-linear regression and sigmoidal dose–response, constraining the top to 100% and the bottom to 0% and allowing variable slope, using GraphPad Prism version 5.01. SEM (standard error of the mean) was calculated from the individual IC_{50}s of each replicate.

4.54. PDE4 enzyme assay

PDE4 enzyme was purified from U937 human monocytic cells by gel filtration chromatography, and phosphodiesterase reactions were carried as previously described.[50] Briefly, reactions were carried out in 96-well deep well plates in 50 mM Tris–HCl pH 7.5, 5 mM $MgCl_2$, 1 μM cAMP, plus 10 nM [^{3}H]-cAMP for 30 min at 30 °C, terminated by boiling, treated with 1 mg/ml snake venom, and separated using AG-1X8 ion exchange resin (BioRad). Reactions consumed less than 15% of available substrate. IC_{50}s and SEM were calculated as described above.

4.55. PBMC TNF- production assay

Peripheral blood mononuclear cells (PBMC) were obtained from leukocyte units (Blood Center of New Jersey, East Orange, NJ) by Ficoll Hypaque (Pharmacia, Piscataway, NJ, USA) density centrifugation. Cells were cultured in R10 medium (RPMI) (Life Technologies, Grand Island, NY, USA) supplemented with 10% AB+ human serum (Gemini Bio-products, Woodland, CA, USA), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Life Technologies)). PBMC (2×10^5 cells) were plated in 96-well flat-bottom Costar tissue culture plates (Corning, NY, USA) in triplicate. Compounds were dissolved in DMSO (Sigma) and further dilutions were done in culture medium immediately before use. The final DMSO concentration in all samples was 0.25%. Compounds were added to cells 1 h before stimulation. Cells were stimulated with LPS (from *Salmonella abortus equi*, Sigma, St.Louis, MO, USA) at 1 ng/ml in the absence or presence of compounds. Cells were incubated for 18–20 h at 37 °C in 5% CO_2 and supernatants were then collected, diluted with culture medium and assayed for TNF-α levels by ELISA (Endogen, Boston, MA, USA) according to the manufacturer's instructions. IC_{50}s and SEM were calculated as described above.

4.56. Tubulin polymerization assay

Tubulin polymerization was measured in a cell-free spectrophotometric assay system, as previously described.[30]

4.57. Solubility determination

Solubility was determined using 0.5 mL of a ~1 mg/mL suspension of the appropriate samples in pH 5.0 buffer (Tritisol 9882–2) or pH 7.4 buffer (Metrepak CAS#270–7.40) in a sealed matrix tube containing a stir flea. Each sample was set on stir plate set at 400 rpm for 4 h. After 4 h, the samples were filtered using a 0.45 mm filter. Samples were analyzed using a Spectramax UV–vis Plate Reader (200–400 nm) and compared with standard curves generated (range 0.25–100 mg/mL) for each compound to determine the concentration in buffer solution. Replicate measurements were made for buffer 5.0 determinations.

4.58. Human plasma stability

Analogs were incubated in human plasma at 37 °C for 0, 0.5, 1, 2, 3, 4, 6, 8, and 24 h. At each time point, 60 μL of each plasma sample was added into 120 μL of acetonitrile containing 0.1% formic acid and internal standard. After centrifugation at $\geqslant$3000*g*, the supernatants were transferred for LC–MS/MS analysis.

A Shimadzu HPLC system interfaced with an Applied System Sciex 4000 QTrap mass spectrometer was used for analysis of samples. The LC separation was carried out on a Phenomenex Onyx Monolithic C18 column (50×2.0 mm) with gradient of aqueous acetonitrile containing 0.1% formic acid. The optimized MRM tandem mass spectrometry was used to detect the analytes and IS. The percentage remaining data of each compound were calculated using the peak area ratios (analyte/IS) of the samples at the designated time points and those at time zero.

4.59. Docking study

Compound **28** was docked into the colchicine binding site of the tubulin crystal structure (PDB code 1SA0)[57] using MOE software.[58]

After aligning the ligand with colchicine, the ligand and protein residues with atoms within 5 Angstroms of the bound inhibitor were minimized using the standard parameters in MOE/LigX. Residues outside the 5 Angstrom radius remained fixed.

4.60. Human tumor xenograft model

Female SCID mice (6–8 weeks old) were obtained from the Charles River Laboratory (Wilmington, MA) and maintained in microisolator cages under sterile conditions. The right hind legs of the mice were inoculated s.c. with HCT-116 cells suspended in sterile PBS (2×10^6 cells per mouse). On day 8, tumors of all mice were measured with a digital caliper and volumes were calculated with a formula of $W^2 \times L / 2$, where W is width (short axis) and L is length (long axis). Mice bearing tumor size ranging between 75 and 125 mm^3 were pooled together and randomly distributed into cages. The mice were then ear tagged and cages were randomly assigned to treatment groups. On day 8, the tumors were measured and considered as starting volumes. The mice were then dosed ip with either vehicle (CMC-Tween: 0.5% Carboxymethylcellulose and 0.25% Tween 80 in water), Compound **28** (20 mg/kg), Compound **45** (20 mg/kg) or positive control Camptosar (5 mg/kg; Pfizer, Inc., New York, NY). Compound **28** and Compound **45** were given once daily administrations (qd). Camptosar was administered once every four days (q4d). Mice were monitored daily for health status as well as tumor growth. Tumors were measured twice weekly.

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Supplementary data

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