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Design and synthesis of triarylacrylonitrile analogues of tamoxifen with improved binding selectivity to protein kinase C

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ABSTRACT

The clinical selective estrogen receptor modulator tamoxifen is also a modest inhibitor of protein kinase C, a target implicated in several untreatable brain diseases such as amphetamine abuse. This inhibition and tamoxifen's ability to cross the blood brain barrier make it an attractive scaffold to conduct further SAR studies toward uncovering effective therapies for such diseases. Utilizing the known compound **6a** as a starting template and guided by computational tools to derive physicochemical properties known to be important for CNS permeable drugs, the design and synthesis of a small series of novel triarylacrylonitrile analogues have been carried out providing compounds with enhanced potency and selectivity for PKC over the estrogen receptor relative to tamoxifen. Shortened synthetic routes compared to classical procedures have been developed for analogues incorporating a β-phenyl ring, which involve installing dialkylaminoalkoxy side chains first off the α and/or α′ rings of a precursor benzophenone and then condensing the resultant ketones with phenylacetonitrile anion. A second novel, efficient and versatile route utilizing Suzuki chemistry has also been developed, which will allow for the introduction of a wide range of β-aryl or β-heteroaryl moieties and side-chain substituents onto the acrylonitrile core. For analogues possessing a single side chain off the α- or α′-ring, novel 2D NMR experiments have been carried out that allow for unambiguous assignment of *E*- and *Z*-stereochemistry. From the SAR analysis, one compound, **6c**, shows markedly increased potency and selectivity for inhibiting PKC with an IC_{50} of 80 nM for inhibition of PKC protein substrate and >10 μM for binding to the estrogen receptor α (tamoxifen IC_{50} = 20 μM and 222 nM, respectively). The data on **6c** provide support for further exploration of PKC as a druggable target for the treatment of amphetamine abuse.

1. Introduction

Protein kinase C (PKC) is a pivotal enzyme in cell signaling pathways and has been implicated in numerous brain diseases such as Parkinson's disease,[1] Alzheimer's disease[2]] bipolar disease,[3,4] and substance abuse disorder.[5,6] Although targeting PKC as a therapeutic target for these diseases has been proposed,[7–9] in vivo validation has been difficult due to lack of a PKC inhibitor that is permeable to the central nervous system. The only known PKC inhibitor that is permeable across the blood brain barrier is the selective estrogen receptor modulator (SERM) tamoxifen[10] (Fig. 1), which inhibits cellular PKC activity reasonably potently, including that of PKCβ.[11,12] Tamoxifen has been utilized to provide in vivo validation in rodents of PKC inhibition toward reducing the effects of amphetamine, which is a model for bipolar mania,[13–15] and clinically has demonstrated efficacy in the treatment of this disorder.[16] The blockade of amphetamine behavioral effects can also be achieved by other traditional PKC inhibitors, but not by the selective estrogen receptor inhibitors medroxyprogesterone or clomiphene[17] (Fig. 1). Inhibition of PKC reduces amphetamine-stimulated dopamine efflux through the dopamine transporter[18,19], as well as amphetamine-stimulated locomotor[20] and rewarding activities.[21] Although the exact mechanism is not known, the dopamine transporter is a substrate for PKC[22] and amphetamine-stimulated dopamine efflux is regulated by transporter phosphorylation.[23,24] Development of a potent PKC inhibitor that is permeable across the blood brain barrier would enhance

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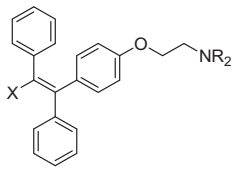


R = Me, X = Et: tamoxifen
R = Et, X = Cl: clomiphene

Figure 1. Clinical triphenylethylene SERMS.

exploration of the effect of PKC on numerous behavioral functions and could prove therapeutically useful.

Despite its wide use, tamoxifen is a drug with many sites of action. Its most common use is as a SERM to treat the recurrence of estrogen receptor positive breast cancer.[25] In addition to the estrogen receptor and PKC, other identified binding sites for tamoxifen include calmodulin,[26] voltage-dependent Ca^{2+} channels[27] and acyl-CoA:cholesterol acyl transferase.[28] Binding to all of these sites occurs at micromolar levels, which is greater than that required for binding to the estrogen receptor.

Evidence suggests that although tamoxifen binds weakly to the catalytic subunit of PKC,[29] its functional binding site is the Ca^{2+} and phospholipid-binding C2 regulatory subunit.[30] Inhibition of PKC by tamoxifen requires Ca^{2+} and phospholipid,[12,31] and is competitive with phospholipids and noncompetitive with Ca^{2+}.[31,32] Tamoxifen inhibits PKC more potently in the presence of diolein and phorbol myristate acetate (PMA), but is not competitive with them.[32] The crystallographic structure of the phorbol ester and PKC regulatory site has been reported, but the mode of interaction between tamoxifen and its derivatives with PKC remains to be fully elucidated.[33–35]

There have been extensive structure–activity relationship (SAR) studies of the tamoxifen scaffold to dissect structural features that confer selective binding to the estrogen receptor relative to other targets such as PKC.[36–38] While tamoxifen can serve as an in vivo inhibitor of PKC, its high affinity for the estrogen receptor and low affinity for PKC compromise its utility to selectively target PKC for brain disorders. To that end, our goal has been to use the triphenylethylene core of tamoxifen as a starting point to design analogues with increased affinity for PKC and decreased affinity for the estrogen receptor. A systematic study by Bignon et al.[38] showed that PKC activity could be enhanced by substituting the tamoxifen ethyl moiety with a cyano function. This paper delineates further SAR of this core change toward the design and synthesis of a small series of novel triarylacrylonitrile derivatives with enhanced selectivity for PKC, and which have the potential for improved permeability across the blood brain barrier.

2. Analogue design

Our goal was to design tamoxifen analogues that display enhanced selectivity for PKCβ versus estrogen receptor binding and exhibit good CNS permeability. Compound **6a** (Table 1), previously synthesized and tested for PKC and ER binding,[39–41] became our starting template for further SAR exploration. Our focus was to expand on **6a** with a small series of triarylacrylonitrile derivatives, listed in Table 1, that could dissect out structural features contributing to selectivity and potency for PKC over ER without a concomitant loss of molecular transport into the brain. The initial selection of **6a** and analogues was guided by computational tools (ChemAxon) to derive properties known to be important for CNS permeable drugs. The calculations for several key physicochemical descriptors are shown in Table 1, and reveal that our targeted analogues possess many of the critical parameters that track fairly closely with those for marketed CNS drugs.[42,43] While molecular weights tend to be greater than found for typical CNS drugs, *c*Log*P* and topological polar surface area (tPSA) values trend toward those favoring CNS penetration.

3. Chemistry

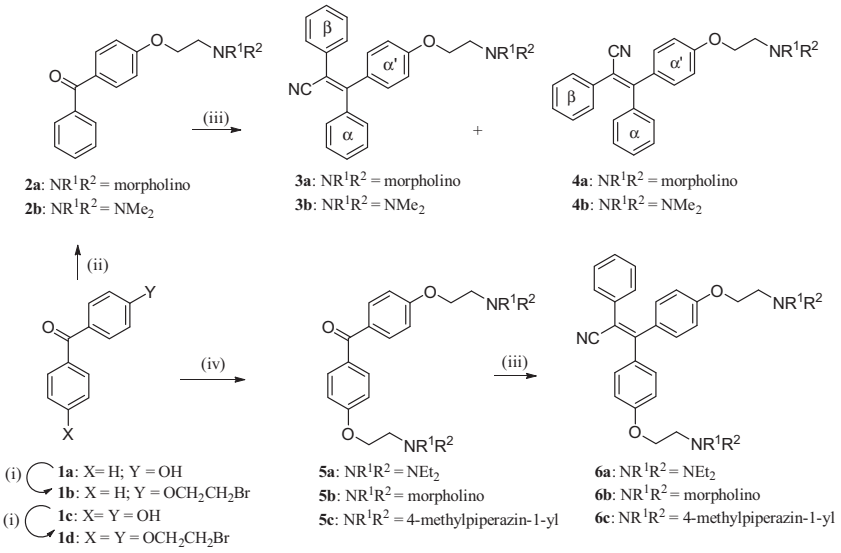
Our synthetic strategy to construct triphenylacrylonitrile compounds with variable aqueous solubilizing dialkylaminoalkoxy side chains is shown in Scheme 1. The classical procedure to construct such compounds is through condensation of a methoxy benzophenone precursor and phenylacetonitrile anion, generated either with NaH or sodium amide in refluxing benzene, followed by pyridinium hydrochloride demethylation[44] and phenolic alkylation with an appropriate dialkylaminoalkyl halide.[41] In order to shorten the sequence and provide the option of introducing variable β-ring aryl or heteroaryl moieties, we decided to install our dialkylaminoalkoxy side chains first off the α and/or α′ rings and then condense the resultant ketones with a phenylacetonitrile anion. Toward that end, we generated a small set of mono- and bis-(dialkylaminoalkoxy)benzophenones either through a one-step phenolic alkylation of **1a** or **1c** with readily available dialkylaminoalkyl halides to give **2a**, **5a**, **5b** in 85–92% yield, or in two steps via mono bromo displacement with excess 1,2-dibromoethane to give **1b** and **1d**, followed by a second bromo displacement with a chosen dialkylamine to give **2b** and **5c** in an overall ~55% yield. The latter method, while longer, is especially suited toward installing a wide range of distal amino headpieces onto the alkoxy side chain, which otherwise would not be readily accessible from aminoalkyl halides. We then examined condensation of these elaborated benzophenones with phenylacetonitrile by screening a range of anion forming conditions. Notably, reaction of **5a** with 1–5 equiv of NaH under a variety of solvent (THF, *p*-dioxane, toluene, DMSO) and temperature (25 –110 °C) conditions resulted in recovery of starting ketone or the generation of complex mixtures showing only trace amounts of product **6a**. Reaction with potassium *t*-butoxide in DMSO at 25 °C left starting material. We then progressed to stronger bases such as *n*-BuLi and LDA at low temperature. Anion generation in THF at −78 °C with 5 equiv of *n*-BuLi followed by addition of ketone **5a** and warming to 25 °C provided the desired product **6a** contaminated with a small amount of by-product, whereas the use of LDA under the same conditions resulted in a cleaner condensation. Optimization of reaction conditions, utilizing 20 equiv of LDA, and application to ketones **2a**, **2b**, **5a**–**5c** provided condensation products (**3a**–**4b**, **6a**–**6c**) in 86–100% yields prior to crystallization. Unsymmetrical ketones **2a** and **2b** generated mixtures of *E* and *Z* isomers (**3a**/**4a** and **3b**/**4b**) in an *E*/*Z* ratio of 9:1 to 5:1 by HPLC. Partial separation of isomers **3a** and **4a** was achieved through fractional crystallization/trituration of the free base. Further fractional crystallization of the formed hydrochloride salts of each enriched mixture then provided individual isomers in ⩾94% purity by HPLC. No attempt was made to purify each isomer of the **3b**/**4b** mixture, which was tested as such.

Having developed condensation conditions to add phenylacetonitrile to a range of dialkylaminoalkoxy-substituted benzophenones, we were interested in applying the same anion generating conditions to provide target compounds in which the β-aryl moiety is derived from representative heterocyclic acetonitriles. Thus, LDA treatment of 2- or 4-pyridylacetonitrile or 2-thienylacetonitrile under the optimum conditions discussed above followed by addition of ketone **5c** resulted either in recovered starting ketone (for pyridylacetonitriles) or a very low yield of product (for 2-thienylacetonitrile), along with intractable side products. This

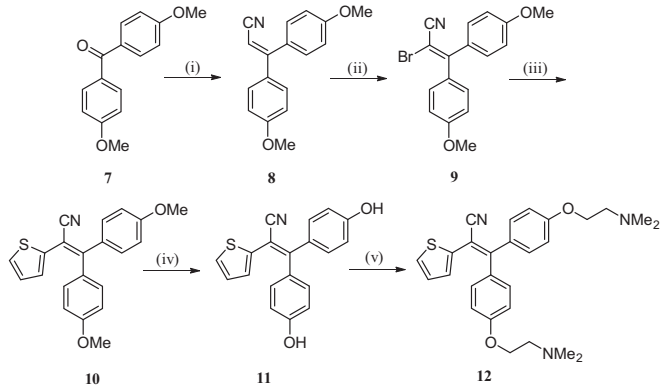
Table 1
Computed physicochemical properties and binding of compounds to PKC and estrogen receptor

Compound	Physicochemical descriptor[a]			Inhibition of PKC-specific MARCKS Phosphorylation (% inhibition ± SEM), $n = 3$		ERα Binding IC_{50} (nM) [95% CI], $n = 2–3$[b]
	MW[c]	*c*Log*P*	tPSA	3 μM	10 μM	
Tamoxifen	371.52	6.74	12.47	27 ± 9	52 ± 7	222 [48–1035]
3a·HCl	410.52	5.57	45.49	43 ± 13	57 ± 9	224 [38–1326]
4a·HCl	410.52	5.57	45.49	37 ± 18	70 ± 11	97 [14–665]
3b/**4b**·HCl (17:3)	368.48	5.91	36.26	22 ± 10	67 ± 16	86 [18–405]
6a	511.71	7.01	48.73	12 ± 5	68 ± 8	>10,000
6b·2 HCl	539.68	4.96	67.19	62 ± 8	72 ± 7	553 [101–3015]
6c·2.5 HCl[d]	565.76	5.24	55.21	83 ± 4	78 ± 13	>10,000
12	461.62	5.46	48.73	39 ± 7	68 ± 5	>10,000

[a] Calculations utilizing ChemAxon/Marvin Sketch software.
[b] For comparison, β-estradiol binding to ERα has $IC_{50} = 4.4$ nM [95% CI, 1–17]; $n = 10$.
[c] Free base.
[d] PKC $IC_{50} = 80$ nM; $n = 6$.



Scheme 1. Reagents and conditions. (i) excess $BrCH_2CH_2Br$, Cs_2CO_3, ACN, reflux, 1–2 d (63–70% yield); (ii) for **2a**: **1a**, 4-(2-chloroethyl)morpholine·HCl, Cs_2CO_3, ACN, reflux, 21 h (85% yield). For **2b**: **1b**, $NHMe_2$·HCl, K_2CO_3, acetone, reflux, 16 h (76% yield); (iii) 20 equiv PhCHLiCN, THF, −78 °C to rt, 20–48 h (86–100% yield); (iv) for **5a**: **1c**, $Et_2NCH_2CH_2Cl$·HCl, Cs_2CO_3, ACN, reflux, 18 h (92% yield). For **5b**: **1c**, 4-(2-chloroethyl)morpholine·HCl, Cs_2CO_3, ACN, reflux, 18 h (90% yield). For **5c**: **1d**, *N*-methylpiperazine, ACN, reflux, 2 h (86% yield).



Scheme 2. Reagents and conditions. (i) NaH, $(EtO)_2P(O)CH_2CN$, THF, reflux, 20 h (89% yield); (ii) Br_2, 1,2-DCE, −25 °C to rt, 4 h (59% yield); (iii) thiophen-2-ylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, 1:1 toluene/2-propanol, reflux, 2.5 d (87%); (iv) BBr_3, DCM, rt, 16 h (98% yield); (v) $Me_2NCH_2CH_2Br$·HBr, Cs_2CO_3, ACN, reflux, 18 h (42% yield).

necessitated the development of a completely novel approach ('Suzuki strategy') for this type of scaffold. Its reduction to practice, which is exemplified with a test heteroaryl boronic acid, is shown in Scheme 2.

Accordingly, Horner–Wadsworth–Emmons (HWE) reaction of diethyl (cyanomethyl)phosphonate with benzophenone **7** proceeded under literature conditions[45] to provide the elaborated acrylonitrile **8** in 89% yield. Selective olefin bromination of **8** was patterned after an analogous literature reaction[46] to give **9** in 59% yield. Heteroarylation of **9** with thiophen-2-ylboronic acid proceeded under standard Suzuki conditions[47] to give the core scaffold **10** in 87% yield incorporating the β-heteroaryl moiety (similar reaction of furan-2-ylboronic acid proceeded also in high yield). Installation of the bis-(2-dimethylamino)ethoxy side chains was then accomplished by a standard sequence of methoxy ether demethylation (BBr_3) followed by alkylation with 2-bromo-*N*,*N*-dimethylethylamine to give target compound **12** in 41% yield. Attempts to further shorten the sequence were evaluated with elaborated ketone **5c** and found to be unsuccessful. While HWE reaction proceeded successfully, attempted bromination of the resultant product under several conditions left only starting cyano olefin.

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Most compounds could be rigorously purified by flash chromatography and/or crystallization, except for except for **6a**–**6c**. Each of these shows a spot on silica gel TLC that overlaps with its respective precursor ketone **5**, and requires an extremely polar eluant (95:5 methanol: concentrated ammonium hydroxide) to develop the plate to a reasonable R_f (~0.35). Hence, standard flash chromatography or preparative thick layer chromatography was not useful, so hydrochloride salts were formed and crystallized for further purification. Structural assignments for all compounds were supported by diagnostic peaks in the ^{1}H NMR spectra and by mass spectrometry. For purified unsymmetrical *E*- and *Z*-isomers, **3a** and **4a** hydrochloride salts, respectively, structural assignments were based on 1D ^{1}H, 1D ^{13}C, 2D ^{1}H–^{1}H TOCSY and 2D ^{1}H–^{13}C HSQC experiments. Chemical shift analysis revealed that **3a** is the *E*-isomer while **4a** is the *Z*-isomer. These assignments are based on significant differences between proton chemical shifts for protons in the α-ring of each isomer (Fig. 2). In **4a** (*Z*-isomer) the C-1 proton of the α-phenyl ring has a strong upfield shift (6.95 ppm) due to its location above the plane of either the α′- or β-phenyl ring. By contrast, the C-1 proton of the α-phenyl ring in **3a** shows a downfield shift (~7.4 ppm), which is consistent with *E*-stereochemistry. Such a strong conformational effect on chemical shifts allows for unambiguous assignment of *Z*- and *E*-isomers, which is more convenient than the classical methods of isomeric assignments by x-ray cystallography.[44]

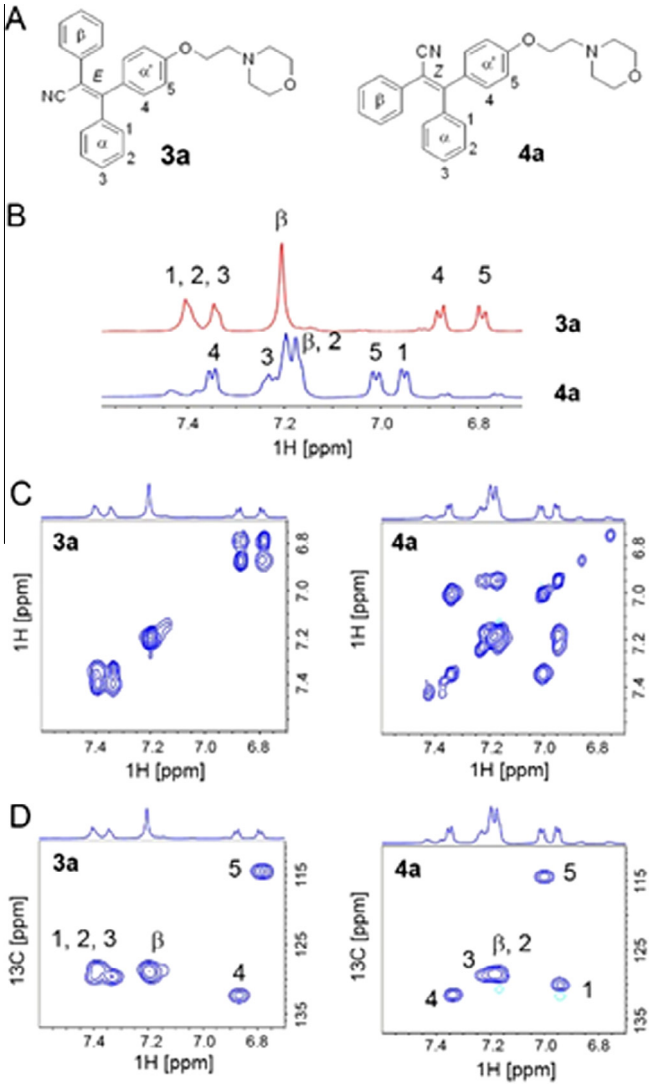

Figure 2. Assignment of aromatic regions of NMR spectra for compounds **3a** and **4a** hydrochloride salts. (A) Structures of **3a** and **4a** with labeled aromatic protons; (B) Assigned ^{1}H aromatic regions for **3a** and **4a**; (C) 2D ^{1}H–^{1}H TOCSY spectra for **3a** (left) and **4a** (right); (D) 2D ^{1}H–^{13}C HSQC spectra for **3a** (left) and **4a** (right).

4. Results and discussion

Bignon et al. carried out a systematic study of a series of triphenylacrylonitrile derivatives for their effects on PKC.[38] One sub-series of compounds, substituted with at least one basic dialkylaminoethoxy side chain, inhibited type α, β, and γ PKC subspecies activated by Ca^{2+} and phosphatidylserine (PS) at micromolar concentrations, with or without diolein, but did not inhibit protamine sulfate phosphorylation. One compound (**6a**, Table 1) was one of the most potent tested (IC_{50} ~3 μM with PS; tamoxifen ~75 μM).[38] Based on an earlier study in which **6a** also displayed a lowered binding affinity to calf uterus cytosolic estrogen receptor relative to tamoxifen,[40] we decided to utilize it as a starting point for further SAR investigation.

To determine the potency of compounds against PKC, SHSY5Y cells were pre-incubated with vehicle or two concentrations (3 μM and 10 μM) of tamoxifen or triarylacrylonitrile analogue at 37 °C followed by a 15 min treatment with the phorbol ester PMA. These concentrations were chosen because in cellular models, tamoxifen inhibits PKC with an IC_{50} of approximately 1–10 μM.[12,48] Therefore, we tested tamoxifen and its analogues in our PKC activity assay at both 3 μM and 10 μM to rapidly evaluate whether the analogues had improved PKC inhibitory activity compared to tamoxifen. The inhibition of phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS), a known PKC target, was quantified using Western blotting. To assess effects against the estrogen receptor, a complex of full length estrogen receptor α (ERα) and a proprietary fluorescent estrogen ligand were added to various concentrations of estradiol, tamoxifen and triarylacrylonitrile analogue for up to 4 h. Relative binding affinities were determined from changes in fluorescence polarization.

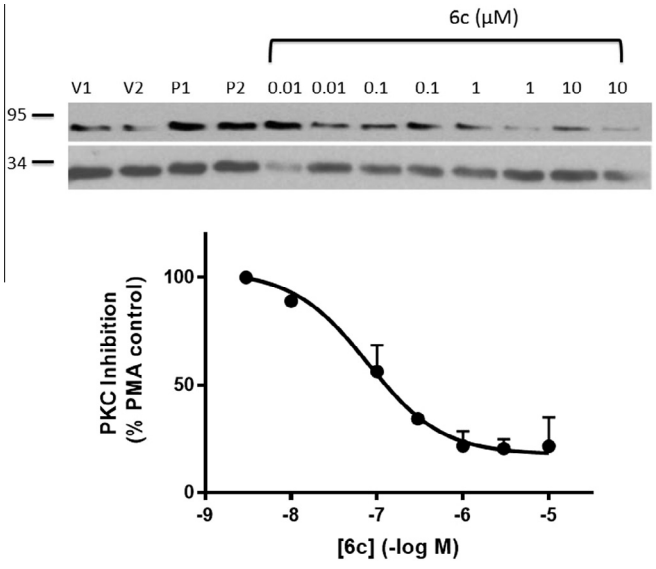

Figure 3. Compound **6c** dose dependently inhibits PMA-stimulated MARCKS phosphorylation. (A) Representative Western blot of pMARCKS (top row) with GAPDH loading control (bottom row). Concentrations in μM are given above the lanes. V1 and V2 are vehicle; P1and P2 are PMA control. The molecular weight markers of 95 and 34 kDa are shown. (B) Dose response curve calculated from pMARCKS Western blot analysis. PMA control is calculated as PMA values minus vehicle control and set at 100%. n = 4–6.

Inhibition data against PKC and ERα for synthesized triarylacrylonitrile analogues versus tamoxifen as control are shown in Table 1. The results are displayed in Table 1 as percent inhibition of PMA-stimulated PKC activity. Representative Western blots for all compounds except for **6c**, which is shown in Figure 3, are shown in Figure S1 of Supplemental Information. In our ERα binding assay, tamoxifen displaced estradiol binding with an IC_{50} of 222 nM. Additionally, we observed a 27 ± 9% and 52 ± 7% inhibition of PKC activity by tamoxifen at 3 μM and 10 μM, respectively. This was nearly equivalent to the inhibition of PKC by the isomeric compounds **3a** and **4a**, which possess a single morpholinoethoxy side chain, with each showing nearly equivalent inhibition of PKC relative to tamoxifen at the two concentrations tested. These compounds also display essentially equivalent affinity for binding to ERα, which is within the same range as tamoxifen. The same pattern holds for the direct nitrile congener of tamoxifen, **3b**, which was tested as a mixture highly enriched in the *E*-isomer. More specifically, **3b**/**4b** caused a 22 ± 10% and 67 ± 16% reduction in PKC activity at 3 μM and 10 μM, respectively. Analogues **6a**–**6c** with solubilizing dialkylaminoalkoxy side chains attached to both the α and α′ rings show a different pattern of inhibition. In general, there is a trend for greater potency toward inhibition of PKC at both concentrations tested relative to tamoxifen, and/or reduced affinity to the ERα. Compound **6a** with the (diethylamino)ethoxy side chains shows essentially equivalent potency to tamoxifen for inhibition of PKC, but with negligible binding to ERα. In contrast, compound **6b** with the less basic morpholinoethoxy side chains shows much greater sensitivity toward PKC, with 3 μM and 10 μM of **6b** causing a 62 ± 8% and 72 ± 7% decrease in PKC activity respectively. However, **6b** displays equivalent potency for ERα relative to tamoxifen. Compared to **6a**, **6b** showed significantly more inhibition of PKC at 3 μM (one-way ANOVA, $^{*}p < 0.05$; see Table 1). Compound **6c** with the more basic (4-methylpiperazin-1-yl)ethoxy side chains shows the best selectivity profile relative to tamoxifen for all analogues synthesized with excellent potency toward inhibition of PKC and undetected binding to ERα. Similar to **6b**, **6c** inhibited PKC more significantly at 3 μM when compared to **6a** (one-way ANOVA, $^{**}p < 0.01$; see Table 1). Titration shows that it inhibits PKC activity with an IC_{50} of 80 nM, but does not bind ERα at concentrations up to at least 10 μM. A representative blot with a calculated dose response curve demonstrating the inhibition of PMA-stimulated MARCKS phosphorylation is shown in Figure 3. By comparison, tamoxifen in our cell-based PKC inhibition assay has an IC_{50} of 20 μM against PKC and an IC_{50} of 222 nM for binding to ERα.

The data for a single congener, **12**, in which the β-phenyl ring has been replaced with a thiophen-2-yl ring, show a similar selectivity pattern to **6c** but with reduced potency for inhibition of PKC. More specifically, unlike **6c** which causes a 83 ± 4% reduction in PKC activity at 3 μM, **12** inhibits only at 39 ± 7%. More work needs to be carried out to fully map out a β-heteroaryl ring SAR.

The overall SAR trends for our small series of triarylacrylonitriles with respect to PKC activity are in general alignment with those summarized in a review in 2004,[36] showing that the introduction of an additional basic side chain of sufficient length off the 4-position of the α-phenyl ring and the nature of the terminal amine head group markedly increase potency for PKC inhibition relative to tamoxifen.

5. Conclusions

Utilizing the known compound **6a** as a starting template, we have designed and synthesized a small series of novel triarylacrylonitrile analogues with some possessing enhanced potency and selectivity for PKC over the estrogen receptor. For analogues incorporating a β-phenyl ring, we have shortened the classical synthetic route by installing dialkylaminoalkoxy side chains first off the α- and/or α′-rings of a precursor benzophenone, and then condensing the resultant ketones with phenylacetonitrile anion. Additionally, we have developed a completely novel, efficient, and versatile route utilizing Suzuki chemistry, which will allow for the introduction of a wide range of β-aryl or β-heteroaryl moieties and side-chain substituents onto the acrylonitrile scaffold. For analogues possessing a single side chain off the α- or α′-ring, we have developed novel 2D NMR experiments that allow for unambiguous assignment of *E*- and *Z*-stereochemistry. From our SAR, we have successfully uncovered a compound, **6c**, with markedly increased potency and selectivity for inhibiting PKC and reduced estrogen-receptor binding compared to tamoxifen. Future publications will detail studies which show that **6c** significantly inhibits amphetamine-induced dopamine release using both in vitro and in vivo models. Additional studies investigating the effects of **6c** on AMPH reinforcement using self-administration in rats as well as current studies to determine CNS penetration will also be reported. These, in addition to the binding data reported herein, support further SAR exploration of the triphenylacrylonitrile scaffold, and heteroaryl congeners, toward the development of potential clinical agents to treat amphetamine abuse.

6. Experimental

6.1. General chemistry procedures

All starting materials were obtained from commercial suppliers and were used without further purification. Routine ^{1}H NMR spectra were recorded at 400 or 500 MHz on a Varian 400 or 500 instrument, respectively, with $CDCl_3$, CD_3OD, or DMSO-d_6 as solvent. ^{13}C NMR were recorded in DMSO-d_6 at 126 MHz on a Varian 400 instrument. Chemical shift values are recorded in δ units (ppm). The 1D ^{1}H, 1D ^{13}C, 2D ^{1}H–^{1}H TOCSY and 2D ^{1}H–^{13}C HSQC experiments were measured at 25 °C using a 600 MHz Bruker spectrometer equipped with a cryogenic probe. Compounds were dissolved either in DMSO-d_6 or a 1:1 DMSO-d_6:CD_3OD mixture. Mass spectra were recorded on a Micromass TofSpec-2E Matrix-Assisted, Laser-Desorption, Time-of-Flight Mass Spectrometer in a positive ESI mode ($TOFES^{+}$) unless otherwise noted. High resolution mass spectrometry (HRMS) analysis was performed on an Agilent Q-TOF system. Analytical HPLC was performed on an Agilent 1100 series instrument with an Agilent Zorbax Eclipse Plus C18 (4.6 mm × 75 mm, 3.5 μm particle size) column with the gradient 10% ACN/water (1 min), 10–90% ACN/water (6 min), and 90% ACN/water (2 min) flow = 1 mL/min. Thin-layer chromatography (TLC) was performed on silica gel GHLF plates (250 microns) purchased from Analtech. Column chromatography was carried out in the flash mode utilizing silica gel (220–240 mesh) purchased from Silicycle. Extraction solutions were dried over $MgSO_4$ prior to concentration.

6.1.1. (4-(2-Bromoethoxy)phenyl)(phenyl)methanone (1b)[49]

A stirred suspension of 4-hydroxybenzophenone (**1a**; 700 mg, 3.53 mmol), 1,2-dibromoethane (3.04 mL, 35.3 mmol), cesium carbonate (2.3 g, 7.1 mmol) and acetonitrile (35 mL) was heated at reflux for 48 h. The mixture was diluted with 250 mL of water and extracted with dichloromethane (3×). The combined extracts were washed with water, sat. brine, dried, and concentrated to a solid that was purified by flash silica gel chromatography, eluting with chloroform. Product fractions were combined and concentrated to leave **1b** (750 mg, 70%) as a white solid, mp 74–75 °C. R_f 0.44 (chloroform). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.76–7.60 (m, 5H), 7.52 (t, J = 7.5 Hz, 2H), 7.10 (d, J = 8.7 Hz, 2H), 4.41 (t,

J = 5.4 Hz, 2H), 3.83 (t, J = 5.3 Hz, 2H). MS TOFES$^+$: m/z 305.0, 307.0 $(M+H)^+$.

6.1.2. Bis(4-(2-bromoethoxy)phenyl)methanone (1d)[50]

A stirred suspension of 4,4′-dihydroxybenzophenone (**1c**; 1.93 g. 9 mmol), 1,2-dibromoethane (15.5 mL, 180 mmol), cesium carbonate (11.77 g, 36.1 mmol) and acetonitrile (66 mL) was heated at reflux for 22 h. The suspension was filtered and the salts washed well with dichloromethane. The combined filtrate was filtered through a small pad of flash silica gel, washing the pad well with dichloromethane. The filtrate was concentrated to a semisolid that was diluted with 2-propanol. The suspension was heated for ~5 min and allowed to cool. The resulting solids were collected, washed with 2-propanol, and dried to leave 2.3 g of **1d**, mp 125–127 °C. Upon standing for several days, additional product crystallized from the mother liquor and was collected to give 130 mg of **1d**, mp 120–125 °C. Total yield = 2.43 g (63%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.73–7.64 (m, 4H), 7.13–7.04 (m, 4H), 4.45–4.37 (m, 4H), 3.87–3.79 (m, 4H). MS TOFES$^+$: m/z 427.9 (M^+), 428.9 $(M+H)^+$.

6.1.3. (4-(2-Morpholinoethoxy)phenyl)(phenyl)methanone (2a)[51]

A stirred suspension of 4-hydroxybenzophenone (**1a**; 750 mg, 3.8 mmol), 4-(2-chloroethyl)morpholine hydrochloride, (739 mg, 4 mmol), cesium carbonate (3.7 g, 11.4 mmol) and acetonitrile (30 mL) was heated at reflux for 21 h. The mixture was poured into 250 mL of water and stirred overnight. The formed suspension was collected, washed with water, and dried to give **2a** (1.0 g, 85%) as an off-white powder, mp 64–66 °C. R_f 0.61 (85:15:2 ethyl acetate/methanol/triethylamine. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.75–7.60 (m, 5H), 7.53 (t, J = 7.5 Hz, 2H), 7.08 (d, J = 8.6 Hz, 2H), 4.17 (t, J = 5.7 Hz, 2H), 3.55 (t, J = 4.6 Hz, 4H), 2.70 (t, J = 5.7 Hz, 2H); remaining protons overlap DMSO peak. MS TOFES$^+$: m/z 312.1 $(M+H)^+$, 334.1 $(M+Na)^+$.

6.1.4. (4-(2-(Dimethylamino)ethoxy)phenyl)(phenyl)methanone (2b)[51]

A stirred mixture of (4-(2-bromoethoxy)phenyl)(phenyl) methanone (**1b**; 750 mg, 2.5 mmol), dimethylamine hydrochloride (301 mg, 3.7 mmol), potassium carbonate (1.36 g, 9.8 mmol), and acetone (10 mL) was heated at reflux for 16 h. The mixture was concentrated to a solid residue that was partitioned between ethyl acetate and water. The aqueous layer was further extracted with ethyl acetate and the combined organic phases were washed sequentially with water and sat. brine, dried and concentrated to an oil that was purified by flash silica gel chromatography eluting with 4:1 dichloromethane/methanol. Concentration of product fractions left **2b** (0.50 g, 76%) as a colorless syrup; R_f 0.25 (85:15:2 ethyl acetate/methanol/trimethylamine). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.71 (d, J = 8.3 Hz, 2H), 7.69–7.59 (m, 3H), 7.53 (t, J = 7.4 Hz, 2H), 7.07 (d, J = 8.3 Hz, 2H), 4.13 (t, J = 5.7 Hz, 2H), 2.63 (t, J = 5.8 Hz, 2H), 2.20 (s, 6H). MS TOF-ES$^+$: m/z 270.2 $(M+H)^+$.

6.1.5. (*E* and *Z*)-3-(4-(2-Morpholinoethoxy)phenyl)-2,3-diphenylacrylonitrile, hydrochloride (3a and 4a)

Run 1: The anion of phenylacetonitrile (32.1 mmol) in THF (30 mL) was generated as described below for the synthesis of **6c**. A solution of (4-(2-morpholinoethoxy)phenyl)(phenyl)methanone (**2a**; 500 mg, 1.6 mmol) in THF (5 mL) was added over a period of 5 min. After 30 min the cooling bath was removed and the mixture warmed gradually to room temperature. After stirring for 48 h, the mixture was poured into 150 mL of 2 N aq HCl and further worked up as described below for **6c** below to leave a crude mixture by NMR of **3a** and **3b** (570 mg, 86%) as a syrup; R_f 0.44 (85:15:2 ethyl acetate/methanol/trimethylamine); R_f 0.18 (ethyl acetate). MS TOFES$^+$: m/z 411.1 $(M+H)^+$. Upon standing at room temperature (~1 month) the syrup crystallized. The solids were triturated in a few mL of ethanol with sonication, collected, washed with ethanol, and dried to leave an isomeric mixture of products (150 mg, 23%) as a cream-colored powder, mp 135–142 °C, shown by HPLC to be a 91:9 mixture of **3a**/**4a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.47 (m), 7.43–7.36 (m), 7.33–7.21 (m), 6.88–6.82 (m), 6.81–6.75 (m), 4.01 (t, J = 5.7 Hz), 3.53 (t, J = 4.7 Hz), 2.62 (t, J = 5.7 Hz), 2.41 (t, J = 4.7 Hz), remaining protons hidden under DMSO signal. The mother liquor was concentrated to leave ~400 mg of an isomeric mixture for further processing. *Run 2*: The above reaction was repeated on starting ketone **2a** (550 mg, 1.8 mmol) to give crude product (700 mg, 97%) that was processed as above to leave 164 mg (23%) of a powder, mp 136–142°, shown by HPLC to be a 82:18 mixture of **3a**/**4a**. The mother liquor was concentrated to leave ~530 mg of an isomeric mixture for further processing. To a stirred solution of 100 mg (0.24 mmol) of the 82:18 mixture of **3a**/**4a** from Run 2 in 5:1 ethanol/dichloromethane (6 mL) was added HCl in ether (0.26 mL of 1 M solution). After 3 h the mixture was concentrated to a glassy residue that eventually crystallized after treatment with a few drops of methanol. The solids were collected, washed with 2-propanol, and dried to give **3a** hydrochloride (54 mg, 50%) as a white powder; mp 200–202 °C; R_f 0.85 (97:3 methanol/conc. ammonium hydroxide); R_f 0.66 (85:15:2 ethyl acetate/methanol/triethylamine). HPLC rt 6.1 min (98%), 6.3 min (2%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.48 (m, 3H), 7.43–7.36 (m, 2H), 7.33–7.22 (m, 5H), 6.91 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 4.33 (t, J = 4.7 Hz, 2H), 3.93 (d, J = 13.0 Hz, 2H), 3.73 (t, J = 12.2 Hz, 2H), 3.55–3.39 (m, 4H), 3.15 (d, J = 5.2 Hz, 2H); ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.02–3.18 (m, 2H, $(CHH)_2$N–), 3.29–3.48[a] (m, 2H, $(CHH)_2$N–)), 3.38–3.52[a] (m, 2H, NCH_2CH_2O), 3.69–3.98 (m, 4H, $(CH_2)_2O$), 4.25–4.44 (m, 2H, NCH_2CH_2O), 6.68–6.84 (m, 2H, $Ar_{\alpha'}H$), 6.84–6.93 (m, 2H, $Ar_{\alpha'}H$), 7.15–7.32 (m, 5H, $Ar_{\beta}H$), 7.33–7.51 (m, 5H, $Ar_{\alpha}H$); ^{1}H NMR (600 MHz, DMSO-d_6:CD_3OD, 1:1 v:v): δ 3.08–3.17 (m, 2H, $(CHH)_2$N–), 3.36–3.46 (m, 2H, $(CHH)_2$N–)), 3.46–3.52 (m, 2 H, NCH_2CH_2O), 3.65–3.78 (m, 2H, $(CHH)_2O$), 3.85–3.98 (m, 2H, $(CHH)_2O$), 4.22–4.28[a] (m, 2H, NCH_2CH_2O), 6.80 (d, J = 8.1 Hz, 2H, $Ar_{\alpha'}H$), 6.88 (d, J = 8.1 Hz, 2H, $Ar_{\alpha'}H$), 7.21 (m, 5H, $Ar_{\beta}H$), 7.31–7.49 (m, 5H, $Ar_{\alpha}H$); ^{13}C NMR (150 MHz, DMSO-d_6): δ 51.7, 54.9, 62.3, 63.2, 110.0, 114.4, 119.9, 128.5, 128.6, 128.8, 129.4, 129.6, 129.9, 131.2, 132.1, 134.7, 140.3, 157.1, 157.9; ([a]peaks overlapped with solvent, determined from HSQC). The sticky semisolid from the above combined mother liquors (~930 mg) was triturated in ethanol to leave solids that were collected, washed well with ethanol, and dried to leave 250 mg of a different mixture of isomers from above, as shown by TLC (95:5 dichloromethane/methanol), as an off-white powder; mp 123–135 °C. The mixture was dissolved in 5 mL of 4:1 methanol/dichloromethane, and anhydrous HCl in ether (0.7 mL of 1 M solution) was added. The mixture was stirred at room temperature for 18 h and concentrated to a solid residue, which was triturated in several mL of 2-propanol, sonicated briefly, and stored overnight. The solids were collected, washed with 2-propanol, and dried to leave enriched **4a** (165 mg), mp 144–169 °C; R_f 0.72 (97:3 methanol/conc. ammonium hydroxide); R_f 0.55 (95:5 dichloromethane/methanol). The product was recrystallized from 2–3 mL of ethanol to leave highly pure **4a**, hydrochloride (45 mg) as a beige powder; mp 144–146 °C. HPLC: rt 6.1 min (6%), 6.3 min (94%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.35 (d, J = 8.6 Hz, 2H), 7.27–7.17 (m, 8H), 7.04 (d, J = 8.4 Hz, 2H), 6.97 (dt, J = 6.8, 1.5 Hz, 2H), 4.25–4.15 (m, 2H), 3.65–3.58 (m, 4H), remaining protons hidden under DMSO signal. ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.38–2.62[a] (m, 4H, $(CH_2)_2$N–), 2.64–2.88 (m, 2H,NCH_2CH_2O), 3.50–3.84 (m, 4H, $(CH_2)_2O$), 4.03–4.47 (m, 2H, NCH_2CH_2O), 6.95–7.04 (m, 2H, $Ar_{\alpha}H$), 7.05–7.12 (m, 2H, $Ar_{\alpha'}H$), 7.18–7.34 (m, 8H, $Ar_{\alpha}H$, $Ar_{\beta}H$), 7.35–7.44 (m, 2H, $Ar_{\alpha'}H$); ^{1}H NMR (600 MHz, DMSO-d_6:CD_3OD, 1:1

v:v): δ 2.70–2.86 (m, 4H, $(CH_2)_2N$–), 2.94–3.04 (m, 2H, NCH_2CH_2O), 3.60–3.73 (m, 4H, $(CH_2)_2O$), 4.17–4.25[a] (m, 2H, NCH_2CH_2O), 6.95 (d, J = 7.0 Hz, 2H, $Ar_\alpha H$), 7.01 (d, J = 8.4 Hz, 2H, $Ar_{\alpha'}H$), 7.12–7.26 (m, 8H, $Ar_\alpha H$, $Ar_\beta H$), 7.34 (d, J = 8.4 Hz, 2H, $Ar_{\alpha'}H$); ^{13}C NMR (150 MHz, DMSO-d_6): δ 53.1[b], 56.5[b], 64.6[b], 65.5[b], 109.7, 114.6, 120.2, 128.4, 128.4, 128.7, 129.1, 129.4, 130.4, 131.3, 132.1, 134.8, 138.9, 157.6; ([a]peaks overlapped with solvent, determined from HSQC, [b]due to broad signals the chemical shifts have been extracted from the HSQC experiment); MS TOFES$^+$: m/z 411.1 $(M+H)^+$; TOFES$^-$: m/z 409.2 $(M-H)^+$.

6.1.6. (*E* and *Z*)-3-(4-(2-(Dimethylamino)ethoxy)phenyl)-2,3-diphenylacrylonitrile, hydrochloride (3b and 4b)[52]

The anion of phenylacetonitrile (33.4 mmol) in THF (45 mL) was generated as described below for the synthesis of **6c**. After 30 minutes at 0–5 °C, the anion was cooled to −78 °C and a solution of the ketone **2b** (450 mg, 1.7 mmol) in THF (15 mL) was added over a period of 5 min. Cooling was removed and the red-brown mixture was stirred at room temperature for 5 d. The mixture was poured into ice-cold 3 N aq HCl and further worked up as described for the preparation of **6c** below to leave a solid residue (600 mg, 97%) that was triturated in 2-propanol, collected, washed with ether and dried to leave crude **3b**, **4b** (98 mg, 16%), confirmed by NMR and MS, as a tan powder. The combined mother liquor and washes were concentrated to a residue that was dissolved in methanol and treated with an excess of anhydrous 1 N HCl in ether. After stirring for 20 h the solution was concentrated leaving a glassy residue that was triturated in 2-propanol. The precipitate was collected and dried to leave an 84:16 mixture (by HPLC) of **3b**/**4b** hydrochloride (0.14 g, 20%) as a cream-colored powder, mp 217–230 °C. R_f 0.69 (99:1 dichloromethane/methanol). ^1H NMR (400 MHz, DMSO-d_6): δ 10.47 (s, 1H), 7.49–6.83 (m, 14H), 4.42, 4.29 (m, 2H), 3.51, 3.44 (m, 2H), 2.83, 2.78 (s, 6H). MS TOFES$^+$: m/z 369.1 $(M+H)^+$.

6.1.7. Bis(4-(2-(diethylamino)ethoxy)phenyl)methanone (5a)[53]

A mixture of bis(4-hydroxyphenyl)methanone (**1c**; 1.07 g, 5 mmol), 2-chloro-*N*,*N*-diethylethylamine hydrochloride (1.76 g, 10.2 mmol), cesium carbonate (8 g, 24.6 mmol) and acetonitrile (52 mL) was stirred at reflux for 18 h. The mixture was poured into 500 mL of water and then extracted with ethyl acetate (3×). The combined extracts were washed with sat. brine, dried and concentrated to leave 1.93 g (92%) of **5a** as a free-flowing pale orange oil, 91% pure by HPLC, that solidified in the refrigerator. The compound was used directly in the next step. ^1H NMR (400 MHz, DMSO-d_6): δ 7.71–7.61 (m, 4H), 7.09–7.00 (m, 4H), 4.09 (t, J = 6.1 Hz, 4H), 2.78 (t, J = 6.1 Hz, 4H), 2.53 (q, J = 7.1 Hz, 8H), 0.95 (t, J = 7.1 Hz, 12H); MS TOFES$^+$: m/z 413.3 $(M+H)^+$.

6.1.8. Bis(4-(2-morpholinoethoxy)phenyl)methanone (5b)

A stirred mixture of 4,4′-dihydroxybenzophenone (**1c**; 500 mg, 2.3 mmol), 4-(2-chloroethyl)morpholine hydrochloride (864 mg, 4.6 mmol), cesium carbonate (3.69 g, 11.3 mmol) and acetonitrile (25 mL) was heated at reflux for 18 h. The mixture was diluted with 250 mL of water and the resulting solution was stirred at room temperature for 18 h. The precipitated solids were collected, washed with water, and dried to leave **5b** (0.9 g, 90%) as a white powder, mp 119–120 °C. R_f 0.33 (ethyl acetate/methanol/triethylamine, 85:15:2). ^1H NMR (400 MHz, DMSO-d_6): δ 7.67 (d, J = 8.7 Hz, 4H), 7.06 (d, J = 8.7 Hz, 4H), 4.17 (t, J = 5.6 Hz, 4H), 3.59–3.52 (m, 8H), 2.70 (t, J = 5.6 Hz, 4H); remaining protons overlap DMSO peak. MS TOFES$^+$: m/z 441.2 $(M+H)^+$.

6.1.9. Bis(4-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl) methanone (5c)

A suspension of the bis-bromoethoxy compound (**1d**; 1.3 g, 3.0 mmol), *N*-methylpiperazine (1.52 mL, 13.7 mmol), and acetonitrile (6 mL) was stirred at reflux for 2 h. The mixture was cooled and distributed between 5% aq $NaHCO_3$ and dichloromethane, using NaCl to break up the emulsion. The layers were separated and the aqueous phase was further extracted with dichloromethane (2×). The combined extracts were dried and concentrated to a semisolid that was dissolved in a minimum volume of hot 2-propanol (5–6 mL). The solution was refrigerated for several hours and the precipitated solids were collected, washed with 2-propanol, and dried to leave 1.17 g of **5c**, mp 129–130 °C. Concentration of the mother liquor and further processing as above gave 45 mg of a second crop, mp 129–130 °C. Total yield = 1.22 g (86%). ^1H NMR (400 MHz, DMSO-d_6): δ 7.68 (d, J = 8.8 Hz, 4H), 7.08 (d, J = 8.9 Hz, 4H), 4.17 (t, J = 5.7 Hz, 4H), 2.71 (t, J = 5.6 Hz, 4H), 2.32 (m, 6H), 2.14 (s, 6H), remaining protons hidden under solvent signal. MS TOFES$^+$: m/z 467.3 $(M+H)^+$.

6.1.10. 3,3-Bis(4-(2-(diethylamino)ethoxy)phenyl)-2-phenylacrylonitrile, dihydrochloride (6a)[41]

A solution of commercially available lithium diisopropylamide (1 M in THF/hexanes, 30 mL, 30 mmol) under nitrogen at −78 °C was treated dropwise with phenylacetonitrile (3.46 mL, 30 mmol) over ~5 min. The cooling bath was removed and the temperature was allowed to come to 0–10 °C. The deep yellow anion suspension was re-cooled to −78 °C and diluted with THF (17 mL). Ketone **5a** (619 mg, 1.5 mmol) in 5 mL THF was added over a ~1 min and the resultant suspension was maintained at −78 °C for 3–3.5 h (beige suspension) and then allowed to slowly warm to room temperature overnight. After stirring for a total of 19 h from the point of ketone addition, the violet mixture was poured into 2 N aq HCl (125 mL), stirred for 2.5 h, and extracted with ethyl acetate (2×). The combined organic extracts were discarded. The acidic aqueous phase was ice-cooled and treated portionwise with 10.5 g of NaOH dissolved in minimal water. The cloudy aqueous solution (pH ~12) was extracted with ethyl acetate (3×), with small aliquots of aq NaOH added to keep the aqueous phase basic. The combined extracts were washed with sat. brine, dried, and concentrated to a viscous oil that was pumped in vacuo 2 h to leave 700 mg (91%) of **6a** as a pale orange viscous oil, shown by HPLC to be 94% pure. Processing a small amount of product by re-dissolving it in 2 N aq HCl followed by further treatment as above provided **6a** that was 96% pure by HPLC. R_f ~0.35 (95:5 methanol/conc. ammonium hydroxide). ^1H NMR (400 MHz, DMSO-d_6): δ 7.36–7.16 (m, 7H), 7.04–6.96 (m, 2H), 6.91–6.80 (m, 2H), 6.79–6.71 (m, 2H), 4.11–3.96 (m, 2H), 3.93 (t, J = 6.1 Hz, 2H), 2.78 (t, J = 6.1 Hz, 2H), 2.69 (t, J = 6.1 Hz, 2H), 2.59–2.43 (m, 8H), 0.94 (dt, J = 18.2, 7.1 Hz, 12H). ^1H NMR (500 MHz, CD_3OD): δ 7.39 (d, J = 8.8 Hz, 2H), 7.25 (s, 5H), 7.02 (d, J = 8.8 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 6.77 (d, J = 8.8 Hz, 2H), 4.19 (t, J = 5.6 Hz, 2H), 4.07 (t, J = 5.6 Hz, 2H), 3.07–2.95 (m, 2H), 2.95–2.84 (m, 2H), 2.75 (m, 4H), 2.69 (m, 4H), 1.14 (t, J = 7.2 Hz, 6H), 1.09 (t, J = 7.2 Hz, 6H); The dihydrochloride salt was made as follows: **6a** free base (90 mg) was dissolved in minimal dichloromethane and the solution was treated with 800 μL of anhydrous 1 N HCl in ether. The mixture was stirred for 10 min and then filtered through a cotton plug to remove a few insolubles. The filtrate was concentrated to a residue that was redissolved in dichloromethane/hexane and then concentrated to a yellow solid that was triturated in hexane. The solids were collected and dried to leave 100 mg (97%) of **6a** dihydrochloride. ^1H NMR (400 MHz, DMSO-d_6): δ 10.02 (s, 2H), 7.37 (d, J = 8.2 Hz, 2H), 7.30–7.19 (m, 5H), 7.08 (d, J = 8.3 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 4.39 (t, J = 5.8 Hz, 2H), 4.28 (t, J = 5.2 Hz, 2H), 3.51 (t, J = 6.7 Hz, 2H), 3.43 (t, J = 6.2 Hz, 2H), 3.25–3.08 (m, 8H), 1.25–1.17 (m, 12H). MS TOFES$^+$: m/z 512.4 $(M+H)^+$.

6.1.11. 3,3-Bis(4-(2-morpholinoethoxy)phenyl)-2-phenylacrylonitrile, dihydrochloride (6b)

The anion of phenylacetonitrile (36.2 mmol) was generated in THF (45 mL) as described below for the synthesis of **6c**. The ketone **5b** (800 mg, 1.8 mmol) in THF (15 mL) was added over a period of 5 min, the solution allowed to gradually warm to room temperature over 2–3 h and maintained there for 18 h. The mixture was poured into 100 mL of ice-cold 3 N aq HCl, stirred for 30 min, and washed with ether (2×). The aqueous phase was made strongly basic with 15% aq NaOH, and extracted with ethyl acetate (3×). The combined extracts were washed with water and then sat. brine, dried, and concentrated to leave a clear amber syrup (960 mg, 98%), which crystallized upon standing at room temperature over several days. The solids were triturated in ethanol with sonication, collected, washed with ethanol, and dried to leave **6b** (0.54 g, 55%) as a pale yellow powder, mp 130–131 °C. R_f 0.15 (85:15:2 ethyl acetate/methanol/trimethylamine). HPLC: rt 4.9 min (96% purity). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.35–7.17 (m, 7H), 7.02 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 6.78 (d, J = 8.6 Hz, 2H), 4.13 (t, J = 5.6 Hz, 2H), 4.01 (t, J = 5.6 Hz, 2H), 3.60–3.45 (m, 8H), 2.69 (t, J = 5.6 Hz, 2H), 2.62 (t, J = 5.7 Hz, 2H); 2.47–2.40 (m, 8H). MS TOFES$^+$: m/z 540.0 (M+H)$^+$. The dihydrochloride salt was prepared as follows: A solution of **6b** (100 mg, 0.19 mmol) in dichloromethane at room temperature was treated dropwise with anhydrous HCl (0.39 mL, 1 M in ether) and the resulting gummy suspension was concentrated. The residue was triturated in ether to give a glassy solid that was collected, rinsed thoroughly with ether and dried to leave **6b** dihydrochloride (0.10 g, 82%) as a yellow powder and solvated with ~0.6 equiv of ether. R_f 0.77 (95:5 methanol/conc. ammonium hydroxide). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.22 (br s, 3H), 7.40–7.21 (m, 7H), 7.09 (d, J = 8.5 Hz, 2H), 6.91–6.84 (m, 4H), 4.51–4.32 (m, 6H), 4.08–3.65 (m, 8H), 3.60–3.00 (remaining protons overlapping water peak).

6.1.12. 3,3-Bis(4-(2-(4-methylpiperazin-1-yl)ethoxy)phenyl)-2-phenylacrylonitrile, 2.5 hydrochloride salt (6c)

To a solution of diisopropylamine (10.03 mL, 71.6 mmol) in THF (50 mL) under nitrogen at −78 °C was added dropwise n-BuLi (44.7 ml of 1.6 M solution in hexane, 71.6 mmol). The solution was stirred for 10 min and then treated dropwise with phenylacetonitrile (8.26 mL, 71.6 mmol) over 20 min. The bath was removed and the temperature was allowed to come to ~0 °C. The pale yellow anion suspension was recooled to −78 °C and diluted with THF (40 mL). The solid ketone (**5c**; 1.67 g, 3.6 mmol) was added all at once and the resultant suspension was maintained at −78 °C for 2–2.5 h and then allowed to slowly warm to room temperature. During this time there was a deepening orange suspension, which became a deep purple solution that remained while the solution was stirred at room temperature for 18 h. The solution was poured into ice-cold 2 N aq HCl (300 mL), stirred for 1.5 h, and extracted with ethyl acetate (2×). The combined extracts were washed with sat. brine and discarded. The brine was combined with the aq acid phase, the solution ice-cooled and treated portion-wise with 25 g of NaOH dissolved in minimal water. The cloudy aqueous solution (pH ~12) was extracted with ethyl acetate (3×), checking after each extraction to ensure the aqueous phase was basic. The combined extracts were washed with sat. brine, dried, and concentrated to a viscous oil that was pumped in vacuo overnight to leave 2 g (100%) of partially crystalline **6c** as a golden solid. R_f ~0.35 (95:5 methanol/conc. ammonium hydroxide). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.50–7.18 (m, 6H), 7.09–6.85 (m, 3H), 6.85–6.70 (m, 4H), 4.12 (t, J = 5.7 Hz, 2H), 3.99 (t, J = 5.7 Hz, 2H), 2.69 (t, J = 5.8 Hz, 2H), 2.61 (t, J = 5.7 Hz, 2H), 2.50–2.16 (m, 9H), 2.13 (s, 3H), 2.11 (s, 3H), remaining protons hidden under solvent signal; MS TOFES$^+$: m/z 566.2 (M+H)$^+$. The residue was dissolved in minimal 2-propanol and while stirring vigorously the solution was treated with anhydrous HCl (12 mL, 1 N in ether) resulting in precipitation of a gum. After stirring for 18 h, the supernatant liquid was decanted and the residue washed once with ether by decantation. The residue was then immersed in fresh ether and stirred vigorously at room temp for 20 h leaving a fine filterable pale yellow solid that was collected, washed with portions of ether, and then once with 1% methanol in dichloromethane, resulting in conversion to a thick gummy syrup, which was collected and dissolved in methanol. The solution was concentrated in vacuo and the resulting glass was immersed in ether and stirred vigorously at room temperature overnight. The resulting yellow solid was collected, rinsed with ether and dried in vacuo over P_2O_5 at 55–60 °C for 36 h to leave **6c** 2.5 hydrochloride salt (1.35 g, 53%) as a pale yellow slightly hygroscopic powder, mp >135 °C. HPLC: rt 4.8 min (90% purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.36 (bs, 3H), 7.35–7.18 (m, 7H), 7.03 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 6.79 (d, J = 8.5 Hz, 2H), 4.15 (bs, 2H), 4.02 (bs, 2H), 2.99 (m, 8H), 2.80–2.60 (m, 10H); remaining protons overlap DMSO peak; ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.48, 135.57, 132.71, 131.93, 131.40, 129.79, 129.17, 128.64, 120.92, 114.85, 114.71, 108.69, 55.93, 52.72, 49.95, 42.51. Anal. Calcd. for $C_{35}H_{43}N_5O_2{\cdot}2.5$ HCl$\cdot$3.3 H_2O (MW 716.36): C, 58.68; H, 7.33; N, 9.78; Cl$^-$, 12.37. Found: C, 59.06; H, 7.20; N, 9.60; Cl$^-$, 12.17.

6.1.13. 3,3-Bis(4-methoxyphenyl)acrylonitrile (8)

A solution of diethyl (cyanomethyl)phosphonate (8.77 g, 49.5 mmol) in THF (10 mL) was added dropwise to a stirred suspension of sodium hydride (1.98 g of 60 wt%, 49.5 mmol) in THF (50 mL) under nitrogen at room temperature. After 30 min a solution of 4,4′-dimethoxybenzophenone (**7**; 2.0 g, 8.3 mmol) in THF (30 mL) was slowly added, and the resulting solution was heated at reflux for 20 h. The mixture was poured into 300 mL of ice water, stirred, and acidified with 4 N aq HCl. After 1 h the mixture was concentrated to ~75% volume and extracted with ethyl acetate (3×). The combined extracts were washed with sat. aq $NaHCO_3$, then sat. brine, dried and concentrated to a solid that was triturated in 2-propanol. The solids were collected, washed with 2-propanol and then hexane, and dried to leave **8** (1.96 g, 89%) as a white powder, mp 104–106 °C. R_f 0.68 (98:2 dichloromethane/ethyl acetate). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 8.6 Hz, 2H), 7.18–6.93 (m, 2H), 6.88 (d, J = 8.3 Hz, 2H), 5.55 (s, 1H), 3.87 (s, 3H), 3.84 (s, 3H). MS TOFES$^+$: m/z 266.0 (M+H)$^+$.

6.1.14. 2-Bromo-3,3-bis(4-methoxyphenyl)acrylonitrile (9)

A solution of bromine (0.55 mL, 10.7 mmol) in 1,2-dichloroethane (12 mL) was added dropwise to a stirred solution of 3,3-bis(4-methoxyphenyl)acrylonitrile (**8**; 1.9 g, 7.2 mmol) in 1,2-dichloroethane (15 mL) at −25 °C. After a few minutes the mixture was warmed slowly to room temperature where it was stirred for 4 h. The mixture was diluted with dichloromethane and then washed successively with sat. aq $NaHCO_3$, aq sodium thiosulfate, sat. brine, and dried. Concentration left a solid that was recrystallized from 3–4 mL of ethanol to give **9** (1.45 g, 59%) as an off-white powder; mp 105–107 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.23 (m, 4H), 6.93–6.84 (m, 4H), 3.85 (s, 3H), 3.84 (s, 3H). MS TOFES$^+$: m/z 344.0, 346.0 (M+H)$^+$.

6.1.15. 3,3-Bis(4-methoxyphenyl)-2-(thiophen-2-yl)acrylonitrile (10)

A stirred mixture of 2-bromo-3,3-bis(4-methoxyphenyl)acrylonitrile (**9**; 500 mg, 1.5 mmol), thiophen-2-ylboronic acid (195 mg, 1.5 mmol), $Pd(PPh_3)_4$ (84 mg, 7.3 mmol), potassium carbonate (1.0 g, 7.3 mmol), toluene (7.3 mL), and 2-propanol (7.3 mL) was heated at reflux for 42 h, and then treated with additional Pd catalyst (84 mg). After heating 18 h more, the mixture

was diluted with water and extracted with ethyl acetate. The combined organic phases were washed with sat. brine, dried and concentrated to an oil that was purified by flash silica gel chromatography, eluting with 4:1 hexane/ethyl acetate. Product fractions were combined and concentrated to give **10** (440 mg, 87%) as a yellow syrup. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.45 (dd, J = 5.1, 1.2 Hz, 1H), 7.33 (d, J = 8.8 Hz, 2H), 7.17 (dd, J = 3.7, 1.2 Hz, 1H), 7.09 (d, J = 8.7 Hz, 2H), 7.02–6.89 (m, 5H), 3.79 (s, 3H), 3.76 (s, 3H). MS TOFES$^+$: m/z 348.0 (M+H)$^+$, 370.0 (M+Na)$^+$.

6.1.16. 3,3-Bis(4-hydroxyphenyl)-2-(thiophen-2-yl)acrylonitrile (11)

BBr_3 (0.86 mL of a 1 M solution in dichloromethane) was added to a stirred solution of 3,3-bis(4-methoxyphenyl)-2-(thiophen-2-yl)acrylonitrile (**10**; 100 mg, 0.3 mmol) in dichloromethane at room temperature. The mixture was stirred for 16 h, and then poured into water, stirred vigorously for 5 min, and extracted with ether (2×). The combined extracts were washed with sat. brine, dried and concentrated to leave **11** (90 mg, 98%) as an amber syrup. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.99 (s, 1H), 9.89 (s, 1H), 7.42 (d, J = 3.9 Hz, 1H), 7.22 (d, J = 8.6 Hz, 2H), 7.13 (dd, J = 3.7, 1.3 Hz, 1H), 6.95 (d, J = 8.7 Hz, 2H), 6.79 (d, J = 8.7 Hz, 2H), 6.87–6.78 (m, 3H). MS TOFES$^+$: m/z 320.1 (M+H)$^+$.

6.1.17. 3,3-Bis(4-(2-(dimethylamino)ethoxy)phenyl)-2-(thiophen-2-yl)acrylonitrile (12)

A stirred mixture of 3,3-bis(4-hydroxyphenyl)-2-(thiophen-2-yl)acrylonitrile (**11**; 30 mg, 0.1 mmol), 2-bromo-*N*,*N*-dimethylethylamine hydrobromide (328 mg, 1.4 mmol), cesium carbonate (612 mg, 1.9 mmol), and acetonitrile (3 mL) was heated at reflux for 18 h. The mixture was diluted with water and extracted with ethyl acetate (3×). The combined extracts were washed successively with water and sat. brine, dried and concentrated to a syrup that was purified by flash silica gel chromatography, eluting first with 4–5 column volumes of 3:1 dichloromethane/methanol and then with 95:5 methanol/conc. ammonium hydroxide to elute the product. Combined product fractions were concentrated to leave **12** (18 mg, 42%) as an amber gum. HPLC: rt 5.1 min (89% purity). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.46 (d, J = 5.2 Hz, 1H), 7.32 (d, J = 8.9 Hz, 2H), 7.17 (d, J = 2.5 Hz, 1H), 7.08 (d, J = 8.8 Hz, 2H), 7.03–6.87 (m, 5H), 4.15–3.97 (m, 4H), 2.65–2.53 (m, 4H), 2.20 (s, 12H). MS TOFES$^+$: m/z 462.1 (M+H)$^+$.

6.1.18. Preparation of compounds for biological testing

Stock solutions of tamoxifen and triarylacrylonitrile analogues (12.5 or 25 mM) were made up in DMSO and stored at 20 °C for no longer than 3 weeks.

6.1.19. PKC assay

To evaluate the ability of tamoxifen and the triarylacrylonitrile analogues to inhibit PKC activity, SHSY5Y cells were incubated in Krebs Ringer HEPES (KRH) buffer (25 mM HEPES, 125 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO4, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, pH 7.4) at 37 °C for 15 min followed by a 1 h treatment with 3 and 10 μM of each compound in KRH. PKC was activated by adding a final concentration of 333 nM phorbol 12-myristate 13-acetate (PMA) in KRH to the samples for 15 min and the reaction quenched with 1 mL cold KRH. The samples were pelleted at 3000 rpm for 2 min. The pellets were washed twice in cold KRH and lysed in solubilization buffer (1% Triton X-100, 50 mM Tris HCl, 150 mM NaCl, pH 7.4). Lysates were rotated at 4 °C for 1 h and centrifuged at 14000 rpm for 15 min to remove debris. Protein assays were conducted using the Biorad DC Protein Assay Kit. PKC activity was quantified using Western Blot analysis. To obtain the IC_{50} for **6a**, the PKC assay was carried out by treating the SHSY5Y cells with 0.1–10 μM **6a** in KRH for 1 h.

6.1.20. Western blot analysis

Lysates were resolved (50 μg/lane) on a 12% polyacrylamide gel using SDS-PAGE. The proteins were transferred onto a nitrocellulose membrane at 0.1 A for 12–16 h. Membranes were incubated in blocking buffer (5% w/v milk, 150 mM NaCl, 10 mM Tris, 0.05% Tween 20). The membranes were probed with either anti-phospho-MARCKS Ser 152/156 antibody (1:1000, catalogue # 2741, Cell Signaling Technology Inc, Danvers, MA) and anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) 14C10 (1:10000, catalogue # 2118, Cell Signaling Technology Inc, Danvers, MA) antibodies for 24 h at 4 °C. Primary phospho-MARCKS antibody binding was detected using goat-anti rabbit antibody (1:2000, catalogue # sc-2054, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 1 h at room temperature and ECL Western Blotting Substrate (catalogue #32106, ThermoFisher Scientific, Waltham, MA). Primary GAPDH antibody binding was detected using donkey-anti rabbit antibody (1:20000, catalogue # sc-2054, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 1 h at room temperature and Chemiluminescent Western Substrate (catalogue # WBKLS0500, EMD Millipore, Darmstadt, Germany). Band densities were quantified using Image J software. PKC activity of each compound was calculated as the ratio of phosphorylated MARCKS to GAPDH as a percentage of that ratio for the PMA control sample. The results are displayed in Table 1 as percent inhibition of PMA-stimulated PKC activity.

6.1.21. Estrogen receptor α (ER α) binding assay

ER binding of tamoxifen and triarylacrylonitrile analogues were evaluated using a commercially available competitive binding assay (PolarScreen™ ER Beta Competitor Assay Kit, Green, catalogue # 15883, ThermoFisher Scientific, Waltham, MA). Compounds were loaded in triplicates.

6.1.22. Statistical analysis

Statistical differences were determined by one-way analysis of variants (ANOVA) using GraphPad Prism 6. Statistical significance was set at $p \leqslant 0.05$.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.09.002. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Zhang, D.; Anantharam, V.; Kanthasamy, A.; Kanthasamy, A. G. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 913.
2. Garrido, J. L.; Godoy, J. A.; Alvarez, A.; Bronfman, M.; Inestrosa, N. C. *FASEB J.* **2002**, *16*, 1982.
3. Wang, H. Y.; Friedman, E. *Biol. Psychiatry* **1996**, *40*, 568.
4. Manji, H. K.; Lenox, R. H. *Biol. Psychiatry* **2000**, *48*, 518.
5. Schmitt, K. C.; Reith, M. E. *Ann. N. Y. Acad. Sci.* **2010**, *1187*, 316.
6. Olive, M. F.; Messing, R. O. *Mol. Neurobiol.* **2004**, *29*, 139.
7. Battaini, F. *Pharmacol. Res.* **2001**, *44*, 353.
8. Manji, H. K.; Chen, G. *Mol. Psychiatry* **2002**, *7*, S46.
9. Mochly-Rosen, D.; Das, K.; Grimes, K. V. *Nat. Rev. Drug Disc.* **2012**, *11*, 937.
10. Zarate, C. A., Jr.; Singh, J. B.; Carlson, P. J.; Quiroz, J.; Jolkovsky, L.; Luckenbaugh, D. A.; Manji, H. K. *Bipolar Disord.* **2007**, *9*, 561.
11. Saraiva, L.; Fresco, P.; Pinto, E.; Goncalves, J. *J. Enzyme Inhib. Med. Chem.* **2003**, *18*, 475.

12. O'Brian, C. A.; Liskamp, R. M.; Solomon, D. H.; Weinstein, I. B. *Cancer Res.* **1985**, *45*, 2462.
13. Einat, H.; Yuan, P.; Szabo, S. T.; Dogra, S.; Manji, H. K. *Neuropsychobiology* **2007**, *55*, 123.
14. Dluzen, D. E.; McDermott, J. L.; Anderson, L. I. *J. Neuroendocrinol.* **2001**, *13*, 618.
15. Sabioni, P.; Baretta, I. P.; Ninomiya, E. M.; Gustafson, L.; Rodrigues, A. L. S.; Andreatini, R. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2008**, *32*, 1927.
16. Armani, F.; Andersen, M. L.; Galduroz, J. C. *Psychopharmacology* **2014**, *231*, 639.
17. Pereira, M.; Martynhak, B. J.; Baretta, I. P.; Correia, D.; Siba, I. P.; Andreatini, R. *Neurosci. Lett.* **2011**, *500*, 95.
18. Kantor, L.; Gnegy, M. E. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 592.
19. Zestos, A. G.; Mikelman, S. R.; Kennedy, R. T.; Gnegy, M. E. *ACS Chem. Neurosci.* **2016**, *7*, 757.
20. Browman, K. E.; Kantor, L.; Richardson, S.; Badiani, A.; Robinson, T. E.; Gnegy, M. E. *Brain Res.* **1998**, *814*, 112.
21. Aujla, H.; Beninger, R. J. *Behav. Brain Res.* **2003**, *147*, 41.
22. Moritz, A. E.; Foster, J. D.; Gorentla, B. K.; Mazei-Robison, M. S.; Yang, J. W.; Sitte, H. H.; Blakely, R. D.; Vaughan, R. A. *J. Biol. Chem.* **2013**, *288*, 20.
23. Wang, Q.; Bubula, N.; Brown, J.; Wang, Y.; Kondev, V.; Vezina, P. *Neurosci. Lett.* **2016**, *622*, 78.
24. Khoshbouei, H.; Sen, N.; Guptaroy, B.; Johnson, L.; Lund, D.; Gnegy, M. E.; Galli, A.; Javitch, J. A. *PLoS Biol.* **2004**, *2*, E78.
25. Jordan, V. C. *Nat. Rev. Drug Disc.* **2003**, *2*, 205.
26. O'Brian, C. A.; Ioannides, C. G.; Ward, N. E.; Liskamp, R. M. *Biopolymers* **1990**, *29*, 97.
27. Kuo, J. R.; Wang, C. C.; Huang, S. K.; Wang, S. J. *Neurochem. Int.* **2012**, *60*, 105.
28. de Medina, P.; Payre, B. L.; Bernad, J.; Bosser, I.; Pipy, B.; Silvente-Poirot, S.; Favre, G.; Faye, J. C.; Poirot, M. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 1165.
29. O'Brian, C. A.; Housey, G. M.; Weinstein, I. B. *Cancer Res.* **1988**, *48*, 3626.
30. Bignon, E.; Pons, M.; Gilbert, J.; Nishizuka, Y. *FEBS Lett.* **1990**, *271*, 54.
31. Su, H. D.; Mazzei, G. J.; Vogler, W. R.; Kuo, J. F. *Biochem. Pharmacol.* **1985**, *34*, 3649.
32. Gundimeda, U.; Chen, Z.-H.; Gopalakrishna, R. *J. Biol. Chem.* **1996**, *271*, 13504.
33. Szallasi, Z.; Bogi, K.; Gohari, S.; Biro, T.; Acs, P.; Blumberg, P. M. *J. Biol. Chem.* **1996**, *271*, 18299.
34. Wang, S.; Kazanietz, M. G.; Blumberg, P. M.; Marquez, V. E.; Milne, G. W. A. *J. Med. Chem.* **1996**, *39*, 2541.
35. Ochoa, W. F.; Garcia-Garcia, J.; Fita, I.; Corbalan-Garcia, S.; Verdaguer, N.; Gomez-Fernandez, J. C. *J. Mol. Biol.* **2001**, *311*, 837.
36. de Medina, P.; Favre, G.; Poirot, M. *Curr. Med. Chem. Anticancer Agents* **2004**, *4*, 491.
37. Ohta, K.; Chiba, Y.; Kaise, A.; Endo, Y. *Bioorg. Med. Chem.* **2015**, *23*, 861.
38. Bignon, E.; Pons, M.; Dore, J.-C.; Gilbert, J.; Ojasoo, T.; Miquel, J.-F.; Raynaud, J.-P.; Crastes de Paulet, A. *Biochem. Pharmacol.* **1991**, *42*, 1373.
39. Bignon, E.; Ogita, K.; Kishimoto, A.; Gilbert, J.; Abecassis, J.; Miquel, J. F.; Nishizuka, Y. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 1377.
40. Bignon, E.; Pons, M.; Crastes de Paulet, A.; Dore, J.-C.; Gilbert, J.; Abecassis, J.; Miquel, J.-F.; Ojasoo, T.; Raynaud, J.-P. *J. Med. Chem.* **1989**, *32*, 2092.
41. Dore, J.-C.; Gilbert, J.; Bignon, E.; Crastes de Paulet, A.; Ojasoo, T.; Pons, M.; Raynaud, J.-P.; Miquel, J.-F. *J. Med. Chem.* **1992**, *35*, 573.
42. Pan, D.; Iyer, M.; Liu, J.; Li, Y.; Hopfinger, A. J. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 2083.
43. Abraham, M. H.; Takacs-Novak, K.; Mitchell, R. C. *J. Pharm. Sci.* **1997**, *86*, 310.
44. Gilbert, J.; Miquel, J. F.; Precigoux, G.; Hospital, M.; Raynaud, J. P.; Michel, F.; Crastes de Paulet, A. *J. Med. Chem.* **1983**, *26*, 693.
45. Muller, G. W.; Payvandi, F.; Zhang, L.H.; Robarge, M. J.; Chen, R.; Man, H.-W.; Ruchelman, A. L. US Patent 0107339, 2005.
46. Suzuki, K.; Kobayashi, T.; Nishikawa, T.; Hori, Y.; Hagiwara, T. US Patent 6455720B1, 2002.
47. Lone, A. M.; Bhat, B. A. *Org. Biomol. Chem.* **2014**, *12*, 242.
48. Horgan, K.; Cooke, E.; Hallett, M. B.; Mansel, R. E. *Biochem. Pharmacol.* **1986**, *35*, 4463.
49. Chen, J.; Wang, Y.; Li, W.; Zhou, H.; Li, Y.; Yu, C. *Anal. Chem. (Washington, DC, U. S.)* **2014**, *86*, 9866.
50. Tang, B.; Hong, Y.; Chen, S.; Kwok, R. T. K. US Patent 0172296 A1, 2012.
51. Meegan, M. J.; Hughes, R. B.; Lloyd, D. G.; Williams, D. C.; Zisterer, D. M. *J. Med. Chem.* **2001**, *44*, 1072.
52. Lashley, M. R.; Dicus, C. W.; Brown, K.; Nantz, M. H. *Org. Prep. Proced. Int.* **2003**, *35*, 231.
53. Palopoli, F. P.; Holtkamp, D. E.; Schaar, J. L. US Patent 3243461, 1966.

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