Available online at www.sciencedirect.com

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry

Bioorganic & Medicinal Chemistry 12 (2004) 2553–2570

ERβ Ligands. Part 2: Synthesis and structure–activity relationships of a series of 4-hydroxy-biphenyl-carbaldehyde oxime derivatives

Cuijian Yang,[a] Richard Edsall, Jr.,[a] Heather A. Harris,[b] Xiaochun Zhang,[b] Eric S. Manas[a] and Richard E. Mewshaw[a,*]

[a] *Chemical Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*
[b] *Women's Health Research Institute, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*

Received 27 October 2003; revised 4 March 2004; accepted 14 March 2004

Abstract—A series of biphenyl carbaldehyde oximes (**6**) was prepared and shown to have significant selectivity for the estrogen receptor-β (ERβ). The exploitation of the oxime moiety as a hydrogen bond donating group, which mimicked the C-ring of genistein makes these compounds unique. Molecular modeling studies showed the oxime moiety hydrogen bonding to the histidine residue, which was supported by the structure–activity relationships. The most potent compounds in this study had IC_{50} values in a radioligand binding assay of between 8–35 nM. Among the most selective compounds were **6i** and **6s** (49- and 31-fold ERβ selective, respectively).

1. Introduction

Estrogens are known to play an important role in the growth, development and maintenance of a diverse range of tissues. They exert numerous biologic effects via estrogen receptors (ER), which function as a ligand-activated transcriptional regulator. Until recently, these effects were attributed to a single ER cloned in 1986.[1] The unexpected discovery of a second ER, termed ERβ,[2] has added a new level of uncertainty to our understanding of the role of estrogen and prompted intense interest in the respective functions of each isoform. While the utility of estrogen based therapies such as oral contraceptives and hormone replacement is well established, the role of ERβ is still being unraveled. Because ERβ is widely expressed, but is not the dominant ER in the uterus, there is much optimism about ERβ as a drug target.[3] Defining the function of ERβ by identifying ERβ selective ligands may reveal its therapeutic value. ERα and ERβ share modest overall sequence identity with little homology between the two N-terminal domains but well conserved DNA- and ligand binding domains.[4] Crystal structures of the ERα and ERβ binding domains have been reported in the literature.[5–7] Although the overall tertiary structure of both proteins remains relatively invariant, the size and shape of their respective ligand binding pockets show considerable variability, which is apparently why a range of ligands bind with similar affinities.[8] Though the estrogens, including the endogenous ligand estradiol (**1**, Fig. 1), were found to have equipotent affinity at both subtypes, many of the phytoestrogens, such as genistein (**2**), were found to have modest selectivity for ERβ.[9] This

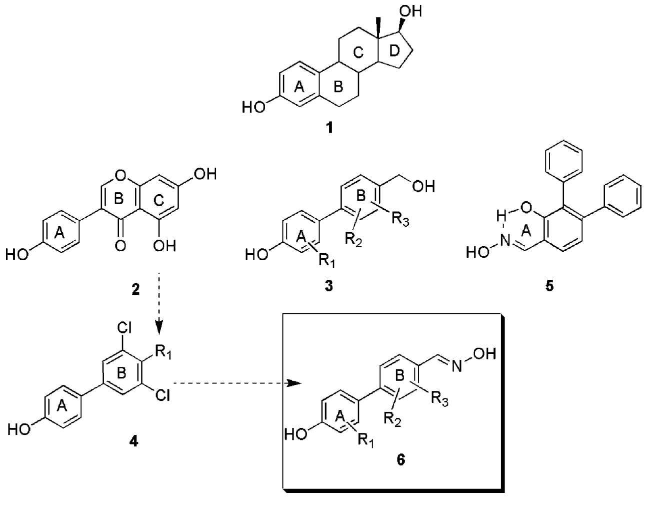


Figure 1. Compounds of interest.

Keywords: ERβ; Biphenyl; Biphenyl oximes; Oximes.

* Corresponding author. Tel.: +1-484-865-2910; fax: +1-484-865-9399; e-mail: mewshar@wyeth.com

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.03.028

selectivity may be due to the two conservative amino acid changes within the binding cavity of the two isoforms. The leucine present at position 384 (ERα) is replaced by a methionine in ERβ (i.e., Met336) while the methionine at position 421 (ERα) is replaced by an isoleucine in ERβ (i.e., Ile373).[7]

In our quest toward understanding the therapeutic role of ERβ, we sought to design selective ligands by incorporating the pharmacophoric groups of genistein (**2**) within a much simpler biaryl scaffold. Being aware that the phenolic group was acting as the A-ring mimic and therefore an essential pharmacophoric element for genistein's affinity, we decided to focus on simplifying the chromenone moiety and conserve the phenolic group. The biphenyl template was chosen as the most obvious starting point for further investigation. Biphenyl derivatives have previously been reported being exploited as surrogates of the steroid backbone by Lesuisse et al.[10] A series of 4-hydroxy-4′-hydroxymethyl-biphenyl derivatives (**3**) were prepared, which demonstrated excellent binding to ERα, whereby the hydroxymethyl group was used as a mimic of the 17β-OH group in the D-ring of estradiol. In our initial investigation toward identifying simple biphenyls as ERβ selective ligands (Part 1[11]), we discovered that 4-OH-biphenyl derivatives could achieve ERβ selectivity on the order of 20–70-fold by attaching substituents at the appropriate positions of the biphenyl framework. Data from our laboratories suggest that at least a 50-fold ERβ selectivity is needed to achieve our desired in vivo profile (unpublished results). The most interesting 4-OH-biphenyls of Part 1 had the 3,5-dichloro substitution pattern (i.e., **4**) and IC_{50} (ERβ) values were approximately 100 nM. In order to further improve ERβ affinity of biphenyls **4**, we wanted to append a hydrogen bond donating group that could potentially mimic the C-ring of genistein. The oxime moiety recently has been used as a bioisosteric replacement of the aromatic A-ring phenol of typical ER ligands with the synthesis of oxime **5**[12] and its analogs.[13] Depicted in Figure 2 is a simple 4′-OH-1,1′-biphenyl-4-carbaldehyde oxime (i.e., **6a**) superimposed with genistein (**2**), showing that the oxime moiety clearly overlays well with the C-ring hydroxyl group of **2**. As an extension of our previous findings, herein we report a novel series of 4′-OH-1,1′-biphenyl-4-carbaldehyde oxime derivatives (i.e., **6**) that exploit the oxime moiety for the first time as a potential means to improve ER affinity by mimicking the C-ring of genistein (**2**).

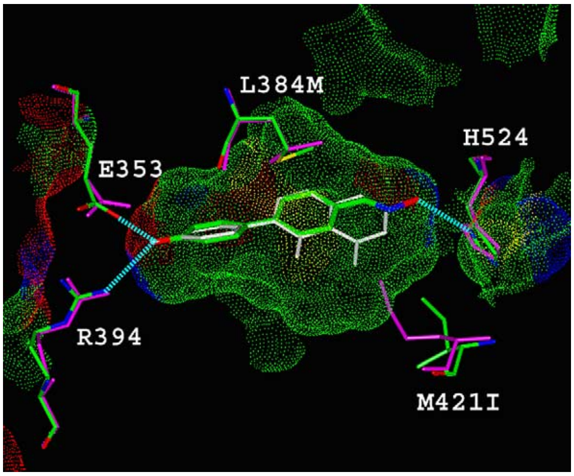
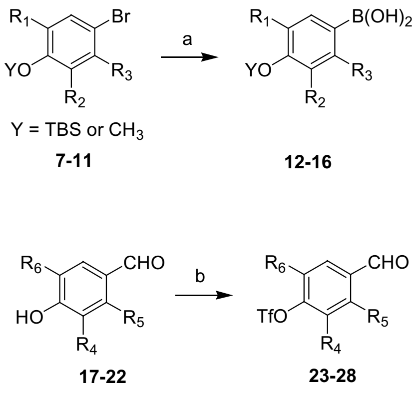


Figure 2. 4′-Hydroxy-biphenyl-4-carbaldehyde oxime (colored by atom type), docked to the ERβ/genistein pocket, and overlayed with ERα/diethylstilbestrol (magenta). Only key residues, including a Connolly surface of the ERβ binding site, are shown for simplicity. The residue numbering scheme for ERα has been used, also for simplicity. Hydrogen bond monitors are shown as turquoise dotted lines. Genistein is shown in white.

2. Chemistry

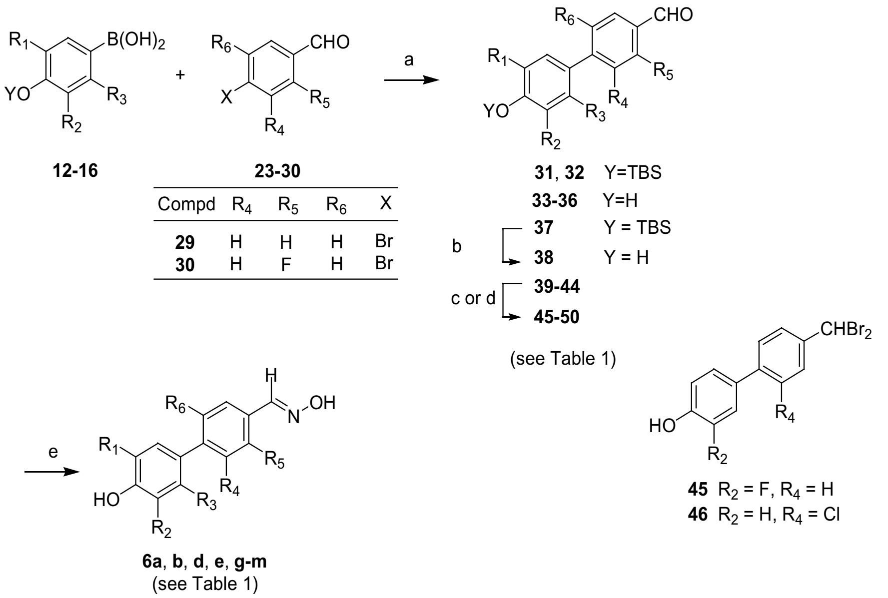
As depicted in Scheme 1, the methyl or TBS protected 4-bromo phenols **7**–**11** were treated with *n*-BuLi followed by triisopropylborate to afford phenyl boronic acids **12**–**15** (method A). Triflation of hydroxy benzaldehydes **17**–**22** provided compounds **23**–**28** (method B). Shown in Scheme 2 is the general synthetic strategy used to prepare compounds **6a**,**b**,**d**,**e**,**g**–**m**. Briefly, benzaldehydes **23**–**30** were coupled to a variety of phenyl boronic acids **12**–**16** using Suzuki coupling protocol[14] (method C) to afford the corresponding protected or deprotected



Compd	R_1	R_2	R_3	Y
12	H	H	H	TBS
13	H	F	H	Me
14	H	H	Me	Me
15	F	F	H	TBS
16	H	H	H	Me

Compd	R_4	R_5	R_6
23	H	Cl	H
24	F	H	H
25	Cl	H	H
26	H	OMe	H
27	Me	H	H
28	Cl	H	Cl

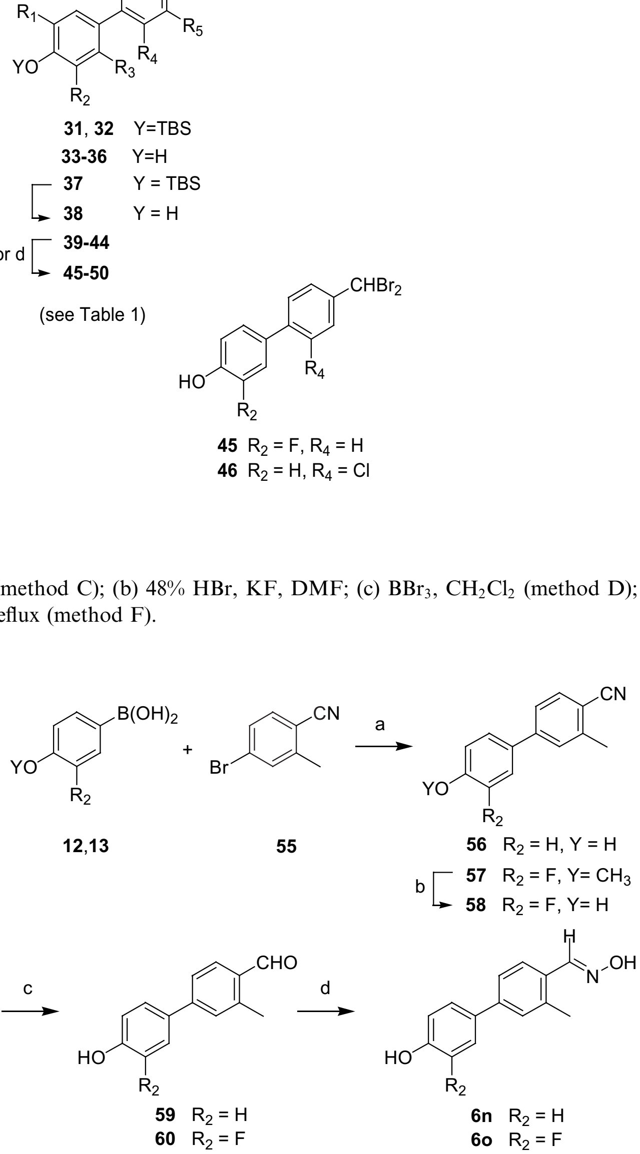
Scheme 1. Reagents and conditions: (a) (i) *n*-BuLi, THF, −78 °C (ii) $B(Oi\text{-}Pr)_3$ (iii) 1 N HCl, 0 °C (method A); (b) TfO_2, pyridine, CH_2Cl_2 (method B).



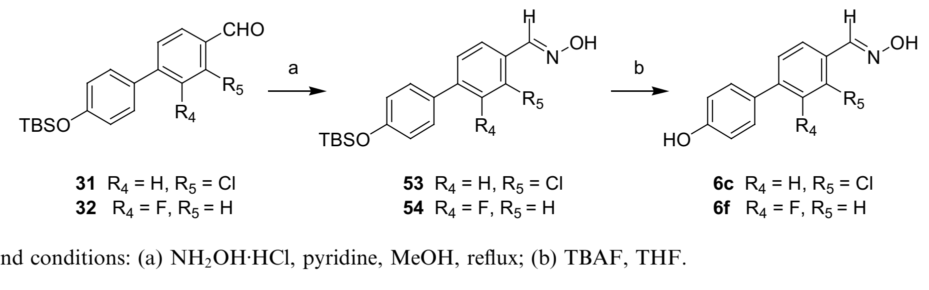
Compd	R_4	R_5	R_6	X
29	H	H	H	Br
30	H	F	H	Br

Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, DME, reflux (method C); (b) 48% HBr, KF, DMF; (c) BBr_3, CH_2Cl_2 (method D); (d) pyridine·HCl, ~195 °C (method E); (e) $NH_2OH·HCl$, pyridine, MeOH, reflux (method F).

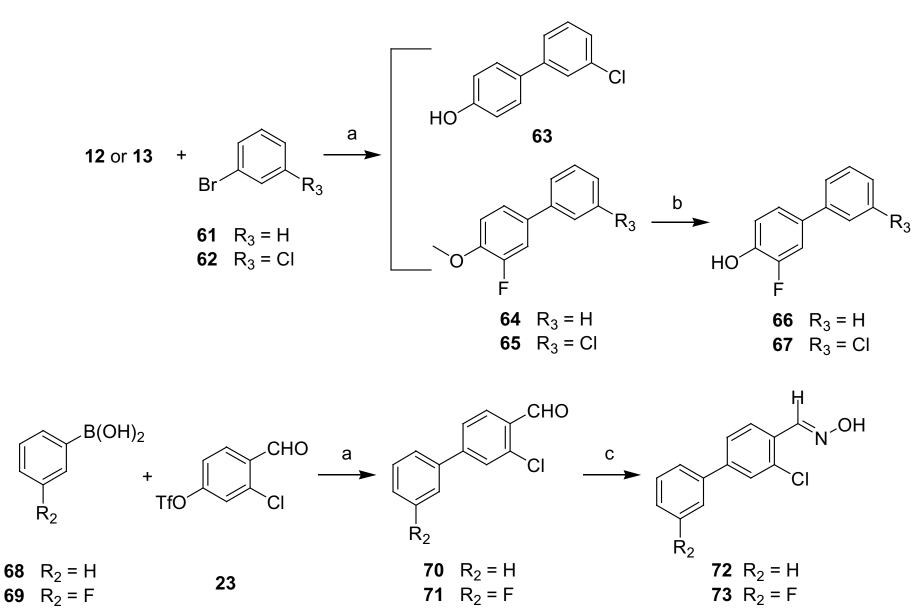
biphenyl carbaldehydes. The TBS protected derivatives were treated with 48% HBr/KF[15] while the methoxy derivatives were deprotected using BBr_3 (method D) or pyridinium hydrochloride (method E). In the case where aldehydes **39** and **40** were deprotected with BBr_3, the dibromomethyl derivatives **45** and **46** resulted but could still be used in the final oxime formation. Formation of oximes **6a**,**b**,**d**,**e**,**g–m** was accomplished by reacting compounds **33–36**, **38**, and **45–50** with hydroxyamine (method F). Treating aldehydes **31** and **32** with hydroxylamine, followed by deprotection with TBAF afforded oximes **6c**,**f** (Scheme 3). The synthesis of oximes **6n**,**o** could be carried out in three steps from commercially available 4-bromo-2-methylbenzonitrile **55** and boronic acids **12**, and **13** as depicted in Scheme 4. The synthesis of target molecules **63**, **66**, **67**, **72**, and **73** was carried out using previously described methods (Scheme 5). Compounds **87** and **88** are commercially available. Oxime **6p** was prepared in a straightforward fashion in five steps from commercially available **74** as depicted in Scheme 6. Dichloro derivative **77** was also used to prepare oxime **6q** via dibromide **83** (Scheme 7), along with an ester by-product (**86**). Aldehyde **84** was used to prepare the corresponding benzyl alcohol (**85**). Oximes **6r**,**s** were prepared from the corresponding known aldehydes using method F.[11] The (*E*)-geometry was assigned to all the oximes (i.e., **6a–s**, **72**, and **73**) based on the chemical shift values of the oxime protons, which ranged from δ 8.17 to 8.41.[16] An NOE study of **6p** also confirmed the (*E*)-geometry (Scheme 6).



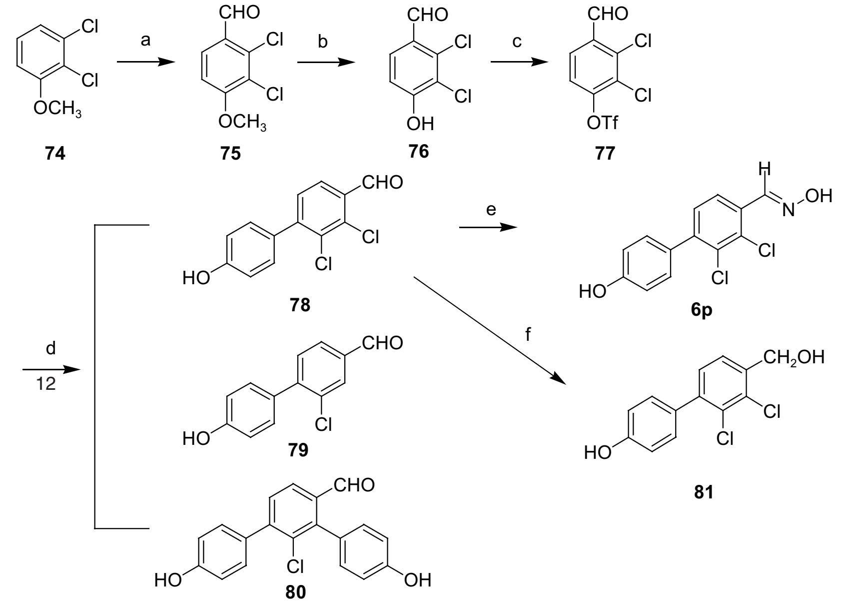
Scheme 4. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, DME, reflux; (b) pyridine·HCl, ~195 °C; (c) $(i\text{-Bu})_2AlH$, −78 °C to rt; (d) $NH_2OH·HCl$, pyridine, MeOH, reflux.



Scheme 3. Reagents and conditions: (a) $NH_2OH·HCl$, pyridine, MeOH, reflux; (b) TBAF, THF.



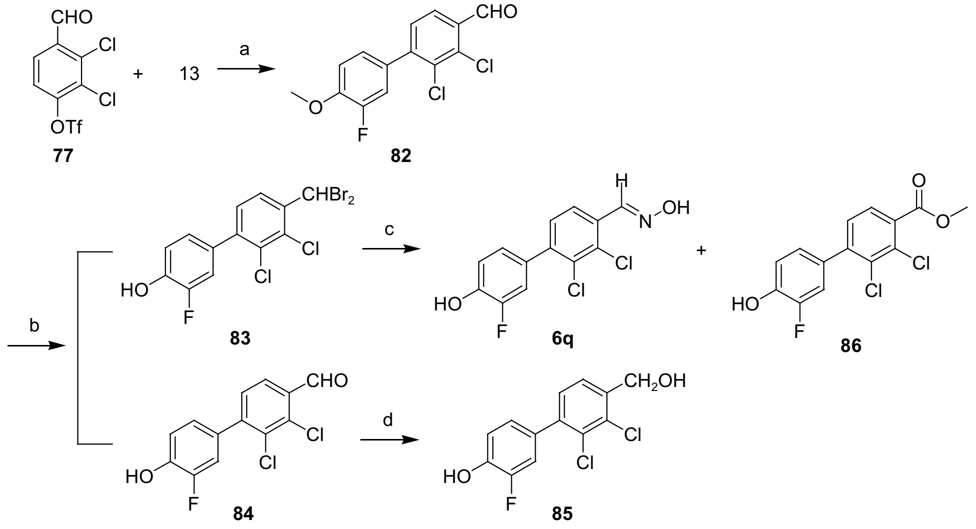
Scheme 5. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, DME, reflux; (b) pyridine·HCl, ~195 °C; (c) $NH_3OH·HCl$, pyridine, MeOH, reflux.



Scheme 6. Reagents and conditions: (a) $TiCl_4$, Cl_2CHOCH_3, CH_2Cl_2; (b) BBr_3, CH_2Cl_2; (c) TfO_2, pyridine, CH_2Cl_2; (d) $Pd(PPh_3)_4$, Na_2CO_3, DME, reflux; (e) $NH_2OH·HCl$, pyridine, MeOH, reflux; (f) $NaBH_4$, THF, MeOH.

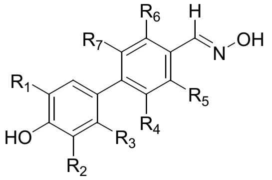
3. Results and discussion

The purpose of this investigation was to evolve from and improve potency of our recently reported ERβ selective 4-OH-biphenyls.[11] The ability to mimic the 'A-ring,' present in natural estrogens (e.g., **1**), seems to be an essential requirement for synthetic ER ligands to possess good binding. Early studies[17] suggested that the 3-OH group of the A-ring of **1** contributes an average of 1.9 kcal/mol to the binding free energy versus only 0.6 kcal/mol for the 17β-OH group. Though the strongest attractive polar interaction between ligand and the ER occurs through the formation of a hydrogen bond network that includes the 3-OH group of the A-ring, a bound water molecule and two amino acid residues of the ER ligand binding domain (Glu353 and Arg394),[4,18] compounds lacking a '17β-OH group' mimic are not completely optimized structures. Genistein (**2**) binds well to ERβ ($IC_{50} = 9.7$ nM, Table 1) and successfully mimicks estradiol by utilizing two phenolic groups within its framework. In order to be able to mimic some of the pharmacophoric elements of genistein, we simplified its structure to a 4-OH-biphenyl template in Part 1. In this study we decided to attach an oxime moiety to mimic the phenolic group in the C-ring of genistein (**2**) to further increase ER affinity with the ultimate goal of



Scheme 7. Reagents and conditions: (a) $Pd(PPh_3)_4$, Na_2CO_3, DME, reflux; (b) BBr_3, CH_2Cl_2; (c) $NH_2OH{\cdot}HCl$, pyridine, MeOH, reflux; (d) $NaBH_4$, THF, MeOH.

Table 1. 4′-Hydroxy-biphenyl-carbaldehyde oxime derivatives



Compound[a]	R_1	R_2	R_3	R_4	R_5	R_6	R_7	ERβ IC_{50} (nM)[b]	ERα IC_{50} (nM)[b]	Ratio (ERα/ERβ)
1								3.6 ± 1.6	3.20 ± 1.0	1
2								9.7 ± 4.3	395 ± 181	41
6a	H	H	H	H	H	H	H	3275 ± 799	>5000	>1
6b	H	F	H	H	H	H	H	660	>5000	8
6c	H	H	H	H	Cl	H	H	83 ± 38	2167 ± 1270	26
6d	H	H	H	Cl	H	H	H	98 ± 35	398 ± 98	4
6e	H	H	H	H	F	H	H	161 ± 16	1720 ± 85	11
6f	H	H	H	F	H	H	H	386 ± 298	3450 ± 2093	9
6g	H	H	H	H	OMe	H	H	882 ± 766	7990	9
6h	H	H	H	Cl	H	H	Cl	34 ± 1	156 ± 32	5
6i	H	F	H	H	Cl	H	H	54 ± 14	2613 ± 1219	49
6j	H	H	Me	H	Cl	H	H	35 ± 12	201 ± 38	6
6k	F	F	H	H	Cl	H	H	268 ± 52	>5000	>19
6l	H	F	H	Cl	H	H	Cl	29 ± 8	84 ± 33	3
6m	H	H	H	Me	H	H	H	607 ± 265	4523 ± 1806	7
6n	H	H	H	H	Me	H	H	163 ± 72	3193 ± 1572	20
6o	H	F	H	H	Me	H	H	122 ± 8	>5000	>43
6p	H	H	H	Cl	Cl	H	H	49 ± 11	851 ± 134	18
6q	H	F	H	Cl	Cl	H	H	81 ± 28	1957 ± 205	24
6r	H	H	H	H	Cl	Cl	H	8 ± 1	64 ± 18	8
6s	H	F	H	H	Cl	Cl	H	9 ± 2	270 ± 157	31

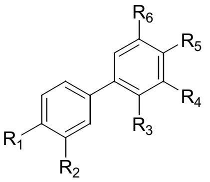
[a] Compounds **37**–**50** use the same substitution pattern as depicted above for **6a**–**s**.

[b] IC_{50} values are the means of at least two experiments ± STD (performed in triplicate, determined from eight concentrations). Values without STDs are for a single determination only.

identifying ERβ selective ligands. From our previous investigation, we learned that to attain affinity and selectivity from the 4-OH-biphenyl motif appropriate substituents on the B-ring's 3′, 4′, and 5′ positions (i.e., **4**, Fig. 1) were required. Having this information in hand, we performed a limited study to verify that the SAR from the simple 4-OH-biphenyls could translate over to the 4-OH′-1,1′-biphenyl-4-carbaldehyde oxime series. Once the crucial phenolic A-ring pharmacophoric group binds to ER, the oxime derivatives can extend much further toward and interact with ERβ His475. Therefore any change in orientation could lend itself to an inconsistent trend in SAR between the oxime and desoxime derivatives. However, in agreement to the SAR discovered in Part 1, the 3-position of the 4′-OH-1,1′-biphenyl template (R5 in Table 1) was again found to be the most crucial position to influence ERβ selectivity (i.e., **6c** vs **6d** and **6m** vs **6n**). Another consistent trend observed was that, in general, a slight improvement in ERβ selectivity was observed when the biphenyls utilized an *o*-fluoro phenolic group for the A-ring when compared to its desfluoro analogs (**6a** vs **6b**; **6c** vs **6i**; **6n** vs **6o**; **6p** vs **6q**; **6r** vs **6s**). Though the unsubstituted biphenyl oxime **6a** had poor affinity for the ERs, upon the addition of substituents, a dramatic increase in ERβ affinity was observed, a trend also noted in our previous report.[11] For example, attaching a chloro group to the 3-position of the parent unsubstituted oxime resulted in a 40-fold increase in affinity for ERβ (**6a** vs **6c**). The most potent oximes had IC_{50}'s ranging from 8 to 54 nM (i.e., **6h–j**,**l**,**p**,**r**,**s**). In fact, oxime **6s** was observed to have almost identical affinity and selectivity to genistein (**2**). Also in agreement with our earlier report is that two *ortho* fluorines attached to the phenolic A-ring had a detrimental effect on binding (**6i** vs **6k**). This is consistent with the fact that such a substitution pattern would force one of the fluorines to be in close proximity to ERα Glu353/ERβ Glu305, leading to electrostatic repulsion. Since it has recently be shown that the oxime moiety can act as a biosteric replacement for the phenolic 'A-ring' of estrogens,[12,13] these 4-OH-biphenyl oximes could potentially be exploiting multiple orientations. Shown in Table 2 are several compounds that were prepared in order to better understand the role the phenol and oxime groups was playing within this series of 4-OH-1,1′-biphenyl carboxaldehyde oximes. Upon removal of the oxime moiety, a 5–19-fold loss in affinity for the ERβ was observed (i.e., **6c** vs **63**; **6i** vs **67**; **6r** vs **89**; **6s** vs **90**). However, removal of the phenolic hydroxyl groups had a much more dramatic (53–157-fold) loss in ERβ affinity (**72** vs **6c**; **73** vs **6i**). This suggests that, although the oxime moiety was having an influencial role in binding to the ERs, the phenolic hydroxyl group was making a much higher contribution to the overall binding. The above SAR study suggests that the phenolic hydroxyl group of these 4-OH-1,1′-biphenyl carbaldehyde oximes tends to act as the A-ring and the oxime moiety as the C-ring of genistein. This is in contrast to the recent report where an oxime moiety was used as a bioisosteric replacement of the phenolic A ring as in compound **5** (Fig. 1), and demonstrates that the carbaldehyde oxime moiety can be used to mimic the A-ring of estradiol or the C-ring of genistein, depending on how the oxime and phenolic hydroxyl moieties are presented to the binding pocket.

A subtle divergence of SAR was observed between the 2,3-dichloro and the 3,5-dichloro derivatives. The 2,3-dichlorobenzyl alcohols had higher affinity than their corresponding aldehydes (i.e., **78** vs **81**; **84** vs **85**). This

Table 2. Substituted biphenyl derivatives of interest



Compound[a]	R_1	R_2	R_3	R_4	R_5	R_6	ERβ IC_{50} (nM)[b]	ERβ IC_{50} (nM)[b]	Ratio (ERα/ERβ)
6a	OH	H	H	H	CHNOH	H	3275 ± 799	>5000	1
6c	OH	H	H	Cl	CHNOH	H	83 ± 38	2167 ± 1270	26
6i	OH	F	H	Cl	CHNOH	H	54 ± 20	2613 ± 1219	49
6r	OH	H	H	Cl	CHNOH	Cl	8 ± 1	64 ± 18	8
6s	OH	F	H	Cl	CHNOH	Cl	9 ± 2	270 ± 157	31
63	OH	H	H	Cl	H	H	401 ± 305	7230 ± 2390	18
66	OH	F	H	H	H	H	3330 ± 340	27,000	8
67	OH	F	H	Cl	H	H	271 ± 42	6670 ± 156	25
72	H	H	H	Cl	CHNOH	H	4420	>30,000	>7
73	H	F	H	Cl	CHNOH	H	8500	>30,000	>3
78	OH	H	Cl	Cl	CHO	H	111 ± 45	3710 ± 1824	33
81	OH	H	Cl	Cl	CH_2OH	H	42 ± 28	1033 ± 675	24
84	OH	F	Cl	Cl	CHO	H	122 ± 7	2189 ± 847	18
85	OH	F	Cl	Cl	CH_2OH	H	22 ± 45	671 ± 216	33
87	OH	H	H	H	OH	H	2640 ± 737	41,500	16
88	OH	H	H	H	H	H	8415 ± 5070	78,000	9
89[b]	OH	H	H	Cl	H	Cl	83 ± 25	2013 ± 356	24
90[b]	OH	F	H	Cl	H	Cl	173 ± 89	2595 ± 346	15
91[b]	OH	H	H	Cl	CHO	Cl	69 ± 18	5007 ± 1729	72
92[b]	OH	F	H	Cl	CHO	Cl	89 ± 26	>5000	>56
93[b]	OH	H	H	Cl	CH_2OH	Cl	122 ± 35	2529 ± 950	21
94[b]	OH	F	H	Cl	CH_2OH	Cl	82 ± 3	3765 ± 191	46

[a] Compounds **37–50** use the same substitution pattern as depicted above for **6a–s**.

[b] Previously reported in Ref. 11.

was in contrast to the 3,5-dichloro analogs (i.e., **91** vs **93**; **92** vs **94**) in which the aldehydes and benzyl alcohols showed similar affinity. Interestingly, the dichloro benzyl alcohols appeared to be optimized with the 2,3-dichloro substitution pattern versus the 3,5-dichloro substitution pattern (**81** and **85** vs **93** and **94**, respectively). However, once attaching the oxime moiety the 3,5-dichloro substitution pattern (i.e., **6r** and **6s**) becomes favored over the 2,3-dichloro (i.e., **6p** and **6q**) and 2,6-dichloro (i.e., **6h** and **6l**) substitution patterns. This divergence in SAR suggests that the torsion angle between the phenyl groups as well as the conformation of the CH_2OH and the oxime moieties both act to influence the position of the hydrogen bond donating groups of the CH_2OH and the oxime moieties. Quantum chemical calculations[19] suggest that the minimum energy dihedral angle for the biphenyl changes from approximately 36° for the 3,5-dichloro compound **6r**, to 57° for the 2,3-dichloro compound **6p**.[20] In addition, the oxime moiety for both **6r** and **6p** tends to remain in the plane of the phenyl ring, whereas benzyl alcohols tend to have their alcohol moiety 90° from the plane of the phenyl ring.[21,22] Therefore, it is possible that a more co-planar arrangement in the biphenyl framework is more optimal when utilizing an oxime moiety as the hydrogen bond donating group than when a CH_2OH moiety is used. However, the most significant contribution to the divergence in SAR likely arises because the 2,3-dichloro substitution pattern actually tends to destabilize the oxime conformation that mimicks the genistein scaffold (see Fig. 2). This is because the oxime nitrogen is in a lower energy state when it adopts an *anti* configuration relative to the 3-Cl of **6p**, which acts to alleviate repulsion between the two moieties. Therefore, **6p** would pay an energetic penalty to adopt a conformation analogous to that of the docked conformation of **6r**, as the oxime moiety rotates to a *syn* configuration. This is not an issue when the oxime is replaced by CH_2OH, since benzyl alcohols tend to remain out of the plane of the phenyl ring and so *ortho* substituents would not be expected to greatly influence the conformation of the CH_2OH moiety.

To further discern the binding orientation and rationalize the ERβ selectivity of these novel 4-OH-biphenyl oximes, **6s** was docked into the ERβ pocket. 3-D coordinates for the protein were obtained from an in-house X-ray structure of ERβ complexed with genistein (see Method Section). As shown in Figure 3, the phenol moiety of oxime **6s** is able to mimic the A-ring of genistein, and the oxime moiety (*E*-isomer) is able to hydrogen bond to the histidine residue (ERα His524/ERβ His475) and mimic the C-ring of genistein. As discussed earlier, only two residues are different within the ligand binding pocket. ERα Leu384 corresponds to ERβ Met336 and ERα Met421 corresponds to ERβ Ile373. The 3′-Cl group is clearly in close proximity to Met421/Ile373 residues. In particular, it appears likely that the ∼3 Å distance between the 3′-Cl and the sulfur of ERα Met421 represents a repulsive interaction, which may be mimicking the behavior of the genistein 5′-OH group, and limiting the conformational space explored by the methionine side chain. In contrast, the 3′-Cl is about 4.1 Å from ERβ Ile373, which is not expected to result in an unfavorable interaction.

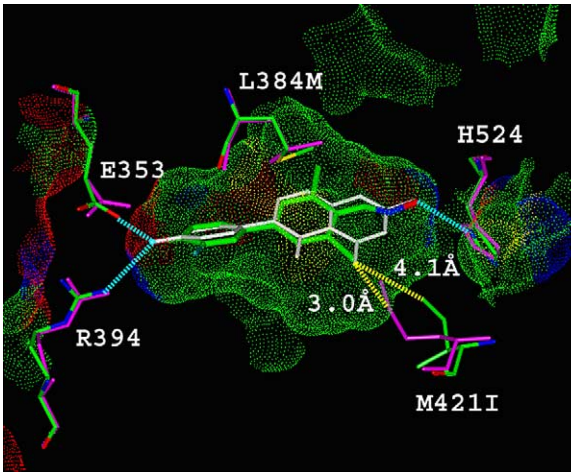


Figure 3. Compound **6s** (colored by atom type), docked to the ERβ/genistein pocket, and overlayed with ERα/diethylstilbestrol (magenta). As in Figure 2, only key residues and a Connolly surface of the ERβ binding site are shown, for simplicity, and the residue numbering scheme for ERα has been used. Hydrogen bond monitors are shown as turquoise dotted lines. Genistein is shown in white. Distance monitors (yellow) show that the 3′-Cl is in close proximity to M421I.

In order to determine the activity of compounds in a cell-based transcriptional assay, regulation of human keratin19 (KRT19) mRNA was measured. This gene is upregulated by estradiol in human prostate cancer cells (LNCaPLN3) engineered to express either ERβ or ERα and thus can be used to determine whether compounds have agonist or antagonist activity. Estradiol regulates KRT19 mRNA via ERβ and ERα with approximately the same potency ($EC_{50} = 0.1$–0.4 nM; data not shown). To measure agonist activity, compounds were tested at 1 μM. To measure antagonist activity, 1 μM of test compound was co-administered with 10 nM 17β-estradiol. As shown in Table 3 (mean of two experiments), the compounds all exhibited some degree of agonist activity via ERβ and ERα. Oxime **6s** was the most selective compound as its efficacy equaled 17β-estradiol via ERβ, but was <25% with respect to 17β-estradiol when cells expressed ERα. None of the compounds had marked antagonist activity.

Table 3. Compound regulation of KRT19 mRNA in LNCaPLN3 cells expressing either ERα or ERβ

Compound	ERα		ERβ	
	Agonist mode[a]	Antagonist mode[b]	Agonist mode[a]	Antagonist mode[b]
6i	8	105	32	110
6s	21	105	100	111
85	8	114	16	98
92	6	87	20	82
94	11	86	13	88

[a] Percent activity of 17β-estradiol when compound tested alone.

[b] Percent activity of 17β-estradiol when compound tested in combination with 17β-estradiol.

4. Conclusion

In this report we have exploited the oxime moiety as a phenolic bioisostere for the C-ring of genistein. Attachment of a second hydrogen bond donating group as an oxime moiety to the 4-position of the 4-OH-biphenyl template led to a significant increase in ERβ affinity. Oxime **6s** had almost identical affinity and selectivity to genistein (**2**). SAR and modeling studies suggested that the phenol of **6s** was acting as the 'A-ring' and the oxime moiety was acting as the 'C-ring' of genistein. The 3-position of the 4′-OH-1,1′-biphenyl carbaldehyde oxime template was shown to influence ERβ selectivity. A similar trend in SAR was observed with these oxime derivatives as their 4-OH-biphenyl desoxime prototypes. Modeling studies revealed a similar binding orientation of the previously reported 4-OH-biphenyls to their corresponding oxime analogs in this study. A select group of compounds were tested to determine their agonist/antagonist activity in a cell-based transcriptional assay and observed to have some degree of agonist activity via ERβ and ERα with no significant antagonist activity. Studies are ongoing in our laboratories to further exploit the oxime moiety within novel estrogenic ligands.

5. Experimental

5.1. General

Melting points were measured on a Mel-Temp II (Laboratory Device Inc., USA) melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX300, Varian INOVA 400, or Varian INOVA 500 instrument. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane in $CDCl_3$, DMSO-d_6, or acetone-d_6. Infrared (IR) spectra were recorded on a Mattson Galaxy 3020 Infrared Spectrophotometer and are reported in reciprocal centimeters (cm^{-1}). Electrospray (ESI) mass spectra were recorded using a Hewlett–Packard 5989B MS engine or Waters Alliance-ZMD mass spectrometer. Electron impact ionization (EI, EE = 70 eV) mass spectra were recorded on a Finnigan Trace mass spectrometer. Elemental analyzes were carried out on a modified Perkin–Elmer model 2400 series II CHN analyzer or sent to Robertson Microlit. Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel, 60 F-254), and spots were visualized with UV light and stained either in an iodine or with an alcohol solution of phosphomolybdic acid. HPLC purification was performed on a preparative Gilson HPLC system using CombiPrep Pro C18 column with acetonitrile (0.1% TFA) and water (0.1% TFA) as solvents at a flow rate of 20 mL/min. Solvents and reagents were used as purchased.

5.1.1. *tert*-Butyl(4-bromo-2,6-difluoro-phenyloxy)dimethylsilane (10). To a solution of 4-bromo-2,6-difluoro-phenol (10.54 g, 50.4 mmol) and TBDMS-Cl (12.65 g, 80.6 mmol) in DMF (100 mL) was added imidazole (9.26 g, 136 mmol). The solution was stirred overnight, poured into 300 mL water, and extracted with EtOAc (3 ×). The combined organic layers were washed with water, dried over anhydrous magnesium sulfate, filtered, and the solvent evaporated to yield 14.61 g (90%) of a colorless oil: ^{1}H NMR ($CDCl_3$): δ 0.17 (6H, s), 0.99 (9H, s), 7.02 (H, d, $J = 7.13$ Hz).

5.2. Method A

5.2.1. 4-*tert*-Butyl-dimethylsilyoxyphenylboronic acid (12). To a solution of (4-bromo-phenoxy)-*tert*-butyl-dimethylsilane (**7**) (20 mL, 23.48 g, 82 mmol) in THF (200 mL) at −78 °C was slowly added *n*-butyl lithium (39.2 mL of 2.5 M solution in hexane, 98 mmol) over 10 min. The solution was stirred for 1 h and triisopropyl borate (66.2 mL, 54.0 g, 0.29 mol) was added by syringe at −78 °C. The solution was stirred for 1 h at −78 °C and then allowed to warm to room temperature overnight. The reaction was cooled to 0 °C followed by the sequential addition of water (20 mL) and 2 N HCl (20 mL). After 5 min an additional 2 N HCl (360 mL) was added and stirred for 10 min. The mixture was extracted with EtOAc (3 × 250 mL) and the combined organic layers were concentrated to a thick oil (50 mL). Crystallization with cold hexane yielded 14.5 g (70%) of the title compound as a white solid: ^{1}H NMR (DMSO-d_6): δ 0.19 (6H, s), 0.94 (9H, s), 6.80 (2H, d, $J = 8.4$ Hz), 7.69 (2H, d, $J = 8.36$ Hz), 7.87 (2H, s).

Compounds **13–15** were prepared in a similar manner using method A.

5.2.2. 3-Fluoro-4-methoxyphenylboronic acid (13). The title compound was prepared by reacting 4-bromo-2-fluoroanisole (**8**) (10.0 g, 49.0 mmol) with *n*-butyl lithium (23.4 mL of 2.5 M solution in hexane, 59 mmol) followed by triisopropyl borate (45.2 mL, 36.9 g, 196 mmol) according to method A to yield 7.1 g (85.2%) of a white solid: ^{1}H NMR (DMSO-d_6): δ 3.84 (3H, s), 7.13 (1H, t, $J = 8.40$ Hz), 7.54 (1H, dd, $J = 12.59$ Hz, $J = 1.41$ Hz), 7.57 (1H, d, $J = 8.43$ Hz), 8.03 (2H, br s); MS (ESI) m/z 169 $(M-H)^-$.

5.2.3. 4-Methoxy-2-methylphenylboronic acid (14). The title compound was prepared by reacting 4-bromo-3-methylanisole (**9**) (10.0 g, 50.0 mmol) with *n*-butyl lithium (24 mL of 2.5 M solution in hexane, 55 mmol) followed by triisopropyl borate (57.7 mL, 47.0 g, 250 mmol) according to method A to yield 5.7 g (69%) of a white solid: MS (ESI) m/z 313 $(2M-H_2O-H)^-$.

5.2.4. 3,5-Difluoro-4-*tert*-butyldimethylsilyoxyboronic acid (15). The title compound was prepared by reacting **10** (12.98 g, 40.19 mmol) with *n*-butyl lithium (27.6 mL of 1.6 N solution, 44.2 mmol) followed by triisopropyl borate (37.8 g, 200 mmol) according to method A to yield 4.38 g (38%) of a white solid: ^{1}H NMR

(DMSO-d_6): δ 0.25 (6H, s), 1.06 (9H, s), 7.54 (2H, d, $J = 7.93$ Hz); MS (ESI) m/z 287 $(M-H)^-$.

5.3. Method B

5.3.1. Trifluoro-methanesulfonic acid 3-chloro-4-formyl-phenyl ester (23). To a solution of 2-chloro-4-hydroxy benzaldehyde (**17**) (1.31 g, 8.4 mmol) and pyridine (1.1 mL, 13.4 mmol) in 80 mL of dichloromethane at 0 °C was added trifluoromethanesulfonic anhydride (1.84 mL, 3.08 g, 10.9 mmol). The solution was allowed to warm slowly to room temperature and stirred for 2.5 h. The solution was cooled to 0 °C and stirred with ice water to decompose any excess anhydride. The mixture was made slightly basic by the addition of saturated sodium bicarbonate solution. The resulting layers were separated and the aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered, and the solvent removed under vacuum to afford an orange oil, which was purified by silica chromatography (5% EtOAc–hexane) to yield 1.83 g (76%) of the title compound as a clear, colorless oil: ^{1}H NMR (DMSO-d_6): δ 7.73 (1H, dd, $J = 2.35$ Hz, $J = 8.64$ Hz), 8.04–8.07 (2H, m), 10.30 (1H, s).

5.3.2. Trifluoro-methanesulfonic acid 2-fluoro-4-formyl-phenyl ester (24). The title compound was prepared by reacting 3-fluoro-4-hydroxybenzaldehyde (**18**) (1.2 g, 8.56 mmol) with trifluoromethanesulfonic anhydride (1.87 mL, 3.14 g, 11.1 mmol) according to method B to yield a yellow oil, which was used directly in the next step without purification: ^{1}H NMR (CDCl$_3$): δ 7.53–7.58 (1H, m), 7.77–7.85 (2H, m), 10.01 (1H, d, $J = 1.45$ Hz).

5.3.3. Trifluoro-methanesulfonic acid 2-chloro-4-formyl-phenyl ester (25). The title compound was prepared by reacting 3-chloro-4-hydroxybenzaldehyde (**19**) (5 g, 31.9 mmol) with trifluoromethanesulfonic anhydride (7.0 mL, 11.7 g, 41.5 mmol) according to method B to yield 9.15 g (100%) of a yellow oil, which was used directly in the next step without purification: ^{1}H NMR (DMSO-d_6): δ 7.92 (1H, d, $J = 8.48$ Hz), 8.07 (1H, dd, $J = 8.51$ Hz, $J = 1.93$ Hz), 8.30 (1H, d, $J = 1.92$ Hz), 10.04 (1H, s).

5.3.4. Trifluoro-methanesulfonic acid 4-formyl-3-methoxy-phenyl ester (26). The title compound was prepared by reacting 4-hydroxy-2-methoxybenzaldehyde (**20**) (3 g, 19.7 mmol) with trifluoromethanesulfonic anhydride (4.3 mL, 7.2 g, 25.6 mmol) according to method B to yield a brown syrup, which was used in the next step without purification: ^{1}H NMR (DMSO-d_6): δ 3.97 (3H, s), 7.21 (1H, dd, $J = 8.64$ Hz, $J = 2.17$ Hz), 7.47 (1H, d, $J = 2.24$ Hz), 7.87 (1H, d, $J = 8.64$ Hz), 10.31 (1H, s).

5.3.5. Trifluoro-methanesulfonic acid 4-formyl-2-methyl-phenyl ester (27). The title compound was prepared by reacting 4-hydroxy-3-methylbenzaldehyde (**21**) (2.5 g, 18.4 mmol) with trifluoromethanesulfonic anhydride (4.0 mL, 6.75 g, 23.9 mmol) according to method B to yield a brown oil, which was used directly in the next step without purification.

5.3.6. Trifluoro-methanesulfonic acid 2,6-dichloro-4-formyl-phenyl ester (28). The title compound was prepared by reacting 3,5-dichloro-4-hydroxybenzaldehyde (**22**) (1.08 g, 5.65 mmol) with trifluoromethanesulfonic anhydride (3.2 g, 11.3 mmol) according to method B to yield 1.47 g (80%) of a clear yellow oil: ^{1}H NMR (CDCl$_3$): δ 7.96 (2H, s), 9.96 (1H, s); MS (EI) m/z 322 $(M)^+$. Anal. Calcd for $C_8H_3Cl_2F_3O_4S$: C: 29.74, H: 0.94. Found: C: 29.55, H: 1.08.

5.4. Method C

5.4.1. 4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-1,1′-biphenyl-4-carbaldehyde (37). A mixture of 4-bromobenzaldehyde (**29**) (0.73 g, 3.97 mmol), **12** (1.3 g, 5.16 mmol), sodium carbonate (6.0 mL of 2 M aqueous solution, 11.9 mmol), tetrakis(triphenylphosphine)-palladium (0.23 g, 0.20 mmol), and ethylene glycol dimethyl ether (25 mL) was heated to reflux for 3.5 h. The mixture was cooled to room temperature and poured into water, then extracted with EtOAc (3 ×), washed with brine, dried over anhydrous sodium sulfate, filtered, and the solvent evaporated. Purification by silica chromatography (5% EtOAc–hexane) yielded 0.60 g (48%) of the title compound as a white solid: ^{1}H NMR (DMSO-d_6): δ 0.24 (6H, s), 1.01 (9H, s), 6.94 (2H, d, $J = 8.54$ Hz), 7.53 (2H, d, $J = 8.56$ Hz), 7.72 (2H, d, $J = 8.19$ Hz), 7.93 (2H, d, $J = 8.20$ Hz), 10.04 (1H, s).

5.4.2. 3′-Fluoro-4′-methoxy-1,1′-biphenyl-4-carbaldehyde (39). The title compound was prepared by reacting 4-bromobenzaldehyde (**29**) (2.17 g, 11.7 mmol) with **13** (2.99 g, 17.6 mmol) according to method C to yield 2.35 g (88%) of a white solid: mp 84–85 °C; ^{1}H NMR (CDCl$_3$): δ 3.95 (3H, s), 7.06 (1H, app.t, $J = 8.71$ Hz), 7.37–7.41 (2H, m), 7.68–7.71 (2H, m), 7.92–7.95 (2H, m), 10.05 (1H, s); MS (EI) m/z 230.3 $(M)^+$. Anal. Calcd for $C_{14}H_{11}FO_2$: C: 73.03, H: 4.82. Found: C: 72.75, H: 4.79.

5.4.3. 4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-3-chloro-1,1′-biphenyl-4-carbaldehyde (31). The title compound was prepared by reacting **23** (1.78 g, 6.18 mmol) with **12** (2.03 g, 8.03 mmol) according to method C to yield 0.92 g (43%) of a white solid: mp 38–39 °C; ^{1}H NMR (DMDO-d_6): δ 0.23 (6H, s), 0.97 (9H, s), 6.98 (2H, d, $J = 8.79$ Hz), 7.74 (2H, d, $J = 8.79$ Hz), 7.81 (1H, d, $J = 7.81$ Hz), 7.89 (1H, d, $J = 1.95$ Hz), 7.91 (1H, d, $J = 7.81$ Hz), 10.34 (1H, s); MS (ESI) m/z 231/233 $(M-H)^-$ (deprotected product ion). Anal. Calcd for

$C_{19}H_{23}ClO_2Si$: C: 65.78, H: 6.68. Found: C: 65.59, H: 6.60.

5.4.4. 2-Chloro-4′-methoxy-1,1′-biphenyl-4-carbaldehyde (40). The title compound was prepared by reacting **25** (5.0 g, 17.4 mmol) with 4-methoxyphenylboronic acid (**16**) (3.44 g, 22.6 mmol) according to method C to yield 3.83 g (89%) of a white solid: mp 85–87 °C; ^{1}H NMR (DMSO-d_6): δ 3.82 (3H, s), 7.07 (2H, d, $J = 8.83$ Hz), 7.46 (2H, d, $J = 8.45$ Hz), 7.63 (1H, d, $J = 7.87$ Hz), 7.92 (1H, dd, $J = 7.87$ Hz, $J = 1.56$ Hz), 8.07 (1H, d, $J = 1.49$ Hz); ^{13}C NMR (DMSO-d_6): δ 55.14, 113.72, 127.80, 129.72, 130.42, 130.82, 132.15, 132.21, 136.11, 144.90, 159.33, 191.73; IR 1692 cm^{-1} ; MS (EI) *m*/*z* 246/248 $(M)^+$. Anal. Calcd for $C_{14}H_{11}ClO_2$: C: 68.16, H: 4.49. Found: C: 67.76, H: 4.36.

5.4.5. 3-Fluoro-4′-methoxy-1,1′-biphenyl-4-carbaldehyde (41). The title compound was prepared by reacting **30** (3.0 g, 14.8 mmol) with **16** (2.70 g, 17.8 mmol) according to method C to yield 3.2 g (94%) of a white solid: mp 85–86 °C; ^{1}H NMR (DMSO-d_6): δ 3.83 (3H, s), 7.06–7.09 (2H, m), 7.70–7.75 (2H, m), 7.79–7.82 (2H, m), 7.88 (1H, t, $J = 7.96$ Hz), 10.22 (1H, s); IR 1681 cm^{-1}; MS (ESI) *m*/*z* 231 $(M+H)^+$. Anal. Calcd for $C_{14}H_{11}FO_2$: C: 73.03, H: 4.82. Found: C: 72.99, H: 4.73.

Compound **24** (2.1 g, 7.72 mmol) was reacted with **12** (2.14 g, 8.49 mmol) according to method C to produce the following two compounds:

5.4.6. 4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-fluoro-1,1′-biphenyl-4-carbaldehyde (32). A waxy yellowish solid (1.62 g, 63%): ^{1}H NMR (acetone-d_6): δ 0.28 (6H, s), 1.02 (9H, s), 7.02–7.06 (2H, m), 7.57–7.60 (2H, m), 7.71–7.78 (2H, m), 7.85 (1H, dd, $J = 7.91$ Hz, $J = 1.51$ Hz), 10.07 (1H, d, $J = 1.73$ Hz); ^{13}C NMR (acetone-d_6): δ 4.29, 18.80, 26.00, 116.82 (d, $J = 23.90$ Hz), 121.15, 126.69 (d, $J = 3.25$ Hz), 128.42 (d, $J = 1.21$ Hz), 131.30 (d, $J = 3.48$ Hz), 132.20 (d, $J = 3.44$ Hz), 135.27 (d, $J = 13.61$ Hz), 138.09 (d, $J = 6.64$ Hz), 157.23, 160.68 (d, $J = 248.56$ Hz), 191.51; IR 1692 cm^{-1}; MS (ESI) *m*/*z* 331 $(M+H)^+$, 372 $(M+H+ACN)^+$. Anal. Calcd for $C_{19}H_{23}FO_2Si$: C: 69.06, H: 7.02. Found: C: 69.71, H: 7.34.

5.4.7. 2-Fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde. A yellowish solid (0.32 g, 19%): mp 149–150 °C; ^{1}H NMR (acetone-d_6): δ 7.08–7.13 (2H, m), 7.62–7.67 (2H, m), 7.80–7.91 (2H, m), 7.94 (1H, dd, $J = 7.92$ Hz, $J = 1.58$ Hz), 8.93 (1H, s), 10.16 (1H, d, $J = 1.73$ Hz); IR 1669 cm^{-1}; MS (ESI) *m*/*z* 215 $(M-H)^{-1}$. Anal. Calcd for $C_{13}H_{19}FO_2\cdot 0.14H_2O$: C: 71.39, H: 4.28. Found: C: 71.38, H: 4.12.

5.4.8. 4′-Hydroxy-3-methoxy-1,1′-biphenyl-4-carbaldehyde (33). The title compound was prepared by reacting **26** (11.0 mmol) with **12** (3.50 g, 13.86 mmol) according to method C to yield 0.88 g (35%) of a yellowish solid: mp 159–161 °C; ^{1}H NMR (DMDO-d_6): δ 4.01 (3H, s), 6.89 (2H, d, $J = 8.62$ Hz), 7.31 (1H, d, $J = 8.16$ Hz), 7.36 (1H, d, $J = 1.15$ Hz), 7.66 (2H, d, $J = 8.63$ Hz), 7.72 (1H, d, $J = 8.09$ Hz), 9.80 (1H, s), 10.34 (1H, s); mp 159–161 °C; IR 1660 cm^{-1}; MS (ESI) *m*/*z* 227 $(M-H)^-$, 229 $(M+H)^+$. Anal. Calcd for $C_{14}H_{12}O_3$: C: 73.67, H: 5.30. Found: C: 73.44, H: 4.99.

5.4.9. 2,6-Dichloro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde (34). The title compound was prepared by reacting **28** (0.66 g, 2.04 mmol) with **12** (0.78 g, 3.10 mmol) according to method C to yield 0.33 g (61%) of white crystals: mp 140–141 °C; ^{1}H NMR (DMSO-d_6): δ 6.88 (2H, d, $J = 8.56$ Hz), 7.11 (2H, d, $J = 8.54$ Hz), 8.05 (2H, s), 9.78 (1H, br s), 10.00 (1H, s); MS (ESI) *m*/*z* 265/267/269 $(M-H)^-$. Anal. Calcd for $C_{13}H_8Cl_2O_2$: C: 58.46, H: 3.02. Found: C: 58.42, H:2.91.

5.4.10. 3-Chloro-3′-fluoro-4′-methoxy-1,1′-biphenyl-4-carbaldehyde (42). The title compound was prepared by reacting **23** (2.8 g, 9.62 mmol) with **13** (1.8 g, 10.6 mmol) according to method C to yield 1.87 g (74%) of a white solid: mp 116–118 °C; ^{1}H NMR (DMSO-d_6): δ 3.91 (3H, s), 7.30 (1H, t, $J = 8.79$ Hz), 7.66–7.68 (1H, m), 7.79 (1H, dd, $J = 12.91$ Hz, $J = 2.47$ Hz), 7.85–7.87 (1H, m), 7.91 (1H, d, $J = 8.24$ Hz), 7.96 (1H, d, $J = 1.65$ Hz), 10.34 (1H, s); IR 1688 cm^{-1}; MS (ESI) *m*/*z* 265/267 $(M+H)^+$. Anal. Calcd for $C_{14}H_{10}ClFO_2$: C: 63.53, H: 3.81. Found: C: 63.29, H: 3.63.

Compound **23** (3.78 g, 13.12 mmol) was reacted with **14** (2.61 g, 15.7 mmol) according to method C to produce the following two compounds:

5.4.11. 3-Chloro-4′-methoxy-2′-methyl-1,1′-biphenyl-4-carbaldehyde (43). A white solid (2.18 g, 64%): mp 77–79 °C; ^{1}H NMR (DMSO-d_6): δ 2.26 (3H, s), 3.79 (3H, s), 6.88 (1H, dd, $J = 8.41$ Hz, $J = 2.66$ Hz), 6.93 (1H, d, $J = 2.50$ Hz), 7.23 (1H, $J = 8.40$ Hz), 7.50 (1H, dd, $J = 7.93$ Hz, $J = 1.32$ Hz), 7.58 (1H, d, $J = 1.53$ Hz), 7.91 (1H, d, $J = 7.57$ Hz), 10.36 (1H, s); IR 1682 cm^{-1}; MS (ESI) *m*/*z* 261/263 $(M+H)^+$. Anal. Calcd for $C_{15}H_{13}ClO_2$: C: 69.10, H: 5.03. Found: C: 69.13, H: 4.73.

5.4.12. 4,4″-Dimethoxy-2,2″-dimethyl-1,1′,3′,1″-terphenyl-4′-carbaldehyde. A colorless syrup (0.56 g, 12%). ^{1}H NMR (DMSO-d_6): δ 2.10 (3H, s), 2.29 (3H, s), 3.78 (3H, s), 3.80 (3H, s), 6.85–6.88 (2H, m), 6.91 (1H, d, $J = 2.38$ Hz), 6.95 (1H, d, $J = 2.38$ Hz), 7.18 (1H, d, $J = 8.32$ Hz), 7.24–7.25 (2H, m), 7.52 (1H, dd, $J = 7.74$ Hz, $J = 1.78$ Hz), 7.95 (1H, d, $J = 8.33$ Hz), 9.68 (1H, s); IR 1679 cm^{-1} ; MS (ESI) *m*/*z* 347 $(M+H)^+$. Anal. Calcd for $C_{23}H_{22}O_3\cdot 0.05H_2O$: C: 79.54, H: 6.41. Found: C: 79.25, H: 6.05.

5.4.13. 3-Chloro-3′,5′-difluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde (35). The title compound was prepared by reacting **23** (1 g, 3.5 mmol) with **15** (1.1 g, 3.8 mmol) according to method C to yield 0.56 g (60%) of a yellow solid: mp 233–235 °C; ^{1}H NMR (DMSO-d_6): δ 7.64 (2H, d, $J = 7.85$ Hz), 7.85–7.91 (2H, m), 7.98 (1H, s), 10.33 (1H, s), 10.70 (1H, br s); IR 1665 cm^{-1}; MS (ESI) m/z 267/269 $(M-H)^-$. Anal. Calcd for $C_{13}H_7ClF_2O_2$: C: 58.12, H: 2.63. Found: C: 57.79, H: 2.72.

5.4.14. 2,6-Dichloro-3′-fluoro-4′-methoxy-1,1′-biphenyl-4-carbaldehyde (44). The title compound was prepared by reacting **28** (0.69 g, 2.14 mmol) with **13** (0.55 g, 3.20 mmol) according to method C to yield 0.33 g (52%) of a clear oil: NMR (DMSO-d_6): δ 3.91 (3H, s), 7.08–7.12 (1H, m), 7.27 (1H, dd, $J = 12.08$ Hz, $J = 2.05$ Hz), 7.31 (1H, t, $J = 8.72$ Hz), 8.08 (2H, s), 10.01 (1H, s). Anal. Calcd for $C_{14}H_{19}Cl_2FO_2$: C: 56.21, H: 3.03. Found: C: 57.31, H: 3.18.

5.4.15. 4′-Hydroxy-2-methyl-1,1′-biphenyl-4-carbaldehyde (36). The title compound was prepared by reacting **27** (9.3 mmol) with **12** (2.35 g, 9.3 mmol) according to method C to yield 1.12 g (57%, over two steps) of a yellowish solid: mp 94–96 °C; ^{1}H NMR (DMSO-d_6): δ 2.33 (3H, s), 6.86 (2H, d, $J = 8.55$ Hz), 7.22 (2H, d, $J = 8.51$ Hz), 7.39 (1H, d, $J = 7.81$ Hz), 7.61 (1H, d, $J = 7.82$ Hz), 7.81 (1H, s), 9.65 (1H, s), 10.00 (1H, s); IR 1670 cm^{-1}; MS (ESI) m/z 211 $(M-H)^-$, 213 $(M+H)^+$. Anal. Calcd for $C_{14}H_{12}O_2{\cdot}0.25H_2O$: C: 77.58, H: 5.81. Found: C: 77.49 H: 5.67.

5.4.16. 4′-Hydroxy-3-methyl[1,1′-biphenyl]-4-carbonitrile (56). The title compound was prepared by reacting 4-bromo-2-methylbenzonitrile **55** (1.8 g, 9.18 mmol) with **12** (3.0 g, 11.9 mmol) according to method C to yield 1.81 g (94% over two steps) of a yellowish solid: mp 177–178 °C; ^{1}H NMR (DMSO-d_6): δ 2.52 (3H, s), 6.88 (2H, d, $J = 8.50$ Hz), 7.60 (3H, d, $J = 8.49$ Hz), 7.71 (1H, s), 7.77 (2H, d, $J = 8.12$ Hz), 9.79 (1H, s); IR 2220 cm^{-1} ; MS (ESI) m/z 208 $(M-H)^-$. Anal. Calcd for $C_{14}H_{11}NO$: C: 80.36, H: 5.30, N: 6.69. Found: C: 79.91, H: 5.27, N: 6.57.

5.4.17. 3′-Fluoro-4′-methoxy-3-methyl-1,1′-biphenyl-4-carbonitrile (57). The title compound was prepared by reacting **55**(2.16 g, 11.0 mmol) with **13** (2.81 g, 16.5 mmol) according to method C to yield 2.08 g (79%) of a white solid: mp 103–105 °C; ^{1}H NMR ($CDCl_3$): δ 2.60 (3H, s), 3.94 (3H, s), 7.05 (1H, app.t, $J_{HF} = J_{HH} = 8.72$ Hz), 7.30–7.35 (2H, m), 7.42 (1H, ddd, $J = 8.09$ Hz, $J = 1.82$ Hz, $J = 0.56$ Hz), 7.46–7.47 (1H, m), 7.63 (1H, d, $J = 8.09$ Hz, $J = 7.64$ Hz); MS (EI) m/z 241.1 $(M)^+$. Anal. Calcd for $C_{15}H_{12}FNO$: C: 74.68, H: 5.01, N: 5.81. Found: C: 74.40, H: 5.05, N: 5.78.

5.4.18. 4′-Hydroxy[1,1′-biphenyl]-4-carbaldehyde (38). A mixture of **37** (0.32 g, 1.03 mmol), anhydrous KF (0.12 g, 2.06 mmol) and 48% aqueous HBr (35 μL, 0.31 mmol) in 6 mL dry DMF was stirred at room temperature under N_2 for 1 h. TLC indicated starting material present and therefore more 48% aqueous HBr (35 μL, 0.31 mmol) was added into the reaction and the mixture was continued to stir for another 1.5 h. Upon cooling, the mixture was then poured into 1 N aqueous HCl (30 mL). The aqueous mixture was extracted with EtOAc (3 ×) and the combined extracts were washed with saturated NaCl solution, and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the product (100% yield) was used directly in the next step. ^{1}H NMR (DMSO-d_6): δ 6.89 (2H, d, $J = 8.43$ Hz), 7.63 (2H, d, $J = 8.46$ Hz), 7.83 (2H, d, $J = 8.16$ Hz), 7.94 (2H, d, $J = 8.02$ Hz), 9.79 (1H, s), 10.01 (1H, s).

5.5. Method D

5.5.1. 3-Fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde (47). To a mixture of **41** (0.986 g, 4.29 mmol) in methylene chloride (35 mL) at 0 °C was slowly added boron tribromide (10.7 mL of 1 N solution in CH_2Cl_2, 10.7 mmol). The mixture was allowed to warm slowly to room temperature and stirred overnight. Water (4 mL) was added to the mixture with stirring in an ice-water bath. The resulting mixture was poured into water and extracted with EtOAc (3 ×). The combined organic layers were washed with brine, dried over sodium sulfate, and filtered. Evaporation of the solvent and purification by silica column chromatography (15–30% EtOAc–hexane) afforded 0.15 g (16%) of the title compound as a white solid. An analytical sample was obtained by reverse-phase preparative HPLC: mp 164–166 °C; ^{1}H NMR (acetone-d_6): δ 6.98–7.01 (2H, m), 7.56 (1H, dd, $J = 12.49$ Hz, $J = 1.64$ Hz), 7.63–7.66 (1H, m), 7.67–7.70 (2H, m), 7.89 (1H, t, $J = 7.85$ Hz), 8.88 (1H, br s), 10.31 (1H, s); MS (ESI) m/z 215 $(M-H)^-$. Anal. Calcd for $C_{13}H_{19}FO_2$: C: 72.22, H: 4.20. Found: C: 71.83, H: 4.04.

5.5.2. 3-Chloro-3′-fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde (48). The title compound was prepared by reacting **42** (0.99 g, 3.75 mmol) with boron tribromide (11.25 mL of 1 N solution in CH_2Cl_2, 11.25 mmol) according to method D to yield 0.94 g (100%) of a yellowish solid. The compound was used in the next step without purification. An analytical sample was obtained by HPLC purification: mp 206–208 °C; ^{1}H NMR (DMSO-d_6): δ 7.06 (1H, t, $J = 8.79$ Hz), 7.51–7.53 (1H, m), 7.71 (1H, dd, $J = 2.47$ Hz, $J = 12.63$ Hz), 7.81–7.83 (1H, m), 7.89 (1H, d, $J = 7.96$ Hz), 7.91 (1H, d, $J = 1.66$ Hz), 10.329 (1H, s), 10.330 (1H, s); IR 1667 cm^{-1}; MS (ESI) m/z 249/251 $(M-H)^-$. Anal. Calcd for $C_{13}H_8ClFO_2$: C: 62.29, H: 3.22. Found: C: 61.97, H: 3.21.

5.5.3. 2,6-Dichloro-3′-fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde (50). The title compound was prepared by reacting **44** (0.30 g, 1.00 mmol) with boron tribromide (3.0 mL of 1 N solution in CH_2Cl_2, 3.0 mmol) according to method D to yield 0.13 g (46%) of a white solid: mp 130–131 °C; ^{1}H NMR (DMSO-d_6): δ 6.91–6.95 (1H, m), 7.08 (1H, t, $J = 8.68$ Hz), 7.17 (1H, dd, $J = 11.88$ Hz,

$J = 2.02$ Hz), 8.06 (2H, s), 10.00 (1H, s), 10.23 (1H, s); MS (ESI) m/z 283/285/287 $(M-H)^-$. Anal. Calcd for $C_{13}H_7Cl_2FO_2\cdot 0.15H_2O$: C: 54.25, H: 2.56. Found: C: 54.16, H: 2.44.

5.5.4. 4′-(Dibromomethyl)-3-fluoro-1,1′-biphenyl-4-ol (45). The title compound was prepared by reacting **39** (1.90 g, 8.26 mmol) with boron tribromide (25 mL of 1 N solution in CH_2Cl_2, 25.0 mmol) according to method D to yield 2.40 g (87%) of a light yellow solid: mp 76–77 °C; ^{1}H NMR (DMSO-d_6): δ 5.21 (1H, d, $J = 4.17$ Hz), 6.69 (1H, s), 7.08 (1H, t, $J = 8.60$ Hz), 7.26–7.34 (2H, m), 7.50–7.53 (2H, m), 7.61–7.64 (2H, m); MS (ESI) m/z 215 $(M-H)^-$, m/z 217 $(M+H)^+$. Anal. Calcd for $C_{13}H_{19}Br_2FO$: C: 43.37, H: 2.52. Found: C: 43.41, H: 2.46.

5.5.5. 2′-Chloro-4′-(dibromomethyl)-1,1′-biphenyl-4-ol (46). The title compound was prepared by reacting **40** (0.80 g, 3.25 mmol) with boron tribromide (8.1 mL of 1 N solution in CH_2Cl_2, 8.13 mmol) according to method D to yield 0.60 g (50%) of a yellowish solid: mp 107–108 °C; ^{1}H NMR (DMSO-d_6): δ 6.86 (2H, d, $J = 8.52$ Hz), 7.29 (2H, d, $J = 8.50$ Hz), 7.43 (1H, s), 7.45 (1H, d, $J = 8.12$ Hz), 7.64 (1H, dd, $J = 8.07$ Hz, $J = 1.83$ Hz), 7.74 (1H, d, $J = 1.79$ Hz), 9.71 (1H, br s); ^{13}C NMR (DMSO-d_6): 40.66, 114.94, 125.66, 127.34, 128.14, 130.39, 130.87, 131.80, 140.95, 142.14, 157.36; MS (ESI) m/z 373/375/377/379 $(M-H)^-$. Anal. Calcd for $C_{13}H_{19}Br_2ClO$: C: 41.48, H: 2.41. Found: C: 41.64, H: 2.14.

5.6. Method E

5.6.1. 3-Chloro-4′-hydroxy-2′-methyl-1,1′-biphenyl-4-carbaldehyde (49). Compound **43** (1.0 g, 3.84 mmol) and pyridinium HCl (6.0 g) in a sealed tube were heated at 195 °C with stirring for 1 h. The reaction mixture was cooled to room temperature and stirred with 2 N HCl solution and EtOAc. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 ×). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and evaporation of the solvent provided (1.0 g) dark green solid. The product was used without any further purification. An analytical sample was obtained as a white solid by HPLC purification: mp 125–127 °C; ^{1}H NMR (DMSO-d_6): δ 2.21 (3H, s), 6.68–6.72 (2H, m), 7.11 (1H, d, $J = 8.15$ Hz), 7.47 (1H, d, $J = 7.89$ Hz), 7.55 (1H, d, $J = 1.48$ Hz), 7.89 (1H, d, $J = 7.98$ Hz), 9.62 (1H, s), 10.35 (1H, s); MS (ESI) m/z 245/247 $(M-H)^-$, 247/249 $(M+H)^+$. Anal. Calcd for $C_{14}H_{11}ClO_2\cdot 0.1H_2O$: C: 67.67, H: 4.54. Found: C: 67.63, H: 4.43.

5.6.2. 3′-Fluoro-4′-hydroxy-3-methyl-1,1′-biphenyl-4-carbonitrile (58). Compound **57** (1.89 g, 7.88 mmol) was treated with pyridinium HCl (45 g) according to method E to yield 1.61 g of a light yellow solid: mp 174–178 °C; ^{1}H NMR (DMSO-d_6): δ 2.52 (3H, s), 7.05 (1H, app.t, $J_{HF} = J_{HH} = 8.84$ Hz), 7.42–7.45 (1H, m), 7.61 (1H, dd, $J = 12.94$ Hz, $J = 2.18$ Hz), 7.63 (1H, dd, $J = 8.65$ Hz, $J = 1.86$ Hz), 7.76 (1H, br s), 7.78 (1H, d, $J = 8.20$ Hz), 10.22 (1H, s); MS (ESI) m/z 226 $(M-H)^-$. Anal. Calcd for $C_{14}H_{10}FNO$: C: 74.00, H: 4.44, N: 6.16. Found: C: 73.98, H: 4.35, N: 6.23.

5.6.3. 4′-Hydroxy-3-methyl[1,1′-biphenyl]-4-carbaldehyde (59). A solution of **56** (0.5 g, 2.39 mmol) in dry toluene was cooled to −78 °C and diisobutylaluminum hydride (4.0 mL of 1.5 M solution in toluene, 5.98 mmol) was added in one portion. The reaction mixture was allowed to warm to room temperature over a period of 3.5 h. Methanol (1.2 mL) was added followed by water (1.2 mL) at 0 °C and the mixture was stirred at room temperature for 20 min. HCl (1 N) solution was added with stirring until pH <7. The mixture was extracted with EtOAc (3 ×), washed with brine, dried over sodium sulfate, filtered, and the solvent evaporated. Purification by silica chromatography (15–25% EtOAc–hexane) to produce 0.46 g (91%) of the title compound as a white solid: mp 161–162 °C; ^{1}H NMR (DMSO-d_6): δ 2.67 (3H, s), 6.88 (2H, d, $J = 8.41$ Hz), 7.59–7.65 (4H, m), 7.85 (1H, d, $J = 8.03$ Hz), 9.78 (1H, s), 10.22 (1H, s); IR 1680 cm^{-1}; MS (ESI) m/z 211 $(M-H)^-$. Anal. Calcd for $C_{14}H_{12}O_2$: C: 79.23, H: 5.70. Found: C: 78.79, H: 5.90.

5.6.4. 3′-Fluoro-4′-hydroxy-3-methyl-1,1′-biphenyl-4-carbaldehyde (60). The title compound was prepared by reacting **58** (1.35 g, 5.95 mmol) with diisobutylaluminum hydride (5.95 mL of 1.5 M solution in toluene, 8.93 mmol) according to the procedure used for **6n** to afford 0.60 g (44%) of a light yellow solid: mp 126–128 °C; ^{1}H NMR (DMSO-d_6): δ 2.67 (3H, s), 7.06 (1H, dd, $J = 9.17$ Hz, $J = 8.52$ Hz), 7.46 (1H, ddd, $J = 8.46$ Hz, $J = 2.43$ Hz, $J = 0.9$ Hz), 7.62 (1H, dd, $J = 12.94$ Hz, $J = 2.18$ Hz), 7.64 (1H, d, $J = 2.18$ Hz), 7.68 (1H, dd, $J = 8.07$ Hz, $J = 1.67$ Hz), 7.85 (1H, d, $J = 8.07$ Hz), 10.19 (1H, s), 10.23 (1H, s); MS (ESI) m/z 229 $(M-H)^-$, m/z 231$(M+H)^+$. Anal. Calcd for $C_{14}H_{11}FO_2$: C: 73.03, H: 4.82. Found: C: 72.89, H: 4.86.

5.7. Method F

5.7.1. 4′-Hydroxy[1,1′-biphenyl]-4-carbaldehyde oxime (6a). A mixture of 4′-hydroxy[1,1′-biphenyl]-4-carbaldehyde **38** (1.0 mmol), hydroxylamine hydrochloride (0.14 g, 2.0 mmol) and pyridine (0.16 mL, 2.0 mmol) in 10 mL absolute methanol was heated to reflux for 3.5 h. The solvent was removed under reduced pressure and the residue was purified by silica chromatography (20–30% EtOAc–hexane) to yield 0.19 g (79%) of the title compound as a yellowish solid: mp 207–210 °C; ^{1}H NMR (DMSO-d_6): δ 6.85 (2H, d, $J = 8.37$ Hz), 7.53 (2H, d, $J = 8.39$ Hz), 7.62 (4H, s), 8.15 (1H, s), 9.62 (1H, s), 11.21 (1H, s); MS (ESI) m/z 212 $(M-H)^-$. Anal. Calcd for $C_{13}H_{11}NO_2$: C: 73.23, H: 5.20, N: 6.57. Found: C: 72.78, H: 5.41, N: 6.38.

5.7.2. 3′-Fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6b). The title compound was prepared by reacting **45** (2.17 g, 6.03 mmol) with hydroxylamine hydrochloride (0.81 g, 11.6 mmol) according to method F to yield 0.81 g (58%) of a white solid: mp 188–190 °C; ^{1}H NMR (DMSO-d_6): δ 7.03 (1H, dd, $J = 9.16$ Hz, $J = 8.52$ Hz), 7.36–7.38 (1H, m), 7.52 (1H, $J_{\mathrm{HF}} = 12.87$ Hz, $J_{\mathrm{HH}} = 2.24$ Hz), 7.62–7.67 (4H, m), 8.16 (1H, s), 10.04 (1H, s), 11.23 (1H, s). Anal. Calcd for $C_{13}H_{10}FNO_2$: C: 67.53, H: 4.36, N: 6.06. Found: C: 67.17, H: 4.37, N: 5.70.

5.7.3. 2-Chloro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6d). The title compound was prepared by reacting **46** (0.270 g, 0.722 mmol) with hydroxylamine hydrochloride (0.185 g, 2.66 mmol) according to method F to yield 0.160 g (90%) of a white solid: mp 178–179 °C; ^{1}H NMR (DMSO-d_6): δ 6.85 (2H, d, $J = 8.54$ Hz), 7.28 (2H, d, $J = 8.51$ Hz), 7.39 (1H, d, $J = 7.98$ Hz), 7.60 (1H, dd, $J = 7.65$ Hz, $J = 1.42$ Hz), 7.72 (1H, d, $J = 1.34$ Hz), 8.18 (1H, s), 9.67 (1H, s), 11.45 (1H, s); MS (ESI) m/z 246/248 $(M-H)^-$. Anal. Calcd for $C_{13}H_{10}ClNO_2$: C: 63.04, H: 4.07, N: 5.66. Found: C: 63.07, H: 3.99, N: 5.67.

5.7.4. 3-Fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6e). The title compound was prepared by reacting **47** (0.154 g, 0.713 mmol) with hydroxylamine hydrochloride (0.099 g, 1.43 mmol) according to method F to yield 0.156 g (95%) of a yellowish solid: mp 181–183 °C; ^{1}H NMR (DMSO-d_6): δ 6.84–6.87 (2H, m), 7.48–7.53 (2H, m), 7.57–7.60 (2H, m), 7.76 (1H, t, $J = 8.14$ Hz), 8.22 (1H, s), 9.74 (1H, s), 11.56 (1H, s); MS (ESI) m/z 230 $(M-H)^-$, 232 $(M+H)^+$. Anal. Calcd for $C_{13}H_{10}FNO_2$: C: 67.53, H: 4.36, N: 6.06. Found: C: 68.10, H: 4.28, N: 6.01.

5.7.5. 4′-Hydroxy-3-methoxy-1,1′-biphenyl-4-carbaldehyde oxime (6g). The title compound was prepared by reacting **33** (0.225 g, 0.986 mmol) with hydroxylamine hydrochloride (0.137 g, 1.97 mmol) according to method F to yield 0.210 g (88%) of a yellowish solid: mp 228–230 °C; ^{1}H NMR (DMSO-d_6): δ 3.91 (3H, s), 6.85 (2H, d, $J = 8.29$ Hz), 7.18 (1H, d, $J = 8.08$ Hz), 7.21 (1H, s), 7.56 (2H, d, $J = 8.59$ Hz), 7.68 (1H, d, $J = 7.98$ Hz), 8.28 (1H, s), 9.63 (1H, s), 11.19 (1H, s); MS (ESI) m/z 242 $(M-H)^-$, 244 $(M+H)^+$. Anal. Calcd for $C_{14}H_{13}NO_3$: C: 69.12, H: 5.39, N: 5.76. Found: C: 68.87, H: 5.29, N: 5.64.

5.7.6. 2,6-Dichloro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6h). The title compound was prepared by reacting **34** (0.19 g, 0.71 mmol) with hydroxylamine hydrochloride (0.10 g, 1.4 mmol) according to method F to yield 0.12 g (60%) of a white solid: mp 148–149 °C; ^{1}H NMR (DMSO-d_6): δ 6.86 (2H, d, $J = 8.45$ Hz), 7.07 (2H, d, $J = 8.33$ Hz), 7.74 (2H, s), 8.18 (1H, s), 9.66 (1H, s), 11.64 (1H, s); MS (ESI) m/z 280/282/284 $(M-H)^-$, 282/284/286 $(M+H)^+$. Anal. Calcd for $C_{13}H_{19}Cl_2NO_2$: C: 55.35, H: 3.22, N: 4.96. Found: C:55.51, H: 3.25, N: 4.75.

5.7.7. 3-Chloro-3′-fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6i). The title compound was prepared by reacting **48** (1.52 mmol) with hydroxylamine hydrochloride (0.233 g, 3.34 mmol) according to method F to yield 0.260 g (66%) of a yellowish solid: mp 198–200 °C; ^{1}H NMR (DMSO-d_6): δ 7.03 (1H, t, $J = 8.79$ Hz), 7.41–7.43 (1H, m), 7.60 (1H, dd, $J = 12.91$ Hz, $J = 2.20$ Hz), 7.64–7.66 (1H, m), 7.77 (1H, d, $J = 1.92$ Hz), 7.84 (1H, d, $J = 8.24$ Hz), 8.36 (1H, s), 10.16 (1H, br s), 11.68 (1H, br s); MS (ESI) m/z 264/266 $(M-H)^-$, 266/268 $(M+H)^+$. Anal. Calcd for $C_{13}H_{19}ClFNO_2$: C: 58.77, H: 3.41, N: 5.27. Found: C: 58.67, H: 3.65, N: 4.99.

5.7.8. 3-Chloro-4′-hydroxy-2′-methyl-1,1′-biphenyl-4-carbaldehyde oxime (6j). The title compound was prepared by reacting **49** (0.500 g, 2.03 mmol) with hydroxylamine hydrochloride (0.425 g, 6.10 mmol) in anhydrous THF (50 mL) and methanol (20 mL) according to method F to yield 0.35 g (57%) of a white solid: mp 169–171 °C; ^{1}H NMR (DMSO-d_6): δ 2.19 (3H, s), 6.65–6.70 (2H, m), 7.06 (1H, d, $J = 8.14$ Hz), 7.31 (1H, d, $J = 8.05$ Hz), 7.41 (1H, d, $J = 1.40$ Hz), 7.83 (1H, d, $J = 8.08$ Hz), 8.38 (1H, s), 9.51 (1H, s), 11.69 (1H, s); MS (ESI) m/z 260/262 $(M-H)^-$, 262/264 $(M+H)^+$. Anal. Calcd for $C_{14}H_{12}ClNO_2$: C: 64.25, H: 4.62, N: 5.35. Found: C: 63.87, H: 4.43, N: 5.31.

5.7.9. 3-Chloro-3′,5′-difluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6k). The title compound was prepared by reacting **35** (0.200 g, 0.746 mmol) with hydroxylamine hydrochloride (0.156 g, 2.24 mmol) according to method F to yield 0.100 g (47%) of a white solid: mp 216–219 °C; ^{1}H NMR (DMSO-d_6): δ 7.54 (2H, d, $J = 8.51$ Hz), 7.70 (1H, d, $J = 7.97$ Hz), 7.84–7.86 (2H, m), 8.37 (1H, s), 10.52 (1H, s), 11.74 (1H, s); MS (ESI) m/z 282/284 $(M-H)^-$, 284/286 $(M+H)^+$. Anal. Calcd for $C_{13}H_8ClF_2NO_2$: C: 55.05, H: 2.84, N: 4.94. Found: C: 54.96, H: 3.02, N: 4.75.

5.7.10. 2,6-Dichloro-3′-fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6l). The title compound was prepared by reacting **50** (0.12 g, 0.42 mmol) with hydroxylamine hydrochloride (0.058 g, 0.84 mmol) according to method F to yield 0.058 g (46%) of a white solid: mp 158–159 °C; ^{1}H NMR (DMSO-d_6): δ 6.90 (1H, dd, $J = 8.27$ Hz, $J = 1.35$ Hz), 7.04 (1H, t, $J = 8.71$ Hz), 7.11 (1H, dd, $J = 11.85$ Hz, $J = 1.99$ Hz), 7.75 (2H, s), 8.18 (1H, s), 10.12 (1H, s), 11.67 (1H, s); MS (ESI) m/z 298/300/302 $(M-H)^-$, m/z 300/302/304 $(M+H)^+$. Anal. Calcd for $C_{13}H_8Cl_2FNO_2$: C: 52.03, H: 2.69, N:4.67. Found: C: 51.82, H: 2.58, N: 4.55.

5.7.11. 4′-Hydroxy-2-methyl-1,1′-biphenyl-4-carbaldehyde oxime (6m). The title compound was prepared by reacting **36** (0.35 g, 1.65 mmol) with hydroxylamine hydrochloride (0.23 g, 3.30 mmol) according to method

F to yield 0.36 g (96%) of a white solid: mp 169–171 °C; ^{1}H NMR (DMSO-d_6): δ 2.25 (3H, s), 6.82 (2H, d, $J = 8.52$ Hz), 7.16 (2H, d, $J = 8.45$ Hz), 7.18 (1H, d, $J = 7.84$ Hz), 7.44 (1H, d, $J = 7.93$ Hz), 7.47 (1H, s), 8.11 (1H, s), 9.52 (1H, s), 11.19 (1H, s); MS (ESI) *m*/*z* 226 $(M-H)^-$, 228 $(M+H)^+$. Anal. Calcd for $C_{14}H_{13}NO_2$: C: 73.99, H: 5.77, N: 6.16. Found: C: 73.72, H: 5.63, N: 6.37.

5.7.12. 4′-Hydroxy-3-methyl[1,1′-biphenyl]-4-carbaldehyde oxime (6n). The title compound was prepared by reacting **59** (0.31 g, 1.46 mmol) with hydroxylamine hydrochloride (0.203 g, 2.92 mmol) according to method F to yield 0.177 g (53%) of a yellowish solid: mp 195–197 °C; ^{1}H NMR (DMSO-d_6): δ 2.43 (3H, s), 6.84 (2H, d, $J = 8.48$ Hz), 7.42–7.45 (2H, m), 7.52 (2H, d, $J = 8.51$ Hz), 7.66 (1H, d, $J = 8.00$ Hz), 8.32 (1H, s), 9.60 (1H, s), 11.25 (1H, s); ^{13}C NMR (DMSO-d_6): δ 19.62, 115.60, 123.40, 126.60, 127.56, 127.99, 129.05, 130.03, 136.34, 140.43, 146.80, 157.24; MS (ESI) *m*/*z* 226 $(M-H)^-$, 228 $(M+H)^+$. Anal. Calcd for $C_{14}H_{13}NO_2$: C: 73.99, H: 5.77, N: 6.16. Found: C: 73.81, H: 5.75, N: 6.04.

5.7.13. 3′-Fluoro-4′-hydroxy-3-methyl-1,1′-biphenyl-4-carbaldehyde oxime (6o). The title compound was prepared by reacting **60** (0.31 g, 1.34 mmol) with hydroxylamine hydrochloride (0.19 g, 2.68 mmol) according to method F to yield 0.20 g (61%) of a white solid: mp 193–194 °C; ^{1}H NMR (DMSO-d_6): δ 2.43 (3H, s), 7.02 (1H, dd, $J = 9.10$ Hz, $J = 8.58$ Hz), 7.36 (1H, app.dt, $J = 8.33$ Hz, $J = 1.16$ Hz), 7.47 (1H, dd, $J = 8.33$ Hz, $J = 1.86$ Hz), 7.50 (1H, s), 7.51 (1H, $J = 12.87$ Hz, $J = 2.24$ Hz), 7.66 (1H, d, $J = 8.07$ Hz), 8.32 (1H, s), 10.02 (1H, s), 11.26 (1H, s); MS (ESI) *m*/*z* 244 $(M-H)^-$, *m*/*z* 246 $(M+H)^+$. Anal. Calcd for $C_{14}H_{12}FNO_2{\cdot}0.30H_2O$: C: 67.09, H: 5.07, N: 5.59. Found: C: 66.94, H: 4.70, N: 5.26.

5.7.14. 4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-3-chloro[1,1′-biphenyl]-4-carbaldehyde oxime (53). The title compound was prepared by reacting **31** (0.405 g, 1.17 mmol) with hydroxylamine hydrochloride (0.154 g, 2.22 mmol) according to method F to yield a yellowish oil, which was used in the next step without purification: MS (ESI) *m*/*z* 362/364 $(M+H)^+$.

5.7.15. 4′-{[*tert*-Butyl(dimethyl)silyl]oxy}-2-fluoro-[1,1′-biphenyl]-4-carbaldehyde oxime (54). The title compound was prepared by reacting **32** (0.45 g, 1.36 mmol) with hydroxylamine hydrochloride (0.19 g, 2.73 mmol) according to method F to yield a white solid, which was used in the next step without purification: MS (ESI) *m*/*z* 344 $(M-H)^-$, 346 $(M+H)^+$.

5.7.16. 3-Chloro-4′-hydroxy[1,1′-biphenyl]-4-carbaldehyde oxime (6c). Tetrabutylammonium fluoride (1.29 mL of 1.0 M solution in THF, 1.29 mmol) was added into a solution of **53** (1.17 mmol) in 10 mL THF. The mixture was stirred at room temperature for 10 min then poured into EtOAc and water. The resulting layers were separated and the aqueous layer was extracted with EtOAc (3 ×). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and the solvent removed under vacuum. Purification by silica chromatography (20–30% EtOAc–hexane) provided 0.186 g (64%) of the title compound as a yellowish solid: mp 187–189 °C; ^{1}H NMR (DMSO-d_6): δ 6.86 (2H, d, $J = 8.55$ Hz), 7.56–7.63 (3H, m), 7.71 (1H, d, $J = 1.60$ Hz), 7.84 (1H, d, $J = 8.26$ Hz), 8.36 (1H, s), 9.75 (1H, s), 11.66 (1H, s); MS (ESI) *m*/*z* 246 $(M-H)^-$, 248 $(M+H)^+$. Anal. Calcd for $C_{13}H_{10}ClNO_2$: C: 63.04, H: 4.07, N: 5.66. Found: C: 62.96, H: 4.10, N: 5.42.

5.7.17. 2-Fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6f). The title compound was prepared by reacting **54** (1.02 mmol) with tetrabutylammonium fluoride (1.12 mL of 1.0 M solution in THF, 1.12 mmol) according to the procedure as described in **6c** to yield 0.198 g (84%) of a white solid: mp 178–180 °C; ^{1}H NMR (DMSO-d_6): δ 6.84–6.89 (2H, m), 7.39–5.54 (5H, m), 8.17 (1H, s), 9.71 (1H, s), 11.43 (1H, s); MS (ESI) *m*/*z* 230 $(M-H)^-$, 232 $(M+H)^+$. Anal. Calcd for $C_{13}H_{10}FNO_2$: C: 67.53, H: 4.36, N: 6.06. Found: C: 67.13, H: 4.11, N: 6.00.

5.7.18. 3′-Chloro-1,1′-biphenyl-4-ol (63). Prepared as previously described in Refs. 23 and 24.

5.7.19. 3-Fluoro-4-methoxy-1,1′-biphenyl (64). The title compound was prepared by reacting bromobenzene (**61**) (2.77 g, 17.6 mmol) with **13** (4.2 g, 24.7 mmol) according to method C to yield 3.00 g (84%) of a white solid: mp 76–77 °C; ^{1}H NMR (CDCl$_3$): δ 3.93 (3H, s), 7.00–7.05 (1H, m), 7.29–7.35 (3H, m), 7.40–7.44 (2H, m), 7.51–7.54 (2H, m); MS (EI) *m*/*z* 202.2 $(M)^+$. Anal. Calcd for $C_{13}H_{11}FO$: C: 77.21, H: 5.48. Found: C: 76.96, H: 5.33.

5.7.20. 3′-Chloro-3-fluoro-4-methoxy-1,1′-biphenyl (65). The title compound was prepared by reacting 3-bromochloro-benzene (**62**) (2.22 g, 11.59 mmol) with **13** (2.8 g, 16.2 mmol) according to method C to yield 2.55 g (93%) of a clear oil: ^{1}H NMR (CDCl$_3$): δ 3.93 (3H, s), 7.02 (1H, t, $J = 8.70$ Hz), 7.25–7.36 (4H, m), 7.38–7.40 (1H, m), 7.50–7.51 (1H, m); MS (EI) *m*/*z* 236/238 $(M)^+$. Anal. Calcd for $C_{13}H_{10}ClFO$: C: 65.97, H: 4.26. Found: C: 65.90, H: 3.95.

5.7.21. 3-Fluoro-1,1′-biphenyl-4-ol[25] (66). Compound **64** (2.05 g, 10.13 mmol) was treated with pyridinium HCl (30 g) according to method E to yield 1.54 g of a white solid (81%): mp 102–105 °C; ^{1}H NMR (DMSO-d_6): δ 7.03 (1H, t, $J = 8.84$ Hz), 7.28–7.34 (2H, m), 7.39–7.44 (2H, m), 7.47 (1H, dd, $J = 12.82$ Hz, $J = 2.18$ Hz), 7.59–7.62 (2H, m), 9.96 (1H, s); MS (ESI) *m*/*z* 187 $(M-H)^-$.

Anal. Calcd for $C_{12}H_{19}FO$: C: 76.58, H: 4.82. Found: C: 76.44, H: 4.67.

5.7.22. 3′-Chloro-3-fluoro-1,1′-biphenyl-4-ol (67). Compound **65** (2.15 g, 9.07 mmol) was treated with pyridinium HCl (30 g) according to method E to yield 1.87 g (92%) of the title compound as a white waxy solid. Further purification by reverse-phase HPLC afforded a white solid: mp 50–51 °C; ^{1}H NMR (DMSO-d_6): δ 7.03 (1H, t, $J = 8.84$ Hz), 7.35–7.39 (2H, m), 7.44 (1H, t, $J = 7.82$ Hz), 7.55 (1H, dd, $J = 12.95$ Hz, $J = 2.35$ Hz), 7.59 (1H, d, $J = 7.81$ Hz), 7.68 (1H, m), 10.11 (1H, s); MS (ESI) m/z 221/223 $(M-H)^-$. Anal. Calcd for $C_{12}H_8ClFO$: C: 64.74, H: 3.62. Found: C: 64.45, H: 3.72.

5.7.23. 3-Chloro-1,1′-biphenyl-4-carbaldehyde (70). The title compound was prepared by reacting **23** (1.30 g, 4.50 mmol) with phenylboronic acid (**68**) (0.77 g, 6.3 mmol) according to method C to yield 0.53 g (54%) of a yellow oil: mp 82–83 °C; ^{1}H NMR ($CDCl_3$): δ 7.44–7.52 (3H, m), 7.60–7.63 (3H, m), 7.69 (1H, d, $J = 1.57$ Hz), 8.00 (1H, d, $J = 8.12$ Hz), 10.51 (1H, s); mp 82–83 °C; MS (EI) m/z 216/218 $(M)^+$. Anal. Calcd for $C_{13}H_{19}ClO \cdot 0.15H_2O$: C: 71.18, H: 4.27. Found: C: 71.17, H: 4.14.

5.7.24. 3-Chloro-3′-fluoro-1,1′-biphenyl-4-carbaldehyde (71). The title compound was prepared by reacting **23** (1.20 g, 4.15 mmol) with 3-fluorophenylboronic acid (**69**) (0.81 g, 5.8 mmol) according to method C to yield 0.47 g (48%) of a white solid: mp 92–93 °C; ^{1}H NMR ($CDCl_3$): δ 7.14 (1H, app.tdd, $J_{HH} = J_{HF} = 8.30$ Hz, $J_{HH} = 2.49$ Hz, $J_{HH} = 1.04$ Hz), 7.31 (1H, ddd, $J_{HF} = 9.75$ Hz, $J_{HH} = 2.49$ Hz, $J_{HH} = 2.07$ Hz), 7.39 (1H, app.dt, $J = 7.88$ Hz, $J = 1.24$ Hz), 7.46 (1H, app.td, $J = 8.09$ Hz, $J = 5.81$ Hz), 7.59 (1H, ddd, $J = 8.09$ Hz, $J = 1.87$ Hz, $J = 0.83$ Hz), 7.67 (1H, d, $J = 1.87$ Hz), 8.00 (1H, d, $J = 8.09$ Hz), 10.51 (1H, s); MS (EI) m/z 234.1/236.1 $(M)^+$. Anal. Calcd for $C_{13}H_8ClFO$: C: 66.54, H: 3.44. Found: C: 65.49, H: 3.50.

5.7.25. 3-Chloro-1,1′-biphenyl-4-carbaldehyde oxime (72). The title compound was prepared by reacting **70** (0.13 g, 0.602 mmol) with hydroxylamine hydrochloride (0.084 g, 1.2 mmol) according to method F to yield 0.10 g (71%) of a white solid: mp 134 °C; ^{1}H NMR (DMSO-d_6): δ 7.40–7.44 (1H, m), 7.48–7.51 (2H, m), 7.69–7.72 (1H, m), 7.72–7.77 (2H, m), 7.82 (1H, d, $J = 1.66$ Hz), 7.90 (1H, d, $J = 8.20$ Hz), 8.39 (1H, s), 11.72 (1H, s); MS (ESI) m/z 232/234 $(M+H)^+$. Anal. Calcd for $C_{13}H_{10}ClNO$: C: 67.40, H: 4.35, N: 6.05. Found: C: 67.13, H: 4.31, N: 5.81.

5.7.26. 3-Chloro-3′-fluoro-1,1′-biphenyl-4-carbaldehyde oxime (73). The title compound was prepared by reacting **71** (0.12 g, 0.513 mmol) with hydroxylamine hydrochloride (0.070 g, 1.0 mmol) according to method F to yield 0.10 (78%) of a white solid: mp 131–132 °C; ^{1}H NMR (DMSO-d_6): δ 7.23–7.28 (1H, m), 7.50–7.56 (1H, m), 7.60–7.65 (2H, m), 7.73–7.75 (1H, m), 7.88 (1H, d, $J = 1.79$ Hz), 7.90 (1H, d, $J = 8.20$ Hz), 8.39 (1H, s), 11.76 (1H, s); MS (ESI) m/z 248/250 $(M-H)^-$, m/z 250/252 $(M+H)^+$. Anal. Calcd for $C_{13}H_{19}ClFNO$: C: 62.54, H: 3.63, N: 5.61. Found: C: 62.24, H: 3.47, N: 5.45.

5.7.27. 2,3-Dichloro-4-methoxybenzaldehyde (75). To a solution of 2,3-dichloroanisole (**74**) (10.00 g, 56.6 mmol) in anhydrous dichloromethane (45 mL) was added $TiCl_4$ (10.5 mL, 96.1 mmol) quickly. α,α′-dichloromethyl methyl ether (5.1 mL, 56.6 mmol) was then added slowly and the internal temperature was maintained between 15 and 20 °C. The mixture was stirred at room temperature for 5 h, slowly poured into crushed ice, extracted with dichloromethane (3 ×), washed with saturated sodium bicarbonate until pH = 7, then washed with brine. The organic layer was dried over anhydrous sodium sulfate, concentrated to give 11.34 g (98%) of a white solid. An analytical sample was obtained by reverse-phase preparative HPLC: mp 112–113 °C; ^{1}H NMR ($CDCl_3$): δ 4.01 (3H, s), 6.97 (1H, d, $J = 8.77$ Hz), 7.90 (1H, d, $J = 8.82$ Hz), 10.36 (1H, s); MS (ESI) m/z 205/207/209 $(M+H)^+$. Anal. Calcd for $C_8H_6Cl_2O_2$: C: 46.86, H: 2.95. Found: C: 46.92, H: 2.70.

5.7.28. 2,3-Dichloro-4-hydroxybenzaldehyde (76). The title compound was prepared by reacting **75** (10 g, 49 mmol) with boron tribromide (147 mL of 1 N solution in CH_2Cl_2, 147 mmol) according to method D to yield 11.3 g of a dark gray solid, which is mainly the desired product as indicated by ^{1}H NMR. Upon trituration with 30% $CHCl_3$ in hexane, 3.6 g of a gray solid was obtained. An analytical sample was obtained as a white solid by reverse-phase preparative HPLC: mp 176–178 °C; ^{1}H NMR (DMSO-d_6): δ 7.10 (1H, d, $J = 8.68$ Hz), 7.74 (1H, d, $J = 8.67$ Hz), 10.16 (1H, s), 11.95 (1H, s); MS (ESI) m/z 189/191/193 $(M-H)^-$, 193/191/195 $(M+H)^+$. Anal. Calcd for $C_7H_4Cl_2O_2$: C: 44.02, H: 2.11. Found: C:43.87, H: 1.67.

5.7.29. Trifluoro-methanesulfonic acid 2,3-dichloro-4-formyl-phenyl ester (77) . The title compound was prepared by reacting **76** (2.50 g, 13.2 mmol) with trifluoromethanesulfonic anhydride (2.88 mL, 4.8 g, 17.1 mmol) according to method B to yield 3.69 g (82%) of brown crystals, which were used directly in the next step without purification. An analytical sample was obtained as white solid by silica chromatography (5% EtOAc–hexane): mp 44–45 °C; ^{1}H NMR (DMSO-d_6): δ 7.88 (1H, d, $J = 8.74$ Hz), 8.02 (1H, d, $J = 8.81$ Hz), 10.28 (1H, s); MS (EI) m/z 322/324/326 $(M)^+$. Anal. Calcd for $C_8H_3Cl_2F_3O_4S$: C: 29.74, H: 0.94. Found: C: 30.19, H: 0.86.

Compound **77** (1.39 g, 4.33 mmol) was reacted with **12** (1.20 g, 4.76 mmol) according to method C to produce the following three compounds:

5.7.30. 2,3-Dichloro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde (78). White solid (0.31 g, 27%): mp 172–174 °C; ^{1}H NMR (DMSO-d_6): δ 6.86–6.91 (2H, m), 7.32–7.35 (2H, m), 7.53 (1H, dd, $J = 7.94$ Hz, $J = 0.45$ Hz), 7.85 (1H, d, $J = 8.01$ Hz), 9.85 (1H, s), 10.35 (1H, s); MS (ESI) *m/z* 265/267/269 (M−H)$^-$. Anal. Calcd for $C_{13}H_8Cl_2O_2$: C: 58.46, H: 3.02. Found: C: 57.75, H: 2.82.

5.7.31. 2-Chloro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde (79). An off white solid (0.090 g, 9%): mp 114–116 °C; ^{1}H NMR (DMSO-d_6): δ 6.85–6.90 (2H, m), 7.32–7.36 (2H, m), 7.61(1H, d, $J = 7.87$ Hz), 7.89 (1H, dd, $J = 7.90$ Hz, $J = 1.55$ Hz), 8.05 (1H, d, $J = 1.52$ Hz), 9.79 (1H, s), 10.02 (1H, s); MS (ESI) *m/z* 231/233 (M−H)$^-$, 233/235 (M+H)$^+$. Anal. Calcd for $C_{13}H_{19}ClO_2$: C: 67.11, H: 3.90. Found: C: 67.44, H: 3.87.

5.7.32. 2′-Chloro-4,4″-dihydroxy-1,1′:3′,1″-terphenyl-4′-carbaldehyde (80). An off white solid (0.13 g, 9%): mp 222–223 °C; ^{1}H NMR (DMSO-d_6): δ 6.85–6.88 (2H, m), 6.88–6.91 (2H, m), 7.17–7.20 (2H, m), 7.31–7.35 (2H, m), 7.52 (1H, dd, $J = 7.96$ Hz, $J = 0.89$ Hz), 7.84 (1H, d, $J = 8.07$ Hz), 9.55 (1H, d, $J = 0.77$ Hz), 9.73 (1H, s), 9.74 (1H, s); MS (ESI) *m/z* 323/325 (M−H)$^-$, 325/327 (M+H)$^+$. Anal. Calcd for $C_{19}H_{13}ClO_3$: C: 70.27, H: 4.03. Found: C: 69.80, H: 3.88.

5.7.33. 2,3-Dichloro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6p). The title compound was prepared by reacting **78** (0.14 g, 0.526 mmol) with hydroxylamine hydrochloride (0.11 g, 1.58 mmol) according to method F to yield 148 mg (100%) of an off white solid: mp 208–210 °C; ^{1}H NMR (DMSO-d_6): δ 6.84–6.87 (2H, m), 7.26–7.29 (2H, m), 7.36 (1H, d, $J = 7.94$ Hz). 7.80 (1H, d, $J = 8.20$ Hz), 8.41 (1H, s), 9.72 (1H, s), 11.83 (1H, s); MS (ESI) *m/z* 280/282/284 (M−H)$^-$, 282/284/286 (M+H)$^+$. Anal. Calcd for $C_{13}H_{19}Cl_2NO_2$: C: 55.35, H: 3.22, N: 4.96. Found: C: 55.78, H: 3.59, N: 4.44.

5.7.34. 2′,3′-Dichloro-4′-(hydroxymethyl)-1,1′-biphenyl-4-ol (81). Sodium borohydride (0.060 g, 0.226 mmol) was added into a solution of **78** (0.017 g, 0.451 mmol) in THF (5 mL) and methanol (5 mL) and the mixture was stirred at room temperature under nitrogen for 1 h. Water (2 mL) was added to the mixture and solvent was removed under reduced pressure. The mixture was extracted with EtOAc (3 ×), washed with brine, dried over anhydrous sodium sulfate, filtered, and the solvent evaporated to yield 0.061 g (100%) of the title compound as an off white solid: mp 194–196 °C; ^{1}H NMR (acetone-d_6): δ 4.62 (1H, t, $J = 5.76$ Hz), 4.77 (2 H, d, $J = 6.08$ Hz), 6.91–6.95 (2H, m), 7.27–7.30 (2H, m), 7.34 (1H, d, $J = 8.01$ Hz), 7.63 (1H, dt, $J = 8.02$ Hz, $J = 0.84$ Hz), 8.59 (1H, s); MS (ESI) *m/z* 267/269/271 (M−H)$^-$. Anal. Calcd for $C_{13}H_{10}Cl_2O_2$: C: 58.02, H: 3.75. Found: C: 58.05, H: 4.04.

5.7.35. 2,3-Dichloro-3′-fluoro-4′-methoxy-1,1′-biphenyl-4-carbaldehyde (82). The title compound was prepared by reacting **77** (1.9 g, 5.90 mmol) with **13** (1.3 g, 7.67 mmol) according to method C to yield 0.84 g (47%) of a white solid: mp 160–164 °C; ^{1}H NMR (DMDO-d_6): δ 3.90 (3H, m), 7.28–7.30 (2H, m), 7.41–7.43 (1H, m), 7.57 (1H, d, $J = 8.30$ Hz), 7.87 (1H, d, $J = 7.81$ Hz), 10.35 (1H, s); MS (EI) *m/z* 298/300/302 (M)$^+$. Anal. Calcd for $C_{14}H_{19}Cl_2FO_2$: C: 56.21, H: 3.03. Found: C: 57.55, H: 2.97.

Compound **82** (0.72 g, 2.42 mmol) was reacted with boron tribromide (7.25 mL of 1 N solution in CH_2Cl_2, 7.25 mmol) according to method D to produce the following two compounds:

5.7.36. 2′,3′-Dichloro-4′-(dibromomethyl)-3-fluoro-1,1′-biphenyl-4-ol (83). A gray thick syrup (0.13 g, 13%): ^{1}H NMR (DMSO-d_6): δ 7.04 (1H, t, $J = 8.54$ Hz), 7.11 (1H, dd, $J = 8.41$ Hz, $J = 1.97$ Hz), 7.32 (1H, dd, $J = 12.18$ Hz, $J = 1.94$ Hz), 7.51 (1H, d, $J = 8.27$ Hz), 7.54 (1H, s), 7.95 (1H, d, $J = 8.23$ Hz), 10.23 (1H, s); MS (ESI) *m/z* 425/427/429 (M−H)$^-$. Anal. Calcd for $C_{13}H_7Br_2Cl_2FO$: C: 36.40, H: 1.65. Found: C: 37.60, H: 1.69.

5.7.37. 2,3-Dichloro-3′-fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde (84). A white solid (0.14 g, 20%): mp 170–171 °C; ^{1}H NMR (DMSO-d_6): δ 7.07 (1H, t, $J = 8.60$ Hz), 7.15 (1H, dd, $J = 8.40$ Hz, $J = 1.79$ Hz), 7.35 (1H, dd, $J = 12.15$ Hz, $J = 1.87$ Hz), 7.56 (1H, d, $J = 7.97$ Hz), 7.86 (1H, d, $J = 8.09$ Hz), 10.31 (1H, s), 10.35 (1H, s); MS (ESI) *m/z* 283/285/287 (M−H)$^-$. Anal. Calcd for $C_{13}H_7Cl_2FO_2$: C: 54.77, H: 2.47. Found: C: 54.93, H: 2.18.

Compound **83** (0.085 g, 0.20 mmol) was reacted with hydroxylamine hydrochloride (0.376 g, 5.39 mmol) and pyridine (0.43 mL, 5.34 mmol) for 8 days according to method F to produce the following two compounds:

5.7.38. 2,3-Dichloro-3′-fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6q). A white solid (0.018 g, 30%): mp 225–227 °C; ^{1}H NMR (DMSO-d_6): δ 7.04 (1H, t, $J = 8.54$ Hz), 7.10 (1H, dd, $J = 8.35$ Hz, $J = 1.88$ Hz), 7.28 (1H, dd, $J = 12.22$ Hz, $J = 2.01$ Hz), 7.39 (1H, d, $J = 8.14$ Hz), 7.81 (1H, d, $J = 8.15$ Hz), 8.41 (1H, s), 10.18 (1H, s), 11.87 (1H, s); MS (ESI) *m/z* 298/300/302 (M−H)$^-$, 300/302/304 (M+H)$^+$. Anal. Calcd for $C_{13}H_8Cl_2FNO_2{\cdot}0.09TFA$: C: 51.00, H: 2.63, N: 4.51. Found: C: 50.97, H: 2.37, N: 4.33.

5.7.39. Methyl 2,3-dichloro-3′-fluoro-4′-hydroxy-1,1′-biphenyl-4-carboxylate (86). A white solid (0.017 g, 27%): mp 152–154 °C; ^{1}H NMR (DMSO-d_6): δ 3.90 (3H, s), 7.05 (1H, t, $J = 8.54$ Hz), 7.11 (1H, dd, $J = 8.41$ Hz, $J = 1.55$ Hz), 7.31 (1H, dd, $J = 12.16$ Hz, $J = 1.68$ Hz), 7.47 (1H, d, $J = 8.02$ Hz), 7.76 (1H, d, $J = 8.64$ Hz); MS (ESI) *m/z* 313/315/317 (M+H)$^+$. Anal. Calcd for $C_{14}H_{19}Cl_2FO_3$: C: 53.36, H: 2.88. Found: C: 51.94, H: 2.54.

5.7.40. 2′,3′-Dichloro-3-fluoro-4′-(hydroxymethyl)-1,1′-biphenyl-4-ol (85). The title compound was prepared by reacting **84** (0.060 g, 0.211 mmol) with sodium borohydride (0.016 g, 0.422 mmol) according to the procedure used for **81** to afford 0.060 g (99%) of a white solid: mp 168–169 °C; ^{1}H NMR (DMSO-d_6): δ 4.60 (2H, d, $J = 4.35$ Hz), 5.58 (1H, t, $J = 5.25$ Hz), 6.99–7.08 (2H, m), 7.21–7.26 (1H, m), 7.38 (1H, d, $J = 7.96$ Hz), 7.55 (1H, d, $J = 8.00$ Hz), 10.14 (1H, s); MS (ESI) *m/z* 285/287/289 $(M-H)^-$. Anal. Calcd for $C_{13}H_{19}Cl_2FO_2$: C: 54.38, H: 3.16. Found: C: 55.22, H: 3.31.

5.7.41. 3,5-Dichloro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6r). The title compound was prepared by reacting 3,5-dichloro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde[11] (0.17 g, 0.637 mmol) with hydroxylamine hydrochloride (0.089 g, 1.27 mmol) according to method F to yield 0.16 g (89%) of a white solid: mp 183–186 °C; ^{1}H NMR (DMSO-d_6): δ 6.86 (2H, d, $J = 8.79$ Hz), 7.63 (2H, d, $J = 8.79$ Hz), 7.76 (2H, s), 8.25 (1H, s), 9.81 (1H, s), 11.78 (1H, s); MS (ESI) *m/z* 280/282/284 $(M-H)^-$, 282/284/286 $(M+H)^+$.

5.7.42. 3,5-Dichloro-3′-fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde oxime (6s). The title compound was prepared by reacting 3,5-dichloro-3′-fluoro-4′-hydroxy-1,1′-biphenyl-4-carbaldehyde[11] (0.29 g, 1.02 mmol) with hydroxylamine hydrochloride (0.14 g, 1.27 mmol) according to method F to yield 0.26 g (85%) of a white solid: mp 185–190 °C; ^{1}H NMR (DMSO-d_6): δ 7.03 (1H, t, $J = 8.86$ Hz), 7.46–7.49 (1H, m), 7.68 (1H, dd, $J = 12.74$ Hz, $J = 2.26$ Hz), 7.82 (2H, s), 8.25 (1H, s), 10.24 (1H, s), 11.80 (1H, s); MS (ESI) *m/z* 298/300/302 $(M-H)^-$, 300/302/304 $(M+H)^+$. Anal. Calcd for $C_{13}H_8Cl_2FNO_2{\cdot}0.2H_2O$: C: 51.41, H: 2.79, N: 4.61. Found: C: 51.70, H: 2.75, N: 4.21.

5.8. Computational methodology

Coordinates for ERα complexed with diethylstilbestrol were obtained from the protein data bank X-ray crystal structure 3ERD.[6] Coordinates for the ERβ-genistein complex were obtained from an in-house X-ray structure, which was found to be in good agreement with previously published results.[8,26] All docking calculations were performed using the QXP software package.[27] After adding hydrogens using the hadd/hopt utilities, the X-ray ligand was minimized in the active site. The resulting structure was used as input to a series of 10 constrained simulated annealing dynamics calculations. This was done to relieve any artifacts of the X-ray refinement perceived as unfavorable interactions or strain by the QXP forcefield. During the dynamics simulation, all atoms were allowed to move in a 0.1 Å radius flat potential, after which a 20 kJ/mol/Å^2 quadratic constraint penalty was applied. Once the binding site model was generated, docking of analogs was performed using the QXP Monte Carlo docking algorithm mcdock. In general, 1000 Monte Carlo steps was sufficient for the poses and their energy scores to converge. Visualization of X-ray structures and docking results was performed using the InsightII software package (Accelrys, Inc., San Diego, CA).

5.9. Ligand binding competition experiments

A solid phase radioligand binding assay was used to measure interaction of compounds with ERα and ERβ.[28] Briefly, the ligand binding domains of human ERα and ERβ were expressed in *E. coli* and a crude lysate prepared. Using high binding microtiter plates, eight concentrations of compounds were combined with 2 nM [^{3}H]-17β-estradiol and binding measured to ERα and ERβ after incubation at room temperature for 5–18 h. The assay buffer was 1 × Dulbecco's phosphate buffered saline (pH 7.4) supplemented with 1 mM EDTA. The results were plotted as measured DPM versus concentration of test compound. For dose-response curve fitting, a four parameter logistic model on the transformed, weighted data was fit and the IC_{50} was defined as the concentration of compound decreasing maximum [^{3}H]-17β-estradiol binding by 50%.

5.10. Cell culture, cell infection, and cell treatment

Human prostate cancer cell line LNCaPLN3 cells (passage 19–35) were plated at densities of 1×10^6 cells per well in 6-well plates in phenol red free RPMI 1640 medium supplemented with 10% charcoal/dextran-treated (stripped) FBS and 1% Pen/Strep 24 h before infection. The media used for the remainder of the experiment was phenol red free RPMI 1640 medium containing 2% stripped serum and antibiotics. Cells were infected with an adenovirus coding for either human ERα or ERβ for 30 min at room temperature. Medium containing virus was aspirated, and the cells were washed once. Cells were allowed to recover in fresh medium for 4 h at 37 °C. Cells were then treated with various test compounds (1 μM) for about 20 h at 37 °C. DMSO (0.1%) was used as a vehicle control and 10 nM 17β-estradiol was used as positive control.

5.11. RNA Preparation and real-time quantitative-PCR

Total RNA was isolated from cells using RNeasy kits, followed by DNase treatment. The RNA concentration was adjusted to 0.05 mg/mL for real-time quantitative-PCR on an ABI PRISM 7700 Sequence Detection System. The sequences of primers and labeled probe used for human KRT19 (NM_002276) mRNA detection were as follows: forward primer, 5′-CAGTGTGGAGGTGGATTCCG-3′, reverse primer, 5′-GCTTCGCATGTCACTCAGGA-3′, probe, 5′FAM-CGGGCACCGATCTCGCCAAG-TAMRA3′. Expression data were normalized to input RNA (based on A_{260}) and plotted as a function of cell treatment. Fold regulation was calculated based on KRT19 expression in vehicle-treated cells. Percent of 17β-estradiol regulation was calculated by subtracting vehicle expression values from all treatments, then dividing the test compound expression level by that of 17β-estradiol treated cells.

References and notes

1. (a) Green, S.; Walter, P.; Kumar, V.; Krust, A.; Bornert, J. M.; Argos, P.; Chambon, P. *Nature* **1986**, *320*, 134–139; (b) Green, G. L.; Gilna, P.; Waterfield, M.; Baker, A.; Hort, Y.; Shine, J. *Science* **1986**, *231*, 1150–1154.
2. Kuiper, G. G. J. M.; Enmark, E.; Markku, P.-H.; Nilsson, S.; Gustafuson, J.-A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 5925–5930.
3. Nilsson, S.; Kuiper, G.; Gustafsson, J. A. *Trends Endocrinol. Metab.* **1998**, *9*, 387–395.
4. (a) Fawell, S. E.; Lees, J. A.; White, R.; Parker, M. G. *Cell* **1990**, *60*, 953–962; (b) Karimont, B. D.; Wagner, R. L.; Apriletti, J. W.; Stallcup, M. R.; Kushner, P. J.; Baxter, J. D.; Fletterick, R. L.; Yamamoto, K. R. *Genes Dev.* **1998**, *12*, 3343–3356.
5. Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J.-A.; Carlquist, M. *Nature* **1997**, *389*, 753–758.
6. Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. *Cell* **1998**, *95*, 927–937.
7. Pike, A. C. W.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A.-G.; Engstrom, O.; Ljunggren, J.; Gustafsson, J.-A. *EMBO* **1999**, *18*, 4608–4618.
8. Duiper, G. G. J. M.; Carlsson, B.; Grandien, J.; Enmark, E.; Haggblad, J.; Nilsson, S.; Gustafsson, J.-A. *Endocrinology* **1997**, *138*, 863–870.
9. Kuiper, G. G.; Lemmen, J. G.; Carlsson, B.; Corton, J. C.; Safe, S. H.; van der Saag, P. T.; van der Burg, B.; Gustafsson, J. A. *Endocrinology* **1998**, *139*, 4252–4263.
10. Lesuisse, D.; Albert, E.; Bouchoux, F.; Cerede, E.; Lefrancois, J.-M.; Levif, M.-O.; Tessier, S.; Tric, B.; Teutsch, G. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1709–1712.
11. Edsall, R. J., Jr.; Harris, H. A.; Manas, E.; Mewshaw, R. E. *Bioorg. Med. Chem. Lett.* **2003**, *11*, 3457–3474.
12. Minutolo, F.; Bertini, S.; Papi, C.; Carlson, K. E.; Katzenellenbogen, J. A.; Macchia, M. *J. Med. Chem.* **2001**, *44*, 4288–4391.
13. Minutolo, F.; Antonello, M.; Bertini, S.; Rapposelli, S.; Rossello, A.; Sheng, S.; Carlson, K. E.; Katzenellenbogen, J. A.; Macchia, M. *Bioorg. Med. Chem.* **2003**, *11*, 1247–1257.
14. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
15. Sinhababu, A. K.; Kawase, M.; Borchardt, R. T. *Synthesis* **1988**, 710–712.
16. Karabatsos, G. J.; His, N. *Tetrahedron* **1967**, *23*, 1079–1095.
17. Anstead, G. M.; Carlson, K. F.; Katzenellenbogen, J. A. *Steroids* **1997**, *62*, 268.
18. Oostenbrink, B. C.; Pitera, J. W.; van Lipzig, M. M. H.; Meerman, J. H. N.; van Gunsteren, W. F. J. *Med. Chem.* **2000**, *43*, 4594–4605.
19. Manas E. S. Unpublished results. Minimum energy geometries for compounds **6r** and **6p** were determined at the B3LYP/6-31G** level of theory, using the Jaguar software package (Jaguar 5.0; Schrodinger, LLC, Portland, OR).
20. McKinney, J. D.; Gottschalk, K. E.; Pedersen, L. J. *Mol. Struct.* **1983**, *104*, 445–450.
21. Im, H.-S.; Bernstein, E. R.; Secor, H. V.; Seeman, J. I. *J. Am. Chem. Soc.* **1991**, *113*, 4422–4431.
22. Abraham, J. R.; Bakke, J. M. *Tetrahedron* **1978**, *34*, 2947–2951.
23. Hajduk, P. J.; Sheppard, G.; Nettesheim, D. G.; Olejniczak, E. T.; Shuker, S. B.; Meadows, R. P.; Steinman, D. H.; Carrera, G. M., Jr.; Marcotte, P. A.; Severin, J.; Walter, K.; Smith, H.; Gubbins, E.; Simmer, R.; Holzman, T. F.; Morgan, D. W.; Davidsen, S. K.; Summers, J. B.; Fesik, S. W. *J. Am. Chem. Soc.* **1997**, *119*, 5818–5827.
24. Hatanaka, Y.; Fukushima, S.; Hiyama, T. *Chem. Lett.* **1989**, *10*, 1711–1714.
25. Misaki, S. *J. Fluorine Chem.* **1982**, *21*, 191.
26. Manas, E. S.; Xu, Z. B. et al. (in press).
27. McMartin, C.; Bohacek, R. S. *QXP: J. Comput. Aided Mol. Des.* **1997**, *11*, 333–344.
28. Harris, H. A.; Bapat, A. R.; Gonder, D. S.; Frail, D. F. *Steroids* **2002**, *67*, 379–384.