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Investigation of (*E*)-3-[4-(2-Oxo-3-aryl-chromen-4-yl)oxyphenyl]acrylic Acids as Oral Selective Estrogen Receptor Down-Regulators

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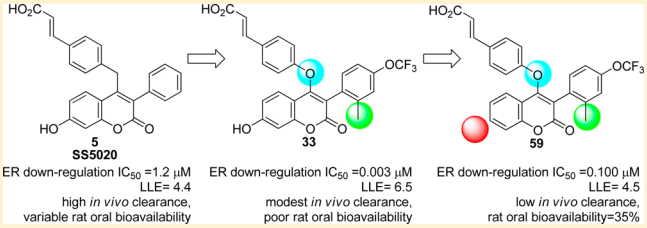
Supporting Information

ABSTRACT: A novel estrogen receptor down-regulator, 7-hydroxycoumarin (**5**, SS5020), has been reported with antitumor effects against chemically induced mammary tumors. Here, we report on our own investigation of 7-hydroxycoumarins as potential selective estrogen receptor down-regulators, which led us to the discovery of potent down-regulating antagonists, such as **33**. Subsequent optimization and removal of the 7-hydroxy group led to coumarin **59**, which had increased potency and improved rat bioavailability relative to SS5020.



INTRODUCTION

The estrogen receptor (ER) has been an oncology target for the treatment of breast cancer since the 1960s.[1] Since then, a number of selective ER modulators (SERMs) and down-regulators (SERDs) have been discovered, and this field has been reviewed extensively.[1−4] Among them, tamoxifen (a SERM, **2a**)[5,6] and fulvestrant (a SERD)[7,8] have provided invaluable treatments for a number of patients. The search for new anti-estrogen agents is still continuing, however, due to emerging drug-resistance mechanisms.[9] More specifically, there is still an unmet medical need for an oral SERD. Most SERMs and SERDs are phenols (mimicking that of estradiol **1**) because a hydroxy group is generally required for potency, but these are also associated with poor oral bioavailability (mostly due to glucuronidation and sulfation of the phenols). In short, the quest for an oral SERD remains a real challenge.

The 7-hydroxycoumarin scaffold has been known to medicinal chemists for over 50 years for its antagonistic properties against the ER.[10,11] Together with its reduced versions, 7-hydroxychromenes[12] and 7-hydroxychromanes,[13−16] they have been reported as selective ER ligands or modulators (SERMs) by many academic groups and pharmaceutical companies, including AstraZeneca.[17] Recently, SS5020 (**5**) had been reported to down-regulate the estrogen receptor with antitumor effects against chemically induced mammary tumors.[18] Structurally (Figure 1), **5** can be viewed as a hybrid of the known SERMs CHF4227 (**4**)[15] and GW7604 (**3b**),[19,20] the latter of which is a descendant of 4-hydroxytamoxifen (**2b**). A closely related analogue of **3b**, SS1020 (**6**), was also reported to be a selective ER down-regulator (SERD) by the same group.[21] **3b**, **5**, and **6** are believed to function as SERDs due to their phenyl acrylic acid moiety, which has now also been used in quinolines, such as **7a** and **7b**,[22] and others more recently.[23,24] The structural basis for the down-regulating mechanism of this phenylacrylic acid has been proposed by Greene et al. and involves a shift in helix 12, ultimately leading to degradation of the receptor.[25]

RESULTS AND DISCUSSION

In our hands, **5** bound strongly to and down-regulated ER albeit with modest potency (IC_{50} = 1.18 μM) and had good overall properties, including notably low lipophilicity (Table 1). However, although it showed efficacy upon repetitive daily oral dosing in rats,[18] the oral exposure for this molecule in both mice and rats was suboptimal and variable and would therefore benefit from further optimization. Although both solubility and permeability (as measured in Caco2 cells) appeared suitable for oral absorption, in vitro metabolism data indicated moderate to high turnover in hepatocytes across all three species of interest. This translated to very high in vivo clearances in both mice and rats. No oral levels were subsequently observed from dosing a 20 mg/kg suspension formulation of **5** in mice or from a 2 mg/kg solution in rats. Poor oral levels were consistent with the observed high in vivo clearances. However, reasonable

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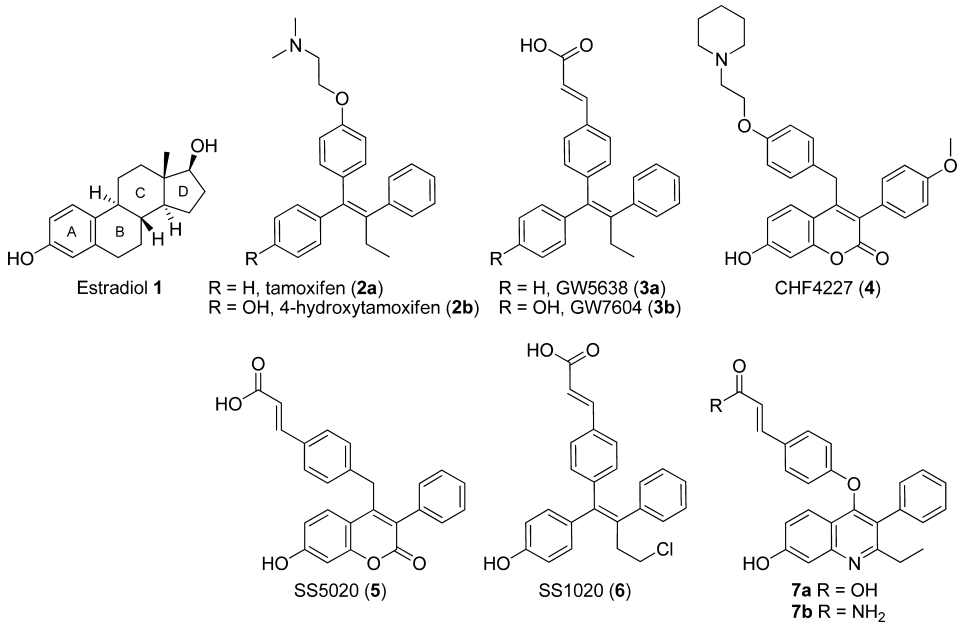

Figure 1. Chemical structures of certain ER ligands, modulators, and down-regulators.

Table 1. Detailed Profile of 3a, 3b, and 5

compound	3a	3b	5
ER binding IC_{50} (μM)[a]	<0.0032	0.00015	0.0081
ER MCF7 down-regulation IC_{50} (μM)[b]	>1.27	0.007	1.18
PR MCF7 antagonism IC_{50} (μM)[c]	4.0	0.012	1.5
PR MCF7 agonism IC_{50} (μM)[d]	>100	>30	>100
MCF7 proliferation EC_{50} (μM)[e]	0.30	nd	0.26
log $D_{7.4}$[f]	3.9	nd	1.5
LLE[g]	<2.0	nd	4.4
pK_a acid/phenol[h]	nd	nd	4.4/7.7
solubility (μM)[i]	3.3	200	470
hERG (μM)[j]	>33	>100	>100
Caco2 A–B P_{app} (10^{-6} cm/s)[k]	nd	nd	10
Hu/mu/rat PPB (% free)[l]	<0.2/6.4/<0.01	nd/nd/0.09	1.2/1.2/0.98
Hu/mu/rat heps CL_{int} (μL/min/10^6 cells)[m]	nd/nd/160	nd/nd/12	18/87/120
Mu/rat CL (mL/min/kg)[n]	6.2/80	nd	210/67
Mu/rat Vss (L/kg)[n]	1.1/0.5	nd	4.7/1.4
Mu/rat bioavailability (%)	62[o]/68[p]	nd	0[q]/0[r], 67[s]

[a]LanthaScreen TR-FRET ERα competitive binding assay (Life Technologies). [b]ERα down-regulation measured in MCF-7 cells. [c]Progesterone receptore (PR) was measured as a biomarker for ERα antagonism in MCF-7 cells. [d]PR was measured as a biomarker for ERα agonism in MCF-7 cells. [e]Decrease in proliferation of MCF-7 cells.[26] [f]Measured using shake-flask methodology with a buffer/octanol volume ratio of 100:1. [g]LLE = down-regulation pIC_{50} − log $D_{7.4}$. [h]pK_a was measured via potentiometric titrations using a Sirius Analytical GLpKa instrument. [i]Thermodynamic solubility of solid samples in a 0.1 M aqueous phosphate buffer at pH 7.4 after 24 h at 25 °C.[27] [j]Inhibition of hERG channel IC_{50} in an electrophysiology (IonWorks) assay.[28] [k]Compound permeability using Caco2 cells.[29] [l]Plasma protein binding (PPB) was assessed by equilibrium dialysis in the appropriate species plasma at 37 °C. Free and bound concentrations were quantified by LC-UV-MS.[27] [m]CL_{int} is the intrinsic clearance of hepatocytes (heps).[30] [n]In vivo parameters were generated in fed CD-1 mice and Alderley Park Han Wistar rats and involved two animals for each study. IV dosing was conducted at 0.5 mg/kg using an aqueous solution of DMSO and cyclodextrin. PO dosing was conducted as follows: [o]A 50 mg/kg 0.5% polysorbate suspension. [p]A 1 mg/kg solution in 5% DMSO, 95% of a 30% aqueous cyclodextrin solution. [q]A 20 mg/kg propylene glycol suspension. [r]A 2 mg/kg propylene glycol solution. [s]A 5 mg/kg HPMC/tween suspension.

plasma levels were detected in rats following oral dosing of a 5 mg/kg suspension. Interestingly, good oral exposure was observed for the closely related compound **3a** lacking a phenolic group in mice with low in vivo clearance and good bioavailability observed. In rats, however, very high in vivo clearance was again observed, consistent with high turnover in rat hepatocytes. Oral exposure in rats was higher than expected given the high clearance of the compound potentially due to the compound undergoing entero-hepatic recirculation.

Although the X-ray structures of **4** and **5** had not been published at the start of our investigation, that of **3a** was available (PDB ID: 1R5K),[25] and we hypothesized that the common phenyl acrylic acids should overlay whereas the phenol of the coumarin core might overlay with the phenol of **2b** (PDB ID: 3ERT), as shown in Figure 2. We thus

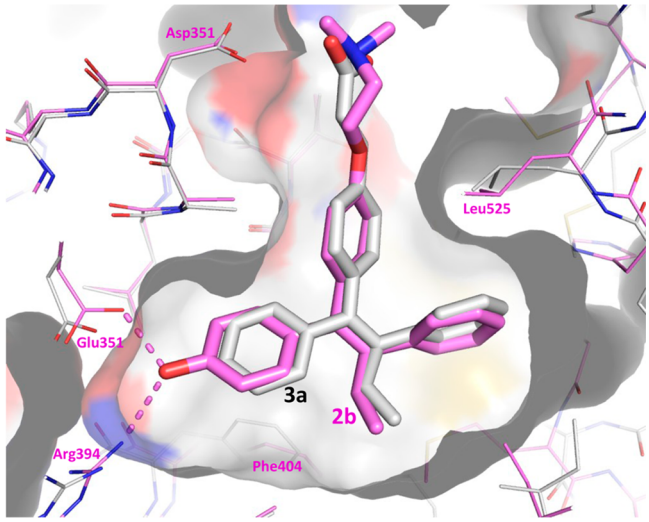

Figure 2. Overlay of **3a** (PDBID: 1R5K, gray carbons) and **2b** (PDB ID: 3ERT, pink carbons). The van der Waals radii of the protein atoms in the 1R5K structure are shown as a continuous surface, highlighting the ligand-binding pocket of the ER. Key protein residues are labeled, and polar interactions are shown as dotted lines.

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hypothesized that the structure−activity relationship (SAR) around the 3-phenyl, assumed to overlay with the D-ring of **1**, might be optimizable based on our findings as well as other groups that have reported beneficial substitutions in this lipophilic pocket.[14,22]

As an initial part of our optimization program, a set of 3-aryl-7-hydroxycoumarins were prepared as shown in Scheme 1. Variation of the 3-aryl unit was achieved via Suzuki coupling of 3-chlorocoumarin intermediate **12** with a range of aryl boronic acids. The core ring system was prepared by condensation of keto ester **9** with resorcinol under acid catalysis. The phenol moiety was then pivaloyl protected to prevent over-reaction of the coumarin core during subsequent chlorination with *N*-chlorosuccinimide (NCS) in acetic acid. Carrying out the chlorination in acetic acid was also found to be advantageous, as it disfavored chlorination of the 4-benzyl CH_2 group. Suzuki coupling proceeded efficiently either on the *tert*-butyl ester intermediate with subsequent deprotection to the acid or by first deprotecting and carrying out the coupling on the deprotected acid intermediate. Other analogues in this set were prepared via an alternative synthesis, as shown later in Scheme 4.

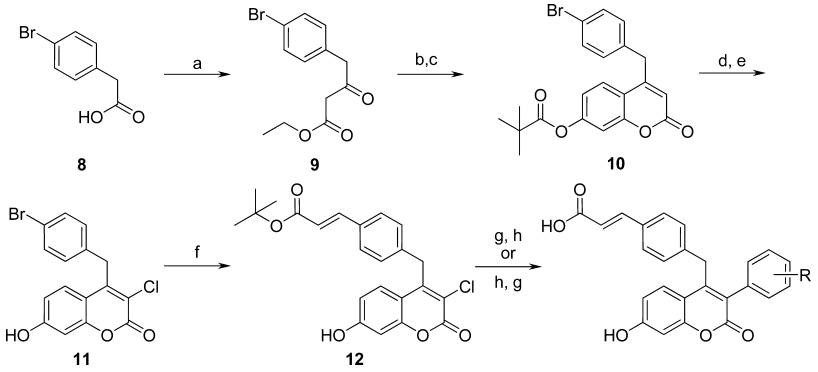
Meta substitution (Table 2) does not appear to particularly favor better potency: the direct meta analogues **13**−**15** offered no advantage over parent SS5020 in contrast to previously reported SARs in analogues of quinoline **7a**, where *m*-trifluoromethyl greatly enhanced cell potency.[22] Lipophilic groups at the para position analogues **16**−**20** offered for the first time single digit nanomolar binders with submicromolar down-regulation. The *p*-trifluoromethoxy analogue **20** proved to be even more potent with a 10-fold improvement in down-regulation (IC_{50} = 52 nM), potentially the result of inserting the trifluoromethyl group deeper into the lipophilic pocket. We observed a similar potency boost with *o*-methyl analogues **21**−**24**, which we attributed to filling a lipophilic hole occupied by the ethyl group of **2a** as well as locking the conformation of this phenyl ring. These two findings could combine to give **26**, a strong binder (1 nM) and potent down-regulator (7 nM). We obtained an X-ray structure of compound **24** in ERα, which validated our initial hypothesis and provided a rationale for the role of *o*-methyl in the observed potency improvement (Figure 3). This *o*-methyl seems to be filling the same small lipophilic hole as the terminal methyl of **3a** and **2a**, and both phenyl acrylic acid down-regulating chains overlay reasonably well. The coumarin core places the phenol in a similar position to that of **2b**, where it interacts with Glu351 and Arg394.

As lipophilicity increased with the introduction of an *o*-methyl group and either a *p*-halogen or a *p*-trifluoromethoxy substituent on the C4-phenyl ring of molecules **24**−**26**, to reduce log *D* and improve LLE, we investigated different linkers between the two aromatic rings (Table 3). *O*- and *N*-linked analogues of 4-benzylcoumarin **24** were prepared as shown in Scheme 2. Acylation of 3-methoxyphenol with substituted phenyl acetic acids (**28**) gave the desired 2-hydroxy-4-methoxyketone intermediates (**29**). These could then be condensed with either diethyl carbonate or ethyl chloroformate to effect conversion to 4-hydroxycoumarins (**30**), which in turn were converted to the key 4-bromocoumarins (**31**) with SEM protection of the 7-phenol group. *O*-Linked compounds were prepared under displacement conditions with (*E*)-*tert*-butyl 3-(4-hydroxyphenyl)acrylate and subsequent deprotection of the *tert*-butyl ester. The corresponding *N*-linked compounds were prepared by Buchwald coupling of **31a** with (*E*)-ethyl-3-(4-aminophenyl)acrylate; intermediate **34** could then be either deprotected to yield **35** or converted to the *N*-methyl derivative with methyl iodide and then deprotected to yield **36**.

The 4-*O*-linked coumarins **32** and **33** proved to be better binders and down-regulators with an additional reduction in lipophilicity, which gave superior LLEs (6.1 and 6.5, respectively) compared to 4-*C*-linked coumarin **24** (LLE = 4.8), 4-*N*-linked coumarins **35** (LLE = 4.5) and **36** (LLE = 4.9), or directly linked coumarin **27** (LLE = 4.5). Table 4 summarizes the profiles of **32** and **33**, which appeared to be much improved versions of the initial hit SS5020 in terms of binding, down-regulation, and antagonistic potencies (**33**: >15-, >420-, and >430-fold, respectively). These translated to excellent inhibition of in vitro proliferation of MCF7 cells (e.g., **33** has an EC_{50} < 0.1 nM). However, pharmacokinetic studies performed on these cells showed no improvement over **5** in terms of oral levels, although clearances were slightly reduced.

To understand the metabolic fate of our 7-hydroxycoumarins, we obtained experimental evidence through metabolite

Scheme 1. Synthesis of 3-Aryl Coumarins via Suzuki Couplings[a]



[a]Reagents and conditions: (a) (i) potassium 3-ethoxy-3-oxopropanoate (2.5 equiv), $MgCl_2$ (3 equiv), Et_3N (5.3 equiv), MeCN, (ii) **8** (1 equiv), CDI (1.25 equiv), MeCN, (iii) 80 °C, 3 h, (iv) 2N aq HCl, rt, 5 min (55%); (b) resorcinol (1 equiv), TsOH (0.1 equiv), toluene, 100 °C, 4 h (61%); (c) PivCl (1 equiv), Et_3N (1.1 equiv), DCM, rt, 10 min (99%); (d) NCS (3.1 equiv), AcOH, 75 °C, 18 h (72%); (e) K_2CO_3 (1.2 equiv), MeOH, rt, 1 h (88%); (f) *t*-butyl acrylate (1.6 equiv), DIPEA (1.2 equiv), (d*t*bpf)$PdCl_2$ (0.07 equiv), dioxane, 90 °C, 15 h (80%); (g) HCO_2H, rt (94%); (h) $ArB(OH)_2$ (1.2 equiv), S-Phos (0.14 equiv), $Pd(OAc)_2$ (0.07 equiv), K_2CO_3 (3 equiv), dioxane, water, 90 °C, 1 h or $ArB(OH)_2$ (1.3 equiv), D*t*BPPS Suzuki mix[31] (0.05 equiv), K_2CO_3 (3 equiv), DMF, water, 65 °C, 18 h (13−96%).

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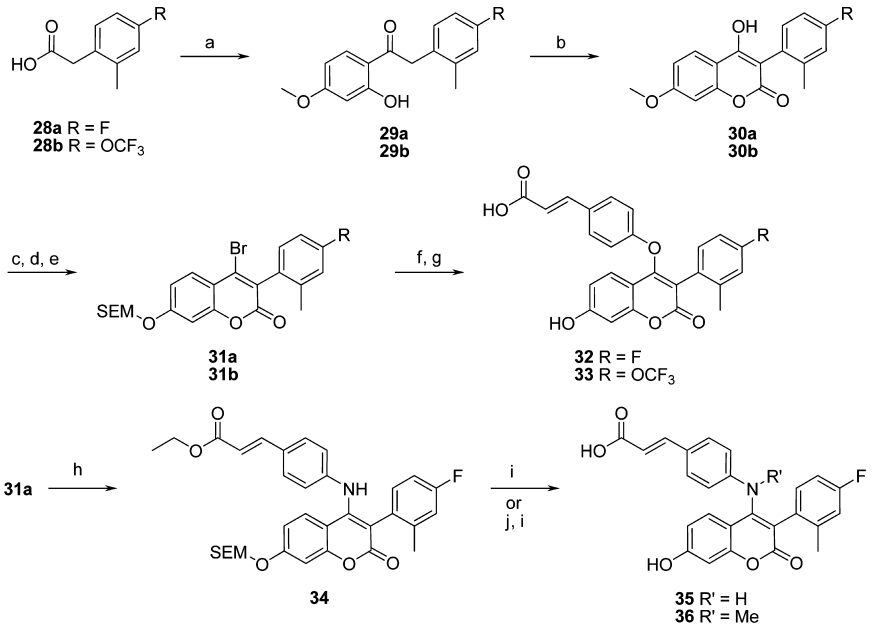
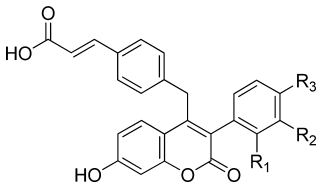
Scheme 2. Synthesis of Various Linkers[a]



[a]Reagents and conditions: (a) 3-methoxyphenol (1 equiv), $BF_3{\cdot}Et_2O$ (4 equiv), chlorobenzene, 100 °C, 2–24 h (44–67%); (b) **29a**, diethylcarbonate (15 equiv), NaH (4 equiv), 0–50 °C, 5 h (84%) or **29b**, ethyl chloroformate (3 equiv), K_2CO_3 (4 equiv), acetone, reflux, 4 h (50%); (c) $POBr_3$ (1.1 equiv), 1,2-dichlorobenzene, 170 °C, 3–24 h (73–81%); (d) 48% aq HBr, AcOH, 110 °C, 1–3 days (80–95%); (e) SEMCl (1.2 equiv), DIPEA (1.2 equiv), DCM, rt, 1–4 h (60–91%); (f) (*E*)-*tert*-butyl 3-(4-hydroxyphenyl)acrylate (1 equiv), Cs_2CO_3 (1.2 equiv), MeCN, 70 °C, 3–4 h (63–66%); (g) **31a** (1) TBAF·H_2O (5 equiv), THF, rt-50 °C, 24 h; (2) TFA, DCM, rt, 3 h (100%) or **31b** TFA, DCM, rt, 2 h (89%); (h) (*E*)-ethyl-3-(4-aminophenyl)acrylate (1.5 equiv), $Pd(P^tBu)_3$ (0.08 equiv), K_3PO_4 (1.5 equiv), dioxane, 125 °C, 2 h (43%); (i) (1) 6N HCl, ^{i}PrOH, MeOH, rt, 2 h (2) 6N NaOH, MeOH, rt, 3 h (**35** 55%; **36** 30% over 2 steps); (j) (1) NaH (1.5 equiv), DMF, 5 min, (2) MeI (6 equiv), rt, 30 min.

Table 2. Effect of the C3-Aryl on ER Binding, Down-Regulation, and Lipophilicity[a]



entry	R_1	R_2	R_3	ER binding IC_{50} (μM)[b]	ER MCF7 down-regulation IC_{50} (μM)[c]	log $D_{7.4}$	LLE
5	H	H	H	0.0081	1.18	1.5	4.4
13	H	Me	H	0.017	3.09	2.2	3.3
14	H	OMe	H	0.041	5.64	1.9	3.3
15	H	OCF_3	H	0.0059	1.71	2.9	2.9
16	H	H	F	0.0090	1.28	nd	nd
17	H	H	Cl	0.0037	0.471	2.4	4.0
18	H	H	CF_3	0.0046	0.531	2.6	3.7
19	H	H	OMe	0.0038	0.350	1.9	4.6
20	H	H	OCF_3	0.0016	0.052	2.8	4.5
21	F	H	F	0.0030	0.660	1.7	4.5
22	Cl	H	F	0.0022	0.178	nd	nd
23	OMe	H	F	0.011	2.26	1.8	3.8
24	Me	H	F	0.0039	0.117	2.1	4.8
25	Me	H	Cl	0.0015	0.030	nd	nd
26	Me	H	OCF_3	0.0010	0.007	3.1	5.1

[a]Experimental assays as per Table 1 unless otherwise specified. [b]All IC_{50} data are the mean of at least n = 2 independent measurements. Each has an SEM ± 0.2 log units. [c]All IC_{50} data are the mean of at least n = 3 independent measurements. Each has an SEM ± 0.3 log units.

identification to show that phenol **16** was extensively conjugated with glucuronides and that sulfates were being formed in rat hepatocytes. Acyl glucuronide was also observed but to a lesser extent. To reduce the formation of these conjugates, we examined how substitutions proximal to the phenol could influence metabolism through lowering lipophilicity by altering the pK_a (fluoro substitutions) or increasing steric hindrance (methyl substitutions).

5-, 6-, and 8-Fluoro analogues **39**, **42**, and **43** were prepared in a similar manner to the syntheses shown in Schemes 1 and 2 starting from the appropriately substituted fluorophenols (Scheme 3). Modification of the procedure was found to be necessary for step d during the synthesis of **43**, as the reaction proved to be very slow under the conditions we had used previously for Friedel–Crafts acylation (four equiv of $BF_3{\cdot}Et_2O$ in chlorobenezene). We found instead that the reaction proceeded more efficiently in neat $BF_3{\cdot}Et_2O$; however, cyclization of the resultant dihydroxyarylketone to coumarin with either diethyl carbonate or ethyl chloroformate was unsuccessful. Monomethylation was thus carried out on the dihydroxyketone to afford intermediate **41b**, which then reacted smoothly to afford the required intermediate coumarin.

The corresponding 6- and 8-methyl substituted analogues with a C4-carbon link to the aryl acrylic acid unit were prepared by a route alternative to that of Scheme 1, introducing the C-3 aryl unit at an earlier stage of the synthesis (Scheme 4). This approach provided efficient, shorter synthesis of the target compounds but without the flexibility of late stage C-3 aryl variation. As this method was successful for the synthesis of compounds **46** and **47** (Table 5), it was also attempted for the preparation of 5-fluoro compound **39** starting from 3-fluoro-5-methoxyphenol. In this case, however, the fluorine substituent was hydrolyzed under the reaction conditions during step c, and the isolated product was the analogous 5,7-dihydroxycoumarin;

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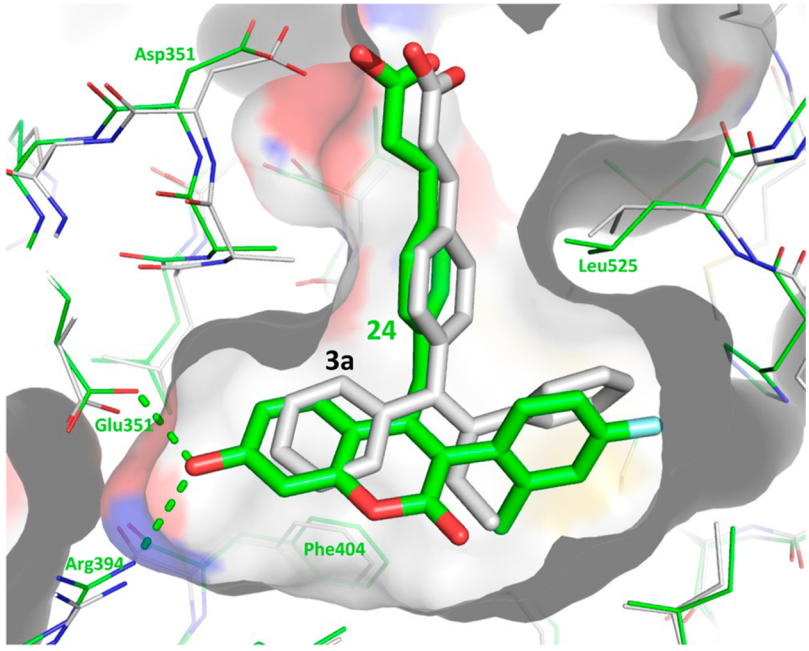
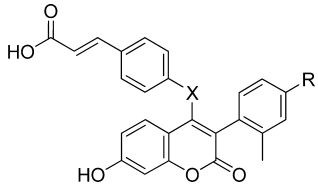


Figure 3. Overlay of **24** (PDB ID: 5AK2, green carbons) and **3a** (PDB ID: 1R5K, gray carbons). The van der Waals radii of the protein atoms in the 1R5K structure are shown as a continuous surface, highlighting the ligand-binding pocket of the ER. Key protein residues are labeled, and polar interactions are shown as dotted lines.

Table 3. Effect of the C4-Linker on ER Binding, Down-Regulation, and Lipophilicity[a]



entry	X	R	ER binding IC_{50} (μM)[b]	ER MCF7 down-regulation IC_{50} (μM)[b]	log $D_{7.4}$	LLE
27		F	0.0058[c]	0.256	2.0	4.6
24	CH_2	F	0.0039	0.117	2.1	4.8
32	O	F	0.0004	0.021	1.6	6.1
33	O	OCF_3	0.0005	0.0027	2.1	6.5
35	NH	F	0.025	1.45	1.3	4.5
36	NMe	F	0.0099	0.060	2.3	4.9

[a]Experimental assays as per Table 1 unless otherwise specified. [b]All IC_{50} data are the mean of at least $n = 3$ independent measurements. Each has an SEM ± 0.1 log units. [c]$n = 1$.

Table 4. Selected Data for *O*-Linked 7-Hydroxycoumarins 32 and 33[a]

compound	32	33
PR MCF7 antagonism IC_{50} (μM)	0.027	0.0039
PR MCF7 agonism IC_{50} (μM)	>100	>3.1
MCF7 proliferation EC_{50} (μM)	0.0045	<0.0001
pK_a acid/phenol	3.9/7.1	nd/8.2
solubility (μM)	>2000	97
hERG (μM)	>100	>100
Caco2 A–B P_{app} (10^{-6} cm/s)	19	15
Hu/Mu/rat PPB (% free)	0.25/2.8/0.51	0.12/0.84/0.37
Hu/Mu/rat heps CL_{int} (μL/min/10^6 cells)	32/68/50	11/9/41
rat CL (mL/min/kg)[b]	35	38
rat Vss (L/kg)[b]	0.9	0.7
rat bioavailability (%)[b]	5	0

[a]Experimental assays as per Table 1 unless otherwise specified. [b]IV dosing was conducted at 0.5 mg/kg using an aqueous solution of DMSO and cyclodextrin. PO dosing was conducted using HPMC/tween suspensions dosed at 5 and 50 mg/kg for **32** and **33**, respectively.

therefore, the route outlined in Scheme 1 was employed instead to provide **39**, as described above (Scheme 3).

Unfortunately, lowering the pK_a of the phenol in an attempt to reduce metabolism seemed to be detrimental to down-regulation with no significant effect on glucuronidation or sulfation as observed by metabolism identification in rat hepatocytes. Notably, 5-fluoro-7-hydroxycoumarin **39** seemed to be the most tolerated in terms of potency and seemed to stop sulfation but not glucuronidation of the phenol, which was identified as the major metabolite. The introduction of fluoro substituents reduced lipophilicity through the expected effect on the phenol's pK_a even in the more distant 5-fluoro analogue. Methyl blockers on each side of the phenol also proved detrimental in terms of potency, and as a result, we decided to probe phenol replacements instead. Our hypothesis was that despite an expected loss of potency improvements in pharmacokinetics in the absence of the 7-hydroxy group may result in compounds with suitable profiles to function as in vivo tools.

Exploration of the C-7 functionality was achieved for several compounds in the C4-carbon linked series by carrying out a variety of palladium-catalyzed reactions on the versatile 7-triflate intermediate bearing the appropriate C-3 aryl substitution, as shown in Scheme 5. In the case of **53**, the triflate was first reacted in a Stille coupling to give alkene **51**. Ozonolysis of this product afforded the bis-aldehyde **52**, which could be converted to the 7-difluoromethyl analogue by reaction with Deoxo-Fluor. The fluorination was found to be selective for the 7-formyl group, presumably due to electronic differences between the two aldehydes, although a smaller amount of the bis-difluoromethyl product was also formed and was separated

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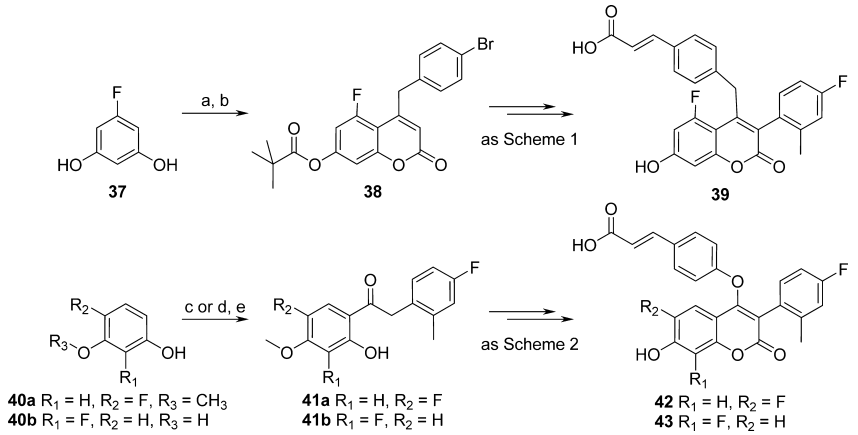
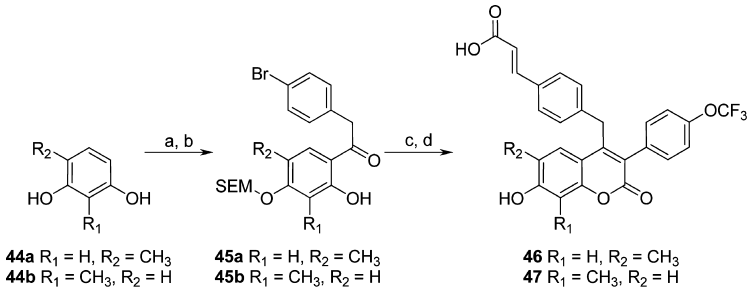
Scheme 3. Synthesis of 5-, 6-, and 8-Fluoro Substituted 7-Hydroxycoumarins[a]



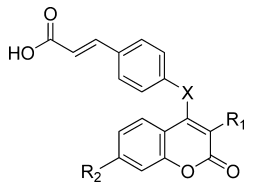
[a]Reagents and conditions: (a) **9**, $MeSO_3H$, 0 °C, rt, 3h (79%); (b) PivCl (1.1 equiv), Et_3N (1.1 equiv), DCM, rt, 1 h (65%); (c) (4-fluoro-2-methylphenyl)acetic acid (1.1 equiv), $BF_3{\cdot}Et_2O$ (4 equiv), chlorobenzene, 100 °C, 20 h (52%); (d) (4-fluoro-2-methylphenyl)acetic acid (1.1 equiv), $BF_3{\cdot}Et_2O$ (40 equiv), 80 °C, 24 h (32%); (e) MeOH (1.05 equiv), DIAD (1.3 equiv), PPh_3 (1.3 equiv), THF, 0 °C, 30 min (38%).

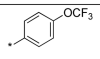
Scheme 4. Synthesis of 6- and 8-Methyl Substituted 7-Hydroxycoumarins[a]



[a]Reagents and conditions: (a) 2-(4-bromophenyl)acetic acid (1.1 equiv), $BF_3{\cdot}Et_2O$ (3.5 equiv), chlorobenzene, 90 °C, 18 h (36−45%); (b) SEMCl (0.95 equiv), DIPEA (2 equiv), DCM, rt, 1 h (44−79%); (c) (i) 2-(4-(trifluoromethoxy)phenyl)acetic acid (1.95 equiv), CDI (2 equiv), DMF, rt, 45 min, (ii) **45a** or **45b**, K_2CO_3 (3 equiv), DMAP (0.2 equiv), 80 °C, 2.5 h, (iii) 5N HCl in ^{i}PrOH, rt, 2 h (46−55%); (d) (i) *tert*-butyl acrylate (1.5 equiv), (d*t*bpf)$PdCl_2$ (0.1 equiv), DIPEA (1.2 equiv), dioxane, 80 °C, 3 h, (ii) TFA, DCM, rt, 16 h (41−61%).

Table 5. Effect of Core Substitutions on ER Binding, Down-Regulation, and in Vitro Properties[a]



Entry	*X*	*R1*	*R2*	*ER binding IC_{50} (μM)*[b]	*ER MCF7 down-regulation IC_{50} (μM)*[c]	*log$D_{7.4}$*	*Hu/Rat heps Clint (μL/min/10^6 cells)*	*Phenol pKa*
24	CH_2		H	0.0039	0.117	2.1	11/81	7.7
32	O		H	0.0004	0.021	1.6	32/50	7.1
39	CH_2		5-F	0.0013	0.055	1.6	14/69	7.2
42	O		6-F	0.0074	>3.0	0.8	28/84	5.8
43	O		8-F	0.0032	0.509	0.6	11/33	6.1
20	CH_2		H	0.0016	0.052	2.8	9/40	8.7
46	CH_2		6-CH_3	0.010	0.320	3.1	17/37	7.6
47	CH_2		8-CH_3	0.280	5.84	3.4	20/39	8.5

[a]Experimental assays as per Table 1 unless otherwise specified. [b,c]All IC_{50} data are the mean of at least n = 3 independent measurements. Each has an SEM ± 0.1 log units.

from the product during chromatographic purification. Reintroduction of the acrylic acid unit was achieved by condensation with malonic acid and subsequent decarboxylation to afford **53**. Other compounds in this set of C-7 variants included the 7-methoxy compound **54** and its fluoro analogue **55**, which were prepared from 3-methoxyphenol or 3-fluorophenol, respectively, in a manner similar to the route shown in Scheme 4.

Variation at C-7 was also carried out in the C-4 *O*-linked series. The 7-ethoxy analogue **64** was prepared via a similar route to that shown in Scheme 2; conversion of 4-hydroxy-7-methoxycoumarin

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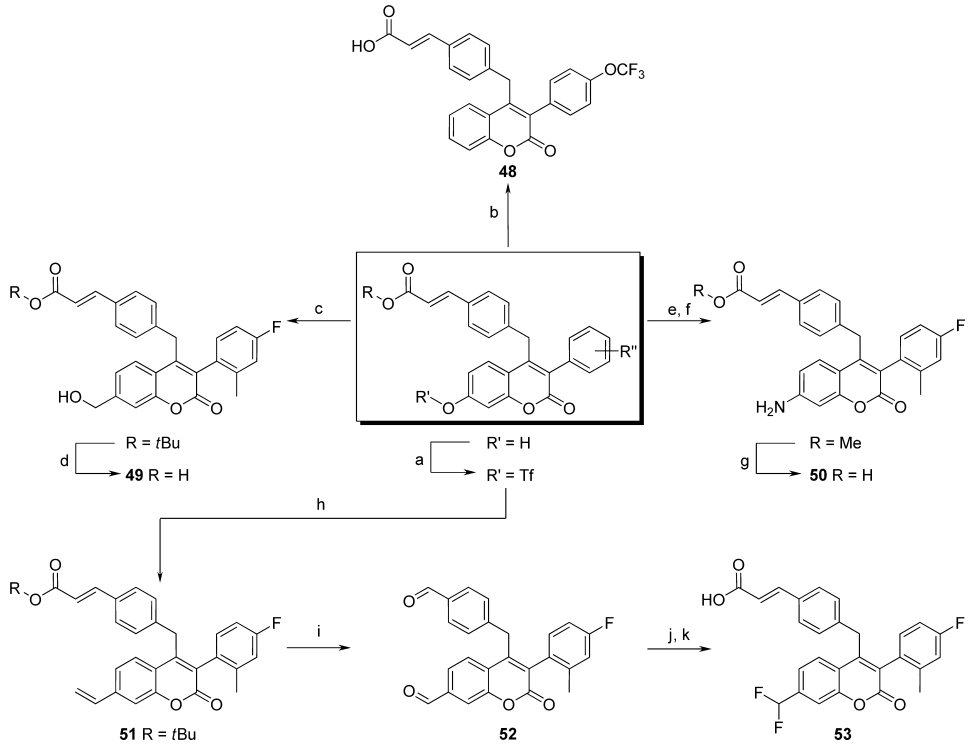
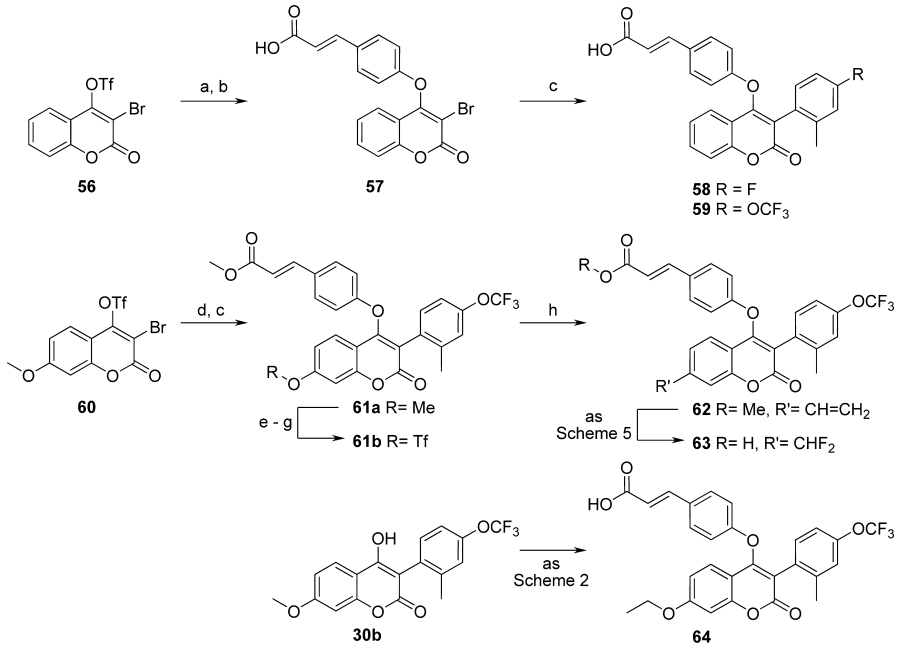
Scheme 5. Synthesis of Various 7-Substituted, 4-*C*-Linked Coumarins[a]



[a]Reagents and conditions: (a) Tf_2NPh (1.2 equiv), Et_3N (1.3 equiv), DCM, rt, 18 h or Tf_2O (1.1 equiv), Et_3N (3 equiv), DCM, 0 °C, rt, 30 min; (b) $Pd(OAc)_2$ (0.2 equiv), PPh_3 (0.4 equiv), Et_3N (3 equiv), HCO_2H, DMF, 60 °C, 1 h (42%); (c) $^tBuOCH_2BF_3K$ (1.3 equiv), dichloro(1,5-cyclooctadiene) Pd(II) (0.05 equiv), dppf (0.1 equiv), K_3PO_4 (7.2 equiv), ^tBuOH, H_2O, 110 °C, 20 h; (d) TFA, DCM, rt, 2 h (28% over 3 steps); (e) $MeCONH_2$ (1.2 equiv), $Pd(OAc)_2$ (0.05 equiv), X-Phos (0.1 equiv), K_3PO_4 (1.6 equiv), toluene, 90 °C, 5 h (95%); (f) 36% aq HCl, THF, MeOH, 65 °C, 3 h (36%); (g) LiOH (1.5 equiv), MeOH, THF, H_2O, 75 °C, 1 h (90%); (h) $Bu_3(CH_2{=}CH)Sn$ (1.2 equiv), $Pd_2(dba)_3$ (0.05 equiv), LiCl (6.5 equiv), PPh_3 (1.01 equiv), NMP, 60 °C, 18 h (88%); (i) (1) $K_2OsO_4.2H_2O$ (0.1 equiv), NMO (3 equiv), THF, rt, 5 h, (2) $NaIO_4$ (5 equiv), H_2O, rt, 20 h (quant); (j) Deoxo-Fluor (2 equiv), DCM, rt, 5 h (40%); (k) malonic acid (2.1 equiv), piperidine (0.3 equiv), pyridine, 90 °C, 2 h (85%).

Scheme 6. Synthesis of Various 7-Substituted 4-*O*-Linked Coumarins[a]



[a]Reagents and conditions: (a) (*E*)-*tert*-butyl 3-(4-hydroxyphenyl)acrylate (1 equiv), K_2CO_3 (5 equiv), dioxane, 50 °C, 4 h (46%); (b) TFA, 0 °C, 2 h (97%); (c) **58** (4-fluoro-2-methylphenyl)boronic acid or **59** [2-methyl-4-(trifluoromethoxy)-phenyl]boronic acid (1.5 equiv), $Pd(OAc)_2$ (0.05 equiv), S-Phos-$SO_3^-Na^+$ (0.1 equiv), Na_2CO_3 (4 equiv), dioxane, H_2O, 100 °C, 2 h (11%); (d) methyl 2-(*E*)-3-(4-hydroxyphenyl)prop-2-enoate (1.1 equiv), K_2CO_3 (2 equiv), MeCN, rt, 4 h (43%); (e) BBr_3 (5 equiv), DCM, −78 °C, rt, 18 h (quant.); (f) H_2SO_4, MeOH, 90 °C, 18 h (77%); (g) Tf_2O (1.3 equiv), Et_3N (3 equiv), DCM, −10 °C, 2 h (80%); (h) $(CH_2{=}CH)BF_3K$ (1.5 equiv), Et_3N (2 equiv), $Pd(dppf)Cl_2$ (0.1 equiv), 100 °C, 4 h (78%).

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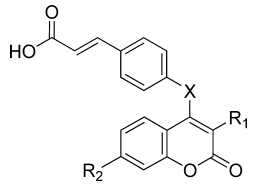
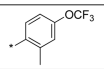
Table 6. Phenol Replacements[a]



Entry	X	R_1	R_2	ER binding IC_{50} (μM)[b]	ER MCF7 down-regulation IC_{50} (μM)[c]	$logD_{7.4}$	Hu/Rat heps Clint (μL/min/10^6 cells)	LLE
48	CH_2		H	0.36	1.3	2.8	8/20	3.1
54	CH_2		OCH_3	1.1	3.6	3.0	23/23	2.4
55	CH_2		F	5.8	10.6	2.5	n.d./4.4	2.5
49	CH_2		CH_2OH	0.100	1.00	1.2	14/81	4.8
50	CH_2		NH_2	n.d.	1.38	1.7	10/18	4.2
53	CH_2		CHF_2	0.39	>2.15	2.2	<3/16	<3.4
58	O		H	0.072	0.248	2.0	9/27	4.6
59	O		H	0.015	0.100	2.5	7/19	4.5
63	O		CHF_2	0.050	0.506	3.0	11/3	3.3
64	O		OCH_2CH_3	0.050	0.253	n.d.	20/21	n.d.

[a]Experimental assays as per Table 1 unless otherwise specified. [b,c]All IC_{50} data are the mean of at least $n = 2$ independent measurements. Each has an SEM ± 0.2 log units.

30b to 4-bromocoumarin and subsequent deprotection with HBr in acetic acid gave the phenol intermediate, which could be alkylated with ethyl iodide. Displacement of the 4-bromo group with (*E*)-*tert*-butyl 3-(4-hydroxyphenyl)acrylate followed by ester hydrolysis then led to final product **64**.

The synthesis of other C-4 *O*-linked C-7 variants is shown in Scheme 6. The 7-H analogue was synthesized from 3-bromo-4-triflate intermediate **56**, prepared according to the method reported by Wu et al.[32] Selective displacement of the triflate with (*E*)-*tert*-butyl 3-(4-hydroxyphenyl)acrylate and deprotection gave acid **57**, which could then be converted to **58** or **59** by Suzuki coupling. A similar approach starting from 7-methoxy-4-triflate **60** gave intermediate **61a**. Conversion of the 7-methoxy group to the triflate was then carried out as shown to provide **61b**, which could then be taken through to 7-difluoromethyl compound **63** via 7-vinyl compound **62** in an analogous route to that for compound **53** (Scheme 5).

Removal of the 7-hydroxy (compounds **48**, **58**, and **59**, Table 6) resulted in significant loss of both ER binding and down-regulation potency. Notably, **59**, carrying an optimized aryl substituent, was the most potent des-phenol synthesized in this scaffold with a down-regulation IC_{50} of 100 nM. Replacement with a fluorine (**55**) was significantly worse than the corresponding hydrogen (**48**) and thus offered no way forward. Similarly, 7-aminocoumarin **50** showed a large loss of down-regulation potency (>100-fold) compared to phenol **24**. The isolipophilic 7-difluoromethyl analogues **53** and **63** also showed reduced potency, whereas the extended hydroxymethyl **49**, although less potent, proved to be the most promising replacement in terms of LLE. However, experimental evidence showed that metabolism then switched to oxidation of the benzylic alcohol to the corresponding carboxylic acid with no observed parent glucuronidation. Capped phenols **54** and **64** as 7-methoxy and 7-ethoxy coumarins, respectively, lost ~100-fold potency in terms of down-regulation. We found that the best compromise was the des-phenol analogues **58** and **59**, which were profiled by pharmacokinetic studies (Table 7).

Pleasingly, in rats, the compounds were characterized by low clearances and much improved bioavailabilities (47% and 35% for **58** and **59**, respectively). Des-phenol **59**, in particular, with a down-regulation potency of 100 nM represented an attractive in vivo tool compound to evaluate the potential of an oral SERD.

Table 7. Detailed Profile of *O*-Linked Coumarins 58 and 59[a]

compound	58	59
PR MCF7 antagonism IC_{50} (μM)	0.33	0.18
PR MCF7 agonism IC_{50} (μM)	>3.1	>3.1
MCF7 proliferation EC_{50} (μM)	nd	0.020
pK_a	5.3	3.7
solubility (μM)	>1500	>470
hERG (μM)	>100	nd
Caco2 A–B P_{app} (10^{-6} cm/s)	47	39
Hu/rat PPB (% free)	0.50/1.4	0.16/0.32
Hu/rat heps CL_{int} (μL/min/10^6 cells)	9/27	7/19
rat CL (mL/min/kg)[b]	23	15
rat Vss (L/kg)[b]	0.47	1.7
rat bioavailability (%)[b]	47	35

[a]Experimental assays as per Table 1 unless otherwise specified. [b]IV dosing was conducted at 0.5 mg/kg using an aqueous solution of DMSO and cyclodextrin. PO dosing was conducted using HPMC/tween suspensions dosed at 1 mg/kg.

CONCLUSION

7-Hydroxycoumarins proved to be very potent SERD antagonists with good overall properties but were prone to metabolism via sulfation and glucuronidation, leading to generally poor oral bioavailability. Through optimization of the scaffold with regard to the linker and aryl substituents, highly potent phenols, such as **33**, were identified, although bioavailability remained poor. Removal of the 7-hydroxy groups in des-phenols **58** and **59** resulted in improvements in pharmacokinetic profiles with reasonable levels of potency being retained. These compounds have the potential to be used as in vivo tools to explore the potential of oral SERDs.

EXPERIMENTAL SECTION

General Procedures. All solvents and reagents were purchased from commercial sources and used without further purification. Flash silica chromatography was typically performed on an Isco Companion, using Silicycle silica gel, 230–400 mesh 40–63 μm cartridges, Grace

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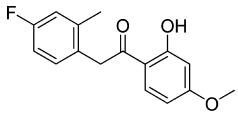
Resolv silica cartridges, or Isolute Flash Si or Si II cartridges. Reverse phase chromatography was performed using a Waters XBridge Prep C18 OBD column (5 μm silica, 19 mm diameter, 100 mm length) using decreasingly polar mixtures of either water containing 1% NH_3 and MeCN or water containing 0.1% formic acid and MeCN as eluents. Analytical LC-MS was performed on a Waters 2790 LC with a 996 PDA and 2000 amu ZQ Single Quadrupole Mass Spectrometer using a Phenomenex Gemini 50 × 2.1 mm, 5 μm C18 column, or UPLC was performed on an Waters Acquity Binary Solvent Manager with Acquity PDA and an SQD mass spectrometer using a 50 × 2.1 mm, 1.7 μm BEH column from Waters. Purities were measured by UV absorption at 254 nm or TIC and are ≥95% unless otherwise stated. NMR spectra were recorded on a Bruker Av400, Av500, or DRX400 spectrometer at 400 or 500 MHz in *d6*-DMSO at 303 or 297 K unless otherwise indicated. ^{1}H NMR spectra are reported as chemical shifts in parts per million (ppm) relative to an internal solvent reference.

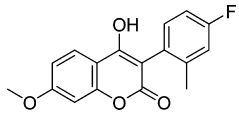
All IC_{50} data are quoted as geometric mean values, and statistical analyses are available in the Supporting Information. All experimental activities involving animals were carried out in accordance with AstraZeneca animal welfare protocols, which are consistent with The American Chemical Society Publications rules and ethical guidelines.

Synthesis of Representative Key Example Compounds 32, 33, 58, and 59. *2-(4-Fluoro-2-methylphenyl)-1-(2-hydroxy-4-methoxyphenyl)ethanone (**29a**).*



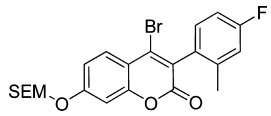
BF_3·Et_2O (53.4 mL, 432.5 mmol) was added dropwise to a stirred mixture of 3-methoxyphenol (11.87 mL, 108.12 mmol) and (4-fluoro-2-methylphenyl)acetic acid (20 g, 118.9 mmol) in chlorobenzene (232 mL) at 100 °C under argon. The resulting mixture was stirred at 100 °C for 20 h. The reaction mixture was quenched with water (340 mL), diluted with EtOAc (200 mL), and washed with brine (2 × 200 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated. The crude product was adsorbed on silica gel and purified by flash chromatography on silica gel eluting with 0 to 10% EtOAc in heptane. The solvent was evaporated to dryness to afford **29a** (19.89 g, 67%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$, 303 K) 2.27 (3H, s), 3.86 (3H, s), 4.23 (2H, s), 6.46 (1H, d), 6.49 (1H, dd), 6.88 (1H, td), 6.95 (1H, dd), 7.09 (1H, dd), 7.77 (1H, d), 12.62 (1H, s); *m*/*z* 275 $[M + H]^+$.

*3-(4-Fluoro-2-methylphenyl)-4-hydroxy-7-methoxy-2H-chromen-2-one (**30a**).*



A solution of 2-(4-fluoro-2-methylphenyl)-1-(2-hydroxy-4-methoxyphenyl)ethanone (19.89 g, 0.65 mol) in diethyl carbonate (119 mL, 0.98 mol) at room temperature was added dropwise to a stirred suspension of sodium hydride (10.44 g, 0.26 mol) in diethyl carbonate (119 mL, 0.98 mol) at 0 °C. The resulting mixture was stirred at 50 °C for 5 h. The reaction mixture was allowed to cool to 0 °C and then quenched with water (100 mL). The remaining diethyl carbonate was extracted into Et_2O. The aqueous layer was carefully acidified to pH 3 with 2N aq HCl and extracted with DCM. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The yellow solid residue was triturated with Et_2O, and the solid product was collected by filtration and dried under vacuum to give **30a** (16.48 g, 84%) as an off-white solid, which was used without further purification. ^{1}H NMR (400 MHz, DMSO, 303 K) 2.13 (3H, s), 3.88 (3H, s), 6.98 (1H, dd), 7.00 (1H, d), 7.04 (1H, td), 7.14 (1H, dd), 7.20 (1H, dd), 7.88 (1H, d), 11.12 (1H, s); *m*/*z* 301 $[M + H]^+$.

*4-Bromo-3-(4-fluoro-2-methylphenyl)-7-((2-(trimethylsilyl)-ethoxy)methoxy)-2H-chromen-2-one (**31a**).*

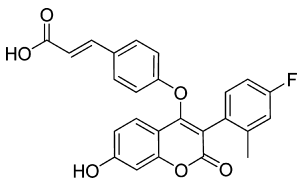


30a (16.48 g, 54.9 mmol) and $POBr_3$ (6.14 mL, 60.4 mmol) in 1,2-dichlorobenzene (288 mL, 2.6 mol) were stirred at 170 °C for 3 h. After cooling, the solvent was removed under vacuum. The residue was diluted with DCM, quenched with saturated aqueous $NaHCO_3$ and extracted with DCM. The combined organic phases were dried over $MgSO_4$, filtered, and concentrated. The residual solid was triturated with petroleum ether and dried under vacuum at 50 °C to afford 4-bromo-3-(4-fluoro-2-methylphenyl)-7-methoxy-2*H*-chromen-2-one (16.12 g, 81%) as a gray solid. ^{1}H NMR (400 MHz, DMSO, 303 K) 2.13 (3H, s), 3.92 (3H, s), 7.05−7.16 (3H, m), 7.19 (1H, dd), 7.27 (1H, dd), 7.82 (1H, d); *m*/*z* 363 $[M + H]^+$.

A suspension of 4-bromo-3-(4-fluoro-2-methylphenyl)-7-methoxy-2*H*-chromen-2-one (14.98 g, 41.25 mmol) and HBr, 48% in H_2O (327 mL, 2.9 mol) in acetic acid (100 mL), was stirred at 110 °C for 3 days. The reaction mixture was allowed to cool to room temperature and concentrated to dryness. Water was added to the residue; the solid was filtered, washed twice with water, and then taken up in EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$, filtered, and concentrated to afford a gray solid. The crude product was adsorbed onto silica and purified by flash silica chromatography eluting with 0 to 10% EtOAc in DCM. The solvent was evaporated to dryness to afford 4-bromo-3-(4-fluoro-2-methylphenyl)-7-hydroxy-2*H*-chromen-2-one (9.59 g, 80%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO, 303 K) 2.12 (3H, s), 6.82 (1H, d), 6.92 (1H, dd), 7.10 (1H, td), 7.18 (1H, dd), 7.25 (1H, dd), 7.74 (1H, d), 10.86 (1H, s); *m*/*z* 349 $[M + H]^+$.

(2-(Chloromethoxy)ethyl)trimethylsilane (3.40 mL, 19.2 mmol) was added dropwise to a stirred solution of 4-bromo-3-(4-fluoro-2-methylphenyl)-7-hydroxy-2*H*-chromen-2-one (5.59 g, 16.0 mmol) and DIPEA (3.35 mL, 19.2 mmol) in DCM (57.3 mL) at 0 °C. The resulting mixture was stirred at room temperature for 1 h. The reaction mixture was quenched with water; the phases were separated, and the organic layer was washed with 0.5 M HCl, brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash silica chromatography eluting with 100% DCM and then further purified by flash silica chromatography with an elution gradient of 0 to 100% DCM in heptane. Product-containing fractions were evaporated to dryness to afford **31a** (6.17 g, 60%) as a pale yellow oil, which solidified upon standing, containing 0.25 equiv of 2-trimethylsilylethanol. ^{1}H NMR (400 MHz, DMSO, 303 K) −0.01 (9H, s), 0.91 (2H, t), 2.13 (3H, s), 3.68−3.82 (2H, m), 5.40 (2H, s), 7.06−7.17 (3H, m), 7.18 (1H, dd), 7.26 (1H, dd), 7.77−7.89 (1H, m); *m*/*z* 479 $[M + H]^+$.

*(E)-3-(4-((3-(4-fluoro-2-methylphenyl)-7-hydroxy-2-oxo-2H-chromen-4-yl)oxy)phenyl)acrylic acid (**32**).*



(*E*)-*tert*-Butyl 3-(4-hydroxyphenyl)acrylate (179 mg, 0.8 mmol) was added to **31a** (390 mg, 0.8 mmol) in MeCN (10 mL). Cs_2CO_3 (318 mg, 1.0 mmol) was added, and the solution was stirred at 70 °C for 4 h. The mixture was allowed to cool, poured into water, and extracted with EtOAc twice. The combined extracts were dried over $MgSO_4$, filtered, and evaporated. The residue was purified by flash silica chromatography, eluting with 1:4 EtOAc/heptane. Product-containing fractions were evaporated to give (*E*)-*tert*-butyl 3-(4-((3-(4-fluoro-2-methylphenyl)-2-oxo-7-((2-(trimethylsilyl)ethoxy)methoxy)-2*H*-chromen-4-yl)oxy)phenyl)acrylate (320 mg, 64%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO, 303 K) 0.00 (9H, s), 0.87 (2H, t),

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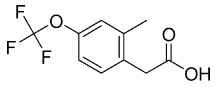
1.48 (9H, s), 2.13 (2H, s), 3.70−3.81 (2H, m), 5.39 (2H, s), 6.36 (1H, s), 6.40 (1H, s), 6.90 (1H, td), 6.96 (1H, s), 6.99 (1H, d), 7.03 (1H, dd), 7.18 (1H, s), 7.19 (1H, d), 7.21 (1H, s), 7.42 (1H, s), 7.46 (1H, s), 7.50 (1H, s), 7.54 (1H, s).

(*E*)-*tert*-Butyl 3-(4-((3-(4-fluoro-2-methylphenyl)-2-oxo-7-((2-(trimethylsilyl)ethoxy)methoxy)-2*H*-chromen-4-yl)oxy)phenyl)-acrylate (100 mg, 0.16 mmol) was added to a solution of TFA (1 mL, 13.5 mmol) in DCM (2 mL) and cooled in an ice bath to 0 °C. The yellow solution was stirred for 1 h, and then the solvent was removed under reduced pressure. Toluene was added to the residue, and the mixture was evaporated to dryness to afford **32** (70 mg, 100%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO, 303 K) 2.11 (3H, s), 6.33 (1H, s), 6.37 (1H, s), 6.76 (1H, d), 6.79 (1H, d), 6.85 (1H, d), 6.93 (1H, d), 6.95 (1H, s), 7.14−7.18 (1H, m), 7.23 (1H, d), 7.37 (1H, d), 7.45 (1H, d), 7.50 (1H, d), 10.72 (1H, s), 12.26 (1H, s); *m*/*z* 433 $[M + H]^+$.

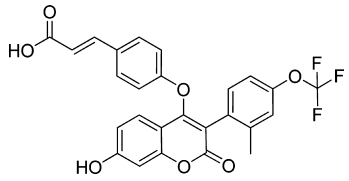
*(2-Methyl-4-(trifluoromethoxy)phenyl)acetic Acid (**28b**).*



$Pd_2(dba)_3$ (1.145 g, 1.25 mmol), 2′-(dicyclohexylphosphino)-*N*,*N*-dimethyl-2-biphenylamine (0.984 g, 2.5 mmol), and 2-methyl-4-(trifluoromethoxy)bromobenzene (4.09 mL, 25.0 mmol) were added to degassed THF (50 mL). (2-*tert*-Butoxy-2-oxoethyl)zinc(II) chloride (0.5 M in Et_2O, 100 mL, 50.0 mmol) was then added, and the reaction mixture was degassed with nitrogen for 5 min before being heated at reflux under nitrogen for 2 h and then allowed to cool to room temperature. Silica was added to the mixture, and then the volatiles were removed under vacuum. The residue was purified via silica chromatography, eluting with 1:30 EtOAc/pentane to give *tert*-butyl 2-(2-methyl-4-(trifluoromethoxy)phenyl)acetate (5.8 g, 80%) as a colorless solid. ^{1}H NMR (400 MHz, DMSO, 303 K) 1.40 (9H, s), 2.26 (3H, s), 3.62 (2H, s), 7.13 (1H, d), 7.18 (1H, d), 7.29 (1H, d); *m*/*z* 277 $[M + H]^+$.

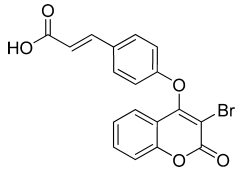
tert-Butyl 2-(2-methyl-4-(trifluoromethoxy)phenyl)acetate (5.8 g, 20.0 mmol) was stirred in DCM (30 mL), and 2,2,2-trifluoroacetic acid (20 mL, 269.24 mmol) was added. The mixture was stirred at room temperature for 3 h. The volatiles were removed under vacuum; toluene was added, and the mixture was evaporated to dryness to give **28b** (4.0 g, 85%) as a light brown solid. ^{1}H NMR (400 MHz, DMSO, 303 K) 2.32 (3H, s), 3.68 (2H, s), 7.18 (1H, d), 7.24 (1H, s), 7.36 (1H, d), 12.43 (1H, s); *m*/*z* 233 $[M - H]^-$.

*(E)-3-(4-((7-Hydroxy-3-(2-methyl-4-(trifluoromethoxy)phenyl)-2-oxo-2H-chromen-4-yl)oxy)phenyl)acrylic Acid (**33**).*



The title compound was prepared from **28b** in a similar manner to that described for **32**. ^{1}H NMR (700 MHz, DMSO, 303 K) 2.14 (3H, s), 6.35 (1H, d), 6.81 (1H, dd), 6.87 (1H, d), 6.93−6.96 (2H, m), 7.03 (1H, d), 7.08 (1H, s), 7.25 (1H, d), 7.43−7.5 (4H, m), 10.76 (1H, s); ^{13}C NMR (176 MHz, DMSO, 303 K) 19.2, 102.3, 107.9, 111.8, 113.4, 116.9, 117.6, 118.2, 119.9 (q), 121.7, 125.3, 129.5, 129.6, 130.0, 132.5, 140.1, 142.8, 147.9, 154.9, 157.2, 159.6, 161.3, 162.1, 167.4; HRMS (ESI$^+$) anal. calcd for $C_{26}H_{17}F_3O_7$ $[M + H]^+$ 499.09991, found 499.09906.

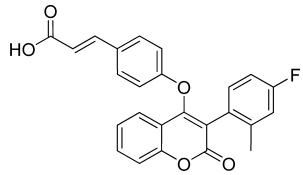
*(2E)-3-(4-((3-Bromo-2-oxo-2H-chromen-4-yl)oxy)phenyl)acrylic Acid (**57**).*



A solution of *tert*-butyl (2*E*)-3-(4-hydroxyphenyl)prop-2-enoate (650 mg, 3.0 mmol) in MeCN was stirred at 45 °C for 3 h and then added dropwise to a solution of 3-bromo-2-oxo-2*H*-chromen-4-yl trifluoromethanesulfonate (1.0 g, 2.7 mmol) in MeCN (100 mL) and K_2CO_3 (1.85 g, 13.4 mmol), and the resulting solution was stirred for another 1 h at 50 °C. The reaction was then quenched by the addition of water (200 mL) and extracted with EtOAc (3 × 100 mL). The organic layers were combined, dried over Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by silica gel chromatography, eluting with 10% EtOAc in petroleum ether. Product fractions were evaporated to afford *tert*-butyl (2*E*)-3-(4-((3-bromo-2-oxo-2*H*-chromen-4-yl)oxy)phenyl)prop-2-enoate (550 mg, 46%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$, 303 K) 1.52 (9H, s), 6.32 (1H, d), 6.96 (2H, d), 7.28−7.30 (2H, m), 7.51−7.66 (5H, m); *m*/*z* 465 $[M + Na]^+$.

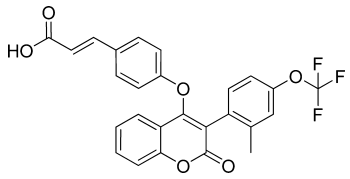
A solution of *tert*-butyl (2*E*)-3-(4-((3-bromo-2-oxo-2*H*-chromen-4-yl)oxy)phenyl)prop-2-enoate (924 mg, 2.08 mmol) in TFA (5 mL) was stirred at 0 °C for 2 h. The resulting mixture was evaporated to dryness under vacuum to afford **57** (781 mg, 97%) as a colorless solid. *m*/*z* 423 $[M + MeCN + H]^+$.

*(2E)-3-(4-[[3-(4-Fluoro-2-methylphenyl)-2-oxo-2H-chromen-4-yl]-oxy]phenyl)acrylic Acid (**58**).*



To a 25 mL round-bottom flask purged and maintained with an inert atmosphere of nitrogen were placed (4-fluoro-2-methylphenyl)boronic acid (104 mg, 0.7 mmol), **57** (200 mg, 0.5 mmol), sodium 2′-dicyclohexylphosphino-2,6-dimethoxy-1,1′-biphenyl-3-sulfonate hydrate (54. Eight mg), a solution of Na_2CO_3 (218 mg, 2.1 mmol) in water (5 mL), and a solution of $Pd(OAc)_2$ (11.60 mg, 0.05 mmol) in 1,4-dioxane (10 mL). The resulting solution was stirred for 2 h at 100 °C in an oil bath and was then diluted with water (20 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated under vacuum. The crude product (292 mg) was purified by preparative HPLC to afford **58** (95 mg, 44%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$, 303 K) 2.10 (3H, s), 6.33 (1H, d), 6.71−6.89 (4H, m), 7.11−7.15 (1H, m), 7.36−7.48 (3H, m), 7.50−7.76 (2H, m), 7.70−7.79 (2H, m); ^{13}C NMR (176 MHz, DMSO, 303 K) 19.2, 112.1 (d), 116.1 (d), 116.1, 116.6, 116.7, 117.0, 118.6, 123.8, 124.5, 126.5, 129.6, 129.7, 132.3 (d), 132.7, 140.1 (d), 142.5, 152.9, 157.2, 158.4, 160.9, 161.7 (d), 167.5; anal. calcd for $C_{25}H_{17}FO_5$ $[M + H]^+$ 417.10881, found 417.11292.

*(2E)-3-(4-((3-(2-Methyl-4-(trifluoromethoxy)phenyl)-2-oxo-2H-chromen-4-yl)oxy)phenyl)acrylic Acid (**59**).*



The title compound was prepared from **57** in a similar manner to that described for **58**. ^{1}H NMR (400 MHz, $CDCl_3$, 303 K) 2.19 (3H, s), 6.32 (1H, d), 6.76 (2H, d), 6.92 (2H, d), 7.10 (1H, d), 7.24−7.38 (3H, m), 7.51 (1H, d), 7.64−7.70 (2H, m), 7.78 (1H, d); *m*/*z* 483 $[M + H]^+$.

Biology. Details for the suite of assays used to support this project have previously been published.[26]

ERα Binding. Test compounds were dispensed into 384 well nonbinding plates to create a 12 point half-log concentration response curve. ERα binding was measured using a LanthaScreen ERα binding assay (Life Technologies), and the TR-FRET emission ratios were acquired on a microplate reader.

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ERα Down-Regulation. MCF-7 cells were plated in reduced serum media in a 384 well assay plate the day prior to the assay. Cells were treated with a 12 point concentration range of each test compound for 24 h before being fixed and immunostained for ERα. The levels of ERα were measured using a high content imaging reader.

PR Antagonism/Agonism. The ERα regulated gene expression of PR was measured as a biomarker for ERα antagonism and agonism. MCF-7 cells were plated in reduced serum media in a 384 well assay plate the day prior to the assay. Antagonism: cells were pretreated for 30 min with 0.1 nM estradiol followed by a 12 point concentration range of each test compound for 24 h before being fixed and immunostained for PR. The levels of PR were detected using a laser scanning imaging cytometer. Agonism: cells were treated with a 12 point concentration range of each test compound for 24 h before being fixed and immunostained for PR. The PR levels were detected using a laser scanning imaging cytometer.

Proliferation. MCF-7 cells were plated in reduced serum media in a 384 well assay plate the day prior to the assay. Cells were treated with a concentration response of each test compound for 7 days before determination of cell number using a nucleic acid stain. The final cell number was measured using a laser scanning imaging cytometer.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Complete experimental details for the syntheses of intermediates and all final compounds are described together with assay statistical analyses and crystallographic information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ER, estrogen receptor; DR, down-regulation; LLE, ligand lipophilicity efficiency; PR, progesterone receptor; SAR, structure−activity relationship; SERM, selective estrogen receptor modulator; SERD, selective estrogen receptor down-regulator; AcOH, acetic acid; CDI, carbonyldiimidazole; DCM, dichloromethane; DIPEA, *N,N*-diisopropylethylamine; DMAP, *N,N*-dimethylpyridin-2-amine; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide; D*t*BPPS, 3-(di-*t*-butylphosphonium)-propanesulfonate; HLPC, high-performance liquid chromatography; NCS, *N*-chlorosuccinimide; SEM, trimethylsilylethoxymethyl; SEMCl, 2-(chloromethoxy)ethyl-trimethyl-silane; TBAF, tetra-*N*-butylammonium fluoride; Tf, triflate; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TsOH, *p*-toluensulfonic acid

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