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Discovery of thiochroman derivatives bearing a carboxy-containing side chain as orally active pure antiestrogens

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Abstract—In order to search for alternatives to the sulfoxide moiety in the long side chain of pure antiestrogens, several molecules that may interact with water in a fashion similar to ICI164,384 were designed and it was found that compounds with the carboxy, the sulfamide, or the sulfonamide instead of the sulfoxide moiety also functioned as pure antiestrogens. Interestingly, the compound possessing the carboxy moiety showed superior antiestrogen activity compared to ICI182,780 when dosed orally. Results of the pharmacokinetic evaluation indicated that the potent antiestrogen activity at oral dosing attributed to both the improved absorption from the intestinal wall and the metabolic stability of the compound in liver.

The effects of pure antiestrogens such as ICI182,780, ICI164,384, and ZM189,154 on estrogen receptor positive breast tumor are well documented.[1–4] In particular, ICI182,780 demonstrated effectiveness in postmenopausal women with advanced breast cancer progression after tamoxifen therapy in clinical trials,[5–7] and was launched in 2002 as an intramuscular injection drug. In the course of our research to develop pure antiestrogens, we found thiochroman and chroman derivatives **1** and **2** also functioned as pure antiestrogens,[8] having in vitro/in vivo antiestrogen activities similar to ICI182,780. One of the structural features of these pure antiestrogens is the presence of the sulfoxide or the amide moiety (Fig. 1), and to our knowledge, no alternative to these moieties in the long alkyl side chain has been reported to date. We speculated that compounds **1**, **2**, ZM189,154, and ICI182,780 would bind to estrogen receptor ligand binding domain (ER-LBD) in a fashion similar to ICI164,384 (PDB entry: 1HJ1),[9] where the side chain would protrude from ER-LBD, the sulfoxide moiety would make hydrophilic interaction with water, and the terminal hydrophobic moiety would be buried in the AF-2 cleft on the surface of ER-LBD.

In this study, several molecules with different moieties in the side chain that might interact with water as alternatives to the sulfoxide or the amide moiety were first designed and evaluated to ascertain whether thiochroman or chroman derivatives having these moieties functioned as pure antiestrogens. After confirming the pure antiestrogen activity of the thiochroman derivative having the carboxy moiety, optimization of the total length of the side chain and the position of the carboxy moiety was carried out.

The thiochroman and chroman derivatives were prepared according to the methods described by this laboratory[10,11] and are summarized in Schemes 1–3. In preparation of thiochroman derivatives **6**, **7**, **10**, and **11** (Scheme 1), a nucleophilic 1,2-addition to ketone **3a**[12] and **3b**[12] with lithiated alkyne derivatives, reduction with $NaBH_3CN$ in the presence of ZnI_2, and subsequent catalytic hydrogenation afforded the 4-alkyl derivatives

Keywords: Pure antiestrogen; Thiochroman; Chroman; Estrogen receptor.
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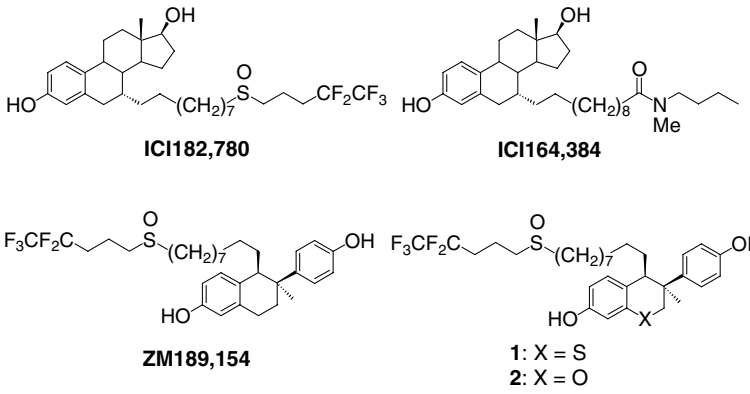
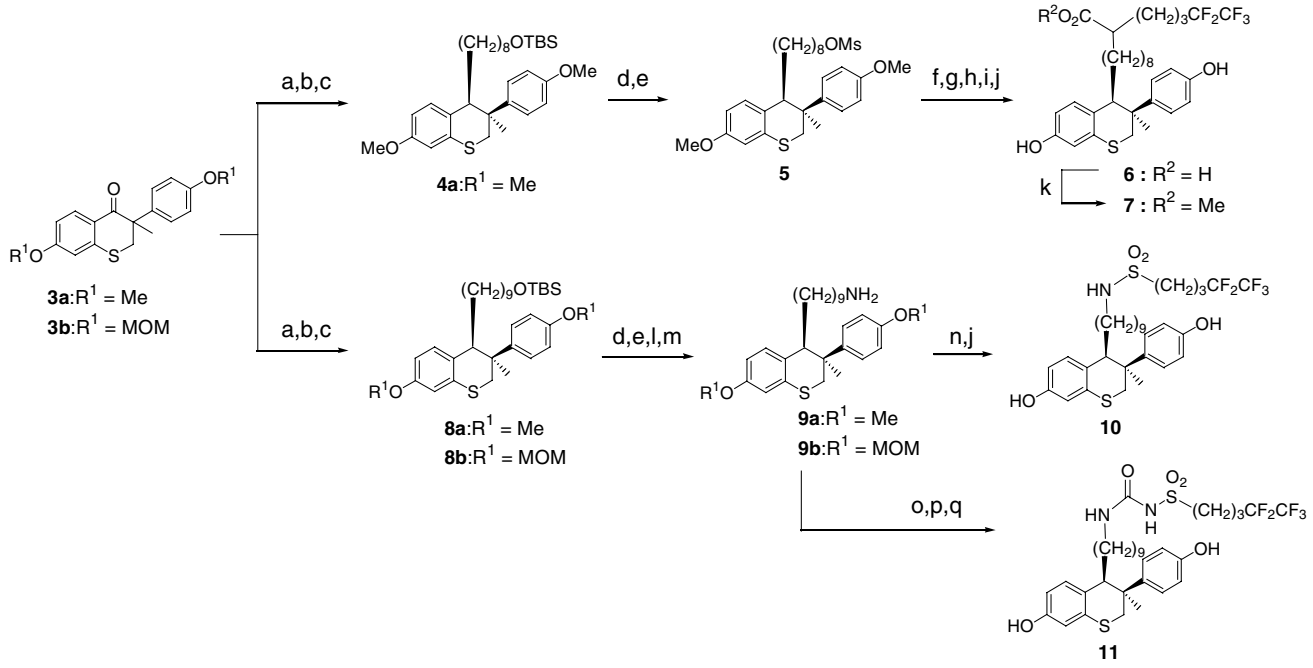
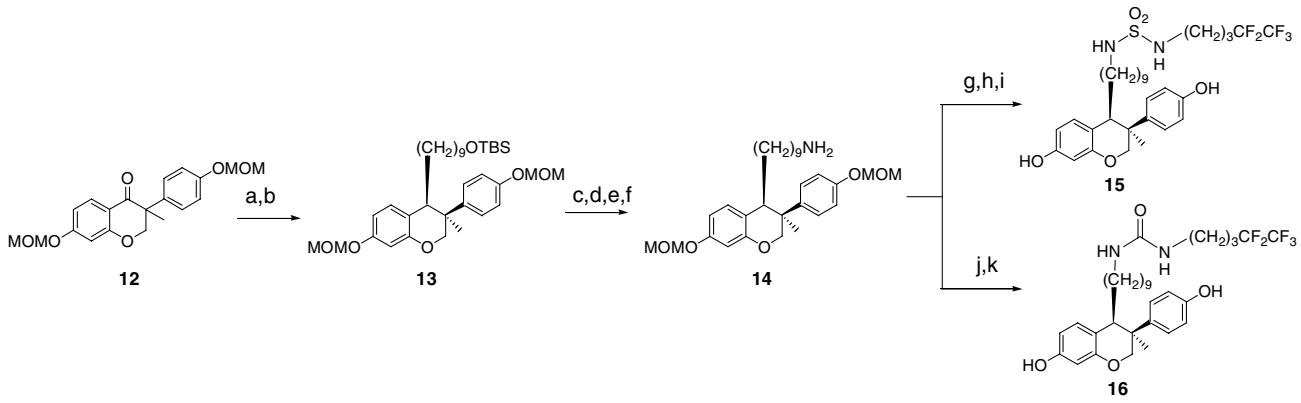


Figure 1. Structure of representative antiestrogens.

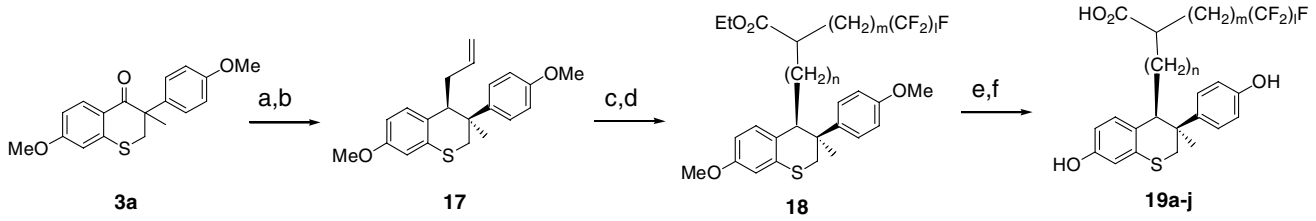


Scheme 1. Reagents and conditions: (a) HC≡C–$(CH_2)_{n-2}$OTBS, *n*-BuLi, THF, −20 to 0 °C; (b) ZnI_2, $NaBH_3CN$, 1,2-dichloroethane, 0 °C to rt; (c) 10% Pd–C, H_2, 0.2 N $NaHCO_3$ aq, THF, rt, then *cis*/*trans* separation with column chromatography; 34–44% from **3a** or **3b**; (d) TBAF, THF, rt, 93%–quant.; (e) MsCl, Et_3N, CH_2Cl_2, rt, 94–98%; (f) NaI, acetone, reflux, 94%; (g) NaH, $CF_3CF_2(CH_2)_3CH(CO_2Et)_2$, THF, 0 °C to rt, 96%; (h) KOH aq, EtOH, reflux, quant.; (i) DMSO, 140 °C, 82%; (j) BBr_3, CH_2Cl_2, −78 to −5 °C (R^1 = Me), 72–89%; (k) diazomethane, Et_2O, 0 °C, 78%; (l) NaN_3, DMF, rt, 86–87%; (m) 10% Pd–C, H_2, MeOH, rt, 97%–quant.; (n) $CF_3CF_2(CH_2)_3SO_2Cl$, Et_3N, CH_2Cl_2, 0 °C, 80%; (o) $ClCO_2Ph$, pyridine, THF, rt; (p) $CF_3CF_2(CH_2)_3SO_2NH_2$, K_2CO_3, DMF, 70 °C; (q) 6 N HCl aq, MeOH, 70 °C (R^1 = MOM), 47% from **9b**.

as a mixture of (3*RS*,4*RS*)- and (3*RS*,4*SR*)-isomers, in which (3*RS*,4*RS*)-isomers **4a**, **8a**, and **8b** were dominant and separated with column chromatography. The relative configuration at the 3- and 4-positions was determined based on NOESY 2D NMR spectroscopy described by this laboratory.[8] Carboxy derivative **6** was obtained as a mixture of diastereomers from mesylate **5** by iodination followed by alkylation with 2-perfluoroalkyl malonate and subsequent decarboxylation and deprotection. Methyl ester **7** was prepared from carboxy derivative **6**. Sulfonamide **10** and sulfonylurea **11** were prepared from amine **9a** and **9b** by condensation with perfluoroalkyl sulfonyl chloride and subsequent deprotection, and by treatment with phenyl chloroformate, reaction with perfluoroalkyl sulfonamide and deprotection, respectively. The chroman derivatives were prepared using a similar procedure (Scheme 2). 4-Alkyl chroman derivative **13**, the (3*RS*,4*RS*)-isomer obtained as the major product from catalytic hydrogenation, was converted to amine **14**. Sulfamide **15** was prepared from amine **14** by treatment with *tert*-butyl *N*-(chlorosulfonyl)carbamate followed by Mitsunobu reaction with perfluoroalkyl alcohol and subsequent deprotection. Urea derivative **16** was prepared by reacting amine **14** with phenyl *N*-(perfluoroalkyl)carbamate followed by deprotection. In optimizing carboxy-containing side chains, the thiochroman derivatives in Table 4 were prepared from ketone **3a** through metathesis as the key reaction[11] (Scheme 3). Reduction of ketone **3a** with LAH followed by allylation with allyl trimethylsilane



Scheme 2. Reagents and conditions: (a) $HC{\equiv}C{-}(CH_2)_7OTBS$, *n*-BuLi, THF, −20 to 0 °C, 94%; (b) 10% Pd–C, H_2, AcOEt, rt, then *cis*/*trans* separation with column chromatography, 57–63%; (c) PPTS, MeOH, rt, quant.; (d) MsCl, Et_3N, CH_2Cl_2, rt, 97%; (e) NaN_3, DMF, 50 °C, 93%; (f) 10% Pd–C, H_2, MeOH, rt, quant.; (g) $ClSO_2NHBoc$ (prepared from $ClSO_2NCO$ and *t*-BuOH), Et_3N, CH_2Cl_2, −78 °C to rt, 36%; (h) $CF_3CF_2(CH_2)_3OH$, PPh_3, DEAD, CH_2Cl_2, rt, 67%; (i) TFA, CH_2Cl_2, rt, 54%; (j) $CF_3CF_2(CH_2)_3NHCO_2Ph$, K_2CO_3, MeCN, 70 °C, 67%; (k) PPTS, MeOH, reflux, 26%.



Scheme 3. Reagents and conditions: (a) LAH, THF, −78 °C; (b) allyl trimethylsilane, ZnI_2, 1,2-dichloroethane, 40 °C to rt, 68% from **3a**; (c) $CH_2{=}CH(CH_2)_{n-3}CH(CO_2Et)(CH_2)_m(CF_2)_lF$, benzylidenebis(tricyclohexylphosphine) dichlororuthenium, CH_2Cl_2, reflux; (d) 10% Pd–C, H_2, AcOEt, rt, 52–72% from **17**; (e) BBr_3, CH_2Cl_2, −78 to 0 °C, 84–92%; (f) NaOH aq, EtOH, reflux, 70–93%.

and ZnI_2 yielded (3*RS*,4*RS*)-4-allyl thiochroman derivative **17**. Ruthenium catalyzed metathesis of allyl thiochroman derivative **17** with a variety of alkene derivatives and subsequent catalytic hydrogenation and deprotection afforded thiochroman derivatives **19a**–**j**.

The thiochroman and chroman derivatives thus prepared were assayed in vitro and in vivo to characterize their biological profiles. In vitro, binding affinity for ERα was determined by displacement of [^{3}H]estradiol with the test compound utilizing human recombinant ERα-LBD. In vivo, estrogen agonist and antagonist activities were measured by the ability of the test compound to increase the uterine weight and to inhibit estrogen-stimulated uterine weight gain in an ovariectomized mouse model, respectively.

Based on our speculation that the sulfoxide moiety of compounds **1** and **2** would make hydrophilic interaction with water in a fashion similar to ICI164,384,[9] several molecules containing the carboxy, the ester, the sulfonamide, the sulfamide, the sulfonylurea, and the urea moieties as alternatives to the sulfoxide moiety were designed. As can be seen in Table 1, subcutaneous administration of compounds **6**, **10**, and **15** at 10 mg/kg almost completely inhibited estrogen-induced uterine weight gain and exhibited no significant uterine weight gain when dosed alone compared to vehicle. These results indicate that compounds **6**, **10**, and **15** functioned as pure antiestrogens, and that the carboxy, the sulfonamide, and the sulfamide moieties could be alternatives to the sulfoxide moiety. Noteworthy is that compound **6** exhibited oral antiestrogen activity superior to compound **1** and ICI182,780 in spite of its lower affinity for ERα. On the other hand, compounds **11** and **16**, which have the sulfonylurea and the urea moiety, respectively, displayed both agonist and antagonist activities with subcutaneous administration, indicating these compounds are partial agonists. We speculated that compounds **6**, **10**, and **15** would bind to ERα-LBD similar to ICI164,384, suggesting these compounds are pure antiestrogens. In contrast, the binding mode of compounds **11** and **16** would be somewhat different, suggesting these compounds to be partial agonists. As has been often observed with estrogen ligands, subtle differences in structure may be critical factors to determine whether the compounds function as pure antiestrogens or as partial agonists.

The remarkable antiestrogen activity of orally administered compound **6** was intriguing because its binding affinity for ERα was less than 1/100 of compound **1** and ICI182,780. To explore the reason for high activity, investigations into the absorption from the intestinal wall and the metabolic stability in liver in a rat model in which the test compound was administered into duodenum were carried out. The areas under the concentration–time curve (AUC) in portal vein and

Table 1. Biological data of thiochroman and chroman derivatives

Compound	RBA[a] (%) E_2 = 100	Antiestrogen activity[b] (% inhibition)			Estrogen activity[c] (% uterine weight gain)
		10 mg/kg sc	10 mg/kg po	50 mg/kg po	10 mg/kg sc
6	0.8	95	77	94[d]	1*
7	7	52	40	n.t.	−1
10	52	99	31	73	3*
11	1.2	33	n.t.	n.t.	8
15	42	100	41	69	1*
16	57	77	1	n.t.	10
1	200	101	51	94	−2*
2	96	94	66	88	1*
ICI 182,780	138	95	54	86	−1*

n.t., not tested.
[a] Relative binding affinities for the recombinant estrogen receptor (ERα), determined by competitive radiometric binding assay with [^{3}H]estradiol.
[b] Inhibition of estrogen-stimulated uterine weight gain by the test compound with subcutaneous (sc) or oral (po) administration.
[c] Stimulation of uterine weight gain by the test compound with sc administration.
[d] 30 mg/kg.
* No significant difference between the test group and the vehicle group at $P < 0.05$ using Student's t test.

peripheral blood within 30 min. after administration were measured (Table 2). The AUC of compound **6** in portal vein was 29- and 45-fold higher than compound **1** and ICI182,780, respectively, and this high AUC level was well maintained in peripheral blood, also. These outstanding parameters in absorption and the metabolic stability were surprising and encouraged us to investigate its pharmacokinetic profile in greater detail. Table 3 summarizes the pharmacokinetic data in rats. As can be seen, compound **6** exhibited 58-fold and 125-fold higher AUC in po, and 1/26 and 1/42 lower clearance in iv than compound **1** and ICI182,780, respectively. These results, together with the results in Table 2, indicated that the absorption from intestinal wall and metabolic stability in liver of compound **6** are superior to compound **1** and ICI182,780, and lead to the remarkable antiestrogen activity of compound **6** administered orally. Factors effecting the absorption and metabolic stability of compound **6** require further investigation. However, it is noteworthy that although compounds **1**, **6**, and ICI182,780 are all highly lipophilic, their Prolog D at pH 7.0 predictions being 8.18, 7.44, and 8.44, respectively, only compound **6** exhibited improved characteristics in absorption and metabolic stability.

Table 2. AUC in portal vein and peripheral blood for 30 min after duodenal administration[a]

Compound	AUC in portal vein[b] (ng × h/mL)	AUC in peripheral blood[c] (ng × h/mL)
6	2509	2041
1	88	13
ICI182,780	56	7

[a] Compound dosed into duodenal in Sprague–Dawley rats as a solution in water/PEG200/EtOH (3:6:1).
[b] AUC of the test compound in portal vein from 0 to 30 min after duodenal dosing of 10 mg/kg.
[c] AUC of the test compound in peripheral blood from 0 to 30 min after duodenal dosing of 10 mg/kg.

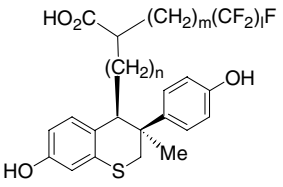
After confirming that the thiochroman derivative having the carboxy moiety exhibited remarkable antiestrogen activity with oral administration, side-chain optimization was next carried out (Table 4). Oral antiestrogen activities of compounds **19a**, **6**, **19g**, and **19j**, which have 7-, 8-, 9-, and 10-methylene between the scaffold and the carboxy moiety, respectively, were first investigated, and 8- and 9-methylene groups were found optimal. Next a variety of perfluoroalkyl moieties into the terminal of compounds **6** and **19g** were incorporated. As can be seen in Table 4, compound **19g** derivatives, **19f**, **19h**, and **19i** showed oral antiestrogen activities similar to compound **19g**, whereas the oral antiestrogen activities of compound **6** derivatives, especially compounds **19b**, **19c**, and **19d**, were approximately 2- or 3-fold superior to that of compound **6**. The clear correlation between the binding affinity for ERα and oral antiestrogen activity was not observed within the compounds prepared. The reason has not been fully investigated; however, it is speculated that thiochroman derivatives **19b–i** may possess high absorption and metabolically stable pharmacokinetic profiles similar to compound **6**, and the potent oral activities might be mainly due to absorption and metabolism rather than their binding affinities. Although the factors effecting oral antiestrogen activities remained to be further investigated, oral antiestrogen activities were improved approximately 2- to 3-fold from the initial lead compound **6** in the course of the side-chain optimization. In addition, it was confirmed

Table 3. Pharmacokinetic data in rats[a]

Compound	iv administration 10 mg/kg		po administration 20 mg/kg	Bioavailability (%)
	AUC (μg × h/mL)	Cltot[b] (mL/h/kg)	AUC (μg × h/mL)	
6	228	47	209	46
1	8.22	1240	3.59	22
ICI 182,780	5.30	1969	1.67	16

[a] Compound dosed in Sprague–Dawley rats as a solution in water/PEG200/EtOH (3:6:1) for iv and po dosing.
[b] Total clearance.

Table 4. Biological data of thiochroman derivatives **6** and **19a–19j**



Compound	*n*	*m*	*l*	RBA[a] (%) E_2 = 100	Antiestrogen activity[b] (% inhibition)		
					3 mg/kg po	10 mg/kg po	30 mg/kg po
19a	7	3	2	0.5	n.t.	20	n.t.
19b	8	2	4	0.6	63	93	96
6	8	3	2	0.8	41	77	94
19c	8	3	4	0.3	61	93	99
19d	8	4	2	1.3	69	87	97
19e	8	5	2	0.8	52	86	94
19f	9	2	4	0.3	38	85	99
19g	9	3	2	0.9	45	81	n.t.
19h	9	3	4	0.4	55	86	100
19i	9	4	2	0.2	53	79	96
19j	10	3	2	0.2	n.t.	62	n.t.
ICI182,780				138	29	54	72

n.t., not tested.
[a] Relative binding affinities for the recombinant estrogen receptor (ERα), determined by competitive radiometric binding assay with [^{3}H]estradiol.
[b] Inhibition of estrogen-stimulated uterine weight gain by the test compound with po administration.

that compound **19b**, one of the most potent antiestrogens, exhibited oral antitumor activity almost equal to the maximum antitumor activity of subcutaneous administration of ICI182,780 in the Br-10 xenograft model,[13] and the chiral isomer of compound **19b** prevented nuclear accumulation of ER in COS-7 cells expressing human ER, suggesting ER downregulation effects (unpublished data).

In summary, it was found that the carboxy, the sulfonamide, and the sulfamide moieties functioned as alternatives to the sulfoxide moiety in the long side chain of pure antiestrogens. Surprisingly, compound **6**, wherein the sulfoxide moiety is replaced with the carboxy moiety, exhibited antiestrogen activity superior to compound **1** and ICI182,780 when administered orally. The outstanding oral antiestrogen activity of compound **6** attributed to its high absorption and metabolically stable profile.

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