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Synthesis and Biological Evaluation of Novel Thio-Substituted Chromanes as High-Affinity Partial Agonists for the Estrogen Receptor

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Abstract—Synthesis of (±)-*cis*-7-hydroxy-3-phenyl-4-(4-(2-piperidinoethanethio)phenyl)chromane (**13**) and (±)-*cis*-7-hydroxy-3-phenyl-4-(4-(2-pyrrolidinoethanethio)phenyl)chromane (**15**) is presented. These compounds are representatives of a novel class of compounds with high in vitro binding affinity for the estrogen receptor (IC_{50} = 7–10 nM), and very low in vitro uterotrophic activity (max stim. = 5–17% rel to moxestrol; EC_{50} = 0.5–1.8 nM).

Estrogen has protective effects against osteoporosis in the post-menopausal woman, coronary heart disease, cognitive deficiency and possibly Alzheimer's disease.[1–5] However, these significant benefits of estrogen replacement therapy are achieved at the expense of an increased risk of endometrial hyperplasia and breast cancer.[6,7] Therefore, several pharmaceutical companies are presently developing tissue-selective estrogen mimetics, initially based on the observation that tamoxifen (**1**) (Scheme 1), an anti-estrogen in breast, shows partial estrogen agonism in other tissues, for example bone and endometrium.[8–11] Raloxifene (**2**) (Scheme 1) was also originally developed for the treatment of breast cancer;[12] however, preclinical findings demonstrated a potential for improving the selectivity of the estrogen effect in bone relative to breast and endometrium.[13] Significantly, in uterine tissue, raloxifene was more effective as an antagonist of the uterotrophic response to estrogen as compared to tamoxifen.[14] Similary, idoxifene (**3**)[15] and levormeloxifene (**4**),[16–19] (Scheme 1) may largely lack the estrogenic activity on the endometrium and they appear to act as anti-resorptives on bone in preclinical and clinical studies.

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Recently, our group demonstrated a group of chromanes to be very potent binders to the estrogen receptor.[20] Among these were (±)-*cis*-7-hydroxy-3-phenyl-4-(4-(2-pyrrolidinoethoxy)phenyl)chromane [(±)-*cis*-(**5**)] and (±)-*cis*-7-hydroxy-3-phenyl-4-(4-(2-piperidinoethoxy)phenyl)chromane [(±)-*cis*-(**6**)], (Scheme 1). Compound (−)-*cis*-(**5**) was further demonstrated to completely prevent the loss of total bone mineral density of the tibiae in the ovariectomized rat. In addition the unwanted uterotrophic changes normally observed with estradiol treatment, and in particular the dangerous proliferative effects on endometrial tissue, were not observed. In the present paper, the synthesis of some novel thio-analogues thereof, along with some in vitro biology data are presented.

Chemistry

The *cis*-racemates of compounds **13**, **14,** and **15** were prepared by the route outlined in Scheme 2. The *cis*-racemate of **7**, previously described by our group,[20] was reacted with *N*,*N*-dimethylthiocarbamoyl chloride in the presence of base to give compound **8**. A Newman–Kwart rearrangement[21] was applied to compound **8**, giving compound **9** in quantitative yield, which was then reduced with lithium aluminium hydride to give the thiophenol **10**. Alkylation of compound **10** using either *N*-(2-chloroethyl)piperidine hydrochloride or *N*-(2-

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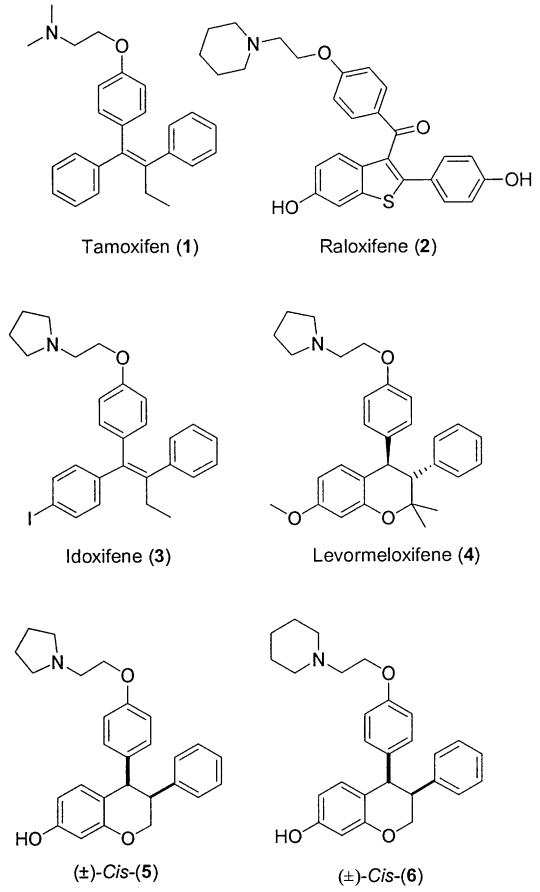
chloroethyl)pyrrolidine hydrochloride gave compounds **11** and **12**, respectively. Compounds **11** and **12** were demethylated by heating with pyridine hydrochloride to give (±)-*cis*-7-hydroxy-3-phenyl-4-(4-(2-piperidinoethanethio)phenyl)chromane **13**[22] and (±)-*cis*-7-hydroxy-3-phenyl-4-(4-(2-pyrrolidinoethanethio)phenyl)chromane **14**, respectively. Compound **14** was isolated as its hydrochloride salt **15**.[23]

Biology

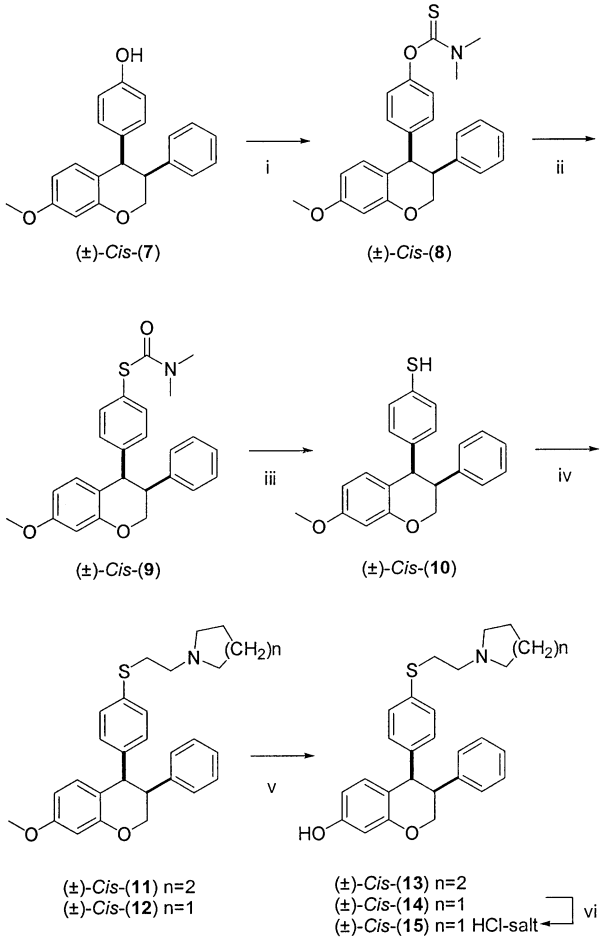
The objective of the present work has been to develop non-steroidal estrogen agonists with high affinity for the estrogen receptor (ER) but without stimulation of endometrial tissue. The ability of the compounds to compete with [^{3}H]-17β-estradiol for binding to the estrogen receptor was determined in ER-rich cytosol from rabbit uterine tissue, and measured by a modified[24] classical ligand binding assay.[25] As shown in Table 1, the binding affinity for compounds **13** (IC_{50} = 9.7 nM) and **15** (IC_{50} = 6.5 nM) is an order of magnitude lower than for 17β-estradiol (IC_{50} = 0.5 nM). However, the binding affinity for compounds **13** and **15** is still quite high, and equipotent to the non-steroidal reference compounds shown in Scheme 1. Of the four chromane analogues, compound **5** (IC_{50} = 19 nM) is the weakest in respect to binding affinity compared to compounds **6** (IC_{50} = 3.1 nM), **13**, and **15** (Table 1).



Scheme 1. Partial estrogen agonists.

The unwanted stimulation of the endometrial tissue was assessed in vitro, based on stimulation of alkaline phosphatase activity[26] in the Ishikawa line[27] of human endometrial adenocarcinoma cells. As shown in Table 1, compounds **5**, **6**, **13** and **15** were all partial agonists in this model showing maximal agonist activity from 5 to 54% relative to moxestrol, and with EC_{50} values from 0.5 to 1.8 nM. In fact, compound **15** has the weakest estrogenic effect on the endometrium, compared to the reference compounds in Table 1.

In conclusion, the novel ethanethio-substituted chromanes, compounds **13** and **15**, showing at the same time very high affinity for the estrogen receptor and very low agonist stimulation of endometrial tissue in vitro, appear to be interesting candidates for a further pharmacological evaluation. As the ethoxy-chromane, compound **5**, previously demonstrated to completely prevent the loss of total bone mineral density of tibiae in the ovariectomized rat, without uterotrophic activity and proliferative effects on the endometrial tissue,[20] the analogues ethanethio-chromanes, compounds **13** and **15**, are predicted as valuable candidates for a further thorough in vivo evaluation in preclinical models of



Scheme 2. Synthesis of two partial estrogen agonists **13** and **15**: (i) NaH, DMF, *N*,*N*-dimethylthiocarbamoyl chloride; (ii) 260 °C, diphenyl ether; (iii) $LiAlH_4$, THF; (iv) *N*-(2-chloroethyl)piperidine hydrochloride (**11**) or *N*-(2-chloroethyl)pyrrolidine hydrochloride (**12**), K_2CO_3, NaI, zinc dust, acetone; (v) pyridine hydrochloride, 155 °C; (vi) HCl, diethyl ether.

Table 1. Estrogen receptor ligand binding affinity (ER-LBA) and endometrial activity in vitro

Compd	ER-LBA[a]	Ishikawa	
	IC_{50} (nM)	EC_{50} (nM)[b]	E_{max} (%)[c]
(±)-*cis*-(**13**)	9.7±2.5	1.8±0.2	17±0.6
(±)-*cis*-(**15**)	6.5±6.4	0.5±0.2	5±2
(±)-*cis*-(**5**)	19.0±1.4	0.9±0.1	36±1
(±)-*cis*-(**6**)	3.1±1.4	0.7±0.4	54±10
Raloxifene (**2**)	7.7±3.5	0.04±0.001	15±0.4
Idoxifene (**3**)	7.5±2.1	7±3	10±0.5
Levormeloxifene (**4**)	73±23	13±0.4	25±2
Moxestrol	2.9±1.7	0.08±0.01	100
17β-Estradiol	0.5±0.3	—	—

All values are presented as mean from two to four separate studies.
[a]ER-LBA (estrogen receptor ligand binding affinity) expressed as IC_{50}, that is the concentration of compound required to displace 50% of the maximal binding for [^{3}H]-17β-estradiol.
[b]Endometrial activity expressed as EC_{50}, that is the concentration of compound required to increase the level of the alkaline phosphatase enzyme 50%.
[c]Endometrial activity expressed as E_{max}, that is the % of maximal stimulation relative to moxestrol. E_{max} of moxestrol is 903±142% of basal proliferation.

post-menopausal osteoporosis and other degenerative disorders. They may turn out to be a significant alternative to the current therapeutics for prevention of post-menopausal osteoporosis.

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22. (±)-*cis*-7-Hydroxy-3-phenyl-4-(4-(2-piperidinoethanethio)-phenyl)chromane **13** was isolated as a white foam and fully characterized: mp 97–106 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.40–1.49 (m, 2H); 1.56–1.65 (m, 4H); 2.40–2.49 (m, 4H); 2.51–2.58 (m, 2H); 2.94–3.02 (m, 2H); 3.55–3.63 (m, 1H); 4.20–4.28 (m, 2H); 4.41 (t, 1H); 5.30 (s, CH_2Cl_2); 6.32 (dd, 1H); 6.41 (d, 1H); 6.51 (d, 2H); 6.63–6.70 (m, 2H); 6.77 (d, 1H); 7.01 (d, 2H); 7.11–7.19 (m, 3H). MS(EI): *m*/*z* 445 (M+). HRMS(EI) calcd for C_{28} H_{31} N_1 O_2 S_1: 445.2076 (M+); found: 445.2074. Elemental analysis calcd for C_{28} H_{31} N_1 O_2 S_1 + 2.94 mol% CH_2Cl_2: C, 73.67; H, 6.88; N, 3.05; S, 6.98%; found: C, 73.7; H, 7.0; N, 3.1; S, 7.0%.
23. (±)-*cis*-7-Hydroxy-3-phenyl-4-(4-(2-pyrrolidinoethanethio)-phenyl)chromane hydrochloride **15** was isolated as a white foam and fully characterised: mp 149–152 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.71–1.88 (m, 4H); 2.50–2.61 (m, 4H); 2.69 (t, 2H); 3.00 (t, 2H); 3.55–3.63 (m, 1H); 4.20–4.28 (m, 2H); 4.41 (t, 1H); 6.35 (dd, 1H); 6.43 (d, 1H); 6.52 (d, 2H); 6.63–6.70 (m, 2H); 6.78 (d, 1H); 7.02 (d, 2H); 7.11–7.20 (m, 3H). MS(EI): *m*/*z* 431 (M+). HRMS(EI) calcd for C_{27} H_{29} N_1 O_2 S_1: 431.1919 (M+); found: 431.1923. Elemental analysis calcd for C_{27} H_{29} N_1 O_2 S_1 + 0.19 mol% HCl: C, 75.00; H, 6.76; N, 3.24; S, 7.41%; found: C, 75.0; H, 6.9; N, 3.2; S, 7.2%.
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