



Published in final edited form as:

Steroids. 2012 May ; 77(6): 596–601. doi:10.1016/j.steroids.2012.02.003.

8-Alkylthio-6-thio-substituted Theophylline Analogues as Selective Progesterone Receptor Antagonists

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Abstract

The progesterone receptor (PR) plays a key role in reproduction and is important in cancers of the reproductive tract. Current PR antagonists usually compete for progestin binding in the PR ligand-binding pocket and often exhibit cross-binding with other members of the steroid receptor family. Using stably transfected cells expressing reporter genes, a set of ~150 theophylline analogues were screened for their ability to inhibit progesterone, estrogen, glucocorticoid and androgen signaling. The structure-activity studies presented here identify branched 8-alkylthio-6-thio-substitutions of theophylline as selective PR inhibitors. 6-thio-8-(2-ethylbutyl)thiotheophylline (**51**), the most extensively studied derivative, does not act by competing with progestins for binding in the ligand-binding pocket of PR. It demonstrated the ability to inhibit the mouse mammary tumor virus (MMTV)-luciferase reporter and endogenous PR-regulated alkaline phosphatase activity in T47D breast cancer cells. Compound **51** is the lead member of a novel class of PR inhibitors that act outside the PR ligand-binding pocket, thus serving as a novel probe to investigate PR action and a lead for further development.

Keywords

Progesterone receptor; Steroid receptor; Small molecule inhibitor; Theophylline analogue; Structure activity relationship

1. Introduction

Progesterone plays a key role in regulating ovulation, uterine contractility, and mammary tissue morphogenesis [1,2]. Most biological actions of progestins result from the activation of the progesterone receptor (PR), a ligand-dependent transcription factor [3]. Hormone

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binding in the PR ligand-binding domain (LBD) induces a conformation that releases the heat shock protein complex, enabling PR to more efficiently enter the nucleus and bind as a homodimer to DNA response elements and regulate the transcription of target genes [4].

Depending on the tissue, estrogens and progestins can work synergistically or antagonistically. While estrogen increases the levels of the two PR isoforms, PR-A and PR-B, progestins binding to PR can suppress estradiol-stimulated estrogen receptor α (ER α) activity [5]. PR antagonists have been developed as potential treatment options for progesterone-dependent gynecological disorders, such as endometriosis and uterine and breast cancers. In addition to their ability to inhibit the effects of endogenous progesterone, PR antagonists repress estrogen-dependent proliferation of the mammary gland and uterus [6,7]. Most antiprogestins, like mifepristone (RU486), are steroidal compounds that compete with progestins binding in the ligand-binding pocket of PR. Classical PR antagonists bind with high affinity and exhibit nanomolar potency, however, they often exhibit significant binding to the glucocorticoid receptor (GR) [8,9].

In a high-throughput screen, our laboratory identified a small molecule inhibitor of steroid receptor transactivation [10], 8-benzylsulfanylmethyl-1,3-dimethyl-3,7-dihydro-purine-2,6-dione (TPBM), that belongs to a family of theophylline derivatives originally synthesized by Dietz & Burgison [11,12]. A second small molecule characterized from this set, 8-((4-(4-fluorophenyl)-4-oxobutyl)thio)-1,3-dimethyl-6-thioxo-6,7-dihydro-1*H*-purin-2(3*H*)-one (TPSF), showed increased potency and a different mode of action than TPBM [13]. While TPBM acts in part by blocking receptor binding to response element DNA, TPSF increases the degradation of ligand-bound ER α . These compounds demonstrate the diversity of action and specificity that this set of analogues present in modulating steroid receptor action. Structure activity relationship (SAR) studies identify correlations between changes in biological activity and molecular structure of a set of similar compounds.

In the course of our SAR studies, we gained the unusual capacity to evaluate the activity of small molecules as inhibitors of transactivation mediated by 4 major steroid hormone receptors: ER, PR, GR, and androgen receptor (AR). In our evaluation of this set of structurally related theophylline derivatives, we identified 8-alkylthio-6-thiotheophyllines that inhibited PR-mediated transactivation better than the parent compound. A subset of small molecules, having saturated carbon-chain branching, exhibited a clear preference for inhibiting PR relative to the other steroid receptors. Here, we describe a novel structural motif that exhibits receptor specificity and describe a small molecule inhibitor of PR that does not act by competing with progestins for binding to PR.

2. Experimental

2.1. Chemistry

2.1.1. General—Theophylline (**1**) was obtained from Sigma (St. Louis, MO). Compounds **2-55** were synthesized as described by Dietz and Burgison [11,12], and obtained from the NCI Developmental Therapeutics Program. Solid compounds were dissolved in dimethyl sulfoxide (DMSO) and stored at -20°C . The lead compound, 6-thio-8-(2-ethylbutyl)thiotheophylline (**51**), has 85% chemical purity as determined by LC/MS.

2.2. Biology

2.2.1. Cell Culture—Unless otherwise indicated, cells were maintained at 37°C in 5% CO_2 in phenol red-free medium containing 1% penicillin/streptomycin and 10% fetal bovine serum (FBS) (Atlanta Biological, Atlanta, GA). T47D/A1-2 breast cancer cells, stably transfected to express additional GR and a mouse mammary tumor virus (MMTV)-

luciferase reporter [14], were maintained in minimum essential medium (MEM) supplemented with 10 mM HEPES, pH 7.4, 2 mM L-glutamine, and 0.2 mg/ml Geneticin (G418). T47D-KBluc cells stably expressing an (ERE)₃-luciferase reporter [15] were maintained in RPMI 1640 supplemented with 10 mM HEPES, pH 7.5, 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, and 1 mM sodium pyruvate. HeLa-AR1C-PSA-Luc-A6 cells stably expressing AR and a prostate-specific antigen (PSA)-luciferase reporter [13] were maintained in MEM supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mg/ml hygromycin B (Roche Applied Science), and 0.5 mg/ml G418. T47D cells were maintained in phenol red MEM supplemented with 10 mM HEPES, pH 7.4, and 2 mM glutamine. MCF-7 breast cancer cells were maintained in MEM supplemented with 10 mM HEPES, pH 7.4, 2 mM glutamine, and 5% FBS. Four days before experiments, cells were transferred to the phenol red-free media described above, containing 10% dextran-charcoal treated (CD)-CS (T47D/A1-2 and HeLa cells) or 10% CD-FBS (T47D-KBluc and T47D cells) with hormone or compound added in DMSO. MCF-7 cells were transferred to 5% CD-FBS and assays were conducted in 10% CD-CS media.

2.2.2. Reporter Gene Assays—Assays for progesterone and glucocorticoid-dependent transactivation were performed in T47D/A1-2 breast cancer cells. Estrogen and androgen-dependent transactivations were assayed in T47D-KBluc and HeLa-A6 cells, respectively. Cells were seeded overnight and then treated with 5 nM of progesterone (P), dexamethasone (DEX), estradiol (E₂), dihydrotestosterone (DHT), or ethanol vehicle - with or without 10 μM of the test compounds in DMSO. After 24 h, cells were washed once with phosphate buffered saline (PBS) and lysed in passive lysis buffer (Promega, Madison WI). Luciferase activity was determined using BrightGlo firefly luciferase reagent from Promega.

2.2.3. Alkaline Phosphatase Assay—T47D cells were seeded overnight and then treated for 24 h with 2.5 nM P, with or without test compounds in DMSO. Cells were washed once with PBS, lysed in harvest buffer (20 mM potassium phosphate, pH 7.8, 5 mM MgCl₂, 0.5% Triton X-100) and frozen at -70°C. 5 μl of thawed supernatant was removed and assayed in a 96-well plate in 25 μl of assay buffer containing 100 mM diethanolamine, pH 9.5, 1 mM MgCl₂, 0.4 mM CSPD substrate (Applied Biosystems/Tropix) and 1X Emerald II enhancer (Applied Biosystems/Tropix). After 1 h at room temperature, luminescence was measured in the visible spectrum (emission max. 542 nm).

2.2.4. Enzyme Inhibition Assays—Assays using purified luciferase protein and the test compounds were performed to rule out direct effects on luciferase enzyme activity. 0.05 ng of purified luciferase protein was added to 5 μl OneGlo Buffer (Promega) in a 96 well plate at a final concentration of 0.01 ng/μl luciferase protein. Each compound was diluted to 10 μM in buffer. 5 μl BrightGlo firefly luciferase reagent was added and incubated for 5 minutes before measuring luciferase activity. To test whether compounds directly affected alkaline phosphatase (AlkP) activity, lysates from cells treated with 2.5 nM P, to induce sufficient AlkP, were aliquoted and treated with 10 μM of each compound for 15 minutes. Assay buffer (described above) was added to the lysate, incubated at room temperature for 1 h, and luminescence was measured.

2.2.5. RNA Isolation and Quantitative PCR—To induce alkaline phosphatase expression, cells were maintained in medium containing 10 nM P and the indicated concentrations of each compound in DMSO for 24 h. Total RNA was isolated using the RNeasy Mini Kit (QIAGEN, Austin, TX) and 1 μg of RNA was reverse transcribed using ProtoScript M-MuLV First Strand cDNA Synthesis Kit (New England BioLabs, Ipswich, MA). Quantitative PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems, United Kingdom). mRNA levels were measured by quantitative RT-

PCR as described [13]. Primers used in qRT-PCR were: AlkP, forward (5'-TCGCTACCAGCTCATGCATAACA) and reverse (5'-TGAAGCTCTTCCAGGTGTCAACGA); and 36B4 internal standard, forward (5'-GTGTTTCGACAATGGCACAT) and reverse (5'-GACACCCTCCAGGAAGCGA).

2.2.6. Receptor Binding Assay—Relative binding affinities were determined in a competitive radiometric assay as previously described [16-18] using 10 nM tritiated tracer, unlabeled promegestone (R5020) as standard, and 10 nM purified full length recombinant human PR-B (Pan Vera/Invitrogen, Carlsbad, CA). Incubations were for 18-24 h at 0°C. Hydroxyapatite (BioRad, Hercules, CA) was used to absorb receptor-ligand complexes, and free ligand was removed by washing with cold buffer. The data were analyzed using Prism 4.0. Binding affinities are expressed as relative binding affinity values with unlabeled R5020 set to 100%. The ³H tracer, [17 α -methyl-³H]-R5020 (Perkin-Elmer, Boston, MA), binds PR with a K_d of 0.4 nM.

2.2.7. Statistical Analysis—Results are expressed as mean \pm standard error (SEM) of at least three independent experiments. Student's t-test was used for comparison of the means between two groups. Significance was established when $p < 0.05$.

3. Results

The ability of 8-thioalkyltheophyllines to inhibit the activity of PR, ER α , GR and AR was assessed using stably transfected cell lines, each expressing receptor-specific hormone response elements linked to a luciferase reporter. PR and GR activities were assayed in T47D/A1-2 breast cancer cells stably transfected to express a mammary tumor virus (MMTV)-luciferase promoter. ER α activity was assayed in T47D-KBluc cells expressing a reporter that contains 3 copies of the consensus estrogen response element (ERE)₃-luciferase. AR activity was assayed in HeLa-A6 cells stably transfected to express AR and a prostate specific antigen (PSA)-luciferase reporter. Compounds were prepared as 10 mM stocks in DMSO and tested at 10 μ M. The final DMSO concentration (0.1%) was below the 0.3% (v/v) concentration associated with cytotoxic effects [10]. Receptor activity was assayed in the presence of progesterone (P) for PR, 17 β -estradiol (E₂) for ER, dexamethasone (DEX) for GR, and dihydrotestosterone (DHT) for AR. Table 1 summarizes the percent transcriptional activity remaining in the presence of theophylline and 54 of its structural derivatives. A table containing similar data for 93 more structurally diverse analogues is provided in the Appendix.

Unmodified theophylline did not inhibit transactivation by any of the steroid receptors, whereas several theophylline derivatives exhibited varying levels of inhibition. To increase lipid solubility, oxygens in the X and Y positions were substituted with sulfurs. The resulting 8-alkylthio-2-thio, 8-alkylthio-6-thio, and 8-alkylthio-2,6-dithiotheophyllines displayed increased inhibition of transactivation, with 6-thio-substituted theophyllines being the most potent inhibitors. For example, compounds **24** and **31** reduced steroid receptor activity to a greater extent than their unsubstituted, 2-thio and 2,6-dithio counterparts (**23-26** and **30-33**, respectively).

The 6-thiotheophyllines with 8-alkylthio-substitutions 5-9 carbons in length (**24**, **31**, and **34**) inhibited PR activity 41-58%, but lacked specificity. Efficacy and specificity for PR was improved with compounds with alkyl branching. For example, **45** and **46**, with a single branched methyl group were moderately potent inhibitors of PR with no activity against ER, GR, and AR. Methyl branching on the terminal carbon improved efficacy, but also inhibited ER, as in the case of **48** and **49**. Increasing the number of methyl groups with compound **49**'s tertiary substitution inhibited PR activity 44%, but it also inhibited ER and GR.

Compounds **51** and **52** had ethyl group side chains and inhibited PR to a greater extent than compounds with methyl side chains. The location of the ethyl side chain was also slightly more favorable in **51** and **52** which contain terminal branching.

Based on the results of the primary screen that indicated $IC_{50}s < 10 \mu M$ and specificity for PR, the alkyl-branched 8-alkylthio-6-thiotheophylline, compound **51**, was selected as the lead compound for further study. Its structure is shown in Figure 1A.

Since most current PR antagonists also inhibit GR, we assessed the potency and specificity of compound **51** in dose-response studies that compared its ability to inhibit PR, ER, GR, and AR (Figure 1B). In the luciferase reporter assays, **51** preferentially inhibited PR, followed by ER, AR, and then GR. Its IC_{50} was $2.3 \mu M$ for PR, which was 2 to 6 fold lower than for the other receptors (Figure 1C). Since PR and GR activities were assessed in a single line of T47D cells, using the same MMTV-luciferase reporter, the absence of GR inhibition is not an artifact due to use of different cells and promoters. In these cells, compound **51** was a 6.1-fold more potent inhibitor of PR than GR, and compounds **48** and **52**, which also contain this novel branched-chain alkyl motif, showed up to 10-fold increased potency on PR than GR (Figure A.1).

We next tested the ability of the lead compound to inhibit progesterone induction of the endogenous alkaline phosphatase (AlkP) gene in T47D human breast cancer cells [19,20]. In these cells, AlkP enzyme activity was induced 23 fold by progesterone, but not by E_2 , DEX, or DHT (data not shown). **51** potently inhibited progesterone induction of AlkP activity with an IC_{50} of $1.2 \mu M$ and complete inhibition by $5 \mu M$ (Figure 2A).

We tested the possibility that the inhibition by **51** was an artifact due to direct inhibition of luciferase or alkaline phosphatase enzyme activity. Compound **51** did not inhibit the enzymatic activity of 0.05 ng of pure luciferase protein ($t > 0.1$). In lysates from cells in which progesterone was used to induce alkaline phosphatase activity (16.5 ± 0.3 fold induction relative to control), treatment with **51** failed to significantly inhibit activity ($t > 0.3$) (data not shown).

The specificity study in Fig. 1 used stably transfected reporter genes. To evaluate the effects of **51** on the expression of an endogenous gene, we used qRT-PCR to investigate progesterone induction of alkaline phosphatase mRNA. The 11.8 ± 1.6 fold induction in mRNA levels was inhibited 74% by $5 \mu M$ of compound **51**, and completely blocked at $10 \mu M$ (Figure 2B).

To compare **51**'s ability to inhibit an ER-regulated endogenous gene, we analyzed pS2 mRNA. We tested the effect of compound **51** on ER at an early time, at which estrogen-ER has not yet induced PR, thus the cells were maintained in medium containing $5 \mu M$ compound **51** for only 2 hours. Under the same conditions in which **51** completely blocked the progesterone induction of AlkP mRNA, it had no effect on the 17β -estradiol induction of pS2 mRNA (Figure A.2). As a positive control, the estrogen antagonist, ICI 182,780 inhibited induction of pS2 mRNA. This data provides additional evidence that compound **51** preferentially inhibits PR-mediated gene expression.

Most PR antagonists compete with progesterone for binding in the ligand-binding domain of the receptor. At functionally significant concentrations, compound **51** had little or no ability to compete with the radio-labeled synthetic progestin, promegestone (R5020), for binding to PR (Figure 3) and bound 4,700 fold less well than R5020. These results indicate that **51** is unlikely to act by competing with progestins for binding in PR's ligand-binding pocket. It

remains possible that binding of **51**, and other thiotheophylline derivatives, to a second site on PR triggers a conformational change that influences the ability of PR to bind progestins.

4. Discussion

Previous studies showed that a set of 8-alkylthiotheophyllines displayed very weak antitumor activity [11]. During a screen of a diversity test library, one of these compounds was identified as an inhibitor of ER α action [10]. This set of small molecules was further explored to identify a structural motif that confers preferential inhibition of steroid hormone receptor activity. Replacing the oxygens in theophylline with sulfurs increased general inhibition of steroid receptor activity. 6-thio substitutions were more effective than 2-thio or 2,6-dithio substitutions. 8-thioalkyl substitution further increased potency, with preferential inhibition of PR as saturated chains 5-9 carbons in length were included. At shorter chain lengths, inhibition was generally weak, while longer chain lengths were not specific for inhibition of PR. Addition of saturated carbon-chain branching increased selectivity and potency for inhibiting PR activity. Our SAR investigation of PR, ER, GR, and AR-responsive reporter genes identified compound **51**, which belongs to a set of saturated branched 8-alkylthio-6-thio-substituted theophyllines that preferentially inhibited PR. Compound **51** demonstrated pure antagonist effects – at concentrations of 30 μ M and higher, there was no observed agonist activity or decrease in antagonism as is often seen with many selective PR modulators like RU486.

The LBDs of steroid receptors share a similar structure with a moderately conserved amino acid sequence. Therefore, PR antagonists, like RU486 that binds in the ligand-binding pocket, tend to discriminate poorly between receptors. Their ability to exert anti-glucocorticoid activity limits the use of many PR antagonists. Our ability to identify small molecules that selectively inhibit PR over GR activity was enhanced by testing them on the same MMTV-luciferase reporter in T47D cells. Since the small molecules were assayed for inhibition of PR and GR on the same promoter in the same cells, it is highly unlikely that general toxicity is responsible for selective inhibition of PR by compound **51**.

Ligand competition studies were used to evaluate the ability of **51** to compete with a progestin for binding to the ligand-binding pocket of PR. Based on these studies, compound **51** and other branched 8-alkylthio-6-thio-substitutions likely exert their actions outside the progestin binding pocket.

Compound **51** inhibited PR-induced enzyme activity of the stably transfected luciferase reporter and endogenous alkaline phosphatase. Using quantitative RT-PCR, we also showed that **51** inhibits the progesterone-PR induction of AlkP mRNA. Thus, compound **51** is a noncompetitive inhibitor of PR-mediated gene expression.

PR, GR and AR are a subgroup of steroid receptors that can bind to the same DNA response element. How target genes are selectively activated by individual receptors is not well understood. Compound **51** may prove useful in elucidating differential effects of PR, GR and AR on gene expression and in distinguishing actions mediated by PR from those mediated by GR and other closely related members of the steroid receptor family.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Ms. Kathryn Carlson and Prof. John Katzenellenbogen (University of Illinois) for conducting the radioligand competition assays.

Appendix A. Supplementary Data

A table reporting the inhibition of steroid receptor action for the remaining 93 analogues of theophylline and the dose response curves of **48** and **52**, compounds closely related to lead compound **51**. This material is available via the internet at <http://www.journals.elsevier.com/steroids/>.

Abbreviations

AlkP	alkaline phosphatase
AR	androgen receptor
ER	estrogen receptor
GR	glucocorticoid receptor
LBD	ligand binding domain
PR	progesterone receptor
SAR	structure activity relationship

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Highlights

- SAR of theophylline analogues against 4 major steroid receptors
- Novel motif preferentially inhibits progesterone receptor activity
- Inhibitor is pure antagonist of PR-mediated reporter and endogenous gene activity
- Compound 51 does not display the properties of a competitive ligand

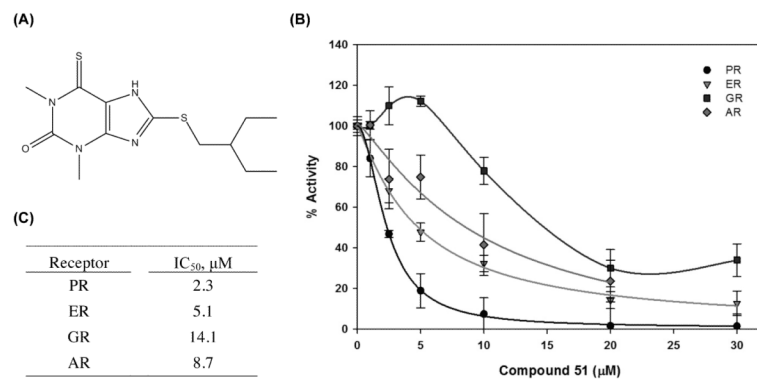


Figure 1. Compound **51** is a selective inhibitor of PR-mediated transactivation. (A) Structure of **51**. (B) Inhibition of transactivation by **51** in reporter gene assays. Reporter assays were performed as described in the Experimental section. For each hormone, activity in the absence of compound **51** was set as 100%. Error bars represent the SEM for at least three experiments. (C) IC₅₀s were calculated using Sigma Plot 11.0.

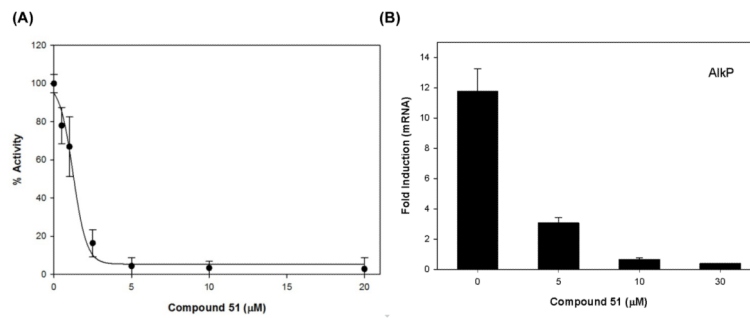


Figure 2. Inhibition of progesterone-induced alkaline phosphatase enzyme activity (A) and mRNA levels (B) by compound **51** in T47D cells. Alkaline phosphatase assays and qRT-PCR were performed as described in the Experimental section. Error bars represent the SEM for at least three experiments. AlkP activity in the absence of **51** was set to 100%. Enzyme activity $IC_{50} = 1.2 \mu\text{M}$. AlkP mRNA in the absence of hormone and **51** was set to 1.0.

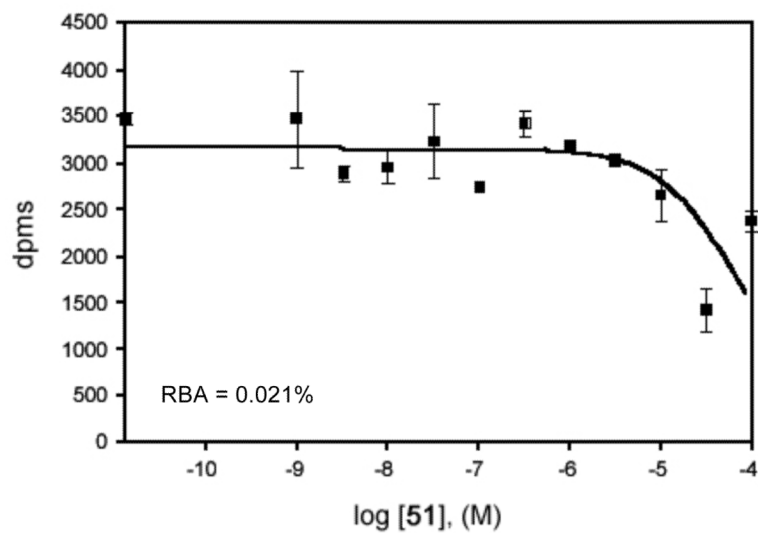
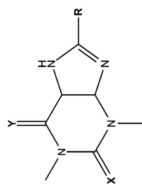


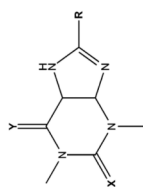
Figure 3. Competitive binding of compound **51** with 10 nM R5020 to the progesterone receptor. Competition assays were performed as described in the Experimental section. Error bars represent the SEM for at least three experiments.

Table 1

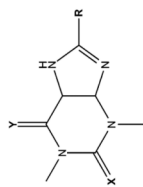
Inhibition of Steroid Receptor Activity by Theophylline Analogues



Cmpd	X	Y	R	Reporter Assay (% Activity) ^d			
				PR	ER	GR	AR
1	O	O	H	105	93	91	95
2	O	O	CH ₂ CH ₃	76	106	120	83
3	O	O	CH ₂ CH ₂ CH ₂ CH ₃	155	88	121	80
4	O	O	CH ₂ Ph	144	94	117	102
5	O	O	CH ₂ CH ₂ Ph	89	82	94	101
6	O	O	SCH ₂ CH ₃	60	98	100	115
7	O	O	SCH ₂ CH ₂ CH ₃	89	111	101	108
8	O	O	SCH ₂ Ph	77	104	127	121
9	O	O	SCH ₂ CH ₂ Ph	86	87	130	101
10	O	O	SCH ₃	9	6	90	119
11	O	S	SCH ₃	96	102	106	76
12	S	O	SCH ₃	114	104	101	76
13	S	S	SCH ₃	107	101	103	75
14	O	S	SCH ₂ CH ₃	89	83	112	80
15	S	O	SCH ₂ CH ₃	147	102	103	97
16	S	S	SCH ₂ CH ₃	123	95	102	86
17	O	S	SCH ₂ CH ₂ CH ₃	104	75	116	95
18	S	O	SCH ₂ CH ₂ CH ₃	202	82	82	104
19	S	S	SCH ₂ CH ₂ CH ₃	64	79	79	78
20	O	S	SCH ₂ CH ₂ CH ₂ CH ₃	63	99	104	84
21	S	O	SCH ₂ CH ₂ CH ₂ CH ₃	117	87	82	150



Cmpd	X	Y	R	Reporter Assay (% Activity) ^d			
				PR	ER	GR	AR
22	S	S	SCH ₂ CH ₂ CH ₂ CH ₃	51	77	91	76
23	O	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₃	58	109	122	89
24	O	S	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₃	57	53	74	120
25	S	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₃	58	87	67	95
26	S	S	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₃	72	72	79	87
27	S	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	76	91	78	85
28	S	S	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	106	94	103	89
29	S	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	115	79	83	79
30	O	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	33	112	92	101
31	O	S	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	59	14	48	109
32	S	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	82	79	91	81
33	S	S	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	97	77	97	86
34	O	S	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	42	23	51	71
35	S	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	79	92	100	123
36	S	S	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	80	100	93	100
37	O	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	72	109	88	107
38	S	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	95	84	90	121
39	S	S	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	66	96	85	85
40	S	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	73	105	95	91
41	O	S	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	76	49	98	104
42	S	O	SCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	87	112	97	91
43	O	S	SCH ₂ CH(CH ₃) ₂	67	93	80	134
44	O	S	SCH(CH ₃)CH ₂ CH ₃	86	85	86	103



Cmpd	X	Y	R	Reporter Assay (% Activity) ^a			
				PR	ER	GR	AR
45	O	S	SCH(CH ₃)/CH ₂ CH ₂ CH ₃	53	94	97	112
46	O	S	SCH(CH ₃)/CH ₂ CH ₂ CH ₂ CH ₃	58	104	105	106
47	O	S	SCH(CH ₃)/CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	65	55	87	97
48	O	S	SCH ₂ CH ₂ CH(CH ₃) ₂	35	65	89	74
49	O	S	SCH ₂ CH ₂ C(CH ₃) ₃	56	45	83	90
50	O	S	SCH(C ₂ H ₅)/CH ₂ CH ₂ CH ₃	51	72	71	78
51	O	S	SCH ₂ CH(C ₂ H ₅) ₂	24	54	89	96
52	O	S	SCH ₂ CH(C ₂ H ₅)/CH ₂ CH ₂ CH ₂ CH ₃	27	51	83	85
53	O	S	SCH ₂ Ph	58	86	156	97
54	O	S	SCH ₂ CH ₂ Ph	15	33	78	114
55	O	S	SCH ₂ CH ₂ CH ₂ Ph	68	37	45	122

^a Activities were determined as a function (%) of maximal luciferase activity (100%) induced by hormone-bound steroid receptors (P:PR, E₂:ER, DEX:GR, or DHT:AR) as described in the Experimental section.