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Substituted 4-hydroxyphenyl sulfonamides as pathway-selective estrogen receptor ligands

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Abstract—The transcription factor nuclear factor-κB (NF-κB) is a key component in the onset of inflammation. We describe here a series of 4-hydroxyphenyl sulfonamide estrogen receptor (ER) ligands that selectively inhibit NK-κB transcriptional activity but are devoid of conventional estrogenic activity.

Overproduction of the cytokine interleukin (IL-6) has been associated with states of chronic inflammation.[1] Production of the IL-6 gene is initiated by the transcription factor nuclear factor κB (NF-κB), a homodimeric and heterodimeric family of the Rel family of proteins which resides in the cytoplasm in a nonactive form bound to the cytoplasmic inhibitory protein-κB (IκB). Although well known for their classic effects on the reproductive tract and action via estrogen response elements in gene promoters, non-selective estrogens such as E2 (17β-estradiol) are also known to have anti-inflammatory activity.[2] This non-classical anti-inflammatory activity of estrogen has been attributed to interference of NF-κB activity through a variety of possible mechanisms.[3]

In endothelial cells, it has been shown that E2 inhibits IL-1β-induced NF-κB reporter activity and IL-6 expression in an estrogen receptor (ER) dependent fashion.[4] This suppression of Il-6 expression correlates with the anti-inflammatory action of E2 in vivo as confirmed in different models of inflammation.[5] The beneficial role of estrogen therapy on bone density, plasma lipid profiles, vasomotor symptoms, and colon cancer has been documented.[6] However, estrogen therapy has also been associated with an increased risk for breast cancer and uterine bleeding. In light of these potential side effects, more selective ligands for ERα have been sought. We thus began an HTS screen for selective ER ligands using an assay developed in HAECT-1 cells.[7] HAECT cells are transfected with human ERα and a reporter gene NFκB-luciferase. These cells are then treated with IL-1β and the test compound. The amount of luciferase present translates directly into the degree of NF-κB transcription. The target profile for a test compound is a $\leqslant$100 nM IC_{50} of NF-κB luciferase reporters with an efficacy (% E2) near 100%, coupled with a lack of classical estrogen receptor activity. Since E2 strongly stimulates creatine kinase (CK) expression, an in vitro assay measuring CK concentration serves as a monitor of unwanted classic estrogenic effects.

Compounds of general type **4** were identified which met the above criteria. The synthesis of these compounds was straightforward as depicted in Scheme 1. The appropriately substituted anilines (**1**) were N-alkylated to compounds **2** by reductive amination with sodium triacetoxyborohydride and an aldehyde.[8] These anilines were coupled with a sulfonyl chloride to give sulfonamides **3**

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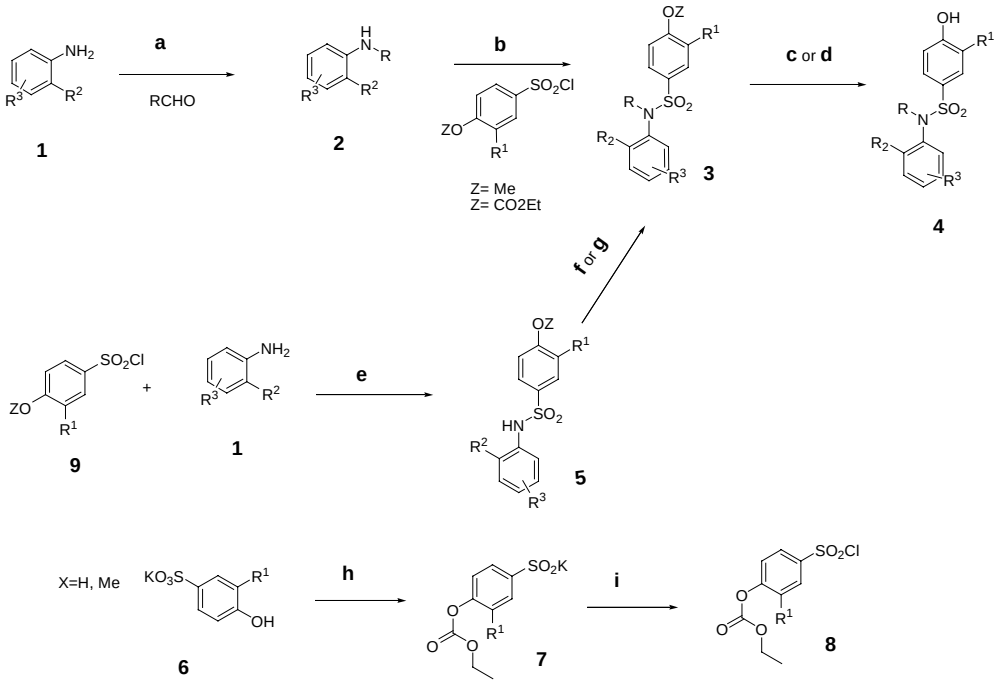
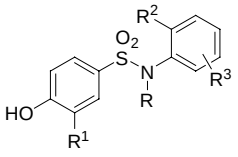


Scheme 1. Reagents and conditions: (a) AcOH/$NaBH(OAc)_3$/DCM (83–89%); (b) Et_3N/DCM (79–87%); (c) BCl_3/*n*-Bu_4NI (for Z = Me) (45–68%); (d) 10% K_2CO_3/MeOH (for Z = CO_2Et) (86–91%); (e) Et_3N/DCM/14 h (78–88%); (f) RX/K_2CO_3/CH_3CN (69–81%); (g) DEAD/PPh_3/THF/ROH (48–67%); (h) $ClCO_2Et$/aq NaOH (78%); (i) SO_2Cl_2/toluene/cat. DMF (81%).

and then deprotected to give the final phenolic target compounds. When a methoxy group was employed as a protecting group for the phenol, de-methylation of sulfonamides **3** was achieved with BCl_3 and *n*-Bu_4NI.[9] The standard procedure employing BBr_3 usually resulted in bromination ortho to the phenol or in some cases, loss of the N-alkyl group. Compound **8** containing a carbonate-protecting group was later employed, allowing deprotection under mild conditions with no side products.[10,11]

Table 1 summarizes the biological results of target molecules **4a**–**4n**. The initial lead **4a** had weak activity in the luciferase assay, however, it provided a template to begin SAR development. The effect of substituents on both rings and the chain length/branching of the R group of the amide nitrogen were two points with which to begin the SAR study. Two noteworthy points quickly established in this series were the need for a substituent R on the amide nitrogen (as exemplified by the inactivity of compound **4b**) and a 4-OH on the aromatic ring containing the sulfonyl group. Attempts to replace this critical OH with Cl, OMe, NO_2 or a phenol mimic (CO_2H, CH_3CONH) resulted in a total loss of activity. Placement of the phenol group was also crucial. Both the 3-phenol and the 3,4-diphenol analogues resulted in a substantial loss of activity.



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Compounds **4e**–**4m** in Table 1 summarize the basic SAR study of the amide nitrogen R group. The optimal group was straight chain C3 (compounds **4f** and **4h**) with little advantage in potency seen in larger chains or branching. Branching on the carbon directly attached to the nitrogen as in isopropyl (**4g**), cyclohexyl (**4k**) or phenyl (**4j**) was particularly detrimental to activity. Surprisingly, addition of a hydroxyl group at the end of the 2 carbon chain (**4n**) resulted in loss of activity.

We next turned our attention to substitution on the aromatic rings. Substitution of a lipophilic group ortho to the phenol could produce a beneficial effect, but was not mandatory for activity. In some cases, an ortho methyl group improved the selectivity. Addition of a methoxy group (**4o**) resulted in a loss of activity. It became apparent that the 3 position might increase selectivity but not potency.

The most dramatic effects were seen by the addition of substituents on the aniline containing aromatic ring. Placing a methoxy group at either the ortho, meta or para position of the aniline containing ring (compounds **4p**–**4r**) demonstrated that electron-donating groups were not favorable. It was noted in these cases that the ortho substitution seemed to have the most activity in this series albeit weak. Nonetheless, the possibility of an 'ortho effect' was investigated. This hypothesis came to fruition in the case of the 2-bromo derivative (compound **4s**). A dramatic increase in potency resulted, producing a compound with a roughly 100 nM activity in the luciferase assay and good selectivity, which fell within project criteria. This significant breakthrough shifted our focus toward the exploration of lipophilic ortho substitution. We concentrated on scaffold **10**, maintaining the

Table 1. Effects of *n*-alkyl analogues of sulfonamides versus E2 on NF-κB, and CK expression in Ad5-wt-ER-infected HAECT-1 cells[a]

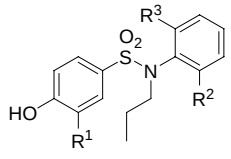
Compound[b]	R^1	R^3	R	ER/NFκB-luc IC_{50} (nM)	% E2-NF/κB	CK EC_{50} (nM)	% E2-CK	Fold selectivity	% E2 selectivity
4a	H	H	Et	404 ± 81	100	1600 ± 525	25	3.9	4
4b	H	H	H	na					
4c	Me	H	Et	328 ± 105	111	2135 ± 805	36	6.5	3.0
4d	H	4-OH	Et	272 ± 105	87	1391	94	5	1.1
4e	H	H	Me	na					
4f	H	H	*n*-Pr	303 ± 138	113	1430 ± 282	88	4.7	.77
4g	H	H	*i*-Pr	871	81	1985	31	2.3	2.6
4h	H	H	Allyl	235	107	599	54	2.5	2.0
4i	H	H	*n*-Bu	317	98	705	25	2.2	3.9
4j	H	H	Phenyl	410	84	1543	45	3.8	1.9
4k	H	H	Cyclohexyl	866	86	534	35	1.6	2.5
4l	H	H	Benzyl	500	79	242	34	2.0	2.3
4m	H	H	neo-Pentyl	1371	92	>2500	35	1.8	2.6
4n	H	H	2-OHEt	na					
4o	OMe	H	Et	na					
4p	H	2-OMe	Et	830	49	2171	54	2.6	.91
4q	H	3-OMe	Et	1973 ± 800	37	830		.42	
4r	H	4-OMe	Et	na					
4s	H	2-Br	Et	90 ± 8	98	378 ± 7	37	4.2	2.6

na = not active.
% E2 = efficacy (relative inhibition of test compound at 10 μM versus E2 at 0.1 nM).
[a] All compounds were ER dependent (active when ER is coexpressed with NFκB-luciferase in the HAECT cells).
[b] R^2 = H.

optimized *n*-propyl group on the amide nitrogen. Table 2 summarizes the biological data for compounds containing an ortho substitution. Compound **10a**, combining the best SAR knowledge to date, produced a lead with 40 nM activity and 10-fold selectivity over CK. We attribute the greater selectivity of **10a** (CK EC_{50} = 480) versus **10b** (CK EC_{50} = 151) to the methyl group ortho to the phenol.

All of the compounds in Table 2 were potent in the luciferase assay at 100 nM or less with the exception of *o*-phenyl (compound **10i**) and *o*-Me (compound **10d**) both of which had activity on a par with the initial leads. The ortho effect was particularly striking in those compounds with di-ortho substitution. Compounds **10j** through **10o** proved to be the most potent with luciferase activities <30 nM and selectivities in most cases more than 10-fold over CK.

With the SAR of the simple chain compounds understood we then explored the effect of having restricted rotation analogues. These would have the benefit of having an N-substituted chain and an ortho substituent as the same moiety. The simple analogue **11** was devoid of activity; however, with the addition of a methyl group alpha to the nitrogen (compound **12**), enhancement of luciferase activity was observed. We also investigated the effect of restricted rotation analogues as exemplified by the benzothiazine **16**. This type of constrained analogue is not easily accessible via commercially available material as in the previous case, so a synthetic route was devised (Scheme 2). Ethyl 3-methoxyphenyl acetate (**13**) was chlorosulfonated para to the methoxy group with chlorosulfonic acid and then coupled with 2-chloro aniline to give sulfonamide **14**. The ester was reduced with lithium aluminum hydride to afford the alcohol, which was cyclized via a Mitsunubo reaction to give **15**. The phenol was deprotected with BCl_3/*n*-Bu_4NI as before to give the target compound **16**. Table 3 summarizes the biological results for the cyclic compounds.



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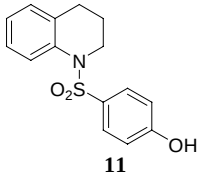

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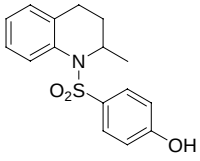

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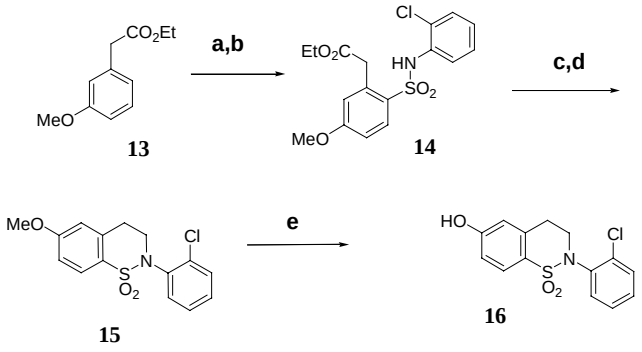
The results clearly show the superior activity of the straight chain analogues versus the cyclic examples. In general, the SAR of the straight chain sulfonamides as exemplified by structure **4** can be summarized in the following manner: (1) a hydroxyl group para to the sulfonyl is mandatory for activity, while the addition of a meta lipophilic group shows an increase in selectivity, (2) smaller N-alkyl chain analogues show the best potency with propyl being the optimal group. Some tolerance of chain extension can be seen but branching at the α-carbon is not tolerated, (3) a lipophilic group ortho to the aniline nitrogen enhancing luciferase activity.

Table 2. Effects of *o*-substituted analogues of *n*-propyl sulfonamides versus E2 on NF-κB, and CK expression in Ad5-wt-ER-infected HAECT-1 cells[a]

Compound	R^1	R^2	R^3	ER/NFκB-luc IC_{50} (nM)	% E2-NF/κB	CK EC_{50} (nM)	% E2-CK	Fold-selectivity	% E selectivity
10a	Me	H	Br	40	97	480	45	12	2.1
10b	H	H	Br	26 ± 10	98	151 ± 14	35	5.8	2.8
10c	CF_3	H	Br	60	120	na			
10d	Me	H	Me	109 ± 2	115	923 ± 57	42	8.4	2.7
10e	H	H	F	74	104	161	63	2.1	1.6
10f	H	H	Cl	42 ± 2	108	144 ± 31	53	3.4	2.0
10g	Me	H	Cl	51 ± 11	79	650	52	12.8	1.5
10h	H	H	CF_3	54 ± 21	138	243	39	4.5	3.5
10i	Me	H	Ph	253	42	>2889		11.4	
10j	H	Me	Cl	24 ± 15	107	63 ± 23	70	2.6	1.5
10k	Me	Me	Cl	26 ± 5	83	153 ± 22	24	5.8	3.5
10l	Me	Et	Et	5 ± 4	72	213 ± 31	42	42.6	1.7
10m	Me	Cl	Cl	16 ± 5	96	378 ± 84	35	23.6	2.7
10n	Me	Me	Me	14 ± 5	90	75 ± 35	30	5.3	3
10o	Me	Me	Et	7 ± 5	53	12	43	1.7	1.2

na = not active.
% E2 = efficacy (relative inhibition of test compound at 10 μM versus E2 at 0.1 nM).
[a] All compounds were ER dependent (active only when ER is coexpressed with NFκB-luciferase in the HAECT cells).



Scheme 2. Reagents and conditions: (a) $ClSO_3H$, neat (41%); (b) 2-Cl aniline/CH_2Cl_2/Et_3N (68%); (c) LAH/ether/0 °C (55%); (d) DEAD/PPh_3/THF (62%); (e) BCl_3/*n*-Bu_4NI (68%).

Table 3. Effects of cyclic sulfonamides versus E2 on NF-κB, and CK expression in Ad5-wt-ER-infected HAECT-1 cells[a]

Compound	ER/NFκB-luc IC_{50} (nM)	% E2 ER/NFκB	CK EC_{50} (nM)	% E2 CK
11	na			
12	479	98	>1000	
16	892	80	na	

na = not active.
% E2 = efficacy (relative inhibition of test compound at 10 μM versus E2 at 0.1 nM).
[a] All compounds were ER dependent (active when ER is coexpressed with NFκB-luciferase in the HAECT cells).

In conclusion, we have demonstrated that a known class of sulfonamides are potent pathway-selective inhibitors of NF-κB induced inflammatory events through ER that are devoid of the traditional unwanted effects of estrogens.[12]

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11. Typical procedures: To a dichloromethane solution of aniline **1** were added 1.1 equiv of proprionaldehyde, 1.1 equiv of AcOH, and 1.5 equiv of $NaBH(OAc)_3$. The reaction mixture was stirred at room temperature overnight and then quenched with 1 N NaOH. The organic phase was separated and the aqueous layer was extracted once more with dichloromethane. The organic layers were combined, dried ($MgSO_4$), and concentrated to yield N-substituted anilines **2**, which could be purified by filtration through a plug of silica gel, eluting with 15% ethyl acetate/hexane. The resulting product was dissolved in dichloromethane and 1.2 equiv of triethylamine added, whereupon a solution of 1.2 equiv of **8** in dichloromethane was added dropwise. Progress of the reaction was monitored by TLC until most of the aniline was consumed. The solution was concentrated and the residue was subjected to silica gel chromatography eluting with 20% ethyl acetate/hexane to provide sulfonamides **3**. The sulfonamide **3** ($Z = CO_2Et$) was dissolved in methanol and a solution of 10% aqueous potassium carbonate added and the reaction monitored by TLC until all the starting material was consumed. The solution was concentrated and the residue extracted 2× with ethyl acetate and the combined extracts dried ($MgSO_4$) and concentrated. The product phenols **4** are crystallized from ethyl acetate/hexane. All compounds had NMR and MS data consistent with the proposed structures.
12. Representative examples of compounds were also screened against ERR, FXR, PPARg, PPARd,LXRa, LXRb, and VDR with no cross-activity observed at 10 uM.