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Tetrahydroquinoline-Based Selective Estrogen Receptor Modulators (SERMs)

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Abstract—A new series of estrogen receptor ligands based on a 6-hydroxy-tetrahydroquinoline scaffold is described, in addition to their binding affinity and functional activity in MCF-7 cells. Several 1,2-disubstituted tetrahydroquinolines bearing a basic side chain were shown to be high affinity ligands and antagonists in the MCF-7 proliferation assay. Compounds lacking the basic side chain were agonists in the MCF-7 assay.

The estrogen receptor is a ligand activated transcription factor which plays an important role not only in the regulation of the female reproductive system, but also in bone, cardiovascular and central nervous system function.[1] Estrogen receptor ligands, and the selective estrogen receptor modulators (SERMs) in particular, are being extensively studied for the treatment of reproductive disorders, estrogen responsive cancers and osteoporosis. A common structural feature of SERMs is a bicyclic core containing a phenolic hydroxyl, with a basic side chain and an aryl ring emanating from the core. Recently described SERMs from this general class include the benzothiophenes (e.g., raloxifene),[2] indoles (e.g., pipendoxifene)[3] and tetrahydronaphthalenes (e.g., lasofoxifene).[4] We herein report the design and synthesis of a series of substituted tetrahydroquinolines, their binding affinity to ERα and ERβ and antagonism of estradiol in MCF-7 breast adenocarcinoma cells.[5]

Although *N*-aryl tetrahydroquinolines which lacked the A-ring phenol have been previously reported to be antifertility agents in rats,[6] their interaction with the estrogen receptor has not been characterized. We felt that the 6-hydroxy-tetrahydroquinoline core would be a suitable structure for the generation of new ER ligands. We not only wished to investigate *N*-aryl derivatives, but also wanted to explore the effect of introducing a carbonyl or methylene linker between the tetrahydroquinoline core and the aminoalkoxyaryl side chain (Fig. 1). In addition, several intermediates lacking the basic side chain were deprotected to liberate the A-ring phenol and were assessed for ER binding and MCF-7 antagonism.

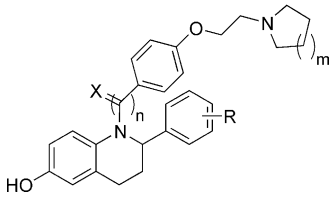

Figure 1. Generic structure of tetrahydroquinoline SERMS.

We first chose to examine analogues where the side chain was connected to the tetrahydroquinoline core via an amide linker. The synthesis began (Scheme 1) with commercially available 6-methoxyquinoline *N*-oxide (**1**) which was arylated at the 2-position using a modification of a literature protocol. Treatment of **1** with methyl chloroformate followed by addition of an aryl Grignard reagent afforded the desired 6-methoxy-2-aryl quinolines **2a–c**.[7] Alternatively, the 2-aryl quinoline could be prepared by addition of an aryllithium to 6-methoxyquinoline;[8] however, the former approach was preferred due to the wider availability of aryl Grignard reagents. Deprotection of the methoxy group of **2c** was readily achieved with BBr_3 in CH_2Cl_2 to afford quinoline **3**. Reduction of the nitrogen-containing ring of **2a–c** was carried out with sodium in ethanol,[9]

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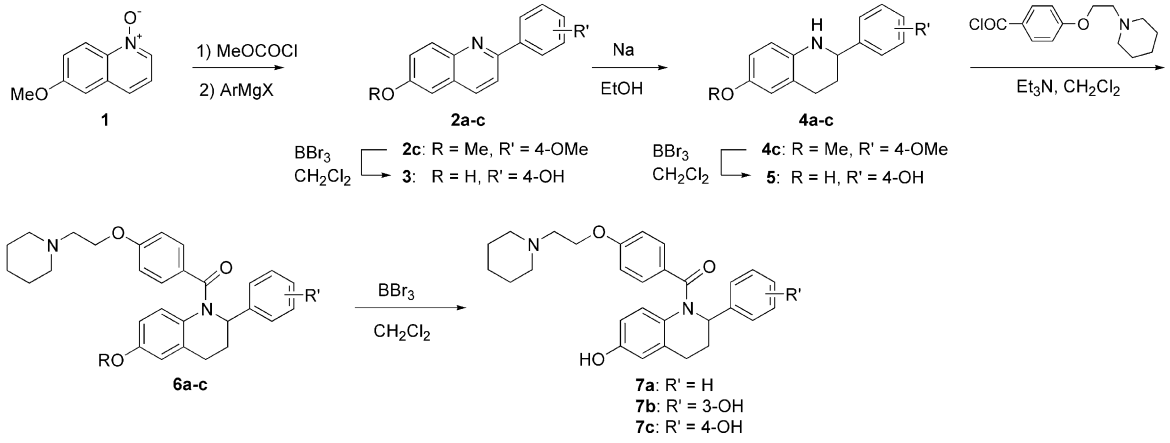


Scheme 1.

thereby yielding tetrahydroquinolines **4a–c**. BBr_3 deprotection of **4c** afforded the free diphenol **5**.

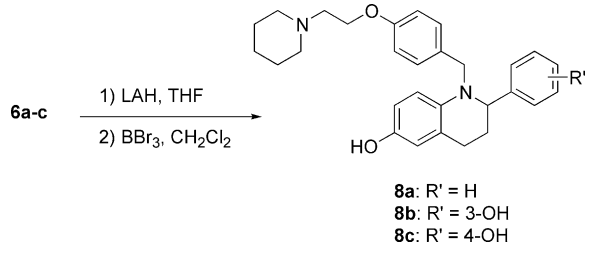
Acylation of the tetrahydroquinoline nitrogen was readily accomplished by treating **4a–c** with 4-(2-piperidinyl-ethoxy)-benzoyl chloride and Et_3N in CH_2Cl_2 to yield amides **6a–c**. Removal of the methoxy protecting group was once again achieved with BBr_3 to afford tetrahydroquinolines **7a–c**.

Introduction of flexibility between the tetrahydroquinoline core and the basic side chain was then explored (Scheme 2). Reduction of amides **6a–c** (R = Me) with LAH in THF followed by BBr_3-promoted deprotection afforded the benzyl substituted tetrahydroquinolines **8a–c**.[10] Pyrrolidine-derived analogue **10** was prepared from intermediate **4c** (Scheme 3). Alkylation of the nitrogen of **4c** was effected with 4-benzyloxybenzyl chloride and phosphazene P_4-*t*-butyl base[11] in THF to yield **9**. Removal of the benzyl protecting group (H_2, Pd/C) followed by alkylation of the phenol with 1-(2-chloro-ethyl)-pyrrolidine and subsequent deprotection (BBr_3) afforded **10**.

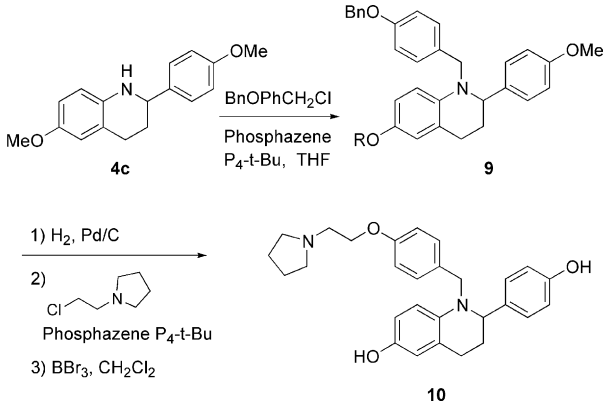
Finally, we wished to investigate compounds where the linker between the tetrahydroquinoline core and the side chain was removed, that is, the effect of aminoalkoxyaryl side chain attachment directly to the N(1) position. The *N*-aryl tetrahydroquinolines were thus prepared using transition metal chemistry[12] by treating **4c** with arylbromide **11**[13] in the presence of $Pd(OAc)_2$, $P(t\text{-}Bu)_3$ and *t*-BuONa in toluene (Scheme 4). Demethylation with BBr_3 afforded *N*-aryl tetrahydroquinolines **12** and **13**.

The compounds were tested as racemates for their ability to compete with ^{3}H-estradiol for binding to both ERα and ERβ.[14] In addition, the compounds were examined for their ability to inhibit estradiol-stimulated proliferation of MCF-7 breast adenocarcinoma cells.[15] Raloxifene and 4-hydroxytamoxifen (4-OHT) have been included for comparison purposes (Table 1).[16]

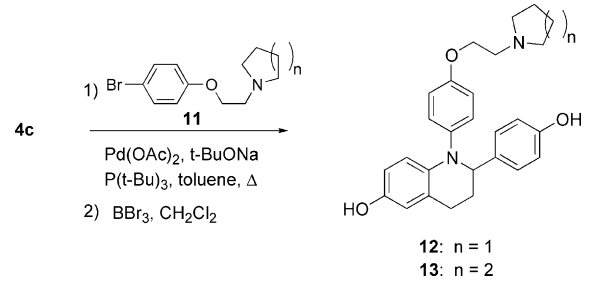
Amide derivative **7a** displayed reasonable binding affinity to ERα (K_i: 29 nM), but considerably weaker affinity to ERβ (K_i: 380 nM). Interestingly, addition of a hydroxyl on the C2-aryl ring failed to increase binding or MCF-7 antagonism. The 4′-OH analogue (**7c**) had weaker binding than the parent, while the 3′-OH congener (**7b**) displayed similar ERα binding and functional activity to the parent (**7a**), but weaker ERβ binding.



Scheme 2.



Scheme 3.



Scheme 4.

Table 1. Binding and MCF-7 inhibition data

Compd	ERα binding K_i, nM	ERβ binding K_i, nM	MCF-7 IC_{50}, nM
Raloxifene	0.4	4.3	0.2
4-OHT	0.5	0.5	2.6
3	310	35	NC
5	170	65	NC
7a	29	380	620
7b	37	>1 μM	590
7c	88	>1 μM	990
8a	1.7	4.0	490
8b	0.6	5.5	390
8c	0.6	4.4	270
10	1.1	4.9	68
12	0.9	3.2	490
13	2.5	8.0	590

NC, not calculated. These compounds were shown to be agonists in this assay.

Reduction of the amide carbonyl to provide the corresponding benzyl-substituted tetrahydroquinolines afforded compounds with significantly higher binding affinity. For example, **8a** is approximately 17-fold more potent than the analogous amide **7a** for ERα and 94-fold more potent for ERβ. The weaker binding affinity of **7a** relative to **8a** may be due to the amide bond forcing the side chain into a less favorable conformation, thereby diminishing binding. An alternative explanation may be that the electronegative amide is not tolerated in this hydrophobic region of the receptor.[17] A similar observation was made for a series of phenanthridines which possessed a lactam moiety in this region.[18] Introduction of a phenol on the C2-aryl ring of **8a** somewhat increased ERα binding affinity but had little effect on ERβ binding. Compounds **8b** and **8c** both had higher affinity to ERα compared to the unsubstituted derivative **8a**. As was observed previously with SERMs from the benzothiophene or indole series,[2,3] there is a weak correlation between ERα binding affinity and MCF-7 inhibition for the tetrahydroquinolines.

Since the pyrrolidine base is a common feature of many SERMs, we wished to investigate the effect of replacing the piperidine with a pyrrolidine. As is commonly seen with SERMs, the base change did not dramatically affect binding affinity, but had a more significant impact on functional activity: Tetrahydroquinoline **10** is a high affinity ER ligand (K_i ERα: 1.1 nM; K_i ERβ: 4.9 nM), and is 4-fold more potent than the piperidine analogue **8c** in the MCF-7 proliferation assay.

N-Aryl tetrahydroquinoline **12** is likewise a high affinity ligand; however, the MCF-7 inhibition is approximately 7-fold weaker than **10**, which contains a methylene spacer between the tetrahydroquinoline ring and the side chain aryl moiety. Piperidine derivative **13** has weaker binding affinity than **12** and is likewise a weak antagonist in the MCF-7 proliferation assay. In general, all of the tetrahydroquinolines displayed weaker MCF-7 antagonism than either raloxifene or 4-hydroxytamoxifen, in spite of a similar binding profile. This may be due to the increased polarity of the tetrahydroquinolines which may limit their cellular penetration relative to the more lipophilic triaryl ethylene backbone of 4-OHT. Alternatively, the three structural classes may differentially recruit cofactors, which may also play a role in functional activity.[19]

Addition of the *N*-benzyl basic side chain is an important feature for regulating receptor affinity. For example, tetrahydroquinoline **10** has >10-fold higher affinity to both ERα and ERβ than compound **5** which lacks the entire side chain moiety. Interestingly, both quinoline **3** and tetrahydroquinoline **5** display modest selectivity for ERβ (2- to 8-fold), which is in contrast to the derivatives bearing a basic side chain which are all α selective (3- to 10-fold). The small, polar nature of **3** and **5** is consistent with previously reported ERβ selective ligands. Both **3** and **5** showed no antagonism in the MCF-7 assay and, in fact, both compounds were shown to be agonists (EC_{50} 15 and 320 nM, respectively) when tested in the absence of 17β-estradiol.

In summary, we have investigated the use of 1,2-disubstituted tetrahydroquinolines as estrogen receptor ligands. Compounds containing an amide linker between the core and basic side chain had weaker binding and somewhat weaker functional activity compared to the other series investigated. Both the *N*-benzyl and *N*-aryl derivatives displayed high binding affinity with modest ERα selectivity. Compound **10** is the most potent inhibitor of MCF-7 proliferation identified in this series (IC_{50} 68 nM).

Acknowledgements

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10. Compound **8b** was also prepared by alkylating **4b** with 1-[2-(4-chloromethyl-phenoxy)-ethyl]-piperidine/phosphazene P_1, followed by BBr_3 deprotection.
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14. The competition binding assay was run in a buffer containing 50 mM Hepes, pH 7.5, 1.5 mM EDTA, 150 mM NaCl, 10% glycerol, 1 mg/mL ovalbumin and 5 mM DTT, using 0.025 μCi per well ^{3}H-Estradiol (NEN #NET517 at 118 Ci/mmol, 1 mCi/mL), 10 ng/well ERα or ERβ receptor (PanVera). Competing compounds were added at 10 different concentrations. Non-specific binding was determined in the presence of 1 μM of 17β Estradiol. The binding reaction (140 μL) was incubated for 4 h at room temperature, then 70 μL of cold DCC buffer was added to each reaction (DCC buffer contains per 50 mL of assay buffer, 0.75 g of charcoal (Sigma) and 0.25 g of dextran (Pharmacia)). Plates were mixed for 8 min on an orbital shaker at 4 °C. Plates were then centrifuged at 3000 rpm at 4 °C for 10 min. An aliquot of 120 μL of the mix was transferred to another 96-well, white flat bottom plate (Costar) and 175 μL of Wallac Optiphase 'Hisafe 3' scintillation fluid was added to each well. Plates were sealed and shaken vigorously on an orbital shaker. After an incubation of 2.5 h, plates were read in a Wallac Microbeta counter. The data was used to calculate an IC_{50} and % inhibition at 10 μM. The K_d for ^{3}H-Estradiol was determined by saturation binding to ERα and ERβ receptors. The IC_{50} values for compounds were converted to K_i using the Cheng–Prusoff equation and the K_d determined by saturation binding assay.
15. MCF-7 breast adenocarcinoma cells (ATCC HTB 22) were maintained in MEM (minimal essential medium, phenol red-free, Gibco BRL) supplemented with 10% fetal bovine serum (FBS) (V/V), L-glutamine (2 mM), sodium pyruvate (1 mM), HEPES ((*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid]10 mM}, non-essential amino acids (0.1 mM) and Penicillin Streptomycin (1×). Seven days prior to assay, MCF-7 cells were switched to assay media which was the same as maintenance medium except supplemented with 10% dextran-coated charcoal-stripped fetal bovine serum (DCC-FBS) assay medium in place of 10% FBS. MCF-7 cells were removed from flasks using 10× Trypsin EDTA (phenol red free, Gibco BRL) and diluted to 1× in (Ca^{++}/Mg^{++} free HBSS (phenol red-free)). Cells were adjusted to 80,000 cells/mL in assay medium. Approximately 8000 cells (100 μL) were added to each well in 96-well Cytostar T scintillation plates (Amersham) and incubated at 37 °C in a 5% CO_2 humidified incubator for 24 h to allow cell adherence and equilibration after transfer. Serial dilutions of drugs were prepared in assay medium at 4× the final desired concentration). A 50 μL aliquot of drug dilutions (at 4× the final assay concentration) was transferred to duplicate wells followed by 50 μL assay medium for the agonist mode or 50 μL of 40 pM of E2 for the antagonist mode to a final volume of 200 μL. For each of the agonist plates, a basal level (media) and a maximum stimulated level (with 1 μM E2) was determined. For each of the antagonist plates, a basal level (media) and an E2 (10 pM) alone control was determined. After an additional 48 h at 37 °C in a 5% CO_2 humidified incubator, 20 μL of assay medium containing 0.01 μCi of ^{14}C-thymidine (52 mCi/mmol, 50 μCi/uL, Amersham) was added to each well. The plates were incubated overnight in the same incubator and then counted on the Wallac Microbeta counter. The data was averaged to calculate an IC_{50} and % inhibition @ 1 μM for the antagonist mode. For the agonist mode, an EC_{50} and % of maximum E2 stimulation and concentration of maximum stimulation was calculated.
16. The compounds were generally tested in duplicate. The minimum significant ratio (MSR) calculated for the ERα and ERβ binding assays were 2.4 and 2.3, respectively. The MSR for the MCF-7 assay was 2.9.
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