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Comparison of 2-phenylspiroindenes and 2-phenylspiroindenediones as estrogen receptor ligands—modeling and binding data don't agree!

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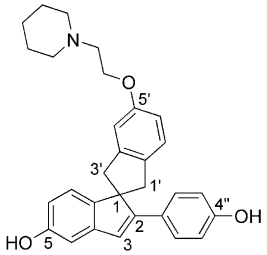
Abstract—A series of 2-phenylspiroindenediones was prepared. The spiroindenediones were found to be less active than the corresponding spiroindenes as estrogen receptor ligands and failed to demonstrate the receptor subtype selectivity that had been anticipated from molecular modeling.

Hormone replacement therapy (HRT) is widely used to treat a variety of conditions, such as hot flashes and osteoporosis, resulting from estrogen deficiency in postmenopausal women.[1] Although HRT is very effective, it is also associated with some serious side-effects, including blood clots and increased risk of cancer. The selective estrogen receptor modulators (SERMs) have generated considerable interest due to their potential ability to provide the benefits of estrogen without the associated liabilities.[2] The clinical utility of SERMs is exemplified by the use of tamoxifen[3] for breast cancer and raloxifene[4] for osteoporosis.[4d,e] The recent discovery of a second ER receptor subtype (ERβ)[5] suggests the possibility of developing subtype selective SERMs. Most of the current SERMs are 'balanced' compounds, that is, they bind both receptor subtypes equally, although several novel classes of subtype selective compounds have recently been described.[6] Due to the variable tissue distribution of the two receptor subtypes, it is possible that selectivity for one receptor over the other might confer clinical advantages.

Recently, we reported a series 2-phenylspiroindenes (e.g., **1**) as estrogen receptor ligands.[7] The spiroindenes were very potent but were not selective for either ERα or ERβ. Molecular modelling studies of **1** and **2** docked



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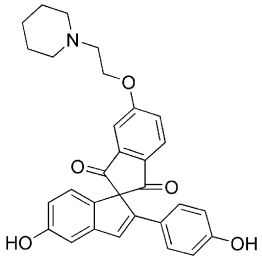

2

in ERα (Fig. 1) suggested that addition of a carbonyl oxygen at C-3′ of the spiroindene system of **1** would result in substantial selectivity for the ERα receptor due to negative steric and electronic clashes with the Met 293 side-chain of ERβ (analogous to Leu 384 of ERα). Although the carbonyl at C-1′ of **2** was predicted to have no effect on selectivity and would merely mimic the carbonyl found in raloxifene, its presence simplified the synthesis somewhat. We thus targeted spiroindenedione **2** for synthesis with the expectation that **2** would be both potent and selective for ERα.

We initially attempted to prepare **2** by modifying our synthetic route to **1**.[7] Synthesis of the acid cyclization precursor **5** proceeded uneventfully (Scheme 1).[8] However, cyclization of **5** to the advanced ketone intermediate **6** was plagued by an unavoidable fragmentation reaction that regenerated dione **3** along with many

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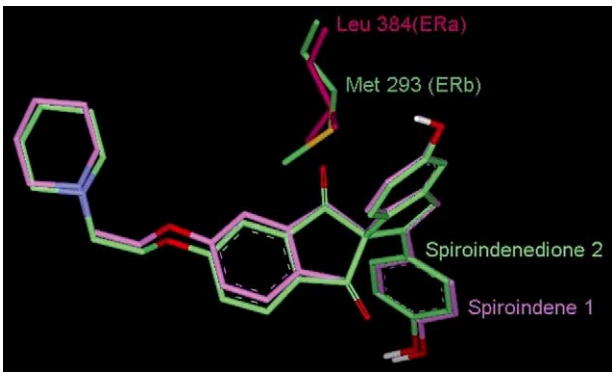
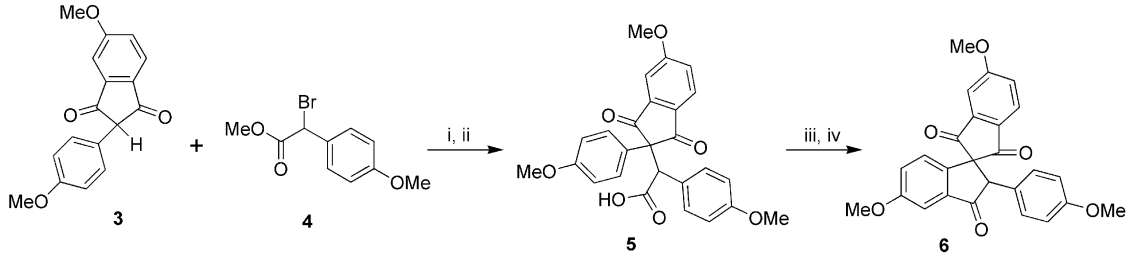


Figure 1. Spiroindenes **1** and **2** were modeled within the context of human ERα using the co-crystallized antagonist raloxifene as a starting point.

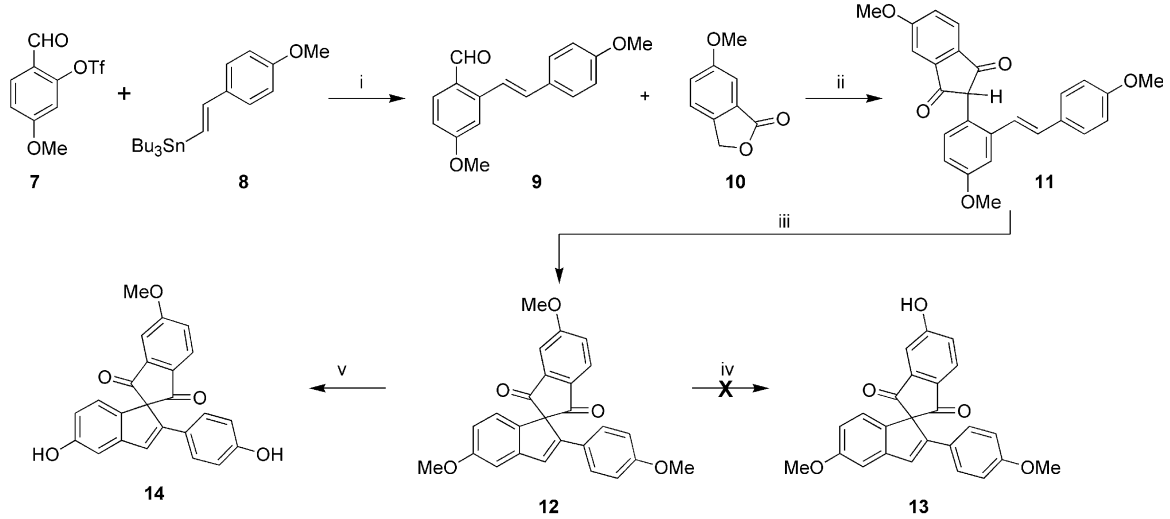
other products. Under some conditions, it was possible to isolate small amounts (~10%) of **6** but the reaction was extremely messy and this was an unacceptably low yield at this relatively early stage of the synthesis so this approach was abandoned.

Several alternative approaches involving bis acylation of an indene anion or oxidation of a spiroindene were similarly unsuccessful, possibly due to product instability. Finally, we turned to an approach based on a palladium mediated intramolecular cyclization of a dione enolate onto an olefin (Scheme 2). The key cyclization step was based on the successful application of a similar reaction by Rao et al. in their studies toward fredericamycin A.[9] The requisite precursors, triflate **7**[10] and stannane **8**,[11] were easily prepared from commercially available starting materials. Palladium mediated coupling of **7** and **8** using conditions developed by Nemoto et al.[10] for vinylation of **8** afforded aldehyde **9** in very good yield. Condensation of **9** with phthalide **10**[12] under the conditions used by Shapiro et al.[13] for a simpler system afforded the key cyclization intermediate **11** in 55% yield.[14] Palladium mediated cyclization of **11** using the Rao conditions[9] cleanly afforded the desired spiroindenedione **12** in 50% yield.[15] Our initial synthetic plan had been to selectively deblock the methyl ether *para* to the ketone of **12** (to afford **13**) then attach the piperidinylethoxy side chain and deprotect the other phenols to afford **2**. In practice, it was not possible to selectively cleave the desired methyl ether, probably due to competing nucleophilic attack at one of the ketone carbonyls. It was, however, possible to remove the other methyl ethers with boron tribromide to afford **14** for biological evaluation.

Unfortunately, **14** was substantially less active in an estrogen receptor binding assay (IC_{50}s: human ERα = 137 nM; ERβ = 153 nM) than we had hoped and, more importantly, it was not selective for ERα. Nevertheless,



Scheme 1. Conditions: (i) K_2CO_3, DMF, 50 °C, 15 m, 94%; (ii) KOH, MeOH, H_2O, 18 h, 58%; (iii) PCl_5, CH_2Cl_2, 0 °C, 1 h; (iv) $AlCl_3$, CH_2Cl_2, 0 °C, 45 m, ~10% from **5**.



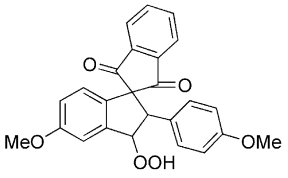
Scheme 2. Conditions: (i) $Pd(PPh_3)_4$, LiCl, THF, reflux, 6 h, 84%; (ii) 4.5 equiv NaOMe, MeOH, ethyl propionate, 82 °C, 50 m, 55%; (iii) NaH, $Pd(OAc)_2$, DMF, rt, 2 h, 50%; (iv) NaSMe, DMF, decomposed; (v) BBr_3, CH_2Cl_2, rt, 75 m, 32%.

since we were finally so close to accomplishing the goal of synthesizing **2**, we decided to complete the synthesis and hope that the addition of the side chain would improve the potency and selectivity.

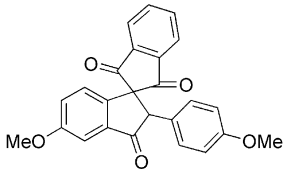
An initial attempt to complete the synthesis of **2** by substituting a MEM protecting group for the troublesome methyl ether failed when the MEM group also proved to be impossible to remove without destroying the spiroindenedione system. In the successful approach the piperidinylethoxy side chain was incorporated in the beginning of the synthesis, thus eliminating the need for a protecting group altogether (Scheme 3). In this sequence, the cyclization precursor **17** was not isolated and purified but was instead cyclized immediately to form the penultimate intermediate **18** which was deprotected to afford the long sought spiroindenedione **2**. Unfortunately, **2** was also only moderately active in the binding assay and was not selective for ERα (Fig. 2).[16]

With the synthetic methodology for synthesizing spiroindenediones in hand, two additional analogues were quickly prepared for direct comparison with previously prepared spiroindenes. Thus, reaction of phthalide **19** with aldehyde **9** afforded the cyclization intermediate **20** in 80% crude yield (Scheme 4). Palladium mediated cyclization of crude **20** afforded the penultimate intermediate **21** which was deblocked uneventfully to afford the spiroindenedione **22**.

Interestingly, **20** was highly susceptible to air oxidation and cyclization to afford primarily the cyclic hydroperoxide **23** along with small amounts of ketone **24** and other unidentified minor products. Indeed, when the condensation of **19** and **9** was run in air rather than under Argon, hydroperoxide **23** was isolated as the main product along with ketone **24** and several unidentified minor byproducts. The structure of **23** was confirmed by extensive NMR studies and by its conversion to a mixture of **22** and **24** upon treatment with *p*-toluenesulfonic acid in hot benzene.



23



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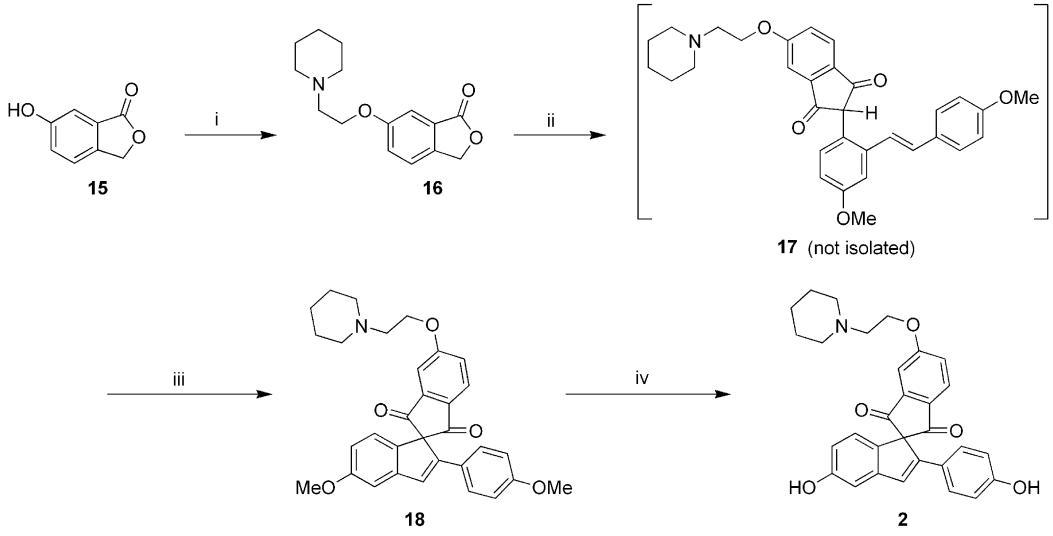
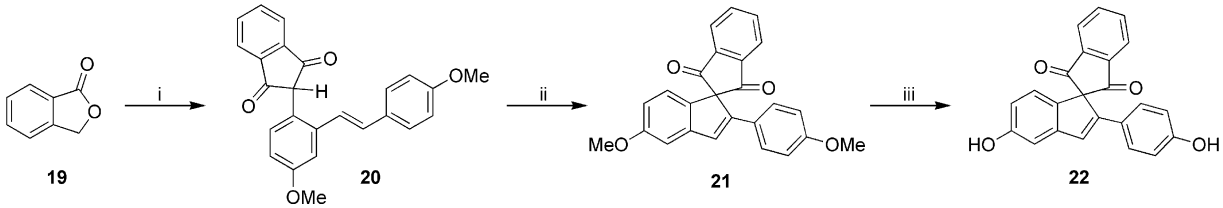
The final spiroindenedione to be prepared was the *meta* isomer **29**. The requisite intermediate aldehyde **26** was prepared from stannane **25**[17] using chemistry analogous to that used to prepare **9**. Application of our standard protocol to aldehyde **26** afforded the cyclization precursor **27** in 95% crude yield. Cyclization and deprotection proceeded uneventfully to afford **29** (Scheme 5).



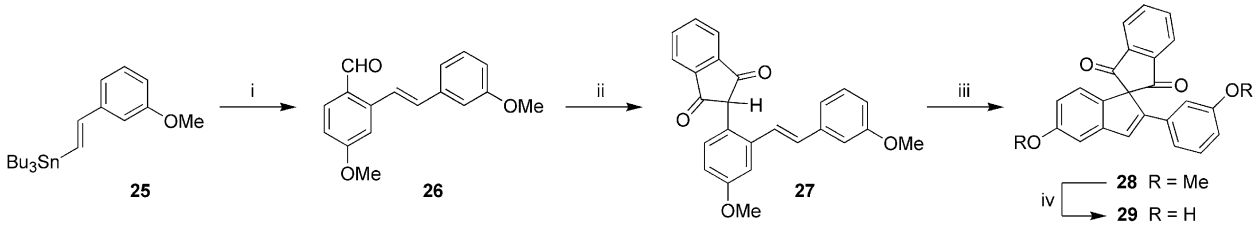
15 **16** **17** (not isolated) **18** **2**

Scheme 3. Conditions: (i) *N*-chloroethylpiperidine, K_2CO_3, MeCN, 40 °C, 37 h, 66%; (ii) **9**, 2.2 equiv NaOMe, MeOH, ethyl propionate, 82 °C, 24 h; (iii) $Pd(OAc)_2$, DMF, rt, 1 h, ~10% from **16**; (iv) HCl, ether, then BBr_3, CH_2Cl_2, rt, 75 m, 27%.



19 **20** **21** **22**

Scheme 4. Conditions: (i) **9**, 4.5 equiv NaOMe, MeOH, ethyl propionate, Argon, 82 °C, 24 h 80% crude; (ii) NaH, $Pd(OAc)_2$, DMF, rt, 90 m, 30%; (iii) BBr_3, CH_2Cl_2, rt, 75 m, 58%.

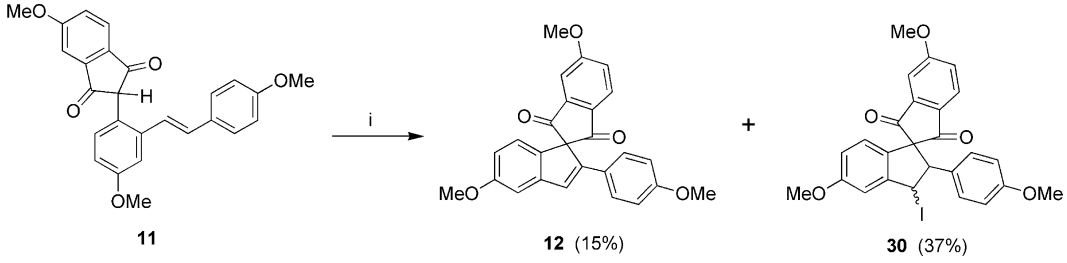


Scheme 5. Conditions: (i) **7**, $Pd(PPh_3)_4$, LiCl, THF, reflux, 7 h, 46%; (ii) 4.5 equiv NaOMe, MeOH, ethyl propionate, Argon, 82 °C, 90 m, 95% crude; (iii) NaH, $Pd(OAc)_2$, DMF, rt, 2 h, 25%; (iv) BBr_3, CH_2Cl_2, rt, 75 m, 78%.

An alternative cyclization method was also briefly explored. Thus, treatment of intermediate **11** with *N*-iodosuccinimide (NIS) in the presence of base afforded a low yield of the desired spiroindenedione **12** along with a substantial amount of the cyclized iodide **30**. Presumably, **30** could be readily converted to **12** although this was not actually done. This approach therefore appears to provide a viable alternative to the palladium-mediated cyclization for preparing spiroindenediones. However, this promising initial result was not followed up since the disappointing results from the evaluation of our spiroindenediones in the ER binding assay did not merit further work in this area (Scheme 6).

The binding data for the spiroindenediones and the corresponding spiroindenes are summarized in Figure 2.[16] Only those compounds which have been prepared in both series are included in Figure 2; analogue **14** (see binding data above) was omitted since the corresponding spiroindene analogue was not prepared. In all cases, introduction of the carbonyl groups at C-1′ and C-3′ of the spiroindene system resulted in a substantial decrease in potency with no gain in selectivity. In contrast to the molecular modeling prediction, the additional carbonyl groups apparently generate an unfavorable interaction with both estrogen receptors rather than with just the beta receptor. Interestingly, this effect is most pronounced in the fully elaborated analogue **2**. This suggests that the range of motion available to the side chain may be more important than was previously realized. Also noteworthy is the observation that **2**, which incorporates the raloxifene side chain, is slightly less active than **22**, which does not have the side chain. This contrasts with the corresponding spiroindene pair (**1** and **31**) wherein introduction of the side chain results in a slight increase in activity. As with the spiroindenes, the analogue with the *para* hydroxy group in the pendant phenol (**22**) is more active than the compound with a meta hydroxy in this ring (**29**).

It is clear that the spiroindenediones are consistently less potent estrogen receptor binders than the spiroindenes and are not selective for either estrogen receptor. In this series, molecular modeling was clearly not successful in predicting the biological consequences of a relatively small chemical structure change despite the availability of a crystal structure of the receptor with a structurally related ligand in the binding site. It remains to be seen whether the introduction of other substituents



Scheme 6. Conditions: (i) NaH, NIS, THF, rt, 5 h.

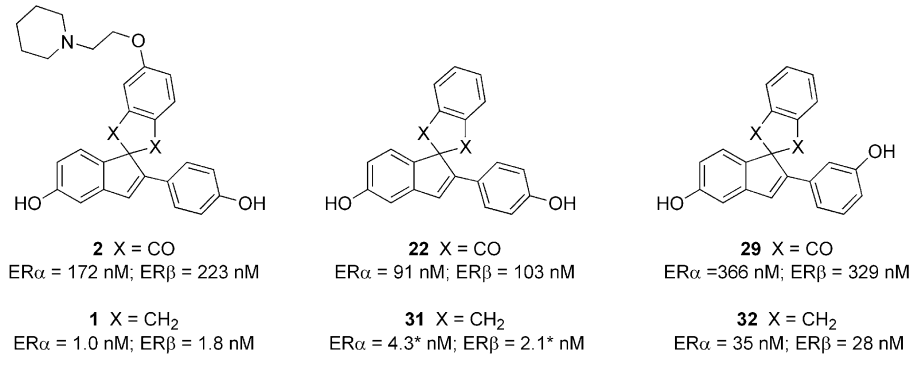


Figure 2. Comparison of estrogen receptor binding affinities (IC_{50}).[16]

at these positions can impart the desired selectivity while retaining potency.

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13. Shapiro et al. *J. Org. Chem.* **1960**, *25*, 1860. The use of ethyl propionate as the cosolvent proved to be critical for the success of this reaction.
14. Note that in principle either 6-methoxyphthalide (**10**) or 5-methoxyphthalide (not shown) could be used in the reaction with **9** to generate **11**.
15. The structure of **12** was confirmed by extensive NMR studies including full assignment of the ^{1}H and ^{13}C NMR spectra using HMQC and HMBC experiments.
16. The IC_{50} values were generated in an estrogen receptor ligand binding assay. This scintillation proximity asssay was conducted in NEN Basic Flashplates using tritiated estradiol and full length recombinant human ERα and ERβ proteins. Most of the data reflects a 3 h incubation time; data marked with an * reflects a 4 h incubation. Compounds were evaluated in duplicate in a single assay. In our experience, this assay provides IC_{50} values that are reproducible to within a factor of 2–3.
17. Prepared in 55% yield by reaction of 3-methoxyphenylacetylene with tributyltin hydride using a procedure analogous to one described by Corey et al.: Corey, E. J.; Wollenberg, R. H. *J. Org. Chem.* **1975**, *40*, 2265.