Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 1417–1421

# Estrogen receptor ligands. Part 1: The discovery of flavanoids with subtype selectivity

Helen Y. Chen,[a,*] Kevin D. Dykstra,[a] Elizabeth T. Birzin,[b] Katalin Frisch,[b] Wanda Chan,[b] Yi T. Yang,[b] Ralph T. Mosley,[a] Frank DiNinno,[a] Susan P. Rohrer,[b] James M. Schaeffer[b] and Milton L. Hammond[a]

[a]*Department of Medicinal Chemistry, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*
[b]*Department of Atherosclerosis & Endocrinology, Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA*

Received 13 November 2003; revised 14 January 2004; accepted 15 January 2004

**Abstract**—A class of flavanoids exhibiting a high degree of selectivity for ERα over ERβ has been discovered. The most active analogue **6** was found to be 66-fold ERα-selective and demonstrated uterine estradiol antagonism.


Owing to a heightened awareness of the adverse effects of hormone replacement therapy, prompted by the Women's Health Initiative study,[1] the search for alternative treatments has intensified. Much of the spotlight has rested on a class of compounds, exemplified by tamoxifen and raloxifene, known as *selective estrogen receptor modulators* (SERMs).[2] These agents have the potential ability to antagonize the proliferative effects of estrogen on uterine and breast tissue while mimicking estrogen's effects on the bone and cardiovascular system. The recent discovery of a second estrogen receptor isoform (ERβ) raises the possibility that receptor subtype selective ligands may offer key advantages. As part of a medicinal chemistry program targeting *selective estrogen receptor subtype modulators* (SERSMs),[2c] we became interested in three naturally occurring leads: genistein, daidzein, and coumestrol, all of which exhibit a moderate selectivity for ERβ (20X at best) and contain a common benzopyran motif (Fig. 1). WS-7528 (Fig. 1), another structurally similar flavanone, isolated from a strain of *Streptomyces*, was reported to have estrogen-like characteristics.[3]

Based on these findings, we sought to identify a novel series of SERSMs centered on the flavanone core structure.[4] Herein, we wish to describe the synthesis and SAR of compounds shown in Table 1.

Initially, flavanones **1**–**8** were prepared according to literature procedures with minor modifications (Scheme 1, Method A).[5] Thus, Knoevenagel condensation of piperidinylethoxy–benzaldehyde with the appropriate ketones[6] **23** yielded a mixture of *cis* and *trans* flavanones. The titer of the *cis* isomer could be increased to 50% simply by epimerization of the 1:4 *cis*/*trans* mixture of TBS-protected flavanones **24** with LiHMDS at −78 °C. Although the two isomers could be separated on silica gel with great care, it became apparent early on that a stereoselective synthesis of *cis* flavanones was needed. Although many synthetic methods for the construction of flavanones are known in the literature, only a few stereospecific syntheses of *cis* flavanones exist.[7]

We therefore decided to pursue a stereospecific synthesis of *cis*-2,3-disubstituted flavanones based on Donnelly's

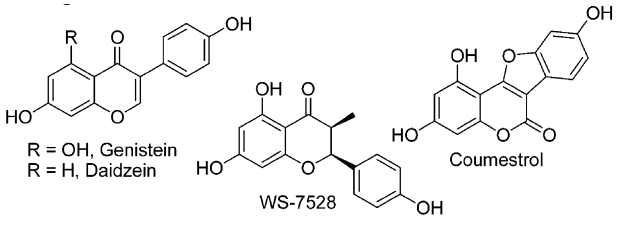
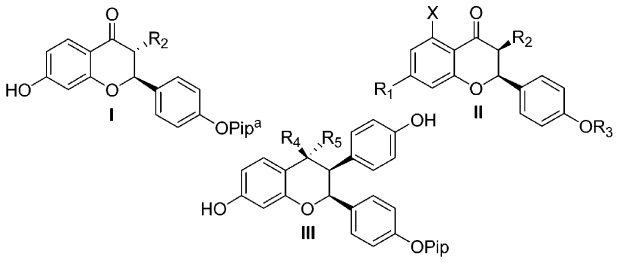



**Figure 1.** Structures of naturally occurring leads.

*Keywords:* SERMs; Flavanoids.
* Corresponding author. Tel.: +1-732-594-5323; fax: +1-732-594-9556; e-mail: helen_chen@merck.com

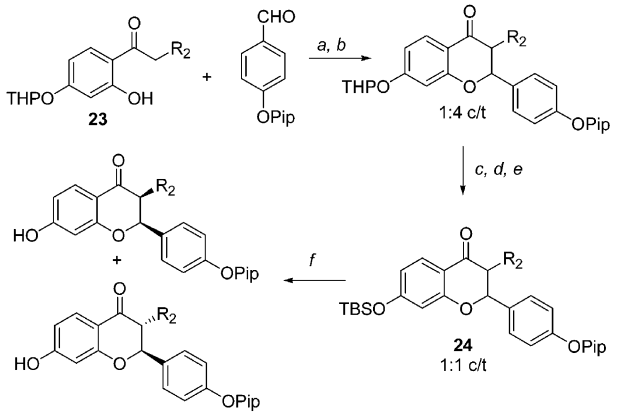
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.01.031

**Table 1.** Flavanones and their derivatives




| Compd | Type[c] | X | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | Method[b] |
|---|---|---|---|---|---|---|---|---|
| **1** | I | — | — | Me | — | — | — | A |
| **2** | I | — | — | *i*Pr | — | — | — | A |
| **3** | I | — | — | 4-Hydroxyphenyl | — | — | — | A |
| **4** | II | H | OH | Me | Pip[a] | — | — | A |
| **5** | II | H | OH | *i*Pr | Pip | — | — | A |
| **6** | II | H | OH | 4-Hydroxyphenyl | Pip | — | — | A or B |
| **7** | II | H | OH | 4-Hydroxyphenyl | H | — | — | A[d] |
| **8** | II | OH | OH | Me | Pip | — | — | A |
| **9** | II | H | OH | Phenyl | Pip | — | — | B[e,f] |
| **10** | II | H | H | 4-Hydroxyphenyl | Pip | — | — | B |
| **11** | II | Me | OH | 4-Hydroxyphenyl | Pip | — | — | B |
| **12** | II | Et | OH | 4-Hydroxyphenyl | Pip | — | — | B |
| **13** | II | Pr | OH | 4-Hydroxyphenyl | Pip | — | — | B |
| **14** | II | Pentyl | OH | 4-Hydroxyphenyl | Pip | — | — | B |
| **15** | II | F | OH | 4-Hydroxyphenyl | Pip | — | — | B[f] |
| **16** | II | Cl | OH | 4-Hydroxyphenyl | Pip | — | — | B[f] |
| **17** | II | H | OH | 2-Methoxy-4-hydroxyphenyl | Pip | — | — | B |
| **18** | II | H | OH | 3-Methoxy-4-hydroxyphenyl | Pip | — | — | B |
| **19** | II | H | OH | 3,5-Dimethyl-4-hydroxyphenyl | Pip | — | — | B |
| **20** | III | — | — | — | — | H | OH | — |
| **21** | III | — | — | — | — | H | H | — |
| **22** | III | — | — | — | — | — | =NOH | — |

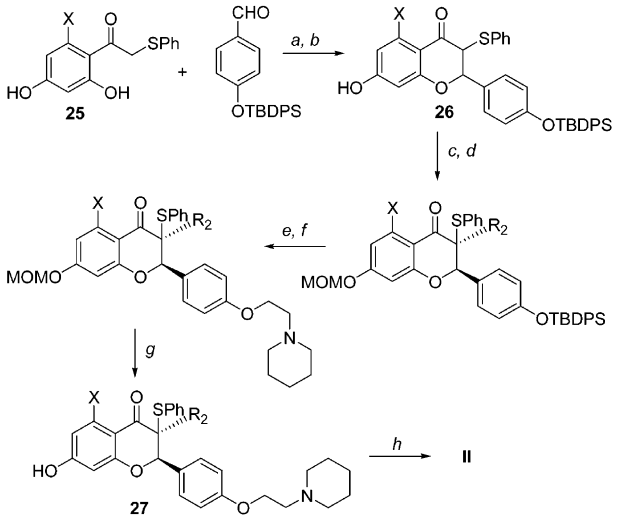
[a] Pip, piperidinoethyl.
[b] Scheme 1 and 2.
[c] Racemic, abs. configuration unknown.
[d] Used 4-hydroxybenzaldehyde in step a.
[e] Used $Ni_2B$ as the reductant in step h.
[f] Used mono-MOM-protected **25** in step a.




**Scheme 1.** Method A, reagents and conditions: (a) piperidine, toluene, 120 °C, overnight; (b) NaOAc, MeOH, 80 °C, 3 h, 41–95% from **23**; (c) 2 N HCl, EtOH, rt, 66–100%; (d) TBSCl, $Et_3N$, $CH_2Cl_2$, 78–100%; (e) LiHMDS, THF, −78 °C, 39–42%; (f) TBAF, AcOH, THF, 0 °C, 62–87%.

work,[8] wherein 3-phenylthio-chroman-4-ones would not only allow for the requisite arylation reaction but the phenylthio group would also serve as our stereocontrol element upon reductive removal. To that end, the flavanone intermediate **26** was prepared via the Knoevenagel condensation of ketones[9] **25** with the TBDPS-protected benzaldehyde according to Scheme 2 (Method B). Stereospecific introduction of the aryl group at C-3[10] was performed by addition of organolead reagents prepared according to literature procedures.[11] Subsequent TBS-deprotection and elaboration to the basic side chain was accomplished with chloroethyl–piperidine and cesium carbonate in refluxing acetone. Treatment with 2 N HCl successfully removed the MOM groups to give the penultimate substrate **27** in good yields. At this time, we explored the reductive removal of the SPh group under a variety of conditions. Following the literature precedent,[8] $Ni_2B$ afforded a mixture of the isomers varying from 2:1 *cis*/*trans* to 10:1 *cis*/*trans* depending on the freshness of the catalyst. Interestingly, with $SmI_2$, a reverse stereochemical outcome of 2:1 *trans*/*cis* ratio was obtained. Success was finally achieved using excess RaNi as the reductant to give 49–88% yield of the desired *cis* isomer. In the case where X = Cl, F (**16** and **15**), some dehalogenation was observed (66% and 10%, respectively).

With the *cis* flavanone **6** in hand, compounds of Type III were readily accessible. Following modified literature procedures,[5b] reduction of the ketone using lithium




**Scheme 2.** Method B, reagents and conditions: (a) piperidine, toluene, 120 °C, overnight; (b) NaOAc, MeOH, 80 °C, 3 h, 30–85% from **25**; (c) MOMCl, DMF, Hunig's base, 47–88%; (d) $R_2Pb(OAc)_3$, pyridine, $CHCl_3$, 40 °C, 52–81%; (e) TBAF, AcOH, THF, 0 °C, 60–96%; (f) $Cs_2CO_3$, 1-(2-chloroethyl)piperidine monohydrochloride, acetone, 60 °C, 79–100%; (g) 2 N HCl, MeOH, 80 °C, 56–100%; (h) RaNi, EtOH, 49–88%.

triethyl borohydride in THF at 0 °C afforded **20** in 76% yield. Compound **20** could be further reduced upon careful treatment with TFA and triethylsilane in methylene chloride to give flavan **21** in 68% yield along with its corresponding chromene. As reported by Donnelly,[12] oximation of flavanone **3** or **6** with hydroxylamine HCl and piperidine in pyridine cleanly gave the *cis* oxime **22** in 61% yield.

Compounds **1**–**22** were tested for potency and selectivity in an ER competitive binding assay with tritiated 17-β estradiol.[13] Agonist and antagonist activities of select compounds were evaluated in vivo using an immature rat uterine weight assay.[14] The results are shown in Table 2.

When we derivatized racemic, synthetic WS-7528 to generate flavanone **8**, we observed a slight shift towards ERα selectivity rather than the anticipated selectivity for ERβ. As the size of the substituent at C-3 was increased to isopropyl (**5**), not only was binding to the ERα receptor enhanced, but selectivity over ERβ was also improved. We found optimal binding and almost 70-fold selectivity with the introduction of a 4-hydroxyphenyl group (**6**).

Although the binding data would suggest that the *trans* isomer differed only slightly from the *cis* isomer, further evaluation in the immature rat uterine assay clearly identified the *cis* isomer as the more pharmacodynamically active antagonist. For instance, at 1 mpk sc, the *trans* flavanone **3** exhibited a 17% inhibition of the estradiol effect in contrast to 50% inhibition with its *cis* isomer **6**. It was thus established early in the project that the *cis* stereochemistry was critical for the development of a SERM.

As with raloxifene, the basic side chain proved to be crucial for in vivo antagonist activity, since **7** displayed

**Table 2.** Binding affinities[13] and in vivo data[14]

| Compd | Human ERα $IC_{50}$ (nM) | Human ERβ $IC_{50}$ (nM) | Selectivity [β]/[α] | Uterine Wt. assay (@1 mpk, sc[a]) |
|---|---|---|---|---|
| **1** | 1415 | >10,000 | 7 | ND[b] |
| **2** | 109 | 930 | 9 | 15% Agonism |
| **3** | 49 | 1947 | 40 | 17% Inhibition |
| **4** | 3563 | 5645 | 2 | ND |
| **5** | 89 | 1200 | 13 | 0% Inhibition |
| **6** | 31 | 2049 | 66 | 50% Inhibition |
| **7** | 490 | 598 | 1 | ND |
| **8** | 551 | 1200 | 2 | ND |
| **9** | 531 | >10,000 | 19 | ND |
| **10** | 179 | 510 | 3 | ND |
| **11** | 14 | 546 | 39 | 46% Inhibition |
| **12** | 68 | 152 | 2 | ND |
| **13** | 652 | 207 | 0.3 | ND |
| **14** | 71 | 454 | 6 | ND |
| **15** | 105 | 5390 | 51 | 17% Agonism |
| **16** | 58 | 1270 | 22 | ND |
| **17** | 149 | 1360 | 9 | ND |
| **18** | 413 | >10,000 | 24 | ND |
| **19** | 1050 | >10,000 | 10 | ND |
| **20** | 929 | 7000 | 8 | ND |
| **21** | 6.7 | 8.9 | 1 | ND |
| **22** | 1040 | 8610 | 8 | ND |
| Raloxifene | 1.8 | 12 | 7 | 96% Inhibition[c] |
| β-Estradiol | 1.3 | 1.1 | 1 | 100% Agonism[d] |
| Genistein | 92 | 4 | 0.04 | 63% Agonism |
| Daidzein | 2160 | 303 | 0.1 | ND |
| Coumestrol | 11 | 2 | 0.2 | ND |

[a] sc = subcutaneous.
[b] ND = not determined.
[c] @ 0.6 mpk.
[d] @ 2 μg/kg.

no antagonist activity in an in vitro coactivation assay.[15] However, the presence of the basic side-chain was not the only requirement for antagonism as evidenced by compound **5** ($R_2$ = *i*-Pr) which was totally devoid of in vivo activity, and thus pointed toward the need for the hydroxyphenyl substituent as well. Both hydroxyls were required for optimal binding as shown by the loss of binding to the receptor upon removal of either hydroxyl at $R_1$ or $R_2$ (**9**, **10**). Similarly, replacement of the carbonyl by an oxime (**22**), diminished the binding as well as the ERα selectivity. Reduction of the carbonyl to the alcohol **20** was likewise detrimental to the binding activity; however, further reduction of **20**, afforded a potent non-selective ligand **21**. In general, substitutions X (**12**–**16**), whether an extended aliphatic chain or a halogen substituent, resulted in a loss of receptor affinity and subtype selectivity, as compared with **6**. One exception to this trend occurred when X = Me (**11**). Although not as selective as **6**, **11** bound with greater affinity to the *alpha* receptor and demonstrated an equal level of antagonism in the uterine weight assay. Further addition of substituents on the 4-hydroxyphenyl group (**17**–**19**) offered no improvements.

As depicted in Figure 2, molecular modeling of **6**, in white against raloxifene in purple, docked in the ER receptors, showed that although structurally quite different, **6** mapped fairly well with raloxifene and maintained the well established interactions in the ligand binding domain.[16,17]

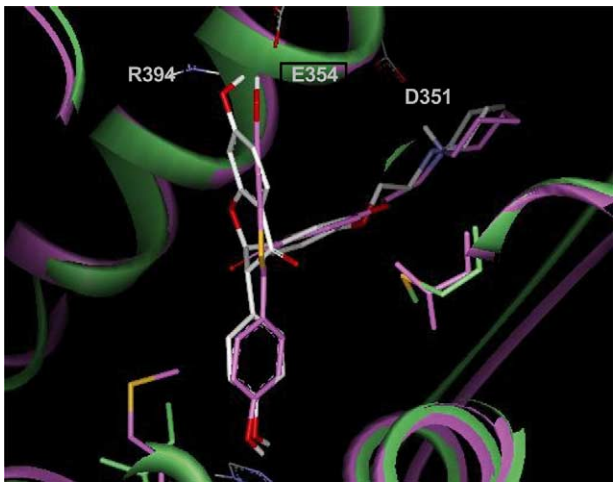



**Figure 2.** Molecular modeling of **6** (white) against Raloxifene (purple). HERα is depicted in purple and hERβ in green. Residue numbering is hERα unless otherwise indicated.

It is postulated that the crucial difference responsible for the *alpha*-selectivity of **6** lies in the interaction of its carbonyl with the two discriminating residues lining the receptor pocket (Fig. 3). In ERβ, there is both a steric and electronic repulsion between the carbonyl oxygen atom of the ligand with the Met 354 residue, which would be expected to be absent in ERα, where the corresponding residue is Leu 384.

In summary, we have created a series of *cis* flavanones which exhibit a greater affinity for ERα over ERβ and in the process, have developed a stereoselective synthesis to these compounds. With compound **6**, we have generated an almost 70-fold ER *alpha* selective ligand with demonstrated in vivo estradiol antagonism on the uterus. We have determined from our SAR that a *cis* relationship is preferred, and both hydroxyls at $R_1$ and $R_2$ are required for optimal binding to the *alpha* receptor. The basic side chain, as demonstrated in other SERM platforms, is required for in vivo antagonism and is optimal with a 4-hydroxyphenyl group at the C-3 position of the isoflavanone. Finally, the carbonyl group of the flavanone is crucial for maintaining subtype selectivity. This work has led to the development of more potent SERSMs[2c] or in this instance, *selective estrogen receptor alpha modulators* (SERAMs) which will be reported in future communications from this laboratory.

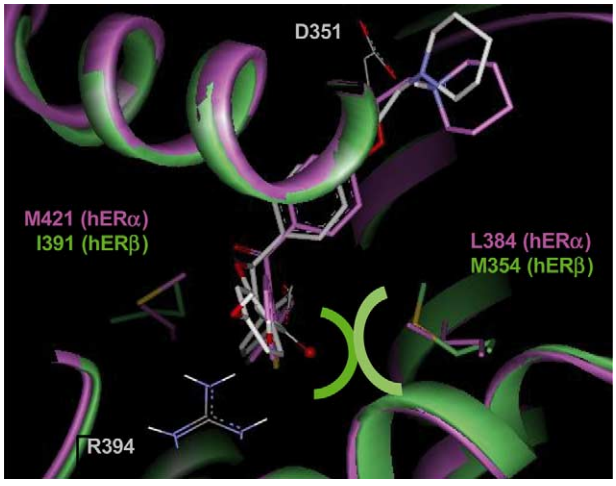



**Figure 3.** Modeling of **6** in the ERα and ERβ receptors. Arcs represent proposed clash between carbonyl O and Met 354 (hERβ) sidechain.

## Acknowledgements

We gratefully acknowledge Professor Barry M. Trost and Professor David Evans for their helpful discussions during their scientific consultations.

## References and notes

1. Writing Group for the WHI Investigators, *JAMA*, **2002**, *288*, 321.
2. (a) Recent reviews on SERMs: Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 883. (b) Jordan, V. C. *J. Med. Chem.* **2003**, *46*, 1081. (c) Meegan, M. J.; Lloyd, D. *G. Curr. Med. Chem.* **2003**, *10*, 181. (d) Miller, C. P. *Curr. Pharm. Design* **2002**, *8*, 2089. (e) Bryant, H. U. *Endocrine and Metabolism Disorders* **2002**, *3*, 231.
3. Nakayama, O.; Yagi, M.; Tanaka, M.; Kiyoto, S.; Uchida, I.; Hashimoto, M.; Okuhara, M.; Kohsaka, M. *J. Antibiotics XLIII* **1990**, 1394.
4. (a) For recent examples of synthetic flavanoid ligands, see: Miller, C. P.; Collini, M. D.; Harris, Heather, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2399. (b) Kim, Y.-W.; Mobley, J. A.; Brueggmeier, R. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1475. (c) Pouget, C.; Lauthier, F.; Simon, A.; Fagnere, C.; Basly, J.-P.; Delage, C.; Chulia, A.-J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3095.
5. (a) Saeed, A.; Sharma, A.; Durani, N.; Jain, R.; Durani, S.; Kapil, R. S. *J. Med. Chem.* **1990**, *33*, 3210. (b) Saeed, A.; Sharma, A.; Durani, S.; Kapil, R. S. *J. Med. Chem.* **1990**, *33*, 3222.
6. (a) The ketones **23** in Scheme 1, where commercially unavailable, were generally prepared by Friedel–Crafts acylation of the appropriate acid chloride or carboxylic acid with resorcinol according to literature procedures: Wahala, K.; Hase, T. *J. Chem. Soc., Perkin Trans. 1* **1991**, 3005. (b) Chiba, K.; Sonoyama, J.; Tada, M. *J. Chem. Soc., Perkin Trans 1* **1996**, 1435. (c) Chandra, H.; Zilliken, F.; Offermann, W.; Breitmaier, E. *Can. J. Chem.* **1981**, *59*, 2266. (d) Selective protection with THP was accomplished using established methodology: Kapil, R. S. *J. Med. Chem.* **1990**, *33*, 3222.
7. (a) Donnelly, D. M. X.; Keenan, A. K.; Leahy, T.; Philbin, E. M. *Tetrahedron* **1972**, *28*, 2545. (b) Fujise, S.; Fujise, Y.; Hishida, S. *Nippon Kagaku Zasshi* **1963**, *84*, 78. (c) *Chem. Abstr.* **1964**, *60*, 5444c. (d) Bognar, R.; Rakosi, M.; Litkei, Gy. *Acta Chim. Engl.* **1962**, *34*, 253. (e) Clark-Lewis, J. W.; Spotswood, T. M.; Williams, L. R. *Aust. J. Chem.* **1963**, *16*, 107. (f) McKervey, A. M.; Ye, T. *J. Chem. Soc., Chem. Commun.* **1992**, 823.
8. Donnelly, D. M. X.; Fitzpatrick, B. M.; O'Reilly, B. A.; Finet, J.-P. *Tetrahedron* **1993**, *49*, 7967.
9. The ketones **25** in Scheme 2 were generally prepared by Friedel–Crafts acylation of 3-substituted resorcinols with commercially available (phenylthio) acetyl chloride using $SnCl_4$ as the Lewis acid. In the case where X = H, the desired ketone was obtained by displacement of the Houben–Hoesch reaction product of resorcinol and chloroacetonitrile with NaSPh. A representative procedure for the

Friedel–Crafts acylation is given below for X = F: To a mixture of 5-fluoro-1,3-dimethoxybenzene (0.5 g, 3.2 mmol) in $CH_2Cl_2$ (5 mL) was added (phenylthio) acetyl chloride (0.48 mL, 3.2 mmol) at 0 °C under a nitrogen atmosphere, followed by dropwise addition of a 1 M solution of $SnCl_4$ in $CH_2Cl_2$ (4.2 mL, 4.2 mmol). After stirring for 30 min, the reaction was allowed to warm to ambient temperature and stirred for another 1 h. The reaction was partitioned between ethyl acetate and 2 N HCl. The organic layer was washed with brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude material contained a 2:1 mixture of the desired product and its other isomer. Purification by silica gel chromatography with 20% ethyl acetate in hexane as the eluant afforded the desired product in 32% yield. Standard deprotection of the methoxy groups was accomplished using $BBr_3$ to give the desired ketone **25** in 40% yield.

10. An X-ray crystal structure determination of a compound related to **27**, where X is H, $R_2$ is 4-hydroxyphenyl, the MOM group is replaced by a methyl group, and the TBDPS group is replaced by a hydrogen atom revealed that the phenyl groups at C-2 and C-3 were *trans* to one another.
11. Kozyrod, R. P.; Morgan, J.; Pinhey, J. T. *Aust. J. Chem.* **1985**, *38*, 1147.
12. Donnelly, D. M. X.; Keenan, A. K.; Leahy, T.; Philbin, E. M. *Tetrahedron* **1972**, *28*, 2545.
13. The $IC_{50}$ values were generated in an estrogen receptor ligand binding assay. This scintillation proximity assay was conducted in NEN Basic Flashplates using tritiated estradiol and full length recombinant human ER-alpha and ER-beta proteins, with incubation times of 3–23 h. In our experience, this assay provides $IC_{50}$ values that are reproducible to within a factor of 2–3. Most compounds are single point determinations. The binding results for **6** reflect an average of three determinants with incubation times of 3 and 15 h. For estradiol, the binding data reflects an average of over 100 determinants at 3 h of incubation.
14. 20-day old intact female Sprague–Dawley rats were treated (sc) with test compounds for 3 days at 1 mpk. The uteri wet weights were determined on day 4 and dry weights were determined after air-drying the tissue samples for 3 days. The anti-estrogenic activity of compounds was determined by co-administration of the compound with a subcutaneous injection of 17-beta-estradiol and reported as % inhibition. The estrogenic activity (partial agonism) of the compounds was determined by administering the test compound without estradiol and reported as % control.
15. Mitra, S. W.; Cai, S.; Wilkinson, H.; Salzmann, G.; Zhou, G.; Hayes, E.; Dashkevicz, M.; Cummings, R.; Smith, R.; Schaeffer, J. M. Abstracts, 80th Annual Meeting of the Endocrine Society, New Orleans, June, 1998, P1-562, p 235.
16. (a) Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. *Cell* **1998**, *95*, 927. (b) Brzozowski, A. M.; Pike, A. C. W.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J. A.; Carlquist, M. *Nature* **1997**, *389*, 753.
17. Our docking and energy minimization approach, which will be described elsewhere by Ralph Mosley in due course, identified a binding mode of compound **6** with the absolute configuration of [2*S*,3*R*] as being the most likely to explain the SAR.