Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 1919–1922

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Novel Estrogen Receptor Ligand Template

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Received 13 December 2002; accepted 14 February 2003

Abstract—Three synthetic routes towards a novel estrogen receptor ligand template based on a rigid bicyclo-[3.3.1]-nonene core have been investigated. The prototype compound exhibits potent binding at the ERβ receptor and promising estrogen receptor subtype selectivity.

Estrogen receptors (ER) have quickly emerged as attractive targets for therapeutic intervention in a wide variety of diseases, including osteoporosis[1] and cancer.[2] The marketed drug raloxifene[3] (**1**, Fig. 1) shows potent binding at both ERα and ERβ nuclear receptors, and combines unique pharmacological and pharmacokinetic properties. Recently, a large number of programs have focused on core modifications.[4] As part of a discovery effort to find novel estrogen receptor modulator templates, the bicyclic ether **2**[5] was identified by high throughput screening.[6]

Subsequently, we observed that ether **2** is acid-sensitive (presumably due to easy formation of a benzylic cation), which makes it a poor lead template structure for an orally administered drug. We decided to embark on the preparation of a simplified bicyclo-[3.3.1]-nonene **3** (Fig. 2), possessing a similar rigid template.

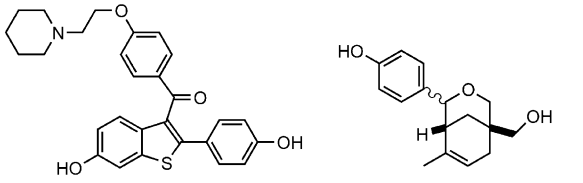

Figure 1. Estrogen receptor modulators.

We predicted that the correct relative stereochemistry of the phenyl and the hydroxymethylene groups should be *trans* as in estradiol **4**.

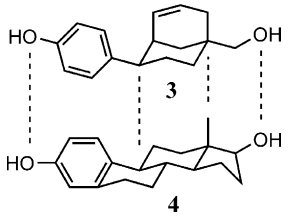

Figure 2. Stable analogue of bicyclic ether **2**.

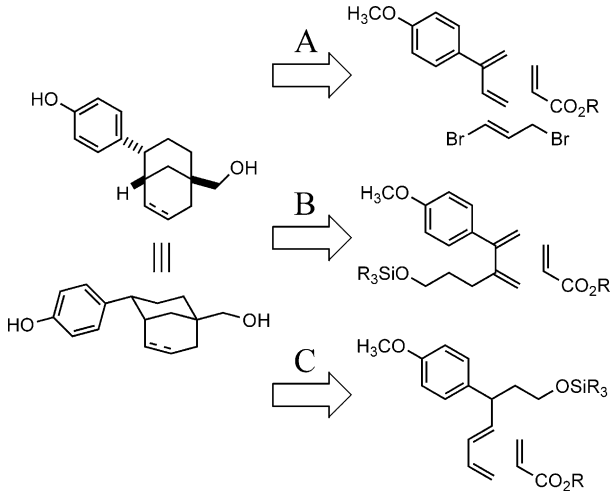

Figure 3. Proposed synthetic routes to bicyclo-[3.3.1]-nonenes.

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doi:10.1016/S0960-894X(03)00307-X

Three synthetic routes to the bicyclic core of **3** were successively investigated (Fig. 3).

Results and Discussion

The three routes differ by the nature of the bond formed by trans-annular cyclization. Route A is the shortest, and relies on a radical cyclization (Fig. 4).

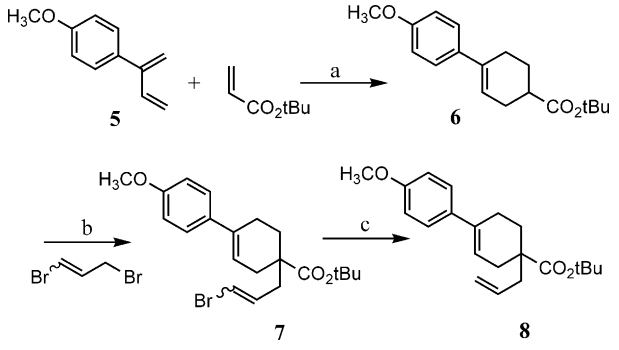


Figure 4. Route A: (a) $PhCH_3$, reflux, 38%; (b) LDA, THF, −78 °C to rt, 38%; (c) Bu_3SnH, AIBN, *t*BuOH.

The known diene **5**[7] was reacted with *tert*-butyl acrylate to form only cyclohexene **6** and none of the undesired regioisomer.[8] Alkylation of ester **6** with *E*/*Z* 1,3-dibromopropene using standard conditions affords **7** as a mixture of isomers. This mixture was not separated, as the *E*- and *Z*-vinyl radicals are known to interconvert. Unfortunately, when we subjected **7** to typical radical cyclization conditions, only the reduced analogue **8** could be isolated, suggesting an intramolecular hydrogen transfer. This route was therefore abandoned.

Route B uses an intramolecular enolate cyclization, resulting in the formation of a bicyclo-[3.3.1]-nonane framework (Fig. 5). Bromoolefin **9**[9] was coupled with stannane **10**[10] using standard palladium-catalyzed reaction conditions, in moderate to good yield. The use of a trimethyl stannane instead of a tributyl stannane shortened the reaction time and improved the overall yield. Diene **11** is stable at room temperature, and smoothly reacts with methyl acrylate in refluxing toluene, to afford cyclohexenes **12a** and **12b** in a circa 2:3 ratio. This mixture, which could not be separated, was hydrogenated, affording cyclohexane **13** as a mixture of isomers. The latter was deprotected and then converted to the corresponding mesylate **15a** using a standard protocol. Alternatively, bromide **15b** was also obtained. Unfortunately, intramolecular cyclization of either mesylate **15a** or bromide **15b** using LDA in THF with or without HMPA did not proceed, as only dimeric products could be isolated.

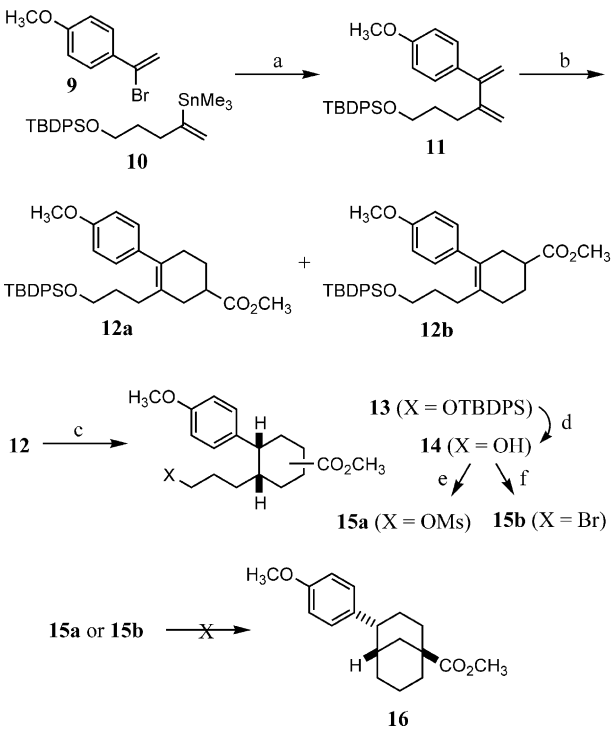


Figure 5. Route B: (a) $Pd_2(dba)_3$, NMP, $P(Ph)_3$, 0 °C to rt, 25%; (b) methyl acrylate, $PhCH_3$, reflux, 64%; (c) H_2, cat Pd/C, EtOH, 47%; (d) TBAF, THF, rt, 96%; (e) MsCl, pyridine, CH_2Cl_2, rt, 57%; (f) CBr_4, Ph_3P, CH_2Cl_2, rt, 80%.

Route C was finally investigated, despite the fact that it is not stereoselective, because it forms the other six-membered ring as a key step (Fig. 6).

The enolate derived from ester **17** was alkylated with oxirane, cyclized into a lactone, and reduced with diisobutyl aluminum hydride to afford lactol **18**. The latter

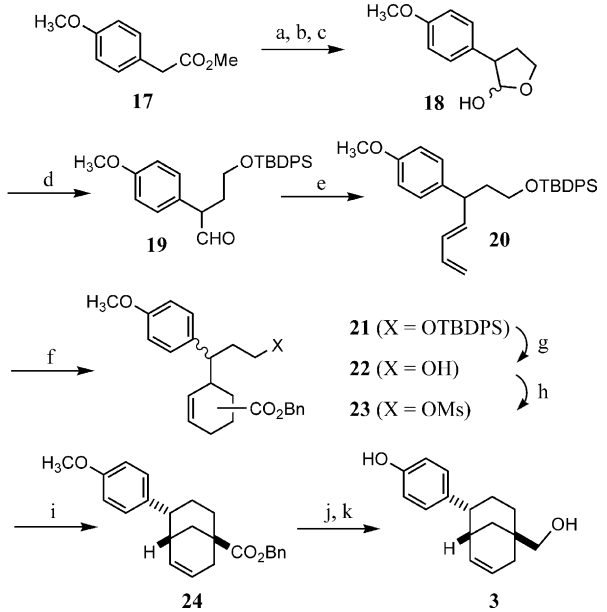
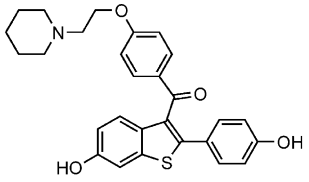
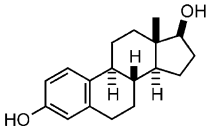


Figure 6. Route C: (a) LDA, THF, oxirane, BF_3–OEt_2, −78 to 10 °C, 31%; (b) PTSA, $PhCH_3$, reflux, 20 min, quant; (c) DIBAL-H, THF, −78 to −15 °C, 43%; (d) TBDPSCl, DMF, cat DBU, imidazole, 0 °C to rt, 57%; (e) allyltriphenyl phosphonium chloride, KO*t*Bu, THF, −78 °C to rt, 63%; (f) benzyl acrylate, *o*-xylene, reflux, 30 h, 75%; (g) TBAF, THF, rt, 16 h, 62%; (h) MsCl, pyridine, CH_2Cl_2, 0 °C to rt, 95%; (i) LDA, HMPA, THF, −78 °C to rt, 4%; (j) LAH, THF, 0 °C to rt, quant; (k) BBr_3, CH_2Cl_2, −80 °C to rt, 35%.

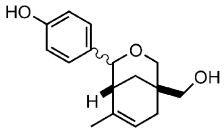
Table 1. In vitro pharmacology of compounds **1**–**4**



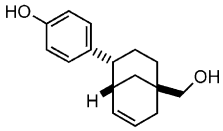
Raloxifene **1**



Estradiol (E2) **4**



2



3

	1	**2**	**3**	**4**
ERα (IC_{50}, nM)	7	3200	550	2
ERβ (IC_{50}, nM)	470	850	75	2
ERα/ERβ	0.015	3.8	7.3	1
pS2 expression in MCF-7 (EC_{50}, nM)	ND	20	<10	0.01
pS2 expression in MCF-7 (% agonist compared to E2)	0	149	139	100

did not react with allyl-triphenyl phosphonium chloride under Wittig conditions, and was therefore opened and protected as the silyl ether **19**, which in turn was converted into diene **20**. When diene **20** was subjected to benzyl acrylate in refluxing benzene, a very complex mixture of isomers was formed, and carried on in the next three steps (desilylation, mesylation, and intramolecular cyclization). No attempt was made to separate these mixtures. We expected to obtain a mixture of isomeric bicyclo-[3.3.1]-nonanes and bicyclo-[4.3.0]-nonanes from the key cyclization step. This, however, did not turn out to be the case, as the desired cyclization product **24** was the only monomeric product observed among a mixture of dimerization products. Furthermore, product **24** could easily be isolated by flash chromatography, albeit in very low yield. It can be hypothesized that the relative success of this cyclization is due to a favorable chair-like transition state. Ester **24** was converted to our target molecule **3** by reduction with lithium aluminum hydride followed by demethylation with boron tribromide. The relative stereochemistry of racemic alcohol **3** was established by NMR.[11] Bicyclic analogue **3** exhibits a very intriguing in vitro pharmacological profile (Table 1).

According to our data,[12] bicyclic alcohol **3** shows improved binding to ERβ, when compared to our original analogue **2**. This may be due to increased lipophilicity, or possibly an unfavorable interaction of the methyl group in **2** with the receptor. In addition, there is an apparent reversal of ERα/ERβ selectivity by **3** compared to raloxifene **1**.[13] Unlike raloxifene, both analogues **2** and **3** show full agonistic activity in MCF-7 cells[14] (pS2[15] gene induction with bDNA detection).

In conclusion, we have prepared a new estrogen receptor ligand prototype, featuring a novel bicyclo-[3.3.1]-nonene skeleton, using an 11-step route. While the preparation of our first analogue is not stereoselective and remains impractical on large scale, the existence of this new series provides an important starting point to future studies focusing on estrogen receptor subtype selectivity, and its impact on pharmacology.

Acknowledgements

We would like to thank J. Brice for HPLC purification of bicyclic analogue **3**, E. Medvedeff for the preparation of a mouse monoclonal ERβ antibody, as well as D. Brittelli, B. Dixon, and Prof. D. Curran for helpful discussions.

References and Notes

1. Gowen, M.; Emery, J. G.; Kumar, S. *Emerg. Drugs* **2000**, *5*, 1.
2. Dhingra, K. *Invest. New Drugs* **1999**, *17*, 285.
3. Hagmeyer, K. O.; Meyer, T. K. *J. Pharm. Technol.* **1999**, *15*, 37.
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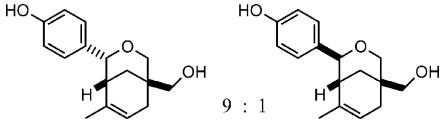
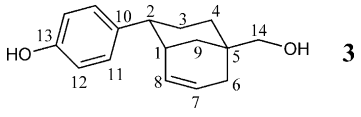
5. ^{1}H NMR analysis of bicyclic ether **2** shows that this sample consists of a 9:1 mixture of two isomers. Stereochemical considerations suggest that the major isomer is also the active ingredient.



6. Estrogen receptors (ER) α and β were purchased from PanVera Corporation (Madison, WI, USA). [2,4,6,7,16,17-^{3}H] Estradiol (157 Ci/mmol) and antimouse antibody binding scintillation proximity assay (SPA) beads were purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Mouse monoclonal ER antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA) and mouse monoclonal ERβ antibody was prepared in-house. SPA microtitre plates were purchased from Wallac (Gaithersburg, MD, USA). Using an antibody binding SPA format, reactions were conducted in 100 μL of assay buffer (50 mM HEPES pH 7.4, 0.5% BSA, 0.2% Tween 20, 1.25% glycerol, 150 mM NaCl) in the presence or absence of test compounds. Test compounds were prepared in 5% DMSO, delivered to the assay plate at final concentrations ranging from 1 to 10,000 nM in 0.25% DMSO (final concentration). For ERα binding assays the reaction mixture contained ERα (0.02 μg/well), mouse monoclonal ER antibody (0.2 μg/well), and [2,4,6,7,16,17-$^3H_6$] estradiol (2 nM). ERβ binding assays contained ERβ (0.03 mg/well), mouse monoclonal ERβ antibody (0.03 μg/well), and [2,4,6,7,16,17-$^3H_6$] estradiol (4.8 nM). Antimouse antibody SPA binding beads were then added to each well (0.25 mg/well), the reaction mixture was equilibrated (3 h), the plate centrifuged (2000 rpm, 10 min), and counted in a Microbeta scintillation counter (Wallac, Inc.). Raloxifene **1** was used as a positive control, unlabeled estradiol **4** was used as a non-specific binding control.

7. Crenshaw, R. R.; Luke, G. M.; Jenks, T. A.; Partyka, R. A.; Bialy, G.; Bierwagen, M. E. *J. Med. Chem.* **1973**, *16*, 813.
8. When using benzyl or methyl acrylate as the dienophile, the ratio of the desired regioisomer **6** to the undesired was 4:1 in each case.
9. Rappoport, Z.; Gal, A. *J. Chem. Soc., Perkin Trans. 2* **1973**, 301.
10. Stannane **10** is prepared from known 2-bromo-pent-1-ene-5-ol in two steps (tBuPh$_2$SiCl, DMF, imidazole, 75 °C, 2 h, then t-BuLi, THF, Me$_3$SnCl, −78 °C to rt).
11. NMR spectra of **3** were acquired on a Bruker DMX-500 spectrometer equipped with a three-axis-gradient inverse triple resonance probe, with the exception of the ^{13}C spectrum, which was obtained using a heteronuclear broadband probe. The sample was dissolved in DMSO-d_6, and the chemical shifts were referenced to the solvent signals. A gradient enhanced COSY spectrum was employed to elucidate the network of coupled protons. One-bond ^{1}H–^{13}C correlations were obtained from a phase-sensitive HMQC experiment. Long-range ^{1}H–^{13}C couplings from a gradient enhanced HMBC experiment were utilized to confirm the assignments. For a qualitative estimate of interproton distances, a transverse NOE experiment was collected in phase sensitive mode using time proportional phase incrementation.



3

	^{1}H (ppm)	^{13}C (ppm)
1	1.88 m	48.6
2	2.57 m	51.8
3a	1.83 m	33.3
3b	1.59 m	
4a	1.97 m	35.3
4b	1.25 m	
5		43.7
6a	1.94 m	22.0
6b	1.86 m	
7	5.62 m	126.1
8	5.36 m	128.1
9a	1.68 m	29.0
9b	1.33 m	
10		133.8
11	7.00 d (J = 8.5 Hz)	127.9
12	6.66 d (J = 8.5 Hz)	115.0
13		155.5
14a	3.27 dd (J = 5.5 and 10.5 Hz)	65.6
14b	3.20 dd (J = 5.5 and 10.5 Hz)	
13 OH	9.13 s	
14 OH	4.56 t (J = 5.5 Hz)	

Elucidation of relative stereochemistry was based on NOE data in comparison with molecular models. Molecular mechanics calculations were performed on a Silicon Graphics Indy workstation using Tripos force fields and the Gasteiger and Hückel method for charges as implemented in SYBYL version 6.6 (Tripos, St. Louis, MO, USA). The basis for the stereochemical assignment of bicyclic analogue **3** is the observation of cross-peaks between the olefinic proton H8 and the aromatic proton H11 in the ROESY spectrum, indicating spatial proximity in agreement with distance calculations. The presence of a strong cross-peak between protons H2 and H9a provides further evidence for the assigned stereochemistry.

12. Leatherbarrow, R. J. *Grafit,* Version 4.0; Erithacus Software: Staines, UK, 1998. Calculated IC_{50} values showed an error of $\leq$15%.
13. While our data indicate that raloxifene is approximately 70-fold selective for ERα over ERβ, the number has historically been 10- to 20-fold: see: Miller, C. P. *Curr. Pharm. Des.* **2002**, *8*, 2089. Miller, C. P.; Komm, B. S. *Ann. Rep. Med. Chem.* **2001**, *36*, 149, and references cited therein for information on the binding of raloxifene and other SERMs to ERα and ERβ.
14. MCF-7 cells were obtained from the ATCC and grown in Growth Medium (GM, see below). The cells are grown to 80% confluency and trypsinized and washed to remove phenol red, then plated at 10,000 cells/well in Starve Medium (SM, see below). After 2 days, the medium is removed from the cells and either fresh SM or SM plus test compound is added (100 mL total volume). The next day the bDNA assay is started by adding Capture Hybridization Buffer to each capture well of the bDNA plate (all solutions supplied with QuantiGene kit). Subsequently, medium is removed from the 96-well plate containing cells and then the solution of lysis buffer and oligomers (designed by ProbeDesigner 1.0 for the gene of interest) is added to the 96-well plate (100 μL/well). The cell and lysis/oligomer mixture is incubated for 15 min at 53 °C and then vortexed for 1 min. The lysate is then transferred to the bDNA capture plate and mixed. The plate is then sealed and incubated at 53 °C for 16 h. The next day, the plate is cooled for 10 min at room temperature and the Amplifier Reagent mixture is prepared. The wells are then washed twice with 200 μL of Wash Solution A. Amplifier Reagent is added (50 μL/well) and the plate is sealed again and incubated at 53 °C for 30 min. The plate is then cooled for 10 min at room temperature (rt) and the Label Probe Reagent is prepared. The plate is washed twice with 200 μL of Wash Solution A and 50 μL/well of Label Probe Reagent is added. The plate is sealed and incubated at 53 °C for 15 min. The plate is cooled for 10 min at rt and the Substrate Mixture is prepared. The plate is again washed twice with 200 μL Wash Solution A and then twice with Wash Solution D. The Substrate Mixture is then added (50 μL) and the plate is sealed. The plate is incubated at 37 °C for 30 min and then read on the Quantiplex luminometer. Raloxifene is used as a positive control in this assay, and behaves as a full antagonist of estrogen-induced pS2 expression.

Growth Medium (GM): MEM (GIBCO) with 10% HyClone heat inactivated fetal bovine serum (FCS), 1 mM sodium pyruvate solution (GIBCO), 0.1 mM non-essential amino acid solution (GIBCO), 2 mM L-Glutamine (GIBCO), 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate and 0.25 μg/mL amphotericin B (GIBCO).

Starve Medium (SM): MEM (Without Phenol Red, GIBCO) with 5% HyClone charcoal/Dextran Treated Fetal bovine serum (the remainder of the medium is identical to GM above).
15. pS2 is an estrogen-responsive gene identified in breast cancer cell lines. Rio, M. C.; Chambon, P. *Cancer Cells* **1990**, *2*, 269. Davidson, N. E.; Bronzert, D. A.; Chambon, P.; Gelmann, E. P.; Lippman, M. E. *Cancer Res.* **1986**, *46*, 1904.