Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 902–906
Bioorganic & Medicinal Chemistry Letters

ERβ ligands. Part 5: Synthesis and structure–activity relationships of a series of 4′-hydroxyphenyl-aryl-carbaldehyde oxime derivatives

Richard E. Mewshaw,[a,*] S. Marc Bowen,[a] Heather A. Harris,[b] Zhang B. Xu,[c] Eric S. Manas[d] and Stephen T. Cohn[e]

[a]*Chemical and Screening Sciences, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*
[b]*Women's Health and Musculoskeletal Biology, Wyeth Research, 500 Arcola Road, Collegeville, PA 19426, USA*
[c]*Structural Biology and Computational Chemistry, Wyeth Research, 200 Cambridge Park Drive, Cambridge, MA 02140, USA*
[d]*Computational, Analytical and Structural Sciences, GlaxoSmithKline, 709 Swedeland Road, King of Prussia, PA 19406, USA*
[e]*Encysive Pharmaceuticals, 7000 Fannin Suite 1920, Houston, TX 77030, USA*

Received 11 October 2006; revised 13 November 2006; accepted 20 November 2006
Available online 1 December 2006

Abstract—A series of 4′-hydroxyphenyl-aryl-carbaldehyde oximes (**5b**) was prepared and found to have high affinity (4 nM) and modest selectivity (39-fold) for estrogen receptor-β (ERβ). Substitution of one of the core rings of the scaffold based around these novel ligands further expanded our knowledge in the quest toward achieving high affinity and selectivity for ERβ. An X-ray co-crystal of structure **11** revealed that the oxime moiety was mimicking the C-ring of genistein, as previously predicted by SAR and docking studies.

Estrogens are known to play important roles in the regulation of growth, development, and maintenance of a diverse range of tissues.[1] Two forms of the estrogen receptor (ER) have been identified, designated ERα and ERβ, which are major mediators of the biologic effects of estrogens.[2] While the utility of estrogen-based therapies such as oral contraceptives and hormone replacement is well-established, the role of ERβ is still being unraveled.[3] Crystal structures of the ERα and ERβ binding domains reported in the literature[4–6] reveal two conservative residue substitutions in the binding site: ERα $Leu_{334} \rightarrow$ ERβ Met_{336} and ERα $Met_{421} \rightarrow$ ERβ Ile_{373}. Several laboratories have exploited these residue substitutions, resulting in a wide variety of structures and ranges of ERβ selectivity.[7] Recently developed non-steroidal selective ERβ agonists in our laboratories [i.e., ERB-041 (**1**)[3,8] and WAY-202196 (**2**)[9] (Fig. 1)] have demonstrated remarkable anti-inflammatory activities but lack classical estrogenic activity in a variety of models. One strategy that our laboratory has embraced was to try to mimic the pharmacophore of genistein (**3**) by employing either the simpler phenylnaphthalene[9,10] (e.g., **2**) or the biphenyl scaffold[11,12] (e.g., **5a**) in place of the isoflavone moiety. Modeling studies predicted that an oxime moiety attached to the biphenyl scaffold could potentially mimic

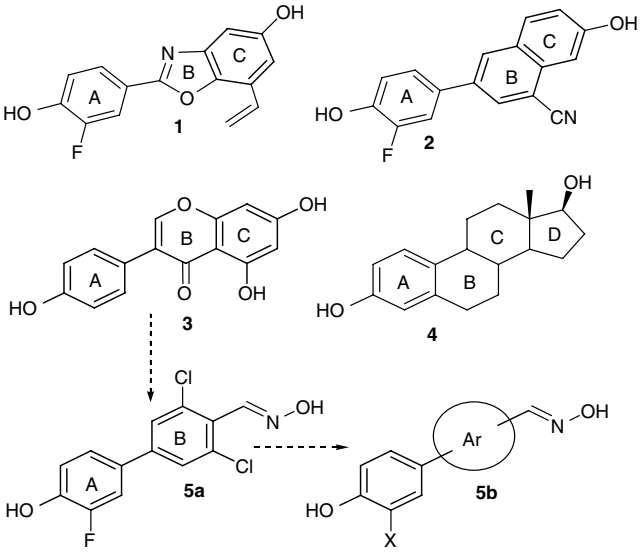
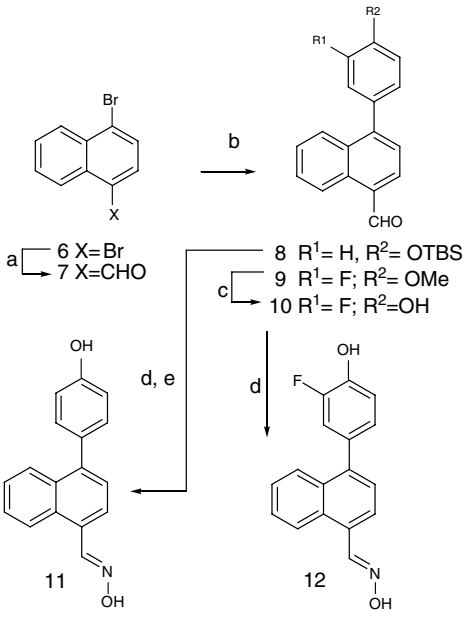


Figure 1. Compounds of interest.

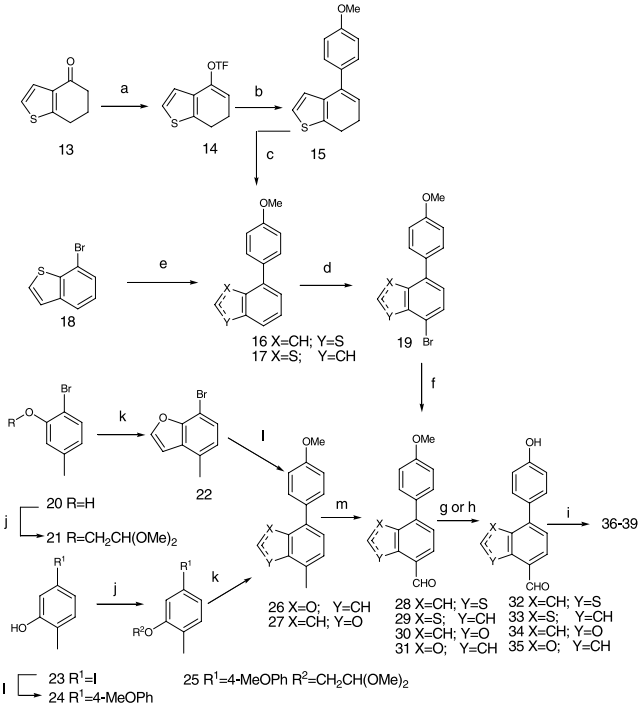
Keywords: Estrogen receptor ligands; Oximes.
* Corresponding author. Tel.: +1 484 865 2910; fax: +1 484 865 9399; e-mail: mewshar@wyeth.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.11.066



Scheme 1. Reagents and conditions: (a) i—*n*-BuLi, ii—DMF; (b) $Pd[P(Ph_3)]_4$, ArBr, Na_2CO_3, DME/H_2O, 85 °C; (c) $C_6H_5N{\cdot}HCl$, 190 °C; (d) $NH_2OH{\cdot}HCl$, MeOH; (e) TBAF/THF.

the hydroxyl group on the C-ring of genistein. Though, the oxime moiety has been recently employed as a mimic of the A-ring phenol of estradiol (**4**),[13] it had never been utilized as a mimic of the D-ring of estradiol (i.e., C-ring



Scheme 2. Reagents and conditions: (a) Tf_2O, DCM, 2,6-lutidine, 0 °C; (b) $Pd_2(dba)_3$, ArBr, KF, $P(Cy)_3$, 60 °C; (c) MnO_2/toluene; (d) Br_2 DCM, −20 °C; (e) $Pd[P(Ph_3)]_4$, ArBr, Na_2CO_3, DME/H_2O, 85 °C; (f) i— *n*-BuLi; ii—DMF; (g) BBr_3, DCM, −78 °C, rt; (h) $C_6H_5N{\cdot}HCl$, 190 °C; (i) $NH_2OH{\cdot}HCl$, MeOH; (j) $BrCH_2CH(OMe)_2$, NaOEt/EtOH; (k) PPA, ClC_6H_5; (l) $Pd[P(Ph_3)]_4$, ArBr, Na_2CO_3, DME/H_2O, 85 °C; (m) i—NBS, AIBN; ii—K_2SeO_2, K_2HPO_4.

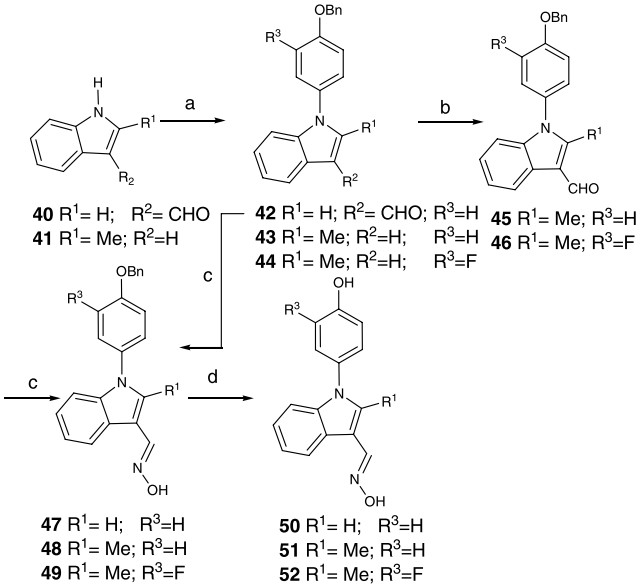
of genistein) until our report in Part 2.[12] In fact, oxime **5a** was discovered to have similar ERβ affinity (IC_{50} = 9 nM) and selectivity (31-fold selective) to those of genistein.

In order to further expand our knowledge-based around these novel oxime ligands, we report in this letter our results toward replacement of the B-ring of the previously reported biphenyl oximes with other aryl groups (i.e., **5b**). Our strategy was to attempt to induce ERβ selectivity using the larger aryl rings that project toward the ERα Met_{421} → ERβ Ile_{373} residue substitution.

The synthesis of naphthalene oximes **11** and **12** is shown in Scheme 1 beginning with dibromo **6**. Formylation, followed by Suzuki coupling,[14] afforded the aldehydes **8** and **9**. Compound **8** was reacted with hydroxylamine and deprotected to afford **11**. Deprotection of **9** with pyridine hydrochloride followed by treatment with hydroxylamine led to **12**.

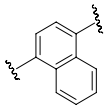
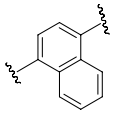
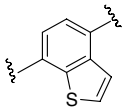
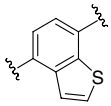
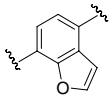
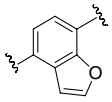
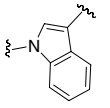
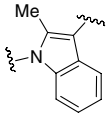
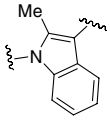
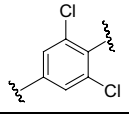
Benzofurans and benzothiophenes were prepared according to Scheme 2. Triflation of the commercially available ketone **13** led to **14**, which upon Suzuki coupling and aromatization afforded **16**. The corresponding isomer was prepared from the known benzothiophene **18** using Suzuki conditions. Both **16** and **17** were brominated and formylated to afford aldehydes **28** and **29**, which were deprotected and treated with hydroxylamine to afford oximes **36** and **37**. The benzofurans were prepared beginning with the known phenols **20**[15] and **23**[16] that were converted into their respective regioisomers **26** and **27** in two steps. Oxidation[17] to the corresponding aldehydes followed by demethylation and treatment with hydroxylamine gave the phenolic oximes **38** and **39**.

Indoles **50**–**52** were prepared in a straight-forward manner as depicted in Scheme 3. Briefly, commercially



Scheme 3. Reagents and conditions: (a) K_2CO_3, NMP, CuBr, ArBr; (b) $POCl_3$, DMF, 80 °C; (c) $NH_2OH{\cdot}HCl$, MeOH; (d) i—$Pd(OAc)_2$, Et_3SiH, TEA; ii—TBAF/THF.

Table 1. Biaryl carboxaldehyde oximes of interest

Compound	X	Y	ER_β IC_{50}[a] (nM)	ER_α IC_{50} [a] (nM)	α/β
11	H		5 ± 2	95 ± 12	20
12	F		4 ± 1	148 ± 145	39
36	H		4	20	5
37	H		4 ± 1	37 ± 1	10
38	H		10 ± 1	155 ± 21	15
39	H		457 ± 122	1330 ± 198	3
50	H		202 ± 124	1905 ± 997	9
51	H		12 ± 1	270 ± 12	23
52	F		8.05	298	37
3		Genistein	9.7 ± 4	395 ± 181	41
4		Estradiol	3.6 ± 1.6	3.2 ± 1.0	1
5[12]			9 ± 3	270 ± 157	31

[a] Values are means of three experiments SEM (performed in triplicate, determined from eight concentrations).

available indoles **40** and **41** were exposed to Ullman arylation conditions by reacting with the appropriately substituted phenyl iodide to afford **42**–**44**. Formylation of **43** and **44** led to aldehydes **45** and **46**, respectively. Aldehydes **42**, **45**, and **46** were converted to their respective oximes and the phenols were unmasked using a debenzylation sequence employing palladium acetate and triethylsilane followed by desilylation to afford indole oximes **50**–**52**. As discussed in our previous paper, the (*E*)-geometry was assigned to all of the oximes.[12]

Shown in Table 1 are the affinities (as measured by IC_{50}s) and selectivities for human ERα or ERβ LBD of all compounds of interest using a competitive radioligand binding assay.[18] In our previous oxime study, employing the biphenyl motif allowed us to identify **5a** as one of the most interesting oximes (ERβ IC_{50} = 9 nM).[11] However, as noted above replacing the substituted phenyl group with an unsubstituted naphthalene moiety resulted in a twofold increase in ERβ affinity (i.e., **11** and **12**) and similar ERβ selectivity to **5a**.

As observed in earlier studies,[8–12,19] the ortho-fluoro substituent resulted in a slight improvement in selectivity (e.g., **11** versus **12**). Replacement of the naphthalene nucleus with the benzothiophene moiety gave similar

affinity for ERβ (**36** and **37** versus **1**). However, ERβ selectivity was not maintained due to an improvement in ERα affinity. A slight loss in affinity was observed for benzofuran **38** (versus **11**, **36**, and **37**). In contrast, the isomeric benzofuran **39** had a dramatic loss in affinity compared to its isomer **38**. One possible explanation is that intramolecular hydrogen bonding may be occurring between the oxygen of the furan ring of **39** and the hydroxyl of the oxime moiety, preventing hydrogen bonding with the His_{475} moiety of ERβ. Incorporation of the unsubstituted indole ring as part of the scaffold (i.e., **50**) led to only modest ERβ affinity. However conformational analysis (using the MMFF94 forcefield and a GBSA solvation model, as implemented in Macromodel; Schrödinger, LLC) suggested that the oxime moiety spends the greatest amount of time (by roughly 5-fold) in an alternate conformation where it is unable to access ERβ His475. This led us to attach a 2-methyl substituent, in an attempt to energetically stabilize the bioactive conformation relative to the alternate one. While this resulted in a 17-fold increase in ERβ affinity (i.e., **51** versus **50**), the improvement may be the result of several factors in addition to conformational preferences of the oxime, including increased lipophilicity and/or a change in the electronic nature of the indole ring. Upon attachment of an ortho-fluoro substituent to **51**, a slight increase in ERβ selectivity was again achieved (i.e., **51** versus **52**).

From our previous report in Part 2,[12] SAR suggested that the phenol moiety, and not the oxime moiety, was mimicking the A-ring of estradiol. In order to further support this argument, an X-ray co-crystal structure of naphthalene **11** was obtained,[19] using methods described in Ref. 20. As shown in Figure 2, the phenolic hydroxyl group of **11** is involved in a hydrogen-bonding interaction with both Glu_{305} and Arg_{346}, while the oxime moiety forms a hydrogen bond to His_{475} corroborating our original hypothesis that the oxime moiety is mimicking the D-ring of estradiol (i.e., C-ring of genistein).

Compound **11** was further evaluated in a cell-based transcriptional assay measuring its ability to regulate human keratin19 (KRT19) mRNA. This gene is upregulated by 17β-estradiol in human prostate cancer cells (LNCaPLN3) engineered to express either ERα or ERβ and thus can be used to determine agonist activity.[11] Analog **11** was tested at 1 μM and was compared to that of 10 nM 17β-estradiol. It was 24% as efficacious as 17β-estradiol via ERα and was about 50% as efficacious as 17β-estradiol via ERβ. The results thus suggest that **11** is a weak partial agonist for both ERs at 1 μM.

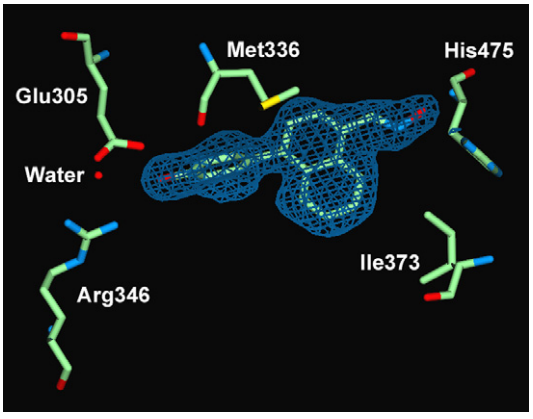


Figure 2. Unbiased $2f_o–f_c$ electron density difference map, indicating the binding mode of **11** in the ERβ LBD.

In summary, we have expanded our knowledge of the type of scaffolds that can be employed to identify novel ER ligands, which embrace the oxime moiety as a C-ring mimic of genistein. The most selective compounds (i.e., **12** and **52**) had a slight improvement in both ERβ affinity and selectivity with respect to genistein. Finally, an X-ray co-crystal structure of **11** verified that the oxime moiety was hydrogen bonded to the His_{475} residue and mimicking the D-ring of estradiol, as predicted from our previous modeling studies. Efforts are continuing in our laboratories to discover novel ERβ scaffolds and understand their modes of binding in order to rationally design highly selective ligands to further unravel the precise roles of ERβ.

References and notes

1. (a) *Estrogens and Antiestrogens: Basic and Clinical Aspects* by Jennings, T. S.; Creasman, W. T Lindsay. R.; Dempster, D. W.; Jordan, V. C., Eds.; Lippencott-Raven: Philadelphia, 1997, pp.223; (a)*Estrogens and Antiestrogens: Basic and Clinical Aspects*; Jennings, T. S., Creasman, T. S., Lindsay, W. T., Dempster, D. W., Jordan, V. C., Eds.; Lippencott-Raven: Philadelphia, 1997, p. 223; (b)*Selective Estrogen Receptors Modulators*; Manni, A., Verderame, M. F., Eds.; Humana: Totowa, NJ, 2002.
2. (a) Mosselman, S.; Polman, J.; Dijkema, R. *FEBS Lett.* **1996**, *392*, 49; (b) Kuiper, G. G.; Enmark, E.; Pelto-Huikko, M.; Nilsson, S.; Gustafsson, J.-A. *Proc. Natl. Acad. Sci. U.S.A* **1996**, *93*, 5925.
3. Harris, H. A.; Albert, L. M.; Leathurby, Y.; Malamas, M. S.; Mewshaw, R. E.; Miller, C. P.; Kharode, Y. P.; Marzolf, J.; Komm, B. S.; Winneker, R. C.; Frail, D. E.; Hederson, R. A.; Zhu, Y.; Keith, J. C., Jr. *Endocrinology* **2003**, *144*, 4241.
4. 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.
5. Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. *Cell* **1998**, *95*, 927.
6. Pike, A. C. W.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A. G.; Engstrom, O.; L-junggren, J.; Gustafsson, J. A.; Carlquist, M. *EMBO J.* **1999**, *18*, 4608.
7. Veeneman, G. H. *Curr. Med. Chem.* **2005**, *12*, 1077.
8. Malamas, M. S.; Manas, E. S.; McDevitt, R. E.; Gunawan, I.; Xu, Z. B.; Collini, M. D.; Miller, C. P.; Dinh, T.; Henderson, R. A.; Keith, J. C., Jr.; Harris, H. A. *J. Med. Chem.* **2004**, *47*, 5021.
9. Mewshaw, R. E.; Edsall, R., Jr.; Yang, C.; Manas, E. S.; Zhang, B. X.; Henderson, R. A.; Keith, J. C., Jr.; Harris, H. A. *J. Med. Chem.* **2005**, *48*, 3953.
10. Vu, A. T.; Cohn, S. T.; Manas, E. S.; Harris, H. A.; Mewshaw, R. E. *Bioorg. Med. Chem.* **2005**, *15*, 4520.
11. Edsall, R. J.; Harris, H. A.; Manas, E. S.; Mewshaw, R. E. *Bioorg. Med. Chem.* **2003**, *11*, 3457.

12. Yang, C. J.; Edsall, R.; Harris, H. A.; Zhang, X. C.; Manas, E. S.; Mewshaw, R. E. *Bioorg. Med. Chem.* **2004**, *12*, 2553.
13. Minutolo, F.; Antonello, M.; Bertini, S.; Rapposelli, S.; Rossello, A.; Sheng, S.; Carlson, K. E.; Katzenellenbogen, J. A.; Macchia, M. *Bioorg. Med. Chem.* **2003**, *11*, 1247.
14. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.
15. Ronald, R. C.; Gewali, M. B.; Ronald, B. P. *J. Org. Chem.* **1980**, *45*, 2224.
16. Hodgson, H. H.; Moore, F. H. *J. Chem. Soc.* **1926**, 2036.
17. Syper, L.; Mlochowski, J. *Synthesis* **1984**, *9*, 747.
18. Harris, H. A.; Bapat, A. R.; Gonder, D. S.; Frail, D. E. *Steroids* **2002**, *67*, 379.
19. Compound **11** X-ray coordinates have been deposited with the RCSB Protein Data Bank (Code # RCSB040341; PDB code # 2NV7).
20. Manas, E. S.; Unwalla, R. J.; Xu, Z. B.; Malamas, M. S.; Miller, C. P.; Harris, H. A.; Hsiao, C.; Akopian, T.; Hum, W.-T.; Malakian, K.; Wolfrom, S.; Bapat, A.; Bhat, R. A.; Stahl, M. L.; Somers, W. S.; Alvarez, J. C. *J. Am. Chem. Soc.* **2004**, *126*, 15106.