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7-Substituted 2-phenyl-benzofurans as ERβ selective ligands

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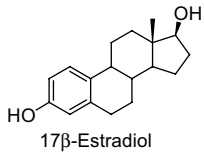
Abstract—A series of 2-(4-hydroxy-phenyl)-benzofuran-5-ols with relatively lipophilic groups in the 7-position of the benzofuran was prepared and the affinity and selectivity for ERβ was measured. Many of the analogues were found to be potent and selective ERβ ligands. Additional modifications at the benzofuran 4-position as well as at the 3′-position of the 2-phenyl group were found to further increase selectivity. Such modifications led to compounds with <10 nM potency and >100-fold selectivity for ERβ.

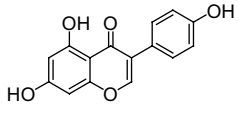
The estrogen receptor (ER) is a member of the nuclear receptor family, which functions as a ligand activated transcription factor. Binding of an ER agonist such as 17β-estradiol to the receptor leads to gene regulation in various tissues.[1] Many tissues throughout the body are affected by estrogens, especially mammary gland, bone and uterine tissues. It has been shown that ERα mediates many of the well documented activities associated with estrogens on these tissues.[2] Since the discovery of ERβ in 1996,[3] there has been much interest in finding potent and selective ligands for this receptor.[4] While the functional characterization of ERβ is still under active investigation, the recent report that an ERβ selective agonist demonstrated potent and efficacious action in two rat models of inflammation confirmed the potential of ERβ as a viable drug target.[5] In view of these recent findings as well as the promise of yet more to come, the desire for additional potent and selective ERβ ligands remains strong.

Although the ligand binding domains of ERα and ERβ share only 56% homology, the ligand binding cavities differ by only two amino acids (ERα Leu384 → ERβ Met336; ERα Met421 → ERβ Ile373).[6] Many of the known ER ligands such as 17β-estradiol bind both subtypes equally well. Among the initial reported selective ligands for ERβ reported were isoflavones such as genistein **1**, which has a binding affinity (IC_{50}) for ERβ of 10 nM and a selectivity for ERβ over ERα of 41-fold (Table 1). Many ER ligands like genistein have two OH groups, which can overlay with the OH groups of estradiol. In addition, genistein also contains an OH group at the 5-position of the chromenone moiety. It has been reported that an OH group at this position is expected to form an intramolecular hydrogen bond with the C-4 carbonyl group.[7] This increases the combined effective lipophilicity of the two groups,[8] allowing these polar substituents to occupy a relatively hydrophobic region of the ER binding pocket.[6]

2-(4-Hydroxy-phenyl)-benzofuran-5-ol **2** is a compound found to have a binding affinity (IC_{50}) for ERβ of 6 nM and a selectivity of 30-fold. Co-crystallization studies of



17β-Estradiol



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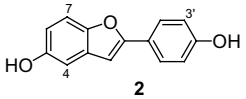

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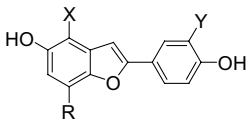
Table 1. Binding affinities (IC_{50}) for human ERα and ERβ ligand binding domain (mean + SD)

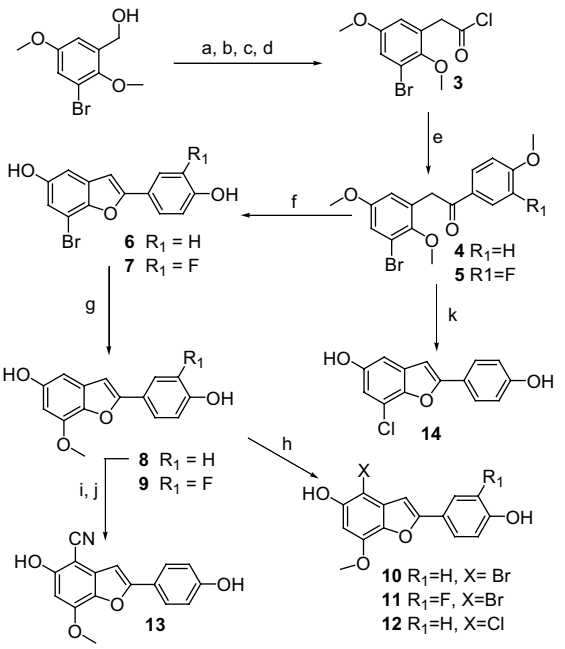


Example	R	X	Y	ERβ IC_{50} (nM)	ERα IC_{50} (nM)	Fold selectivity for ERβ
17β-Estradiol				3.6 (±1.6) $n = 144$	3.2 (±1) $n = 144$	1
Genistein **1**				10 (±4) $n = 79$	395 (±181) $n = 80$	41
2	H	H	H	6 (±2) $n = 32$	176 (±76) $n = 31$	30
6	Br	H	H	2 $n = 1$	1.5 $n = 1$	9
7	Br	H	F	0.35 (±0.4) $n = 2$	12 (±3) $n = 2$	36
8	OCH_3	H	H	10 (±3) $n = 5$	483 (±152) $n = 5$	49
9	OCH_3	H	F	10 (±2) $n = 6$	990 (±380) $n = 6$	99
10	OCH_3	Br	H	0.5 (±0.2) $n = 10$	21 (±5) $n = 11$	50
11	OCH_3	Br	F	3.3 (±1.4) $n = 5$	335 (±151) $n = 4$	99
12	OCH_3	Cl	H	2.5 (±0.7) $n = 3$	114 (±32) $n = 3$	45
13	OCH_3	CN	H	32 (±14) $n = 2$	1650 (±537) $n = 2$	51
14	Cl	H	H	1.6 $n = 1$	6.8 (±2.5) $n = 2$	4
19	CH_3	H	H	5.6 (±1) $n = 3$	114 (±22) $n = 3$	20
20	CH_3	H	F	4.0 (±1) $n = 2$	109 (±25) $n = 2$	26
21	CH_2CN	H	H	14 (±3) $n = 8$	1152 (±558) $n = 8$	80
22	CH_2CN	H	F	10 (±3) $n = 5$	1056 (±141) $n = 4$	108
23	CH_2CN	Br	H	2.0 (±1) $n = 5$	209 (±40) $n = 5$	104
24	CH_2CN	Cl	H	7.9 (±6) $n = 4$	625 (±383) $n = 4$	79
27	$COCH_3$	H	H	6.0 (±1) $n = 2$	103 (±4) $n = 2$	17
28	COEt	H	H	3.2 (±0) $n = 2$	49 (±11) $n = 2$	15
29	CHO	H	H	6.6 (±2) $n = 4$	263 (±27) $n = 4$	40
30	CN	H	H	2.2 (±1) $n = 9$	46 (±0.6) $n = 7$	21
31	CN	H	F	1.1 (±0) $n = 2$	23 (±5) $n = 2$	22

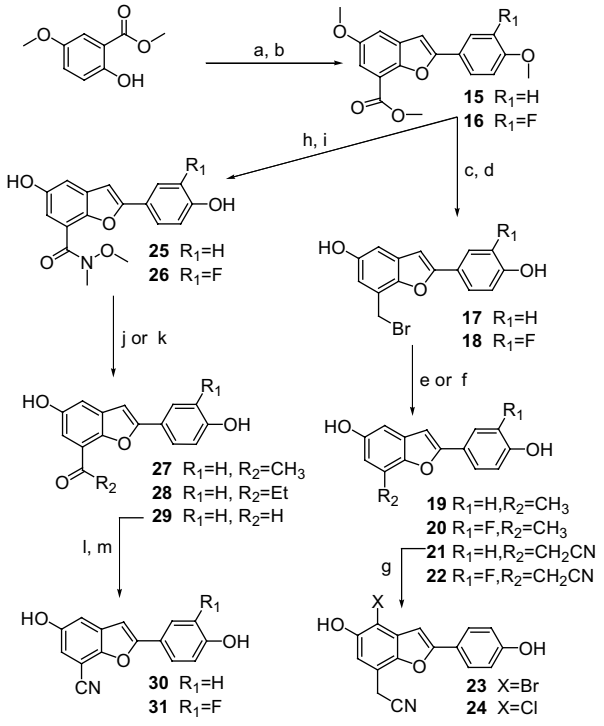
this ligand complexed to ERβ indicated that the benzofuran 7-position would overlay well with the genistein 5-position when bound to ERβ.[9] However, **2** lacks any group that could mimic the genistein 5-OH group, which we felt was contributing to the modest selectivity of genistein via interactions with ERα Met421/ERβ Ile373.[10] Therefore, to increase the potency and selectivity of compound **2**, a series of benzofurans was synthesized that contained relatively lipophilic groups at the benzofuran 7-position including halogen, alkyl, cyano, methoxy, CH_2CN, or various carbonyl containing groups. The 7-methoxy or 7-acetonitrile benzofurans were further substituted with halogens or cyano at the 4-position because docking studies showed that these groups would help direct the groups at the 7-position toward ERα Met421/ERβ Ile373 and reduce residual motion in the binding pocket (cpds **10**–**13**, **23**–**24**). In addition, several compounds with a fluoro group at the 3′-position of the 2-phenyl group were prepared and tested.

The compounds were prepared either by the reaction sequences shown in Schemes 1 or 2. The synthesis in Scheme 1 starts with 3-bromo-2,5-dimethoxy-benzyl alcohol, which can be made by a three step procedure.[11] The acid chloride **3** is prepared via a four step procedure from the benzyl alcohol by chlorination with $SOCl_2$, conversion to the nitrile and hydrolysis to give the carboxylic acid, which is then converted to the acid chloride by $SOCl_2$. A Friedel–Craft reaction between an anisole and **3** with $AlCl_3$ gave a 1,2-diphenyl-ethanone **4** or **5**, which was then deprotected with pyridine hydrobromide at high temperature to give compounds **6** or **7**. When pyridine hydrochloride was used instead of pyridine hydrobromide, the bromo was displaced by chloro to give **14**. The methoxy compounds **8** and **9** were made from a CuBr coupling of **6** or **7** with $NaOCH_3$. Bromination with NBS or chlorination with NCS led to compounds **10**–**12**. Treatment of compound **8** with $POCl_3$/DMF gave the 4-formyl group, which was converted to an oxime and dehydrated to give the nitrile compound **13**.

Benzofurans synthesized by the Sonogashira coupling (Scheme 2) start with methyl 4-methoxysalicylate which is iodinated to give methyl 2-iodo-4-methoxysalicylate and then coupled by a Sonogashira type reaction between either 4-methoxy-phenylacetylene or 3-fluoro-4-methoxyphenylacetylene to give the benzofuran **15** or **16**. Reduction followed by BBr_3 demethylation led to benzyl bromides **17** or **18**. Hydrogenation of **17** or **18** led to the methyl analogues **19** or **20**, while NaCN displacement gave the CH_2CN compounds **21** or **22**. Treatment of **21** with NBS or NCS gave the 4-halo substituted compounds **23** or **24**. Deprotection of **15** or **16** with pyridine hydrochloride converted the methoxy groups to phenols and the ester to a carboxylic acid. The acid was coupled with N,O-dimethylhydroxyl amine using EDCI to give the Weinreb amide **25** or **26**. Addition of methyl or ethyl Grignard reagents gave ketones **27** or **28**. Reduction of **25** or **26** with $LiAlH_4$ gave the formyl analogue **29** or the fluoro derivative, which was converted to a cyano group by forming the oxime followed by dehydration with pyridine hydrochloride at 200 °C to give the nitriles **30** or **31**.



Scheme 1. Reagents and conditions: (a) $SOCl_2$, THF; (b) NaCN, DMF; (c) H_2SO_4, AcOH, H_2O; (d) $SOCl_2$, CH_2Cl_2; (e) anisole or 2-fluoroanisole, $AlCl_3$; (f) pyridine hydrobromide; (g) $NaOCH_3$ CuBr; (h) NBS or NCS, CH_3CN; (i) $POCl_3$, DMF; (j) NH_2OH then Burgess reagent; (k) pyridine hydrochloride.



Scheme 2. Reagents and conditions: (a) I_2, KOH, MeOH; (b) $Pd(PPh_3)_2Cl_2$, CuI, $HNEt_2$/DMF, 4-methoxy-phenylacetylene or 3-fluoro 4-methoxyphenylacetylene; (c) $LiAlH_4$, THF; (d) BBr_3, CH_2Cl_2; (e) H_2Pd/C, MeOH; (f) NaCN, DMF; (g) NBS or NCS, CH_3CN; (h) pyridine hydrochloride 200 °C; (i) EDCI, CH_3NHOCH_3, DMF; (j) CH_3MgI or CH_3CH_2MgI, THF; (k) $LiAlH_4$, THF; (l) NH_2OH; (m) pyridine hydrochloride.

Table 1 presents the binding affinities (IC_{50}) for this series of benzofurans for the human ERβ and ERα ligand binding domains.[12] It is clear that the groups incorporated at the 7-position of compound **2** are well tolerated in ERβ. The ERβ binding affinities for most of the compounds were at least as potent as **2** with some of the analogues, for example, **7** and **10**, having an $IC_{50} < 1$ nM. Interestingly, the ERβ binding affinity appears to be relatively insensitive to the identity of the 7-substituent for this series of compounds. For example, the 7-bromo, 7-chloro and 7-cyano compounds **6**, **14**, and **30** were all potent, with an $IC_{50} < 3$ nM each, while the 7-methoxy **8**, and 7-acetonitrile **21** were slightly less potent.

Much more noticeable SAR is revealed when considering the binding selectivity for ERβ relative to ERα. For example, the ERβ selectivities of the 7-chloro compound **14** and 7-bromo compound **6** are only 4-fold and 9-fold, respectively, so selectivity has actually been reduced compared to the parent benzofuran compound **2**. In contrast, groups such as 7-methoxy and 7-acetonitrile exhibit significant improvements in ERβ selectivity relative to **2**: the 7-methoxy compound **8** is 49-fold selective for ERβ while the 7-acetonitrile compound **21** is 80-fold selective. The 7-cyano compound **30** was more potent, but not as selective as the 7-acetonitrile compound **21**. Finally, the 7-carbonyl derivatives **27**–**29** were also potent, but the ketones exhibited decreased ERβ selectivity, while the formyl group displayed selectivity similar to compound **2**. The above SAR is consistent with the results our docking calculations, which indicate that groups at the 7-position are likely to occupy the pocket near ERα Met421/ERβ Ile373, as intended. Therefore, we are probing a region of the binding site that is different when comparing the two receptor isoforms, and thus significant variations in selectivity are likely.

Since the 7-methoxy compound **8** and 7-acetonitrile compound **21** were the most selective compounds in the series so far, we decided to incorporate substituents at the benzofuran 4-position, as described above. The 4-bromo, 7-methoxy derivative **10** exhibited improved ER binding affinity, but there was no increase in selectivity relative to ERα, compared with **8**. A similar result was seen when the bromo was replaced by a chloro **12** or a cyano **13**. In contrast, **21** was halogenated at the 4-position, and the 4-bromo, 7-acetonitrile compound **23** exhibited a modest gain in both ERβ binding affinity (from 14 to 2 nM) and selectivity (from 80-fold to 104-fold). The 4-chloro, 7-acetonitrile compound **24** did not gain as much in ERβ binding affinity, and had no increase in ERβ selectivity.

The final group of compounds tested were the fluoro analogues **7**, **9**, **11**, **20**, **22**, and **31**. The addition of the fluoro group *ortho* to the phenolic OH group led to modest increases in selectivity for all the compounds except for the CN compound **31**. Selectivity enhancement due to such *ortho*-fluoro substitution has been reported previously by our laboratories.[10] The most selective fluoro compound was the 7-acetonitrile **22**, which had an ERβ IC_{50} of 10 nM and a selectivity of 108-fold relative to ERα. Compounds **22** and **23** were the most

selective compounds in this series. An interesting compound would be a derivative of **23**, which contained the 3′-fluoro group to ascertain whether the selectivity gains seen in **22** and **23** compared with **21** would be additive in the 7-acetonitrile series and would be a promising basis for future work.

To assess whether the compounds prepared were agonists or antagonists, the compounds were tested in a cell-based transcriptional assay and their effect on IGFBP4 (insulin-like growth factor binding protein-4) mRNA levels were assessed and compared to that of 17β-estradiol. Estradiol is able to upregulate IGFBP4 in SAOS-2 human osteosarcoma cells via ERβ.[2] Examples were tested at 1 μM and 17β-estradiol was used at 10 nM. As shown in Table 2, all compounds tested regulated IGFBP4 mRNA almost to the same extent as 17β-estradiol.

Examples **10**, **11**, and **21** were tested in vivo for their ability to increase uterine wet weight, a standard estrogenic bioassay. Sexually immature mice were dosed subcutaneously for 3 or 4 days with 50 mg/kg of compound in a vehicle of 5% DMSO/95% corn oil. In contrast to the reference estrogen, which increased organ weight 4-fold, examples **10**, **11**, and **21** did not significantly increase uterine weight. As the rodent uterus expresses primarily ERα, these data suggest these compounds are functionally selective for ERβ in vivo.

In order to further understand the mechanism of ERβ selectivity, compound **21** was co-crystallized with ERβ.[9] Figure 1 shows the resulting structure overlaid with that of ERβ complexed with genistein, so that the binding of these two compounds can be compared. It is clear from these structures that the 7-acetonitrile does indeed occupy a position analogous to that of the genistein 5-OH group, confirming our earlier hypothesis and the results of our docking studies. It can be seen from Figure 1 that the 7-acetonitrile group of compound **21** and the 5-OH of genistein are both in close proximity to ERβ Ile373, which is substituted by Met421 in ERα. This observation is consistent with the enhanced

Table 2. Effect of examples on ERβ mediated-IGFBP4 mRNA regulation in SAOS-2 cells

Example (1 μM)	% Activity of 10 nM 17β-estradiol
1	105
2	126
10	80
11	100
20	120
21	105
22	130
23	100
24	113
27	120
28	100
29	122
30	142
31	90

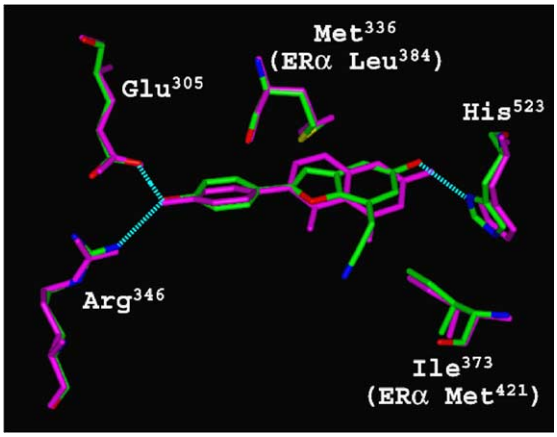


Figure 1. X-ray structure of ERβ co-crystallized with **21** (colored by atom type), overlaid with an X-ray of ERβ/genistein, only key residues shown for simplicity.

ERβ selectivity of **21** relative to **2**. It is also instructive to compare the selectivities of **21** and **30**. Placing a methylene spacer between the benzofuran ring and the nitrile leads to a compound with twice the ERβ selectivity, which is also consistent with the fact that the methylene spacer allows the nitrile group to penetrate more deeply into the ERβ Ile373/ERα Met421 pocket. The exact mechanism by which the 7-acetonitrile and other groups enhance ERβ selectivity via specific interactions with these residues will be addressed in other venues.[9]

In conclusion, placing various groups at the 7-position of 2-(4-hydroxy-phenyl)-benzofuran-5-ol **2** to mimic the 5-OH of genistein resulted in compounds that were more potent as well as more selective than genistein. A variety of groups led to potent binding affinities for ERβ, but the 7-methoxy and 7-acetonitrile groups resulted in the greatest enhancement in selectivity for ERβ over ERα. Incorporating additional groups at the 4-position and/or a fluoro group *ortho* to the 2-phenyl hydroxyl was found to further increase selectivity, and led to the most selective compound of the series (**22**), with an ERβ IC_{50} of 10 nM and 108-fold selectivity relative to ERs. Crystallography and molecular modeling studies[9] demonstrated that the benzofuran 7-position provides access to ERα Met421/ERβ Ile373, thus playing a key role in the enhancement of ERβ selectivity.

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References and notes

1. (a) Kuiper, G. G. J. M.; VandenBemd, G. J. C. M.; Van Leeuwen, J. P. T. M. *J. Endocrinol. Invest.* **1999**, *22*, 594; (b) Ritzen, E. M.; Nilsson, O.; Grigelioniene, G.; Holst, M.; Savendahl, L.; Wroblewski, J. J. *Steroid Biochem. Mol. Biol.* **2000**, *74*, 383.

2. Harris, H. A.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Endocrinology* **2002**, *143*, 4172.
3. Kuiper, G. G.; Enmark, E.; Pelto-Huikko, M.; Nilsson, S.; Gustafsson, J. A. *Proc. Nat. Acad. Sci. U.S.A.* **1996**, *93*, 5925.
4. (a) Meyers, M. J.; Sun, J.; Carlson, K. E.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **1999**, *42*, 2456; (b) Meyers, M. J.; Sun, J.; Carlson, K. E.; Gwendolyn, M. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2001**, *44*, 4230; (c) Schopfer, U.; Schoeffter, P.; Bischoff, S. F.; Nozulak, J.; Feuerbach, D.; Floersheim, P. J. *J. Med. Chem.* **2002**, *45*, 1399.
5. 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.; Henderson, R. A.; Zhu, Y.; Keith, J. C., Jr. *Endocrinology* **2003**, *144*, 4241.
6. Pike, A. C. W.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A.; Engstrom, O.; Ljunggren, J.; Gustafsson, J.; Carlquist, M. *EMBO J.* **1999**, *18*, 4608.
7. (a) Kozerski, L.; Kamienski, B.; Kawecki, R.; Urbanczyk-Lipowska, Z.; Bocian, W.; Bednarek, E.; Sitkowski, J.; Zakrzewska, K.; Nielsen, K. T.; Hansen, P. E. *Org. Biomol. Chem.* **2003**, *1*, 3578; (b) Nishiyama, T.; Ogura, K.; Nakano, H.; Kaku, T.; Takahashi, E.; Ohkubo, Y.; Sekine, K.; Hiratsuka, A.; Kadato, S.; Watabe, T. *Drug Metabol. Pharmacokinet.* **2002**, *3*, 221.
8. (a) Ferte, J.; Kuhnel, J. M.; Chapuis, G.; Rolland, Y.; Lewin, G.; Scwaller, M. A. *J. Med. Chem.* **1999**, *42*, 478; (b) Michalak, K.; Hendrich, A. B.; Wesolowska, O.; Pola, A. *Cell Biol. Mol. Lett.* **2001**, *6*, 362.
9. Manas, E. S. et al., submitted for publication.
10. (a) Edsall, R. J.; Harris, H. A.; Manas, E. S.; Mewshaw, R. E. *Bioorg. Med. Chem.* **2003**, *11*, 3457; (b) Yang, C.; Edsall, R.; Harris, H. A.; Zhang, X.; Manas, E. S.; Mewshaw, R. E. *Bioorg. Med. Chem.* **2004**, *12*, 2553.
11. Evano, G.; Schaus, J. V.; Panek, J. S. *Org. Lett.* **2004**, *6*, 525.
12. The binding assay is described in: Harris, H. A.; Bapat, A. R.; Gonder, D. S.; Frail, D. F. *Steroids* **2002**, *67*, 379.