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Triazolo-tetrahydrofluorenones as selective estrogen receptor beta agonists

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Abstract—Several tetrahydrofluorenones with a triazole fused across C7–C8 showed high levels of ERβ-selectivity and were found to be potent ERβ-agonists. As a class they demonstrate improved oral bioavailability in the rat over a parent class of 7-hydroxy-tetrahydrofluorenones. The most selective agonist displayed 5.7 nM affinity and 333-fold selectivity for ERβ.

The diminishing levels of circulating estrogen that occur during menopause leads to a variety of physiologic changes including hot flashes, bone loss, and cognitive impairment.[1] To relieve these symptoms, many women have turned to hormone (estrogen) replacement therapy (HRT) despite an increased risk of breast and uterine cancer as well as coronary artery disease and stroke.[2] The natural ligand, 17β-estradiol, for the two known estrogen receptors, termed ERα and ERβ, binds with equal affinity to each. However, in both females and males, the two receptors are expressed in differing amounts throughout the body. The differing tissue distribution of the receptors suggests differing roles for ERα and ERβ.[3,4] The potential that subtype-selective compounds might lead to new therapies, as well as to further the understanding of estrogen biology, has lead a number of groups to pursue ERα-selective[5] and ERβ-selective agents.[6–16]

We have previously reported that compounds derived from the phenolic tetrahydrofluorenone framework (**I**), as exemplified by compound **1**, are subtype-selective agonists of ERβ.[17] While many compounds of this class performed well in vitro in receptor binding and cell-based transactivation assays, as a class they displayed poor pharmacokinetic properties characterized by rapid clearance and low oral bioavailability. A likely explanation for the observed poor pharmacokinetics is first pass glucuronidation of the C7-phenol followed by rapid elimination. One approach to address this liability has been to replace the 7-hydroxy functionality with heterocycles which might serve as phenol bioisosteres.[18] Recently, we have described tetrahydrofluorenones upon which a pyrazole ring is fused at C7 and C8 (**II**).[19] In this report, we extend these efforts to the analogous triazolo-tetrahydrofluorenones, (**III**)[20] (See Fig. 1).

Based on our results with phenolic analogs, we focused our synthetic efforts on triazolo-tetrahydrofluorenones

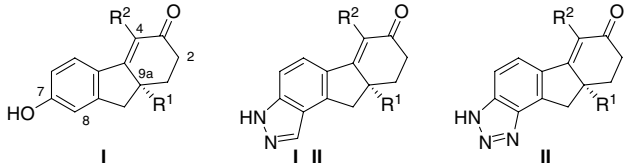

Figure 1. ERβ ligands based on tetrahydrofluorenones.

Keywords: Tetrahydrofluorenones; SERMs; ERβ-agonists; Bioisosteres.
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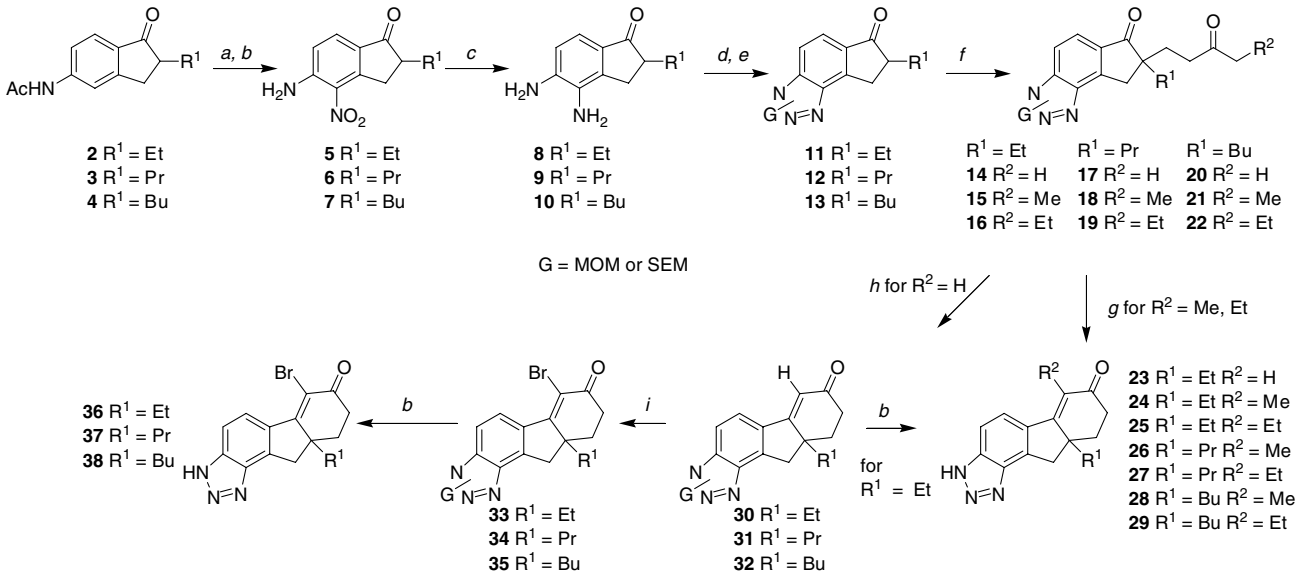


Scheme 1. Reagents and conditions: (a) HNO_3, 0 °C, ~20% for desired regioisomer; (b) 6 N HCl, MeOH, 80 °C, quant.; (c) H_2, 10% Pd/C, EtOH/EtOAc, rt, ~90%; (d) aq $NaNO_2$, EtOH, HCl, 0 °C, ~95%; (e) NaH, MOMCl or SEMCl, DMF, rt, ~85%; (f) DBU, methyl, ethyl or propyl vinyl ketone, THF, rt, −55 °C, ~90%; (g) 6 N HCl, AcOH, 100 °C, ~75%; (h) pyrrolidine, AcOH, $PhCH_3$, 100 °C, ~75%; (i) Br_2, $NaHCO_3$, CCl_4, 0 °C, 25–70%.

to analogs where R^1 = Et, Pr, or Bu and limited our exploration of R^2 to substituents with known effects in the phenolic series. Several synthetic approaches to the preparation of the requisite triazolo-tetrahydrofluorenone were investigated. However, the majority of derivatives described here were prepared by a route which features early introduction of the triazole ring (Scheme 1). This route begins with alkylated 5-acetamido-indanones **2**–**4** which were prepared in one step by reductive alkylation from a commercial material.[19] Nitrations of **2**–**4** were not selective, and the desired 4-nitro regioisomers were isolated chromatographically.[21] The acetyl group was then removed under acidic conditions to give **5**–**7**, respectively. Hydrogenation of the nitro group gave **8**–**10**. Treatment with sodium nitrite in aqueous acid gave the triazolo-indanones which were protected with either MOMCl or SEMCl to give **11**–**13** each as a mixture of N-regioisomers. Michael reaction with the appropriate alkyl vinyl ketone (methyl, ethyl or propyl) followed. The Robinson annulation was completed under different conditions for compounds where R^2 = H than for compounds where R^2 = Me or Et. Where R^2 = Me or Et (**15**, **16**, **18**, **19**, **21**, and **22**), the aldol condensation was best accomplished under strongly acidic conditions which also removed the protecting group giving triazolo-tetrahydrofluorenones **24**–**29**. Where R^2 = H (**14**, **17**, and **20**), the aldol-type condensation could be carried out under milder conditions providing triazolo-tetrahydrofluorenones **30**–**32** with the protection intact. These three intermediates were further manipulated to give additional test compounds. The protio compound **30** was deprotected to give **23**. Bromination of compounds **30**–**32** gave **33**–**35** which were deprotected to give R^2-bromo analogs **36**–**38**.

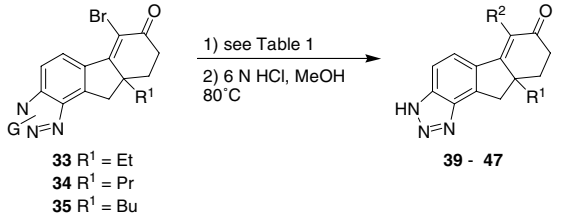
Additional R^2-variation was accomplished in good yields by appropriate coupling reactions (Table 1). Compounds **39** and **40** were prepared by a known trifluoromethylation reaction.[22] The remaining compounds (**41**–**47**) were prepared by palladium-catalyzed couplings in toluene at 100 °C.

Chiral HPLC techniques allowed the resolution of many of these triazolo-tetrahydrofluorenones into pairs of enantiomers, generally as the final step.[24]

In order to improve on the unfavorable nitration selectivity observed with our initial procedures, subsequent studies employed a different approach which is summarized in Scheme 2 for the preparation of **29**. This strategy begins by Robinson annulation of **4** to provide tetrahydrofluorenone **48**. Bromination of **48** proceeded regioselectively at the C6-position (in approx. 85:15 vs C8) thereby blocking this position in the subsequent nitration step. A mild nitration using tetrabromo-4-methyl-4-nitrocyclohexadienone provided **49**.[25] The bromo protection was removed and the nitro reduced to amine in one pot by hydrogenolysis. The resulting diamino compound **50** was resolved into enantiomers by chiral HPLC. The synthesis was completed for each enantiomer by treatment with sodium nitrite to give **29**. This strategy worked well for compound **29** and was also used for the preparation of **25**, both where R^2 = Et.[26]

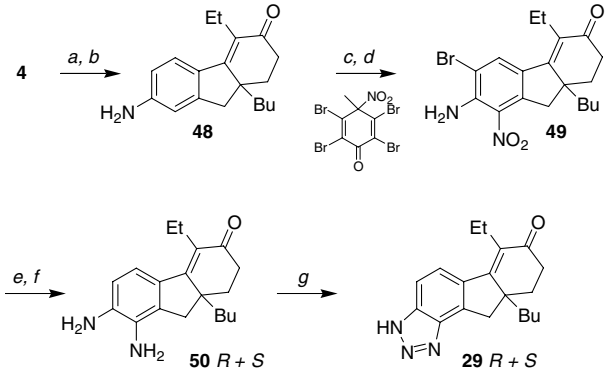
All of the triazolo-tetrahydrofluorenone compounds described above were tested in a primary binding assay to measure affinity for the estrogen receptors.[27] Additionally, most were evaluated in a cell-based transactivation assay utilizing HEK 293 cells which were stably cotransfected with human ERα or ERβ and an alkaline phosphatase reporter gene.[28] The transcriptional activity of triazolo-tetrahydrofluorenone analogs was

Table 1. Conditions for C4 substituted compounds



Entry	R^1	R^2	Conditions	Product
1	Et	CF_3	CuI, MFSDA, *i*-Pr_2NEt, DMF, 80 °C[a]	**39**
2	Bu	CF_3		**40**
3	Et	Pr	(a) $AllylSnBu_3$, $PdCl_2(PPh_3)_2$; (b) H_2, 10% Pd/C EtOH	**41**
4	Pr	Pr		**42**
5	Bu	Pr		**43**
6	Et	4-HOC_6H_4	$MOMOC_6H_4SnBu_3$, 0.05 $Pd(PPh_3)_4$	**44**
7	Et	*n*-Bu	*n*-Bu-stannatrane,[b] $Pd(PPh_3)_4$	**45**
8	Bu	2-Furyl	2-$FurylSnBu_3$, LiCl, $PdCl_2(PPh_3)_2$	**46**
9	Bu	2-Thienyl	2-$ThienylSnBu_3$, LiCl, $PdCl_2(PPh_3)_2$	**47**

[a] See Ref. 22.
[b] See Ref. 23 for *n*-Bu-stannatrane.



Scheme 2. Reagents and conditions: (a) propyl vinyl ketone, NaOMe, MeOH, 70 °C, quant.; (b) 6 N HCl, AcOH, 100 °C, 72%; (c) NBS, DMF, −35 °C, 60%; (d) nitrating reagent,[25] TFA, 0°, 61%; (e) H_2, 10% Pd/C, KOAc, EtOAc, EtOH, rt, 96%; (f) Chiralcel OJ, 25% EtOH/heptane; (g) aq $NaNO_2$, EtOH, HCl, 0 °C, 97%.

determined and compared as a percent response of 17β-estradiol. The results for these two assays are summarized in Table 2.

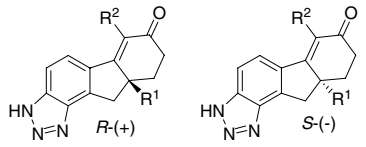
Several of the structure–activity trends previously observed in the phenolic series[17] are recapitulated in the corresponding triazoles. While R^2-substitution is required for activity, there is a narrow window of substituents which demonstrate potent ERβ-agonism. While the protio analog **23** (entry 1) is essentially inactive, methyl, ethyl, bromo, and trifluoromethyl substitution at R^2 gives analogs which are potent selective ERβ-agonists (entries 2, 3, 5, 9–12, 14, 15, 17, 19, and 20). However, certain larger substituents such as propyl (entries 6, 13, and 18), butyl (entry 7), and 4-hydroxyphenyl (entry 8) tend to diminish binding selectivity and ER-agonism. An example of the particular requirement of R^2 is the contrasting results for R^2 = 2-furyl and R^2 = 2-thienyl. While furyl analog **46** is a potent selective ERβ-agonist, the thienyl analog **47** showed diminished selectivity and only partial agonism for ERβ (entries 21 and 22). Generally, R^1-butyl analogs display the greatest combination of potency, selectivity, and ERβ-agonism. Certainly it appears the ERβ-potency trend for R^1 is Bu > Et > Pr, and selectivity Bu > Et > Pr. Several of the triazolo-tetrahydrofluorenones shown in Table 2 are single enantiomers obtained by chiral HPLC separations. As had been seen in the phenolic-tetrahydrofluorenones, one enantiomer is much more active than the other.[17,29] A comparison of *rac*-**25** to (+)-**25** and (−)-**25**, and of (+)-**29** to (−)-**29** (entries 3–5 and 16 and 17, respectively) illustrates this difference.

While generally less potent, the triazolo-tetrahydrofluorenones are more selective for ERβ than the corresponding phenolic analogs. Further, many display single-digit nanomolar ERβ affinity (entries 5, 17, 19, and 20–22). Notably, several triazole analogs are greater than 100-fold selective for ERβ (entries 5, 10, 15, 19, 20, and 21). One of the most potent, selective compounds in the binding assay (−)-**38**, which displayed 5.7 nM affinity and 333-fold selectivity for ERβ, was also the most potent, selective ERβ-agonist in the transactivation assay (entry 19).

Initial pharmacokinetic evaluation was conducted in the rat on a selection of triazolo-tetrahydrofluorenones (Table 3).[30] Generally the triazoles demonstrate modest to excellent oral bioavailability resulting in good systemic exposure albeit with relatively high plasma clearances. The analog with the best overall PK profile was the direct comparator to **1**, compound (−)-**38** where R^1 = Bu and R^2 = Br. Interestingly, while the racemic analog (±)-**29** (where R^1 = Bu and R^2 = Et) showed an encouraging profile, the individual enantiomers showed strikingly different pharmacokinetics.

The less ER-active enantiomer (+)-**29** accounts for nearly all of the desirable PK profile observed for the

Table 2. Human estrogen receptor binding and selectivity and transactivation data for triazolo-tetrahydrofluorenones



Entry	Compound	Stereo	R^1	R^2	Binding assay IC_{50} nM			Transactivation assay EC_{50} nM (% ag.)		
					hERα	hERβ	α/β	hERα	hERβ	α/β
1	**23**	*rac*	Et	H	>10,000	3041	3	1233 (10%)	475 (71%)	3
2	**24**	*rac*	Et	Me	1900	24	79	671 (62%)	20 (86%)	34
3	**25**	*rac*	Et	Et	1322	18	76	460 (88%)	7.3 (90%)	63
4	**25**	*R*-(+)	Et	Et	>10,000	1524	7	ND[a]	ND	
5	**25**	*S*-(−)	Et	Et	647	5.7	113	140 (85%)	5.8 (99%)	24
6	**41**	*rac*	Et	Pr	352	11	31	794 (76%)	14 (78%)	57
7	**45**	*rac*	Et	Bu	1127	43	26	681 (45%)	60 (39%)	11
8	**44**	*rac*	Et	HOC_6H_4	81	11	7	901 (37%)	4.3 (24%)	210
9	**36**	*rac*	Et	Br	5170	60	87	1267 (61%)	46 (90%)	28
10	**39**	*S*-(−)	Et	CF_3	1678	11	151	241 (78%)	4.9 (76%)	49
11	**26**	*rac*	Pr	Me	6173	81	76	449 (84%)	17 (89%)	26
12	**27**	*rac*	Pr	Et	612	18	35	120 (74%)	7.7 (90%)	16
13	**42**	*rac*	Pr	Pr	344	23	15	ND	ND	
14	**37**	*rac*	Pr	Br	3301	43	77	561 (93%)	18 (82%)	32
15	**28**	*rac*	Bu	Me	5982	27	218	ND	20 (96%)	
16	**29**	*R*-(+)	Bu	Et	4753	242	20	391 (29%)	28 (88%)	14
17	**29**	*S*-(−)	Bu	Et	220	5.1	43	30 (78%)	0.4 (91%)	75
18	**43**	*rac*	Bu	Pr	1249	58	22	1178 (52%)	96 (77%)	12
19	**38**	*S*-(−)	Bu	Br	1906	5.7	333	519 (80%)	5.5 (85%)	94
20	**40**	*S*[b]	Bu	CF_3	896	3.2	279	169 (81%)	2.3 (83%)	73
21	**46**	*rac*	Bu	2-Furyl	1119	7.4	151	870 (44%)	6.5 (82%)	134
22	**47**	*S*-(−)	Bu	2-Thienyl	34	2.3	15	79 (23%)	0.96 (55%)	82
23	Estradiol				1.3	1.1	1			
24	**1**	*rac*	Bu	Br	141	1.8	76	94 (68%)	4 (81%)	24

[a] ND, not determined.
[b] Rotation not obtained for this compound, but compound is a single enantiomer.

Table 3. Rat pharmacokinetic parameter for selected compounds

Compound	iv AUC (μM h)	CL_p (mL/min/kg)	$T_{1/2}$ (h)	*F* (%)
(−)-**25**	1.0	64.8	0.3	70
(−)-**39**	0.6	108	0.4	59
(±)-**29**	1.7	31.6	2.9	85
(+)-**29**	3.0	18.3	1.2	99
(−)-**29**	0.9	62	0.4	28
(−)-**38**	1.9	24.4	2.9	36
(±)-**1**	0.5	102	1.5	0

racemate. In contrast the more ERβ-active enantiomer (−)-**29** displays a much higher clearance, much lower bioavailability, and generally worse PK than the data for the racemate would suggest.[31]

In conclusion, we have prepared a series of triazolo-tetrahydrofluorenones in an effort to address the pharmacokinetic shortcomings of the corresponding phenols. Indeed, many of these triazole analogs displayed improved oral bioavailability, and in addition yielded a number of analogs with excellent and selective ERβ affinity, while retaining full ERβ-agonism. For example, compound (−)-**38** combined potent affinity for ERβ (IC_{50} = 5.7 nM) and excellent selectivity versus ERα (>300-fold) with a substantially improved PK profile over the parent compound **1**.

References and notes

1. Stearns, V.; Ullmer, L.; Lopez, J.; Smith, Y.; Isaacs, C.; Hayes, D. *Lancet* **2002**, *360*, 1851.
2. Women's Health Initiative. *JAMA* **2002**, *288*, 321.
3. Harris, H. A.; Katzenellenbogen, J. A.; Katzenellenbogen, B. S. *Endocrinology* **2002**, *143*, 4172.
4. Enmark, E.; Pelto-Huikko, M.; Grandien, K.; Lagercrantz, S.; Fried, G.; Nordenskjold, M.; Gustafsson, J.-A. *J. Clin. Endocrinol. Metab.* **1997**, *82*, 4258.
5. Here is one example: Kim, S.; Wu, J. Y.; Birzin, E. T.; Frisch, K.; Chan, W.; Pai, L. Y.; Yang, Y. T.; Mosley, R. T.; Fitzgerald, P. M. D.; Sharma, N.; Dahllund, J.; Thorsell, A.-G.; DiNinno, F. D.; Rohrer, S. A.; Hammond, M. L. *J. Med. Chem.* **2004**, *47*, 2171.
6. Meyers, M. J.; Sun, J.; Carlson, K. E.; Marriner, G. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2001**, *44*, 4230.
7. Schopfer, U.; Schoeftter, P.; Bischoff, S. F.; Nozulak, J.; Feuerbach, D.; Floersheim, P. *J. Med. Chem.* **2002**, *45*, 1399.
8. Henke, B. R.; Consler, T. G.; Go, N.; Hale, R. L.; Hohman, D. R.; Jones, S. A.; Lu, A. T.; Moore, L. B.; Moore, J. T.; Orband-Miller, L. A.; Robinett, R. G.; Shearin, J.; Spearing, P. K.; Stewart, E. L.; Turnbull, P. S.; Weaver, S. L.; Williams, S. P.; Wisely, G. B.; Lambert, M. H. *J. Med. Chem.* **2002**, *45*, 5492.
9. Yang, W.; Wang, Y.; Ma, Z.; Golla, R.; Stouch, T.; Seethala, R.; Johnson, S.; Zhou, R.; Gungor, T.; Feyen, J.

H. M.; Dickson, J. K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2327.
10. 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.; Harris, H. A. *J. Med. Chem.* **2004**, *47*, 5021.
11. Collini, M. D.; Kaufman, D. H.; Manas, E. S.; Harris, H. A.; Henderson, R. A.; Xu, Z. B.; Unwalla, R. J.; Miller, C. P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4925.
12. Vu, A. T.; Cohn, S. T.; Manas, E. S.; Harris, H. A.; Mewshaw, R. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4520.
13. Chesworth, R.; Wessel, M. D.; Heyden, L.; Mangano, M.; Zawistoski, M.; Gegnas, L.; Galluzzo, D.; Lefker, B.; Cameron, K. O.; Tickner, J.; Lu, B.; Castleberry, T. A.; Peterson, D. N.; Brault, A.; Perry, P.; Ng, O.; Owen, T. A.; Pan, L.; Ke, H. Z.; Brown, T. A.; Thompson, D. D.; DaSilva-Jardine, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5562.
14. Angelis, M. D.; Stossi, F.; Waibel, M.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *Bioorg. Med. Chem.* **2005**, *13*, 6529.
15. Blizzard, T. A.; Gude, C.; Morgan, J. D., II; Chan, W.; Birzin, E. T.; Mojena, M.; Tudela, C.; Chen, F.; Knecht, K.; Su, Q.; Kraker, B.; Mosley, R. T.; Holmes, M. A.; Sharma, N.; Fitzgerald, P. M. D.; Rohrer, S. P.; Hammond, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 834.
16. Sun, W.; Cama, L.; Birzin, E. T.; Warrier, S.; Locco, L.; Mosley, R.; Hammond, M. L.; Rohrer, S. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1468.
17. Wilkening, R. R.; Ratcliffe, R. W.; Tynebor, E. C.; Wildonger, K. J.; Fried, A. K.; Hammond, M. L.; Mosley, R. T.; Fitzgerald, P. M. D.; Sharma, N.; McKeever, B. M.; Nilsson, S.; Carlquist, M.; Thorsell, A.; Locco, L.; Katz, R.; Frisch, K.; Birzin, E. T.; Wilkinson, H. A.; Mitra, S.; Cai, S.; Hayes, E. C.; Shaeffer, J. M.; Rohrer, S. P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3489.
18. Wright, J. L.; Gregory, T. F.; Kesten, S. R.; Boxer, P. A.; Serpa, K. A.; Meltze, L. T.; Wise, L. D.; Espisitia, S. A.; Konkoy, C. S.; Whittermore, E. R.; Woodward, R. M. *J. Med. Chem.* **2000**, *43*, 3408–3419.
19. Wilkening, R. R.; Ratcliffe, R. W.; Fried, A. K.; Meng, D.; Sun, W.; Colwell, L.; Lambert, S.; Greenlee, M.; Nilsson, S.; Thorsell, A.; Mojena, M.; Tudela, C.; Frisch, K.; Chan, W.; Birzin, E. T.; Rohrer, S. P.; Hammond, M. L. *Bioorg. Med. Chem. Lett.* in press.
20. Triazoles fused across C6–C7 were inactive. Additionally, C7–C8 imidazoles appear much less promising than the C7–C8 triazoles we now report. D. Parker, R. Wilkening, unpublished results.
21. The nitration gave between 1:1 and 2:1 selectivity for the undesired 6-nitro regioisomer.
22. Chen, Q.-Y.; Wu, S.-W. *J. Chem. Soc., Chem. Commun.* **1989**, 705, These conditions were modified by the addition of Hunigs base to prevent protecting group removal and subsequent triazole methylation (by methyl iodide which is generated in situ in this reaction).
23. Vedejs, E.; Haight, A. R.; Moss, W. O. *J. Am. Chem. Soc.* **1992**, *114*, 6556.
24. Most often a Chiralcel OJ column was employed using a mixture of EtOH and heptane as the eluent. Compounds (−)-**40** and (−)-**47** were prepared from (−)-**38** (via reprotection and further elaboration).
25. Lemaire, M.; Guy, A.; Roussel, J.; Guette, J.-P. *Tetrahedron* **1987**, *43*, 844.
26. However, it proved not to be a general solution. Further examination with other substrates showed that the selectivity of brominations and nitrations of 7-amino-tetrahydrofluorenones was governed by the electronic nature of R^2-substituent, often providing no selectivity or increased selectivity for the undesired regioisomer. Unfortunately in several cases the nitration selectivity opposed the bromination selectivity. Future utility of a halo-protection strategy will require a starting material with a pre-installed bromine.
27. 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 ERα and ERβ proteins, with incubation times of 3 and 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. For estradiol, the binding data reflect an average of over 100 determinations at 3 h of incubation.
28. Barkhem, T.; Carlsson, B.; Nilsson, Y.; Enmark, E.; Gustafsson, J.-A.; Nilsson, S. *Mol. Pharmacol.* **1998**, *54*, 105.
29. A crystal structure of compound **1** co-crystallized with ERβ supported the configurational assignment of the active enantiomer as *S*. (see Ref. 17). By analogy we have assigned the more active enantiomer of the triazolotetrahydrofluorenones described herein to also be *S*. The active enantiomer was found to have a negative rotation in each case determined.
30. Female Sprague–Dawley rats were dosed at 1 mpk iv ($n = 2$) and 2 mpk po ($n = 3$) using a 1.0 mg/mL solution of compound dissolved in EtOH/PEG/H_2O (2:3:5). Plasma samples were mixed with acetonitrile, centrifuged, and analyzed by LC–MS/MS on an Applied Biosystems MDS SCIEX API tandem mass spectrometer/HPLC system.
31. The difference between the PK of the individual enantiomers of **29** was much more dramatic than we expected based on results for a closely related pyrazole analog (Ref. 19). While many other racemic analogs were examined for rat pharmacokinetics, given the significant difference in the PK profiles for the individual enantiomers, we have not included data for these analogs.