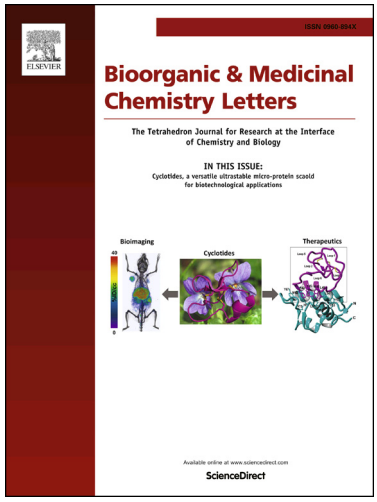

Design, synthesis, and evaluation of simple phenol amides as ERRγ agonists

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ABSTRACT

Herein we report the design and synthesis of a series of simple phenol amide ERRγ agonists based on a hydrazone lead molecule. Our structure activity relationship studies in this series revealed the phenol portion of the molecule to be required for activity. Attempts to replace the hydrazone with more suitable chemotypes led to a simple amide as a viable alternative. Differential hydrogen-deuterium exchange experiments were used to help understand the structural basis for binding to ERRγ and aid in the development of more potent ligands

The nuclear receptor (NR) superfamily of ligand regulated transcription factors has proven to be a rich source of targets for the development of therapeutics for a wide range of human diseases. The NR3B subfamily known as the estrogen-related receptor (ERRα[NR3B1], ERRβ[NR3B2] and ERRγ[NR3B3]) regulate several physiological processes, including mitochondrial function, glucose and lipid metabolism, and muscle fiber type determination.[1] The ERR's are constitutively active orphan nuclear receptors, and while ERRα and ERRβ are more ubiquitously expressed,[1-3] ERRγ is more restricted to metabolically active and highly vascularized tissues such as heart, kidney, brain and skeletal muscle.[4,5] ERRγ$^{-/-}$ mice fail to thrive shortly after birth due to abnormal heart and spinal cord development, but haploinsufficient ERRγ$^{+/-}$ mice are viable and phenotypically normal in the absence of stress. ERRγ$^{+/-}$ mice exhibit decreased exercise capacity and muscle mitochondrial function compared to their WT littermates. In mice, muscle-specific forced expression of ERRγ increased oxygen consumption, treadmill endurance, mitochondrial function and these animals were resistant to diet-induced weight gain.[6] Interestingly, repression of ERRγ expression in db/db mice ameliorated hyperglycemia via inhibition of hepatic gluconeogenesis. Synthetic modulators of ERRγ including agonists, antagonists or inverse agonists may hold utility in the treatment of a myriad of human disorders, including obesity and type-2 diabetes, cardiovascular disease and muscle atrophy.[7]

The *in vivo* functions of ERRγ using genetically engineered mice has provided valuable insight into the *in vivo* role of ERRγ in the context of overexpression or depletion of the receptor; however, pharmacological modulation of ERRγ using selective small molecule chemical probes would complement and validate these data in a more translational context.

Although endogenous ligands for the ER's have shown no activity at ERRs, some synthetic ligands have demonstrated activity towards both ER's and ERRs. Diethylstilbesterol, a synthetic ER agonist, has been demonstrated to function as an inverse agonist for all three ERRs.[8] 4-hydroxytamoxifen, a synthetic ER antagonist, functions as an inverse agonist of ERRβ and ERRγ, but displays no activity at ERRα.[9,10] GSK5182 functions as a dual ERRγ /ERα inverse agonist.[11,12] A few ERR selective ligands have also been identified: XCT790[13] is an ERRα selective inverse agonist and GSK4716[14] an ERRβ/γ selective agonist. Given the receptors specific tissue distribution and important physiological role, the identification of ERRγ-selective small molecules would be valuable as chemical probes and pharmacological tools.

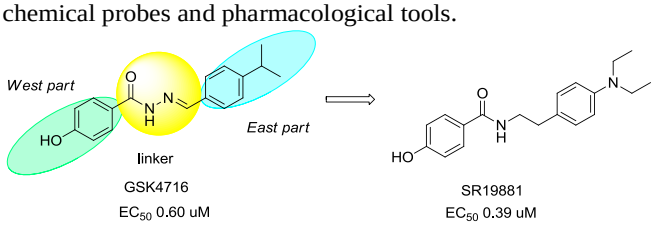
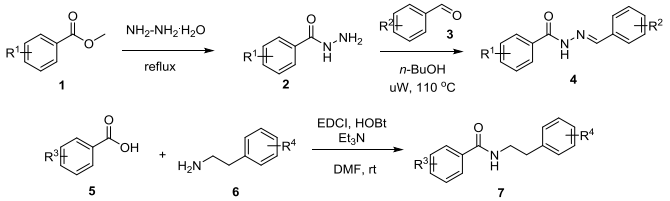


Figure 1

In the search for ERRγ agonists as *in vivo* probes, GSK4716 was the only identified agonist reported in the primary literature (Figure 1). It was discovered via a combination of diversity screening and structure guided array synthesis. GSK4716 is >50-fold selective over the classic ERs. Although, GSK4716 can substantially potentiate the transcriptional activity of ERR with moderate potency, it is far from optimal as an *in vivo* probe since it suffers from poor metabolic stability likely due to the hydrolytically unstable hydrazide moiety.[15,16] Additionally, the phenol group may be subject to phase two metabolism and

excretion. Herein we describe our efforts to date to optimize the potency and metabolic stability of this ligand by modifications to all portions of the molecule.

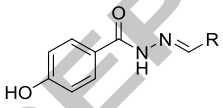
The synthesis of analogs described herein is shown in Scheme 1. Esters **1** were treated with hydrazine to give acylhydrazides **2**, which were then treated with aldehydes **3** under microwave heating to provide acylhydrazones **4**. The amide compounds **7** were simply obtained by coupling acids **5** with amines **6** in the presence of EDCI and HOBt.



Scheme 1. Synthesis of acylhydrazones and amides

Compounds were initially screened in a FRET-based peptide recruitment assay using α-HisSUMO-ERRγ-LBD, FITC-RIP140 peptide and a terbium-labelled α-HIS antibody. The FRET signal was measured by excitation at 340 nm and emission at 520 nm for fluorescein and 490 nm for terbium using a Perkin Elmer ViewLux ultra HTS microplate reader. The fold change over DMSO was calculated by the 520nm/490nm ratio. Graphs were plotted in GraphPad Prism as fold change of FRET signal for each compound over DMSO-only control. While the intent was always to find a suitable replacement for the hydrazone moiety, initial structure-activity relationship studies (SAR) began with examining both sides of the N-acylhydrazone to see what groups were tolerated (Tables 1 and 2). A couple of quick replacement analogs of the isopropylphenyl ring revealed a 7-fold boost in activity by incorporating a tert-butyl group at the para-position of the phenyl ring (SR209906). Other substitutions indicated a need for something of size at the para-position, as SR205163 was completely inactive. Naphthyl analog SR9861 was moderately potent, as was dimethylamine analog SR106447 leaving open the options for possible substitution patterns down the road.

Table 1. 4-hydroxybenzohydrazones as ERRγ agonists

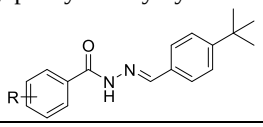


No.	R	ERRγ EC_{50} (μM)
GSK4716	4-*i*PrC_6H_4	0.60
SR209906	4-*t*-BuC_6H_4	0.084
SR205163	2-FC_6H_4	NA
SR9861	2-Naphthyl	2.0
SR106447	4-$Me_2N$$C_6H_4$	2.15 (PA)

NA = not active; PA = partial agonist; All assay results are reported as the arithmetic mean of at least two separate runs.

Given the potency enhancing effect of the 4-tert-butyl group, this substitution was incorporated into the Eastern portion of the molecule for further SAR investigations (Table 2). Removal of the phenol group (SR19822) or moving it from the para-position to the ortho-position (SR19552) or led to significant loss of activity. The meta-substituted phenol (SR19538) and the bis-hydroxy analog SR19553 were both 10 times less potent than SR209906. Methylation of the phenol (SR19537) was also not tolerated, indicating a hydrogen bond donor at the para-position was likely required for activity. A carboxyl group (SR19638) was not a suitable replacement for the phenol group. It is perhaps too bulky, or of the wrong pKa. A secondary N-methylamide (SR19541), is also perhaps too bulky and results in loss of activity. The primary amide SR19639 is slightly better, and the acetamide (SR19539) a bit more active, although both are partial agonists. Nonetheless, it was quite difficult to find a suitable replacement for the phenolic group on the benzoyl ring.

Table 2. 4-tert-Butylphenyl N-acylhydrazones

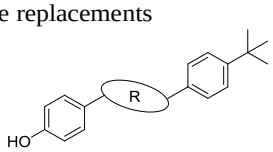


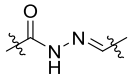
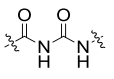
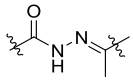
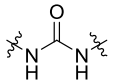
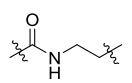
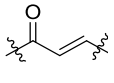
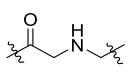
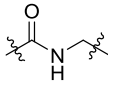
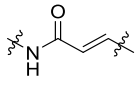
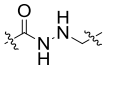
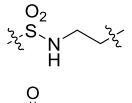
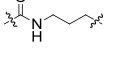
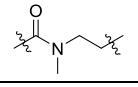
SR	R	ERRγ EC_{50} (μM)	SR	R	ERRγ EC_{50} (μM)
209906	4-OH	0.084	19537	4-OMe	NA
19822	H	7.6	19638	4-CO_2H	NA
19552	2-OH	NA	19541	4-CONHMe	NA
19538	3-OH	0.82	19639	4-$CONH_2$	6 (PA)
19553	3,4-di-OH	0.81 (PA)	19539	4-NHAc	3.1 (PA)

NA = not active; PA = partial agonist; All assay results are reported as the arithmetic mean of at least two separate runs.

The last portion of the molecule to investigate was the *N*-acyl hydrazone (Table 3). It was unclear whether the *N*-acyl hydrazone moiety was simply a linker required to hold in place the t-butyl phenyl and phenol rings, or whether it was indeed required for activity. Numerous attempts were made to replace the hydrazone with other bioisosteres of the same or similar length, but most led to substantial losses in potency. The only viable substitution was the simple phenethylamine found in SR19797. While there is a 10-fold loss in activity, we have successfully replaced the metabolically labile *N*-acyl hydrazone residue with something potentially much more tolerable.

Table 3. Hydrazone replacements

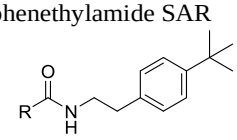


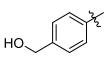
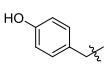
SR	R	ERRγ EC_{50} (μM)	SR	R	ERRγ EC_{50} (μM)
209906		0.084	19819		NA
19430		2.3	19820		NA
19797		0.70	19817		NA
19798		NA	19879		NA
19818		NA	19640		6.2 (PA)
20248		NA	20739		4.4
20121		3.2			

NA = not active; PA = partial agonist; All assay results are reported as the arithmetic mean of at least two separate runs.

Efforts then focused on optimizing SR19797 to build back the potency lost from replacing the hydrazone linkage (Tables 4 & 5). Similar to the SAR observed in the hydrazone series (Table 2), movement of the para-substituted phenol residue was not well tolerated. In fact, movement to the ortho-position as in SR19886 seemed to lead to a switch in pharmacology conferring ERRγ inverse agonism. The phenol residue could not be replaced by a phenyl ring (SR20010), 4-pyridyl ring (SR19889) or aniline (SR19887) without significant loss in potency. Neither aminopyridine analog (SR19888) or hydroxy-pyridine analogs (SR20123, 20253) were tolerated nor was a hydroxy-thiophene analog (SR20255). Benzyl alcohol analog SR19895 were also inactive, however its isostere SR19894 was essentially equipotent to SR19797. Attempts to replace the 4-phenol ring with various indoles were not successful (SR19890, 19891, 19892). The saturated versions of the 4-phenolic group, 4-hydroxy cyclohexane, were not viable replacements (SR20256, 20257). Finally, substituted phenols were equipotent to SR19797, when the substitutent was meta to the phenol group as in SR20011 and 20012, but 10 times less potent when substituted ortho to the phenol (SR20122, 20338). These results highlight the importance of the phenol ring hydroxy group to its activity and its resistance to replacement.

Table 4. tert-Butyl phenethylamide SAR

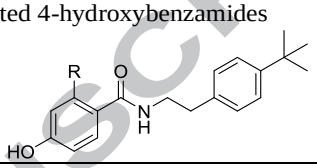


SR	R	ERRγ EC_{50} (μM)	SR	R	ERRγ EC_{50} (μM)
19797		0.70	19890		NA
19886		2.9[a]	19891		NA
20010		7.9(PA)	19892		NA
19889		NA	20256		NA
19887		9.1	20257		NA
19888		NA	20120		NA
20123		NA	20122		6.2
20253		NA	20338		7.0
20255		NA	20011		0.76
19895		NA	20012		0.95
19894		0.95			

[a] inverse agonist; NA = not active; PA = partial agonist; All assay results are reported as the arithmetic mean of at least two separate runs.

Inspection of the X-ray crystal structure of GSK-4716 with ERRγ indicates an additional hydrophobic pocket orthogonal to the binding of the molecule adjacent to the hydrazone amide group.[17] In an attempt to reach this binding pocket and take advantage of hydrophobic interactions in this region, addition analogs were prepared incorporating substitution meta to the phenol group (Table 5). Virtually all attempts at substitution including dimethylamine, acetamide, methoxy, hydroxyl, cyanide, florine, trifloromethyl, nitro, different phenyl rings, and pyridines ledd to loss of activity. The 2-aminosubstituted compound was slightly more potent as a partial agonist. Its possible SR19797 which lacks the rigid hydrazone group found in GSK4716, adopts a slightly different conformation within the receptor and R-substitution ortho to the amide group cannot reach this hydrophobic pocket.

Table 5. Substituted 4-hydroxybenzamides

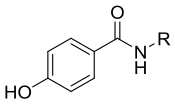


SR	R	ERRγ EC_{50} (μM)	SR	R	ERRγ EC_{50} (μM)
19797	H	0.70	20252	CN	NA
20011	CH_3	0.76	20238	NO_2	NA
20012	Cl	0.95	20244	C_6H_5	NA
20239	NH_2	0.30(PA)	20246	2-$CH_3C_6H_4$	NA
20240	Me_2N	NA	20242	3-$CH_3C_6H_4$	NA
20241	AcNH	NA	20237	4-$CH_3C_6H_4$	NA
20247	MeO	NA	20243	4-$AcNHC_6H_4$	NA
20254	OH	NA	20245	3-$AcNHC_6H_4$	NA
20339	F	8.9	20249	3-Py	NA
20340	CF_3	5.3	20250	4-Py	NA

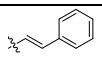
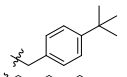
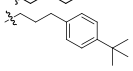
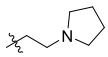
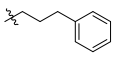
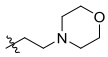
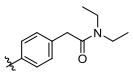
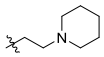
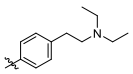
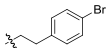
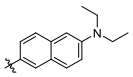
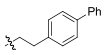
NA = not active; PA = partial agonist; All assay results are reported as the arithmetic mean of at least two separate runs.

Finding it difficult to modify the phenolic ring, we turned our attention back to the Eastern portion of the molecule (Table 6 and Table 7). Attempts to simplify the molecule by incorporating simpler amides were not tolerated (SR21580-21585). Bromine and phenyl substitutions were not viable replacements for the tert-butyl group and led to loss of activity (SR19878, 19884). The more rigid amide (SR19885) was not active although it also lacked para-substitution. Shortening the linker between the amide and phenyl ring by one carbon atom (SR19879) led to loss of activity, whereas a one carbon extension led to ~6x drop in potency (SR20739). Unexpectedly, the one carbon extension in a compound lacking the tert-butyl group was only 2-fold less potent than the lead, so there may be room for additional modifications here (SR19887). Attempts to shorten the linker between the amide nitrogen atom and the phenyl ring, at the same time increasing the length of the substituent at the para-position of the phenyl ring wasn't tolerated (SR20118, 20119). However, rigidifying the linker in the amide side chain by incorporating it into a ring led to a compound with similar potency to SR19737 (SR20043).

Table 6. Eastern Portion - Amide SAR



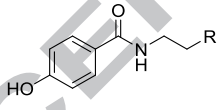
SR	R	ERRγ EC_{50} (μM)	SR	R	ERRγ EC_{50} (μM)

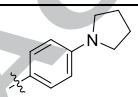
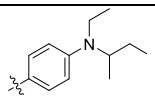
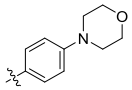
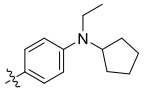
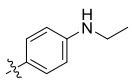
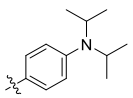
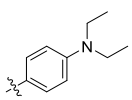
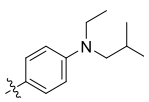
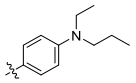
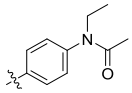
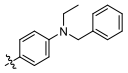
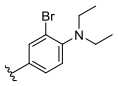
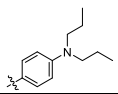
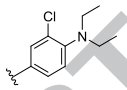
21580		NA	19885		NA
21581		NA	19879		NA
21582		NA	20739		4.4
21583		NA	19887		1.3
21584		NA	20118		NA
21585		NA	20119		NA
19878		NA	20043		1.3
19884		NA			

NA = not active; All assay results are reported as the arithmetic mean of at least two separate runs

Given that dimethylamine substitution was tolerated as in SR106447 (Table 1), we investigated hydrophobic amine substitutions as replacements for the tert-butyl group (Table 7). Cyclic amines (SR19880, 19882) or simple mono-substituted anilines (SR20037) were not active, nor was an acetylated version (SR20039). The slightly bulkier diethylamine variant (SR19881) is twice as potent as the tert-butyl varient (SR19797) and the most potent full agonist identified in our SAR studies. Increasing the size of the alkyl substituents on the nitrogen led to some analogs with modest potency (SR20038, 19883, 20042, 20007), but too big, and potency was lost (SR20043, 20044). The isobutyl ethylamine substituted analog SR20041 was very potent, but as a partial agonist. Incorporation of a halogen ortho to the para-amine group (SR20737, 20738) was not tolerated and led to a 10-fold drop in potency.

Table 7. *p*-Amino substitutions



SR	R	ERRγ EC_{50} (μM)	SR	R	ERRγ EC_{50} (μM)
19880		NA	20042		3.5
19882		NA	20043		NA
20037		NA	20044		NA
19881		0.39	20041		0.21(PA)
20038		1.14	20039		NA
19883		1.71(PA)	20737		4.3
20007		3.0	20738		5.9

NA = not active; All assay results are reported as the arithmetic mean of at least two separate runs

The lead hydrazone GSK4716 was reported to be an ERRβ/γ-selective agonist with no activity at ERRα. To confirm selectivity was maintained in this series, a few best-in-class molecules were counterscreened in a FRET-based peptide recruitment assay using either α-HisSUMO-ERRα-LBD or α-HisSUMO-ERRβ-LBD, FITC-RIP140 peptide and a terbium-labelled α-HIS antibody (data not shown). All analogs tested (GSK4716, SR19797, SR19881, SR19894, SR20041, SR20043) showed no activity vs ERRα. Activity vs ERRβ was more interesting. GSK4716 is 5-fold less potent on ERRβ than on ERRγ (ERRβ EC_{50}=3.0μM). SR19881 and SR19894 show a slight preference for ERRγ vs ERRβ (SR19881 ERRβ EC_{50}=0.63μM; SR19894 ERRβ EC_{50}=2.7μM). Other analogs showed increased selectivity (7-8-fold) for ERRβ vs ERRγ (SR19797 ERRβ EC_{50}=0.090μM; SR20239 ERRβ EC_{50}=0.045μM). Finally, SR20011 and SR20012 showed 30-40-fold selectivity for ERRβ vs ERRγ (SR20011 ERRβ EC_{50}= 0.020μM; SR20012 ERRβ EC_{50}= 0.031μM). These data may pave the way to designing truly (>100-fold) ERRγ-selective and ERRβ-selective agonists.

To further confirm pharmacology of the compounds *in vitro*, GSK4716 and SR19797 were screened in HEK293T cells in a Gal4-ERRγ::UAS-Luc reporter assay with counterscreening against Gal4-VP16::UAS-Luc. Concentration-response curves (CRCs) are shown in Figure 2. Both compounds present as agonists in this cell-based assay, but GSK4716 exhibits some non-specific activation of VP16 which might be contributing to its activity whereas SR19797 does not.

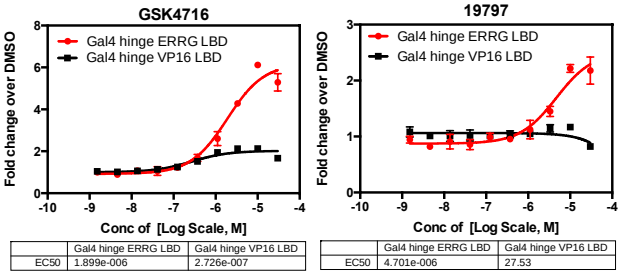
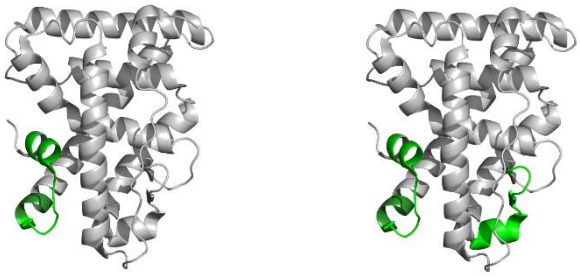


Figure 2. *In vitro* characterization of synthetic ERRγ agonist.

To better understand the structural basis of binding of lead SR19797 and the diethylamine analog SR19881 to ERRγ, we performed differential hydrogen/deuterium exchange (HDX) mass spectrometry (Figure 3) using purified ERRγ ligand binding domain (LBD). HDX data show a clear difference in structural perturbations between the apo receptor and the liganded complexs indicating that both SR19797 and SR19881 bind to the LBD. HDX revealed that helix 11 and helix 12 regions show increased protection from solvent exchange (interpreted as stabilization, in green) with both ERRγ ligands tested, suggesting common sites of interaction within the ligand-binding pocket (LBP) of ERRγ. Moreover, presence of the diethylamine substituent (H-bond acceptor) in SR19881 but not the parent compound (SR19797), showed additional protection in helix 2

and β-sheet regions indicating ancillary hydrogen bond interactions that likely contributes to its increased binding potency.

Figure 3. HDX analysis of selected ERRγ agonists



SR-19797 SR-19881

Selected compounds were tested for stability in human, rat and mouse liver microsomes (Table 8). All analogs inlcuding GSK4716 had short half-lives. Nonetheless, we have successfully replaced the acylhydrazone linkage with a saturated amide and maintained potency and comparable metabolic stability to the lead GSK4716. The phenethyl linkage is likely a metabolic soft spot in the saturated analogs. We will continue to explore substitutions here to improve stability. While not necessarily important to microsomal stability, the phenol group will likely be subject to phase 2 metabolism. Future SAR efforts will continue to search for suitable replacements for the phenol group to reduce this liability.

Table 8: Metabolic stability

Half life in 1 mg/ml heptatic microsomes

	Species ($T_{1/2}$ in minutes)		
Compound ID	Human	Mouse	Rat
GSK4716	6.4	2.3	2.2
SR20041	3.0	1.6	1.5
SR20012	2.9	1.2	1.1
SR20011	3.6	1.5	1.5
SR19881	3.1	2.2	3.9
SR19797	6.8	3.3	1.6

In summary, we have identified a series of amides as ERRγ agonists with suitable potency for further development. Our SAR studies of this chemotype identified SR19881 as the most potent full agonist of ERRγ with an EC_{50}=0.39 μM in a binding assay and an EC_{50}=4.7 μM in a cell-based assay. SR19881 was also equipotent on ERRβ with an EC_{50}=0.63 μM making it an equipotent dual agonist of ERRβ/γ. HDX studies help explain how subtle changes to the molecule translate into improved potency by increased stabilization of the receptor. These studies provided valuable information and an opportunity to expand the series in search of more potent, selective, and druglike molecules. Further investigations of new chemotypes and pharmacokinetic properties continue in an effort to explore the role of ERRγ *in vitro* and *in vivo*. These results will be reported in due course.

Acknowledgments

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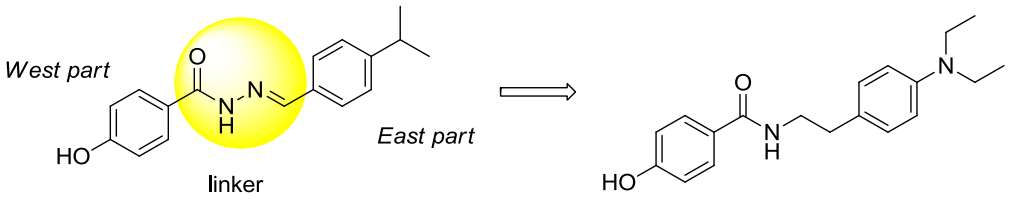
References and notes

1. Giguère, V. *Endocr. Rev.* **2008**, *29*, 677.
2. Audet-Walsh, E; Giguère, V. *Acta. Pharmacol. Sin.* **2015**, *36*, 51.
3. Huss, J. M.; Garbacz, W. G.; Xie, W. *Biochim. Biophys. Acta.* **2015**, *1852*, 1912.
4. Eudy, J.D. Yao, S.; Weston, M. D.; Ma-Edmonds, M.; Talmadge, C. B.; Cheng, J. J.; Kimberling, W. J.; Sumegi, J. *Genomics* **1998**, *50*, 382.
5. Jagannath M.; Kim, D. -K.; Choi, H.-S., *Trends Endocrin. Met.* **2017**, *28*, 261.
6. Narkar, V. A.; Fan, W.; Downes, M.; Yu, R. t.; Jonker, J. W.; Alaynick, W. A.; Banayo, E.; Karunasiri, M. S.; Lorca, S.; Evans, R. M. *Cell Metab.* **2011**, *13*, 283.
7. Yang, X.; Downes, M.; Yu, R. T.; Bookout, A. L.; He, W.; Straume, M.; Mangelsdorf, D. J.; Evans, R. M. *Cell* **2006**, *126*, 801.
8. Tremblay, G. B.; Kunath, T.; Bergeron, D.; Lapointe, L.; Champigny, C.; Bader, J-A.; Rossant, J.; Giguère, V. *Genes. Dev.* **2001**, *15*, 833.
9. Coward, P.; Lee, D.; Hull, M. V.; Lehmann, J. M. *PNAS.* **2001**, *98*, 8880.
10. Tremblay, G. B.; Bergeron, D.; Giguere, V. *Endocrinology* **2001**, *142*, 4572.
11. Chao, E. Y. H.; Collins, J. L.; Gaillard, S.; Miller, A. B.; Wang, L. P.; Orband-Miller, L. A.; Nolte, R. T.; McDonnell, D. P.; Willson, T. M.; Zuercher, W. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 821.
12. Kim, J.; Chin, J.; Im, C. Y.; Yoo, E. K.; Woo, S.; Hwang, H. J.; Cho, J-H.; Seo, K.; Song, J.; Hwang, H.; Kim, K-H.; Kim, N. D.; Yoon, S. K.; Jeon, J-H.; Yoon, S-Y.; Jeon, Y. H.; Choi, H-S.; Lee, I-K.; Kim, S. H.; Cho, S. *J. Eur. J. Med. Chem.* **2016**, *120*, 338.
13. Busch, B. B.; Stevens, W. C. Jr.; Martin, R.; Zhou, S.; Sapp, D. W.; Horlick, R. A.; Mohan, R. *J. Med. Chem.* **2004**, *47*, 5593.
14. Zuercher, W. J.; Gaillard, S.; Orband-Miller, L. A.; Chao, E. Y. H.; Shearer, B. G.; Jones, D. G.; Miller, A. B.; Collins, J. L.; McDonnell, D. P.; Willson, T. M. *J. Med. Chem.* **2005**, *48*, 3107.
15. Bhat, V. T.; Caniard, A. M.; Luksch, T.; Brenk, R.; Campopiano, D. J.; Greaney, M. F. *nat. chem.* **2010**, *2*, 490.
16. Ji, K.; Lee, C.; Janesko, B. G.; Simanek, E. E. *Mol. Pharmaceutics* **2015**, *12*, 2924.
17. Wang, L.; Zuercher, W. J.; Consler, T. G.; Lambert, M. H.; Miller, A. B.; Orband-Miller, L. A.; McKee, D. D.; Willson, T. M.; Nolte, R. T. *J. Bio. Chem.* **2006**, *281*, 37773.



GSK4716
EC_{50} 0.60 uM

SR19881
EC_{50} 0.39 uM