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Synthesis of the PPARβ/δ-selective agonist GW501516 and C4-thiazole-substituted analogs

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Abstract—Sequential, position-selective, Pd-catalyzed cross-coupling reactions of 2,4-dibromo-5-hydroxymethylthiazole provided the scaffold for the synthesis of GW501516, the most potent PPARβ/δ agonist yet described, and equally selective analogs at the thiazole-C4 position.

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily.[1] Upon activation by a ligand, these proteins act as transcription factors, regulating multiple physiological pathways, including reproduction, growth, differentiation, development, energy metabolism, and homeostasis.[1b,c] The PPAR subfamily comprises three subtypes (α, γ, and β/δ) that exhibit different tissue distribution and physiological functions, serving as dietary lipid sensors for the control of fatty acid and carbohydrate metabolism.[2] PPARα is highly enriched in the liver and, upon binding its ligands, such as the fibrates, modulates lipid metabolism. PPARγ is mostly expressed in adipose tissue and activates adipogenesis when bound to natural [(*S*)-15-deoxy-$\Delta^{12,14}$-PGJ$_2$] or synthetic (thiazolidinedione or glitazone) ligands. Together, the α and γ subtypes regulate the balance between catabolism and storage of long-chain fatty acids. Interestingly, the PPARβ/δ subtype, widely expressed in brain, colon, and skin, can be a potent transcriptional repressor,[3] inhibiting the ligand-induced transcriptional activity of the α and γ subtypes. The anti-lipid oxidation and anti-adipogenic role of PPARβ/δ holds considerable promise for the therapeutic control of obesity and type II diabetes through ligand design.[3]

Compared to the α and γ subtypes, relatively few ligands for PPARβ/δ have been described.[2] In common with the other subtypes, a variety of polyunsaturated fatty acids (arachidonic acid, linoleic acid, and eicosapentaenoic acid) and their metabolites bind PPARβ/δ at micromolar concentrations, whereas the semisynthetic carbaprostacyclin shows higher affinity.[4] Synthetic ligands have been discovered which show selectivity for the β/δ subtype, in particular those built around thiazole and oxazole rings.[5] GW501516 **1a** (Scheme 1) is the most potent ($K_i = 1.1 \pm 0.1$ nM) and selective (>1000-fold selective for PPARβ/δ over the other subtypes) PPARβ/δ agonist.[3] GW501516 also promotes reverse cholesterol transport, an effect of potential interest for the prevention of cardiovascular diseases.[3]

The synthesis of GW501516 has recently been described[6] and consists of a linear sequence in which the thiazole ring **3** is constructed by a Hantzsch-type condensation of thiobenzamide **5** and 2-chloroacetoacetate **4** (Scheme 1).

We considered an alternative synthesis that would allow the incorporation of a variety of substituents on the thiazole scaffold. To this end, dihalogenated thiazole derivatives, such as **7** or **11**, were selected, with the purpose of exploiting the differential reactivity of the

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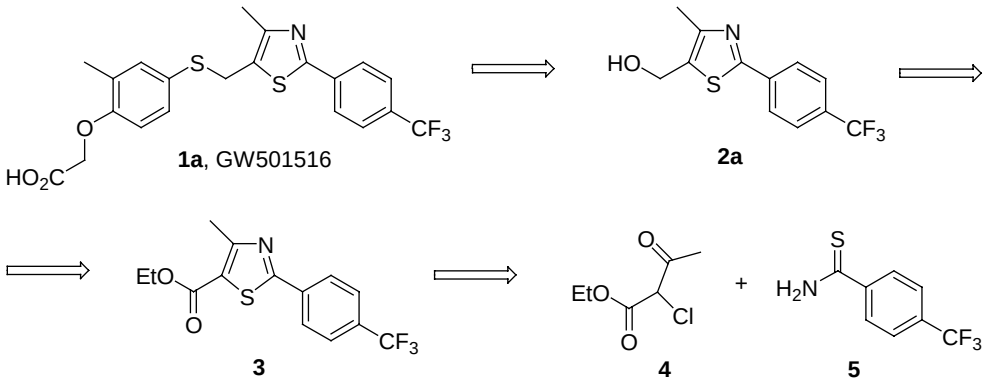


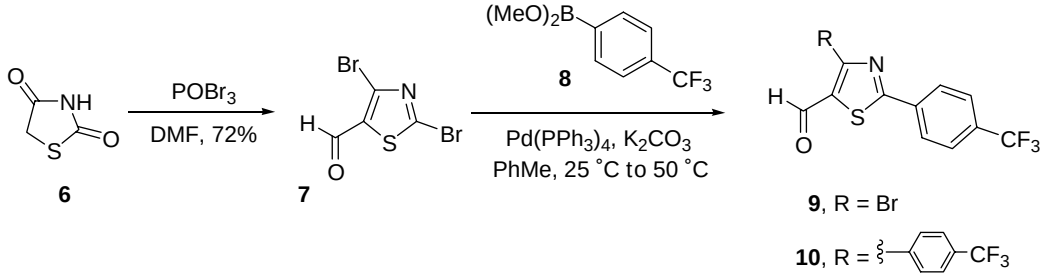
Scheme 1.

halogens using palladium-catalyzed cross-coupling reactions[7] (Schemes 2 and 3).

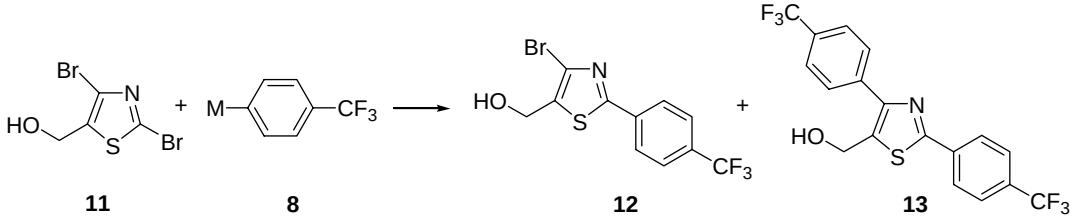
The premise that site-selective reactions of **7** or **11** are possible was based on previously reported sequential halogen replacement reactions in (un)substituted dibromoheteroarenes (thiophenes,[8,9] furans,[10] and thiazoles[11]) at the most electron-deficient position. Sequential replacement of 2,4-dibromothiazole with organozinc compounds, first at C2 and then at C4, has been described.[11e] The halogen-selective Pd-catalyzed cross-coupling reaction of 2-bromo-5-chlorothiazole-4-carboxylate has been described,[11g] but the site-selective cross-coupling of a functionalized dibromothiazole is unprecedented. We show that this approach for building functional diversity at the thiazole scaffold is indeed feasible, and describe a new total synthesis of GW501516 as well as a group of derivatives that incorporate aryl and heteroaryl substituents at C4 using commercially available organometallic compounds.

The required 2,4-dibromo-5-formylthiazole **7**, Scheme 2, can be readily prepared from commercially available thiazolidinedione **6**.[12] Given the enhanced reactivity expected at the most electron-deficient C2 position of **7**, a metal derivative **8**—prepared from commercially available 4-bromotrifluoromethylbenzene[13]—for transferring a 4-trifluoromethylphenyl group was used. However, under the Suzuki reaction conditions optimized for the selective coupling of dibromothiophenes,[8] boronate **8** [M = B(OMe)$_2$] led to mixtures of mono- (**9**) and di-substituted (**10**) derivatives together with unreacted starting material. The electron-withdrawing nature of both the formyl and trifluoromethylphenyl substituents in the thiazole ring of monosubstituted derivative **9** combines to induce a second coupling at C4 at a rate which competes with that of **7**.

Position-selective replacement of the bromine at C2 was achieved on the less electron-deficient hydroxymethyl dibromothiazole **11**,[12] using either the Stille or the Suzuki cross-coupling reactions (Scheme 3) under carefully optimized conditions.[8] The organotin derivative **8** (M = SnBu$_3$, 1.3 mol equiv) coupled at 70 °C for 24 h using Farina's catalytic system[14] [Pd$_2$(dba)$_3$/AsPh$_3$ as palladium source/ligand combination in NMP] to



Scheme 2.



Scheme 3.

provide derivative **12** in 68% yield. Increasing the reaction temperature to 90 °C led to erosion of selectivity (71:29 **12/13**). The Suzuki reaction with **8** [M = $B(OMe)_2$, 1.3 mol equiv] [$Pd(PPh_3)_4$, K_2CO_3, toluene][15] required a higher temperature (100 °C) but the selectivity was also complete (87% yield).[16]

For the second metal-catalyzed coupling, more forcing reaction conditions were required (Scheme 4, Table 1) to reach completion. The Stille reaction was optimized for coupling **12** with tetramethyltin **14a** (entry 1, 70%), tri-*n*-butyl-2-furyltin **14b** (entry 3, 70%), and tri-*n*-butylvinyltin **14c** (entry 6, 69%), whereas Suzuki cross-coupling served well for the reaction with phenylboronic acid **14d** (entry 8, 78%), to furnish the trisubstituted thiazoles **2a**–**d**, respectively. NOE experiments at this stage in derivative **2a** revealed the proximity of the methyl and methylene substituents, thus supporting the anticipated outcome of the sequential cross-coupling reactions.

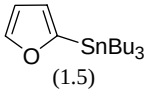
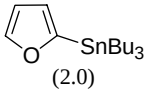
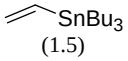
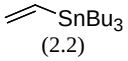
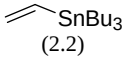
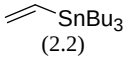
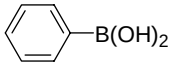
The synthesis of GW501516 and its C4-substituted analogs was completed as shown in Scheme 5, following the previously described protocol.[6b] Alcohols **2** were converted into the corresponding chlorides **15** using MsCl and Et_3N. Substitution of the chloride by aryl thiols **16**[6] and **22**[6b] (Scheme 6) using Cs_2CO_3 in CH_3CN proceeded at room temperature to give esters **17a**,[17] **17b**, **17d**, **23a**, **23b**, and **23d**. However, the vinyl derivatives **17c** and **23c** could not be isolated. Reaction of **15c** led to a complex mixture including ester **17c**, which may be present in an approximate 45% yield, as estimated by ^1H NMR, thiol **16**, or its disulfide derivative, and the product of addition of the thiol to the vinyl group, **18**. Only the addition product **18** could be isolated from this mixture in 20% yield. Its formation during the reaction course is not totally unexpected due to the known precedents for the addition of thiols onto carbon–carbon double bonds in the presence or absence of an acidic catalyst.[18]

The synthetic route was completed with the saponification of esters **17** and **23** with potassium carbonate to afford the desired targets **1a**,[17] **1b**, **1d**, **24a**, **24b**, and **24d**.

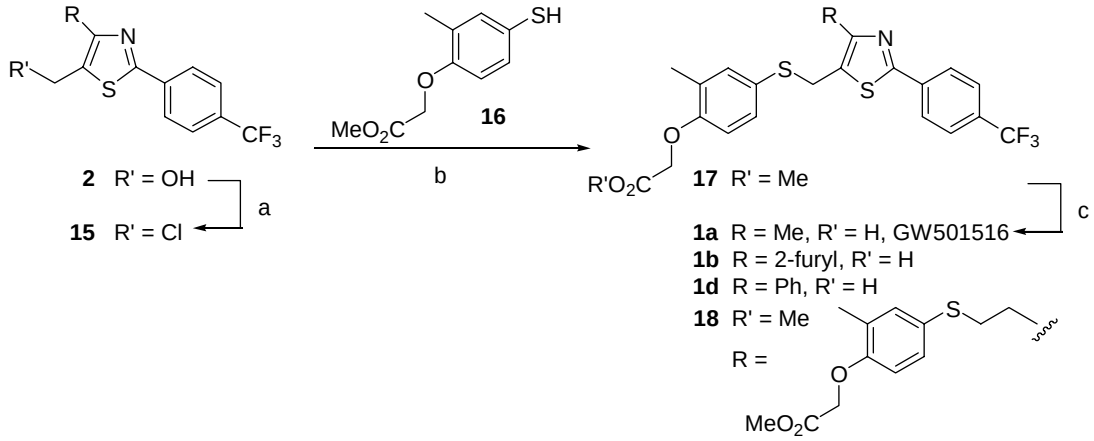
The transcriptional activity of the newly synthesized compounds has been investigated using a PPARβ/δ 'reporter' cell line and compared to the activity of the

Scheme 4.

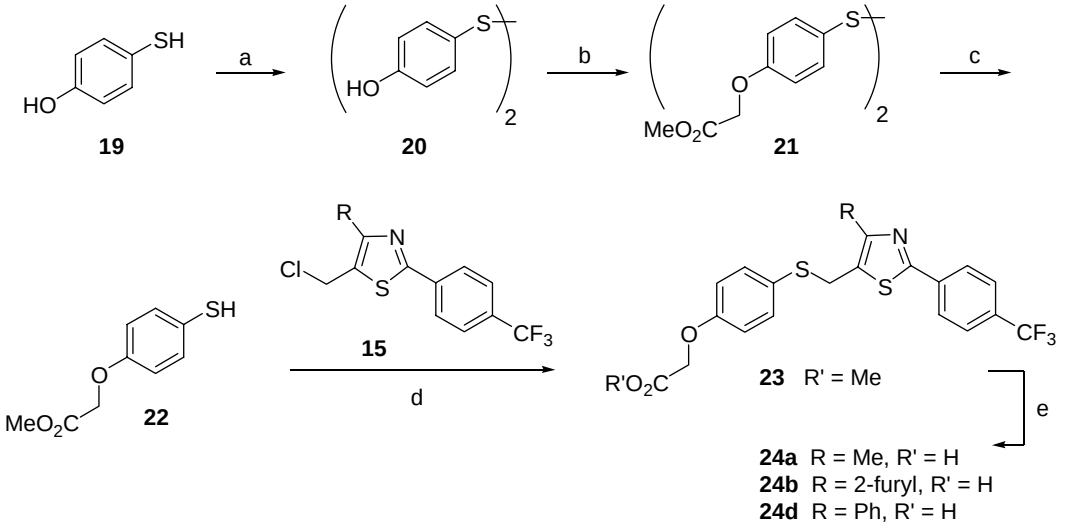
Table 1. Palladium-catalyzed cross-coupling reactions of bromothiazole **12**

Entry	R–M (mol equiv)	Reaction conditions	*T* (°C)	*t* (h)	**2** (%)	**12** (%)
1	Me_4Sn (2.0)	$PdCl_2(PPh_3)_2$ (15%), DMA	140	18	70	0
2	 (1.5)	$Pd_2(dba)_3$ (3%), $AsPh_3$ (20%), NMP	115	17	68	7
3	 (2.0)	$Pd_2(dba)_3$ (3%), $AsPh_3$ (20%), NMP	115	16	70	0
4	 (1.5)	$Pd_2(dba)_3$ (3%), $AsPh_3$ (20%), NMP	120	24	24[a]	49[a]
5	 (2.2)	$Pd_2(dba)_3$ (3%), $AsPh_3$ (20%), NMP	120	24	18[a]	52[a]
6	 (2.2)	$Pd_2(dba)_3$ (7%), $AsPh_3$ (47%), NMP	120	20	69	0
7	 (2.2)	$Pd_2(dba)_3$ (1.5%), P^tBu_3 (6%), CsF, dioxane	100	20	47	0
8	 (1.5)	$Pd(PPh_3)_4$ (5%), K_2CO_3, PhMe	115	16	78	0

[a] Yield was determined by ^1H NMR integration of the mixture.



Scheme 5. Reagents: (a) $ClSO_2Me$, Et_3N, CH_2Cl_2; (b) Cs_2CO_3, CH_3CN (**17a**, 87%; **17b**, 75%; **17d**, 87%; combined yields); (c) K_2CO_3, MeOH (**1a**, 96%; **1b**, 91%; **1d**, 92%).



Scheme 6. Reagents: (a) DMSO (93%); (b) 1—NaH, DMF, 2—$BrCH_2CO_2Me$ (82%); (c) Zn, HCl 10%, CH_2Cl_2 (84%); (d) Cs_2CO_3, CH_3CN (**23a**, 37%; **23b**, 97%; **23d**, 69%); (e) K_2CO_3, MeOH (**24a**, 97%; **24b**, 92%; **24d**, 86%).

previously identified potent agonist GW501516 **1a**. At high concentrations (10^{-7} M) all studied compounds are quite similar in their ability to activate reporter gene transcription through PPARβ/δ (Fig. 1). None of the compounds displayed any detectable activity with either PPARα or PPARγ (data not shown), demonstrating a PPARβ/δ selectivity similar to that of GW501516 **1a**. Dose–response curves with increasing ligand concentrations were performed to assess the cellular potency of the compounds to PPARβ/δ. Similar curves were obtained with GW501516 **1a** and both its derivatives **1b** and **1d**, indicating comparable transcriptional activities (Fig. 2). However, EC_{50} derived from our experimental data indicate that GW501516 **1a** is slightly more effective than both **1b** and **1d** (5×10^{-10}, 4×10^{-9}, and 2×10^{-9} M, respectively). Series 1 exhibited a higher cellular potency than series 24, because the compounds of series 24 required, in general, higher concentrations to attain the same transcriptional outcome as series 1 (see the right shift of the curves in Fig. 3 compared to Fig. 2). The EC_{50} determined for series 24 are 4×10^{-9} M for **24a**, 3×10^{-8} M for **24b**, and 9×10^{-9} M for **24d**. Thus, addition of furyl and phenyl groups does not significantly affect the potencies of GW501516 **1a** and **24a** to PPARβ/δ, whereas removal of the methyl group in series 1 (converting series 1 into series 24) provoked a loss of efficacy. Increasing the bulkiness at the thiazole C4-position by introducing a phenyl group reduced the agonist activity of the ligand.

In summary, the potent PPARβ/δ agonist GW501516 **1** and analogs have been synthesized from a trisubstituted thiazole scaffold **2** itself obtained by position-selective consecutive palladium-catalyzed cross-coupling reaction of 2,4-dibromo-5-hydroxymethylthiazole **11** and organometallic derivatives. Structural variations of the organometallic components (as shown for the second cross-coupling) add potential to the synthetic scheme and lead to a diverse range of thiazole C4-analogs built

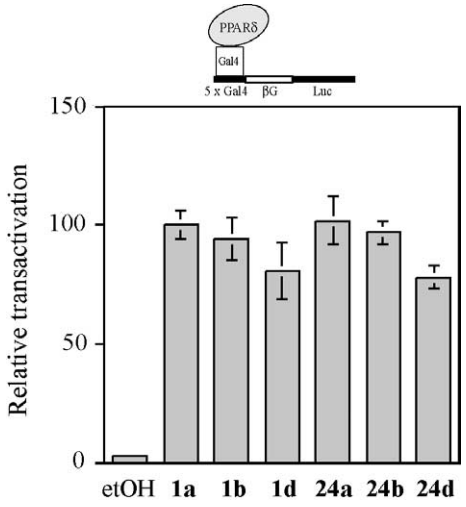


Figure 1. Transactivation studies were performed to assess PPARβ/δ activity of synthetic compounds using stably transfected HeLa cells expressing chimeric proteins containing the GAL4 DNA-binding domain fused to the ligand binding domain of PPARβ/δ and a luciferase gene driven by a pentamer of the Gal4 recognition sequence ('17 m') in front of the β-globin promoter, as illustrated at the top. This reporter system is unaffected by the presence of endogenous receptors as they cannot recognize the Gal4 binding.[19] Cells were incubated with various synthetic compounds at 0.1 μM.

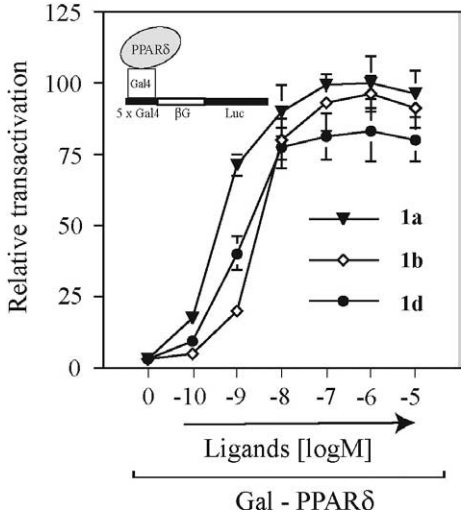


Figure 2. Transactivation studies to assess PPARβ/δ activity of compounds **1**. Gal-PPARβ/δ reporter cells were incubated with increasing concentrations of **1a** (closed triangles), **1b** (open diamonds), or **1d** (closed circles) for 16 h.

around the same scaffold that retains agonist activity and subtype selectivity. Inspired by the structural analysis of the agonist–antagonist switch of retinoids described previously,[20] an increase in the bulkiness of the added substituent could generate PPARβ/δ ligands with antagonistic activity. We are currently exploring this possibility.

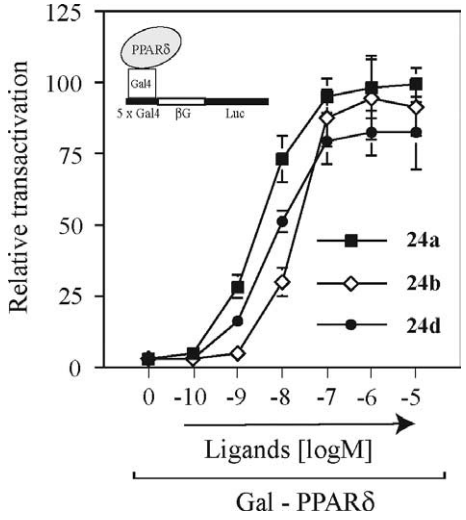


Figure 3. Transactivation studies as in Figure 2. Gal-PPARβ/δ reporter cells were incubated with increasing concentrations of **24a** (closed squares), **24b** (open diamonds), or **24d** (closed circles) for 16 h.

Acknowledgments

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