Insights into the mechanism of the site-selective sequential palladium-catalyzed cross-coupling reactions of dibromothiophenes/dibromothiazoles and arylboronic acids. Synthesis of PPARβ/δ agonists†

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A reactivity study, aided by NMR spectroscopy, allowed a mechanistic rationale to be postulated for the palladium-catalyzed regioselective coupling of arylboronic acid (and arylstannane where feasible) at the position next to the sulfur atom in functionalized dibromothiophenes and dibromothiazoles. The analysis of the NMR spectra (using ^{19}F from the boronic acid CF_3 group and ^{31}P from the phosphine of the catalyst as probes) of the entire reaction starting from the dibromoheterocycles allowed the qualitative proposal that the transmetalation is the rate-limiting step for both sequential substitution processes. The extremely facile oxidative addition at the C–Br bond next to the sulfur atom of the heterocycle instead determines the positional selectivity. An additional Stille reaction then replaced the second halogen, providing the trisubstituted heterocyclic scaffolds of PPAR ligands, which displayed PPARβ/δ agonist activity, as revealed by reporter assays in living cells.

Introduction

In recent years, there has been a growing interest in the study of site-selective palladium-catalyzed cross-coupling reactions of dihaloheteroarenes.[1–4] These halogenated heterocycles have proved to be suitable precursors for the synthesis of a plethora of carbon-substituted heterocycles with interesting biological activities.

We have recently described a new total synthesis of thiazole derivative GW501516 **1a**, a potent PPARβ/δ selective agonist, based on the palladium-catalyzed site-selective cross-coupling of a functionalized dibromothiazole and organometallics.[5] This approach serves as an attractive alternative to the classical Hantzsch reaction commonly used for thiazole formation, and facilitates the preparation of a group of derivatives with increased structural diversity by incorporating aryl and heteroaryl substituents at the thiazole C4-position (Scheme 1).

We envisioned that the synthetic methodology thus developed could be successfully applied to the preparation of some other substituted heterocyclic compounds with promising biological activity. For instance, the case of highly substituted thiophenes, which find wide application in materials science and medicinal chemistry. In fact, the thiophene analogues of GW501516, namely **3a**, **3b**,[6] **4a** and **4b** are interesting synthetic targets as potential PPARβ/δ selective agonists, an activity that has been proven for **3b**[6] (Fig. 1). Analogues **3–4** can be efficiently prepared using the same methodology, namely site-selective palladium-catalyzed cross-coupling reactions of the appropriate starting materials (**5a**, **5b**) with organometallics. We report a new total synthesis of compounds **3–4** based on site-selective palladium-catalyzed

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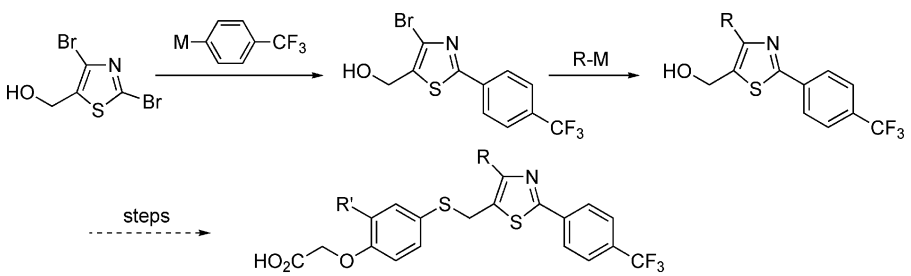
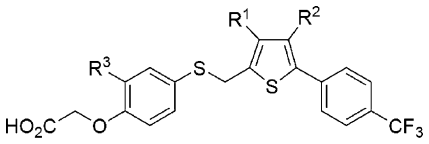
† Electronic supplementary information (ESI) available: NMR monitoring experiments, experimental procedures, spectroscopic characterization data for selected compounds, ^1H and ^{13}C NMR spectra of synthesized products. See DOI: 10.1039/b612235c



1a R = Me, R' = Me, GW501516 **2a** R = Me, R' = H
1b R = 2-furyl, R' = Me **2b** R = 2-furyl, R' = H
1c R = Ph, R' = Me **2c** R = Ph, R' = H

Scheme 1



3a R^1 = H, R^2 = Me, R^3 = Me
4a R^1 = H, R^2 = Me, R^3 = H
3b R^1 = Me, R^2 = H, R^3 = Me
4b R^1 = Me, R^2 = H, R^3 = H

Fig. 1

cross-coupling reactions with organometallic compounds, and the study of their biological activity as PPAR ligands.

From a mechanistic point of view, palladium-catalyzed cross-coupling reactions have been thoroughly studied. The complex reaction mechanism can be simplified to include three key steps: oxidative addition, transmetalation and reductive elimination (Fig. 2). In close dependence with the substrate and the reaction conditions employed for the cross-coupling process either the oxidative addition, the transmetalation or the reductive elimination have been proposed as the rate-determining step of the catalytic cycle. Site-selectivity, *i.e.* the differentiation in favour of a certain position in di- or polyhalogenated electrophiles can conceivably occur at any of these three steps. A detailed study on the regioselectivity of the palladium-catalyzed cross-coupling reactions of substituted dibromothiophenes and dibromothiazoles with organometallics and a mechanistic investigation of the site-selectivity observed in the reaction with arylboronic acids will also be presented. Our findings support that transmetalation is the rate-determining step in these coupling reactions.

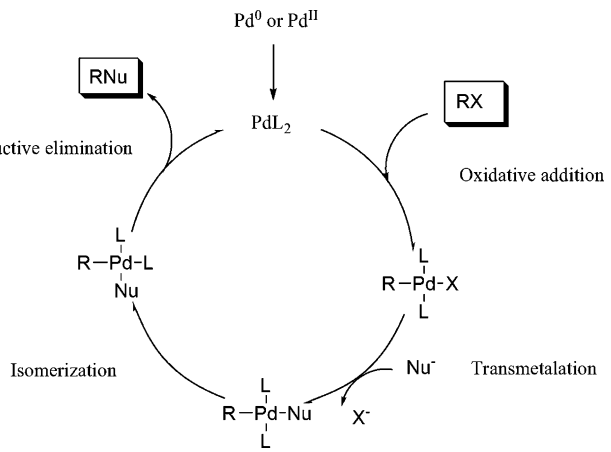


Fig. 2

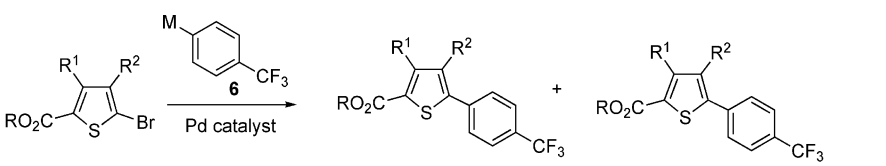
Results and discussion

Regioselectivity on the palladium catalyzed cross-coupling of 5a and 5b

With the focus on the potential PPARβ/δ targets, we selected methyl 4,5-dibromothiophene-2-carboxylate (**5a**[7]) and ethyl 3,5-dibromothiophene-2-carboxylate (**5b**,[8] Scheme 2) as substrates. These are positional isomers of dibromothiophene carboxylate which keep the ester and the bromine vicinal to the sulfur atom, but differ on the location of the second halogen. They are readily prepared from commercially available 4,5-dibromothiophene-2-carboxylic acid and 2,3,5-tribromothiophene,[9] respectively. Aimed at establishing the optimal experimental conditions for the regioselective cross-coupling reactions of substrates **5a** and **5b**, a thorough investigation of their reactions with different organometallics was carried out. Previous reports on the reactivity of dibromothiophenes have shown regioselective reactions occurring on the carbon atom close to the sulfur. The disubstituted derivative was also produced as the result of a competitive process.[2d,f,g,h,j] Whereas these precedents might anticipate position-selective replacement of the Br at C5 in **5a** by the aryl group, the presence of an electron-withdrawing substituent vicinal to the C3–Br bond in **5b** was expected to alter the reactivity at this position, making the prediction on selectivity less reliable.

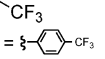
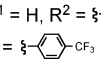
In keeping with the anticipated enhanced reactivity at C5, the first organometallic component of the sequence was chosen to be the 4-trifluoromethylphenyl metal derivative **6** *en route* to targets **3** and **4**. We surveyed the common metal derivatives **6** prepared from commercially available 4-bromotrifluoromethylbenzene.[10] The results of the palladium-catalyzed cross-coupling reactions of **5a** and **5b** with **6** are listed in Table 1. The electron-withdrawing nature of the trifluoromethyl substituent reduces the reactivity of the organometal partner **6** relative to the unsubstituted analogue, and temperatures in excess of 70 °C were required for efficient coupling.

The organotin derivative **6** (M = $SnBu_3$) coupled at 80 °C with dibromothiophene **5a** using the conditions developed by Farina[11] [$Pd_2(dba)_3$–$AsPh_3$ as palladium–ligand combination in NMP] to provide a 96 : 4 mixture of the monocoupled and bis-coupled derivatives **7a** and **8a**, respectively, in 78% overall yield (entry 1). However, use of the same reaction conditions for **5b** at 60 °C provided a disappointing 57 : 43 mixture of the mono-coupled **7b** and bis-coupled **8b** derivatives in unacceptable yield (40%), thus proving the anticipated greater reactivity of the second bromine of **5b** relative to **5a** (entry 2). Attempts at reducing the reaction temperature by employing the modification described by Fu, with the bulky P^tBu_3 [$Pd_2(dba)_3$–P^tBu_3, CsF, dioxane, 25 °C],[12] proved unrewarding (entries 3 and 4). The



5a R = Me, R^1 = H, R^2 = Br
5b R = Et, R^1 = Br, R^2 = H

7a R = Me, R^1 = H, R^2 = Br
7b R = Et, R^1 = Br, R^2 = H

8a R = Me, R^1 = H, R^2 = 
8b R = Et, R^1 =  , R^2 = H

Scheme 2

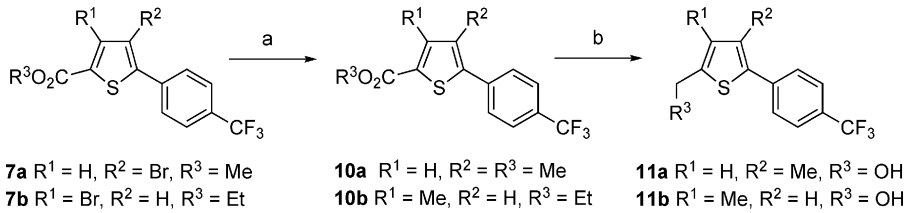
Table 1 Position-selective Pd-catalyzed cross-coupling of dibromothiophenes **5a** and **5b**

Entry	M in **6** (mol equiv.)	Substrate	Reaction conditions	T/°C	t/h	Selectivity **7** : **8**	Yield (%) **7**	**8**	**5**
1	$SnBu_3$ (1.5)	**5a**	$Pd_2(dba)_3$, $AsPh_3$, NMP	80	5	96 : 4[a]	75	3	—
2	$SnBu_3$ (1.3)	**5b**	$Pd_2(dba)_3$, $AsPh_3$, NMP	60	4	57 : 43[b]	40[c]	—	
3	$SnBu_3$ (1.3)	**5a**	$Pd_2(dba)_3$, P^tBu_3, CsF, dioxane	25 to 60	25	92 : 8[a]	44	4	52
4		**5b**				50 : 50[b]	28[c]	71	
5	ZnCl (1.6)	**5a**	$Pd(PPh_3)_4$, THF	70	21	—	—[d]	100	
6		**5b**				—	—[d]	100	
7	$B(OMe)_2$ (2)	**5a**	$Pd(PPh_3)_4$, Tl_2CO_3, THF	100	2	—	—[d]	100	
8		**5b**				—	—[e]	—	
9	$B(O^iPr)_2$ (2)	**5a**	$Pd(PPh_3)_4$, Tl_2CO_3, THF	100	2	—	—[f]	—	
10		**5b**				—	—[f]	—	
11	$B(OMe)_2$ (2)	**5a**	$Pd(PPh_3)_4$, K_2CO_3, PhMe	100	18	95 : 5[a]	82	4[g]	—
12		**5b**				75 : 25[b]	50[c,h]		18
13[i]	$B(OMe)_2$ (2)	**5a**	$Pd(PPh_3)_4$, K_2CO_3, PhMe	70	24	36 : 64[a]	31	54	—
14[i]		**5b**		100	19	70 : 30[b]	70[c,j]	—	
15[i]	$B(OMe)_2$ (1.6)	**5a**	$Pd(PPh_3)_4$, K_2CO_3, PhMe	70	25	100 : 0[a]	90	<1	—
16[i]		**5b**				78 : 22[b]	89[c]	—	
17[i]	$B(OMe)_2$ (1.2)	**5a**	$Pd_2(dba)_3$, P^tBu_3, Cs_2CO_3, dioxane	25 to 60	25	89 : 11[a]	57	7	36
18[i]		**5b**				71 : 29[b]	41[c]	59	
19[i,k]	$B(OMe)_2$ (2)	**5b**	$Pd(PPh_3)_4$, K_2CO_3, PhMe	50	72	~100 : 0[b]	90[c]	—	
					96	60 : 40[b]			
20[i]	$B(OMe)_2$ (1.2)	**5b**	$Pd(PPh_3)_4$, K_2CO_3, PhMe	50	96	100 : 0[b]	40	—	44
21[i]	$B(OMe)_2$ (1.2)	**5b**	$Pd(PPh_3)_4$, K_2CO_3, PhMe	70	24	100 : 0[b]	90	—	—

[a] Selectivity was determined after isolation of the substituted thiophenes by chromatography. [b] Selectivity was determined by ^{1}H NMR integration of the crude mixture. [c] Yield refers to the inseparable mixture of the substituted thiophenes. [d] The homocoupling product 4,4′-bistrifluoromethylbiphenyl **9** was isolated in 100% yield. [e] Mixture of the esters (Me and Et) of the starting dibromide and the substituted thiophene. [f] Unidentifiable mixture of products. [g] The homocoupling product 4,4′-bistrifluoromethylbiphenyl **9** was isolated in 20% yield. [h] The homocoupling product 4,4′-bistrifluoromethylbiphenyl **9** was isolated in 17% yield. [i] The reaction mixture was thoroughly degassed with freeze–thaw cycles (3×). [j] Trace amounts of the homocoupling product **9** were detected. [k] Reaction progress was monitored by ^{1}H NMR.

reaction appears to stop at about 30–50% conversion and seems unaffected by higher reaction temperatures (up to 60 °C). Likewise, the reaction of the organozinc derivative [$Pd(PPh_3)_4$, THF][13] or the dimethyl organoboronate under thallium carbonate rate-acceleration conditions [$Pd(PPh_3)_4$, Tl_2CO_3, THF][14] led to recovery of the starting dibromide accompanied by the homocoupling product, 4,4′-bis-trifluoromethylbiphenyl **9** (entries 5, 6 and 7), or scrambling of ester groups both in the recovered starting material and in the product (entry 8). The use of the di-isopropyl organoboronate under the same reaction conditions (entries 9 and 10) led to an intractable mixture of products. Changing the base of the Suzuki reaction to K_2CO_3 and using toluene as solvent[15] provided a better ratio (**5a** 95 : 5, entry 11; **5b** 75 : 25, entry 12) of the above reaction mixture, in particular for **5a**, but the homocoupling product **9** (entries 11 and 12) and recovered starting material (entry 12) were also isolated. Mindful of precedents showing a direct influence of oxygen in the formation of homocoupling products in Pd-catalyzed reactions,[16] the reaction flasks were thoroughly degassed. Only trace amounts of the secondary product **9** were now detected under these conditions. The disubstituted thiophene **8a** was the main product (36 : 64 ratio) when two equiv. of the boronate were employed with substrate **5a** (entry 13). Furthermore, lowering the quantities of boronate to 1.6 equivalents, and heating the degassed mixture for 25 h at 70 °C proved optimal, and a 90% yield of compound **7a**, the bromothiophene-2-carboxylate selectively substituted at the C5 position, was obtained (entry 15). However, for the positional isomer **5b**, the ratio of **7b** to **8b** did not improve even when the reaction temperature or the amounts of organoborane were lowered (entries 14 and 16). Attempts at accelerating the cross-coupling with P^tBu_3–Cs_2CO_3 faced incomplete conversion at room temperature, with no further evolution observed upon heating up to 60 °C (entries 17 and 18).[17] Interestingly, at the lowest reaction temperatures required for detectable coupling of **5b** with excess organoborane (50 °C) we observed complete selectivity albeit slow conversion (72 h). Upon extended reaction time (96 h) selectivity was lost (entry 19). This result, however, was encouraging and suggested that selectivity could be achieved through a precise control of the reaction variables. Lowering the amount of boronate to 1.2 equivalents and heating for the same time period (96 h) returned 44% of starting dibromide, although position-selectivity was maintained (entry 20). Upon further optimization, conditions were developed (entry 21) that afforded the desired product **7b** in high yield and complete position-selectivity using a slight excess of organoborane (1.2 equivalent) and heating to 70 °C for 24 h.

The positional selectivity of the cross-coupling was determined by NOE experiments on advanced intermediate **11** of the planned synthetic sequence (Scheme 3). A second Stille cross-coupling of compounds **7a** and **7b** with tetramethyltin in DMA at 90 °C afforded the trisubstituted thiophene derivatives **10a** and **10b** in 92% and 83% yield, respectively. Reduction of the esters to the alcohols using LAH furnished the thiophene carbinols **11a** and **11b** in 93% and 79% yield, respectively. The latter compound is an intermediate in the previously reported synthesis of analog **3b**.[6] NOE experiments on **11a** revealed cross-peaks at the C3-H and ArC-H signals upon irradiation of the methyl substituent, whereas **11b** showed cross-peaks at the -CH_2OH and C4-H signals on irradiating its methyl group. These experiments confirm the



Scheme 3 *Reagents and conditions*: (a) Me_4Sn, $PdCl_2(PPh_3)_2$, DMA, 90 °C, 7 h, 92% (**10a**); 15 h, 83% (**10b**); (b) $LiAlH_4$, THF, 0 °C, 2.5 h, 93% (**11a**); 2 h, 79% (**11b**).

substitution pattern depicted in structures **11a** and **11b** (and hence, that of precursors **7a** and **7b**) and discard the alternative constitutional isomer, the product of sequential cross-coupling at C4 or C3 and then at C5.

None of the conditions listed in Table 1 led to the monosubstituted thiophene isomer by coupling at C4 of **5a** or C3 of **5b**. The activating nature of the 4-trifluoromethyl group in **7** does accelerate a second coupling reaction at C4 or C3 but the bis-coupled product **8** only competes when excess organometallic reagent is present in the reaction media. The presence of an electron-withdrawing group at the neighboring position to the C3–Br bond in **5b** increases the reactivity at this position, calling for a strict control of the reaction conditions in order to achieve selectivity.

In accordance to our published results on the synthesis of the thiazole derivatives GW501516 and analogues,[5] appropriate experimental conditions for the selective synthesis of the C-5 monosubstituted thiophene derivatives **7a** and **7b** have been designed, completely avoiding the other possible monosubstituted products (at C-4 or C-3, respectively), which are not isolated or even detected.

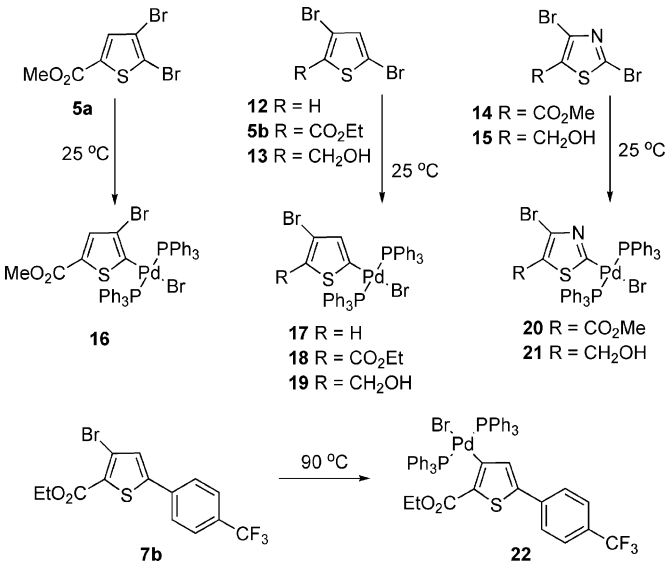
Mechanistic studies

Mechanistic studies of cross-coupling reactions using different techniques (^{31}P NMR, ^{19}F NMR, UV, cyclic voltammetry, *etc.*) have been applied to all key steps of the catalytic cycle (oxidative addition,[18] transmetalation or reductive elimination[19]) as well as to the entire process.[20]

More recently, the tools of computational chemistry have also added a new view on the metal-catalyzed reaction through the characterization of reaction profiles of the catalytic cycles, including transition structures.[21]

In order to gain insights into both the origin of the disubstituted product and the nature of the rate-determining step of the cross-coupling processes, the reactions of **5a** and **5b** were carefully monitored by NMR using the ^{31}P and ^{19}F signals for the catalyst and the organometallic reagent, respectively, as probes. In addition, since similar regioselectivity had been previously noticed for the dibromothiazole analogues,[5] the study was extended to include both types of dibromoheteroarenes with two functional groups of different electronic properties.

A great number of studies have focused on the oxidative addition of aromatic halides to palladium complexes.[18,22] Some lines of evidence suggest that, in many cases, the oxidative addition is the rate-limiting step of the cycle.[23] Strikingly, the reaction of methyl 4,5-dibromothiophene-2-carboxylate **5a** with stoichiometric quantities of $Pd(PPh_3)_4$ in toluene proceeded at ambient temperature to afford exclusively the palladium complex **16** resulting from oxidative addition on the carbon atom at position C5 (Scheme 4), which was isolated and fully characterized.[24]



Scheme 4

Dibromoheteroarenes **5b**, **12**, **13**,[25] **14** and **15**[26] were converted in a similar fashion into the C-5 (C-2 for the thiazole derivatives) palladium insertion complexes in good yields (Scheme 4).

Complexes **16**, **17**, **20** and **21** have been characterized by X-ray analysis and drawings of their molecular structures are given in Fig. 3. They all show a square-planar geometry with the heteroaryl group and the bromine atom in *trans* disposition, and two triphenylphosphine ligands completing the coordination sites. The finding that only the complexes resulting from oxidative addition on the carbon–halogen bond close to the sulfur were isolated not only corroborates the reactivity pattern experimentally observed throughout this study, but also confirms previous reports by different authors.[2,5]

In turn, preparation of the oxidative addition palladium complex **22** derived from coupling product **7b** required the reaction temperature to be raised to 90 °C. The molecular structure of **22** was also elucidated by single-crystal X-ray analysis and a plot with thermal ellipsoids is displayed in Fig. 4. The more drastic conditions employed for oxidative addition of monosubstituted thiophene **7b** further confirm the significant difference in reactivity between positions C-3 and C-5 of the dibromoheteroarene. This in turn suggests that the selectivity in the palladium catalyzed

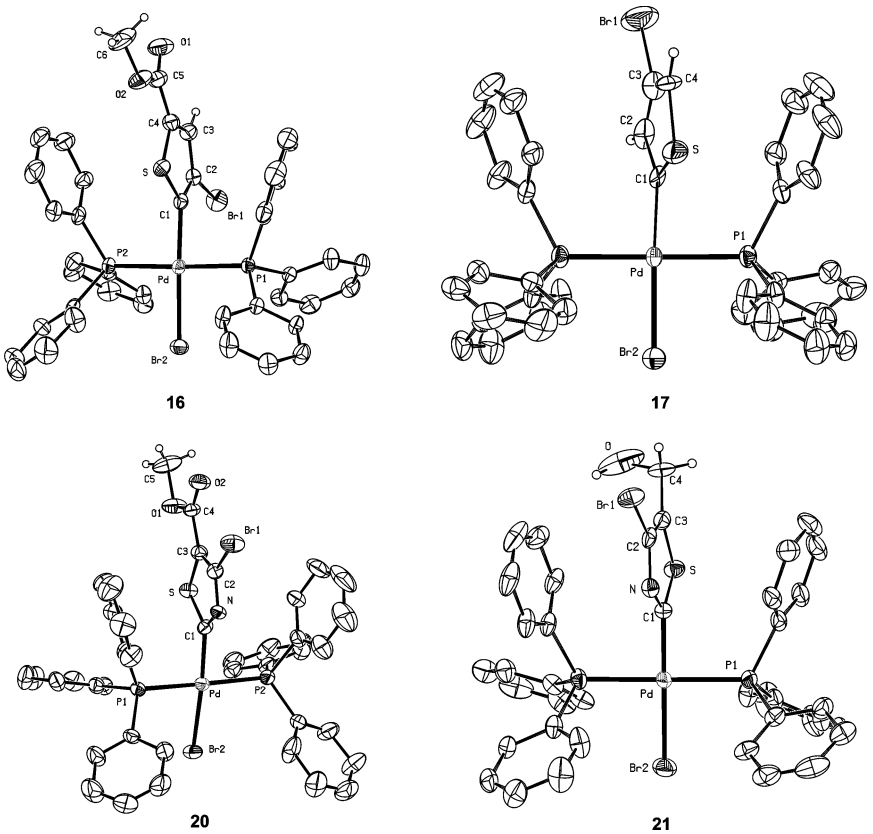


Fig. 3 Representation of molecular structures of **16**, **17**, **20** and **21**. Ellipsoids are shown at the 30% probability level. Cocrystallized solvent molecules in **16** and **20**, hydrogen atoms of the PPh_3 ligands and one set of disordered ligands in **17** and **21** have been omitted for clarity.

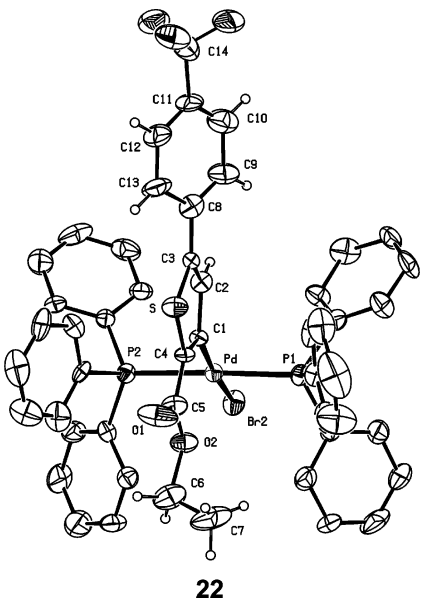


Fig. 4 Representation of molecular structure of **22**. Ellipsoids are shown at the 30% probability level. Hydrogen atoms of the PPh_3 ligands and the minor component of the disordered CF_3 group have been omitted for clarity.

cross-coupling reactions of dibromothiophene derivatives[27] is primarily due to this rate difference. To confirm this assumption, the experiment was performed at the temperatures required for the C–C bond forming process. The reaction of **5b** and boronic acid **6** was monitored by ^{31}P NMR spectroscopy using increasingly higher reaction temperatures. A similar study (not shown: Supporting Information†) was carried out for the remaining analogues depicted in Scheme 4 with comparable findings.

The oxidative addition of palladium to the C–Br bond of **5b** is complete at room temperature after just a few minutes (spectra acquisition time). The ^{31}P NMR spectrum of the mixture reveals the presence of three signals: a singlet for complex **18** at δ 23.9 ppm, a second singlet at δ − 4.2 ppm, characteristic of free triphenylphosphine, and a third peak at δ 24.2 ppm, tentatively assigned (*vide infra*) to some palladium–triphenylphospine species (Supporting Information†). Signals for other substrate–phosphorus-containing species were conspicuously absent, which appears to discard alternative oxidative additions on the C–Br bond more distant from the sulfur atom either alternatively or in addition to (double oxidative addition) that at C5.

The reaction between monobromothiophene derivative **7b** and $Pd(PPh_3)_4$ was also monitored by ^{31}P NMR. The signals can be attributed to complex **22** (singlet at δ 23.8 ppm) and the unidentified $Pd(PPh_3)_n$ species at δ 24.2 ppm (Supporting Information†). The presence of this Pd(0) species in solution starting also from a monobrominated substrate confirms that these signals resonating at δ 24.2 ppm are due to some species comprising only palladium and triphenylphosphine. On the other hand, heating to 90 °C for 3 h afforded a spectrum exhibiting a peak at δ 23.8 ppm,

which corresponds to **22**, the intensity of which is notably smaller than that attributed to the palladium species, and this leads to the conclusion that the oxidative addition process is considerably slower at C-3 than at C-5.

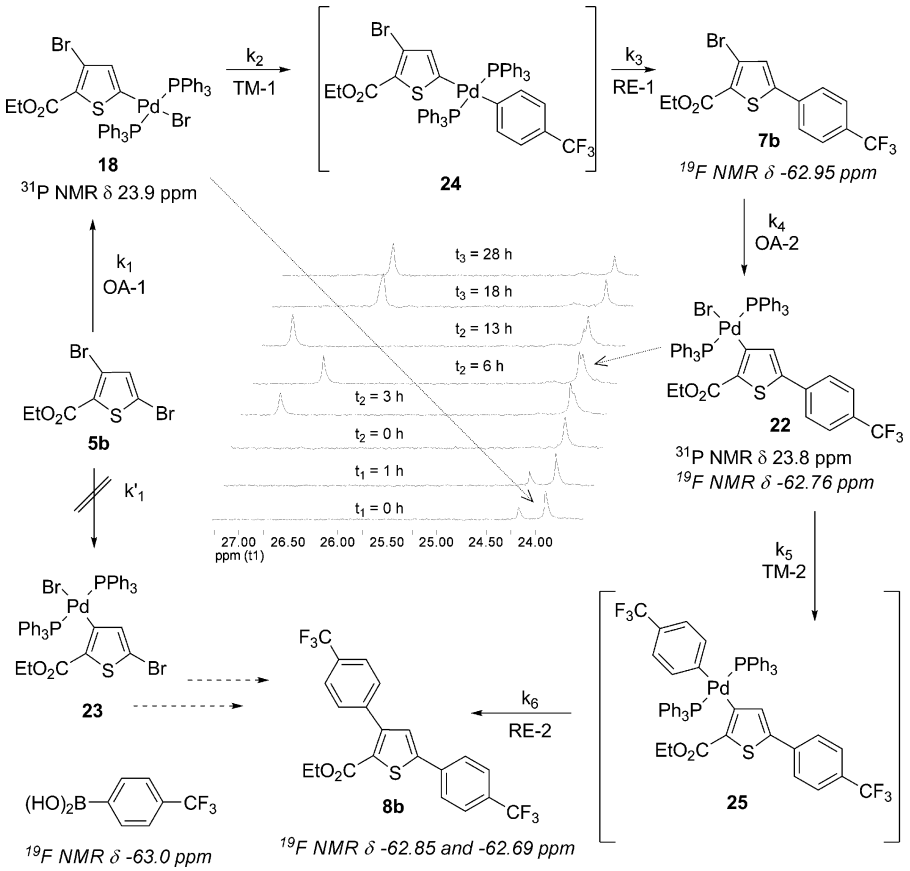
The transmetalation step of the catalytic cycle has received considerable attention, especially in the context of the Stille reaction.[28] In many cases, this has proven to be the rate-limiting step,[29] a consideration that also extends to the Suzuki reaction.[30] The characterization of the intermediate palladium complexes **18** and **22** resulting from the oxidative addition of **5b** and **7b** allowed the remaining steps of the catalytic cycle to be investigated in order to elucidate which of them is responsible for the observed selectivity and which, if different, is the rate-determining step.

Scheme 5 shows possible reaction pathways leading to the disubstituted product **8b**, with indication of chemical shifts (^{31}P NMR and ^{19}F NMR resonances) for the selected nuclei determined previously on known isolated intermediates.

Judging from the spectroscopic characterization of the products resulting from the oxidative addition step just discussed, and the absence of the alternative oxidative addition complexes **23** (OA-1, $k_{1'}$ negligible) in solution, we concluded that the disubstituted product **8b** must arise from two coupling reactions occurring in a sequential manner: first at position C-5 and then at position C-3. Monitoring the reaction by ^{31}P and ^{19}F NMR confirmed this assumption and further clarified which of the remaining steps of the cycle, transmetalation and reductive elimination, must be rate-limiting.

The reaction of the product substituted at C5 **7b** with $Pd(PPh_3)_4$ at 90 °C produces, as seen before by ^{31}P NMR, complex **22** and the $Pd(0)–(PPh_3)_n$ species. After addition of 1 mol equivalent of boronic acid (and base), the latter peak at δ 24.2 ppm disappears in favour of a broad signal at lower field, confirming the nature of these substances as Pd(0) species in equilibrium with free triphenylphosphine in the reaction medium. On the other hand, the peak corresponding to complex **22** progressively diminishes its intensity until the reaction is complete, which occurs after 15 h (Supporting Information†). The failure to detect any additional signal that could be assigned to the proposed transmetalation intermediate **25** (Scheme 5) seems to confirm that transmetalation (TM-2) is the rate-limiting step of the process.

The entire coupling process starting from ethyl 3,5-dibromothiophene-2-carboxylate **5b** and an equimolar amount of $Pd(PPh_3)_4$ at 90 °C in toluene was then monitored by NMR (^{31}P and ^{19}F). Signals corresponding to complex **18** (δ 23.9 ppm, ^{31}P NMR, Scheme 5) arose rapidly. After addition of 1 mol equivalent of *p*-trifluoromethylphenylboronic acid **6** and base (t_2), the peak at δ 24.2 ppm shifted to a downfield position, varying its location throughout the analysis in accordance to its proposed nature as equilibrating Pd(0) species. The new peak that resonates at δ 23.8 ppm increases its intensity with time (the reaction requires 13 h at 90 °C to reach completion), and corresponds to complex **22**, resulting from the oxidative addition at C-3 of the monosubstituted product **7b**. Signals for additional species corresponding to other reaction intermediates that could be



Scheme 5

accumulating were absent, thus confirming that transmetalation is likely the rate-determining step. Consequently, for the first coupling process, OA-1 and RE-1 are the rapid steps leading to monosubstituted product **7b**, and TM-1 is the slow, limiting event. Addition of the second molar equivalent of boronic acid and base (t_3) induces slow disappearance (the second coupling requires more than 15 h) of the signal corresponding to complex **22**, pointing to the transmetalation TM-2 as the rate-limiting step of the second cross-coupling as well (Scheme 5).

The entire experiment starting from dibromide **5b** was likewise monitored by ^{19}F NMR spectroscopy (Supporting Information†). ^{19}F NMR analysis of the cross-coupling process shows evidence for formation of the monosubstituted product **7b** (δ −62.95 ppm), since the peak corresponding to the boronic acid **6** (δ −63.0 ppm) diminishes in intensity, concomitantly with the oxidative addition of $Pd(PPh_3)_4$ to thiophene **7b** to provide complex **22** (δ −62.76 ppm). A second equivalent of boronic acid **6** leads to a complete transformation of **7b** into complex **22** and the latter into disubstituted product **8b** (signals at δ −62.85 ppm and δ −62.69 ppm are already present at early stages of the process, t_1 = 5 h, albeit their intensity is low). Like the experiment followed by ^{31}P NMR, monitoring the reaction by ^{19}F NMR reveals solely the oxidative addition species and corroborates that transmetalation is the limiting process for both cross-coupling reactions, with a qualitative estimate of k_2 (TM-1) slightly higher than k_5 (TM-2) judging from the reaction times required for completion.

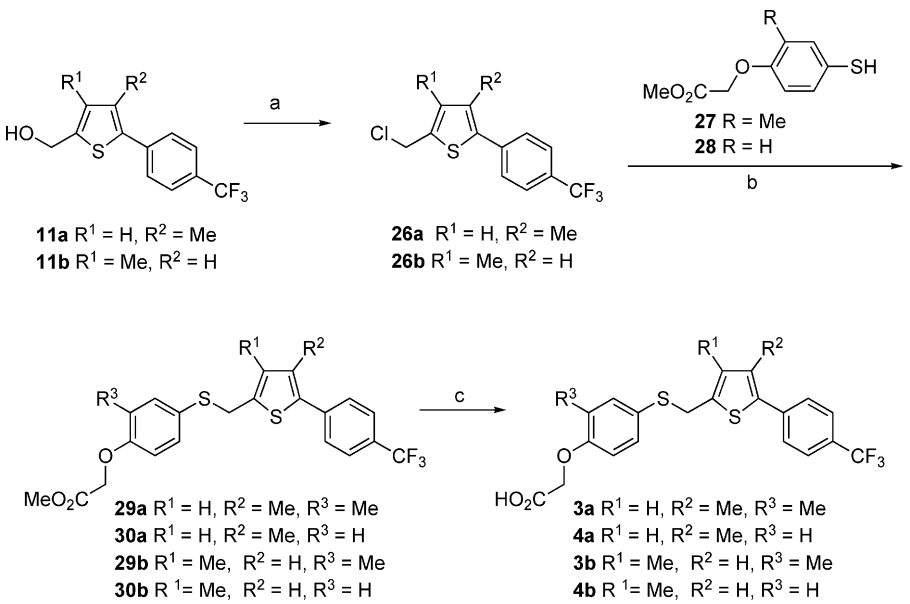
With regard to the clear preference for monosubstitution observed in the first cross-coupling reaction of all the dibromoheteroarenes under study, we interpret this as a consequence of the pronounced difference in reaction rates for the two sequential oxidative additions: k_1 (OA-1) $\gg$ k_4 (OA-2). Nevertheless, although the transmetalation is proposed to be the limiting step, the high temperature required for promoting the coupling turns the second oxidative addition (OA-2) into a competitive process. In consequence, the control of the reaction temperature and the quantities of the organometal counterpart are crucial for the selective synthesis of the monosubstituted coupling products at the position next to the sulfur atom in both (functionalized) dibromothiophenes and dibromothiazoles.

Synthesis of PPARβ/δ agonists

Compound **11b** is an intermediate in the previously reported synthesis of these analogues.[6] With the patent synthesis intercepted at this stage, the remaining steps followed the sequence depicted in Scheme 6. Alcohols **11a** and **11b** were converted into the corresponding chlorides **26a** and **26b** upon treatment with mesyl chloride in Et_3N. Aryl thiols **27**[6,31] and **28**[31] displaced the chlorides at room temperature when treated with Cs_2CO_3 in CH_3CN, and the esters were then saponified to the desired targets, **3b** and its yet undescribed analogs **3a**, **4a** and **4b**.

Biological studies

The transcriptional activity of the newly synthesized compounds has been studied using a PPARβ/δ 'reporter' cell line and compared to the activity of the previously identified potent agonist GW501516 **1a**. At 1 μM all investigated compounds (**3a**, **3b**, **4a**, **4b**) are quite similar in their ability to activate reporter gene transcription through PPARβ/δ and work as full agonists (Fig. 5). Dose–response curves with increasing ligand concentrations were performed to assess the cellular potency of the synthesized compounds to PPARβ/δ (Figs. 6 and 7). Similar curves were obtained with all four analogues (**3a**, **3b**, **4a**, **4b**), albeit all of them exhibited higher EC_{50} values than the reference compound GW501516 **1a** (5×10^{-10}, 3×10^{-7}, 9×10^{-6}, 4×10^{-7}, and 1×10^{-7} M, respectively for **1a**, **3a**, **3b**, **4a** and **4b**).



Scheme 6 *Reagents and conditions*: (a) $ClSO_2Me$, Et_3N, CH_2Cl_2, 0 °C, 2 h, then 25 °C, 16 h, 97% (**26a**); 90% (**26b**); (b) **27** or **28**, Cs_2CO_3, CH_3CN, 25 °C, 2 h, 87% (**29a**); 67% (**30a**); 77% (**29b**); 80% (**30b**); (c) K_2CO_3, MeOH, 80 °C, 1 h, 92% (**3a**); 89% (**4a**); 88% (**3b**); 91% (**4b**).

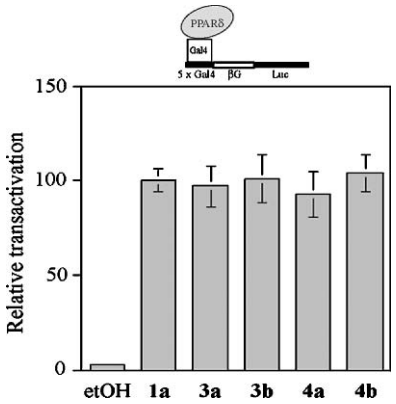


Fig. 5 Transactivation studies were performed to assess PPARβ/δ activity of synthetic compounds using stably transfected HeLa cells expressing chimeric proteins containing the GAL4 DNA-binding domain fused to the ligand binding domain of PPARβ/δ and a luciferase gene driven by a pentamer of the Gal4 recognition sequence ('17m') in front of the β-globin promoter, as illustrated at the top. This reporter system is unaffected by the presence of endogeneous receptors as they cannot recognize the Gal4 binding. Cells were incubated with various synthetic compounds at 1 μM.

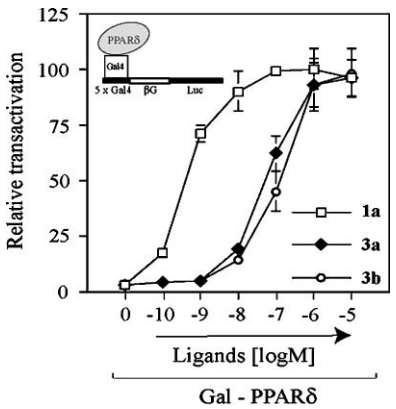


Fig. 6 Transactivation studies to assess PPARβ/δ activity of compounds **3a** and **3b**. Gal-PPARβ/δ reporter cells were incubated with increasing concentrations of **1a** (□), **3a** (♦), or **3b** (○) for 16 hours.

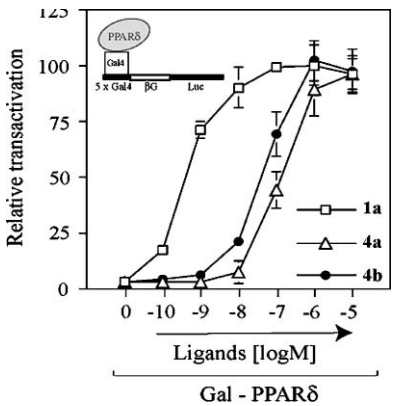


Fig. 7 Transactivation studies for compounds **4a** and **4b**. Gal-PPARβ/δ reporter cells were incubated with increasing concentrations of **1a** (□), **4a** (△), or **4b** (●) for 16 hours.

Conclusion

Control of the reaction variables, at a particular temperature and equivalents of arylstannane or arylboronic acid, allowed the regioselective (or position-selective) coupling at the position next to the sulfur atom in functionalized dibromothiophenes and dibromothiazoles. An additional Stille reaction under more drastic conditions then replaced the second halogen, providing trisubstituted heterocyclic scaffolds of interest for the diversity-oriented preparation of PPAR ligands, as shown by the synthesis of agonists **3** and **4**. Mechanistic insights were gleaned from the analysis of the NMR spectra (^{19}F and ^{31}P as probes) of the entire reaction starting from the dibromoheterocycles once the signals for the putative intermediates were previously assigned and the oxidative addition products properly characterized by X-ray diffraction. We conclude that the transmetalation is likely to be the rate-limiting event for both steps of the cycle, whereas the positional selectivity is determined by the extremely favorable oxidative addition at the C–Br bond next to the sulfur atom of the heterocycle, which then enters the transmetalation step at a rate comparable to that of the oxidative addition at the alternative C–Br bond. Using the isolated oxidative addition intermediate as starting material, it was noticed that the coupling to the boronic acid releases Pd(0) which then inserts into the alternative Pd–Br bond to complete the second palladium-catalyzed cross-coupling leading to the doubly substituted derivative.

Experimental

General

Solvents were dried according to published methods and were distilled before use. All other reagents were commercial compounds of the highest purity available. Analytical thin-layer chromatography (TLC) was performed using Merck silica gel (60 F-254) plates (0.25 mm) precoated with a fluorescent indicator. Column chromatography was performed using Merck silica gel 60 (particle size 0.040–0.063 μm). Melting points (mp) were measured on a Gallenkamp apparatus and a Stuart SMP10 apparatus and are uncorrected. Magnetic resonance spectra (NMR) were recorded on a Bruker AMX-400 [400 MHz for ^{1}H, 100 MHz for ^{13}C, 162 MHz for ^{31}P and 376 MHz for ^{19}F] Fourier transform spectrometer, and chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane (TMS, 0 ppm) or chloroform ($CHCl_3$, 7.26 ppm for ^{1}H and 77.00 ppm for ^{13}C) as internal reference, or 85% H_3PO_4 (^{31}P NMR) or $CFCl_3$ (^{19}F NMR) as external standard. ^{13}C multiplicities (s, singlet; d, doublet; t, triplet; q, quartet) were assigned with the aid of the DEPT pulse sequence. Infrared spectra (IR) were obtained on a MIDAC Prospect Model FT-IR spectrophotometer. Absorptions are recorded in wavenumbers (cm^{-1}). Low-resolution mass spectra were taken on an HP59970 instrument operating at 70 eV. High-resolution mass spectra were taken on a VG Autospec M instrument. Elemental analyses were obtained on a Carlo Erba Elemental Analyzer EA1108.

X-Ray diffraction

Crystallographic data were collected on a Bruker Smart 1000 CCD diffractometer at CACTI (Universidade de Vigo) at 20 °C using

graphite monochromated Mo Kα″ radiation (λ = 0.71073 Å), and were corrected for Lorentz and polarisation effects. The frames were integrated with the Bruker SAINT[32] software package and the data were corrected for absorption using the program SADABS.[33] The structures were solved by direct methods using the program SHELXS97.[34] All non-hydrogen atoms were refined with anisotropic thermal parameters by full-matrix least-squares calculations on F^2 using the program SHELXL97.[35] Hydrogen atoms were inserted at calculated positions and constrained with isotropic thermal parameters. Drawings were produced with PLATON.[36] Crystal data and structure refinement parameters are summarized in Table 2 (see Electronic Supplementary Information). Selected bond distances and angles are listed in Table 3 (see Electronic Supplementary Information).‡

Preparation of methyl 4-bromo-5-(4-trifluoromethylphenyl)-thiophene-2-carboxylate (7a). General procedure for Stille reactions

To a solution of $Pd_2(dba)_3$ (0.05 g, 0.05 mmol) in NMP (0.5 mL) was added, in one portion, $AsPh_3$ (0.1 g, 0.33 mmol), followed by a solution of methyl 4,5-dibromothiophene-2-carboxylate **5a** (0.5 g, 1.67 mmol) in NMP (2 mL). After stirring at 25 °C for 10 min, a solution of tributyl(4-trifluoromethylphenyl)stannane **6** (1.1 g, 2.5 mmol) in NMP (2.5 mL) was added and the resulting mixture was heated to 80 °C for 5 h. After cooling down to 25 °C, a KF saturated solution was added and the mixture was stirred for 30 min. It was then extracted with TBME (3×), the combined organic extracts were washed with H_2O (2×) and KF saturated solution, dried (Na_2SO_4) and evaporated. Purification of the residue by chromatography (SiO_2, 98 : 2 hexane–AcOEt) afforded 0.46 g (75%) of a white solid (mp 62 °C, hexane) identified as **7a** and 0.02 g (3%) of **8a** as a white solid (mp 102 °C, hexane–AcOEt). *Spectroscopic data for* **7a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (s, 3H, CO_2CH_3), 7.72 (d, J = 8.3 Hz, 2H, H3′ + H5′), 7.76 (s, 1H, H3), 7.79 (d, J = 8.3 Hz, 2H, H2′ + H6′) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.6 (q, CO_2CH_3), 109.0 (s, C4), 123.8 (s, CF_3, $^1J_{C-F}$ = 272.3 Hz), 125.7 (d, 2C, C3′ + C5′, $^3J_{C-F}$ = 3.7 Hz), 129.4 (d, 2C, C2′ + C6′), 131.1 (s, C4′, $^2J_{C-F}$ = 32.7 Hz), 133.0 (s), 135.5 (s), 137.2 (d, C3), 143.1 (s), 161.4 (s, CO_2Me) ppm. IR (NaCl) ν 2956 (w, C–H), 1724 (s, C=O), 1616 (m), 1532 (w), 1513 (m), 1448 (m), 1326 (s), 1289 (s), 1249 (s), 1169 (s), 1129 (s), 1073 (s), 1017 (m), 830 (s), 750 (m) cm^{-1}. MS m/z (%) 368 ([M + 2]$^+$ [^{81}Br], 5), 366 (M$^+$ [^{81}Br], 97), 365 ([M + 1]$^+$ [^{79}Br], 15), 364 (M$^+$ [^{79}Br], 91), 335 ([M − OMe]$^+$ [^{81}Br], 100), 333 ([M − OMe]$^+$ [^{79}Br], 95), 226 (54), 69 (22). HRMS (EI$^+$) calcd for $C_{13}H_8{}^{81}BrF_3O_2S$ 365.9360 and $C_{13}H_8{}^{79}BrF_3O_2S$ 363.9380, found 365.9358 and 363.9379. Anal. Calcd for $C_{13}H_8BrF_3O_2S$: C, 42.76; H, 2.21; S, 8.78. Found: C, 43.04; H, 2.27; S, 8.70%. *Spectroscopic data for methyl 4,5-bis(4-trifluoromethylphenyl)thiophene-2-carboxylate* (**8a**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (s, 3H, CO_2CH_3), 7.35 (d, J = 8.1 Hz, 2H, ArH), 7.40 (d, J = 8.1 Hz, 2H, ArH), 7.58 (d, J = 8.2 Hz, 4H, ArH), 7.85 (s, 1H, H3) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 52.4 (q, CO_2CH_3), 123.7 (s, CF_3, $^1J_{C-F}$ = 272.4 Hz), 123.8 (s, CF_3, $^1J_{C-F}$ = 272.4 Hz), 125.6 (d, 2C, $^3J_{C-F}$ = 3.7 Hz), 125.7 (d, 2C, $^3J_{C-F}$ = 3.7 Hz), 129.1 (d, 2C), 129.4 (d, 2C), 129.7 (s, $^2J_{C-F}$ = 33.8 Hz), 130.5 (s, $^2J_{C-F}$ = 32.6 Hz), 132.8 (s), 135.7 (d, C3), 136.3 (s), 138.1 (s), 138.4 (s), 144.2 (s), 162.1 (s, CO_2Me) ppm. IR (NaCl) ν 2956 (w, C–H), 1718 (s, C=O), 1617 (w), 1451 (w), 1326 (s), 1294 (m), 1252 (m), 1168 (m), 1124 (s), 1069 (s), 839 (m) cm^{-1}. MS m/z (%) 432 ([M + 2]$^+$, 7), 430 (M$^+$, 100), 411 (12), 400 (15), 399 ([M − OCH_3]$^+$, 71), 327 (25), 302 (15). HRMS (EI$^+$) calcd for $C_{20}H_{12}F_6O_2S$ 430.0462, found 430.0461. Anal. Calcd for $C_{20}H_{12}F_6O_2S$: C, 55.82; H, 2.81; S, 7.45. Found: C, 55.77; H, 2.78; S, 7.40%.

Preparation of 7a. General procedure for Suzuki reactions

To a cold (−78 °C) solution of 4-bromotrifluoromethylbenzene (0.05 g, 0.22 mmol) in THF (1 mL) was added dropwise *n*-BuLi (0.16 mL, 1.44 M in hexane, 0.23 mmol), and the mixture was stirred for 30 min at −78 °C. Then $B(OMe)_3$ (0.03 mL, 0.23 mmol) was added dropwise. After stirring for an additional 30 min, the mixture was allowed to warm to 25 °C. The solvent was removed and toluene (1 mL) was added. The solution was transferred to a Schlenk tube charged with methyl 4,5-dibromothiophene-2-carboxylate **5a** (0.05 g, 0.17 mmol) and $Pd(PPh_3)_4$ (0.01 g, 0.008 mmol) in toluene (0.5 mL). After addition of K_2CO_3 (0.11 mL, 3 M in H_2O, 0.33 mmol), the reaction mixture was thoroughly degassed with three freeze–thaw cycles, the Schlenk tube was sealed, placed in a 70 °C oil bath and stirred for 25 h. After cooling to room temperature, water was added and the mixture was extracted with TBME (3×). The combined organic extracts were dried (Na_2SO_4), and the solvent was evaporated. Purification of the residue by chromatography (SiO_2, 98 : 2 hexane–AcOEt) yielded 0.05 g (90%) of thiophene **7a** as a white solid (mp 62 °C, hexane).

Preparation of methyl 4-methyl-5-(4-trifluoromethylphenyl)-thiophene-2-carboxylate (10a). General procedure for the second Stille coupling

A Schlenk tube was charged with methyl 4-bromo-5-(4-trifluoromethylphenyl)thiophene-2-carboxylate (**7a**) (0.42 g, 1.16 mmol), $PdCl_2(PPh_3)_2$ (0.04 g, 0.06 mmol), $SnMe_4$ (0.41 g, 2.32 mmol) and DMA (5 mL), sealed and placed in a 90 °C oil bath. After stirring for 7 h, the reaction mixture was cooled to 25 °C. A NH_4Cl saturated aqueous solution was then added and the mixture was extracted with TBME (3×). The combined organic extracts were dried (Na_2SO_4), and the solvent was evaporated. Purification of the residue by chromatography (SiO_2, 95 : 5 hexane–AcOEt) afforded 0.32 g (92%) of methyl 4-methyl-5-(4-trifluoromethylphenyl)thiophene-2-carboxylate (**10a**) as a white solid (mp 65 °C, hexane–TBME). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32 (s, 3H, C4-CH_3), 3.89 (s, 3H, CO_2CH_3), 7.58 (d, J = 8.2 Hz, 2H, H2′ + H6′), 7.64 (s, 1H, H3), 7.69 (d, J = 8.2 Hz, 2H, H3′ + H5′) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9 (q, C4-CH_3) 52.2 (q, CO_2CH_3), 124.0 (s, CF_3, $^1J_{C-F}$ = 272.3 Hz), 125.7 (d, 2C, C3′ + C5′, $^3J_{C-F}$ = 3.7 Hz), 129.2 (d, 2C, C2′ + C6′), 130.1 (s, C4′, $^2J_{C-F}$ = 32.5 Hz), 131.5 (s), 135.1 (s), 137.0 (d, C3), 137.3 (s), 143.3 (s), 162.5 (s, CO_2Me) ppm. IR (NaCl) ν 2955 (m, C–H), 2927 (m, C–H), 2852 (w, C–H), 1717 (s, C=O), 1615 (m), 1551 (w), 1447 (s), 1325 (s), 1292 (s), 1251 (s), 1193 (s), 1168 (s), 1127 (s), 1073 (s), 1016 (m), 842 (s), 753 (m) cm^{-1}. MS m/z (%) 302 ([M + 2]$^+$, 5),

‡ CCDC reference numbers 612416–612420 for compounds **16**, **17**, **20**, **21** and **22**, respectively. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b612235c

301 ([M + 1]$^+$, 15), 300 (M$^+$, 100), 197 (39), 171 (20), 128 (28), 115 (21), 69 (17). HRMS (EI$^+$) calcd for $C_{14}H_{11}F_3O_2S$ 300.0432, found 300.0430. Anal. Calcd for $C_{14}H_{11}F_3O_2S$: C, 55.99; H, 3.70; S, 10.67. Found: C, 55.56; H, 3.64; S, 11.03%.

Preparation of [4-methyl-5-(4-trifluoromethylphenyl)thien-2-yl]methanol (11a). General procedure for the reduction of esters to alcohols

A solution of methyl ester **10a** (0.2 g, 0.67 mmol) in diethyl ether (1 mL) was added to a cold (0 °C) suspension of $LiAlH_4$ (0.03 g, 0.80 mmol) in Et_2O (1 mL). The reaction mixture was stirred at 0 °C for 2.5 h, after which time a mixture of MeOH–H_2O (90 : 10) was added, followed by a 10% NH_4Cl aqueous solution. After extraction with TBME (3×), the combined organic extracts were washed with H_2O, dried (Na_2SO_4) and evaporated. Purification of the residue by column chromatography (SiO_2, 80 : 20 hexane–AcOEt) afforded 0.17 g (93%) of a colourless oil identified as [4-methyl-5-(4-trifluoromethylphenyl)thien-2-yl]methanol (**11a**). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 (t, J = 6.0 Hz, 1H, OH), 2.30 (s, 3H, C4′-CH_3), 4.80 (d, J = 6.0 Hz, 2H, 2H1), 6.87 (s, 1H, H3′), 7.55 (d, J = 8.6 Hz, 2H, H2″ + H6″), 7.66 (d, J = 8.6 Hz, 2H, H3″ + H5″) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9 (q, C4′-CH_3), 59.9 (t, C1), 124.1 (s, CF_3, $^1J_{C-F}$ = 272.0 Hz), 125.4 (d, 2C, C3″ + C5″, $^3J_{C-F}$ = 3.8 Hz), 128.9 (d, 2C, C2″ + C6″), 129.0 (s, C4″, $^2J_{C-F}$ = 32.5 Hz), 129.7 (d, C3′), 134.1 (s), 136.1 (s), 138.3 (s), 142.9 (s) ppm. IR (NaCl) ν 3600–3000 (br, O–H), 2927 (w, C–H), 1614 (m), 1408 (w), 1326 (s), 1166 (m), 1115 (s), 1070 (m), 1015 (m), 840 (m) cm^{-1}. MS m/z (%) 274 ([M + 2]$^+$, 6), 273 ([M + 1]$^+$, 16), 272 (M$^+$, 100), 271 ([M − 1]$^+$, 15), 255 (36), 243 (51), 239 (17), 86 (12), 84 (18). HRMS (EI$^+$) calcd for $C_{13}H_{11}F_3OS$ 272.0483, found 272.0481.

General procedure for the synthesis of palladium complexes 16, 17, 18, 19, 20, 21 and 22

A mixture of $Pd(PPh_3)_4$ (1 mmol) and bromo heterocycle (1 mmol) in toluene (0.1 M) was deoxygenated and stirred overnight in a Schlenk flask under argon at room temperature for **16**, **17**, **18**, **19**, **20**, **21** and 90 °C for **22**. The resultant yellow solution or suspension was evaporated to dryness *in vacuo*. The solid residue was triturated with Et_2O and the ether solution discarded. Washing was repeated twice and the residual product purified by recrystallization from $CHCl_3$-hexane or CH_2Cl_2–hexane. Data collection and refinement parameters for X-ray structures of complexes **16**, **17**, **20**, **21** and **22** are given in Table 2 (See Electronic Supplementary Information). For the spectroscopic characterization of complexes **16–22** see also ESI.†

^{31}P NMR monitoring of the heteroarenes cross-coupling reaction

The heteroarene (1 mmol) and $Pd(PPh_3)_4$ (0.1 mmol) were added to d_8-toluene (0.75 mL) in an NMR tube under an argon atmosphere. The tube was warmed to 90 °C (0.5 h or 1 h). ^{31}P NMR data were obtained at this temperature. Then the organometallic (1 mmol) and base (2 mmol) were added, and the reaction was monitored by ^{31}P NMR at 90 °C until the organometallic reagent was consumed. New portions of organometallic (1 mmol) and base (2 mmol) were subsequently added and ^{31}P NMR spectroscopic data were collected at various reaction times.

Preparation of 5-chloromethyl-3-methyl-2-(4-trifluoromethylphenyl)thiophene (26a). General procedure for the preparation of chlorides from alcohols

Methanesulfonyl chloride (0.05 mL, 0.88 mmol) was added dropwise to the solution of [4-methyl-5-(4-trifluoromethylphenyl)thien-2-yl]methanol **11a** (0.06 g, 0.22 mmol) and Et_3N (0.12 mL, 0.88 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After 2 h at 0 °C, the mixture was stirred at 25 °C for 16 h. Then CH_2Cl_2 (3 mL) was added and the mixture was washed with saturated $NaHCO_3$ aqueous solution (2×) and water (2×). Drying (Na_2SO_4) and evaporation of the solvent afforded 0.62 g (97%) of chloride **26a** as a yellow oil, which was used in the next step without further purification, due to its instability. A small sample was purified by column chromatography (SiO_2–C_{18}, CH_3CN) for characterization purposes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.29 (s, 3H, C3-CH_3), 4.77 (s, 2H, CH_2Cl), 6.95 (s, 1H, H4). 7.55 (d, J = 8.2 Hz, 2H, H2′ + H6′), 7.66 (d, J = 8.2 Hz, 2H, H3′ + H5′) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9 (q, C3-CH_3), 40.4 (t, CH_2Cl), 124.1 (s, CF_3, $^1J_{C-F}$ = 271.7 Hz), 125.5 (d, 2C, C3′ + C5′, $^3J_{C-F}$ = 3.7 Hz), 129.0 (d, 2C, C2′ + C6′), 129.4 (s, C4′, $^2J_{C-F}$ = 32.2 Hz), 131.9 (d, C4), 134.2 (s), 137.6 (s), 137.9 (s), 138.9 (s) ppm. IR (NaCl) ν 2927 (w, C–H), 1615 (m), 1407 (w), 1325 (s), 1259 (m), 1167 (s), 1126 (s), 1069 (s), 1016 (m), 840 (s), 678 (w) cm^{-1}. MS m/z (%) 292 (M$^+$ [^{37}Cl], 7), 291 ([M + 1]$^+$ [^{35}Cl], 3), 290 (M$^+$ [^{35}Cl], 19), 257 (5), 256 (16), 255 ([M − Cl]$^+$, 100), 185 (3). HRMS (EI$^+$) calcd for $C_{13}H_{10}{}^{35}ClF_3S$ 290.0144 and $C_{13}H_{10}{}^{37}ClF_3S$ 292.0114, found 290.0149 and 292.0118.

Preparation of methyl (2-methyl-4-[4-methyl-5-(4-trifluoromethylphenyl)thien-2-ylmethylthio]phenoxy)acetate (29a). General procedure for the nucleophilic displacement of chlorides by thiols

Cs_2CO_3 (0.23 g, 0.70 mmol) was added to a solution of chloride **26a** (0.09 g, 0.32 mmol) and methyl (4-mercapto-2-methylphenoxy)acetate **27** (0.09 g, 0.42 mmol) in CH_3CN (2 mL). After stirring the mixture at 25 °C for 2 h, water was added and the solution was extracted with TBME (3×). The combined organic extracts were washed with brine (2×), dried (Na_2SO_4) and evaporated. Purification of the residue by chromatography (SiO_2, 87 : 10 : 3 hexane–AcOEt–Et_3N) afforded 0.13 g (87%) of thiophene **29a** as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.24 (s, 3H, Ar-CH_3) 2.26 (s, 3H, Ar-CH_3), 3.79 (s, 3H, CO_2CH_3), 4.16 (s, 2H, SCH_2), 4.64 (s, 2H, 2H2), 6.62 (d, J = 8.4 Hz, H6′), 6.66 (s, 1H, H3″), 7.19 (dd, J = 8.4, 2.1, 1H, H5′), 7.23 (br s, 1H, H3′), 7.52 (d, J = 8.1 Hz, 2H, H2‴ + H6‴), 7.64 (d, J = 8.1 Hz, 2H, H3‴ + H5‴) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9 (q, Ar-CH_3), 16.0 (q, Ar-CH_3), 35.4 (t, CH_2S), 52.1 (q, CO_2CH_3), 65.4 (t, C2), 111.4 (d, C6′), 124.0 (s, CF_3, $^1J_{C-F}$ = 271.7 Hz), 125.3 (d, 2C, C3‴ + C5‴, $^3J_{C-F}$ = 3.3 Hz), 126.5 (s), 128.1 (s), 128.6 (d, 2C, C2‴ + C6‴), 128.7 (s, C4‴, $^2J_{C-F}$ = 32.5 Hz), 130.5 (d, C3″), 130.8 (d, C5′), 133.9 (s), 135.0 (d, C3′), 135.3 (s), 138.2 (s), 140.2 (s), 155.7 (s), 169.2 (s, C1) ppm.

Preparation of (2-methyl-4-[4-methyl-5-(4-trifluoromethylphenyl)thien-2-ylmethylthio]phenoxy)acetic acid (3a). General procedure for the hydrolysis of esters

A solution of methyl ester **29a** (0.11 g, 0.24 mmol) and 3 M K_2CO_3 (0.8 mL, 2.4 mmol) in MeOH (2.5 mL) was heated to 80 °C for 1 h.

After cooling to 25 °C, the mixture was acidified with 10% HCl (pH ≈ 3). It was then extracted with AcOEt (5×), and the extracts were dried (Na_2SO_4) and evaporated. Purification of the residue by recrystallization (hexane–$CHCl_3$) afforded 0.10 g (92%) of acid **3a** as a white solid (mp 100 °C). ^{1}H NMR (400 MHz, CD_3COCD_3) δ 2.05 (s, 3H, Ar-CH_3), 2.08 (s, 3H, Ar-CH_3), 4.10 (s, 2H, SCH_2), 4.58 (s, 2H, 2H2), 6.59 (s, 1H, H3″), 6.66 (d, J = 8.5, 1H, H6′), 7.06 (d, J = 8.5, 1H, H5′), 7.10 (s, 1H, H3′), 7.48 (d, J = 8.0 Hz, 2H, H2‴ + H6‴), 7.58 (d, J = 8.0 Hz, 2H, H3‴ + H5‴) ppm. ^{13}C NMR (100 MHz, CD_3COCD_3) δ 15.2 (q, Ar-CH_3), 16.2 (q, Ar-CH_3), 35.2 (t, CH_2S), 65.5 (t, C2), 112.6 (d, C6′), 125.3 (s, CF_3, $^1J_{C-F}$ = 270.7 Hz), 126.4 (d, 2C, C3‴ + C5‴, $^3J_{C-F}$ = 3.7 Hz), 127.0 (s), 128.4 (s), 129.0 (s, C4‴, $^2J_{C-F}$ = 32.2 Hz), 129.7 (d, 2C, C2‴ + C6‴), 131.3 (d, C5′), 131.8 (d, C3″), 135.1 (s), 135.1 (d, C3′), 135.6 (s), 139.5 (s), 141.9 (s), 156.8 (s), 170.1 (s, C1) ppm. IR (NaCl) ν 3400 − 2700 (br, O–H), 2925 (w, C–H), 1734 (s, C=O), 1614 (m), 1490 (s), 1325 (s), 1228 (m), 1124 (s), 1069 (s), 1016 (w), 840 (m) cm^{-1}. MS m/z (%) 453 ([M + 1]$^+$, 4), 452 (M$^+$, 17), 257 (18), 256 (47), 255 ([M–$C_9H_9O_3S$]$^+$, 100), 139 (10). HRMS (EI$^+$) calcd for $C_{22}H_{19}F_3O_3S_2$ 452.0728, found 452.0732. Anal. Calcd for $C_{22}H_{19}F_3O_3S_2$: C, 58.39; H, 4.23; S, 14.17. Found: C, 58.06; H, 4.16; S, 14.15%.

Cell culture

HeLa cells were routinely maintained in DMEM supplemented with 5% FCS and ligand assays were performed in the same medium.

Stable reporter cell lines

A stable Gal4-hPPARβ/δ cell line was generated as described previously to facilitate assessment of agonistic activity at PPARβ/δ with minor modifications.[37] Briefly, HeLa cells were cotransfected with Gal4-hPPARβ/δ and $(17m)_5$-βglobin-Luc-Neo reporter gene. At 48 h post-transfection, the cell line was selected with Geneticin at 0.8 mg mL^{-1} and puromycin at 0.3 μg mL^{-1}. Clones expressing PPARβ/δ agonist-inducible luciferase activity were selected as described.

Assessment of agonistic activity

To test the different compounds, identical aliquots of the Gal-hPPARβ/δ reporter cell line were seeded and exposed in parallel to the known agonist GW501516 **1a**, the test ligand, and vehicle for 16 h. Cells were lysed in 100 μL of lysis buffer (25 mM Tris phosphate [pH 7.8], 2 mM EDTA, 1 mM DTT, 10% glycerol, and 1% Triton X-100). A 100 μL portion of luciferin buffer (20 mM Tris phosphate [pH 7.8], 1.07 mM $MgCl_2$, 2.67 mM $MgSO_4$, 1 mM EDTA, 33.3 mM DTT, 0.53 mM ATP, 0.47 mM luciferin, and 0.27 mM coenzyme A) was added prior to direct photon counting.

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