A novel biphenyl-based chemotype of retinoid X receptor ligands enables subtype- and heterodimer-preference

Julius Pollinger et al.

- Supplementary Information –

Table of Contents

Supplementary Figures

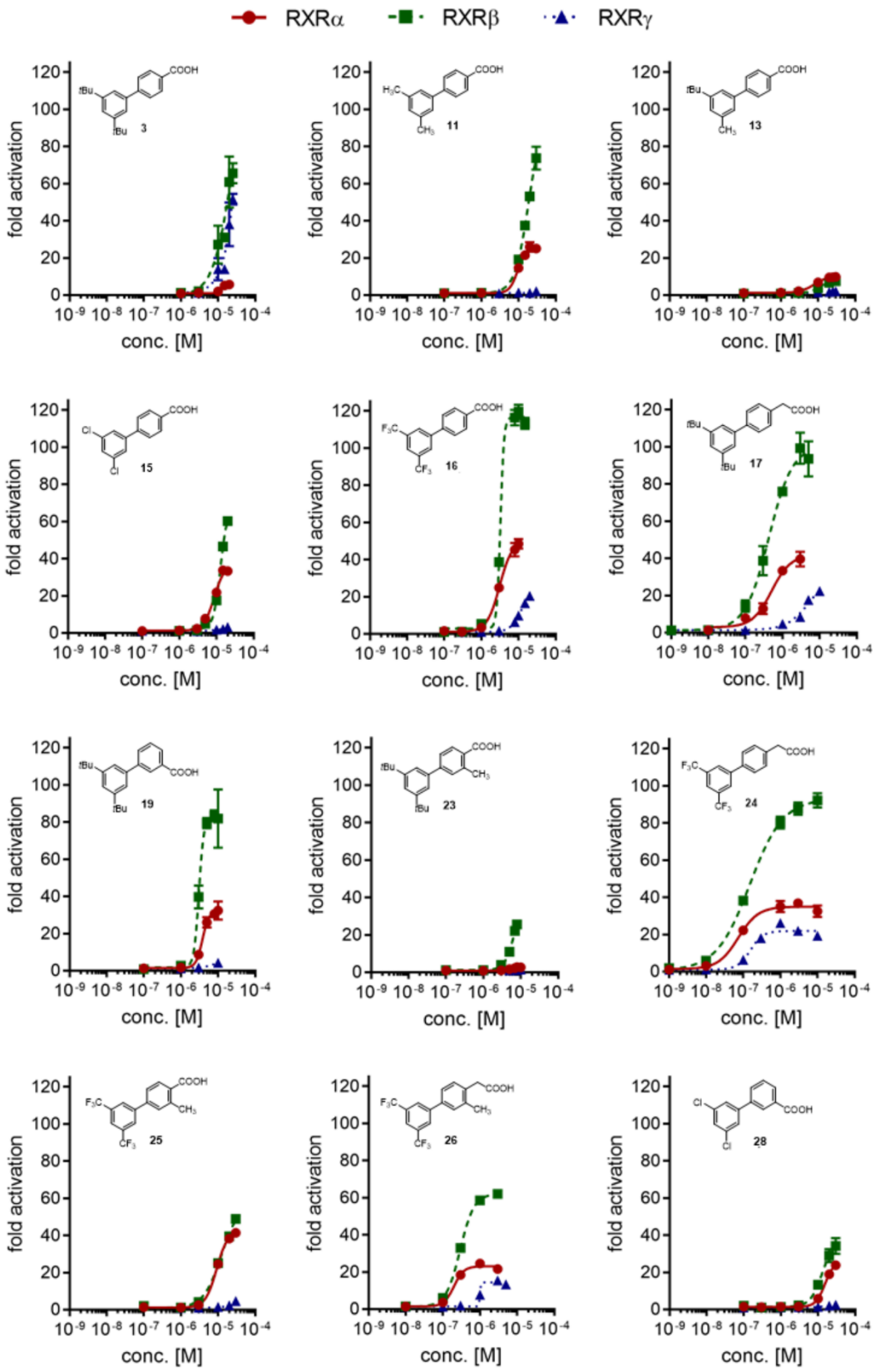

Figure S1. Activity profiles (dose-response) of important biphenylcarboxylate based RXR ligands on RXRα (red), RXRβ (green) and RXRγ (blue). Data are the mean ± S.E.M.; n≥3.

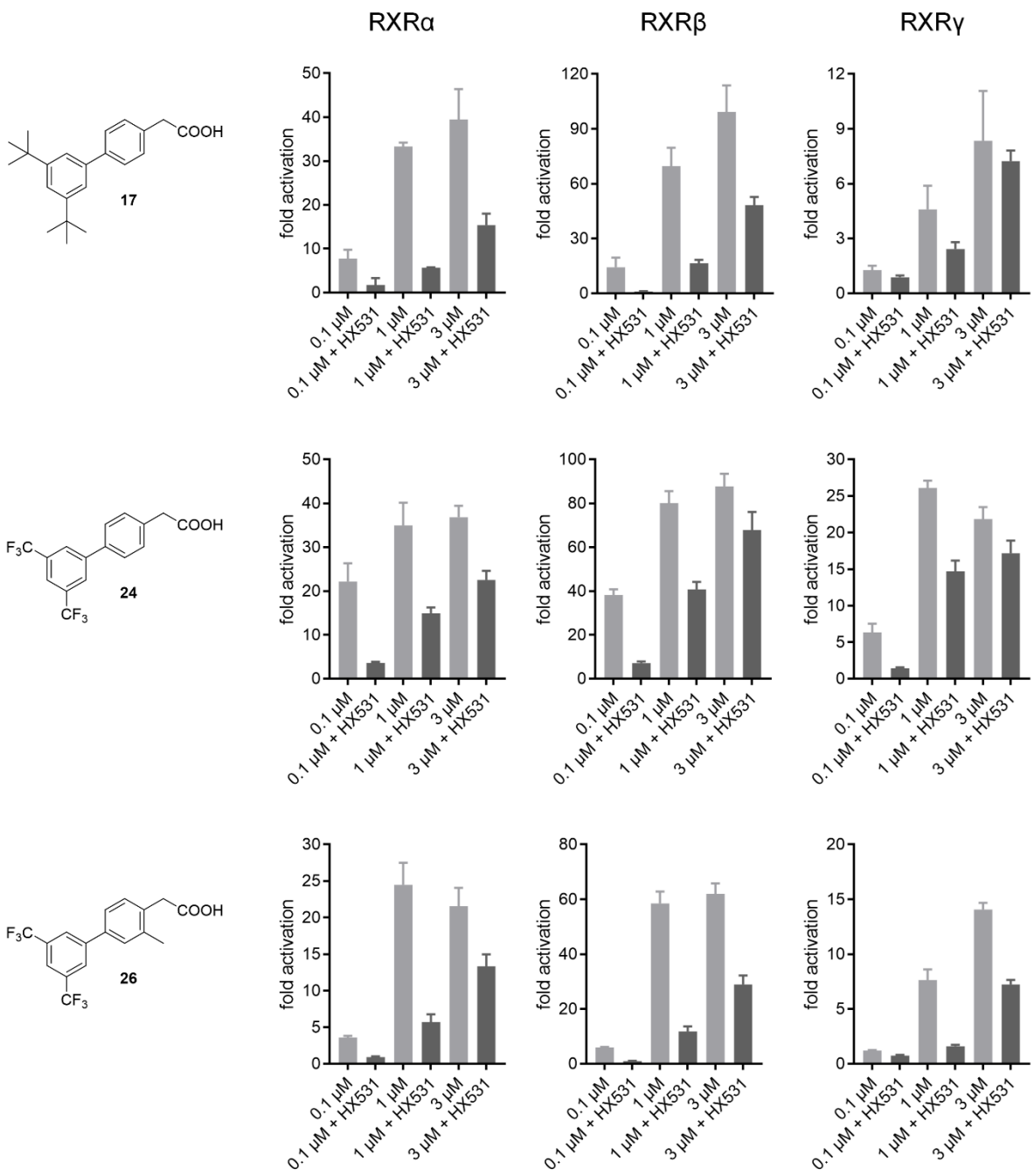

Figure S2. Activity profiles (dose-response) of the most potent biphenylcarboxylate based RXR ligands on RXRα, RXRβ and RXRγ alone and in competition with 1 μM of RXR antagonist HX531. Data are the mean ± S.E.M.; n=3.

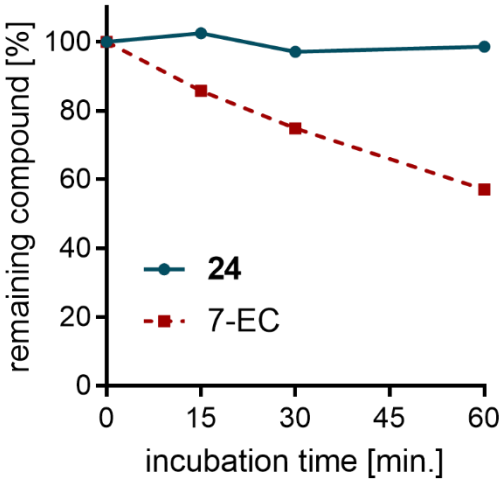

Figure S3. Stability of **24** against microsomal degradation by Wistar rat liver microsomes. 7-Ethoxycoumarin (7-EC) as control. Data are the mean ± S.E.M., n=3.

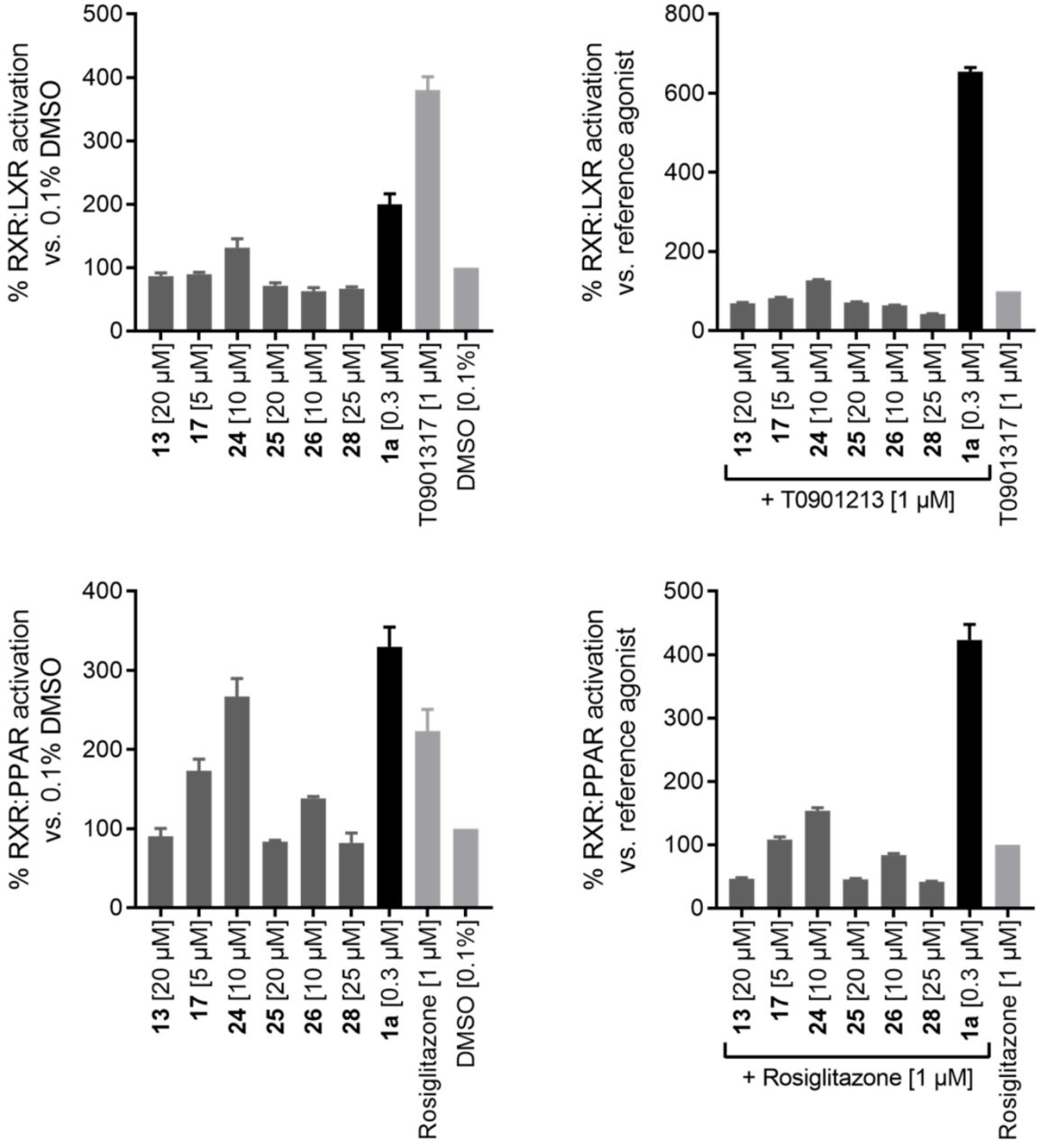

Figure S4. Activity of biphenylcarboxylate RXR agonists (**13**, **17**, **24**-**26** and **28**; dark grey) and RXR agonist bexarotene (**1a**; black) in two different full-length reporter gene assays compared to reference agonists rosiglitazone (PPAR) or T0901317 (LXR) or compared to 0.1% DMSO (light grey) as negative control. RXR agonists were studied alone and in combination with rosiglitazone or T0901317, respectively. Data are the mean ± S.E.M., n≥3.

Table S1. Compounds **5**-**27** and their corresponding starting materials/building blocks for Suzuki coupling. Synthesis of **9, 13** and **21** additionally involved alkaline ester hydrolysis.

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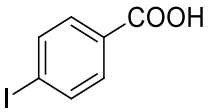
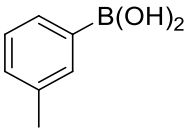
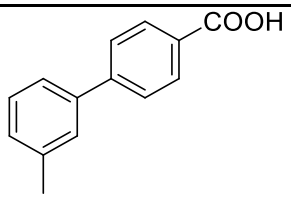
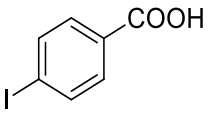
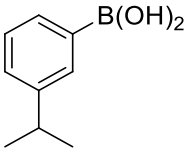
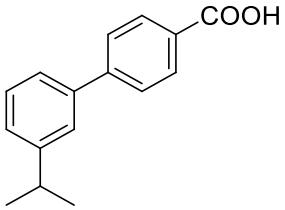
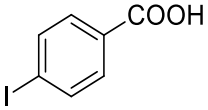
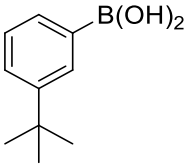
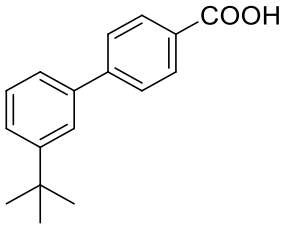
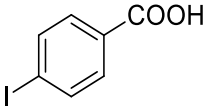
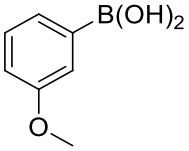
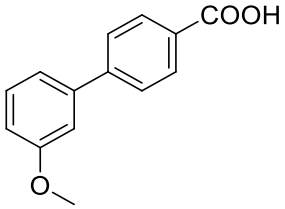
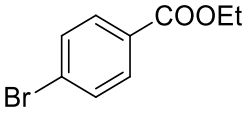
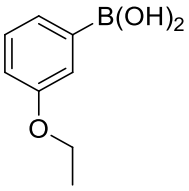
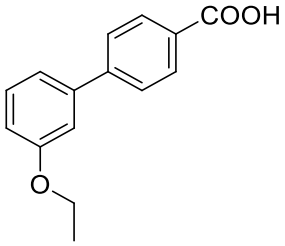
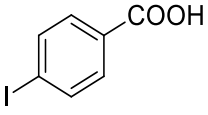
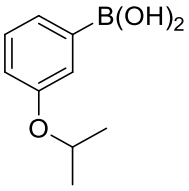
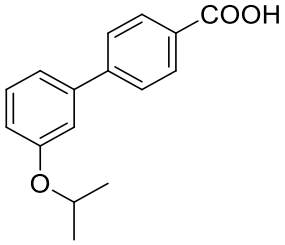
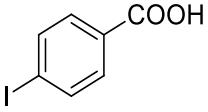
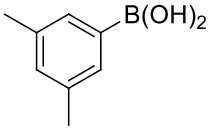
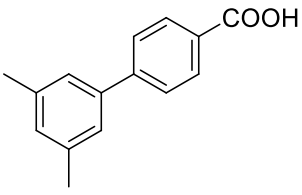
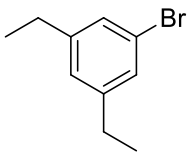
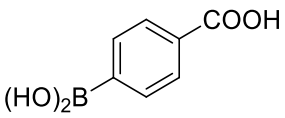
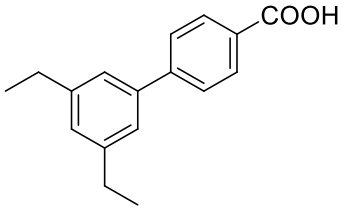
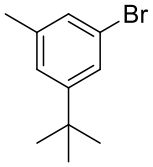
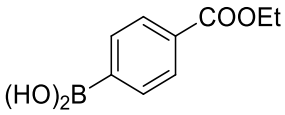
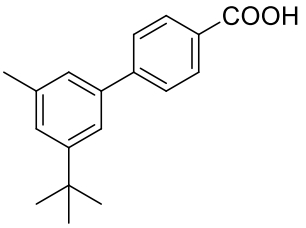
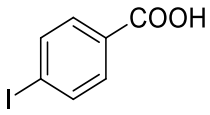
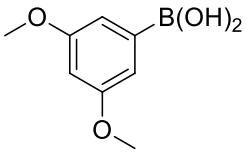
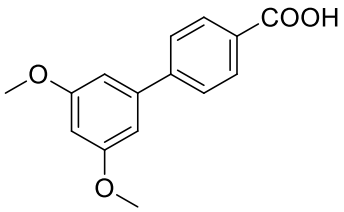
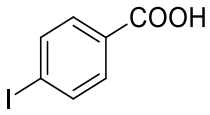
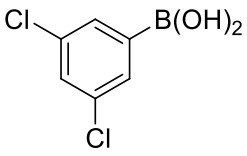
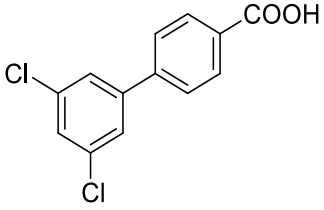
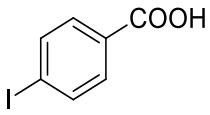
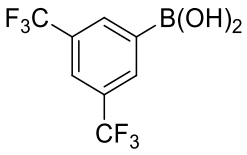
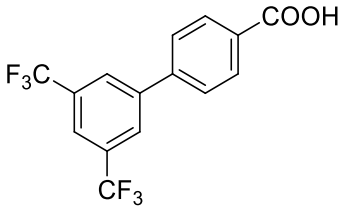
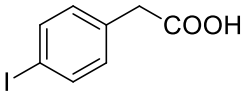
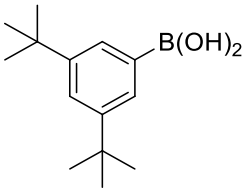
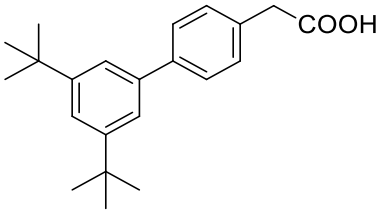
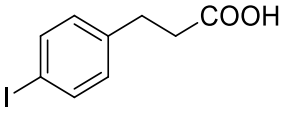
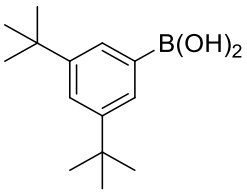
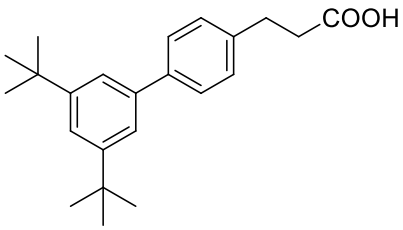
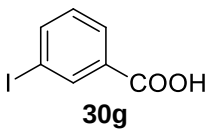
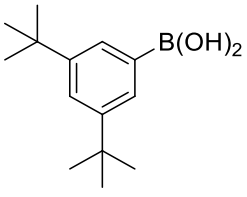
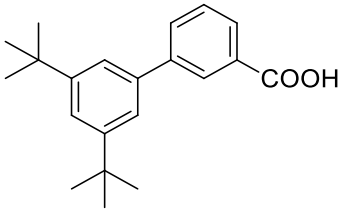
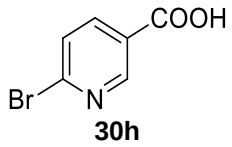
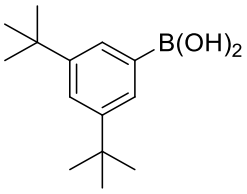
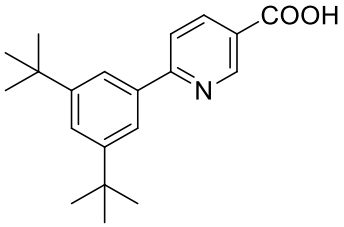
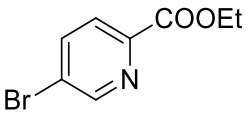
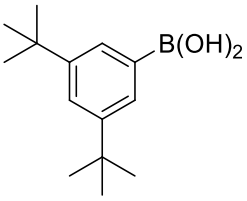
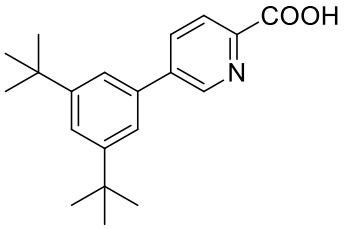
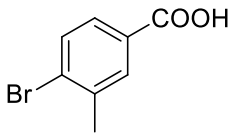
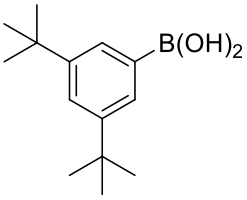
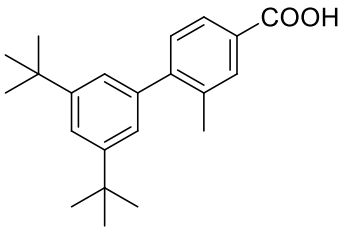
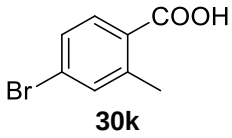
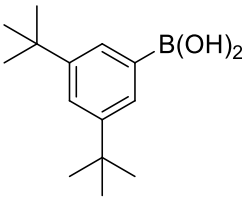
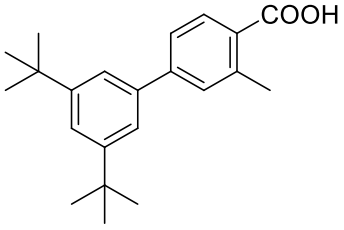
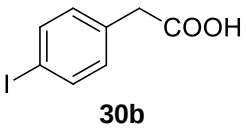
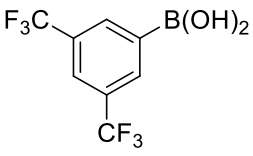
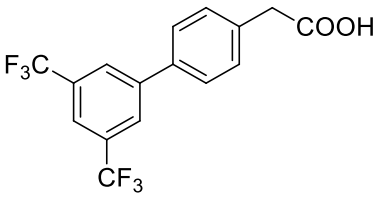
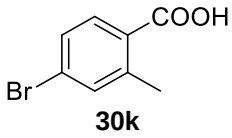
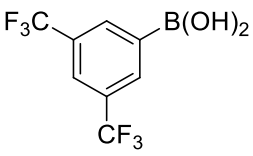
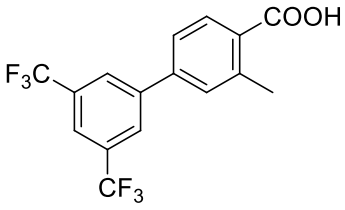
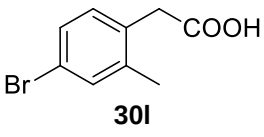
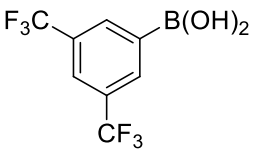
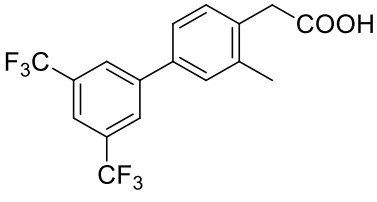
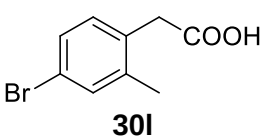
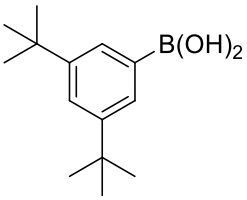
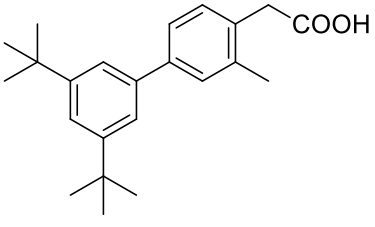
5	 **30a**	 **29a**	
6	 **30a**	 **29b**	
7	 **30a**	 **29c**	
8	 **30a**	 **29d**	
9	 **30d**	 **29k**	
10	 **30a**	 **29e**	
11	 **30a**	 **29f**	

ID	Arylhalide building block	Arylboronic acid building block	Structure
12	 **30e**	 **29l**	
13	 **30f**	 **29m**	
14	 **30a**	 **29g**	
15	 **30a**	 **29h**	
16	 **30a**	 **29i**	
17	 **30b**	 **29j**	
18	 **30c**	 **29j**	
19	 **30g**	 **29j**	

ID	Arylhalide building block	Arylboronic acid building block	Structure
20	 **30h**	 **29j**	
21	 **30i**	 **29j**	
22	 **30j**	 **29j**	
23	 **30k**	 **29j**	
24	 **30b**	 **29i**	
25	 **30k**	 **28i**	
26	 **30l**	 **29i**	
27	 **30l**	 **29j**	

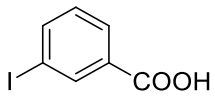
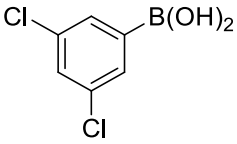
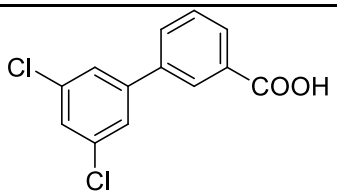
ID	Arylhalide building block	Arylboronic acid building block	Structure
28	 **30g**	 **29h**	

Table S2. Data collection and refinement statistics for DYRK1A-FC162 structure.

Complex	**RXRα-24**
PDB accession code	**6SJM**
Data Collection	
Resolution[a] (Å)	45.25-2.52 (2.66-2.52)
Spacegroup	*P* $4_3 2_1 2$
Cell dimensions	*a* = b = 64.0, *c* = 109.6 Å *α, β, γ* = 90.0°
No. unique reflections[a]	8,101 (1,164)
Completeness[a] (%)	99.2 (100.0)
I/σI[a]	12.7 (2.1)
R_{merge}[a] (%)	0.071 (0.992)
CC (1/2)	0.998 (0.777)
Redundancy[a]	7.3 (7.7)
Refinement	
No. atoms in refinement (RXR/pep/L/O)[b]	1,674/ 106/ 24/ 4
B factor (RXR/pep/L/O)[b] ($Å^2$)	80/ 89/ 84/ 63
R_{fact} (%)	20.5
R_{free} (%)	24.7
rms deviation bond[c] (Å)	0.010
rms deviation angle[c] (°)	1.2
Molprobity Ramachandran	
Favour (%)	96.4
Allowed (%)	0

[a] Values in brackets show the statistics for the highest resolution shells.
[b] RXR/pep/L/O indicate RXRα protein, coactivator peptide, ligand molecules presented in the active sites, and other (water and solvent molecules), respectively.
[c] rms indicates root-mean-square.

Chemistry

General

All chemicals and solvents were of reagent grade and used without further purification unless otherwise specified. All reactions were conducted in oven-dried glassware under argon atmosphere and in absolute solvents. NMR spectra were recorded on a Bruker AV 300, Bruker AV 400 or a Bruker AV 500 spectrometer (Bruker Corporation, Billerica, MA, USA). Chemical shifts (δ) are reported in ppm relative to TMS as reference; approximate coupling constants (*J*) are shown in Hertz (Hz). Mass spectra were obtained on a VG Platform II (Thermo Fischer Scientific, Inc., Waltham, MA, USA) using electrospray ionization (ESI). High resolution mass spectra were recorded on a MALDI LTQ ORBITRAP XL instrument (Thermo Fisher Scientific). Compound purity was analyzed on a Waters 600 Contoller HPLC using a Waters 2487 Dual Absorbance Detector and Waters 717 plus Autosampler or Hitachi Chromaster with a 5160 pump system, using a DAD 5430 and 5260 Autosampler both equipped with a MultoHigh100 RP18-5 µ 250x4 mm column (CS-Chromatographie Service GmbH, Langerwehe, Germany) using a gradient (H_2O+0.1% formic acid/MeOH 80:20 isocratic for 5 min to MeOH after additional 45 min and MeOH for additional 10 min) at a flow rate of 1 mL/min and UV-detection at 245 nm and 280 nm (method A) or using a gradient (H_2O+0.1% formic acid/MeOH 60:40 isocratic for 5 min to MeOH after additional 25 min and MeOH for additional 10 min) at a flow rate of 1 mL/min and UV-detection at 245 nm and 280 nm (method B).

All final compounds for biological evaluation had a purity of >95% according to HPLC−UV analysis at wavelengths 245 and 280 nm.

Synthesis and analytical characterization

3'-Methyl-(1,1'-biphenyl)-4-carboxylic acid (5)

4-Iodobenzoic acid (**30a**, 0.30 g, 1.2 mmol, 1.0 eq), 3-methylbenzeneboronic acid (**29a**, 0.20 g, 1.4 mmol, 1.2 eq) and sodium carbonate (0.40 g, 3.6 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.069 g, 0.060 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 14 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (86:12:2) as mobile phase to obtain **5** as colorless solid (60 mg, 24%). R_f (hexane/EtOAc/HOAc = 86:12:2) = 0.34. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 13.00 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.55 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.23 (d, *J* = 7.5 Hz, 1H), 2.39 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.18, 144.43, 139.00, 138.30, 129.93, 129.58, 128.99, 128.94, 127.61, 126.79, 124.09, 21.09. HRMS (MALDI): *m/z* calculated 212.08318 for $C_{14}H_{12}O_2$ found 212.08295 ([M*]).

3'-*iso*-Propyl-(1,1'-biphenyl)-4-carboxylic acid (6)

4-Iodobenzoic acid (**30a**, 0.30 g, 1.2 mmol, 1.0 eq), 3-isopropylbenzeneboronic acid (**29b**, 0.24 g, 1.4 mmol, 1.2 eq) and sodium carbonate (0.40 g, 3.6 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.069 g, 0.060 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 5.5 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (89:9:2) as mobile phase to obtain **6** as colorless solid (0.20 g, 69%). R_f (hexane/EtOAc/HOAc = 89:9:2) = 0.35. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ = 12.96 (s, 1H), 8.02 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.57 (s, 1H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.29 (d, *J* = 7.6 Hz, 1H), 2.97 (hept, *J* = 6.9 Hz, 1H), 1.25 (d, *J* = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ = 167.22, 149.32, 144.68, 139.12, 129.96, 129.52, 129.11, 126.91, 126.29, 125.11, 124.57, 33.57, 23.91. HRMS (MALDI): *m/z* calculated 241.12216 for $C_{16}H_{17}O_2$ found 241.12231 ($[M+H]^+$).

3'-*tert*-Butyl-(1,1'-biphenyl)-4-carboxylic acid (7)

4-Iodobenzoic acid (**30a**, 0.30 g, 1.2 mmol, 1.0 eq), 3-*tert*-butylbenzeneboronic acid (**29c**, 0.26 g, 1.4 mmol, 1.2 eq) and sodium carbonate (0.40 g, 3.6 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.069 g, 0.060 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 5.5 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (95:3:2) as mobile phase to obtain **7** as colorless solid (55 mg, 18%). R_f (hexane/EtOAc/HOAc = 95:3:2) = 0.2. ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ = 12.93 (s, 1H), 8.02 (d, *J* = 8.3 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.69 (t, *J* = 1.6 Hz, 1H), 7.53 - 7.50 (m, 1H), 7.47 – 7.40 (m, 2H), 1.34 (s, 9H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ = 167.20, 151.50, 144.92, 138.89, 129.94, 129.49, 128.84, 126.98, 125.22, 124.29, 123.85, 34.60, 31.15. HRMS (MALDI): *m/z* calculated 254.13013 for $C_{17}H_{18}O_2$ found 254.13017 ([M*]).

3'-Methoxy-(1,1'-biphenyl)-4-carboxylic acid (8)

4-Iodobenzoic acid (**30a**, 0.30 g, 1.2 mmol, 1.0 eq), 3-methoxybenzeneboronic acid (**29d**, 0.22 g, 1.4 mmol, 1.2 eq) and sodium carbonate (0.40 g, 3.6 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.069 g, 0.060 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 5.5 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (87:11:2) as mobile phase to obtain **8** as

white solid (25 mg, 9%). R_f (hexane/EtOAc/HOAc = 87:11:2) = 0.2. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.96 (s, 1H), 8.01 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 8.4 Hz, 2H), 7.41 (t, J = 7.9 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.26 – 7.24 (m, 1H), 7.01 - 6.97 (m, 1H), 3.83 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.16, 159.82, 144.21, 140.55, 130.18, 129.91, 129.77, 126.96, 119.29, 114.00, 112.37, 55.22. HRMS (MALDI): *m/z* calculated 229.08592 for $C_{14}H_{13}O_3$ found 229.008671 ($[M+H]^+$).

3'-Ethoxy-(1,1'-biphenyl)-4-carboxylic acid (9)

Ethyl 3'-ethoxy-(1,1'-biphenyl)-4-carboxylate (**31**, 80 mg, 0.29 mmol, 1.0 eq) was dissolved in a mixture of THF (5.0 mL) and EtOH (1.0 mL). LiOH (0.061 g, 1.5 mmol, 5.0 eq) was dissolved in water (1.5 mL) and added to the solution. The mixture was stirred for 18 h at room temperature. The organic solvents were removed in vacuum and 2 N HCl (5.0 mL) was added to precipitate **9**, which was filtrated and isolated as a white solid (60 mg, 84%). ^{1}H NMR (500 MHz, acetone-d_6): δ = 8.09 (d, J = 8.5 Hz, 2H), 7.76 (d, J = 8.5 Hz, 2H), 7.38 (t, J = 7.9 Hz, 1H), 7.28 - 7.25 (m, 1H), 7.24 – 7.22 (m, 1H), 6.97 - 6.94 (m, 1H), 4.12 (q, J = 7.0 Hz, 2H), 1.38 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, acetone-d_6): δ = 168.29, 160.44, 145.77, 142.09, 131.05, 130.95, 130.87, 127.70, 120.09, 115.01, 113.91, 64.07, 15.06. HRMS (MALDI): *m/z* calculated 243.10157 for $C_{15}H_{15}O_3$ found 243.10157 ($[M+H]^+$).

3'-(Propan-2-yloxy)-(1,1'-biphenyl)-4-carboxylic acid (10)

4-Iodobenzoic acid (**30a**, 0.37 g, 1.5 mmol, 1.0 eq), 3-isopropyloxybenzeneboronic acid (**29e**, 0.30 g, 1.8 mmol, 1.2 eq) and sodium carbonate (0.48 g, 4.5 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.087 g, 0.075 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 6 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (87.5:10.5:2) as mobile phase to obtain **10** as pale yellow solid (0.19 g, 49%). R_f (hexane/EtOAc/HOAc = 87.5:10.5:2) = 0.28. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.02 (d, J = 8.5 Hz, 2H), 7.79 (d, J = 8.5 Hz, 2H), 7.57 (s, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.41 (t, J = 7.6 Hz, 1H), 7.30 (d, J = 7.6 Hz, 1H), 3.02 – 2.92 (m, 1H), 1.25 (d, J = 6.9 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.21, 149.31, 144.66, 139.12, 129.95, 129.53, 129.09, 126.90, 126.28, 125.11, 124.57, 33.56, 23.90. HRMS (MALDI): *m/z* calculated 257.11722 for $C_{16}H_{17}O_3$ found 257.11729 ($[M+H]^+$).

3',5'-Dimethyl-(1,1'-biphenyl)-4-carboxylic acid (11)

4-Iodobenzoic acid (**30a**, 0.37 g, 1.5 mmol, 1.0 eq), 3,5-dimethylbenzeneboronic acid (**29f**, 0.27 g, 1.8 mmol, 1.2 eq) and sodium carbonate (0.48 g, 4.5 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.087 g, 0.075 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 5 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were

separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (89:9:2) as mobile phase to obtain **11** as white solid (80 mg, 24%). R_f (hexane/EtOAc/HOAc = 89:9:2) = 0.34. ^{1}H NMR (500 MHz, acetone-*d*$_6$): δ = 8.11 (d, *J* = 8.5 Hz, 2H), 7.75 (d, *J* = 8.5 Hz, 2H), 7.33 (s, 2H), 7.05 (s, 1H), 2.36 (s, 6H). ^{13}C NMR (126 MHz, acetone-*d*$_6$): δ = 167.65, 146.49, 140.60, 139.25, 131.00, 130.55, 130.06, 127.73, 125.81, 21.37. HRMS (MALDI): *m/z* calculated 227.10666 for $C_{15}H_{15}O_2$ found 227.10655 ($[M+H]^+$).

3',5'-Diethyl-(1,1'-biphenyl)-4-carboxylic acid (12)

1-Bromo-3,5-diethylbenzene (**30e**, 0.27 g, 1.3 mmol, 1.0 eq), 4-boronobenzoic acid (**29l**, 0.25 g, 1.5 mmol, 1.2 eq) and sodium carbonate (0.40 g, 3.8 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.069 g, 0.060 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 3.5 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (89:9:2) as mobile phase to obtain **12** as yellow solid (80 mg, 24%). R_f (hexane/EtOAc/HOAc = 89:9:2) = 0.3. ^{1}H NMR (500 MHz, acetone-*d*$_6$): δ = 8.12 (d, *J* = 8.6 Hz, 2H), 7.78 (d, *J* = 8.6 Hz, 2H), 7.38 (s, 2H), 7.12 (s, 1H), 2.69 (q, *J* = 7.6 Hz, 4H), 1.26 (t, *J* = 7.6 Hz, 6H). ^{13}C NMR (126 MHz, acetone-*d*$_6$): δ = 167.69, 146.71, 145.85, 140.74, 131.01, 129.97, 128.27, 127.80, 124.98, 29.47, 16.18. HRMS (MALDI): *m/z* calculated 255.13796 for $C_{17}H_{19}O_2$ found 255.13810 ($[M+H]^+$).

3'-*tert*-Butyl-5'-methyl-(1,1'-biphenyl)-4-carboxylic acid (13)

Ethyl 3'-*tert*-butyl-5'-methyl-(1,1'-biphenyl)-4-carboxylate (**32**, 0.15 g, 0.49 mmol, 1.0 eq) was dissolved in a mixture of THF (5.0 mL) and EtOH (1.0 mL). LiOH (0.11 g, 2.5 mmol, 5.0 eq) was dissolved in water (1.5 mL) and added to the solution. The mixture was stirred for 15 h at room temperature. The organic solvents were removed in vacuum and 2 N HCl (5.0 mL) was added to precipitate **13**, which was filtrated and isolated as a white solid (0.10 g, 74%). ^{1}H NMR (500 MHz, DMSO-*d*$_6$): δ = 7.96 (d, *J* = 8.3 Hz, 2H), 7.59 (d, *J* = 8.3 Hz, 2H), 7.43 (s, 1H), 7.29 (s, 1H), 7.21 (s, 1H), 2.36 (s, 3H), 1.32 (s, 9H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$): δ = 168.70, 151.23, 141.96, 139.76, 137.61, 129.71, 125.91, 125.22, 124.79, 120.88, 34.44, 31.24, 21.37. HRMS (MALDI): *m/z* calculated 269.15361 for $C_{18}H_{21}O_2$ found 269.15345 ($[M+H]^+$).

3',5'-Dimethoxy-(1,1'-biphenyl)-4-carboxylic acid (14)

4-Iodobenzoic acid (**30a**, 0.37 g, 1.5 mmol, 1.0 eq), 3,5-dimethoxybenzeneboronic acid (**29g**, 0.33 g, 1.8 mmol, 1.2 eq) and sodium carbonate (0.48 g, 4.5 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.087 g, 0.075 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 6 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were

separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (87:11:2) as mobile phase to obtain **14** as brown solid (90 mg, 23%). R_f (hexane/EtOAc/HOAc = 87:11:2) = 0.1. ^{1}H NMR (500 MHz, acetone-d_6): δ = 8.10 (d, *J* = 8.5 Hz, 2H), 7.79 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 2.2 Hz, 2H), 6.55 (t, *J* = 2.2 Hz, 1H), 3.87 (s, 6H). ^{13}C NMR (126 MHz, acetone-d_6): δ = 167.43, 162.37, 146.23, 142.79, 130.96, 130.43, 127.95, 106.09, 100.88, 55.76. HRMS (MALDI): *m/z* calculated 259.09649 for $C_{15}H_{15}O_4$ found 259.09675 ($[M+H]^+$).

3',5'-Dichloro-(1,1'-biphenyl)-4-carboxylic acid (15)

4-Iodobenzoic acid (**30a**, 0.37 g, 1.5 mmol, 1.0 eq), 3,5-dichlorobenzeneboronic acid (**29h**, 0.34 g, 1.8 mmol, 1.2 eq) and sodium carbonate (0.48 g, 4.5 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.087 g, 0.075 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 5.5 hours The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (84:14:2) as mobile phase to obtain **15** as colorless solid (0.16 g, 40%). R_f (hexane/EtOAc/HOAc = 84:14:2) = 0.33. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.02 (d, *J* = 8.3 Hz, 2H), 7.87 (d, *J* = 8.1 Hz, 2H), 7.81 (s, 2H), 7.65 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 166.99, 142.56, 141.18, 134.82, 130.94, 129.97, 127.65, 127.30, 125.76. HRMS (MALDI): *m/z* calculated 266.99741 for $C_{13}H_8Cl_2O_2$ found 266.99759 ($[M+H]^+$).

3',5'-Bis(trifluoromethyl)-(1,1'-biphenyl)-4-carboxylic acid (16)

4-Iodobenzoic acid (**30a**, 0.37 g, 1.5 mmol, 1.0 eq), 3,5-bis(trifluoromethyl)benzeneboronic acid (**29i**, 0.46 g, 1.8 mmol, 1.2 eq) and sodium carbonate (0.48 g, 4.5 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.087 g, 0.075 mmol, 0.050 eq) was added and the mixture was refluxed for 42.5 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (90:8:2) as mobile phase to obtain **16** as colorless solid (0.21 g, 42%). R_f (hexane/EtOAc/HOAc = 90:8:2) = 0.29. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 12.84 (s, 1H), 8.39 (s, 2H), 8.14 (s, 1H), 8.07 - 8.04 (m, 2H), 8.00 (d, *J* = 8.4 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 166.94, 141.64, 140.97, 131.05, 130.03, 127.81, 127.72, 124.40, 122.23, 121.71. HRMS (MALDI): *m/z* calculated 335.05013 for $C_{15}H_9F_6O_2$ found 335.05022 ($[M+H]^+$).

2-(3',5'-Di-*tert*-butyl-(1,1'-biphenyl)-4-yl)acetic acid (17)

2-(4-Iodophenyl)acetic acid (**30b**, 0.26 g, 1.0 mmol, 1.0 eq), 3,5-di-*tert*-butylbenzeneboronic acid (**29j**, 0.28 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.32 g, 3.0 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 17.5 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (84:14:2) as mobile phase to obtain **17** as colorless solid (75 mg, 23%). R_f (hexane/EtOAc/HOAc = 84:14:2) = 0.3. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 7.56 (d, *J* = 8.1 Hz, 2H), 7.39 (s, 3H), 7.34 (d, *J* = 8.1 Hz, 2H), 3.60 (s, 2H), 1.33 (s, 18H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 163.09, 141.12, 130.16, 129.93, 124.31, 120.20, 117.23, 111.34, 111.30, 30.69, 24.98, 21.64. (MALDI): *m/z* calculated 347.19815 for $C_{22}H_{28}O_2Na$ found 347.19830 ($[M+Na]^+$).

3-(3',5'-Di-*tert*-butyl-(1,1'-biphenyl)-4-yl)propanoic acid (18)

3-(4-Iodophenyl)propanoic acid (**30c**, 0.28 g, 1.0 mmol, 1.0 eq), 3,5-di-*tert*-butylbenzeneboronic acid (**29j**, 0.28 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.32 g, 3.0 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 17.5 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (84:14:2) as mobile phase to obtain **18** as colorless solid (0.12 g, 36%). R_f (hexane/EtOAc/HOAc = 84:14:2) = 0.28. ^{1}H NMR (500 MHz, acetone-d_6): δ = 7.57 (d, *J* = 8.1 Hz, 2H), 7.49 - 7.45 (m, 3H), 7.35 (d, *J* = 8.1 Hz, 2H), 2.97 (t, *J* = 7.7 Hz, 2H), 2.67 (t, *J* = 7.7 Hz, 2H), 1.38 (s, 18H). ^{13}C NMR (126 MHz, acetone-d_6): δ = 174.09, 151.82, 141.21, 140.94, 140.79, 129.59, 127.99, 122.05, 121.90, 35.91, 35.49, 31.82, 31.14. (MALDI): *m/z* calculated 338.22403 for $C_{23}H_{30}O_2$ found 338.22410 ($[M]^*$).

3',5'-Di-*tert*-butyl-(1,1'-biphenyl)-3-carboxylic acid (19)

3-Iodobenzoic acid (**30g**, 0.25 g, 1.0 mmol, 1.0 eq), 3,5-di-*tert*-butylbenzeneboronic acid (**29j**, 0.28 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.32 g, 3.0 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 16 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by HPLC chromatography to obtain **19** as colorless solid (60 mg, 20%). R_f (hexane/EtOAc/HOAc = 83:15:2) = 0.32. ^{1}H NMR (500 MHz, acetone-d_6): δ = 8.28 (t,

J = 1.6 Hz, 1H), 8.04 - 8.01 (m, 1H), 7.92 - 7.89 (m, 1H), 7.59 (t, *J* = 7.7 Hz, 1H), 7.55 (t, *J* = 1.7 Hz, 1H), 7.53 (d, *J* = 1.8 Hz, 2H), 1.39 (s, 18H). ^{13}C NMR (126 MHz, acetone-d_6): δ = 167.67, 152.20, 143.43, 140.46, 132.56, 132.03, 129.86, 129.00, 128.97, 122.68, 122.26, 35.56, 31.77. HRMS (MALDI): *m/z* calculated 310.19273 for $C_{21}H_{26}O_2$ found 310.19221 ([M]$^{\bullet}$).

6-(3,5-Di-*tert*-butylphenyl)pyridine-3-carboxylic acid (20)

6-Bromopyridine-3-carboxylic acid (**30h**, 0.20 g, 1.0 mmol, 1.0 eq), 3,5-di-*tert*-butylbenzeneboronic acid (**29j**, 0.28 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.32 g, 3.0 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.05 eq) was added and the mixture was stirred under reflux for 27 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (87:11:2) as mobile phase to obtain **20** as colorless solid (0.12 g, 40%). R_f (hexane/EtOAc/HOAc = 87:11:2) = 0.1. ^{1}H NMR (500 MHz, DMSO-d_6): δ = 9.15 (d, *J* = 1.6 Hz, 1H), 8.31 (dd, *J* = 8.3, 2.2 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.95 (d, *J* = 1.8 Hz, 2H), 7.54 (t, *J* = 1.7 Hz, 1H), 1.35 (s, 18H). ^{13}C NMR (126 MHz, DMSO): δ = 166.37, 160.38, 150.97, 150.39, 137.99, 137.21, 124.86, 123.81, 121.35, 120.33, 34.77, 31.29. HRMS (MALDI): *m/z* calculated 312.19581 for $C_{20}H_{26}NO_2$ found 312.19635 ([M+H]$^+$).

5-(3,5-Di-*tert*-butylphenyl)pyridine-2-carboxylic acid (21)

Ethyl 5-(3,5-di-*tert*-butylphenyl)pyridine-2-carboxylate (**33**, 60 mg, 0.20 mmol, 1.0 eq) was dissolved in a mixture of THF (5.0 mL) and EtOH (1.0 mL). LiOH (0.041 g, 1.0 mmol, 5.0 eq) was dissolved in water (1.5 mL) and added to the solution. The mixture was stirred for 18 h at room temperature. The organic solvents were removed in vacuum and 2 N HCl (5 mL) was added to precipitate **21**, which was filtrated and isolated as a pale yellow solid (40 mg, 73%). ^{1}H NMR (500 MHz, DMSO-d_6): δ = 8.99 (d, *J* = 1.9 Hz, 1H), 8.25 (dd, *J* = 8.1, 2.3 Hz, 1H), 8.11 (d, *J* = 8.1 Hz, 1H), 7.54 (d, *J* = 1.7 Hz, 2H), 7.50 (t, *J* = 1.6 Hz, 1H), 1.35 (s, 18H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 166.16, 151.39, 147.82, 146.88, 139.51, 135.71, 135.49, 124.79, 122.56, 121.60, 34.78, 31.25. HRMS (MALDI): *m/z* calculated 312.19581 for $C_{20}H_{26}O_2N$ found 312.19626 ([M+H]$^+$).

3',5'-Di-*tert*-butyl-2-methyl-(1,1'-biphenyl)-4-carboxylic acid (22)

4-Bromo-3-methylbenzoic acid (**30j**, 0.22 g, 1.0 mmol, 1.0 eq), 3,5-di-*tert*-butylbenzeneboronic acid (**29j**, 0.28 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.32 g, 3.0 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 27 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed

by column chromatography with hexane/EtOAc/HOAc (87:11:2) as mobile phase to obtain **22** as colorless solid (0.11 g, 34%). R_f (hexane/EtOAc/HOAc = 87:11:2) = 0.2. 1H NMR (500 MHz, DMSO-d_6): δ = 7.87 (s, 1H), 7.83 - 7.79 (m, 1H), 7.41 (t, *J* = 1.8 Hz, 1H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.17 (d, *J* = 1.8 Hz, 2H), 2.29 (s, 3H), 1.32 (s, 18H). ^{13}C NMR (126 MHz, DMSO-d_6): δ = 167.32, 150.21, 146.50, 139.49, 135.24, 131.32, 129.96, 129.36, 126.93, 123.07, 120.84, 34.63, 31.28, 20.29. HRMS (MALDI): *m/z* calculated 324.20838 for $C_{22}H_{28}O_2$ found 324.20805 ([M]*).

3',5'-Di-*tert*-butyl-3-methyl-(1,1'-biphenyl)-4-carboxylic acid (23)

4-Bromo-2-methylbenzoic acid (**30k**, 0.22 g, 1.0 mmol, 1.0 eq), 3,5-di-*tert*-butylbenzeneboronic acid (**29j**, 0.28 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.32 g, 3.0 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 24 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (87:11:2) as mobile phase to obtain **23** as colorless solid (0.20 g, 61%). R_f (hexane/EtOAc/HOAc = 87:11:2) = 0.26. ^{1}H NMR (500 MHz, acetone-d_6): δ = 8.05 (d, *J* = 8.1 Hz, 1H), 7.62 (s, 1H), 7.60 - 7.57 (m, 1H), 7.55 (s, 3H), 2.67 (s, 3H), 1.39 (s, 18H). ^{13}C NMR (126 MHz, acetone-d_6): δ = 168.68, 152.12, 146.49, 141.59, 140.26, 132.28, 131.20, 129.13, 125.35, 122.93, 122.37, 35.58, 31.77, 22.07. HRMS (MALDI): *m/z* calculated 324.20838 for $C_{22}H_{28}O_2$ found 324.20701 ([M]*).

3-Methyl-3',5'-bis(trifluoromethyl)-(1,1'-biphenyl)-4-carboxylic acid (25)

4-Bromo-2-methylbenzoic acid (**30k**, 0.22 g, 1.0 mmol, 1.0 eq), (3,5-bis(trifluoromethyl)benzeneboronic acid (**29i**, 0.31 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.38 g, 3.6 mmol, 3.6 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 14 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (83:15:2) as mobile phase to obtain **25** as colorless solid (0.25 g, 72%). R_f (hexane/EtOAc/HOAc = 83:15:2) = 0.38. ^{1}H NMR (500 MHz, acetone-d_6): δ = 8.37 (s, 2H), 8.10 (t, *J* = 6.3 Hz, 1H), 8.08 (s, 1H), 7.86 - 7.83 (m, 1H), 7.80 - 7.77 (m, 1H), 2.70 (s, 3H). ^{13}C NMR (126 MHz, acetone-d_6): δ = 168.41, 143.36, 142.05, 141.77, 132.78, 132.51, 131.51, 131.15, 128.61, 125.63, 122.32, 21.83. HRMS (MALDI): *m/z* calculated 348.05795 for $C_{16}H_{10}O_2F_6$ found 348.05809 ([M]*).

2-(3-Methyl-3',5'-bis(trifluoromethyl)-(1,1'-biphenyl)-4-yl)acetic acid (26)

2-(4-Bromo-2-methylphenyl)acetic acid (**30l**, 0.23 g, 1.0 mmol, 1.0 eq), 3,5-bis(trifluoromethyl)benzeneboronic acid (**29i** 0.31 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.38 g, 3.6 mmol, 3.6 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 48 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by HPLC chromatography to obtain **26** as pale yellow solid (10 mg, 3%) ^{1}H NMR (500 MHz, MeOD-*d*$_4$): δ = 8.15 (s, 2H), 7.92 (s, 1H), 7.54 (s, 1H), 7.49 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 7.9 Hz, 1H), 3.72 (s, 2H), 2.41 (s, 3H). ^{13}C NMR (126 MHz, MeOD-*d*$_4$): δ = 175.09, 144.90, 139.52, 138.11, 135.98, 133.20, 132.42, 129.94, 128.26, 125.88, 123.84, 121.59, 39.53, 19.70. HRMS (MALDI): *m/z* calculated 362.07360 for $C_{17}H_{12}O_2F_6$ found 362.07449 ([M]$^+$).

2-(3',5'-di-tert-butyl-3-methyl-(1,1'-biphenyl)-4-yl)acetic acid (27)

2-(4-Bromo-2-methylphenyl)acetic acid (**30l**, 0.23 g, 1.0 mmol, 1.0 eq), 3,5-di-*tert*-butylbenzeneboronic acid (**29j**, 0.28 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.38 g, 3.6 mmol, 3.6 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 16 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (85:13:2) as mobile phase and in a second column chromatography with cyclohexane/EtOAc/HOAc (83:15:2) as mobile phase to obtain **27** as colorless solid (0.11 g, 32%). R_f (hexane/EtOAc/HOAc = 85:13:2) = 0.22. R_f (cyclohexane/EtOAc/HOAc = 83:15:2) = 0.25. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.47 – 7.40 (m, 5H), 7.31 (d, *J* = 7.8 Hz, 1H), 3.76 (s, 2H), 2.44 (s, 3H), 1.41 (s, 18H). ^{13}C NMR (126 MHz, $CDCl_3$): δ = 178.24, 151.15, 142.19, 140.47, 137.30, 130.85, 130.78, 129.71, 125.48, 121.85, 121.49, 38.79, 35.10, 31.67, 19.90. HRMS (MALDI): *m/z* 338.22403 calculated for $C_{23}H_{30}O_2$ found 338.22402.

3',5'-Dichloro-(1,1'-biphenyl)-3-carboxylic acid (28)

3-Iodobenzoic acid (**30g**, 0.25 g, 1.0 mmol, 1.0 eq), 3,5-dichlorobenzeneboronic acid (**29h**, 0.23 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.38 g, 3.6 mmol, 3.6 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 16 hours. The resulting mixture was cleared over celite 535, 20 ml EtOAc and 10 ml HOAc were added, and the phases were separated. The organic layer was washed with 2 N HCl (10 ml) and dried over $MgSO_4$, and the solvents were removed in vacuum. Further purification was performed by HPLC chromatography to obtain **28** as pale yellow solid (10 mg, 4%). ^{1}H NMR (500 MHz, acetone-*d*$_6$): δ = 8.29 (t, *J* = 1.7 Hz, 1H), 8.10 (dt, *J* = 7.8, 1.3 Hz, 1H), 7.98 (ddd, *J* = 7.8, 1.7,

1.2 Hz, 1H), 7.73 (d, *J* = 1.8 Hz, 2H), 7.66 (t, *J* = 7.8 Hz, 1H), 7.52 (t, *J* = 1.8 Hz, 1H).^{13}C NMR (126 MHz, acetone-d_6): δ = 167.28, 144.43, 139.35, 136.18, 132.51, 132.42, 130.52, 130.31, 129.00, 128.23, 126.64. HRMS (MALDI): *m/z* calculated 266.99741 for $C_{13}H_8O_2Cl_2$ found 266.99793 ($[M+H]^+$).

Ethyl 3'-ethoxy-(1,1'-biphenyl)-4-carboxylate (31)

Ethyl 4-bromobenzoate (**30d**, 0.20 ml, 1.2 mmol, 1.0 eq), 3-ethoxybenzeneboronic acid (**29k**, 0.24 g, 1.4 mmol, 1.2 eq) and sodium carbonate (0.40 g, 3.6 mmol, 3.0 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.069 g, 0.060 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 5.5 hours. The resulting mixture was cleared over celite 535 and 20 ml EtOAc and 10 ml HOAc were added. The organic solvents were washed with 2 N HCl (10 ml). Organic layers were dried over $MgSO_4$ and the solvent was removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc (9:1) as mobile phase to obtain **31** as colorless oil (85 mg, 26%). R_f (hexane/EtOAc = 9:1) = 0.44. ^{1}H NMR (300 MHz, acetone-d_6): δ = 8.11 – 8.06 (m, 2H), 7.80 – 7.76 (m, 2H), 7.42 – 7.36 (m, 1H), 7.28 – 7.22 (m, 2H), 6.97 (ddd, *J* = 8.2, 2.5, 1.0 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 4.13 (q, *J* = 7.0 Hz, 2H), 1.42 – 1.36 (m, 6H). ^{13}C NMR (75 MHz, acetone-d_6): δ = 166.55, 160.58, 146.15, 142.04, 130.91, 130.69, 130.38, 127.87, 120.15, 115.12, 114.08, 64.12, 61.46, 15.14, 14.62. MS (ESI+): *m/z* calculated 293.11 for $C_{17}H_{18}O_3Na$ found 293.03 ($[M+Na]^+$).

Ethyl 3'-*tert*-butyl-5'-methyl-(1,1'-biphenyl)-4-carboxylate (32)

4-Ethoxycarbonylbenzeneboronic acid (**29m**, 0.20 g, 1.1 mmol, 1.2 eq), 3-bromo-5-(*tert*-butyl)toluene (**30f**, 0.20 g, 0.90 mmol, 1.0 eq) and sodium carbonate (0.34 g, 3.2 mmol, 3.6 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 24 hours. The resulting mixture was cleared over celite 535 and 20 ml EtOAc and 10 ml HOAc were added. The organic solvents were washed with 2 N HCl (10 ml). Organic layers were dried over $MgSO_4$ and the solvent was removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc (9:1) as mobile phase to obtain **32** as colorless oil (0.19 g, 71%). R_f (hexane/EtOAc = 9:1) = 0.63. ^{1}H NMR (400 MHz, acetone-d_6): δ = 8.10 - 8.06 (m, 2H), 7.79 - 7.75 (m, 2H), 7.54 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 2H), 4.37 (q, *J* = 7.1 Hz, 2H), 2.41 (s, 3H), 1.43 – 1.35 (m, 12H). ^{13}C NMR (101 MHz, acetone-d_6): δ = 166.61, 152.63, 146.99, 140.43, 138.99, 130.66, 130.06, 127.91, 126.92, 126.01, 122.23, 61.40, 35.30, 31.70, 21.70, 14.64. MS (ESI+): *m/z* calculated 319.16 for $C_{20}H_{24}O_2Na$ found 319.11 ($[M+Na]^+$).

Ethyl 5-(3,5-di-*tert*-butylphenyl)pyridine-2-carboxylate (33)

Ethyl 5-bromopicolinate (**30i**, 0.23 g, 1.5 mmol, 1.0 eq), 3,5-di-*tert*-butylbenzeneboronic acid (**29j**, 0.28 g, 1.2 mmol, 1.2 eq) and sodium carbonate (0.38 g, 3.6 mmol, 3.6 eq) were dissolved in dioxane (10 ml) and water (2.5 ml). The mixture was stirred at room temperature

for 10 min while the solvents were degassed with argon. Then, $Pd(PPh_3)_4$ (0.058 g, 0.050 mmol, 0.050 eq) was added and the mixture was stirred under reflux for 20 hours. The resulting mixture was cleared over celite 535 and 20 ml EtOAc and 10 ml HOAc were added. The organic solvents were washed with 2 N HCl (10 ml). Organic layers were dried over $MgSO_4$ and the solvent was removed in vacuum. Further purification was performed by column chromatography with hexane/EtOAc/HOAc (87:11:2) as mobile phase to obtain **33** as colorless solid (60 mg, 18%). R_f (hexane/EtOAc/HOAc = 87:11:2) = 0.20. ^{1}H NMR (500 MHz, acetone-d_6): δ = 9.00 - 8.98 (m, 1H), 8.23 (dd, *J* = 8.1, 2.3 Hz, 1H), 8.16 - 8.13 (m, 1H), 7.63 – 7.60 (m, 3H), 4.41 (q, *J* = 7.1 Hz, 2H), 1.41 – 1.39 (m, 21H). ^{13}C NMR (126 MHz, acetone-d_6): δ = 165.75, 152.61, 149.08, 147.85, 141.13, 137.17, 135.93, 125.62, 123.75, 122.59, 61.83, 35.65, 31.71, 14.59. MS (ESI+): *m/z* calculated 340.22 for $C_{22}H_{30}NO_2$ found 340.19 ($[M+H]^+$).

Methods for in vitro characterization

Hybrid reporter gene assays

Plasmids: The Gal4-fusion receptor plasmids pFA-CMV-*NR*-LBD[1–3] coding for the hinge region and ligand binding domain (LBD) of the canonical isoform of the respective nuclear receptor have been reported previously. pFR-Luc (Stratagene) was used as reporter plasmid and pRL-SV40 (Promega) for normalization of transfection efficiency and cell growth. *Assay procedure*: HEK293T cells were cultured in DMEM high glucose with 10% FCS, sodium pyruvate (1 mM), penicillin (100 U/ml) and streptomycin (100 µg/ml) were added, at 37 °C and 5% CO_2. 24 h before transfection, HEK293T cells were seeded in 96-well plates ($3*10^4$ cells/well). Before transfection, medium was changed to Opti-MEM without supplements. Transient transfection was carried out using Lipofectamine LTX reagent (Invitrogen) according to the manufacturer's protocol with pFR-Luc (Stratagene), pRL-SV40 (Promega) and the corresponding Gal4-fusion nuclear receptor plasmid. 5 h after transfection, medium was changed to Opti-MEM supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml) and additionally containing 0.1% DMSO and the respective test compound or 0.1% DMSO alone as untreated control. Each concentration was tested in duplicates and each experiment was repeated independently at least three times. After overnight (14-16 h) incubation with the test compounds, cells were assayed for luciferase activity using Dual-Glo™ Luciferase Assay System (Promega) according to the manufacturer's protocol. Luminescence was measured with a Spark 10M luminometer (Tecan Deutschland GmbH). Normalization of transfection efficiency and cell growth was done by division of firefly luciferase data by renilla luciferase data and multiplying the value by 1000 resulting in relative light units (RLU). Fold activation was obtained by dividing the mean RLU of a test compound at a respective concentration by the mean RLU of untreated control. All hybrid assays were validated with reference agonists (PPARα: GW7647; PPARγ: rosiglitazone; PPARδ: L165,041; RXRα/β/γ: bexarotene; RARα/β/γ: tretinoin; LXRα/β: T09021317; FXR: GW4064; CAR: CITCO; VDR: calcitriol). which yielded EC_{50} values in agreement with literature. EC_{50}/fold activation for bexarotene (**1a**) in this system are: RXRα: 3.0±0.5 nM/125±3-fold; RXRβ: 9±2 nM/283±9-fold; RXRγ: 4±2 nM/76±6-fold.

Full-length reporter gene assays for RXR heterodimer activation[4,5]

HEK293T cells were grown in DMEM high glucose, supplemented with 10% FCS, sodium pyruvate (1 mM), penicillin (100 U/mL) and streptomycin (100 µg/mL) at 37 °C and 5% CO_2. The day before transfection, HEK293T cells were seeded in 96-well plates ($3\cdot10^4$ cells/well). Before transfection, medium was changed to Opti-MEM without supplements. Transient transfection was carried out using Lipofectamine LTX reagent (Invitrogen) according to the manufacturer's protocol with the reporter RXR-PPARγ responsive construct PPRE1-pGL3 or the RXR-LXR responsive construct ABCA1-pGL3 as well as pRL-SV40 (Promega). 5 h after transfection, medium was changed to Opti-MEM supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), now additionally containing 0.1% DMSO and the respective test compound or 0.1% DMSO alone as untreated control. Each concentration was tested in duplicates and each experiment was repeated independently at least three times. Following overnight (14-16 h) incubation with the test compounds, cells were assayed for luciferase activity using Dual-Glo™ Luciferase Assay System (Promega) according to the manufacturer's protocol. Luminescence was measured with a Spark 10M (Tecan Deutschland GmbH). Normalization of transfection efficiency and cell growth was done by division of firefly luciferase

data by renilla luciferase data and multiplying the value by 1000 resulting in relative light units (RLU). Fold activation was obtained by dividing the mean RLU of a test compound at a respective concentration by the mean RLU of untreated control.

Determination of aqueous solubility

Aqueous solubility of compound **24** was determined using Whatman Uniprep filters (Whatman plc, Maidstone, UK). 3 mg of each compound and 2 mL H_2O dest. were inserted into the Uniprep vessel and the mixture was shaken at 37 °C for 24 h. The mixture was then pressed through the Uniprep filter and the concentration of dissolved compound in filtrate was quantified by HPLC (Waters 600 Controller and Waters 2487 Dual Absorbance Detector equipped with a MultoHigh100 Phenyl 5 µ 240+4 mm column, CS-Chromatographie Service GmbH) using external calibration.

Metabolism assay

The solubilized test compound **24** (5 µL, final concentration 10 µM in phosphate buffer (0.1 M, pH 7.4)) was pre-incubated at 37 °C in 432 µL of phosphate buffer (0.1 M, pH 7.4) together with a 50 µL NADPH regenerating system (30 mM glucose-6-phosphate, 4 U/mL glucose-6-phosphate dehydrogenase, 10 mM NADP, 30 mM MgCl2). After 5 min, the reaction was started by the addition of 13 µL of microsome mix from the liver of Sprague−Dawley rats (Invitrogen; 20 mg protein/mL in 0.1 M phosphate buffer) in a shaking water bath at 37 °C. The reaction was stopped by addition of 500 µL of ice-cold methanol at 0, 15, 30 and 60 min. The samples were centrifuged at 5000 g for 5 min at 4 °C. The supernatants were analyzed and test compound was quantified by HPLC: mobile phase: MeOH 83%/H2O 17%/formic acid 0.1%; flow-rate: 1 mL/min; stationary phase: MultoHigh Phenyl phase, 5 µm, 250×4, precolumn, phenyl, 5 µm, 20×4; detection wavelength: 330 and 254 nm; injection volume: 50 µL. Control samples were performed to check the stability of **24** in the reaction mixture: first control was without NADPH, which is needed for the enzymatic activity of the microsomes, second control was with inactivated microsomes (incubated for 20 min at 90 °C), third control was without test compound (to determine the baseline). The amount of the test compound **24** was quantified by an external calibration curve, where data are expressed as mean±SEM of single determinations obtained in three independent experiments.

Protein purification, crystallization and structure determination

Recombinant ligand-binding domain of RXRα was expressed as an N-terminal His_6 fusion in *E. coli*, and was initially was purified using Ni^{2+} affinity chromatography. The histidine tag was then removed by treatment with TEV protease, and the cleaved protein was further purified by size exclusion chromatography. The pure protein in 20 mM Tris and 100 mM NaCl was concentrated to 11 mg/ml, and was mixed with **24** and a coactivator peptide (KHKILHRLLQDSSY) at 5 and 1.2 mM, respectively. The complex was crystallized using sitting drop vapor diffusion method at 20 °C and the solution containing 21% PEG 4000, 0.2 M ammonium acetate and 0.1 M tris, pH 7.5. Crystals were cryo-protected using the mother liquor supplemented with 25% glycerol. Diffraction data were collected at SLS beamline X06SA and were processed and scaled with XDS[6] and aimless[7], respectively. Initial structure was obtained by molecular replacement using Phaser[8] and the published coordinates of RXRα (PDB ID: 5LYQ[9]). Manual model rebuilding and structure refinement was performed in COOT[10] and REFMAC[11], respectively. Data collection and refinement statistics are summarized in Table S2.

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