Supplementary material

Antagonists for the orphan G protein-coupled receptor GPR55 based on a coumarin scaffold

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Chemical synthesis of coumarin derivatives

General procedures for the preparation of coumarin derivatives

Under an atmosphere of argon, 1.00 eq. of substituted salicylaldehyde, 1.20 eq. of potassium carbonate, 2.50 eq. of α,β-unsaturated aldehyde and 1.20 eq. of 1,3-dimethylimidazolium dimethylphosphate were suspended in toluene (3.3 mL/mmol salicylaldehyde). The reaction vessel was subjected to microwave irradiation to keep a constant temperature at 110 °C for 50 min (max. 200 watt) while being stirred. After cooling to rt the reaction was quenched by addition of water. The aqueous layer was extracted with EtOAc, the combined organic phases were dried over sodium sulfate and the solvent was removed under reduced pressure. The products were purified by flash column chromatography.

Analytical data of coumarin derivatives

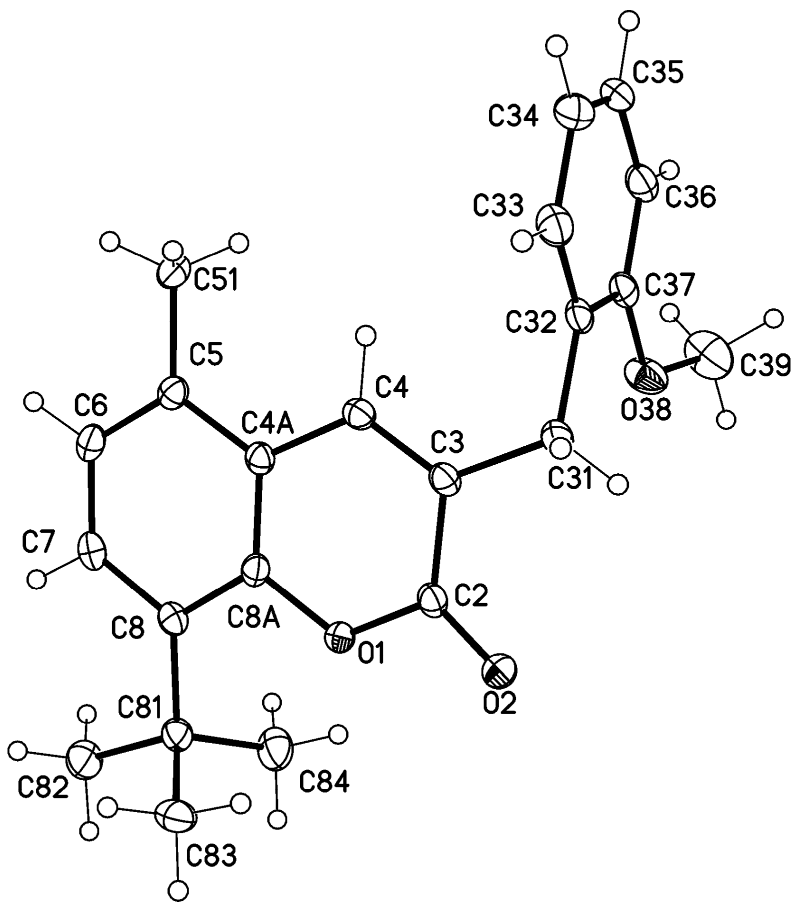


Figure S1. Crystal structure of **14** (displacement parameters are drawn at 50% probability level).

NMR spectral data

NMR spectral data of previously published compounds can be found in references 1 and 2.

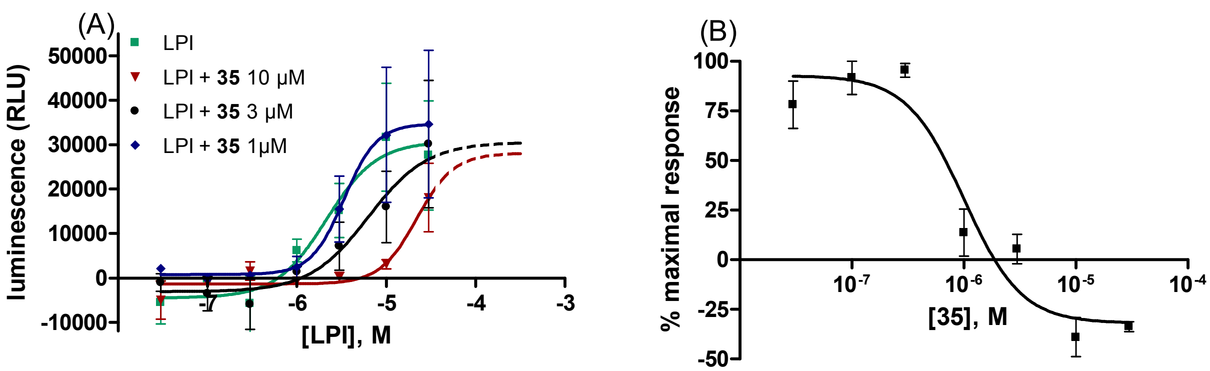


Figure S2. Antagonistic activity of **35** in β-arrestin recruitment assays performed with CHO cells stably expressing the human GPR55. (A) Concentration-dependent β-arrestin recruitment by the agonist LPI in absence and presence of **35** in different concentrations. EC_{50} values: LPI: 2.47 ± 0.89 µM; LPI + **35** (10 µM): 21.8 ± 1.8 µM; LPI + **35** (3 µM): 8.11 ± 2.15 µM; LPI + **35** (1 µM): 3.51 ± 0.46 µM. (B) Concentration-dependent inhibition of LPI (1 µM) effect on β-arrestin recruitment by **35**. IC_{50}: 0.981 ± 0.140 µM. Data points represent means ± SEMs of three independent experiments, performed in duplicates.

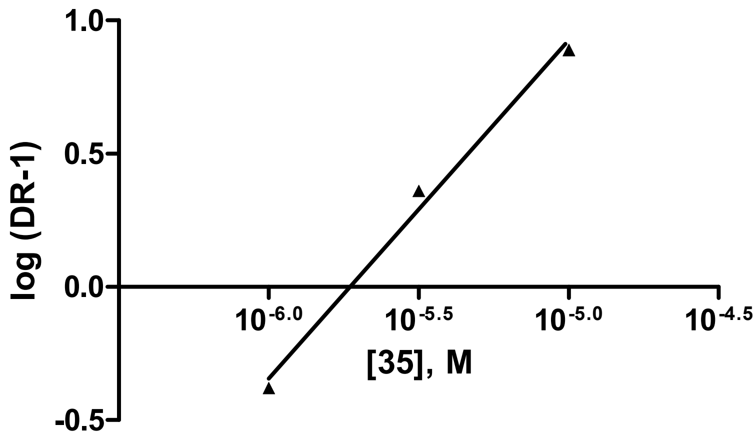


Figure S3. Schild regression for **35** antagonism of LPI-induced β-arrestin recruitment to human GPR55. The regression is linear (r^2: 0.99) with a slope of 1.270 ± 0.121. A K_B-value of 1.87 µM was determined.

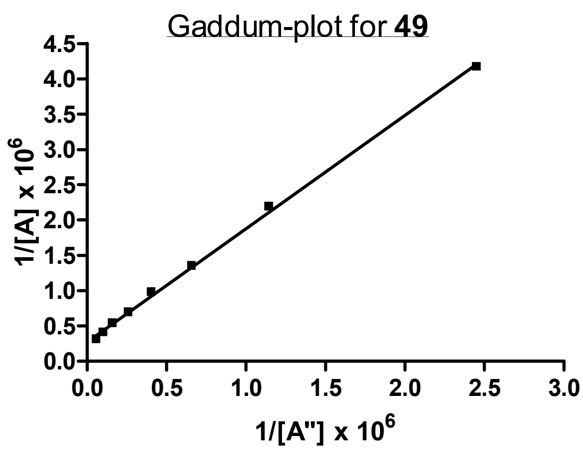


Figure S4. Gaddum plot for **49**. [A] and [A"] represent equiactive concentrations of the agonist LPI in the presence and absence of the allosteric modulator NV-435 (10 µM). The regression is linear (r^2: 0.99) with a slope of 1.605 ± 0.023. A K_B-value of 16.5 µM could be determined by application of the following equation: K_B = [Antagonist]/(slope – 1).

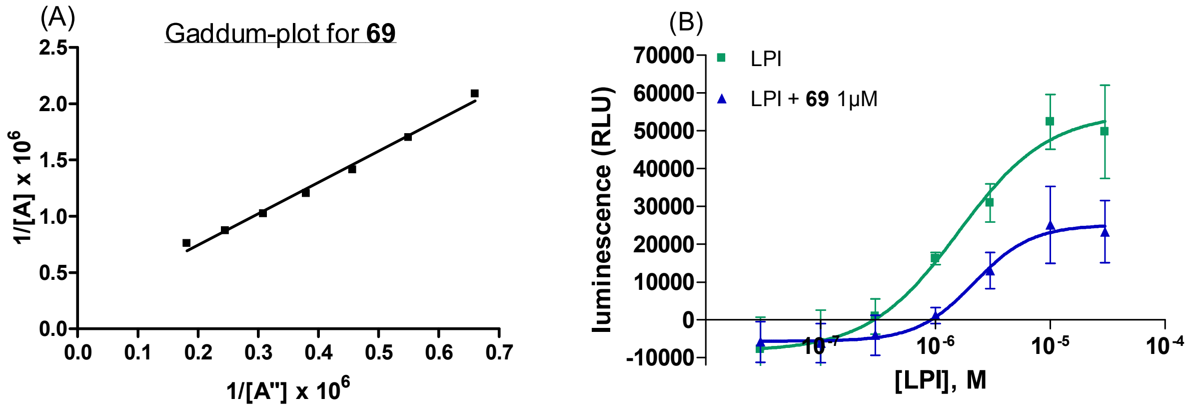


Figure S5. (A) Gaddum plot for **69**. [A] and [A"] represent equiactive concentrations of the agonist LPI in the presence and absence of the allosteric modulator **69** (1 µM). The regression is linear (r^2: 0.99) with a slope of 2.782 ± 0.123. A K_B-value of 0.561 µM was determined for **69** by application of the following equation: K_B = [Antagonist]/(slope – 1). (B) Concentration response curve of the agonist LPI in the absence and presence of the allosteric modulator **69** (1 µM). The determined pA_2-value was 0.483 ± 0.198 µM. The pA_2 value was determined using equiactive agonist concentrations at a level of 30% of the maximal response of the depressed concentration-response curve. Data points represent means ± SEMs of three independent experiments, performed in duplicates.

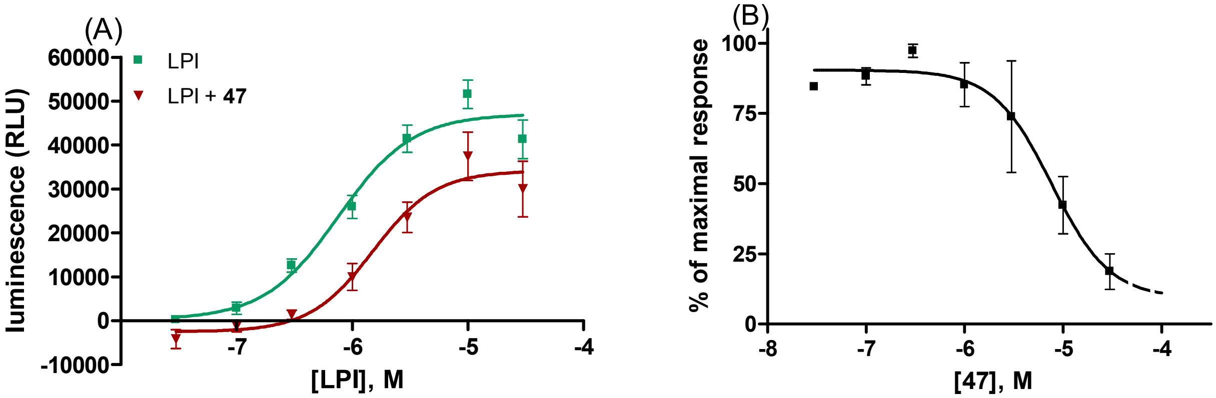


Figure S6. Antagonistic activity of **47** in β-arrestin recruitment assays performed with CHO cells stably expressing the human GPR55. (A) Concentration-dependent β-arrestin recruitment by the agonist LPI in the absence and presence of **47** (10 μM). EC_{50} values: LPI: 0.769 ± 0.056 μM; LPI + **47** (10 μM): 1.51 ± 0.12 μM. A pA_2 value of 11.2 ± 2.5 μM could be determined. (B) Concentration-dependent inhibition of LPI (1 μM) effect on β-arrestin recruitment by **47**. IC_{50}: 6.35 ± 2.66 μM. Data points represent means ± SEMs of three independent experiments, performed in duplicates.

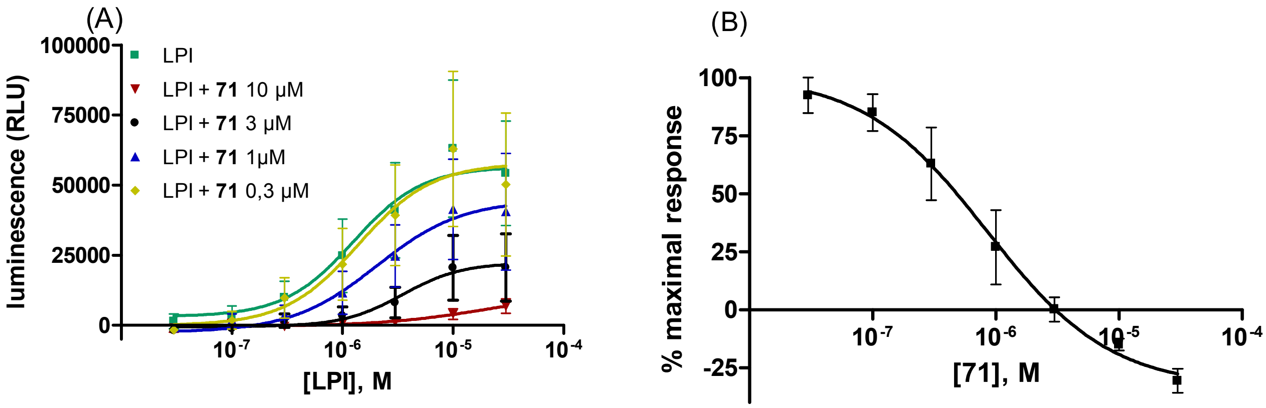
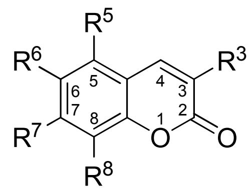


Figure S7. Antagonistic activity of **71** in β-arrestin recruitment assays performed with CHO cells stably expressing the human GPR55. (A) Concentration-dependent β-arrestin recruitment by the agonist LPI in the absence and presence of **71** (different concentrations). EC_{50} values: LPI: 1.59 ± 0.24 µM; LPI + **71** (3 µM): 5.22 ± 2.01 µM; LPI + **71** (1 µM): 2.23 ± 0.09 µM; LPI + **71** (0.3 µM): 1.56 ± 0.24 µM. A pA_2 value of 0.340 ± 0.071 µM could be determined. (B) Concentration-dependent inhibition of LPI (1 µM) effect on β-arrestin recruitment by **71**. IC_{50}: 0.854 ± 0.454 µM. Data points represent means ± SEMs of three independent experiments, performed in duplicates.

Table S1. Intrinsic activities of selected coumarin derivatives at CB_1 and CB_2 receptors[a]



Compd	R^3	R^5	R^6	R^7	R^8	human CB_1	human CB_2
						cAMP accumulation assay $EC_{50} \pm$ SEM (µM) (% of CP55,940 effect)[b]	
Standard agonists and antagonists							
CP55,940						0.00228[1, 23] (100)	0.00100[1, 23] (100)
rimonabant						(0)	(0)
Δ^9-THC						0.00676 ± 0.00361 (67)	0.0140 ± 0.0068 (34)
Coumarin derivatives I: with small 7-substituents							
13	2-methoxy-benzyl	methyl	H	H	methyl	n.d.[c] (7)	n.d.[c] (22)
31	3-methoxy-benzyl	methoxy	H	Br	H	n.d.[c] (0)	n.d.[c] (0)
35 (PSB-SB-258)	2-methoxy-benzyl	methyl	methoxy	methyl	methyl	n.d.[c] (0)	n.d.[c] (55)
43	3-methoxy-benzyl	methoxy	H	bromomethyl	H	n.d.[c] (23)	n.d.[c] (0)
Coumarin derivatives II: 7-pentyl-substitution							
46	4-methoxy-3,5-dimethyl-benzyl	methoxy	H	pentyl	H	n.d.[c] (39)	n.d.[c] (74)
47	2-methoxy-benzyl	methoxy	H	pentyl	H	0.0561[1] (93)	0.0139[1] (106)
48	2-hydroxy-benzyl	hydroxy	H	pentyl	H	n.d.[c] (109)	n.d.[c] (0)
49 (PSB-SB-435)	3-methoxy-benzyl	methoxy	H	pentyl	H	0.430[1] (58)	0.112[1] (93)

50	3-hydroxy-benzyl	hydroxy	H	pentyl	H	n.d.[c] (0)	n.d.[c] (0)
51	benzyl	methoxy	H	pentyl	H	n.d.[c] (58)	n.d.[c] (4)
52	benzyl	hydroxy	H	pentyl	H	n.d.[c] (0)	n.d.[c] (0)
53	2-methyl-benzyl	methoxy	H	pentyl	H	n.d.[c] (0)	n.d.[c] (57)
54	2-methyl-benzyl	hydroxy	H	pentyl	H	n.d.[c] (9)	n.d.[c] (12)
55	3-methyl-benzyl	methoxy	H	pentyl	H	n.d.[c] (0)	n.d.[c] (50)
56	3-methyl-benzyl	hydroxy	H	pentyl	H	n.d.[c] (42)	n.d.[c] (10)
57	2-chloro-benzyl	methoxy	H	pentyl	H	n.d.[c] (6)	n.d.[c] (47)
58	2-chloro-benzyl	hydroxy	H	pentyl	H	n.d.[c] (60)	n.d.[c] (2)
59	3-chloro-benzyl	methoxy	H	pentyl	H	n.d.[c] (0)	n.d.[c] (40)
60	3-chloro-benzyl	hydroxy	H	pentyl	H	n.d.[c] (70)	n.d.[c] (0)
61	4-chloro-benzyl	methoxy	H	pentyl	H	n.d.[c] (11)	n.d.[c] (47)
62	4-fluoro-benzyl	methoxy	H	pentyl	H	n.d.[c] (98)	n.d.[c] (91)
63	4-bromo-benzyl	methoxy	H	pentyl	H	n.d.[c] (17)	n.d.[c] (0)
Coumarin derivatives III: long, branched 7-substituent							
64	benzyl	methoxy	H	1,1-dimethylheptyl	H	n.d.[c] (27)	n.d.[c] (0)
65	benzyl	hydroxy	H	1,1-dimethylheptyl	H	n.d.[c] (84)	n.d.[c] (32)
66	2-methoxy-benzyl	methoxy	H	1,1-dimethylheptyl	H	n.d.[c] (0)	n.d.[c] (30)
67 (PSB-SB-1203)	2-hydroxy-benzyl	hydroxy	H	1,1-dimethylheptyl	H	n.d.[c] (0)	0.0542[1] (76)
69 (PSB-SB-487)	2-hydroxy-benzyl	hydroxy	H	1,1-dimethyloctyl	H	n.d.[c] (0)	n.d.[c] (52)
70	2-methoxy-benzyl	methoxy	H	1-butylcylo-pentyl	H	n.d.[c] (110)	n.d.[c] (3)
71	2-hydroxy-benzyl	hydroxy	H	1-butylcylo-pentyl	H	n.d.[c] (37)	0.0480[1] (106)
73	2-hydroxy-benzyl	hydroxy	H	1-butylcylco-hexyl	H	n.d.[c] (81)	0.179 (76)

[a]all data result from three independent experiments, performed in duplicates.

[b]effect of test compounds (10 µM) on inhibition of forskolin(10 µM)-stimulated cAMP production was related to the effect of the full agonist CP55,940 (=100%). CP55,940 was used in a concentration of 1 µM.

[c]n.d. not determined

Table S2. Intrinsic activities of coumarin derivatives at the orphan GPR18 and GPR55 receptors[a]

compd	β-arrestin recruitment assay			
	human GPR18 **EC_{50} ± SEM** (µM)	**human GPR18** **IC_{50} ± SEM** (µM)	**human GPR55** **EC_{50} ± SEM** (µM)	**human GPR55** **IC_{50} ± SEM** (µM)
Standard agonists and antagonists				
LPI	> 10 (44%)[b]	> 10 (15%)[c]	1.00 ± 0.25	n.d.[e]
CP55,940	> 10 (0%)[b]	5.99 ± 1.88	> 10 (4%)[d]	1.89[3]
rimonabant	> 10 (0%)[b]	10.1 ± 1.3	2.01[3]	n.d.[e]
Δ^9-THC	4.61 ± 0.50	> 10 (0%)[c]	> 10 (0%)[d]	14.2 ± 5.4
Coumarin derivatives I: with small 7-substituents				
10 (PSB-SB-115)	> 10 (10%)[b]	> 10 (11%)[c]	> 10 (0%)[d]	3.45 ± 0.36
11	> 10 (25%)[b]	> 10 (12%)[c]	> 10 (0%)[d]	5.33 ± 1.10
12 (PSB-SB-489)	> 10 (10%)[b]	> 10 (32%)[c]	> 10 (15%)[d]	1.77 ± 0.23
13	> 10 (7%)[b]	> 10 (0%)[c]	> 10 (0%)[d]	7.14 ± 2.66
14	> 10 (12%)[b]	11.3 ± 2.0	> 10 (0%)[d]	5.70 ± 1.62
15	> 10 (27%)[b]	> 10 (0%)[c]	> 10 (37%)[d]	> 10 (46%)[f]
16	> 10 (19%)[b]	> 10 (0%)[c]	> 10 (20%)[d]	> 10 (40%)[f]
17	> 10 (11%)[b]	> 10 (7%)[c]	> 10 (0%)[d]	> 10 (28%)[f]
18	> 10 (9%)[b]	> 10 (9%)[c]	> 10 (0%)[d]	> 10 (13%)[f]
19	> 10 (3%)[b]	> 10 (9%)[c]	> 10 (18%)[d]	~ 10 (54%)[f]
20	> 10 (4%)[b]	> 10 (0%)[c]	> 10 (0%)[d]	> 10 (45%)[f]
21	> 10 (18%)[b]	> 10 (0%)[c]	> 10 (11%)[d]	> 10 (16%)[f]
22	> 10 (8%)[b]	> 10 (0%)[c]	> 10 (10%)[d]	> 10 (7%)[f]
23	> 10 (1%)[b]	> 10 (0%)[c]	> 10 (18%)[d]	> 10 (5%)[f]
24	n.d.[e]	n.d.[e]	> 10 (0%)[d]	2.81 ± 1.16

25	> 10 (31%)[b]	> 10 (9%)[c]	> 10 (0%)[d]	> 10 (28%)[f]
26	> 10 (29%)[b]	> 10 (29%)[c]	> 10 (5%)[d]	> 10 (43%)[f]
27	> 10 (3%)[b]	> 10 (34%)[c]	> 10 (4%)[d]	> 10 (6%)[f]
28	> 10 (0%)[b]	> 10 (4%)[c]	> 10 (26%)[d]	> 10 (2%)[f]
29	> 10 (10%)[b]	> 10 (11%)[c]	> 10 (0%)[d]	9.38 ± 0.58
30	> 10 (0%)[b]	> 10 (13%)[c]	> 10 (0%)[d]	> 10 (54%)[f]
31	> 10 (0%)[b]	> 10 (31%)[c]	> 10 (0%)[d]	3.99 ± 0.75
32	> 10 (3%)[b]	> 10 (18%)[c]	> 10 (0%)[d]	> 10 (45%)[f]
33	> 10 (14%)[b]	> 10 (24%)[c]	> 10 (0%)[d]	6.74 ± 2.04
34	> 10 (9%)[b]	> 10 (14%)[c]	> 10 (39%)[d]	> 10 (42%)[f]
35 (PSB-SB-258)	> 10 (4%)[b]	> 10 (27%)[c]	> 10 (0%)[d]	0.981 ± 0.140
36	> 10 (0%)[b]	≥ 10 (46%)[c]	> 10 (0%)[d]	12.8 ± 3.2
37	> 10 (14%)[b]	> 10 (16%)[c]	> 10 (2%)[d]	9.32 ± 1.05
38	> 10 (0%)[b]	> 10 (32%)[c]	> 10 (0%)[d]	13.5 ± 4.3
39	> 10 (12%)[b]	≥ 10 (47%)[c]	> 10 (3%)[d]	10.3 ± 0.7
40	> 10 (4%)[b]	> 10 (33%)[c]	> 10 (0%)[d]	7.69 ± 1.71
41	> 10 (12%)[b]	> 10 (38%)[c]	> 10 (2%)[d]	5.16 ± 0.73
42	> 10 (13%)[b]	> 10 (25%)[c]	> 10 (1%)[d]	> 10 (13%)[f]
43	> 10 (9%)[b]	> 10 (27%)[c]	> 10 (8%)[d]	> 10 (18%)[f]
44	> 10 (12%)[b]	> 10 (13%)[c]	> 10 (0%)[d]	~ 10 (53%)[f]
45	> 10 (17%)[b]	> 10 (0%)[c]	> 10 (23%)[d]	> 10 (25%)[f]
Coumarin derivatives II: 7-pentyl-substitution				
46	> 10 (9%)[b]	> 10 (27%)[c]	> 10 (0%)[d]	> 10 (44%)[f]
47	> 10 (1%)[b]	> 10 (20%)[c]	> 10 (10%)[d]	6.35 ± 2.66
48	n.d.[e]	n.d.[e]	> 10 (0%)[d]	> 10 (37%)[f]
49 (PSB-SB-435)	> 10 (9%)[b]	> 10 (33%)[c]	> 10 (30%)[d]	3.23 ± 0.31

50	> 10 (0%)[b]	> 10 (37%)[c]	> 10 (25%)[d]	10.6 ± 4.9
51	> 10 (0%)[b]	≥ 10 (46%)[c]	> 10 (22%)[d]	> 10 (36%)[f]
52	> 10 (5%)[b]	> 10 (33%)[c]	> 10 (47%)[d]	> 10 (0%)[f]
53	> 10 (0%)[b]	> 10 (34%)[c]	> 10 (17%)[d]	5.08 ± 1.05
54	> 10 (0%)[b]	> 10 (10%)[c]	> 10 (26%)[d]	> 10 (19%)[f]
55	> 10 (0%)[b]	> 10 (27%)[c]	> 10 (39%)[d]	> 10 (27%)[f]
56	> 10 (0%)[b]	> 10 (33%)[c]	> 10 (19%)[d]	> 10 (19%)[f]
57	> 10 (0%)[b]	> 10 (25%)[c]	> 10 (26%)[d]	9.00 ± 2.44
58	> 10 (11%)[b]	> 10 (0%)[c]	> 10 (41%)[d]	> 10 (0%)[f]
59	> 10 (0%)[b]	≥ 10 (47%)[c]	> 10 (20%)[d]	> 10 (36%)[f]
60	> 10 (0%)[b]	> 10 (44%)[c]	> 10 (54%)[d]	> 10 (30%)[f]
61	> 10 (0%)[b]	> 10 (50%)[c]	> 10 (10%)[d]	3.29 ± 1.30
62	> 10 (0%)[b]	> 10 (35%)[c]	> 10 (0%)[d]	~ 10 (57%)[f]
63	> 10 (0%)[b]	> 10 (30%)[c]	> 10 (0%)[d]	3.76 ± 1.46
Coumarin derivatives III: long, branched 7-substituent				
64	> 10 (0%)[b]	> 10 (31%)[c]	> 10 (26%)[d]	~ 10 (51%)[f]
65	> 10 (0%)[b]	8.10 ± 0.58	> 10 (5%)[d]	0.358 ± 0.089
66	> 10 (1%)[b]	> 10 (26%)[c]	> 10 (57%)[d]	> 10 (25%)[f]
67 (PSB-SB-1203)	> 10 (0%)[b]	15.9 ± 4.9	> 10 (5%)[d]	0.261 ± 0.181
68	> 10 (5%)[b]	> 10 (33%)[c]	> 10 (43%)[d]	> 10 (31%)[f]
69 (PSB-SB-487)	> 10 (0%)[b]	12.5 ± 2.9	> 10 (0%)[d]	0.113 ± 0.020
70	> 10 (3%)[b]	> 10 (25%)[c]	> 10 (13%)[d]	> 10 (30%)[f]
71	> 10 (0%)[b]	≤ 10 (57%)[c]	> 10 (25%)[d]	0.759 ± 0.415
72	> 10 (0%)[b]	> 10 (33%)[c]	> 10 (12%)[d]	> 10 (44%)[f]
73	> 10 (0%)[b]	≤ 10 (59%)[c]	> 10 (7%)[d]	0.961 ± 0.431

[a]all data result from three independent experiments, performed in duplicates.
[b]effect of test compounds (10 µM) on β-arrestin recruitment to human GPR18 related to the effect of Δ^9-THC in a concentration of 10 µM (=100%).

[c]%inhibition of Δ^9-THC (10 μM)-mediated β-arrestin recruitment by test compounds in a concentration of 10 μM.
[d]effect of test compounds (10 μM) on β-arrestin recruitment to human GPR55 related to the effect of LPI in a concentration of 1 μM (=100%).
[e]n.d.=not determined.
[f]%inhibition of LPI (1 μM)-mediated β-arrestin recruitment by test compounds in a concentration of 10 μM.

References

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2. Rempel, V.; Fuchs, A.; Hinz, S.; Karcz, T.; Lehr, M.; Koetter, U.; Müller, C. E., Magnolia extract, magnolol and metabolites: activation of cannabinoid CB_2 receptors and blockade of the related GPR55. *ACS Med. Chem. Lett.* **2012,** *4*, 41-45.