Supplementary Material

Bicyclic imidazole-4-one derivatives: a new class of antagonists for the orphan G protein-coupled receptors GPR18 and GPR55

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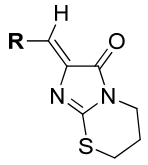
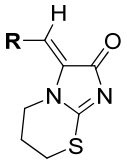
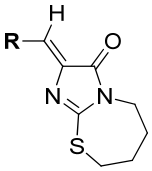
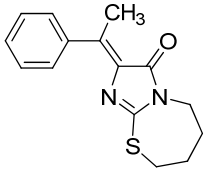
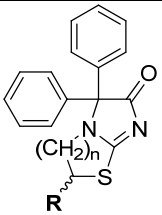
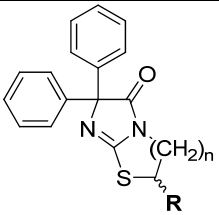
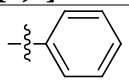
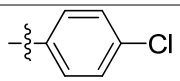
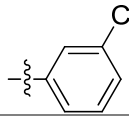
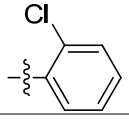
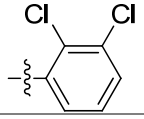
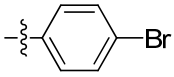
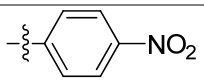
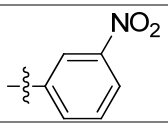
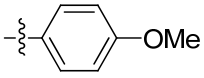
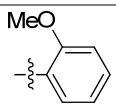
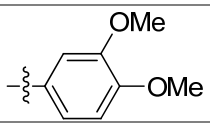
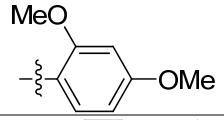
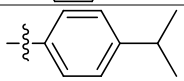
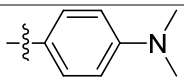
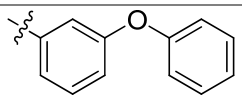
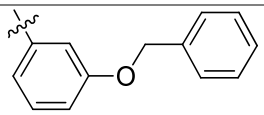
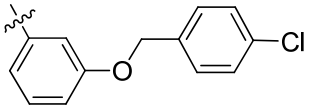
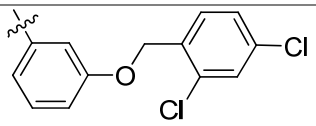
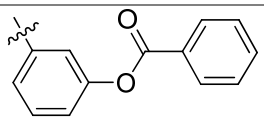
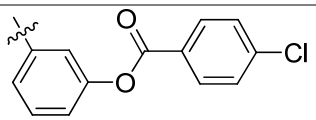
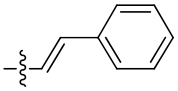
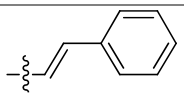
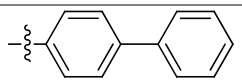
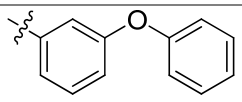
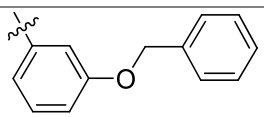
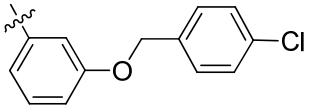
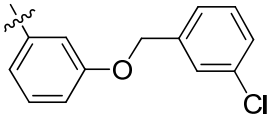
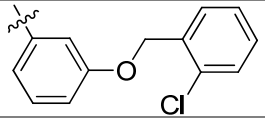
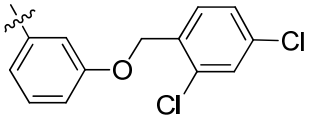
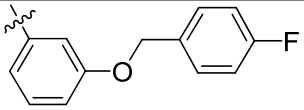
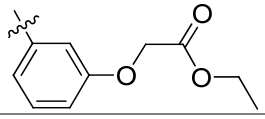
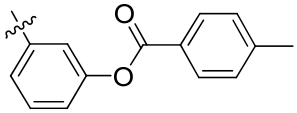
Table S1. Affinities of annelated imidazolone derivatives at human and rat CB_1 receptors[a]

					
A	**B**	**C**	**D**	**E**	**F**
10-38	**39**	**40-49**	**50**	**51**, **52**, **55**, **57**	**53**, **56**, **58**

		radioligand binding assays vs. [^{3}H]CP55,940	
compd.	**R**	**human CB_1** $K_i \pm$ SEM (μM) (% inhibition at 10 μM)[b]	**rat CB_1** $K_i \pm$ SEM (μM) (% inhibition at 10 μM)[b]
Imidazo[2,1-*b*][1,3]thiazin-3-ones (A)			
10		*>10 (40%)*	*>10 (1%)*
11		*>10 (25%)*	*>10 (15%)*
12		*>10 (30%)*	*>10 (6%)*
13		*>10 (0%)*	*>10 (5%)*
14		*>10 (12%)*	*>10 (6%)*
20		*>10 (29%)*	*>10 (21%)*
21		*>10 (0%)*	*>10 (23%)*
22		*>10 (19%)*	*>10 (8%)*
23		*>10 (39%)*	*>10 (5%)*
24		*>10 (4%)*	*>10 (4%)*
25		*>10 (7%)*	*>10 (3%)*

27		*>10 (42%)*	*>10 (12%)*
28		*>10 (12%)*	*>10 (6%)*
29		*>10 (29%)*	*>10 (6%)*
30		*>10 (29%)*	**6.58** ± 1.77
31		*>10 (25%)*	**4.22** ± 1.36
32		> 10 (0%)	**8.24** ± 4.11
34		**2.09** ± 0.08	**0.55** ± 0.22
36		*>10 (2%)*	*>10 (13%)*
37		*>10 (12%)*	*>10 (24%)*
38		*>10 (16%)*	*>10 (18%)*
39		*>10 (26%)*	*>10 (-14%)*
40		*>10 (-5%)*	*>10 (0%)*
41		**1.60** ± 0.42	**3.63** ± 0.21
42		**2.13** ± 0.47	**2.01** ± 2.25

43		**0.25** ± 0.06	**0.82** ± 0.09
44		**2.16** ± 0.07	**2.46** ± 0.21
45		**2.29** ± 0.46	*>10 (37%)*
46		**3.18** ± 0.49	**2.53** ± 0.89
47		**0.85** ± 0.03	**0.73** ± 0.04
48		**4.78** ± 1.70	*>10 (27%)*
49		*>10 (35%)*	*>10 (4%)*
Imidazo[2,1-b][1,3]thiazepin-3-ones (D)			
50	see above for structure	*>10 (10%)*	*>10 (3%)*
Imidazo[2,1-*b*]thiazol-6-ones (E, n = 1)			
51	-H	*>10 (30%)*	*>10 (13%)*
52	-$COOC_2H_5$	*>10 (26%)*	*>10 (0%)*
Imidazo[2,1-*b*]thiazol-5-ones (F, n = 1)			
53	-H	**21.1** ± 4.51	*>10 (15%)*
54	-$COOC_2H_5$	*>10 (2%)*	*>10 (38%)*
Imidazo[2,1-*b*][1,3]thiazin-2-ones (E, n = 2)			
55	-H	*>10 (13%)*	*>10 (4%)*

Imidazo[2,1-*b*][1,3]thiazin-3-ones (F, n = 2)			
56	-H	*>10 (25%)*	**7.68** ± 1.68
Imidazo[2,1-*b*][1,3]thiazepin-2-ones (E, n= 3)			
57	-H	*>10 (34%)*	**21.3** ± 2.17
Imidazo[2,1-*b*][1,3]thiazepin-3-ones (F, n=3)			
58	-H	**1.34** ± 0.37	**3.94** ± 0.10

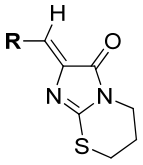
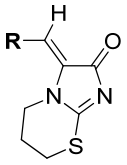
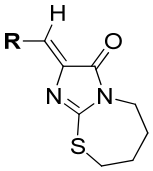
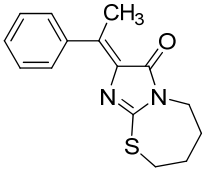
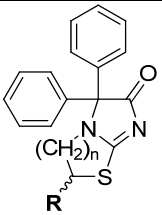
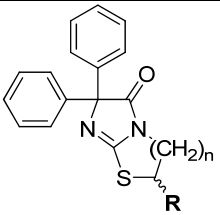
[a]All data result from three independent experiments, performed in duplicates.
[b]Percent inhibition of [^{3}H]CP55,940 binding (0.1 nM)

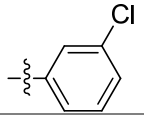
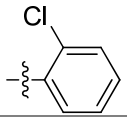
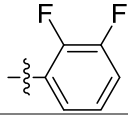
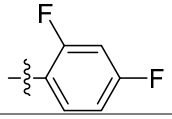
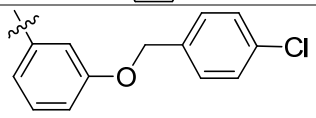
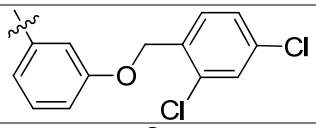
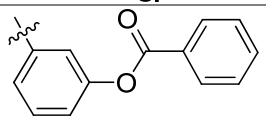
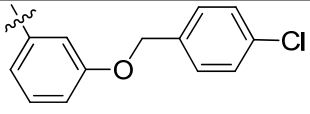
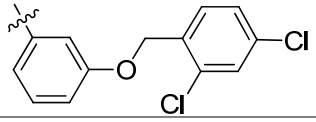
Table S2. Potencies of selected compounds at the human GPR35[a]

compd	**human GPR35** EC_{50} ± SEM (μM) (% of zaprinast activation)[b]	**human GPR35** IC_{50} ± SEM (μM) (% of zaprinast inhibition)[c]
10	>10 (-5%)	>10 (12%)
13	>10 (-22%)	≥10 (49%)
18	>10 (-4%)	>10 (17%)
27	>10 (-7%)	>10 (20%)
32	>10 (-1%)	>10 (3%)
43	>10 (6%)	>10 (1%)
44	>10 (-3%)	>10 (25%)

[a]Data represent means from three independent experiments, performed in duplicates.
[b]Zaprinast was used at a concentration of 30 μM (corresponding to a maximal effect). The measured effect was set as 100%.
[c]Zaprinast was used at a concentration of 5 μM (~EC_{80}).

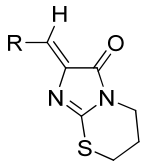
Table S3. Affinities of annelated imidazolone derivatives at GABAa receptors of rat brain cortex[a]

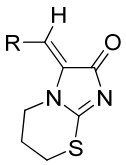
					
A	**B**	**C**	**D**	**E**	**F**
10-38	**39**	**40-49**	**50**	**51**, **52**, **55**, **57**	**53**, **56**, **58**

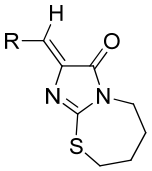
		radioligand binding assays vs. [^{3}H]diazepam
compd	**R**	**GABA$_A$ receptors**, rat brain cortical membranes $K_i \pm$ SEM (μM) (% inhibition)[b]
Imidazo[2,1-*b*][1,3]thiazin-3-ones (A)		
12		~10 (57%)
13		**4.15** ± 1.34
18		>10 (24%)
19		>10 (34%)
32		>10 (-18%)
34		>10 (-35)
36		>10 (-20%)
Imidazo[2,1-*b*][1,3]thiazepin-3-ones (C)		
43		>10 (-11%)
46		>10 (-30)

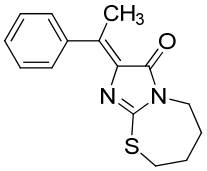
[a]All data result from three independent experiments, performed in duplicates.
[b]Percent inhibition of [^{3}H]diazepam binding (2 nM) by test compounds at a concentration of 10 μM.

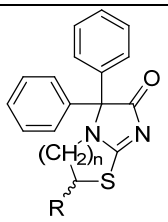
Table S4. Agonistic potencies of investigated compounds at human GPR18 and GPR55[a]

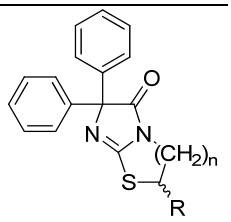

A

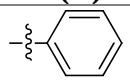
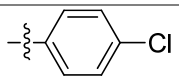
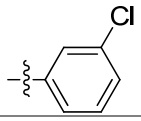
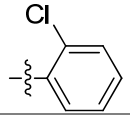
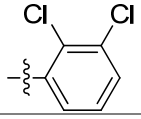
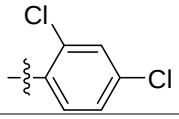
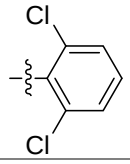
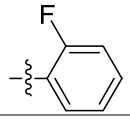
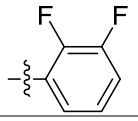
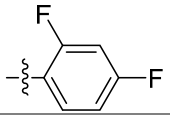

B

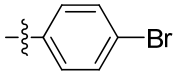
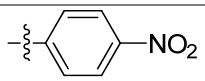
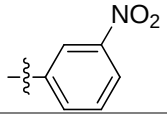
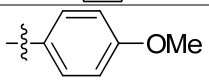
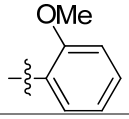
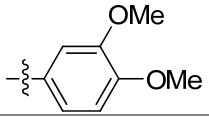
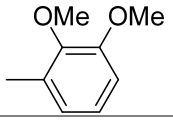
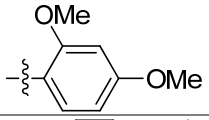
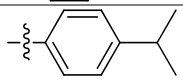
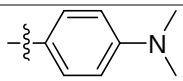
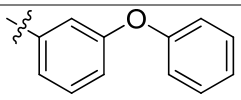
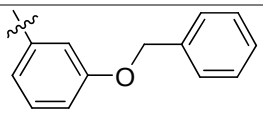
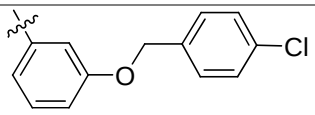
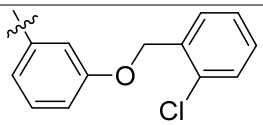

C

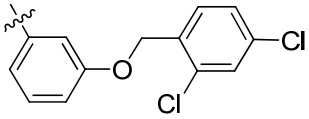
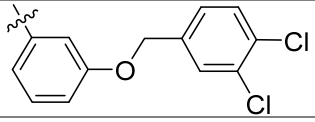
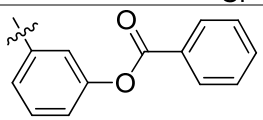
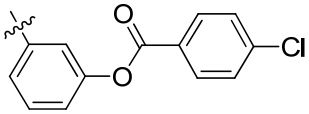
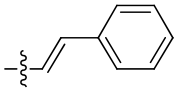
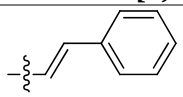
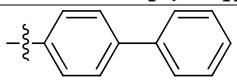
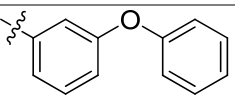
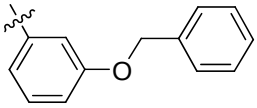
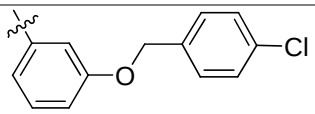
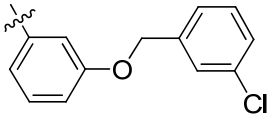
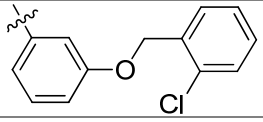

D

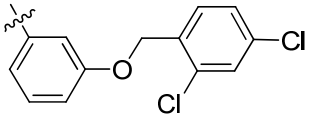
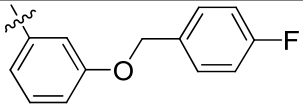
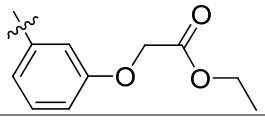
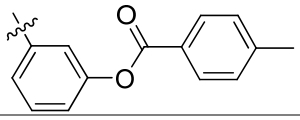

E


F

		β-arrestin recruitment assay	
compd	**R**	**human GPR18** $EC_{50} \pm$ SEM (μM) (% of Δ^9-THC activation)[b]	**human GPR55** $EC_{50} \pm$ SEM (μM) (% of LPI activation)[c]
Imidazo[2,1-*b*][1,3]thiazin-3-ones (A)			
10		>10 (7%)	>10 (32%)
11		>10 (13%)	>10 (0%)
12		>10 (0%)	>10 (9%)
13		>10 (20%)	>10 (27%)
14		>10 (17%)	>10 (3%)
15		>10 (0%)	>10 (0%)
16		>10 (12%)	>10 (15%)
17		>10 (6%)	>10 (0%)
18		>10 (4%)	>10 (0%)
19		>10 (11%)	>10 (0%)

20		>10 (0%)	>10 (26%)
21		>10 (0%)	>10 (39%)
22		>10 (2%)	>10 (21%)
23		>10 (0%)	>10 (37%)
24		>10 (0%)	>10 (12%)
25		>10 (0%)	>10 (6%)
26		>10 (10%)	>10 (0%)
27		>10 (0%)	>10 (0%)
28		>10 (0%)	>10 (13%)
29		>10 (17%)	>10 (20%)
30		>10 (5%)	>10 (13%)
31		>10 (0%)	>10 (37%)
32		>10 (0%)	>10 (21%)
33		>10 (8%)	>10 (10%)

34		>10 (0%)	>10 (12%)
35		>10 (0%)	>10 (4%)
36		>10 (0%)	≥10 (49%)
37		>10 (0%)	>10 (31%)
38		>10 (7%)	>10 (29%)
Imidazo[2,1-*b*][1,3]thiazin-2-ones (B)			
39		>10 (9%)	~10 (59%)
Imidazo[2,1-*b*][1,3]thiazepin-3-ones (C)			
40		>10 (6%)	>10 (30%)
41		>10 (1%)	>10 (5%)
42		>10 (7%)	>10 (4%)
43		>10 (0%)	**10.7** ± 0.3 (70%)
44		>10 (0%)	≥10 (52%)
45		>10 (0%)	>10 (7%)

46		>10 (0%)	>10 (16%)
47		>10 (0%)	>10 (19%)
48		>10 (19%)	>10 (0%)
49		>10 (0%)	>10 (25%)
Imidazo[2,1-b][1,3]thiazepin-3-ones (D)			
50	see above for structure	>10 (14%)	>10 (1%)
Imidazo[2,1-*b*]thiazol-6-ones (E, n = 1			
51	-H	>10 (21%)	≥10 (50%)
52	$-COOC_2H_5$	>10 (7%)	~10 (58%)
Imidazo[2,1-*b*]thiazol-5-ones (F, n = 1)			
53	-H	>10 (12%)	≥10 (50%)
54	$-COOC_2H_5$	>10 (11%)	>10 (39%)
Imidazo[2,1-*b*][1,3]thiazin-2-ones (E, n = 2)			
55	-H	>10 (11%)	~10 (53%)
Imidazo[2,1-*b*][1,3]thiazin-3-ones (F, n = 2)			
56	-H	>10 (14%)	>10 (19%)
Imidazo[2,1-*b*][1,3]thiazepin-2-ones (E, n = 3)			
57	-H	>10 (11%)	>10 (10%)
Imidazo[2,1-*b*][1,3]thiazepin-3-ones (F, n = 3)			

58	-H	>10 (15%)	>10 (54%)

[a]All data result from three independent experiments, performed in duplicates.
[b]THC was used in a concentration of 10 μM. The measured effect was set as 100%.
[c]LPI was used at a concentration of 1 μM. The measured effect was set as 100%.

Table S5. GTPγS binding studies of selected compounds at native rat cannabinoid CB_1 receptors (rat cortex) and human cannabinoid CB_1 or CB_2 receptors expressed in human embryonic kidney (HEK) (n=3)

	intrinsic activity of selected compounds at rat CB_1 receptors normalized with respect to the full agonist **7** set at 100 % (% ± SEM)	intrinsic activity of selected compounds at human CB_1 receptors normalized with respect to the full agonist **7** set at 100 % (% ± SEM)	intrinsic activity of selected compounds at human CB_2 receptors normalized with respect to the full agonist **7** set at 100 % (% ± SEM)
7	100[a]	100[a]	100[a]
6	-42 ± 14[b]	-80 ± 12[c]	n.d.[d]
30	-15 ± 5	- 49 ± 15	n.d.[d]
31	-9 ± 3	-33 ± 16	n.d.[d]
32	-7 ± 3	- 16 ± 9	n.d.[d]
34	-10 ± 3	- 51 ± 15	n.d.[d]
41	- 21 ± 2	- 37 ± 10	n.d.[d]
42	-26 ± 7	- 35 ± 11	n.d.[d]
43	-15 ± 4	- 40 ± 12	-59 ± 7
44	-12 ± 29	- 14 ± 12	n.d.[d]
46	-21 ± 3	- 44 ± 18	n.d.[d]
47	-16 ± 32	- 16 ± 3	n.d.[d]
56	-21 ± 3	- 31 ± 4	n.d.[d]
57	-19 ± 4	- 48 ± 20	n.d.[d]
58	-15 ± 5	- 16 ± 6	n.d.[d]

[a]the full agonist **7** led to a maximal stimulation of 132 ± 3 % at rat CB_1, 161 ± 11 % at human CB_1 and 156 ± 14 % (n=2) at human CB_2 receptors over basal (= 100 %)

[b]the full inverse agonist **6** reduced [^{35}S]GTPγS binding in rat cortical membranes from basal (= 100 %) to 88 ± 4 %

[c]the full inverse agonist **6** reduced [^{35}S]GTPγS binding in human CB_1-transfected human embryonic kidney (HEK293) cells from basal (= 100 %) to 53 ± 7 %

[d]not determined

Compound screening in cAMP accumulation assays at human CB_1 receptors

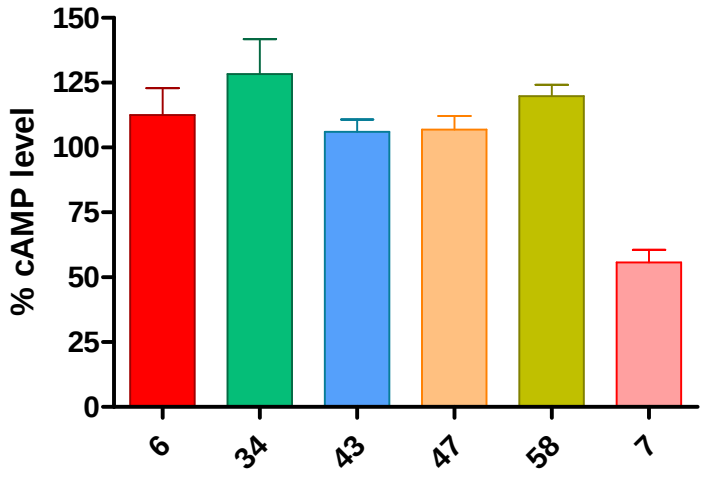


Figure S1. Effects on forskolin (10 µM)-induced cAMP accumulation in CHO cells stably expressing the human CB_1 receptor by test compounds at a concentration of 1 µM. AM281 (**6**) was used at a concentration of 250 nM and CP55,940 (**7**) at a concentration of 1 µM. Data are expressed as means ± SEM of at least three separate experiments performed in duplicates. While the agonist **7** inhibited cAMP accumulation, neither antagonist **6**, nor any of the test compounds led to an inhibition of cAMP accumulation at CB_1 receptors.

Compound screening in cAMP accumulation assays at human CB_2 receptors

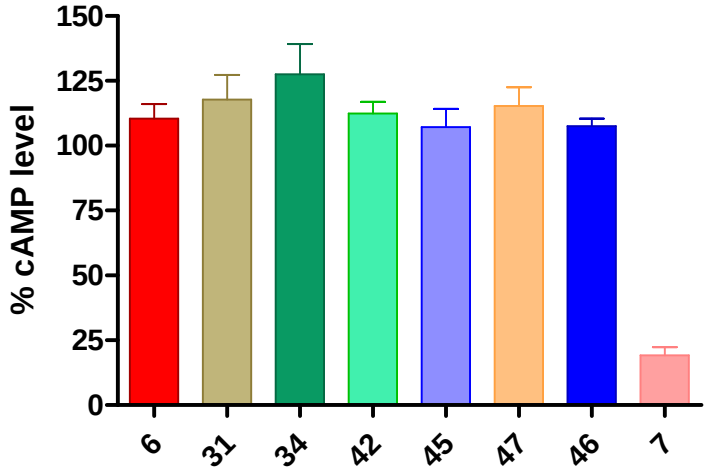


Figure S2. Effects on forskolin (10 µM)-induced cAMP accumulation in CHO cells stably expressing the human CB_2 receptor by test compounds at a concentration of 1 µM. AM281 (**6**) was used at a concentration of 10 µM and CP55,940 (**7**) at a concentration of 1 µM. Data are expressed as means ± SEM of at least three separate experiments performed in duplicates. While the agonist **7** strongly inhibited cAMP accumulation, none of the test compounds led to an inhibition of cAMP accumulation at CB_2 receptors.