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New bicyclic cannabinoid receptor-1 (CB_1-R) antagonists

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Abstract—A series of conformationally constrained bicyclic derivatives derived from SR141716 was prepared and evaluated as hCB_1-R antagonists and inverse agonists. Optimization of the structure–activity relationships around the 2,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one derivative **2a** led to the identification of two compounds with oral activity in rodent feeding models (**2h** and **4a**). Replacement of the PP group in **2h** with other bicyclic groups resulted in a loss of binding affinity.

Endocannabinoids are endogenous fatty acid-derived molecules (e.g., anandamide and 2-arachidonylglycerol) that exhibit a wide range of pharmacological activities such as stimulating appetite, decreasing locomotor activity, increasing pain sensitivity, and enhancing function of the immune system.[1,2] The biological properties of these substances are mediated through two G-protein coupled receptors—cannabinoid receptor type 1 (CB_1-R) which is expressed predominantly in the central nervous system (amygdala, cortex, hippocampus, and hypothalamus) and to a lesser extent in the periphery (gastric tract and adipose tissue) and cannabinoid receptor type 2 (CB_2-R) which is found in the spleen and in cells of the immune system (macrophages and T-lymphocytes).[3] In the central nervous system, endocannabinoids behave as retrograde signaling messengers that stimulate pre-synaptic receptors on neurons. CB_1-R activation results in inhibition of both adenylate cyclase activity and voltage-gated ion channels. Over the past years, numerous cannabinoid receptor modulators have been identified including non-selective CB_1-R/CB_2-R agonists such as CP-55940 and Δ9-tetrahydrocannabinol (a constituent of *Cannabis sativa* L. or marijuana)[4] and selective CB_1-R antagonists and inverse agonists such as SR141716 (**1**, rimonabant, Fig. 1).[5–7]

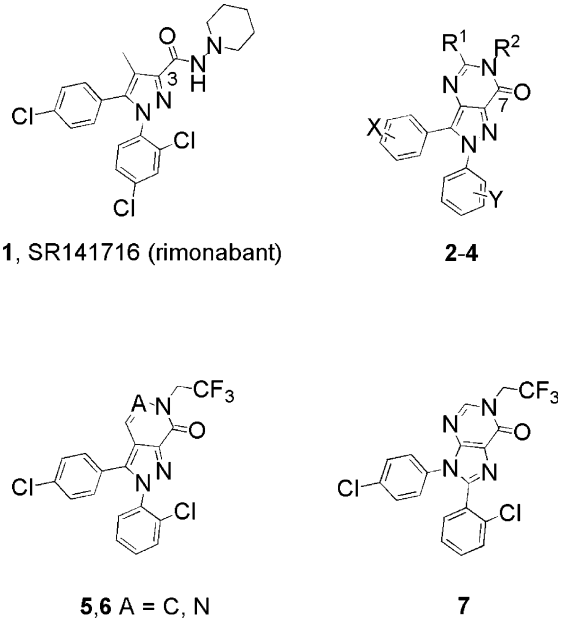

Figure 1. Structures of **1** and bicyclic derivatives **2**–**7**.

Keywords: Cannabinoid receptor antagonists; CB1-R; 2,6-Dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one; 2,6-Dihydro-pyrazolo[3,4-*c*]pyridin-7-one; 2,6-Dihydro-pyrazolo[3,4-*d*]pyridazin-7-one; 1,9-Dihydro-purin-6-one; Obesity; Conformational restriction.
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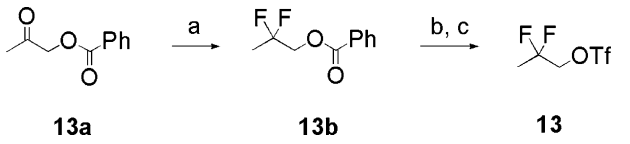
Overactivity of the endocannabinoid system has been implicated in the development of obesity, a disease of excess body weight that is associated with multiple co-morbidities such as hypertension, diabetes mellitus, and dyslipidemia.[8] Modulation of this system using CB_1-R antagonists/inverse agonists represents a promising new approach for reducing body weight, preventing weight re-gain, and decreasing co-morbidities associated with excess body fat.[9] One selective CB_1-R antagonist, SR141716, has recently completed phase III clinical trials, while several other antagonists are in earlier stages of clinical development.

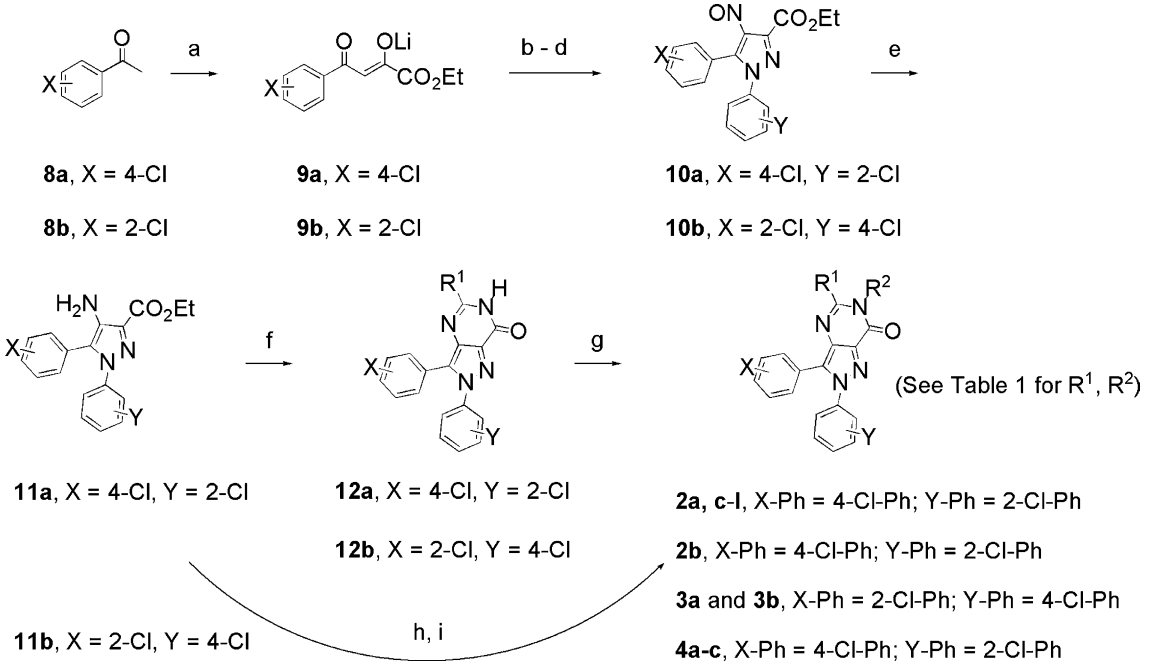
Several conformationally constrained analogs of the pyrazole-3-carboxamide derivative, SR141716, have recently been described.[10] As part of a program to identify new rigid analogs of SR141716 with improved CB_1-R potencies, a series of diaryl compounds containing different bicyclic scaffolds was prepared. Carboxamide groups were incorporated within the framework of various heterocyclic scaffolds, rather than appended to such systems. These compounds were specifically designed to explore alternative spatial arrangements of the key functional groups in SR141716, not to mimic the low-energy conformer. Herein, we describe the syntheses, structure–activity relationships, and biological activities of compounds **2**–**7** containing 2,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one (PP), 2,6-dihydro-pyrazolo[3,4-*c*]pyridin-7-one, 2,6-dihydro-pyrazolo[3,4-*d*]pyridazin-7-one, and 1,9-dihydro-purin-6-one groups (Fig. 1).

Bicyclic PP derivatives **2a**,**c**–**l** and **4a**–**c** were prepared from 4-chloroacetophenone **8a** (Scheme 1).[11] Deprotonation of **8a** with lithium 1,1,1,3,3,3-hexamethyldisilazane ($LiN(SiMe_3)_2$), followed by reaction with diethyl oxalate (EtO_2CCO_2Et) yielded **9a**. A three-step procedure involving nitrosation, 2-chlorophenylhydrazine addition, and cyclocondensation was used to convert **9a** into pyrazole-3-carboxylic acid ethyl ester **10a**. Reduction of the nitroso group in **10a** and treatment of the product **11a** with an appropriately substituted amidine derivative afforded PP **12a**. N-alkylation using commercially available R^2X provided compounds **2a**,**c**–**h**,**j**–**l** and **4a**–**c**. Triflate **13**, prepared in three steps from 1-benzoyloxypropan-2-one **13a**[12] (Scheme 2), was used to synthesize **2i**. Treatment of compound **11a** with *N*-aminopiperidine and trimethylaluminum ($AlMe_3$) and condensation of the resulting hydrazide intermediate with triethyl orthoformate yielded **2b**. PP compounds **3a**,**b** were prepared analogously to **2a**,**h** except for 2-chloroacetophenone **8b**, which was used in the first step and 4-chlorophenylhydrazine in the formation of pyrazole intermediate **10b**.

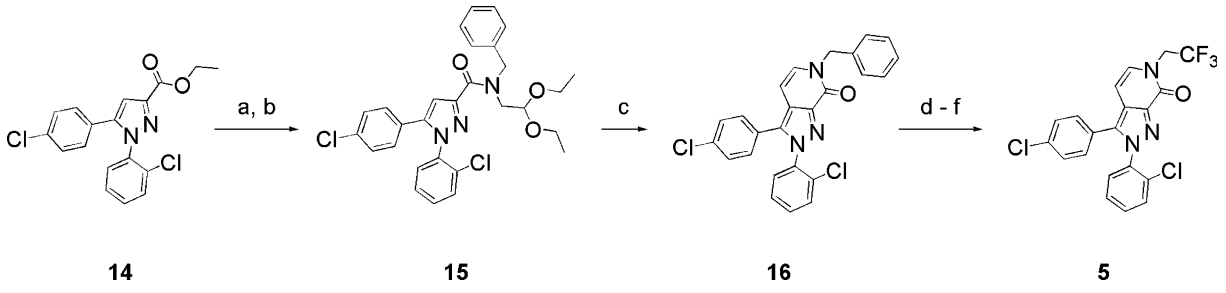
Pyrazole-3-carboxylic acid ester **14**[13] was used to prepare 2,6-dihydro-pyrazolo[3,4-*c*]pyridin-7-one **5** (Scheme 3). Hydrolysis of the ethyl ester group in **14**, followed by coupling of the resulting carboxylic acid moiety with



Scheme 2. Reagents: (a) (diethylamino)sulfur trifluoride, CH_2Cl_2, EtOH (94%); (b) NaOH/MeOH; (c) Tf_2O, DMAP, NEt_3, CH_2Cl_2 (72% from **13b**).



Scheme 1. Reagents: (a) $LiN(SiMe_3)_2$, EtO_2CCO_2Et, Et_2O, −78 °C to rt (100%); (b) $NaNO_2$, HOAc; (c) Y-PhNHNH$_2$·HCl; (d) *i*-PrOH, H_2SO_4 (56–71% from **9**); (e) $Na_2S_2O_4$ (3 equiv), EtOAc, H_2O (57–75%); (f) $R^1C(=NH)NH_2$·HOAc, EtOH, reflux (75–96%); (g) R^2X, Cs_2CO_3, DMF, 100–115 °C (35–55%); (h) *N*-aminopiperidine (1-piperidinamine), 2 M $AlMe_3$ in toluene, $CHCl_3$ (97%); (i) $CH(OEt)_3$, toluene, 0.45 h at 100 °C, 2 h at 120 °C (63%).

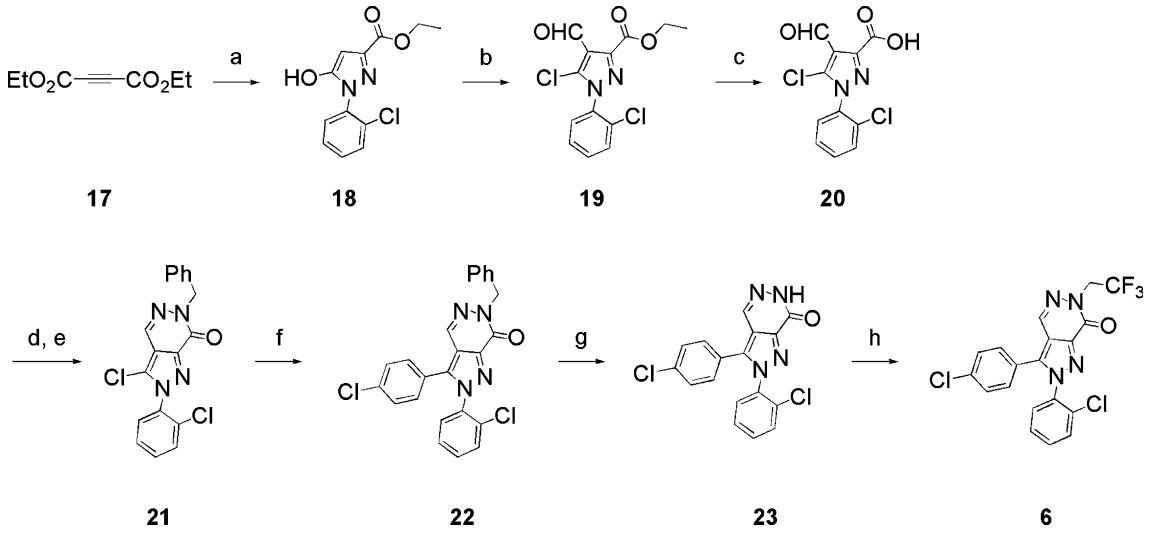


Scheme 3. Reagents and conditions: (a) KOH, EtOH; (b) *N*-benzyl-*N*-(2,2-diethoxyethyl)amine, EDC·HCl, EtN-*i*-Pr_2, CH_2Cl_2; (c) *p*-TsOH, toluene, reflux (16%); (d) $POCl_3$, reflux, 120 h (77%); (e) 3 M HCl, THF, 60 °C (80%); (f) CF_3CH_2OTf, Cs_2CO_3, DMF (79%).

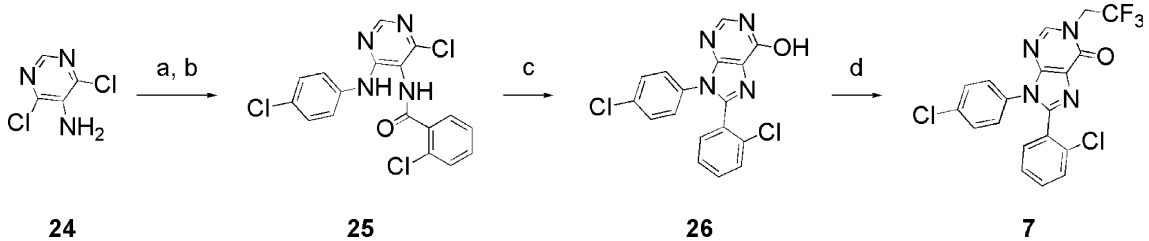
N-benzyl-*N*-(2,2-diethoxyethyl)-amine, yielded the pyrazole-3-carboxamide derivative **15**. Intramolecular cyclocondensation of **15** under acidic conditions, followed by removal of the N-benzyl protecting group and N-alkylation, afforded **5**.

2,6-Dihydro-pyrazolo[3,4-*d*]pyridazin-7-one **6** was prepared from diethylacetylene dicarboxylate (**17**) as shown in Scheme 4. Reaction of **17** with 2-chlorophenylhydrazine afforded the ethyl ester **18**, which was formylated using DMF in phosphorus oxychloride to give **19**. Hydrolysis of the ester group in **19** and reaction of the pyrazole-3-carboxylic acid **20** first with oxalyl chloride and then with benzylamine dihydrochloride provided the bicyclic pyrazolo[3,4-*d*]pyridazin-7-one derivative **21**. Palladium-catalyzed biaryl cross-coupling gave intermediate **22**. Removal of the N-benzyl protecting group in **22** and N-alkylation yielded **6**.

5-Amino-4,6-dichloropyrimidine (**24**) was used to prepare 1,9-dihydro-purin-6-one **7** (Scheme 5). Displacement of a chloro group in **24** with 4-chloro-aniline, followed by acylation of the resulting diamino derivative with 2-chlorobenzoyl chloride, gave intermediate **25**.



Scheme 4. Reagents and conditions: (a) 2-Cl-$PhNHNH_2$, K_2CO_3, EtOH (87%); (b) $POCl_3$, DMF (41%); (c) LiOH·H_2O, 2:1 MeOH/H_2O (100%); (d) ClCOCOCl, DMF; (e) $PhCH_2NHNH_2$·2HCl, NEt_3, CH_2Cl_2 (74% from **20**); (f) 4-Cl-$PhB(OH)_2$, $Pd(P(Ph_3)_4$, aqueous K_2CO_3, toluene/EtOH, 110 °C (97%); (g) $AlCl_3$, $PhNO_2$, 80 °C (90%); (h) CF_3CH_2I, NaH, DMF, rt (23%).



Scheme 5. Reagents and conditions: (a) 4-Cl-$PhNH_2$, concd HCl, H_2O, EtOH, 19 h at 82 °C, 60 h at rt (95%); (b) 2-Cl-PhCOCl, CH_3CONMe_2, 0 °C to rt (100%); (c) H_2SO_4, *i*-PrOH, 8 h at reflux, 16 h at rt (90%); (d) CF_3CH_2I, Cs_2CO_3, DMF, 100 °C (50%).

The synthesis of **7** was completed from **25** by a three-step, two-pot procedure that involved cyclocondensation, hydrolysis, and N-alkylation.

Binding affinities of bicyclic derivatives **1**–**7** were determined with standard competitive binding assays in HEK-293 cells transfected with the human CB_1-R (hCB_1-R) using [^{3}H]-SR141716 (Amersham) as a radioligand and in CHO-K1 cells overexpressing human CB_2-R (hCB_2-R) using [^{3}H]-5-(1,1-dimethyl-heptyl)-2-[5-hydroxy-2-(3-hydroxy-propyl)-cyclohexyl]-phenol as a radioligand. Functional activities of **1**–**7** were determined in GTP-γ-^{35}S binding assays using membranes from CHO-K1 cells stably transfected with hCB_1-R cDNA.

Conformationally constrained analogs **2a**,**b** with bicyclic PP rings were slightly less active in the hCB_1-R binding assay than SR141716 (Table 1). The two PP analogs, which have only hydrogen bond accepting capability, exhibited surprisingly high affinities despite the poor overlap of both the core 2,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one rings and the R^2 side chains with the corresponding pyrazole-3-carboxamide and 1-piperidinyl groups in the low-energy conformer of SR141716 (see Fig. 2). These results suggest that the PP analogs may bind differently than SR141716 in hCB_1-R, with the non-aryl R^1 side chains in **2a**,**b** occupying a separate hydrophobic pocket, distinct from that for the 1-piperidinyl group in SR141716 and the hydrogen bond-accepting groups (i.e., oxygen atoms in the C7 carbonyl moiety) forming different networks of hydrogen bond interactions. It is also possible the rigid PP compounds induce conformational changes in hCB_1-R that allow the receptor to accommodate the R^1 side chains in the same hydrophobic pocket occupied by the 1-piperidinyl group in SR141716 ('induced fit' model of binding). The PP derivatives **2a**,**b** contain alternative spatial arrangements of the key pharmacophoric groups in SR141716 that do not result in any significant loss of binding activity.

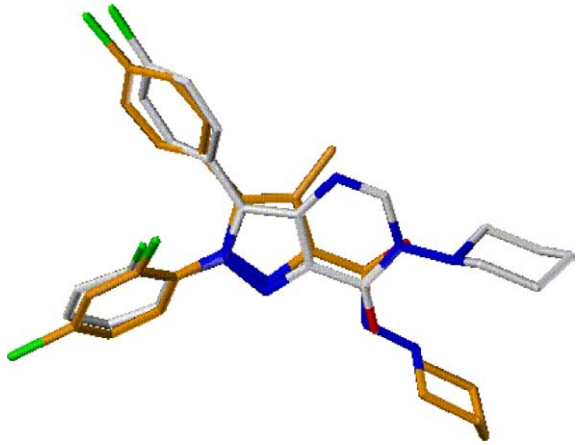


Figure 2. Superimposition of minimized structures **1** and **2b**.[14]

Structure–activity relationships around **2a** were investigated. The isomeric dichloro analog **3a** was less potent than **2a**. Trichloro compounds with the identical substituent pattern on the diaryl groups in SR141716 were not prepared. Replacement of the N6 cyclohexyl group with branched alkyl groups had no effect on activity (**2c**,**d**). Introduction of small alkyl (i.e., ethyl and *n*-propyl), fluorinated alkyl, or benzyl groups increased affinity

Table 1. Binding and functional activities of compounds **1**–**7**

Compound no.	R^1	R^2	hCB_1-R binding K_i (nM)[a,b]	hCB_2-R binding K_i (nM)[a]	GTP-γ-^{35}S binding K_i (nM)[a]
1	—	—	2.1 ± 1.6[c]	[e]	1
2a	H	*c*-Hex	20 ± 10[c]	>10,000	[e]
2b	H	1-Piperidinyl	12 ± 8[c]	>10,000	20
2c	H	$CH(CH_2CH_3)_2$	26	[e]	[e]
2d	H	$CH(CH_3)_2$	21	[e]	[e]
2e	H	CH_2CH_3	4	>10,000	4
2f	H	$CH_2CH_2CH_3$	2	>10,000	2
2g	H	CH_2Ph	5	>10,000	4
2h	H	CH_2CF_3	0.3 ± 0.1[d]	>10,000	0.1
2i	H	$CH_2CF_2CH_3$	0.2	>10,000	0.1
2j	H	$CH_2CH_2CF_3$	1.8 ± 0.6[c]	>10,000	4
2k	H	CH_2CHF_2	0.6	>10,000	1
2l	H	CH_2CH_2F	3	>10,000	5
3a	H	*c*-Hex	38	>10,000	[e]
3b	H	CH_2CF_3	28	>10,000	[e]
4a	Me	CH_2CF_3	0.6 ± 0.1[c]	>10,000	1
4b	Et	CH_2CF_3	1	>10,000	3
4c	*i*-Pr	CH_2CF_3	6	>10,000	13
5	—	—	1	>10,000	1
6	—	—	8	>10,000	4
7	—	—	1	>10,000	1

[a] See Ref. 15 for details.
[b] Data are mean of one independent determination performed in triplicate, except as noted.
[c] Data are means of two independent determinations performed in triplicate.
[d] Data are means of three independent determinations performed in triplicate.
[e] Not measured.

(**2e**–**g**). Compounds **2h**,**i** with 2′,2′,2′-trifluoroethyl and 2′,2′-difluoropropyl groups were 10-fold more potent than the corresponding ethyl and *n*-propyl alkyl derivatives **2e** and **f** and 60-fold more potent than **2a**, the benchmark compound. The position of the fluoro substitution on the N6 alkyl chain was important since no increase in affinity was observed when the *n*-propyl group in **2f** was replaced with a 3′,3′,3′-trifluoropropyl group as in **2j**. Removal of one or two fluorine atoms in **2h** led to a decrease in affinity (**2k**,**l**). The fluorine atoms in **2h**,**i**, and **k** may favorably modulate the electronic character of the PP ring or act as weak hydrogen bond-accepting groups in hCB_1-R. No increase in binding affinity was observed when the N6 cyclohexyl group in **3a** was replaced with a 2′,2′,2′-trifluoroethyl group (**3b**).

Substitution at the C5 position in PP analog **2h** was examined. Introduction of a C5 methyl group did not affect binding affinity (**4a**). However, incorporation of larger alkyl groups (Et and *i*-Pr) resulted in a small loss of activity (**4b**,**c**).

The core bicyclic PP ring in **2h** was replaced with other heterocyclic scaffolds. The 2,6-dihydro-pyrazolo[3,4-*c*]pyridin-7-one **5** and the 2,6-dihydro-pyrazolo[3,4-*d*]pyridazin-7-one **6** were, respectively, 3- and 26-fold less potent in the binding assay than **2h**. The weakly basic derivative, 1,9-dihydro-purin-6-one **7**, was equipotent to **5**. The decreases in potency between these new heterocyclic analogs and **2h** were attributed to differences in the electronic characteristics and lipophilic properties of the bicyclic rings.

Rigid PP analogs **2b**,**e**–**l** and **4a**–**c** and heterocyclic derivatives **5**–**7** were shown to be potent antagonists in the GTP-γ-^{35}S assay, with K_i values less than 20 nM (Table 1). As with binding affinity, the presence of a hydrogen bond donor group was not critical for functional activity. All compounds inhibited basal GTP-γ-^{35}S binding, thus demonstrating inverse agonism activity (data not shown). Compounds **2a**,**b**,**e**–**l**, **4a**–**c**, and **5**–**7** were highly selective for hCB_1-R over hCB_2-R.

Analogs **2h** and **4a** were selected for further evaluation in animal models. These two compounds were first assessed for their ability to reverse CB_1-R agonist-stimulated analgesia and hypothermia in mice, two of the four assays that constitute the 'tetrad'.[16] In these challenge assays, activity is influenced by a combination of factors such as in vitro binding/functional potency, plasma protein binding, plasma half-life, and brain penetration. Both rigid compounds were able to fully reverse CP-55940-stimulated responses at 1 mg/kg sc. Compounds **2h** and **4a** were also examined in a fasting-induced re-feeding model in Sprague–Dawley rats to assess CB_1-R mediated anorectic activity. Both compounds decreased food intake over a 1.5 h time period in a dose-dependent manner (**2h**, −23%, −32%, and −78% change relative to vehicle-treated control animals at 0.3, 1, and 3 mg/kg po; **4a**, −35%, −42%, and −59% change relative to vehicle-treated control animals at 1, 3, 10 mg/kg po).[17] These PP analogs showed good brain penetration (brain to plasma or B/P ratio for **2h** at the 1.5 time point following a dose of 1 mg/kg po = 7.24; B/P ratio for **4a** at the 1.5 h time point following a 3 mg/kg po dose = 1.92).

Table 2. Pharmacokinetic properties of **2h** and **4a**

		Compound **2h**	Compound **4a**
Rat PK[a]	Cl (ml/min/kg)	45	32
	V_d (l/kg)	9.2	7.7
	Half life, $t_{1/2}$ (h)	2.6	4.7
	F (%)	62	7
Dog PK[b]	Cl (ml/min/kg)	0.54	10
	V_d (l/kg)	10	17
	$t_{1/2}$ (h)	223	39

[a] See Ref. 18 for details.
[b] See Ref. 19 for details.

Pharmacokinetic (PK) properties of **2h** and **4a** were measured in rats and dogs (Table 2). Amorphous solids were used in intravenous formulations, crystalline solids for oral delivery. For both compounds, plasma clearances (Cl) were moderate in the rat, but low in the dog. Volumes of distribution (V_d) were high, attributed primarily to the presence of lipophilic 2′,2′,2′-trifluoroethyl groups. Bioavailability (F) in the rat was moderate for **2h**, but very low for **4a**. Good plasma exposure of the amorphous form of **4a** was observed at the 1.5 h time point in the food intake studies (i.e., 209 ng/mL at 3 mg/kg po), suggesting the poor bioavailability of the crystalline form was due to solubility-limited absorption, rather than low intestinal permeability. Introduction of a methyl substituent at the C5 position in **2h** resulted in minimal effects on most PK properties, the only exception being bioavailability.

In conclusion, conformationally constrained derivatives of SR141716 were prepared and evaluated as hCB_1-R antagonists and inverse agonists. Two 2,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-ones **2a**,**b** exhibited high binding affinities despite showing poor overlap with SR141716. These compounds may bind differently than SR141716 in hCB_1-R or may induce conformational changes in the receptor that then allow binding. Analogs of **2a** showed good anorectic activities in a fasting-induced re-feeding model in rats following oral administration. Novel compounds containing other bicyclic ring systems will be the subject of future publications.

Acknowledgments

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17. Overnight fasted male CD rats were dosed the following morning with suspensions of compounds **2h** and **4a** in 0.5% methylcellulose. Food was returned 0.5 h after dosing, and cumulative food intake was assessed 1.5 h later.
18. Male Sprague–Dawley rats were dosed intravenously with **2h** and **4a** via a jugular vein cannula (1 mg/kg). Compound **2h** was formulated in 20% EtOH/80% CD, compound **4a** in in Transcutol P/CD. For oral administration, compounds **2h** and **4a** were formulated in 0.5% methylcellulose. Plasma concentrations were determined by LC–MS/MS following protein precipitation.
19. Male beagle dogs were dosed intravenously with **2h** and **4a** (0.5 mg/kg iv). Compound **2h** was formulated in 6.7% EtOH/60% CD/33.3% PEG 400, compound **4a** in Transcutol P. Plasma concentrations were determined by LC–MS/MS following protein precipitation.