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Tetrahydroindazole derivatives as potent and peripherally selective cannabinoid-1 (CB1) receptor inverse agonists

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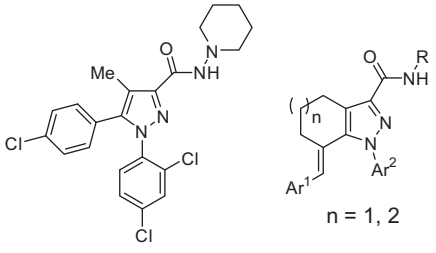
ABSTRACT

A series of potent and receptor-selective cannabinoid-1 (CB1) receptor inverse agonists has been discovered. Peripheral selectivity of the compounds was assessed by a mouse tissue distribution study, in which the concentrations of a test compound in both plasma and brain were measured. A number of peripherally selective compounds have been identified through this process. Compound **2p** was further evaluated in a 3-week efficacy study in the diet-induced obesity (DIO) mouse model. Beneficial effects on plasma glucose were observed from the compound-treated mice.

The cannabinoid receptors are part of the endocannabinoid system, which regulates many important biological processes. Two such receptors have been identified to date as cannabinoid-1 (CB1) receptor[1] and cannabinoid-2 (CB2) receptor.[2] The CB2 receptor is mainly located in the immune system and regulates inflammatory responses.[3] The CB1 receptor is expressed abundantly in the central nervous system (CNS) and in peripheral tissues such as liver, skeletal muscle, adipose tissue, and pancreas.[4] Antagonism of the CB1 receptor leads to decreasing food intake and increasing insulin sensitivity, and has emerged as an attractive approach to treat obesity and related metabolic diseases such as type 2 diabetes. Unfortunately, undesirable psychiatric side effects led to the withdrawal of the only marketed brain penetrating CB1 receptor inverse agonist/antagonist, rimonabant **1**, as well as the termination of the development of other clinical-stage agents in this class. However, in recent years, an increasing amount of evidence suggested that some of the metabolic benefits seen in treatment with CB1 receptor inverse agonists/antagonists may result from actions in the peripheral tissues. Therefore peripherally restricted CB1 receptor inverse agonists/antagonists that do not cross blood–brain barrier (BBB) may be useful therapeutics for metabolic disease without causing CNS-mediated adverse effects.[5–9]

Herein, we report the discovery of a series of potent and peripherally selective CB1 receptor inverse agonists **2** that originated from our earlier brain-penetrant CB1 receptor inverse agonist program (Fig. 1).[10] The synthesis of compound **2** is illustrated in Scheme 1. Cyclohexanone ($n = 1$) or cycloheptanone ($n = 2$) **3** was reacted with aryl aldehyde **4** in aqueous sodium hydroxide solution at elevated temperature to give the condensation product **5**. Treatment of compound **5** with lithium bis(trimethylsilyl)amide at -78 °C followed by diethyl oxalate led to compound **6**. Condensation of **6** with aryl hydrazine **7** was achieved in the presence of trifluoroacetic acid (TFA) in dioxane at elevated temperature to give pyrazole **8**. Hydrolysis of the ethyl ester of compound **8** under basic conditions yielded carboxylic acid **9**, which was then coupled



1 (rimonabant) **2**

Figure 1.

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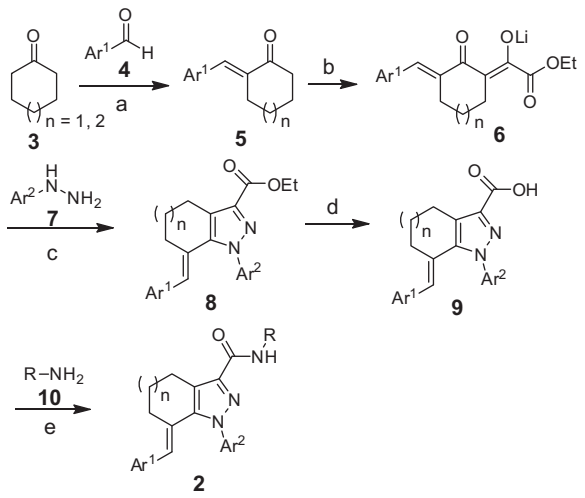


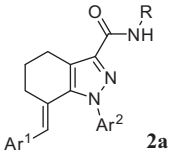
Scheme 1. Reagents and conditions: (a) NaOH, H_2O, 65 °C; (b) (i) LiHMDS, Et_2O, −78 °C; (ii) diethyl oxalate, −78 °C to rt; (c) CF_3CO_2H, 1,4-dioxane; (d) aq NaOH, EtOH; (e) HATU, DIPEA, DMF.

with amine **10** under standard amide formation conditions (HATU, Et_3N) to give final product **2**.

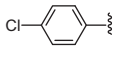
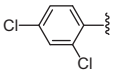
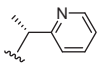
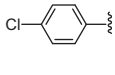
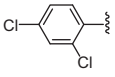
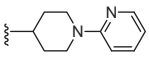
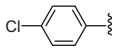
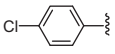
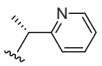
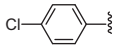
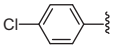
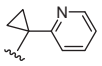
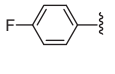
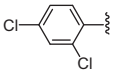
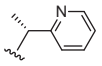
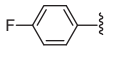
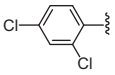
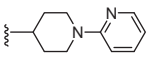
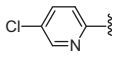
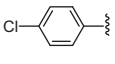
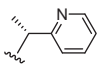
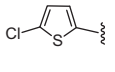
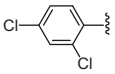
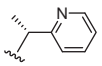
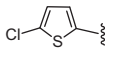
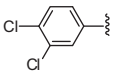
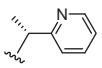
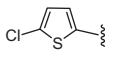
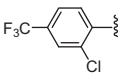
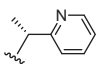
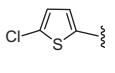
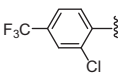
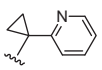
The in vitro CB1 and CB2 receptor inverse agonist activities of compounds **2** were assessed in cell-based functional assays measuring cyclic adenosine monophosphate (cAMP) production.[11] Compounds with acceptable CB1 receptor potency and CB1/CB2 receptor selectivity were then evaluated in a mouse tissue distribution study.[12] In this study, male C57bl/6j mice were dosed orally with a test compound at 20 mg/kg, and the concentrations of the test compound in both plasma and brain were measured at either a 2 h or 4 h time point. The in vitro CB1 and CB2 receptor inverse agonist activity and in vivo tissue distribution data of cyclohexanone-derived compounds **2a**–**k** (6-membered ring series, $n = 1$) are shown in Table 1, and those of cycloheptanone-derived compounds **2l**–**w** (7-membered ring series, $n = 2$) are shown in Table 2. The calculated topological polar surface area (TPSA) and *c*Log*P* data are also included in the tables.

As demonstrated by the data in Table 1, both aryl (**2a**–**f**) and heteroaryl (**2g**–**k**) Ar^1 groups led to good CB1 receptor inverse agonist activity, while substituted aryls are the optimal Ar^2 groups in terms of CB1 potency. Additionally, the R group plays an important role. Compounds with 1-(pyridin-2-yl)piperidin-4-yl as the R

Table 1
In vitro pharmacology and in vivo tissue distribution of compounds **2a**–**k**

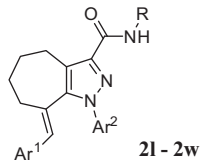


2a - 2k

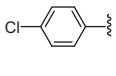
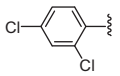
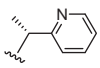
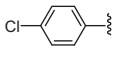
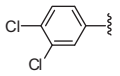
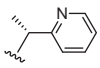
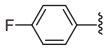
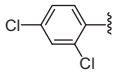
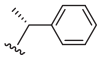
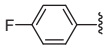
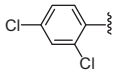
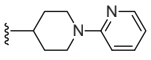
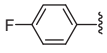
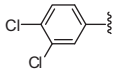
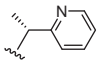
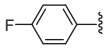
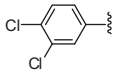
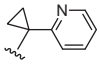
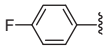
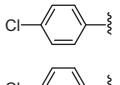
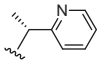
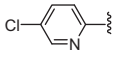
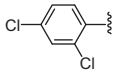
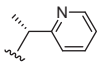
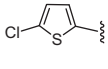
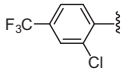
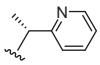
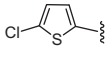
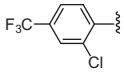
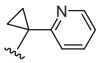
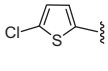
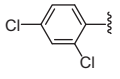
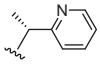
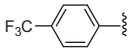
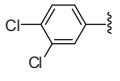
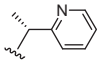
Compd.	Ar^1	Ar^2	R	CB1 EC_{50} (μM)	CB2 EC_{50} (μM)	Plasma Conc. (μM)	Brain Conc. (nmol/g)	B/P ratio	*c*Log*P*	TPSA
2a				0.030	0.854	1.60[a]	0.32[a]	0.20	7.40	59.8
2b				0.034	>10	0.45[a]	0.04[a]	0.09	7.33	63.1
2c				0.127	>10	3.72[b]	0.97[b]	0.26	6.68	59.8
2d				0.007	1.61	1.61[a]	1.28[a]	0.82	6.72	59.8
2e				0.004	>10	1.56[b]	1.39[b]	0.92	6.83	59.8
2f				0.012	>11	1.52[b]	0.035[b]	0.02	7.71	50.2
2g				0.022	>10	2.93[b]	1.91[b]	0.65	5.27	72.7
2h				0.035	3.82	2.96[b]	0.24[b]	0.08	7.32	88.1
2i				0.037	>10	3.18[a]	0.10[a]	0.03	7.20	88.1
2j				0.018	>10	0.46[a]	0.051[a]	0.11	7.56	88.1
2k				0.011	>10	4.63[a]	0.64[a]	0.14	7.59	88.1

[a] Sample was collected at 4 h.
[b] Sample was collected at 2 h. B/P ratio: brain/plasma concentration ratio.

Table 2
In vitro pharmacology and in vivo tissue distribution of compounds **2l–w**



2l - 2w

Compd.	Ar^1	Ar^2	R	CB1 EC_{50} (μM)	CB2 EC_{50} (μM)	Plasma Conc. (μM)	Brain Conc. (nmol/g)	B/P ratio	*c*Log*P*	TPSA
2l				0.007	0.743	0.83[b]	0.13[b]	0.16	7.96	59.8
2m				0.037	>10	2.40[b]	0.05[b]	0.02	7.84	59.8
2n				0.006	5.59	1.29[b]	BLOQ[b]		8.88	46.9
2o				0.028	>10	2.03[b]	0.05[b]	0.03	7.32	63.1
2p				0.035	2.00	1.62[b]	0.07[b]	0.05	7.27	59.8
2q				0.013	0.807	2.41[b]	0.11[b]	0.05	7.30	59.8
2r				0.028	>10	2.47[b]	1.02[b]	0.41	6.67	59.8
2s				0.004	0.56	0.38[b]	0.16[b]	0.48	6.54	72.7
2t				0.008	>10	0.64[a]	0.17[a]	0.28	8.12	88.1
2u				0.008	>10	1.54[a]	0.36[a]	0.24	8.15	88.1
2v				0.002	9.41	3.63[b]	0.56[b]	0.15	7.88	88.1
2w				0.056	>10	4.76[b]	0.05[b]	0.01	8.01	59.8

[a] Sample was collected at 4 h.
[b] Sample was collected at 2 h. B/P ratio: brain/plasma concentration ratio. BLOQ: Below limit of quantitation (LOQ: 5 ng/ml).

Table 3
Efficacy of compound **2p** in 3-week DIO mouse model

Group	OGTT Plasma glucose AUC (mg/dL*min)
2p ($n = 10$)	22000.50 ± 647.03[a]
vehicle ($n = 10$)	29890.50 ± 2516.45[a]

[a] On day 19 from 0 to 120 min after oral glucose (2 g/kg) dosing.

group generally exhibited good peripheral selectivity (**2b** and **2f**). However, when R is (*R*)-1-(pyridine-2-yl)ethyl, good peripheral selectivity was only observed with compounds whose Ar^1 group is 5-chloro-thiophen-2-yl (**2h–j**). There is a correlation between the compound's *c*Log*P* and its brain/plasma ratio. Compounds with *c*Log*P* < 7 showed higher brain/plasma ratio (**2c–e**, **2g**).

The 7-membered ring series (**2l–w**) generally follows the same trend as that of the 6-membered ring series (**2a–k**) in terms of structure–activity relationships (SAR) and peripheral selectivity. Structurally similar analogs from both series showed comparable CB1 potency and peripheral selectivity (i.e. **2a** vs **2l**, **2f** vs **2o**). However, in the case of the Ar^1 group being 5-chloro-thiophen-2-yl, the 7-membered ring compounds (**2t**, **2u** and **2v**) are less peripherally selective than their corresponding 6-membered ring analogs (**2h**, **2j** and **2k**). Interestingly, when the Ar^2 group is 3,4-dichlorophenyl, compounds from both series consistently exhibited good peripheral selectivity (**2i**, **2m**, **2p**, **2q** and **2w**). In addition, replacing the pyridine of the R group with a phenyl led to compound **2n**, which possesses excellent peripheral selectivity with no detectable concentration of the compound in the brain. The correlation between the compound's *c*Log*P* and its brain/plasma ratio largely holds true for this series. Compounds with calculated *c*Log*P* < 7 showed higher degree of brain penetration (**2r** and **2s**). On the other hand, the *c*Log*P* of compound **2n** is 8.88, which may explain its exceptional peripheral selectivity.

In vivo efficacy of compound **2p** in a 3-week diet-induced obesity (DIO) mice model is shown in Table 3.[13] In this chronic efficacy

study, groups of DIO mice were dosed orally with compound **2p** (30 mg/kg, 5 mL/kg), or the vehicle (10% PEG400 + 10% solutol, 5 mL/kg) once a day for 3 weeks. Encouragingly, the DIO mice that were treated with compound **2p** showed statistically significant lower plasma glucose levels in an oral glucose tolerance test (OGTT) compared to the vehicle group.[14] Furthermore, a PK (pharmacokinetic) study of compound **2p** was conducted concurrent with the efficacy study. The PK results indicated that the high peripheral selectivity of **2p** was maintained after the 22-day repeated dosing. Two hours after the last dose, the plasma and brain concentrations of **2p** are 6.62 μM and 0.25 nmol/g respectively, which gave a brain/plasma ratio of 0.037. Compound **2p** had no significant effects on food intake and body weight of the tested DIO mice. In addition, compound **2p** showed no significant centrally-mediated behavioral effects in a rat CNS behavioral assessment (30 mg/kg, 2 h).[15]

In summary, a novel series of CB1 receptor inverse agonists has been investigated to search for peripherally restricted compounds that will not cross the blood–brain barrier (BBB). A large number of compounds that exhibited potent inverse agonist activity against CB1 receptor and good selectivity versus CB2 receptor have been identified. The peripheral selectivity of these compounds was then assessed in a mouse tissue distribution study, which led to the discovery of several highly peripherally selective compounds, such as **2f**, **2i**, **2m**, **2n**, **2o**, **2p**, **2q** and **2w**. Furthermore, compound **2p** was evaluated in a 3-week efficacy study in DIO mice and demonstrated beneficial effects on plasma glucose levels. These analogs can be useful tool compounds to further investigate the therapeutic potential of peripherally restricted CB1 receptor inverse agonists.

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12. Male C57bl/6j mice ($n = 3$) were dosed orally with test compounds at 20 mg/kg. Plasma was collected via retro-orbital bleeding at 1 h and 2 h or 1 h and 4 h after dosing. Whole brain without cerebellum was collected at 2 h or 4 h after dosing. Wet brain weight was recorded before freezing. Brains were homogenized in saline and sent for analysis by LC/MS to determine the concentration of the test compound.
13. Compound **2p** was formulated in 10% PEG400 and 10% solutol. DIO mice received vehicle or compound **2p** at 30 mg/kg daily. On day 19, after an overnight fast, an oral glucose tolerance test (2 g/kg glucose) was performed. Blood glucose was measured at 0, 30, 60 and 120 min after glucose challenge. Blood glucose was measured from the tail vein with a Lifescan glucometer. The dosing was continued until day 22. On day 23, the mice were euthanized, and blood and tissues were collected.
14. One-way ANOVA multiple comparisons.
15. Sprague Dawley rats were dosed orally with vehicle (10% PEG400 and 10% solutol) or compound **2p** (30 mg/kg). Automated recording of stereotypic activity started 15 min post-dose and continued for a total of 2 h.