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Note

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Development of Quinazoline/Pyrimidine-2,4(1*H*,3*H*)-diones as Agonists of Cannabinoid Receptor Type 2

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ABSTRACT: Starting from a prototypical structure **1**, we describe our efforts to design and obtain novel quinazoline/pyrimidine-2,4(1*H*,3*H*)-diones with high CB2 agonist potency and selectivity as well as improved physicochemical characteristics, mainly hydrophilicity. The most potent and selective CB2 agonists, **8** and **36**, in this series were also endowed with lower logP values than that of GW842166X and lead compound **1**. These derivatives appear to be promising lead compounds for the development of future CB2 agonists.

Cannabinoids target two G protein-coupled receptors, cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2).[1] A number of ligands have been developed for CB1 or CB2, with several compounds proceeding to clinical trials. CB1 antagonist SR141617A (Figure S1) was approved as an anti-obesity drug in Europe in 2006[2] but was withdrawn from the market two years later due to serious central nervous system (CNS) side effects associated with CB1 inhibition. In contrast, CB2 has been proposed to be a potential drug target to treat pain and immune-related diseases without CNS problems. Several CB2 ligands, such as JWH-133,[3] HU-308,[4] and AM1241[5] (Figure S1), were developed based on traditional cannabinoids. Other structurally diverse CB2 ligands have also been reported, among which S-777469[6] and GW842166X[7] (Figure S1) have been tested in clinical trials for the treatment of atopic dermatitis and pain associated with osteoarthritis and rheumatoid arthritis, respectively. However, GW842166X turned out to lack efficacy in subjects.[8] Although many CB2 ligands have been discovered,[9-11] no compound has completed clinical development and reached the market. One major issue could be inadequate drug-likeness, which decreases clinical performance.[12] The ClogP value of GW842166X exceeds 4. Hence, reducing the logP value without compromising activity may be a strategy to obtain promising CB2 ligands. Ellsworth et al. have described their efforts to improve the pharmacokinetic properties within their chemical series via reductions in logP.[13]

Recently, a series of quinolone-2,4(1*H*,3*H*)-diones has been reported to be CB2 ligands by our group.[14] Compound **1** (Figure 1), a potent CB2 agonist, has been demonstrated to alleviate clinical symptoms of experimental autoimmune encephalomyelitis and protect the CNS from immune damage. Interestingly, the introduction of substituents at the C7 position of the nucleus reversed the functional profile, as exemplified by compound **2**, which is actually an antagonist of CB2 (Figure 1). Despite their good potency and selectivity for CB2, this class of molecules has drawbacks, including the difficult separation of the two *E*/*Z*-isomers and their high lipophilicity.

Herein, we describe our efforts to obtain new CB2-selective

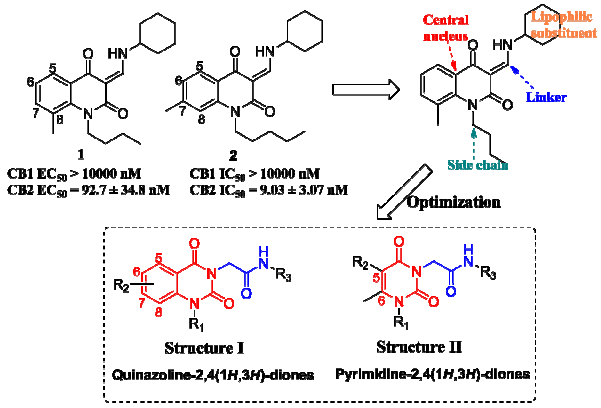


Figure 1. Lead compounds **1** and **2** and diagram for the

agonists while retaining CB2 bioactivity and selectivity, starting from structure **1**. First, to reduce the lipophilicity, the nucleus in **1** was replaced as shown in quinazoline-2,4(1*H*,3*H*)-dione (general structure **I**) and pyrimidine-2,4(1*H*,3*H*)-dione (general structure **II**, Figure 1). Second, to address the difficult separation of the *E*/*Z*-isomers, the enamine moiety was replaced by an acetamide link based on the interaction patterns between the designed compounds and CB2 predicted by molecular simulations (described in the Supporting Information).

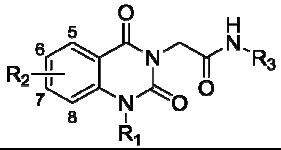
The CB1 and CB2 bioactivities (EC_{50} values) of target compounds **3**-**40** (Tables 1-2) were determined by whole-cell calcium mobilization assays.[15] GW842166X was synthesized based on the reported method[16] and used as the reference standard for a CB2 agonist; several standard cannabinoids were tested for further validation of the bioassays (details in the Supporting Information). Most of the compounds displayed full or partial CB2 agonist activities without activating or inhibiting CB1 (Table S1). Some compounds exhibited higher CB2 agonist potency than GW842166X and lead compound **1**. The EC_{50} values (CB2 agonist) of compounds **7**, **8**, and **11** were below 30 nM, and their

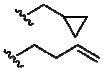
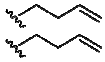
E_{max} values were between 77% and 99%. Compound **8**, with more than 715-fold selectivity against CB1,was 25-times more potent than GW842166X. Encouragingly, the CB2-selective agonist **8** was slightly more potent on CB2 than CP55940, a dual agonist of CB1 and CB2. Compounds **7** and **11** displayed full agonist activity on CB2, with EC_{50} values of 23.53 ± 4.85 nM and 27.69 ± 6.28 nM, respectively, similar to the CB2 activity of CP55940. Notably, among the pyrimidine-2,4(1*H*,3*H*)-diones, **36** had both the highest CB2 potency and CB2 selectivity. Generally, this class of ligands showed lower efficacy on CB2 than the quinazoline-2,4(1*H*,3*H*)-diones.

To evalute the drug-like physicochemical properties of the designed compounds, their ClogPs were calculated using X-logP.[17] Most quinazoline/pyrimidine-2,4(1*H*,3*H*)-diones have an MW of approximately 350 and a ClogP near 3.00. For example, compounds **7**, **8**, **14**, and **36** presented ClogP values of 2.76, 3.30, 2.61, and 2.73, respectively, which were significantly lower than that of the lead compound **1** (ClogP = 4.65) and GW841266X (ClogP = 4.13). The experimental logP values of GW842166X, **1**, **8**, and **36** were also measured at 4.27, 4.35, 2.56, and 2.24, respectively, using the method described in the Supporting Information. Through structural optimization, we developed novel CB2-selective agonists with high potency and selectivity as well as more appropriate logP values compared to both GW842166X and lead compound **1**.

As listed in Table 1, the structure-activity relationships (SARs) related to various R_1 and R_2 groups on the quinazoline-2,4(1*H*,3*H*)-diones were explored. Compounds **4** and **5**, with *n*-butyl and *n*-pentyl groups at R_1, respectively, showed more than 10-fold higher CB2 agonist activity than **3**, with a shorter *n*-propyl at R_1. Among the C5-substituted compounds, compound **8**, with an *n*-pentyl group at R_1, exhibited the best CB2 agonist activity, but truncating the *n*-pentyl group (**6**) or replacing the saturated carbon chain of R_1 with a cyclopropylmethyl group (**12**) or an unsaturated alkyl chain (**14**) led to a decrease in the CB2 agonist activity. The EC_{50} values increased for the R_2 8-methyl derivatives bearing the following R_1 moieties in increasing order: *n*-pentyl (**11**), *n*-butyl (**10**), but-3-en-1-yl (**15**), cyclopropylmethyl (**13**), and *n*-propyl (**9**). These results suggested that the *n*-pentyl group of R_1 was best for generating CB2 agonists. The essential length of the R_1 straight chain could be determined from its interaction with the narrow hydrophobic cavity surrounded by W172, Y190, and W194 in CB2, as observed from molecular simulations (Figure S3). Moreover, the R_2 5-methyl derivatives **6**-**8** and R_2 8-methyl analogues **9**-**11** demonstrated higher potency for CB2 than compounds **3**-**5**, which lacked an R_2 substituent. When the C5 position was substituted with a methoxy group (**25**), the potency dramatically decreased compared to that of **8**. Similarly, compound **11**, with an R_2 8-methyl group, displayed higher CB2 agonist activity than the corresponding 8-chloride (**28**) or 8-methoxy (**29**) analogue. Therefore, for the R_2 positon of quinazoline-2,4(1*H*,3*H*)-diones, 5/8-methyl groups were found to be most beneficial to the CB2 agonist activity and selectivity. In addition, an R_2 5-methyl group was slightly perferable to an 8-methyl group based on comparison of the activities of **6** *vs* **9**, **7** *vs* **10**, and **8** *vs* **11**.

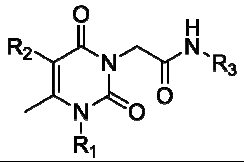
Table 1. Effects of Different Functional Groups on Quinazoline-2,4(1*H*,3*H*)-diones on the Activity Profiles



Cmpd	R_1	R_2	R_3	CB1 EC_{50} (nM)	CB2 EC_{50} [a] (95% CI, nM)	CB2 E_{max} [b] (% of control)	SI [c]
3	*n*-propyl	H	*t*-butyl	>10000	1420 ± 258	75 ± 11	>7
4	*n*-butyl	H	*t*-butyl	>10000	111.9 ± 28.3	101 ± 4	>89
5	*n*-pentyl	H	*t*-butyl	>10000	86.51 ± 25.22	80 ± 3	>116
6	*n*-propyl	5-CH_3	*t*-butyl	>10000	113.3 ± 10.6	113 ± 4	>88
7	*n*-butyl	5-CH_3	*t*-butyl	>10000	23.53 ± 4.85	98 ± 8	>425
8	*n*-pentyl	5-CH_3	*t*-butyl	>10000	13.99 ± 5.36	77 ± 8	>715
9	*n*-propyl	8-CH_3	*t*-butyl	>10000	406.9 ± 96.16	121 ± 10	>25
10	*n*-butyl	8-CH_3	*t*-butyl	>10000	38.22 ± 10.73	102 ± 2	>262
11	*n*-pentyl	8-CH_3	*t*-butyl	>10000	27.69 ± 6.28	99 ± 11	>361
12		5-CH_3	*t*-butyl	>10000	68.33 ± 6.10	106 ± 3	>146
13		8-CH_3	*t*-butyl	>10000	308.8 ± 110.7	110 ± 5	>32
14		5-CH_3	*t*-butyl	>10000	46.79 ± 7.69	103 ± 6	>214
15		8-CH_3	*t*-butyl	>10000	99.57 ± 24.22	90 ± 6	>100
16	*n*-pentyl	H	*n*-butyl	>10000	78.59 ± 16.73	78 ± 3	>127
17	*n*-pentyl	H	*i*-butyl	>10000	370.2 ± 118.3	81 ± 1	>27
18	*n*-pentyl	H	*i*-propyl	>10000	213.7 ± 56.35	65 ± 8	>47
19	*n*-pentyl	H	cyclopropyl	>10000	743.2 ± 307.8	58 ± 4	>13
20	*n*-pentyl	H	cyclohexyl	>10000	>10000	ND[d]	ND
21	*n*-pentyl	5-CH_3	*n*-butyl	>10000	467.6 ± 83.2	83 ± 3	>21
22	*n*-pentyl	5-CH_3	cyclohexyl	>10000	394.9 ± 126.0	56 ± 3	>25
23	*n*-pentyl	5-Cl	*n*-butyl	>10000	147.4 ± 11.8	30 ± 4	>68
24	*n*-pentyl	5-Cl	cyclohexyl	>10000	>10000	ND	ND
25	*n*-pentyl	5-OCH_3	*t*-butyl	>10000	1250 ± 610	88 ± 5	>8
26	*n*-pentyl	5-OCH_3	*n*-butyl	>10000	>10000	ND	ND
27	*n*-pentyl	5-OCH_3	cyclohexyl	>10000	>10000	ND	ND
28	*n*-pentyl	8-Cl	*t*-butyl	>10000	52.71 ± 12.52	118 ± 4	>190
29	*n*-pentyl	8-OCH_3	*t*-butyl	>10000	>10000	ND	ND
GW842166X				>10000	342.8 ± 46.63	97 ± 2	>29
CP55940				30.02 ± 8.40	28.62 ± 6.42	100 ± 8	1

[a]Assay protocols are provided in the Supporting Information. EC_{50} values were obtained from an 8-point experiment with three replicates. CI, confidence interval. [b]E_{max} values displayed as the means ± SEM are relative (%) to the maximal effect of CP55940. [c]SI: selectivity index for CB2, SI = EC_{50}(CB1)/EC_{50}(CB2). [d]ND, not determined.

Table 2. Effects of Different Functional Groups on Pyrimidine-2,4(1*H*,3*H*)-diones on the Activity Profiles



Cmpd	R_1	R_2	R_3	CB1 EC_{50} (nM)	CB2 EC_{50} [a] (95% CI, nM)	CB2 E_{max} [b] (% of control)	SI [c]
30	*n*-butyl	H	*t*-butyl	>10000	>10000	ND[d]	ND
31	*n*-pentyl	H	*t*-butyl	>10000	722.1 ± 27.7	72 ± 3	>13
32	*n*-hexyl	H	*t*-butyl	>10000	766.8 ± 92.8	65 ± 10	>13
33	*n*-pentyl	H	*n*-butyl	>10000	>10000	ND	ND
34	*n*-pentyl	H	cyclohexyl	>10000	>10000	ND	ND
35	*n*-pentyl	Cl	*t*-butyl	>10000	226.8 ± 43.8	65 ± 2	>44
36	*n*-pentyl	Br	*t*-butyl	>10000	48.74 ± 7.88	74 ± 2	>205
37	*n*-pentyl	I	*t*-butyl	>10000	178.2 ± 51.3	78 ± 11	>56
38	*n*-pentyl		*t*-butyl	>10000	269.5 ± 31.7	71 ± 2	>37
39	*n*-pentyl	phenyl	*t*-butyl	>10000	>10000	ND	ND
40	*n*-pentyl	*m*-CH_3-phenyl	*t*-butyl	>10000	>10000	ND	ND

[a]Assay protocols are provided in the Supporting Information. EC_{50} values were obtained from an 8-point experiment with three replicates. CI, confidence interval. [b]E_{max} displayed as the means ± SEM are relative (%) to the maximal effect of CP55940. [c]SI: selectivity index for CB2, SI = EC_{50}(CB1)/EC_{50}(CB2). [d]ND = not determined.

Next, SAR studies of the R_3 substituents in quinazoline-2,4(1*H*,3*H*)-diones were conducted with a fixed *n*-pentyl group at R_1. The R_3 *n*-butyl derivative **16** maintained similar CB2 agonist activity to that of **5** with an *t*-butyl group at R_3. However, when other hydrophobic groups, such as isobutyl (**17**), isopropyl (**18**), and cyclopropyl (**19**), were introduced as the R_3 moiety, significant decreases in potency were observed in comparison with the potency of **5**. Compound **20** with an R_3 cyclohexyl group lost all CB2 agonist activity. Intriguingly, replacing the R_3 *t*-butyl group of **8** with an *n*-butyl (**21**) or cyclohexyl (**22**) group was proved to be exceedingly detrimental to the CB2 agonist activity. In fact, our modeling results predicted that the R_3 *t*-butyl group of **8** makes favorable contact with the aromatic side chains of F91 and F94 (Figure S3B). As indicated by the SAR results for the quinazoline-2,4(1*H*,3*H*)-diones, R_1 *n*-pentyl, R_2 5/8-methyl, and R_3 *t*-butyl groups are optimal for CB2 agonist activity in this series.

For the pyrimidine-2,4(1*H*,3*H*)-diones (Table 2), R_1 *n*-pentyl and *n*-hexyl groups contributed similarly to the CB2 agonist activity (**31** *vs* **32**), while an *n*-butyl at R_1 was competely detrimental, as reflected by compound **30**. Herein, we selected the an R_1 *n*-pentyl group for further structural modification. Replacement of the R_3 *t*-butyl group (**31**) with an *n*-butyl (**33**) or cyclohexyl (**34**) moiety completely lost the CB2 agonist activity. Consequently, the *t*-butyl group was identified as a desirable R_3 moiety in pyrimidine-2,4(1*H*,3*H*)-diones. Based on **31**, compounds **35**-**40** with various R_2 groups were obtained. Three halogen derivatives with 5-chloro (**35**), 5-bromo (**36**), and 5-iodo (**37**) groups displayed improved CB2 agonist activities and selectivities compared with **31**, of which **36** was the most potent. In addition, introducing an R_2 cyclopropyl group resulted in compound **38**, with a 6-fold decreased CB2 agonist activity compared with that of **36**. Significantly, compounds **39** and **40**, with phenyl and *m*-CH_3-phenyl groups at R_2, respectively, lacked any CB2 activity. Thus, an R_2 5-bromo group is optimal for highly potent CB2 agonist activity of pyrimidine-2,4(1*H*,3*H*)-diones.

In summary, starting from lead compound **1**, extensive SARs of novel quinazoline/pyrimidine-2,4(1*H*,3*H*)-diones were developed. Our optimization efforts led to potent and selective CB2 agonists with more suitable logP values. Among the quinazoline-2,4(1*H*,3*H*)-diones **3**-**29**, compounds with 5/8-methyl groups performed better in terms of both potency and selectivity. Compound **8** exhibited the highest CB2 agonist activity without CB1 potency. In addition, structural optimizations around the pyrimidine-2,4(1*H*,3*H*)-dione scaffold, especially at the C5 position, led to the highly potent and selective CB2 agonist **36**. Most importantly, the lipophilicity of target compounds **8** (logP = 2.56) and **36** (logP = 2.24) were significantly improved compared to that of the known CB2 agonist GW842166X (logP = 4.27) and lead **1** (logP = 4.35). The presented data offer an attractive starting point for further optimization.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Molecular simulations to predict the interaction patterns of the designed compounds binding to the CB2 receptor, synthetic procedures for target compounds **3**-**40**, analytical and spectral characterization data of the target compounds, logP measurements of compounds **GW842166X**, **1**, **8**, and **36** in octanol/water, and biological assays information.

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Author Contributions

§ These authors (H.-Y.Q. and Z.-L.W.) contributed equally to this work.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; CNS, central nervous system; SAR, structure-activity relationship

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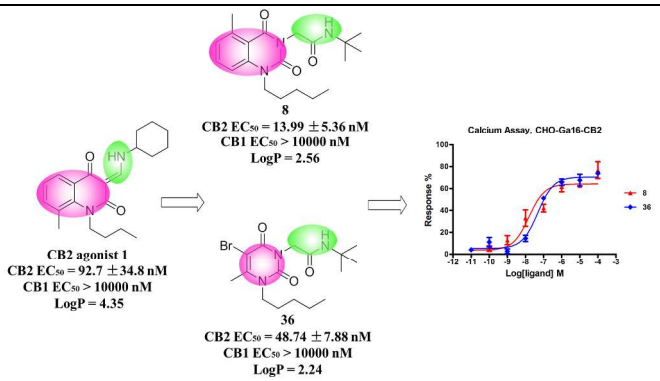

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