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Design, synthesis, and structure–activity relationship study of conformationally constrained analogs of indole-3-carboxamides as novel CB1 cannabinoid receptor agonists

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ABSTRACT

Novel tricyclic indole-3-carboxamides were synthesized as structurally restricted analogs of bicyclic indoles, and found to be potent CB1 cannabinoid receptor agonists. The CB1 agonist activity depended on the absolute configuration of the chiral center of the tricyclic ring. The preferred enantiomer was more potent than the structurally unconstrained lead compound. Structure–activity relationships in the amide side chain of the indole C-3 position were also investigated.

The CB1 cannabinoid receptor is a member of G-protein coupled receptor (GPCR) superfamily, which is characterized by seven-transmembrane receptors. The CB1 receptor is located primarily in the central nervous system but is also expressed on peripheral neurones. Recently many studies revealed that the CB1 receptor is a potential therapeutic target against pain and several other diseases including glaucoma, traumatic brain injury, and multiple sclerosis.[1] Several lines of evidence have been reported regarding the analgesic effects of CB1 agonists in both experimental animal models and clinical studies. Moreover, a couple of CB1 agonists including Δ^9-THC, one of the major bioactive components of cannabis, are used clinically as antiemetics in cancer chemotherapy or appetite stimulants in AIDS patients. However, the classical cannabinoids represented by Δ^9-THC are highly lipophilic and the administration methods are still limited. We have previously described indole-3-carboxamide derivatives as water soluble CB1 agonists which might be suitable for intravenous administration as peri-operative analgesics.[2] We now report a series of conformationally constrained indole derivatives in which the direction of the cyclohexyl group is restricted by the formation of a six-membered ring between indole 1- and 7-position. Generally, unconstrained agonists exist as a mixture of conformers in solution and we hypothesized that limiting the number of conformations of these molecules might improve potency by lowering the entropic contribution to the binding of a particular conformation. Stereoselective synthesis of both enantiomers revealed the structural requirements for highly potent CB1 agonist activity. Further structure–activity relationship studies were also performed to find the optimum amide substituent.

The racemic mixture of tricyclic indole **1**, which has the $-OCH_2-$ linkage, could be synthesized using Fischer indole synthesis methodology[3] as show in Scheme 1. The key precursor **24** was synthesized efficiently by intramolecular reductive amination using **20** which was obtained from 2-nitrophenol and α-bromoketone **19**. The indole ring formation of **24** proceeded in good yield and the subsequent de-carboxylation and regioselective introduction of a piperazinylcarbonyl group afforded desired compound **1**.

The carbon analog **2**, which has an ethylene moiety instead of the $-OCH_2-$ of **1**, was synthesized in a similar manner using 2-cyclohexyl-1,2,3,4-tetrahydroquinoline **25** as a precursor of indole ring formation reaction, which could be obtained by the reaction[4] of quinoline with cyclohexane carboxylic acid followed by the partial reduction of the quinoline ring.[5] The homochiral analogs of **1** could be obtained from commercially available (*S*)- or (*R*)-*N*-Boc-cyclohexyl-glycine **30** as illustrated in Scheme 2. Mitsunobu reaction of 2-bromophenol with the alcohol **31**, which was obtained by the reduction of **30**, afforded aryl ether **32**.

Intramolecular palladium catalyzed amination[6] gave 3,4-dihydro-2*H*-1,4-benzoxazine derivative **33**. After removing the Boc group of **33**, the tricyclic core could be constructed in a similar manner as shown in Figure 1. The subsequent introduction of the

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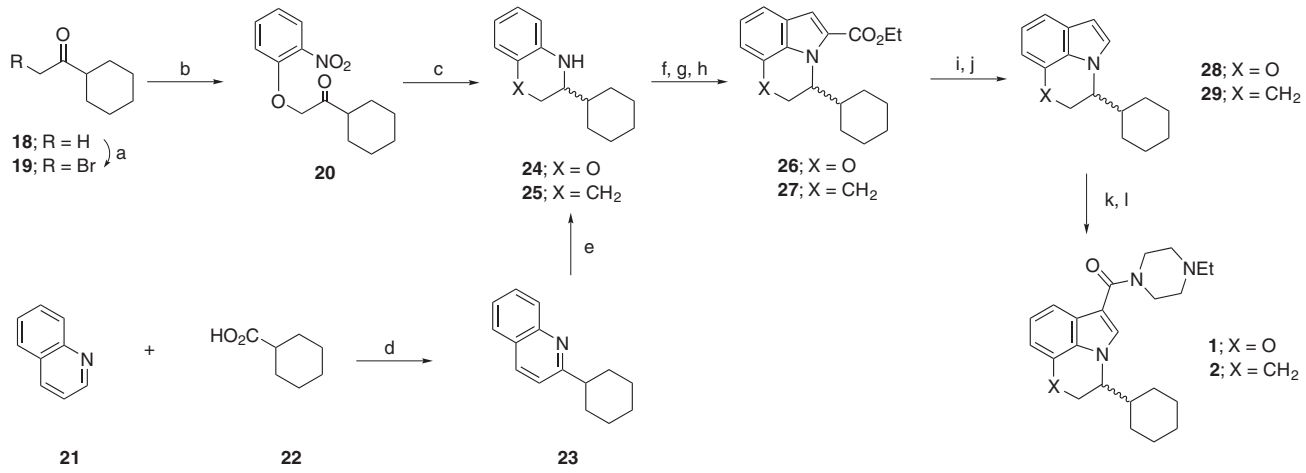
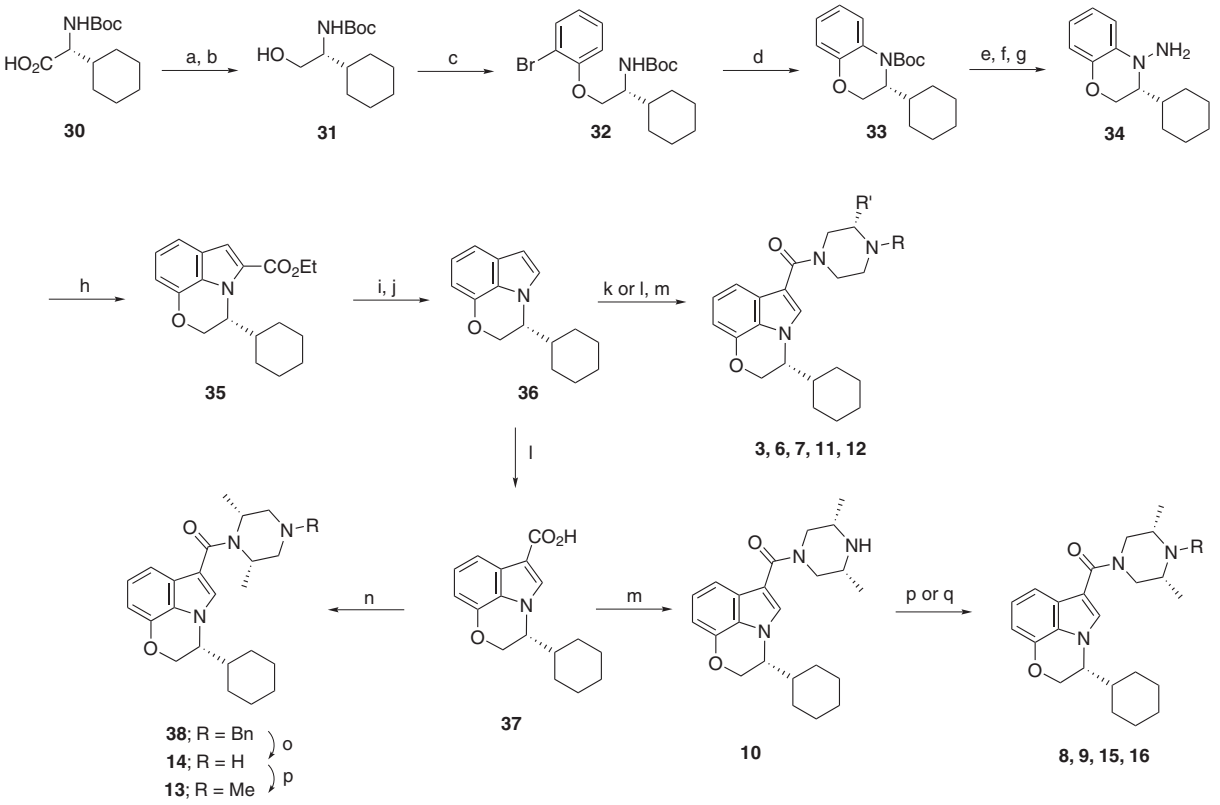


Scheme 1. Reagents and conditions: (a) Br_2, quant.; (b) 2-nitrophenol, K_2CO_3, KI, 62%; (c) H_2, Pd–C, quant.; (d) $AgNO_3$, $(NH_4)_2S_2O_8$, TFA, chlorobenzene, water, 140 °C, 13%; (e) $NaBH_3CN$, AcOH, 59%; (f) $NaNO_2$; (g) LAH, 61% from **24**, 68% from **25**; (h) ethyl pyruvate, 75%; (i) NaOH; (j) Cu, quinoline, 65% from **26**, 48% from **27**; (k) oxalyl chloride, quant.; (l) 1-ethylpiperazine, quant.



Scheme 2. Reagents and conditions: (a) MeI, $NaHCO_3$, DMF, 90%; (b) $NaBH_4$, $CaCl_2$, THF, MeOH, 85%; (c) 2-bromophenol, DIAD, PPh_3, toluene, 0 °C to rt, 46%; (d) $Pd(PPh_3)_4$, *t*-BuONa, toluene, microwave 120 °C, 67%; (e) HCl, EtOH, 70 °C, quant.; (f) $NaNO_2$, DMF, water, 0 °C, 85%; (g) LAH, THF, 0 °C, 70%; (h) ethyl pyruvate, H_2SO_4, EtOH, reflux, 85%; (i) NaOH, EtOH, 70 °C, 92%; (j) Cu, quinoline, 210 °C, 86%; (k) i—oxalyl chloride, 1,1,2,2-tetrachloroethane, 120 °C; ii—amine, Et_3N, two steps 44–52%; (l) i—$(CF_3CO)_2O$, DMF, 0 °C to rt, 75%; ii—NaOH, 1,4-dioxane, water, reflux, quant.; (m) amine, EDCI, HOBt, DMF, 48%; (n) i—oxalyl chloride, DCM; ii—1-benzyl-*cis*-3,5-dimethylpiperazine, DIEA, DCM, two steps 39%; (o) H_2, Pd–C, EtOH, 60%; (p) aldehyde, $NaBH(OAc)_3$, EtOH; (q) alkylbromide, CH_3CN, microwave, 150 °C.

piperazinylcarbonyl moiety was performed either by direct amide formation reaction using oxalyl chloride or by stepwise reaction via carboxylic acid intermediate **37** to afford a variety of substituted piperazine derivatives. The alternative enantiomer **4** could be synthesized by the same route.

The prepared compounds were tested for CB1 agonist activity using CHO cells doubly transfected with human CB1 and a luciferase reporter gene.[7] As shown in Table 1, racemic compounds **1** and **2** were 5- to 6-fold more potent than the non-constrained parent compound **5** (pEC_{50} = 7.5, 7.6, and 6.8, respectively). The result encouraged us to prepare and test both enantiomers of **1**. Interestingly, the (*R*)-isomer **3** retained similar activity to the racemic **1**, though the (*S*)-isomer **4** completely lost activity. This result indicated that the correct orientation of the cyclohexyl group is essential to express potent CB1 agonist activity.

The SAR studies on the piperazine moiety of (*R*)-tricyclic derivatives are summarized in Table 2. The substituent at the 4-position on the piperazine ring showed an important influence on the CB1

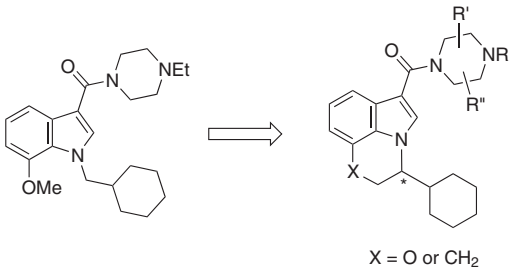
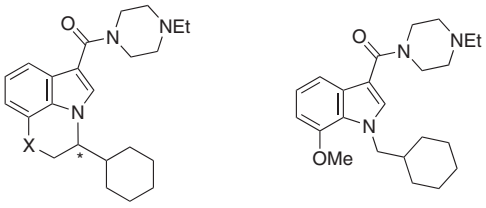


Figure 1. The structure of the original lead compound and newly designed scaffold.

Table 1
CB1 agonist activities for conformationally constrained compounds **1**–**4** and non-constrained compound **5**



1 - 4 **5**

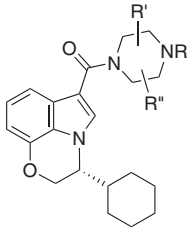
Compound	X	Absolute configuration	pEC_{50}[a]
1	O	Racemic mixture	7.5
2	CH_2	Racemic mixture	7.6
3	O	*R*	7.5
4	O	*S*	<5
5	—	—	6.8

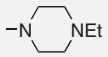
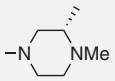
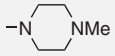
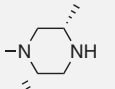
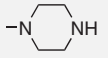
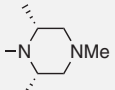
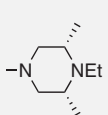
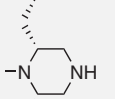
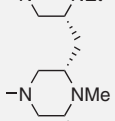
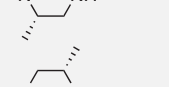
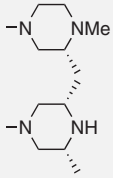
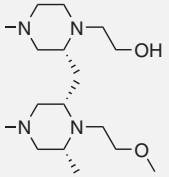
[a] Values are means of three experiments.

agonist activity, that is, replacement of the ethyl group in compound **3** with methyl group slightly attenuated the activity (pEC_{50} = 7.5–7.0), whereas removal of the alkyl group showed considerably reduced potency (**6** and **7**, respectively). Introduction of two methyl groups on 3- and 5-position resulted in significantly improved potency; pEC_{50} values of *cis*-4-ethyl-3,5-dimethyl derivative **8** and *cis*-3,4,5-trimethyl derivative **9** were 8.4 and 8.2, respectively. The activity of 3,4-dimethyl derivative **11** was between that of trimethyl derivative **9** and monomethyl compound **6**. The 2,4,6-trimethyl derivative **13**, a regioisomer of **9**, displayed similar activity to 4-methylpiperazine **6**. Among all of these substituted piperazines, removal of alkyl group on the 4-position resulted in loss of activity (**10**, **12**, and **14**). Interestingly, introduction of a hydrophilic moiety such as hydroxy or methoxy group, that could improve water solubility and PK/PD properties of the molecule, was well tolerated at the piperazine 4-position (pEC_{50} = 8.2 for **15** and 8.3 for **16**, respectively).

CB1 agonist activity of related unconstrained indole derivatives are summarized in Table 3. Interestingly, the SAR of constrained and unconstrained derivatives on the piperazine ring were quite similar, that is, 4-ethyl-3,5-dimethyl piperazine derivatives showed the highest agonist activity in each series, the activity of 4-methyl derivatives were slightly lower than the 4-ethyl derivatives (**17b** vs **17a** and **9** vs **8**, respectively), and 3,4-dimethyl derivatives **17c** and **11** displayed better activity than the corresponding 4-ethyl derivatives (**5** and **3**). When bearing the same substituents on the piperazine ring, the constrained compounds always showed better activity than the unconstrained ones. These results strongly support the hypothesis that limiting the number of conformations of a molecule can improve its CB1 receptor agonist activity.

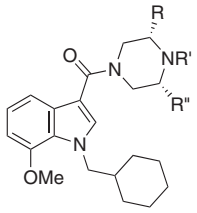
Table 2
CB1 agonist activities for (*R*)-tricyclic indole derivatives



Compound	Piperazine moieties	pEC_{50}[a]	Compound	Piperazine moieties	pEC_{50}[a]
3		7.5	**11**		7.8
6		7.0	**12**		6.3
7		5.4	**13**		7.2
8		8.4	**14**		6.1
9		8.2	**15**		8.2
10		6.9	**16**		8.3

[a] Values are means of three experiments.

Table 3
CB1 agonist activities for unconstrained indole derivatives



Compound	R	R′	R″	pEC_{50}[a]
5	H	Et	H	6.8
17a	Me	Et	Me	8.0
17b	Me	Me	Me	7.6
17c	Me	Me	H	7.6

[a] Values are means of three experiments.

Table 4
Profile of CB1 agonists in in vitro hCB1 and hCB2 binding assays

Compound	CB1 pK_i	CB2 pK_i
3	7.9	8.4
11	8.7	9.4

Table 5
Aqueous solubility of the bicyclic and tricyclic indole derivatives

Compound	MSF solubility (mg/L)	
	Citrate pH 5 (final pH)	PBS pH 7.4 (final pH)
2	3509 (4.9)	114 (7.0)
4	>4000 (4.9)	308 (7.0)
5	3398 (5.0)	216 (6.7)

Table 6
DMPK profile of CB1 agonist **11**

Microsomal stability, mouse CL_{int} (μl/min/mg)	>180
PK (ICR mouse, 0.5 μmol/kg, iv)	
Vehicle	Saline
Plasma C_{max} (ng/ml; t = 0.05 h)	51.5
AUC_{plasma}, iv (h ng/ml)	23.7
Clearance (ml/min/kg)	134
$T_{1/2}$ elimination (h)	0.23
V_{ss} (L/kg)	2.6
Brain penetration (ICR mouse) same studies as above	
Brain C_{max} (ng/g)	76.2
Brain t_{max} (h)	0.17
Brain AUC_{0-1h} (h ng/g)	44.6
Brain:plasma C_{max} ratio	1.48

The results of binding assays for both CB1 and CB2 cannabinoid receptors are listed in Table 4. Compounds **3** and **11** exhibited high affinity for both CB1 and CB2 cannabinoid receptors, as determined by radioligand competition binding assays using [^{3}H]CP 55,940 binding to either hCB1 or hCB2 receptors expressed in insect Sf9 membranes.

It is worthwhile to mention that structural constraint did not negatively influence the aqueous solubility of the molecules. The aqueous solubility of the HCl salt of the tricyclic derivative **4** was >4000 mg/L at pH 4.9 and 308 mg/L at pH 7.0, respectively, whereas solubility of the corresponding bicyclic derivative (**5**) was measured as 3398 mg/L at pH 5.0 and 216 mg/L at pH 6.7, respectively.[2] Although substitution of the oxygen atom in the tricyclic ring for a methylene linker (CH_2) slightly reduced aqueous solubility as shown in Table 5 (compound **2** vs compound **4**), these compounds are still sufficiently soluble for intravenous administration. As expected, hydrophilic moieties like secondary amines or hydroxy groups afforded further solubility improvements (data not shown).

The in vitro and in vivo DMPK profiles of compound **11** are summarized in Table 6. The compound was rapidly metabolized by mouse hepatic microsomes. Mouse brain and plasma levels were determined following intravenous administration of a 0.5 μmol/kg dose (terminal sampling using CO_2). Good CNS penetration was seen, as expected based on the physico-chemical properties of the compound. However, compound **11** was rapidly cleared in vivo, as predicted from the rapid microsomal metabolism.

The antinociceptive activity of compound **11** was determined in the mouse tail flick test[8] after iv administration. The compound significantly increased the tail flick latency; the ED_{50} value was 0.19 μmol/kg.

In summary, a series of conformationally constrained 3-(piperazin-1-ylcabonyl)indole derivatives were synthesized stereoselectively, revealing that one of the stereoisomers was more potent than the non-constrained compound, while the other enantiomer was inactive. A systematic SAR study on the piperazine ring revealed a number of highly potent CB1 receptor agonists with drug like properties. Further studies are in progress to improve metabolic stability within the series by attention to metabolic hot-spots such as the cyclohexane and alkyl piperazine moieties.

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