Bioorganic & Medicinal Chemistry Letters 21 (2011) 506–509

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Design, synthesis, and structure–activity relationships of indole-3-heterocycles as agonists of the CB1 receptor

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ARTICLE INFO

*Article history:*
Received 31 August 2010
Revised 18 October 2010
Accepted 19 October 2010
Available online 25 October 2010

*Keywords:*
Cannabinoid
CB1 receptor
GPCR agonist

ABSTRACT

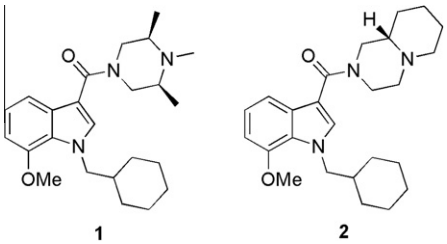
Novel indole-3-heterocycles were designed and synthesized and found to be potent CB1 receptor agonists. Starting from a microsomally unstable lead **1**, a bioisostere approach replacing a piperazine amide was undertaken. This was found to be a good strategy for improving stability both in vitro and in vivo. This led to the discovery of **24**, which had an increased duration of action in the mouse tail flick test in comparison to the lead **1**.



The CB1 cannabinoid receptor is a member of the G-protein coupled receptor (GPCR) superfamily, which is characterized by seven transmembrane helices. The CB1 receptor is located primarily in the central nervous system but is also expressed on peripheral neurones. Recently, many studies have revealed that the CB1 receptor is a potential therapeutic target against pain and several other diseases including glaucoma, traumatic brain injury, multiple sclerosis, and obesity.[1] Several lines of evidence have been reported regarding the analgesic effects of CB1 agonists in both experimental animal models and clinical studies. Moreover, CB1 agonists such as $\Delta^9$-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 $\Delta^9$-THC are highly lipophilic and the administration methods are still limited. We have previously described indole-3-carboxamide derivatives **1** and **2**, Figure 1, as CB1 agonists that displayed potent antinociceptive activity in the mouse tail flick test after iv administration, both of which displayed a fast onset and short duration of action.[2]

In following up these compounds we were keen to explore increasing the duration of action of these types of compounds in the mouse model. Attempts at improving the metabolic stability by introducing conformational constraints and steric blocking groups had failed to deliver any enhancements in metabolic stability as evidenced by the mouse microsomal stabilities of **1** and **2**, Table 1.[2] Therefore, alternative strategies were sought that may lead to more stable analogs.

Replacement of the amide bond with appropriate bioisosteres is a classical approach in medicinal chemistry and in particular the replacement with five-membered ring heterocycles.[3] A simple superposition model of **2** and a generic five-membered heterocycle showed a good overlap of key features.[4] An appropriately positioned heteroatom can mimic the H-bond acceptor functionality of the carbonyl, if required, whilst allowing the amines to occupy the same region. This is exemplified in Figure 2 by the superposition of compounds **1** and **24**. Based on this observation a set of five-membered ring heterocycles bearing two and three heteroatoms




1 2

**Figure 1.** Indole-3-carboxamide CB1 agonists.

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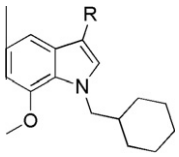
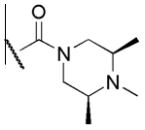
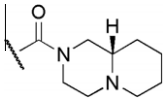
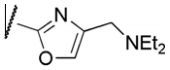
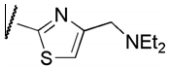
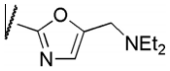
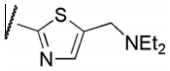
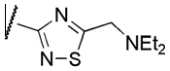
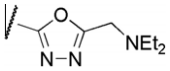
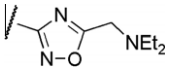
doi:10.1016/j.bmcl.2010.10.093

**Table 1**
CB1 agonist activities and mouse microsomal stabilities for indole-3-heterocycles



| Compound | R | $pEC_{50}$[a] | MLM[b] |
|---|---|---|---|
| **1** |  | 7.6 | >270 |
| **2** |  | 7.9 | >270 |
| **6** |  | 5.8 | 64 |
| **7** |  | 7.1 | 17 |
| **10** |  | 6.9 | nt[c] |
| **11** |  | 6.2 | nt[c] |
| **14** |  | 7.7 | 71 |
| **16** |  | 7.8 | >120 |
| **18** |  | 7.2 | 128 |

[a] Values are means of at least three experiments and values varied by no more than twofold.
[b] MLM: mouse microsomal clearance (μL/min/mg).
[c] nt: not tested.

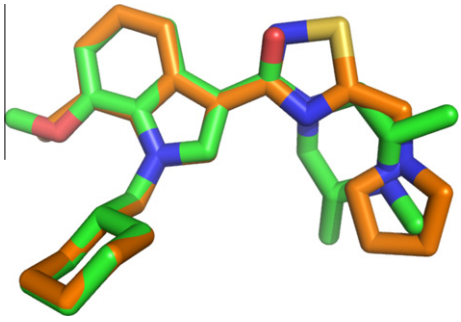
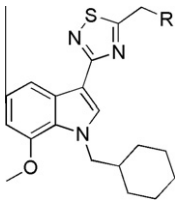


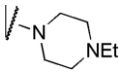
**Figure 2.** Superposition model of **1** (green) and **24** (orange).

and incorporating a simple tertiary amine were targeted for synthesis.

Compounds were synthesised as described in Scheme 1. Briefly, starting from the previously described carboxylic acid **3**,[2a] conversion to the primary amide **4** was achieved via the acid chloride, subsequent treatment with Lawesson's reagent furnished the thioamide **5**. The oxazole **6** and thiazole **7** were readily prepared from the requisite amide or thioamide by initially reacting with 1,3-dichloroacetone to deliver the chloromethyl oxazole **8** and chloromethyl thiazole **9**,[5] followed by simple displacement with diethylamine. The isomeric oxazole **10** and thiazole **11** were

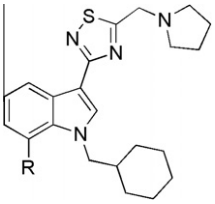
**Table 2**
SAR of amine unit



| Compound | R | $pEC_{50}$[a] | MLM[b] | HLM[c] |
|---|---|---|---|---|
| **1** | — | 7.6 | >270 | >270 |
| **2** | — | 7.9 | >270 | >270 |
| **14** |  | 7.7 | 71 | 89 |
| **20** |  | 7.4 | 53 | 41 |
| **21** |  | 8.7 | 41 | 64 |
| **22** |  | 7.1 | 41 | 49 |
| **23** |  | 6.3 | 14 | 10 |

[a] Values are means of at least three experiments and values varied by no more than twofold.
[b] MLM: mouse microsomal clearance (μL/min/mg).
[c] HLM: human microsomal clearance (μL/min/mg).

**Table 3**
SAR of the indole 7-position

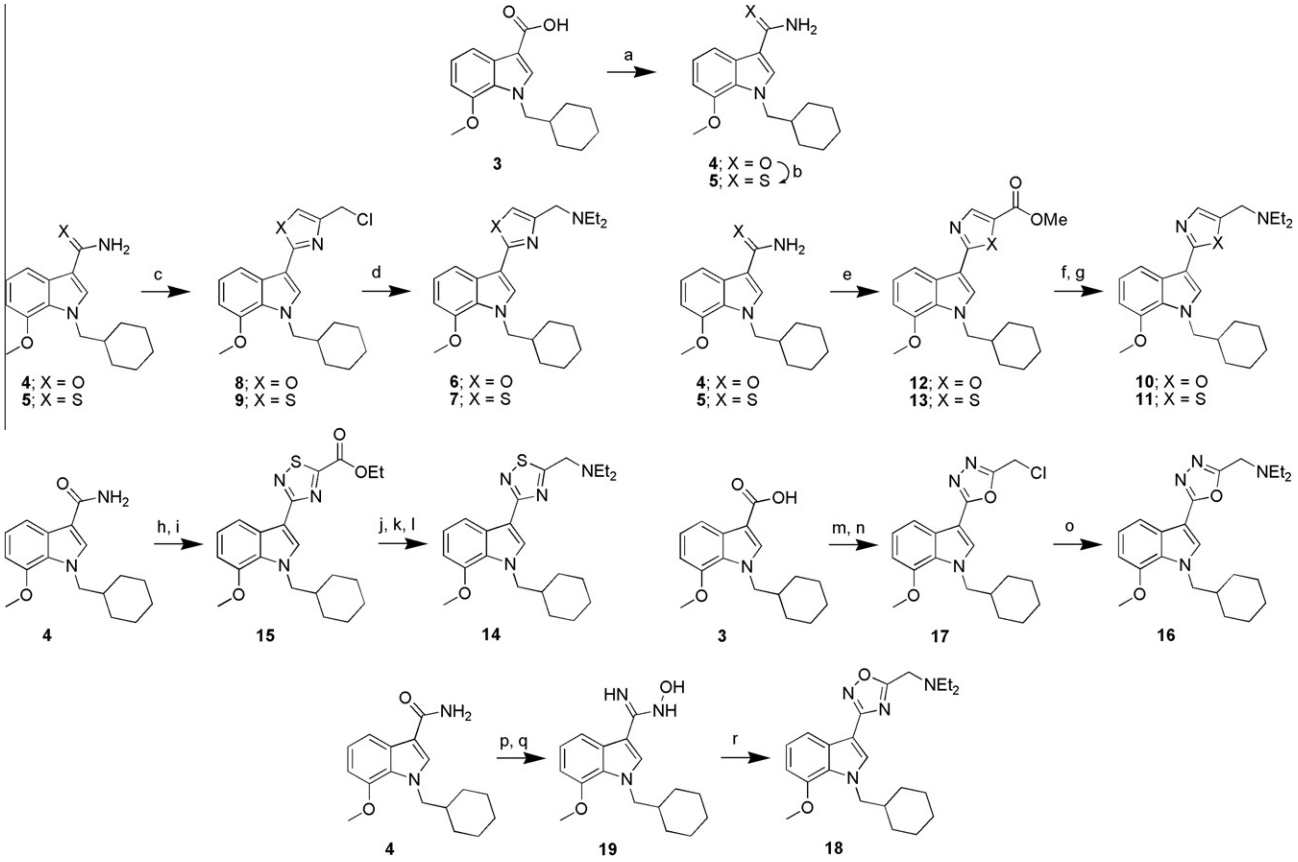


| Compound | R | $pEC_{50}$[a] | MLM[b] | HLM[c] |
|---|---|---|---|---|
| **1** | — | 7.6 | >270 | >270 |
| **2** | — | 7.9 | >270 | >270 |
| **21** | OMe | 8.7 | 41 | 64 |
| **24** | Et | 8.5 | 34 | 74 |
| **25** | Cl | 7.8 | 17 | 35 |
| **26** | F | 7.3 | 24 | 41 |

[a] Values are means of at least three experiments and values varied by no more than twofold.
[b] MLM: mouse microsomal clearance (μL/min/mg).
[c] HLM: human microsomal clearance (μL/min/mg).

prepared in a similar fashion using methylformyl chloroformate as the coupling partner to furnish the ester derivatives, **12** and **13**.[6] Reduction, mesylation, and displacement with diethylamine furnished the desired oxazole **10** and thiazole **11**.

Synthesis of the 1,2,4-thiadiazole **14** was achieved by initial formation of the oxathiazolone, followed by microwave assisted [3+2] cycloaddition in the presence of ethyl cyanoformate to provide the ester derivative **15**.[7] Reduction, mesylation, and displacement with diethylamine provided the thiadiazole **14**. The 1,3,4-oxadiazole **16** was prepared by initial formation of the hydrazide followed by dehydration with Burgess' reagent to provide the chloromethyl derivative **17**,[8] subsequent displacement with diethylamine provided the desired oxadiazole **16**. Finally, the 1,2,4-oxadiazole **18** was synthesized by initial formation of the amidoxime **19**, which was achieved by initial dehydration of the primary amide **4** to



**Scheme 1.** Reagents and conditions: (a) (i) $(COCl)_2$, DCM, (ii) $NH_3$ (gas), DCM, 81%; (b) Lawesson's reagent, toluene, rt, 74%; (c) 1,3-dichloroacetone, toluene, microwave 150 °C, 81% from **4** and 1,3-dichloroacetone, toluene, 40 °C, 61% from **5**; (d) $HNEt_2$, THF, microwave 150 °C, 84% from **8** and $HNEt_2$, MeCN, microwave 150 °C, 51% from **9**; (e) methyl formylchloroacetate, DMA, microwave 150 °C, 76% from **4** and 49% from **5**; (f) $LiAlH_4$, THF, 0 °C, 42% from **12** and 94% from **13**; (g) (i) $CH_3SO_2Cl$, *i*$Pr_2NEt$, DCM, 0 °C, (ii) $HNEt_2$, THF, microwave 150 °C, 20% for **10** over two steps and (i) $CH_3SO_2Cl$, $Et_3N$, DCM, (ii) $HNEt_2$, THF, MeCN, microwave 160 °C, 40% for **11** over two steps; (h) chlorocarbonylsulfonyl chloride, $CHCl_3$, reflux, 56%; (i) ethyl cyanoformate, *m*-xylene, microwave 200 °C, 71%; (j) $NaBH_4$, MeOH, 86%; (k) $CH_3SO_2Cl$, $NEt_3$, DCM, quant.; (l) $HNEt_2$, THF, microwave 150 °C, 61%; (m) (i) $(COCl)_2$, DMF (cat.), DCM, (ii) chloroacetylhydrazide·HCl, $Et_3N$, DCM, 30% over two steps; (n) *N*-(triethylammoniumsulfonyl)carbamate, THF, microwave 150 °C, 71%; (o) $HNEt_2$, THF, microwave 150 °C, 86%; (p) TFAA, $NEt_3$, dioxane, 88%; (q) hydroxylamine hydrochloride, *i*$Pr_2NEt$, EtOH, 85 °C, 66%; (r) benzyl 2-(diethylamino)acetate, NaH, THF, rt to 90 °C, 20%.

the nitrile and subsequent treatment with hydroxylamine·HCl. With the amidoxime **19** in hand the 1,2,4-oxadiazole **18** was readily prepared in one step from benzyl 2-(diethylamino) acetate under basic conditions.

The prepared compounds were tested for CB1 agonist activity using CHO cells doubly transfected with human CB1 and luciferase reporter gene.[9] As shown in Table 1, the hypothesis that these heterocycles would provide bioisosteric replacements for the piperazine amide was shown to be correct. The new compounds did indeed retain CB1 agonist activity and in particular the 1,2,4-thiadiazole **14** and 1,2,4-oxadiazole **16** had comparable potency. Furthermore, and much to our delight, compounds **6**, **7**, and **14** had enhanced metabolic stability in mouse liver microsomes in comparison to the original indole-3-carboxamides.

With these results in hand, we focused our attention on the 1,2,4-thiadiazole motif which offered the best combination of potency and metabolic stability within the series. An SAR study of the amine portion of the molecule was carried out. Synthesis of these compounds was readily achieved using the method outlined in Scheme 1 and replacing the diethylamine with the appropriate amine (scheme not shown). Replacement of the diethyl group for the slightly smaller dimethylamine unit **20**, resulted in a slight drop in potency. Constraining the diethyl unit into a pyrrolidine ring, **21** resulted in a marked 10-fold improvement in potency. Increasing the ring size further to a piperidine **22** or *N*-ethyl piperazine **23** resulted in a drop in potency suggesting that SAR in this region may be tight. With regard to metabolic stability, little change was observed in both mouse and human liver microsomes within this series. However, all had enhanced stability in comparison to the original carboxamide compounds, Table 2. Having optimized the heterocycle and amine portions of the molecule, attention turned to the 7-position of the indole.

Synthesis of these compounds was readily achieved using the method outlined in Scheme 1 and the appropriate 7-substituted indole. Replacement of the 7-methoxy group for the similarly sized ethyl was well tolerated with comparable potency and stability. Replacement with smaller halogen groups resulted in a loss of potency although microsomal stability was improved in both cases, Table 3. With these results in hand, compound **24**, was selected

**Table 4**
DMPK profile of **24**

| | **24** | **1** |
|---|---|---|
| Microsomal stability, mouse $CL_{int}$ (μL/min/mg) | 34 | >270 |
| PK (ICR mouse, 0.5 μmol/kg, iv) | | |
| Vehicle | 5% DMSO/saline | Water |
| Plasma $C_{max}$ (ng/ml; $t$ = 0.05 h) | 667 | 593 |
| $AUC_{plasma}$, iv (h ng/ml) | 124 | 253 |
| Clearance (ml/min/kg) | 28 | 77 |
| $T_{1/2}$ elimination (h) | 1.2 | 0.32 |
| $V_{ss}$ (L/kg) | 1.4 | 2.0 |
| Brain penetration (ICR mouse) same studies as above | | |
| Brain $C_{max}$ (ng/g) | 32 | 1619 |
| Brain $t_{max}$ (h) | 1 | 0.05 |
| Brain:plasma $C_{max}$ ratio | 0.05 | 2.7 |

**Table 5**
Effects of **1** and **24** following intravenous dosing in the tail flick test in the mouse

| Test compound | Dose (μmol/kg) | %MPE[a] | $ED_{50}$ (μmol/kg) | DofA[b] (min) |
|---|---|---|---|---|
| Vehicle (10% Tween 80) | 10 ml/kg | 2.9 ± 2.8 | | |
| Compound **1** | 0.1 | 14.5 ± 3.7 | | |
| Compound **1** | 0.3 | 72.3 ± 9.7[c] | | |
| Compound **1** | 1.0 | 100 ± 0[c] | | |
| Compound **1** | | | 0.2 | 50 |
| Vehicle (10% Tween 80) | 10 ml/kg | 2.01 ± 4.9 | | |
| Compound **24** | 0.35 | 29.3 ± 8.3 | | |
| Compound **24** | 1.17 | 54.1 ± 15.4 | | |
| Compound **24** | 3.52 | 100 ± 0[c] | | |
| Compound **24** | | | 0.8 | 160 |
| CP 55,940 | | | 0.11 | 110 |
| WIN 55,212-2 | | | 1.7 | 90 |

[a] %MPE: %maximum possible effect, see Ref. 11.
[b] Dofa: duration of action, time taken for the tail flick latency to return within the mean plus three standard deviations of the baseline value following a twice $ED_{50}$ dose.
[c] Denotes that the effect was significantly greater than in vehicle treated mice ($P$ <0.01, Dunn's post-test)

for further profiling based on its overall potency and stability in microsomes.

Compound **24** exhibited high affinity for both CB1 ($pK_i$ 8.2) and CB2 ($pK_i$ 8.5) 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. Furthermore, compound **24** was screened at $10^{-5}$ M against a panel of 63 unrelated molecular targets and showed no activity against any other targets.

The in vitro and in vivo DMPK profile of **24** and **1** (for comparison) is summarized in Table 4. In comparison to the carboxamide, **24** is cleared less rapidly as was predicted from the in vitro microsomal stability results. Furthermore, **24** had a slightly lower volume of distribution but overall a longer half-life than **1**. In stark contrast, the brain levels were markedly different with **24** having a much lower B:P ratio and a delayed brain $t_{max}$.

Based on its enhanced stability in vivo, **24** was progressed for testing in the mouse tail flick test.[10,11] Intravenous administration of **24** increased tail flick latency in a dose-dependent manner, Table 5, but with a lower $ED_{50}$ than the starting compound **1**, this may be due to the differences in CNS penetration between the two compounds. Having demonstrated that the compound is efficacious in this model the duration of action was measured, the time taken for the tail flick latency to return within the mean plus three standard deviations of the baseline value following a twice $ED_{50}$ dose. Pleasingly it was found that the duration of action of **24** was 160 min over three times longer than the duration of action observed with **1**, Table 5 and that our strategy of improving metabolic stability by replacement of the carboxamide with an bioisosteric heterocycle had realized the goal of improving duration of action in vivo.

In summary, a series of indole-3-heterocyclic derivatives were synthesized and found to be agonists of the CB1 receptor. In addition, these heterocyclic derivatives had improved mouse microsomal stability when compared to our previously reported indole-3-carboxamides. A systematic SAR study of the heterocycle, amine, and 7-position of the indole identified a highly potent and relatively stable CB1 receptor agonist **24**. This compound was found to have an improved duration of action after intravenous administration in the mouse tail flick test, a preclinical model of nociception.

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4. Superposition analysis was carried out with SYBYL7.3 and GASP as distributed by Tripos Inc., 1699 South Hanley Road, St. Louis, MO 63144-2913, USA. Image created with PyMOL 0.99, DeLano, M. L. The PyMOL Molecular Graphics System (2008) DeLano Scientific LLC, Palo Alto, CA, USA. http://www.pymol.org.
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11. Determination of tail flick latency in mice; male ICR mice weighing 20–34 g were trained to sit still in a tail flick apparatus (Ugo Basile, Italy) whilst tail flick latency was measured. The tail was exposed to a focused beam of radiant heat at a point approximately 2.5 cm from the tip. Tail flick latency was defined as the interval between the appliance of the thermal stimulus and withdrawal of the tail. A 12 second cut-off was employed to prevent tissue damage. Four groups of eight mice were treated with vehicle or one of three doses of the test compound. Tail flick latency was measured before administration of the test compound and at 20, 40, and 60 min after compound administration. Data were plotted as mean ± SEM and expressed as % maximum possible effect (%MPE).

$$\%\text{MPE} = \frac{(\text{post drug latency} - \text{baseline latency})}{(\text{Cut off latency} - \text{baseline latency})} \times 100$$

The time of maximum effect for each mouse in the two top dose groups was determined and these values averaged to calculate the mean time of maximum effect. For analytical purposes $T_{max}$ was defined as the time point closest to this averaged value. $T_{max}$ data were then compared between groups using the Kruskal–Wallis one-way analysis of variance, a non-parametric statistical test. If statistical significance ($P$ <0.05) was observed with this test, the vehicle group and each of the treatment groups were compared using the non-parametric Dunn's test (Unistat 5.0 software).