Bioorganic & Medicinal Chemistry Letters 20 (2010) 7327–7330

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

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

Elizabeth M. Moir [a], Kazuya Yoshiizumi [a], Jim Cairns [a], Phillip Cowley [a], Morag Ferguson [a], Fiona Jeremiah [a], Takao Kiyoi [a], Richard Morphy [a], Jason Tierney [a], Grant Wishart [a], Mark York [a], James Baker [b], Jean E. Cottney [b], Andrea K. Houghton [b], Petula McPhail [b], Andrew Osprey [b], Glenn Walker [b], Julia M. Adam [a,*]

[a] Department of Chemistry, Merck Research Laboratories, MSD Ltd, Newhouse, Lanarkshire ML1 5SH, UK
[b] Department of Pharmacology, Merck Research Laboratories, MSD Ltd, Newhouse, Lanarkshire ML1 5SH, UK

ARTICLE INFO

Article history:
Received 31 August 2010
Revised 13 October 2010
Accepted 14 October 2010
Available online 20 October 2010

Keywords:
Cannabinoid CB1 receptor
GPCR agonist

ABSTRACT

Bicyclic piperazine derivatives were synthesized as conformationally constrained analogs of *N*-alkyl piperazines and were found to be potent CB1 receptor agonists. The CB1 receptor agonist activity was dependent upon the absolute configuration of the chiral center of the bicyclic ring system. Although the conformational constraint did not protect the compounds from metabolism by N-dealkylation, several bicyclic analogs were found to be more potent than the unconstrained lead compound. Compound **8b** demonstrated potent antinociceptive activity in vivo.

The CB1 cannabinoid receptor is a member of G-protein coupled receptor (GPCR) superfamily, which is characterized by seven-transmembrane receptors.[1] The CB1 receptor is located primarily in the central nervous system but is also expressed on peripheral neurones. Activation of the CB1 receptor has been suggested as a potential strategy for the treatment of pain and several other diseases including glaucoma, traumatic brain injury, and multiple sclerosis,[2] while inhibition of CB1 receptors has been explored as a strategy for the treatment of obesity and addiction.[3] Several lines of evidence have been reported regarding the analgesic effects of CB1 receptor agonists in both experimental animal models and clinical studies. While limited in clinical utility by their small therapeutic window with respect to psychotropic side effects, a couple of CB1 receptor agonists including Δ^9-tetrahydrocannabinol (Δ^9-THC, Fig. 1), one of the major bioactive components of cannabis, are used clinically as antiemetics in cancer chemotherapy or appetite stimulants in AIDS patients.[4] Sativex™, a medicinal cannabis extract containing a mixture of Δ^9-THC and cannabidiol, has been recently launched for treatment of multiple sclerosis (MS)- and cancer-associated neuropathic pain, and for MS-associated spasticity. In addition, several lines of research are being progressed toward identifying novel cannabinoid related medicines that avoid or minimize the adverse effects associated with administration of classical cannabinoid agonists.[5] Moreover, the classical cannabinoid agonists represented by Δ^9-THC are highly lipophilic and the administration methods are still limited.

Previous publications described indole-3-carboxamide derivatives as water soluble CB1 receptor agonists suitable for intravenous administration as potential post-operative analgesics.[6,7] This Letter describes a series of bicyclic piperazine analogs in which the piperazine *N*-alkyl substituent is tethered back onto the piperazine ring (Fig. 2). Metabolite identification studies on initial compounds in the mono-cyclic series indicated piperazine N-dealkylation as a major route of metabolism. It was proposed that tethering the *N*-alkyl group back onto the piperazine ring system would favorably affect the metabolic stability of these compounds and, in addition, potentially improve potency within the series. The impact of stereochemistry and effects of substitution in the bicyclic piperazine system on CB1 receptor agonist potency and microsomal stability were investigated.

Compounds were synthesized as described in Scheme 1. Fourteen optically pure bicyclic piperazines **6a–n** were prepared using parallel synthesis techniques, applying the previously reported method for the synthesis (*S*)-1,4-diazabicyclo[4.3.0]nonane.[8] Reaction of amino acid methyl esters **2** and cyclic carboxylic acids **3** afforded amides **4**. Boc deprotection and cyclization followed by reduction of the amide carbonyl afforded bicyclic piperazines **6a–n**. Introduction of the piperazinylcarbonyl moiety to *N*-cyclohexylmethyl-7-methoxyindole **7** was performed by direct amide

* Corresponding author. Tel.: +44 1698 736000; fax: +44 1698 736187.
E-mail address: julia.adam@merck.com (J.M. Adam).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.10.061

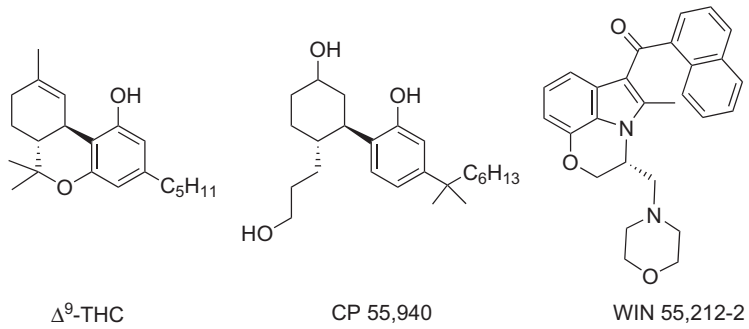


Figure 1. Representative naturally occurring and synthetic cannabinoid receptor agonists.

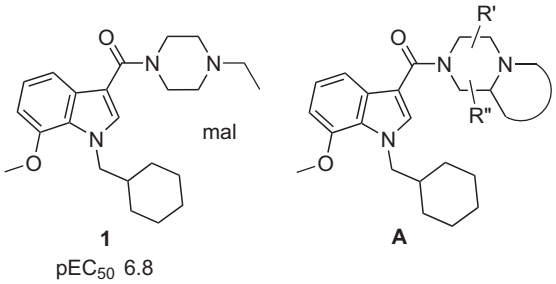


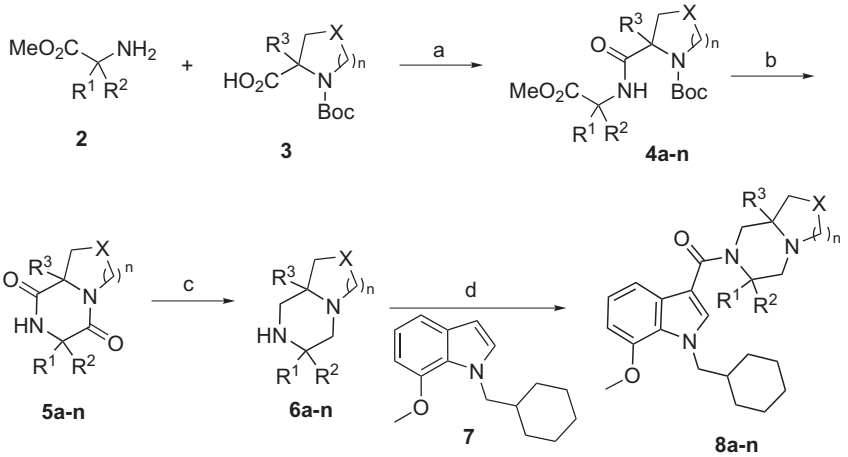
Figure 2. The structure of the original lead compound **1** and newly designed scaffold **A**.

formation[6] using oxalyl chloride in 1,1,2,2-tetrachloroethane at 120 °C for 2 h, followed by addition of bicyclic piperazine **6a–n** and triethylamine, to form the piperazine amides **8a–n**.

The prepared compounds were tested for CB1 receptor agonist activity using CHO cells doubly transfected with human CB1 and a luciferase reporter gene.[9] As shown in Table 1, a number of bicyclic piperazine analogs were more potent at CB1 than the unconstrained *N*-ethyl piperazine **1** (Fig. 2), for example, compound **8b** with $pEC_{50} = 7.9$ versus $pEC_{50} = 6.8$ for compound **1**. In general, for analogs with one chiral center (**) at the bridgehead position, the (*S*)-isomer was more potent than the (*R*)-isomer. This was true for 6,6- and 6,5-bicyclic piperazine ring systems, comparing derivative **8b** with **8a**, and derivative **8d** with **8c**. This general rule also held true for the R^1, R^2 dimethyl analogs, comparing derivative **8j** with **8i**. In cases where there was a single substituent at R^1 and $R^2 = H$ (examples **8e**–**h**), the combined effect of stereochemistry at the two chiral centers (* and **) was less clear cut. Replacement of methyl with the larger isobutyl substituent at R^1 was not tolerated, illustrated by the reduced potency of compounds **8k** and **8l** in comparison with derivatives **8e** and **8f**, respectively. Addition of a methyl substituent at the bridgehead position, R^3, was detrimental to activity (comparing analog **8m** with **8d**). Incorporation of a further heteroatom into the bicyclic piperazine system was tolerated. Indeed for X = S (example **8n**), there was an improvement in potency from $pEC_{50} = 6.3$ for compound **8g** to $pEC_{50} = 7.5$ for **8n**. Where tested, different salt forms (e.g., HCl salt/free base) had no effect on the in vitro potency (data not shown).

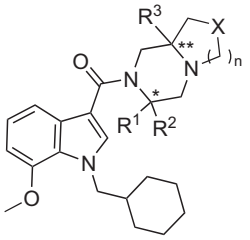
Microsomal stability was determined for all compounds in Table 1 but no improvement in stability over the initial lead, **1**, was observed. Compounds **8a–n** all showed a half-life of <5 min in human and mouse liver microsome preparations.

In order to investigate whether tethering back the *N*-alkyl substituent had perhaps shifted the major site of metabolism to a different region of the molecule, compound **8b** was briefly incubated with human liver microsomes (5 min) and the profiles of the metabolites were analyzed by LC–MS–MS (Scheme 2 and Table 2). To our surprise, the major metabolites were still found to correspond to metabolism in the bicyclic piperazine portion of the molecule, with N-dealkylation remaining a significant metabolic pathway as demonstrated by formation of metabolite **M2**. The enzymes responsible for this metabolism have not been characterized; however,



Scheme 1. Reagents and conditions (reaction yields depicted below were from synthesis of **8b** as a typical example): (a) $(Me)_2N(CH_2)_3N{=}C{=}NEt{\cdot}HCl$, HOBt, NEt_3, DCM, rt, 40 h, 98%; (b) (i) CF_3COOH, rt, 2 h, (ii) NEt_3, MeOH, reflux, 6 h, two steps 52%; (c) $LiAlH_4$, THF, reflux, 3 h, 68%; (d) oxalyl chloride, 1,1,2,2-tetrachloroethane, 120 °C, 2 h, then NEt_3 and **7**, rt, 7 h, 61%.

Table 1
CB1 receptor agonist activities for bicyclic piperazine compounds **8a–n**



8a - n

Compd	*n*	X	R^1	R^2	R^3	*	**	pEC_{50}[a]
8a	2	CH_2	H	H	H	—	*R*	7.0[b]
8b	2	CH_2	H	H	H	—	*S*	7.9[c]
8c	1	CH_2	H	H	H	—	*R*	7.2[b]
8d	1	CH_2	H	H	H	—	*S*	7.7[c]
8e	1	CH_2	CH_3	H	H	*R*	*R*	7.2[c]
8f	1	CH_2	CH_3	H	H	*S*	*S*	7.0[b]
8g	1	CH_2	CH_3	H	H	*S*	*R*	6.3[c]
8h	1	CH_2	CH_3	H	H	*R*	*S*	6.0[c]
8i	1	CH_2	CH_3	CH_3	H	—	*R*	6.2[b]
8j	1	CH_2	CH_3	CH_3	H	—	*S*	7.0[b]
8k	1	CH_2	*i*But	H	H	*R*	*R*	6.5[b]
8l	1	CH_2	*i*But	H	H	*S*	*S*	<5.0[b]
8m	1	CH_2	H	H	CH_3	—	*S*	5.9[c]
8n	1	S	CH_3	H	H	*S*	*R*	7.5[c]
1[6]								6.8[d]
CP 55,940								7.7
WIN 55,212-2								7.3

[a] Values are means of three experiments.
[b] Data for free base.
[c] Data for HCl salt.
[d] Data for maleic acid salt.

compound **8b** was shown to have inhibitory activity at a number of human P450s (IC_{50} values—CYP3A4 6 μM, CYP2C19 10 μM, CYP2C9 12 μM, and CYP2D6 13 μM). It was concluded that relatively high lipophilicity (*c* log *P* = 5.5 for compound **8b**) was likely to be a major contributing factor to the metabolic vulnerability[10] and that more profound changes to the physico-chemical properties of these molecules would be required in order to achieve metabolically stable CB1 receptor agonists.

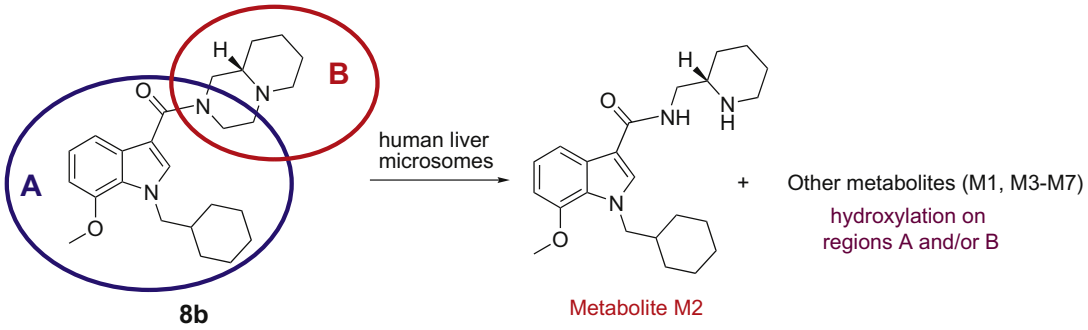
In view of the unexpectedly high potency of compound **2** and the earlier observation[7] that linking the 1- and 7-positions of the indole core improved the potency, we decided to combine these methods. The target compounds **10a** and **10b** were synthesized by the previously described methods using the optically pure tricyclic indole[7] (***R***)-**9** or (***S***)-**9** and the bicyclic amine **6b** (Scheme 3).

As depicted in Table 3, the (*R,S*)-isomer **10a** showed comparable CB1 receptor agonist activity to **8b**. The stereochemistry of the tricyclic indole core was crucial for the activity; the potency of the (*S,S*)-isomer **10b** was more than two log units lower than that of **10a**. Similar effects of this chiral center on CB1 receptor agonist activity were observed in the previously reported tricyclic indole series.[7] As expected, compounds **10a** and **10b** also showed a half-life of <5 min in human and mouse liver microsome preparations.

Based on their high potency, compounds **8b** and **10a** were selected for further evaluation. As shown in Table 4, compounds **8b** and **10a** 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.

The antinociceptive activities of compounds **8b** and **10a** were determined in the tail flick test[6] after iv administration in the mouse. A beam of radiant heat is focused onto the tail of the animal and the time recorded for the mouse to flick it's tail away from the heat source. The compounds significantly increased the tail flick latency, indicating an antinociceptive response, with ED_{50} values of 0.58 and 0.42 μmol/kg, respectively (Table 5).

The antinociceptive profile of compound **8b** was examined further in the formalin model of inflammatory pain, in which dilute formalin is injected into one hind paw of the mouse. The resultant nociceptive behavior is characterized by flinching, licking, lifting,

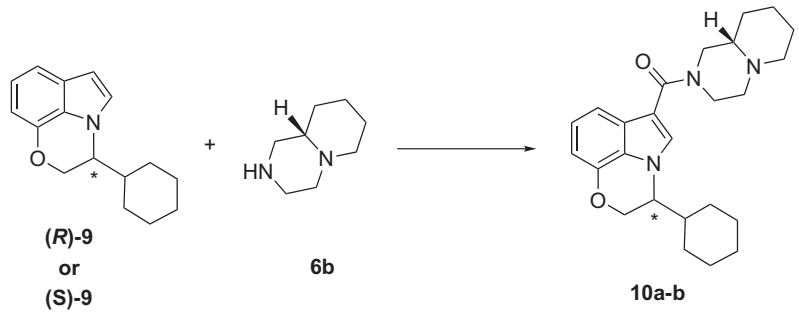


Scheme 2. Metabolism of compound **8b** in human liver microsomes.

Table 2
Metabolites of compound **8b**

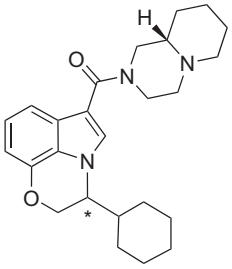
Compd	Retention time (min)	Apparent [M+H]	Metabolic transformation	Proposed site of metabolism	Relative peak area ratio metabolite/parent
M1	6.43	426	Hydroxylation	Region B	0.6
M2	6.14	384	Dealkylation	Region B	0.4
M3	4.54	442	Di-hydroxylation	At least one OH region A	0.2
M4	4.87	426	Hydroxylation	Region A	0.1
M5	4.66	442	Di-hydroxylation	At least one OH region A	0.05
M6	5.10	442	Di-hydroxylation	Unknown	0.05
M7	4.37	426	Hydroxylation	Region A	0.05

Conditions; column; Develosil RP aqueous 150 × 4.6 mm, eluents; 0.1% formic acid in MeCN/0.1% formic acid in water, 10/90 to 100/0, detected by Mariner-TOF.



Scheme 3. Reagents and conditions: oxalyl chloride, 1,1,2,2-tetrachloroethane, 120 °C, 1.5 h, then NEt_3 and **6b**, rt, 7 h, 76% for **10a** and 64% for **10b**.

Table 3
CB1 receptor agonist activities for tricyclic indoles **10a** and **10b**



Compound	*	pEC_{50}[a]
10a	*R*	8.0[b]
10b	*S*	5.7[b]

[a] Values are means of three experiments.
[b] Data for HCl salt.

Table 4
Profiles of CB1 receptor agonists **8b** and **10a** in in vitro hCB1 and hCB2 binding assays

Compound	CB1 pK_i	CB2 pK_i
8b	8.6	8.4
10a	8.8	9.4
CP 55,940	9.5	9.5
WIN 55,212-2	7.9	8.6

Table 5
Effects of CB1 receptor agonists following intravenous dosing in the tail flick test in the mouse

Compound	ED_{50} (μmol/kg)
8b	0.58
10a	0.42
CP 55,940	0.11
WIN 55,212-2	1.7

and biting the affected paw, and occurs in two distinct phases separated by a short period of quiescence. Iv administration of compound **8b** (0.03–3.0 μmol kg^{-1}) demonstrated dose-dependent inhibition of nociceptive behavior in both phases of the formalin response in the mouse.[6] The antinociceptive response was statistically significant from control at doses equal to, and above, 0.3 μmol kg^{-1}. The effect of **8b** in this test was markedly attenuated by pretreatment with the CB1 antagonist, SR-141716A, which indicated that the antinociceptive activity of this compound is mediated via activation of CB1 receptors. Furthermore, compound **8b** showed no genotoxic effects in Vitotox™ assay,[11] which can rapidly assess genotoxicity, at concentrations up to 10^{-4} M.

In summary, a series of conformationally constrained and optically pure bicyclic piperazines were synthesized and tested for CB1 receptor agonist activity and metabolic stability. Although the modifications failed to protect the compounds from metabolism by N-dealkylation, several analogs were found to have improved potency compared to the unconstrained *N*-alkyl piperazines. The selected compounds demonstrated potent antinociceptive activity in the tail flick and formalin paw tests in the mouse.

References and notes

1. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. *Nature* **1990**, *346*, 561.
2. Recent reviews for cannabinoid receptor agonists: (a) Pertwee, R. G. *Expert Opin. Investig. Drugs* **2000**, *9*, 1553; (b) Adam, J.; Cowley, P. M.; Kiyoi, T.; Morrison, A. J.; Mort, C. J. W. In *Progress in Medicinal Chemistry*; King, F. D., Lawton, G., Eds.; Elsevier, 2006; Vol. 44, pp 207–329; (c)*Handbook of Experimental Pharmacology*; Pertwee, R. G., Ed.; Springer, 2005; Vol. 168, (d) Huffman, J. W.; Padgett, L. W. *Curr. Med. Chem.* **2005**, *12*, 1395.
3. (a) Jagerovic, N.; Fernandez-Fernandez, C.; Goya, P. *Curr. Top. Med. Chem.* **2008**, *8*, 205; (b) Bermudez-Silva, F. J.; Viveros, M. P.; McPartland, J. M.; Rodriguez de Fonseca, F. *Pharmacol., Biochem. Behav.* **2010**, *95*, 375.
4. Mackie, K. *Annu. Rev. Pharmacol. Toxicol.* **2006**, *46*, 101.
5. Thakur, G. A.; Tichkule, R.; Bajaj, S.; Makriyannis, A. *Expert Opin. Ther. Patents* **2009**, *19*, 1647.
6. Adam, J. M.; Cairns, J.; Caulfield, W.; Cowley, P.; Cumming, I.; Easson, M.; Edwards, D.; Ferguson, M.; Goodwin, R.; Jeremiah, F.; Kiyoi, T.; Mistry, A.; Moir, E.; Morphy, R.; Tierney, J.; York, M.; Baker, J.; Cottney, J. E.; Houghton, A. K.; Westwood, P. J.; Walker, G. *Med. Chem. Commun.* **2010**, *1*, 54.
7. Kiyoi, T.; York, M.; Francis, S.; Edwards, D.; Walker, G.; Houghton, A. K.; Cottney, J. E.; Baker, J.; Adam, J. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4918.
8. De Costa, B. R.; He, X.; Linders, J. T. M.; Dominguez, C.; Gu, Z. Q.; Williams, W.; Bowen, W. *J. Med. Chem.* **1993**, *36*, 2311.
9. Price, M. R.; Baillie, G. L.; Thomas, A.; Stevenson, L. A.; Easson, M.; Goodwin, R.; McLean, A.; McIntosh, L.; Goodwin, G.; Walker, G.; Westwood, P.; Marrs, J.; Thomson, F.; Cowley, P.; Christopoulos, A.; Pertwee, R. G.; Ross, R. A. *Mol. Pharmacol.* **2005**, *68*, 1484.
10. Yoon, H. C.; Choi, E.; Park, J. E.; Cho, M.; Seo, J. J.; Oh, S. J.; Kang, J. S.; Kim, H. M.; Park, S. K.; Lee, K.; Han, G. *Bioorg. Med. Chem. Lett.* **2010**. doi:10.1016/j.bmcl.2010.08.117.
11. Westerink, W. M.; Stevenson, J. C.; Lauwers, A.; Griffioen, G.; Horbach, G. J.; Schoonen, W. G. *Mutat. Res.* **2009**, *676*, 113.