Synthesis, SAR Evaluation and Molecular Modeling of Modified Phenanthridines: Novel and Selective CB2 Agonists

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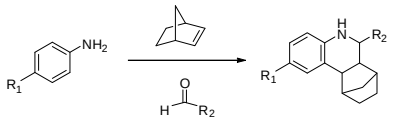
Introduction

Non-selective cannabinoid (CB) agonists are known to exhibit potent analgesic and anti-inflammatory effects. However, these therapeutic benefits are often accompanied by undesirable side effects which restrict their clinical use. Since the unwanted side effects of CB agonists are mostly CNS-dependent and attributed to activation of CB1 receptors, our aim was to identify compounds selective for CB2 receptors.

High throughput screening of our in-house library of compounds resulted in the identification of a number scaffolds that showed potent CB2 agonist activity in the nanomolar range. A modified phenanthridine scaffold was chosen for further exploration, due to ease of synthesis and promising CB2 selectivity in addition to potency. A short SAR program yielded compounds with cAMP EC_{50} < 10 nM for hCB2 and EC_{50} >10 μM for hCB1.

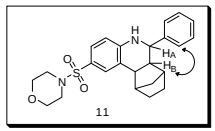
We will describe the synthesis and SAR of this series and provide a rationale for the activity and selectivity, based on molecular modeling comparison studies with known CB2 selective agonists.

Synthesis



The compounds described (**Table 1,2**) were prepared in a one-pot reaction by acid-catalyzed cyclo-condensation of bicyclo[2.2.1]hept-2-ene with imines derived from para-substituted arylamines and aldehydes in acetonitrile [see: Grieco *et al., Tetrahedron Lett.*, *29*, 5855 (1988))]. In some cases, the desired product precipitated from solution and was isolated simply by filtration. Alternatively, crude material was isolated after a basic aqueous extraction and was further purified by column chromatography.

Interestingly, several compounds were isolated as single diastereoisomers (racemates). The relative stereochemistry of **11** was determined by ^{1}H NMR. Coupling constants between the two hydrogen's suggest that the relative stereochemistry is trans (J_{AB} = 10 Hz).



Results

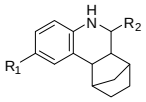


Table 1: Modification of R_1 and R_2

Cmpd #	R_1	R_2	cLogP	cAMP hCB2 EC_{50} nM (%Eff)	cAMP rCB2 EC_{50} nM (%Eff)	cAMP hCB1 EC_{50} nM (%Eff)
1			4.99	19 (84)	8 (78)	>10000
2			4.61	187 (49)	7 (78)	1189 (77)
3		$H_2C{-}CH_3$	3.71	1195 (84)	65 (84)	>10000
4			4.64	288 (73)	13 (70)	2149 (45)
5			4.56	140 (94)	31 (79)	>10000
6			4.42	3 (96)	2 (90)	5279 (52)
7			3.65	1 (99)	3 (89)	>10000
8			2.65	74 (98)	111 (85)	>10000
9			3.17	103 (89)	148 (53)	>10000
10			2.29	8 (95)	14 (91)	>10000
11			3.61	1 (101)	1 (96)	>10000
12			2.25	1 (96)	1 (91)	>10000
13			1.80	2004 (82)	3435 (65)	>10000
14			3.52	37 (96)	36 (84)	>10000
15			2.16	1486 (83)	4830 (96)	>10000

Experimental Methods

Receptor mediated responses were determined in Chinese Hamster Ovary (CHO-K1) cell lines stably expressing hCB2, hCB1 (both from Euroscreen) or rCB2 by measuring changes in intracellular cAMP using LANCE cAMP detection kit (PerkinElmer). Cells were pre-incubated with IBMX (isobutyl methyl xanthine) and stimulated with forskolin to increase basal cAMP levels. Responses (% Eff) were calculated as a percent of maximal cAMP inhibition by CP55,940.

Molecular modeling was performed using the Tripos Benchware3D Suite with Gateiger charges and the Amber 2002 atom force field for local minimization.

Discussion

Main SAR points **Table 1**:

- Reverse amide, urea, or directly attached morpholine all result in loss of potency – suggests requirement for H-bond acceptor (**Compounds # 2,4,5**)
- Amide can be replaced by sulfonamide (# **6-15**)
- R_1 needs lipophilic component – SO_2NH_2 loses potency but gains improved cLogP which results in metabolic stability (HLM>90 min) (# **13**)
- Alkyl R_2 strongly affects hCB2 (but rCB2 less so) (#**3**)
- Phenyl can be replaced by nitrogen-containing heterocycles without compromising potency (**10** and **12**)

Main SAR points **Table 2**:

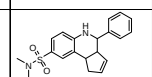
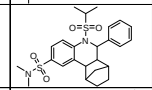
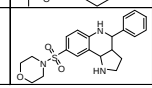
- Deletion of external cyclopentane ring (reaction with cyclopentadiene) abolishes activity (Compound # **16**)
- N5 substitution reduces activity (# **17**)
- Addition of N in cyclopentene ring of **16** gives improved cLogP values, high metabolic stability (HLM >90 min) but weak activity (# **18**)

Overall Summary

- High selectivity for hCB2 over hCB1 was observed in this series.*
- Initial active compounds showed little metabolic stability which may be due, at least in part, to high lipophilicity (cLogP >3).
- Reduction of cLogP values while maintaining hCB2 and rCB2 activity and hCB1 selectivity was accomplished by replacing the phenyl substituent with a heterocycle such as pyrazole.
- Achieving a balance of potency and metabolic stability required for advancement of this series remains a challenge

*Functional cAMP assay results were comparable with a radioligand binding assay data from MDS Pharma Inc. Compounds bind competitively with WIN55,212-2 at hCB2.
Compound **6**, hCB2 Ki = 9.5 nM
Compound **11**, hCB2 96%@1μM
Compound **12**, hCB2 73%@1μM

Table 2: Modifications to the Phenanthridine N5 and the Bridged Bicycle

Cmpd #	Structure	cLogP	cAMP hCB2 EC_{50} nM (%Eff)	cAMP rCB2 EC_{50} nM (%Eff)	cAMP hCB1 EC_{50} nM (%Eff)
16		3.33	>10000	3121 (-138)	1036 (-61)
17		3.88	1500 (81)	2825 (79)	>10000
18		2.22	2048 (82)	232 (49)	4280 (-56)

Modeling

In an effort to explain the observed SAR of this series, **6** was modeled in relation to the known CB2 ligands WIN 55,212-2 and A-796260. A manual overlay of R_1 with the morpholine group and R_2 with the lipophilic region orients the NH of the phenanthridine core with the conserved carbonyl group and gives a reasonable theoretical alignment (Figure 1). The proposed alignment also places the bridged bicycle in the vicinity of the aromatic ring of A796260 which is known to be tolerant to substitution [Frost *et al*. *J Med. Chem*., 51, 1904 (2007)]. The bridge itself occupies unique 3D space with respect to the known cannabinoid ligands.

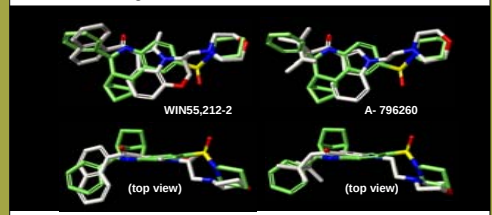


Fig 1: Theoretical alignment of Compound **6** with WIN 55,212-2 (orthosteric, non-selective) & A-796260 (CB2 selective).

The lack of CB1 activity of the bridged phenanthridines can be explained by comparison of **11** with classical CB1 and CB2 ligands (Figure 2). Although all of the ligands adopt the classical cannabinoid "U-shape" conformation (Panel A), there are subtle differences in the way the pendant aromatic substituents interact with the receptor (Panel B). We hypothesize that the unique space occupied by the bridged bicycle is accessible for CB2, but forbidden for CB1.

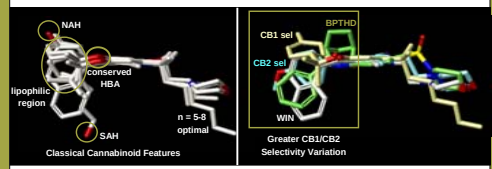


Fig 2: Panel A – Overlay of WIN 55,212-2, A-796260, CP-55,940, Δ9-THC & JWH-133 in a putative bioactive conformation with pendant groups perpendicular to the core. Panel B – Overlay of selective cannabinoids with WIN 55,212-2; HU-210 (CB1), AM630 (CB2) & Compound **11** (CB2).

Conclusions

- A facile, one-pot, stereoselective route to a novel series of bridged phenanthridines was developed.
- Potent hCB2 agonists were identified with minimal hCB1 activity, which can be rationalized by modeling.
- cLogP values were improved while maintaining good activity and selectivity.
- These compounds may be further optimized for drug development, and they may provide useful tools for the investigation of CB2-specific pharmacology.

Contact information

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