Design and Synthesis of Novel Cannabinoid Receptor Modulators

Mary Paige Thrash

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DESIGN AND SYNTHESIS OF NOVEL CANNABINOID RECEPTOR MODULATORS

by
Mary Paige Thrash

A thesis submitted to the faculty of The University of Mississippi in partial fulfillment of the requirements of the Sally McDonnell Barksdale Honors College.

Oxford
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ABSTRACT

Cannabinoid (CB) receptors are validated drug targets in the endocannabinoid signaling system associated with a number of human pathologies, and the development of novel and selective small molecule CB ligands is warranted. A lead molecule HL-010, a member of the $4H$-thieno[3,2-$b$]pyrrole-5-carboxamide class of compounds, was previously identified through CB homology modeling and virtual screening protocols as a potential high affinity ligand for cannabinoid receptors. Validation of the in silico data was realized with the evaluation of both CB$_1$ and CB$_2$ receptor binding and functional activity assessment: HL-010 was found to be a potent (~10 nM) and selective CB$_2$ receptor agonist with more than 100-fold selectivity over CB$_1$ receptors. Unfortunately, its high lipophilicity limited its aqueous solubility, and prevented further evaluation in animal models. The bioisostere, RS-DFA-6-2 containing the $4H$-furo[3,2-$b$]pyrrole-5-carboxamide scaffold, was synthesized and preliminary data showed similar CB$_2$ binding affinity (~18 nM) with modest selectivity (40 fold) compared to HL-010, with slightly improved aqueous solubility. This thesis research discloses the design and synthesis of four additional analogs modified at the carboxamide group (7a-d) using RS-DFA-6-2 as the parent scaffold. These compounds were tested against CB receptors using established radioligand displacement assays. Analog 7a, predicted to have enhanced aqueous solubility, was the only compound that demonstrated reasonable CB$_2$ receptor potency and selectivity when compared to CB$_1$. 
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INTRODUCTION

CANNABINOID RECEPTORS AND THE ENDOCANNABINOID SYSTEM

The endocannabinoid signaling system is implicated in a number of central and peripheral physiologies and pathologies including, but not limited to: inflammation, acute and chronic pain, neurodegenerative diseases, reproductive health, immune regulation, and muscle movement and coordination (1). The endocannabinoid system is composed primarily of two main receptor types known as cannabinoid receptor subtype 1 (CB1) and cannabinoid receptor subtype 2 (CB2). Both CB1 and CB2 are G-protein-coupled receptors (GCPRs) that can adopt multiple active conformations, allowing them to regulate distinct signaling cascades. This process of using multiple active conformations to signal different pathways is referred to as biased signaling (2).

CB1 and CB2 receptors exhibit distinct tissue and cellular expression patterns: CB1 receptors are predominantly expressed in the brain and to a lesser extent in the periphery, while CB2 receptors are prevalent in nearly all immune cells in addition to their overexpression in activated glial cells in the central nervous system (3).

Cannabinoids like Δ⁹-tetrahydrocannabinol (Δ⁹-THC), which agonize both cannabinoid receptor types, result in physiological effects attributed primarily to centrally-expressed CB1 receptors. Studies have also shown CB2 agonists have large potential as therapeutics for neuropathic pain, immune disorders involving inflammation,
osteoporosis, and other pathologies without eliciting the psychoactive effects seen with nonselective cannabinoids (4).

The design and development of selective small-molecule ligands for the CB receptor family has been challenging due to the lack of X-ray structures. However, the X-ray crystal structure of the human CB₁ receptor in complex with the stabilizing antagonist AM6538 has recently been reported (5). Molecular docking studies were also performed to elucidate the binding modes of a diverse set of antagonists and agonists of the CB₁ receptor. Prior to the disclosure of the CB₁ receptor X-ray structure, most drug discovery efforts were based on the development and validation of CB homology models based on related GPCRs like the bovine rhodopsin receptors and beta-adrenergic receptors (6,7). Computational drug discovery efforts based on these homology models enabled researchers to use virtual screening protocols to identify novel small molecule “hit” compounds. These computational strategies served as predictive models to guide drug discovery and development in hit-to-lead campaigns targeting the CB receptors (8).

Through in silico virtual screening in combination with homology modeling conducted at the University of Mississippi (Professor Robert Doerksen), a selective CB₂ agonist known as HL-010 was discovered (Figure 1). This molecule was synthesized and tested in the UM NIH COBRE CORE-NPN in vitro Core for CB receptor binding and functional activity. Based on the data, HL-010 was determined to be a selective (100-fold vs CB₁) and potent (≈10 nM) CB₂ agonist. HL-010 was also tested in vivo in mice and showed no activity in the tetrad behavioral assay which measures locomotor activity, catalepsy latency, change in rectal temperature, and hotplate latency. The absence of activity suggested that this molecule did not produce any collateral CB₁ agonist effects.
These results further demonstrated the potential for selective CB$_2$ agonists as therapeutics without the psychotropic effects. However, the formulation and administration of **HL-010** was difficult due to its limited aqueous solubility, a function of its high LogP. In an effort to mitigate this detrimental physiochemical property, additional analogs were considered. For example, **RS-DFA-6-2**, a bioisostere containing the 4$H$-furo[3,2-$b$]pyrrole-5-carboxamide scaffold, was synthesized and CB receptor binding data showed equivalent CB$_2$ binding affinity (18 nM) with modest selectivity (40 fold vs CB$_1$) when compared to **HL-010**. However, the improvement in aqueous solubility was only marginal.

![Figure 1. Structures of selective CB$_2$ ligands and associated CLogP values.](image)

The aim of this thesis research was to design and synthesize a library of **RS-DFA-6-2** analogs that were predicted to display greater water solubility profiles with a retention of its CB$_2$ affinity, selectivity, and functional activity (agonist). The research described includes the synthesis and structure characterization of four carboxamide analogs of **RS-DFA-6-2**, their corresponding *in vitro* evaluation against CB$_1$ and CB$_2$ receptors, and functional activity assessment for the most promising analog(s).
Methods

General Methods

All reagents and starting materials were purchased from Sigma-Aldrich (St. Louis, MO), Acros Organics/Thermo Fisher Scientific (Waltham, MA). All anhydrous solvents were purchased in bottles with molecular sieves (4Å). Air-free and moisture sensitive reactions were carried out using standard air-free techniques in flame dried glassware under an argon atmosphere. Thin-layer chromatography was utilized to monitor reactions with 225 μm aluminum backed TLC plates coated with silica gel 60 F254 (EMD Millipore, Billerica, MA). TLC plates were visualized with UV light (254 nm) and staining with ethanolic p-anisaldehyde, potassium permanganate, or phosphomolybdic acid stains. Mass spectrometry (low resolution) experiments were performed on a Waters Micromass ZQ single quadrupole mass spectrometer using either ESI positive (ESI+) or ESI negative (ESI-) electrospray ionization. Proton (1H) and carbon (13C) NMR spectra were recorded on Bruker 400 MHz Avance NMR spectrometers and processed using M nova NMR (Mestrelab Research).

(2): Ethyl 2-azidoacetate (Ref 10)

A solution of commercially available ethyl-2-bromoacetate (20 g, 0.12 mol) and acetone (125 mL) was combined under standard conditions and then
cooled to 0°C. While stirring, a solution of sodium azide (19.5 g, 0.3 mol) in water (100 mL) was added dropwise to the ethyl-2-bromoacetate solution. The temperature was then increased to 63°C and remained stirring at 63°C for four hours. The mixture was then cooled to room temperature, placed into a separation funnel, and extracted 7x with 40 mL of ethyl acetate. The combined organic fractions were then washed 3x with 20 mL of sodium bicarbonate and 3x with 10 mL of water. The organic layer was then dried over anhydrous sodium sulfate. Following filtration, the solvent was removed from the filtrate under reduced pressure and the residue was dried under vacuum to yield 15.0 grams (97 %) of 2. 

**MS(ESI+):** m/z = 152.48 [M+Na]^+

**(3a): Methyl (Z)-2-azido-3-(furan-2-yl) acrylate (Ref 11)**

Sodium ethoxide (4.8 g, 0.07 mol) was added to a flask fitted with a pressure equalizer. Air was evacuated from the flask and the system was then kept under an argon atmosphere. Anhydrous ethanol (39 mL) was added to the flask and cooled to -10 °C. The pressure equalizer was charged with a solution containing 2-furaldehyde (1.49 mL, 0.018 mol) and compound 2 (9.0 g, 0.07 mol) dissolved in tetrahydrofuran (75 mL). While the flask remained at -10 °C and with constant stirring, the solution was added slowly over a period of 2 hours. The mixture was then continuously stirred for an additional 2.5 hours at 0 °C. The resulting mixture was combined with ice water, extracted three times with equal amounts of ethyl acetate, and then washed...
with water. The organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure. The residue was purified using silica gel chromatography column with a mobile phase consisting of 10% ethyl acetate/hexanes. The collected fractions containing the product were combined and the solvent was removed under reduced pressure. Mass spectrometry and NMR analysis confirmed the product structure. Storage of the purified product in the refrigerator for 3 days showed significant decomposition.

$^1$H NMR: (400 MHz, Chloroform-\textit{d}) $\delta$ 7.49 (s, 1H), 7.10 (s, 1H), 6.86 (s, 1H), 6.53 (d, $J = 1.6$ Hz, 1H), 3.89 (s, 3H). $^{13}$C NMR: (101 MHz, Chloroform-\textit{d}) $\delta$ 177.81, 152.90, 148.08, 121.14, 112.57, 77.45, 77.14, 76.82, 29.60.

(3b): Methyl (Z)-2-azido-3-(furan-2-yl) acrylate (Ref I1)

Sodium methoxide (1.69 g, 0.031 mol) was added to into a flask fitted with a pressure equalizer. Air was evacuated from the flask and the system was then kept under an argon atmosphere. Anhydrous methanol (24.5 mL) was added to the flask and cooled to -10°C. Over a period of two hours, a mixture of anhydrous tetrahydrofuran (33.4 mL), 2-furaldehyde (0.72 mL, 0.009 mol), and ethyl-2-azidoacetate (4.0 g, 0.03 mol) was added to the flask with constant stirring. The mixture was then continuously stirred for an additional 2.5 hours at 0°C. The resulting mixture was combined with ice water, extracted three times with equal amounts of ethyl acetate, and then washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered, and then the solvent was evaporated under reduced pressure. The residue was purified using silica gel
chromatography column with a 10% ethyl acetate/hexanes solution as the mobile phase. The collected fractions from the column were combined and the solvent was removed under reduced pressure. This yielded 1.2 grams of compound 3.

**MS(ESI+):** m/z= 216.13 [M+Na]+.

**^1H NMR:** (400 MHz, Chloroform-d) δ 7.51, 7.12, 6.88, 6.55, 6.54, 3.90.


An anhydrous toluene (75 mL) solution of compound 3b (1.0 g, 5.18 mmol) was added to a flask of boiling toluene (75 mL) slowly over one hour. The reaction was allowed to reflux for an additional hour and then cooled to room temperature. The product was condensed under reduced pressure and purified using silica gel chromatography with a mobile phase consisting of 25% ethyl acetate/hexanes solvent system. The solvent was removed under reduced pressure, and the product dried under high vacuum. 390 milligrams of compound 4 were obtained.

**MS(ESI+):** m/z= 187.98 [M+Na]+

**^1H NMR:** (400 MHz, Chloroform-d) δ 9.08 (s, 1H), 7.51 (d, J = 2.2 Hz, 1H), 6.80 (dd, J = 1.7, 0.9 Hz, 1H), 6.45 (dd, J = 2.2, 1.0 Hz, 1H), 3.88 (d, J = 0.8 Hz, 3H).

**^13C NMR:** (101 MHz, CDCl₃) δ 162.68, 148.71, 147.98, 128.96, 123.81, 98.91, 96.96, 77.35, 77.04, 76.72, 51.59.
A minimal amount of dimethylformamide (2.5 mL) was used to dissolve compound 4 (200 mg, 1.21 mmol) in a flask and then cooled to 0°C. NaH (60% in mineral oil; 145 mg, 3.63 mmol) was added slowly to the flask while stirring and cooling. The flask was then vacuumed and purged with argon. The mixture was stirred at room temperature for one hour. 4-fluorobenzylchloride (0.36 mL, 3.63 mmol) was then added dropwise and the mixture continued to stir for an additional 3 hours. Water (10 mL) was added to the mixture which was then added to a separatory funnel to be extracted with toluene (3 x 10 mL) and washed with water (3 x 10 mL). The organic layer was dried over anhydrous sodium sulfate and filtered. The solvent was removed under reduced pressure and the crude product was purified using a silica gel chromatography column with a 10% ethyl acetate/hexanes solvent system. This yielded 165 milligrams of compound 5. This procedure was repeated on a 185 milligram scale and produced an additional 90 milligrams of compound 5.

**MS(ESI+):** m/z = 296.33 [M+ Na]^+

**1H NMR:** 1H NMR (400 MHz, Chloroform-d) δ 7.46 (d, J = 2.3 Hz, 1H), 7.24 – 7.10 (m, 2H), 6.99 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 0.8 Hz, 1H), 6.19 (dd, J = 2.2, 0.9 Hz, 1H), 5.60 (s, 2H), 3.81 (s, 3H).
\[ ^{13}C \text{ NMR:} \ (101 \text{ MHz, CDCl}_3) \delta 163.43, 162.39, 160.98, 148.59, 145.97, 133.52, 132.96, 129.06, 128.98, 123.20, 115.60, 115.39, 99.08, 98.56, 77.34, 77.00, 51.19, 50.02. \]

(6): 4-(4-fluorobenzyl)-4\textit{H}-furo[3,2-\textit{b}] pyrrole-5-carboxylic acid

Compound 5 (220 mg, 0.80 mmol) was dissolved in 3mL of tetrahydrofuran and added to a flask with a stir bar. An aqueous solution of KOH (60 mg in 4 mL of water) was added to the flask while stirring. The resulting mixture was allowed to reflux for 10 hours at 83°C. The tetrahydrofuran was removed under reduced pressure. The aqueous solution was acidified with 5% hydrochloric acid and extracted three times with 10mL of ethyl acetate. The compound was then washed with water (3 x 10mL), dried over anhydrous sodium sulfate, and filtered. The solvent was removed under reduced pressure and yielded 172 mg of compound 6.

\[ \text{MS(ESI+): } m/z = 282.36 [\text{M+Na}]^+ \]

\[ ^{1}H \text{ NMR:} \ ^{1}H \text{ NMR (400 MHz, Chloroform-}d\text{)} \delta 7.49 (d, J = 2.2 \text{ Hz, 1H}), 7.32 - 7.10 (m, 2H), 7.07 - 6.90 (m, 3H), 6.17 (dd, J = 2.2, 1.0 \text{ Hz, 1H}), 5.60 (s, 2H). \]

\[ ^{13}C \text{ NMR:} \ (101 \text{ MHz, CDCl}_3) \delta 166.87, 163.49, 161.04, 149.41, 146.01, 134.25, 129.19, 129.11, 115.65, 115.44, 100.75, 98.59, 77.33, 76.69, 50.18. \]
General Procedure 7: Amine Coupling Reaction

A portion of purified 6 (25 mg, 96.44 µmol) was dissolved in anhydrous tetrahydrofuran (2 mL) and stirred in a flask. Each variable amine group (1.5 equivalents) in dichloromethane (1 mL), water soluble carbodiimide (25 mg), HOBT (21 mg), and DIEA (30 µL) were added to the stirring solution. The mixture continued to stir at room temperature for 16 hours. The solvent was removed under reduced pressure, then water (20 mL) was added to the flask. Then, 1N HCl (5 mL) was added dropwise to the flask while cooling. The mixture was extracted with ethyl acetate, washed with water, and washed with brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using a silica gel column with a 50% ethyl acetate/ hexanes solvent system. The product was analyzed using mass spectrometry and NMR spectroscopy prior to CB receptor evaluation.

(7a): 4-(4-fluorobenzyl)-N-((1R,3S,5R,7S)-3-hydroxyadamantan-1-yl)-4H-furo[3,2-b]pyrrole-5-carboxamide

Synthesized via General Procedure 7 with 3-amino-1-hydroxyadamantane (24.2 mg, 144 µmol), yielding 17.0 mg of 7a.

**MS(ESI+):** m/z = 409.52 [M+H]⁺

**¹H NMR:** (400 MHz, Chloroform-d) δ 7.19 (d, J = 5.5 Hz, 2H), 6.98 (t, J = 8.7 Hz, 2H), 6.40 (s, 1H),
6.16 (s, 1H), 5.66 (s, 1H), 5.58 (s, 2H), 2.31 (s, 2H), 2.06 (d, J = 10.2 Hz, 4H), 2.00 (s, 4H), 1.75 (d, J = 12.3 Hz, 4H), 1.65 (s, 2H), 1.26 (d, J = 7.1 Hz, 2H).

**$^{13}$C NMR:** (101 MHz, CDCl$_3$) δ 161.99, 146.97, 145.79, 130.75, 129.33, 128.16, 115.48, 115.27, 98.75, 93.39, 77.33, 76.69, 69.24, 54.56, 49.28, 44.11, 40.48, 34.87, 30.66.

(7b): azepan-1-yl(4-(4-fluorobenzyl)-4H-furo[3,2-b] pyrrol-5-yl) methanone

![Chemical structure of (7b)](image)

Synthesized via General Procedure 7 with azepane (14.3 mg, 16.30 µL, 144.8 µmol) yielding 26.9 mg of 7b.

**MS(ESI+):** m/z= 364.47 [M+Na]$^+$

**$^1$H NMR:** $^1$H NMR (400 MHz, Chloroform-d) δ 7.37 (dd, J = 2.3, 1.1 Hz, 1H), 7.26 (d, J = 1.1 Hz, 0H), 7.20 – 7.09 (m, 2H), 7.01 – 6.90 (m, 2H), 6.30 – 6.19 (m, 1H), 5.35 (s, 2H), 3.57 (t, J = 6.0 Hz, 3H), 1.68 (s, 3H), 1.61 (d, J = 1.1 Hz, 1H), 1.43 (d, J = 1.1 Hz, 3H), 1.25 (s, 3H), 0.86 (d, J = 11.8 Hz, 1H).

(7c): (4-(4-fluorobenzyl)-4H-furo[3,2-b] pyrrol-5-yl)(morpholino) methanone

![Chemical structure of (7c)](image)

Synthesized via General Procedure 7 with morpholine (12.6 mg, 144 µmol) yielding 24.7 mg of 7c.

**MS(ESI+):** m/z= 351.41 [M+Na]$^+$

**$^1$H NMR:** $^1$H NMR (400 MHz, Chloroform-d) δ 7.39 (d, J = 2.2 Hz, 1H), 7.26 (s, 1H), 7.18 – 7.08 (m, 2H),
6.98 (t, $J = 8.7$ Hz, 2H), 6.31 – 6.20 (m, 2H), 5.35 (s, 2H), 3.68 (dd, $J = 5.7$, 3.9 Hz, 4H), 2.04 (s, 2H), 1.26 (t, $J = 7.1$ Hz, 2H).

$^{13}$C NMR: (101 MHz, CDCl$_3$) δ 146.75, 129.28, 129.20, 115.56, 115.34, 98.56, 95.25, 77.32, 77.00, 76.68, 66.84, 49.69.

(7d): $N$-(3,4-dihydroxyphenethyl)-4-(4-fluorobenzyl)-4H-furo[3,2-b] pyrrole-5-carboxamide

Synthesized via General Procedure 7 with 23 mg of compound 6 and 3-hydroxytyramine (20 mg, 133 µmol) yielding 19.4 mg of 7d.

MS(ESI+): m/z = 417.41 [M+Na]$^+$

$^1$H NMR: $^1$H NMR (400 MHz, Chloroform-d) δ 7.39 (d, $J = 2.2$ Hz, 1H), 7.14 (dd, $J = 8.6$, 5.4 Hz, 2H), 6.95 (t, $J = 8.7$ Hz, 2H), 6.78 (d, $J = 8.0$ Hz, 1H), 6.67 (d, $J = 2.0$ Hz, 1H), 6.56 (dd, $J = 8.0$, 2.0 Hz, 1H), 6.37 (s, 1H), 6.17 (d, $J = 2.8$ Hz, 1H), 5.97 (t, $J = 6.0$ Hz, 1H), 5.57 (s, 2H), 4.13 (q, $J = 7.1$ Hz, 1H), 3.54 (q, $J = 6.7$ Hz, 2H), 2.69 (t, $J = 6.9$ Hz, 2H), 1.27 (t, $J = 7.1$ Hz, 1H).

$^{13}$C NMR: (101 MHz, Chloroform-d) δ 163.04, 160.93, 147.49, 145.82, 144.17, 142.89, 133.50, 131.64, 130.87, 129.03, 126.77, 120.78, 115.53, 115.32, 98.78, 94.26, 50.02, 40.89, 34.96.
Radioligand Displacement for Cannabinoid Receptor Subtypes.

Compounds evaluated in this assay were run in competition binding with both cannabinoid receptor subtypes, CB₁ and CB₂. CB receptor binding screening was performed under the following conditions: 10 μM of each compound from independent triplicate dilutions was incubated with 1.70 nM (CB₁) or 1.9 nM (CB₂). The full agonist [³H]-CP 55,940, and 5 μg of CB₁ or 1 μg of CB₂ membrane were incubated for 90 min at 37 °C with gentle agitation in a 96-well plate in a 0.2 mL final volume of 50 mM Tris-HCl, 20 mM EDTA, 154 mM NaCl, and 0.2% radioimmunoassay grade BSA, pH 7.4. The reaction was terminated via rapid vacuum filtration through a UniFilter 96 GF/C filter (PerkinElmer Life Sciences Inc., Boston, MA, USA), presoaked with 0.3% polyethylenimine, followed by 10 washes with 50 mM Tris-HCl, pH 7.4, buffer containing 0.2% BSA. Filters were dried, 25 μL of MicroScint20 was added, and the plates were read using a TopCount NXT microplate scintillation counter (PerkinElmer Life Sciences Inc., Boston, MA, USA). Total binding was defined as binding in the presence of vehicle (1.0 % DMSO). Nonspecific binding was the binding observed in the presence of 10.0 μM CP-55,940. Specific binding was defined as the difference between total and nonspecific binding. Percent displacement was calculated using the following formula: 100-(specific binding of compound - nonspecific binding) x (100/specific binding).
Results and Discussion

Design and Synthesis of RS-DFA-6-2 Analogs

One of the more critical challenges associated with structure-activity-relationship (SAR) studies for GPCR drug design and discovery campaigns is to make judicious changes to the lead molecule without altering its functional activity. This is clearly denoted in Figure 2, where small structural modifications of CB$_2$ receptor ligands results in major reversals in their functional activities (13).

![Figure 2. Consequences of minor structure changes on CB functional activity.](image-url)
Prior to the inclusion of the **RS-DFA-6-2** scaffold in our SAR analysis, lead compound **HL-010** was identified through virtual screening methods, synthesized, and evaluated against CB receptors. From these initial efforts, bioisosteric **RS-DFA-6-2** was synthesized and shown to have similar binding affinity to the CB2 receptor, but its aqueous solubility was only marginally improved. **RS-DFA-6-2** differs from **HL-010** only with the substitution of an oxygen atom for a sulfur atom in the bicyclic ring system.

The synthesis of 4H-furo[3,2-b]pyrrole-5-carboxamide analogs commenced with the initial synthesis of the scaffold as illustrated in **Figure 3**. Treatment of ethyl-2-bromoacetate (1) with sodium azide resulted in the facile substitution reaction, resulting in ethyl 2-azidoacetate (2). Reaction of 2-furaldehyde with 2 in a condensation reaction afforded 2-azido acrylate (3). Heating a solution of 3 in toluene (Hemetsberger-Knittel reaction) resulted in the formation of the fused bicyclic 4H-furo[3,2-b]pyrrole-5-carboxylic acid ester (4). Alkylation of 4 with sodium hydride and 4-fluorobenzyl chloride yielded the substitution product 5. Hydrolysis of the methyl ester of 5 with potassium hydroxide afforded the product scaffold carboxylic acid 6. Mass spectrometry and NMR spectroscopy analysis were employed to confirm the mass and structure of the intermediates and final products.
Figure 3. Synthesis 4-(4-fluorobenzyl)-4H-furo[3,2-b] pyrrole-5-carboxylic acid (6)
Reagents and conditions: (i) NaN₃, Acetone, 63 °C, 4 h. (ii) NaOMe/MeOH, THF, 0 °C, 2.5 h. (iii) Toluene, reflux, 2 h. (iv) DMF, NaH, 25 °C, 3 h. (v) KOH, THF, H₂O, 83 °C, 10 h.

The approach taken to design new analogs included a search of the primary literature, examining molecules that have reported CB₂ selective activity with good aqueous solubility. The successful synthesis of the scaffold 6 allowed for the expedient synthesis of new carboxyamide analogs. Four analogs were proposed for synthesis, and included the reaction of scaffold 6 with various amines using standard coupling reactions (Figure 4).

Figure 4. Synthesis of Analogs. Reagents and Conditions: (vi) amine, THF, DCM, WSC, HOBT, DIEA, 25°C, 16 h.
The four new analogs (7a-7d) synthesized from scaffold 6 used the same general reaction conditions while varying the nature of the amine group, illustrated in Figure 4. Specifically, an amine-carboxylic acid coupling reaction was employed, in the presence of HOBT as an activator to produce the analogs. The compounds were analyzed by mass spectrometry for molecular weight confirmation, and by NMR analysis to confirm their structure.

Table 1 depicts the structures of the analogs, their corresponding molecular weights, and the calculated LogP values. The reduction in lipophilicity is evident from calculated LogP values. Analog 7a, comprising a simple modification of RS-DFA-6-2 with the introduction of a hydroxyl group to the adamantyl ring resulted in a dramatic reduction in lipophilicity by 1.5 log units when compared to RS-DFA-6-2 (CLogP=5.3). Analogs 7b and 7d are structurally dissimilar but also display lower CLogP values relative to the parent molecule. The least lipophilic analog 7c (CLogP=2.2) would be expected to exhibit the greatest aqueous solubility of the synthesized analogs.

<table>
<thead>
<tr>
<th>Analog:</th>
<th>7a</th>
<th>7b</th>
<th>7c</th>
<th>7d</th>
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<tbody>
<tr>
<td>Structure:</td>
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<td><img src="structure_7b.png" alt="Structure" /></td>
<td><img src="structure_7c.png" alt="Structure" /></td>
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<tr>
<td>Molecular Formula:</td>
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<td>C_{20}H_{21}F_{2}N_{2}O_{2}</td>
<td>C_{18}H_{17}F_{2}N_{2}O_{3}</td>
<td>C_{22}H_{19}F_{2}N_{2}O_{4}</td>
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<tr>
<td>Molecular Weight:</td>
<td>408.47</td>
<td>340.40</td>
<td>328.34</td>
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<tr>
<td>CLogP</td>
<td>3.9</td>
<td>3.8</td>
<td>2.2</td>
<td>3.5</td>
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</table>

Table I. Synthesized Analogs and Calculated LogP values
These four analogs were screened for both cannabinoid and opioid receptor binding activity. In the case of cannabinoid activity assessment, a radioligand competitive binding assay was employed to measure the ability of ligands to displace the radioligand $[^3\text{H}]-\text{CP-55940}$ from CB$_1$ and CB$_2$ cannabinoid receptors and is commonly used as an initial step in the assessment of efficacy of a cannabinoid receptor ligand. The binding data presented in Table II is a result of experiments conducted by NIH COBRE CORE-NPN in vitro Core scientists at the University of Mississippi; the assay protocols used were in accordance with previously reported methods conducted by this Core (14).

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB$_1$</th>
<th>CB$_2$</th>
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<tbody>
<tr>
<td>7a</td>
<td>41.3%</td>
<td>92.2%</td>
</tr>
<tr>
<td>7b</td>
<td>43.6%</td>
<td>45.8%</td>
</tr>
<tr>
<td>7c</td>
<td>16.8%</td>
<td>5.4%</td>
</tr>
<tr>
<td>7d</td>
<td>75.7%</td>
<td>40.9%</td>
</tr>
</tbody>
</table>

Table II. Displacement of $[^3\text{H}]-\text{CP55940}$ binding. 10 µM test compound against human CB$_1$ and CB$_2$ receptors, expressed as percentage (%)

The preliminary screening data reveal several important findings. Analog 7a displaced the largest percent displacement against CB$_2$ receptors and reasonable selectivity versus CB$_1$ receptors. Analogs 7b, 7c, and 7d did not meet the threshold of 50% displacement in the CB$_2$ receptor assay, and were not submitted for Ki evaluation. It appears that major changes to the structure of the carboxyamide side chain negatively influences binding to the CB$_2$ receptor, and the presence of the adamantane ring (or bioisostere) is recommended. Based on the CB receptor binding data, analog 7a showed the most promise as a CB$_2$ selective ligand. Since analog 7a exceeded the threshold of
CB₂ percent displacement, additional studies were performed using serial dilutions of 7a using the same assay to establish binding constants. The results are shown in Table III. Analog 7a was approximately 10 fold less effective in binding to CB₂ receptors that the parent molecule RS-DFA-6-2 when the Ki values are compared. It also displays a preferred selectivity for CB₂ receptor binding versus CB₁ receptors, similar to RS-DFA-6-2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CB₁ (Ki, nM)</th>
<th>CB₂ (Ki, nM)</th>
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<tbody>
<tr>
<td>RS-DFA-6-2</td>
<td>682.9</td>
<td>18.1</td>
</tr>
<tr>
<td>CP-55,940</td>
<td>3.0</td>
<td>1.9</td>
</tr>
<tr>
<td>7a</td>
<td>&gt;1000</td>
<td>115.0</td>
</tr>
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</table>

Table III. Ki values for test compounds against cannabinoid receptors. Compound 7a shows high affinity for the CB₂ receptor (Kᵢ = 115.0 ± 21.0 nM).

The results of this research help to define the structural requirements necessary to maintain potent and selective ligand binding to cannabinoid receptors, as it pertains to modification of the lead compound, RS-DFA-6-2. In an attempt to produce an analog with increased aqueous solubility, four analogs were designed and evaluated against both CB₁ and CB₂ receptors. Only one analog, 7a, exhibited reasonable potency and receptor selectivity, with a calculated decrease in lipophilicity. Future research should be focused on analogs related to 7a, namely, with the retention of a carboxyamide group containing an adamantane ring or bioisosteric equivalent.
Appendix

$^1$H and $^{13}$C Nuclear Magnetic Resonance Data
MT-Furo-HL-010-adamantyl-OH.1.fid

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<th>Parameter</th>
<th>Value</th>
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</thead>
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<td>2 Owner</td>
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<tr>
<td>13 Spectral Size</td>
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</table>

$^1$H NMR (400 MHz, Chloroform-d$_6$) $\delta$ 7.19 (d, $J = 5.5$ Hz, 2H), 6.98 (s, $J = 8.7$ Hz, 2H), 6.40 (s, 1H), 6.16 (s, 1H), 5.66 (s, 1H), 5.58 (s, 2H), 2.31 (s, 2H), 2.06 (d, $J = 10.2$ Hz, 4H), 2.00 (s, 4H), 1.75 (d, $J = 12.3$ Hz, 4H), 1.65 (s, 2H), 1.20 (d, $J = 7.1$ Hz, 2H).
Chemical Formula: C_{24}H_{25}FN_{2}O_{3}
Molecular Weight: 408.473

Parameters

<table>
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<th>Parameter</th>
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<td>Solvent</td>
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<td>Nucleus</td>
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</table>

$^{13}$C NMR (100 MHz, CDCl$_3$): 161.99, 146.97, 145.79, 130.75, 129.33, 128.16, 115.48, 115.27, 98.75, 93.39, 77.33, 76.69, 69.24, 54.56, 49.28, 44.11, 40.48, 34.87, 30.66.
Chemical Formula: C_{19}H_{20}F_{2}N_{2}O_{2}
Molecular Weight: 340.40

$^1$H NMR (400 MHz, Chloroform-d): δ 7.37 (d, $J = 2.3$, 1H, H1), 7.26 (d, $J = 1.4$, 1H, 0H), 7.20 – 7.09 (m, 2H), 7.01 – 6.90 (m, 2H), 6.30 – 6.19 (m, 1H), 5.35 (s, 1H), 3.57 (t, $J = 6.0$, 3H), 1.68 (s, 3H), 1.61 (d, $J = 1.1$, 1H), 1.42 (d, $J = 1.1$, 3H), 1.25 (s, 3H), 0.96 (d, $J = 11.8$, 1H).
Chemical Formula: C\textsubscript{20}H\textsubscript{21}FN\textsubscript{2}O\textsubscript{2}
Molecular Weight: 340.40

\begin{table}
\begin{tabular}{|l|l|}
\hline
Parameter & Value \\
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1. Origin & Bruker Biospin GmbH \\
2. Solvent & CDCl\textsubscript{3} \\
3. Temperature & 298.3 \\
4. Pulse Sequence & zgsp30 \\
5. Experiment & 1D \\
6. Number of Scans & 1000 \\
7. Spectrometer Frequency & 100.63 \\
8. Nucleus & 1\textsc{H} \\
\hline
\end{tabular}
\end{table}

\textsuperscript{13}C-NMR (101 MHz, CDCl\textsubscript{3}) \delta 146.22, 146.15, 129.31, 129.23, 127.58, 115.44, 115.23, 98.57, 94.57, 49.71, 29.69.
\[^1\text{H NMR (400 MHz, Chloroform-d\text{̄}) }\delta\]
\[7.39 (d, J = 2.2 \text{ Hz}, 1H), 7.26 (s, 1H), 7.18 - 7.08 (m, 2H), 6.98 (t, J = 8.7 \text{ Hz}, 2H), 6.31 - 6.20 (m, 2H), 5.35 (s, 2H), 3.68 (dd, J = 5.7, 3.9 \text{ Hz}, 4H), 2.04 (s, 2H), 1.20 (t, J = 7.1 \text{ Hz}, 2H).\]
Chemical Formula: C_{18}H_{17}F_{2}N_{2}O_{3}
Molecular Weight: 328.34

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$
146.75, 129.28, 129.20, 115.56, 115.34, 98.56, 95.25, 77.32, 77.00, 76.68, 66.84, 49.69.
$^1$H NMR (400 MHz, Chloroform-d) δ 7.39 (d, J = 2.2 Hz, 1H), 7.14 (dd, J = 8.6, 5.4 Hz, 2H), 6.95 (t, J = 8.7 Hz, 2H), 6.78 (d, J = 8.0 Hz, 1H), 6.67 (d, J = 2.0 Hz, 1H), 6.56 (dd, J = 8.0, 2.0 Hz, 1H), 6.37 (s, 1H), 6.17 (d, J = 2.8 Hz, 1H), 5.97 (t, J = 6.0 Hz, 1H), 5.57 (s, 2H), 4.13 (q, J = 7.1 Hz, 1H), 3.54 (q, J = 6.7 Hz, 2H), 2.69 (t, J = 6.9 Hz, 2H), 1.27 (t, J = 7.1 Hz, 1H).
$^{13}$C NMR (101 MHz, Chloroform-d): δ 163.04, 160.93, 147.49, 145.82, 144.17, 142.89, 133.50, 131.64, 130.87, 129.03, 126.77, 120.78, 115.53, 113.32, 98.78, 94.26, 50.02, 40.89, 34.96.
Bibliography


