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Synthesis of S-2-((S)-3-(4-chlorophenyl)-N'-((4-chlorophenyl)sulfonyl)-4-phenyl-4,5-dihydro-1H-pyrazole-1-carboximidamido)-3-(methyl-d₃)butanamide-d₅, octa-deuterated JD5037

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Abstract

JD5037 (**1**) is a potent and selective, peripherally acting inverse agonist of the cannabinoid (CB₁R) receptor. Peripheral CB₁ receptor antagonists/inverse agonists have great potential in the treatment of metabolic disorders like type 2 diabetes, obesity and NASH. We report the synthesis of octa-deuterated [²H₈]-JD5037 (*S, S*) (**8**) along with its (*S, R*) diastereomer (**13**) from commercially available L-valine-d₈ starting material. The [²H₈]-JD5037 compound will be used to quantitate unlabeled JD5037 during clinical ADME studies and will be used as an LC-MS/MS bioanalytical standard.

TOC image

We report the synthesis of octa-deuterated [²H₈]-JD5037 (*S, S*) (**8**) along with its (*S, R*) diastereomer (**13**) from commercially available L-valine-D8 starting material. The ²H₈-JD5037 compound will be used to quantitate unlabeled JD5037 during clinical ADME studies and will be used as an LC-MS/MS bioanalytical standard.

Keywords

EC; CB₁R; PSA; ADME

Introduction

Endocannabinoids (EC) are endogenous ligands found throughout the body where they bind to two known cannabinoid receptors, CB₁ and CB₂¹. These endogenous ligands, their two G-protein coupled receptors along with several enzymes responsible for their biosynthesis and degradation constitute the endocannabinoid system^{2,3}. The CB₁ receptor (CB₁R) is mainly expressed in the central nervous system, where it is one of the most abundant GPCRs. It is also expressed in a number of peripheral tissues such as the liver, testis and ileum.⁴ The CB₂ receptor is mainly expressed by cells of the immune system, and is involved in the immunomodulatory effects of cannabinoids⁵. The CB₁R gained attention due to its role in regulating appetite and food intake⁶. The modulation of endocannabinoid functions through CB₁R antagonism has been recognized as a viable therapeutic strategy to treat obesity and its associated metabolic complications⁷. The first-in-class and only CB₁R antagonist to be approved as an anti-obesity medication, rimonabant (**2**), was withdrawn from the market soon after its approval in Europe due to the occurrence of neuropsychiatric side effects including depression, anxiety and suicidal ideation. Clinical development of similar brain-penetrant agents, such as ibipinabant (**3**), otenabant (**4**) and taranabant (**5**)⁸ was also terminated. While it was initially thought that the beneficial effects of CB₁R antagonism could not be separated from neuropsychiatric side effects resulting from brain CB₁R occupancy, a new body of evidence suggests that most of the beneficial effects can be retained by sequestering the agents away from the brain and limiting their access to the

periphery.⁹ This justified a ‘re-do’ of the CB₁R antagonism strategy by developing non-brain penetrant versions of compounds, which are in early development¹⁰.

Strategies for designing non-brain penetrant compounds are well documented.¹¹ Thus, in case of CB₁R, agents with low propensity to penetrate the BBB were designed by utilizing the conventional functionalities that offer an increase in polar surface areas. Molecules with a PSA of >120 have poor passive cell membrane permeability indicating low oral bioavailability.¹² For compounds with limited brain penetrance, PSA is generally found to be in the range of 80–120.

One of the first compounds with peripherally selective CB₁R profile was TM38837 (**6**) (structure undisclosed) identified by 7TM Pharma. This compound was tested in a small number of healthy subjects at a dose predicted to be equipotent with rimonabant without eliciting adverse effects; however, limited data is available regarding its structure, *in vitro* and physicochemical properties¹³. Earlier efforts to develop peripherally-restricted CB₁R antagonists focused on the rimonabant scaffold, the team at Jenrin Discovery used the ibipinabant template as a starting point to develop non-brain penetrant CB₁R antagonists. The compounds advanced by this group include JD5037 (**1**) and JD5006 (**7**).¹⁴ Replacement with pendant N-L-valinamide in lieu of N-methyl group in case of ibipinabant was found to be particularly attractive because it resulted in excellent CB₁R affinity, favorable physicochemical parameters as well as peripheral restriction. JD5037 has been extensively studied in various *in vitro* and *in vivo* assays and its therapeutic efficacy in animals models of obesity/metabolic syndrome and type-2 diabetes has been well-documented.^{9,15,16} The compound is highly selective for peripheral CB₁R and has no off-target effects. JD5037 will soon be submitted for IND approval.

Stable isotope labeled standards provide precise quantitation of new drug molecules and human bio-analytical validation. It was rationalized that ²H₈-labeling on the L-valinamide pendant of the molecule would provide such a useful standard for JD5037 quantitation. Toward that end, a viable procedure was developed, based on the reported synthesis of JD5037, starting from L-valinamide-d₈ **9** and intermediate **10** to [²H₈]-JD5037 (**8**).^{14, 17}

Results and Discussion

The initial synthesis involved a two-step procedure to assemble L-valinamide-d₈ (**9**). Treatment of commercially available L-valine-d₈ with SOCl₂/MeOH at 65°C followed by condensation with NH₄OH/MeOH at 65°C afforded L-valinamide-d₈. The L-valinamide-d₈, was used without further purification. The sulfonyl urea **10**¹⁷ was converted into the imidoyl chloride in the presence of PCl₅ and refluxing chlorobenzene. After evaporation of the chlorobenzene, the chloroimidate **11** was treated *in situ* with a mixture of L-valinamide-d₈ and triethylamine in methanolic dichloromethane to yield a mixture of diastereomers **12** (Scheme 1). Flash chromatography purification followed by a recrystallization of the mixture in ethanol yielded [²H₈]-JD5037 with about 80–90% diastereomeric purity. Additional re-crystallization of this enriched mixture in ethanol-dichloromethane mixture (1:1) yielded [²H₈]-JD5037 (**8**) as white crystalline needles with undetectable amounts of compound **13**. The mother liquor was then subjected to further purification under

chromatography conditions to give the diastereomer of [$^2\text{H}_8$]-JD5037 (**13**). The X-ray crystal structure of the deuterium-labeled compound **8** confirmed the (*S*, *S*) stereochemistry as seen in Figure 3.

The identity of the active diastereomer **8** ([$^2\text{H}_8$]-JD5037) was further corroborated by CB₁R radioligand binding studies as illustrated in Figure 4/Table 1.

Conclusions

L-Valine- d_8 (98 atom % D) was used as the stable-isotope label reagent in the labelled synthesis of L-valineamide- d_8 intermediate. This approach allowed for the rapid incorporation of the L-valineamide- d_8 in the synthesis of deuterium labelled JD5037. The [$^2\text{H}_8$]-JD5037 will be used to quantitate unlabeled JD5037 during clinical ADME studies and will be used as an LC-MS/MS bioanalytical standard. The synthesized compound **8** showed isotopic purity of 98% and purity of > 99%.

Experimental

Chemistry

Reagents available commercially were purchased and used as is. Intermediate compounds were synthesized based on literature procedures. D8-L-valine (98% D) was purchased from Cambridge Isotope Laboratories or synthesized based on literature procedures. Proton (^1H NMR) spectra were recorded on a Varian 400 or Bruker 500 MHz spectrometer in CDCl_3 or $\text{DMSO}-\text{d}_6$ (unless otherwise noted) with the values given in ppm (TMS as internal standard) and *J* (Hz) assignments of ^1H resonance coupling. Mass spectra (HRMS) were recorded on a VG 7070E spectrometer or a JEOL SX102a mass spectrometer. Thin layer chromatography (TLC) analyses were carried out on Analtech silica gel GHLF 0.25 mm plates using various gradients of $\text{CHCl}_3/\text{MeOH}$ containing 1% NH_4OH or gradients of $\text{EtOAc}/n\text{-hexane}$. Visualization was accomplished under UV light or by staining in an iodine chamber. Flash column chromatography was performed on Teledyne ISCO Combiflash system with PurIon mass detector. Product yields are un-optimized. Compounds tested in radioligand binding assay had 95% purity. Purity and structural confirmation was analyzed by a combination of TLC, ^1H -NMR, LC/MS, high-resolution mass spec, elemental analysis and X-ray (where indicated). LC-MS detection was carried out on Agilent 1200 using Luna C₁₈ 3 μm (3×75 mm). The mobile phase was 4% to 100% acetonitrile (0.05% TFA) standard gradient. The LC-MS chromatogram showed the correct molecular (MH^+) ion as well as a single peak at UV (254 nm). X-ray results were obtained utilizing the X-ray facility at University of California, San Diego.

2-Amino-3-(methyl- d_3)butanamide- d_5 (L-valinamide- d_8)(**9**)

To a solution of L-valine- d_8 (125 mg, 1.00 mmol), in methanol at -20°C was added drop-wise thionyl chloride (0.37 mL, 5.00 mmol). The mixture was heated to 65°C overnight, cooled to room temperature and solvent evaporated. The residue was taken up in methanolic ammonium hydroxide (2.00 mL), heated at 65°C for 24 h, and the organic solvent was evaporated to dryness to yield compound **9**. The crude product, L-valinamide- d_8 , was used as is in the next step. LRMS (MH^+) for $\text{C}_5\text{H}_5\text{D}_8\text{N}_2\text{O}$ 125.1.

2-((S)-3-(4-chlorophenyl)-N'-((4-chlorophenyl)sulfonyl)-4-phenyl-4,5-dihydro-1H-pyrazole-1-carboximidamido)-3-(methyl-*d*₃)butanamide-*d*₅ ([²H₈]-JD5037) (8**)**

A mixture of compound **10**¹⁷ (200 mg, 0.43 mmol) and PCl₅ (107 mg, 0.50 mmol) in chlorobenzene (5 mL) was refluxed for 1 h to give the imidoyl chloride **11**. The reaction mixture was then cooled and chlorobenzene was removed in vacuo. To the crude imidoyl chloride compound (**11**) dissolved in dichloromethane (10 mL) was added a mixture of compound **9** from the previous step (80 mg, 0.65 mmol) in methanol/dichloromethane (1:1) and Et₃N (0.12 mL, 0.86 mmol) at 0 °C drop-wise. After maintaining at room temperature overnight, the reaction mixture was extracted into dichloromethane, washed with water and purified by flash chromatography using hexanes: EtOAc (6:4) to afford compound **12** as a mixture of diastereomers. (150 mg, 61%). This mixture was dissolved in ethanol and slowly allowed to evaporate overnight to give a crop of crystals which was enriched in one diastereomer (80–90%). The crystals were filtered and dissolved again in an ethanol-dichloromethane (1:1) mixture, warmed to 40 °C, and slowly allowed to cool overnight. The resulting white needles (40 mg/17 % overall) were enriched in single diastereomer **8** (>99%) by HPLC analysis. Thus, [²H₈]-JD5037 was synthesized with an isotopic purity of 98% and diastereomeric purity of > 99%. Mother liquors from the crystallization were purified again by flash chromatography hexane: ethylacetate (1:1) to afford isomer **13** (30 mg, 13%) as white needles (>99%) by HPLC analysis.

Compound 8

¹H-NMR (400 MHz, CDCl₃): δ 7.86 (d, *J* = 8.5 Hz, 2H), 7.51 (d, *J* = 8.3 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.30 (t, *J* = 6.3 Hz, 3H), 7.25 (d, *J* = 7.2 Hz, 2H), 7.11 (d, *J* = 7.0 Hz, 2H), 6.39 (s, 1H), 5.50 (s, 1H), 4.64 (s, 1H), 4.43 (t, *J* = 11.6 Hz, 1H) 4.12–4.09 (m, 1H). LC method: 53% CH₃CN (0.1% TFA) Waters XBridge C₁₈ column 4.6×50mm, 4.824 min detection at 254 nm >99% purity. LC-MS method: Gradient 4–100% CH₃CN (0.05% TFA) over 4 mins Luna C₁₈ 3×75mm MH⁺ 580 detection at 254 nm 100%. HRMS C₂₇H₂₀D₈Cl₂N₅O₃S (MH⁺): Found *m/z* 580.1785, calcd 580.1792. Anal. calcd for C₂₇H₁₉D₈Cl₂N₅O₃S: C, 55.85; H+D, 4.66; N, 12.06; Found: C, 56.09; H+D, 4.60; N, 12.08.

X-Ray Crystallographic Analysis of (8**)**

Suitable crystals of **8** were obtained by re-crystallization of the compound from IPA and water (9:1) by slow evaporation of the solvent. The single crystal X-ray diffraction studies were carried out on a Bruker Kappa APEX-II CCD diffractometer equipped with Cu K_α radiation (λ = 1.5478). A 0.113 × 0.057 × 0.011 mm piece of a colorless plank was mounted on a Cryoloop with Paratone oil. Data were collected in a nitrogen gas stream at 100(2) K using φ and ω scans. Crystal-to-detector distance was 45 mm using variable exposure time (2s–5s) depending on θ with a scan width of 2.0°. Data collection was 99.4% complete to 68.00° in θ. A total of 21118 reflections were collected covering the indices, −22 ≤ h ≤ 23, −6 ≤ k ≤ 6, −30 ≤ l ≤ 29. 4646 reflections were found to be symmetry independent, with a R_{int} of 0.0357. Indexing and unit cell refinement indicated a C-centered, monoclinic lattice. The space group was found to be C2. The data were integrated using the Bruker SAINT software program and scaled using the SADABS software program. Solution by direct methods (SHELXT) produced a complete phasing model consistent with the proposed

structure. All nonhydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-2014). All carbon bonded hydrogen atoms were placed using a riding model. Their positions were constrained relative to their parent atom using the appropriate HFIX command in SHELXL-2014. All other hydrogen atoms (H-bonding) were located in the difference map. Their relative positions were restrained using DFIX commands and their thermals freely refined. The absolute stereochemistry of the molecule was established by anomalous dispersion using the Parson's method with a Flack parameter of 0.027 Atomic coordinates for **8** has been deposited with the Cambridge Crystallographic Data Centre (deposition number: 1536796). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Compound 13

¹H-NMR (400 MHz, CDCl₃): δ 7.82 (d, *J* = 8.4 Hz, 2H), 7.49 (d, *J* = 8.2 Hz, 2H), 7.37 (d, *J* = 8.0 Hz, 2H), 7.25 (td, *J* = 12.4, 7.3 Hz, 6H), 7.06 (d, *J* = 7.1 Hz, 2H), 6.38 (s, 1H), 5.87 (s, 1H), 4.60–4.50 (m, 2H), 3.92–3.89 (m, 1H). LC-MS method: LC method: 53% CH₃CN (0.1% TFA) Waters XBridge C₁₈ column 4.6×50mm, 4.518 min detection at 254 nm >99% purity. Gradient 4–100% CH₃CN (0.05% TFA) over 4 mins Luna C₁₈ 3×75mm MH⁺ 580 detection at 254 nm 100%. HRMS C₂₇H₂₀D₈Cl₂N₅O₃S (MH⁺): Found *m/z* 580.1783, calcd 580.1792. Anal. calcd for C₂₇H₁₉D₈Cl₂N₅O₃S: C, 55.85; H+D, 4.66; N, 12.06; Found: C, 56.09; H+D, 4.60; N, 12.08.

Radioligand Binding Studies

Materials for *in vitro* assays

JD5037 was obtained from Jenrin Discovery, LLC. CP-55,940 was obtained from the National Institute of Drug Abuse Drug Supply Program (Research Triangle Park, North Carolina, USA). [³H] CP55,940 was obtained from Perkin-Elmer (Waltham, MA). All other chemicals were from Sigma–Aldrich (St. Louis, MO).

CB₁R Binding assay

The assay was performed as described previously.¹⁸

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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The chemical structure shows a central 1H-imidazole ring. At position 2, there is a (4-chlorophenyl)(phenylthio)methyl group, where the phenylthio group is attached via a wedge bond and the 4-chlorophenyl group is attached via a dashed bond. At position 4, there is a 2-((4-chlorophenyl)sulfonyl)ethylamine group. The ethyl chain is labeled with 'D' for deuterium at the 2-position and 'D₃C' for a trimethyl-d₃carbamoyl group at the 1-position. The amine group is shown as H₂N. The sulfonyl group consists of a sulfur atom double-bonded to two oxygen atoms (red) and single-bonded to a 4-chlorophenyl group (green chlorine).

Figure 2. Peripherally-restricted CB₁R inverse agonist JD5037 and its d₈ (octa-deuterated) analog

Figure 3.
Single crystal X-Ray structure of Compound 8

	JD5037 (1)	[² H ₈]JD5037-peak 1 (13)	[² H ₈]JD5037-peak 2 (8)
CB ₁ K _i (nM)	0.58	>500	0.59

Figure 4.

In vitro CB₁R binding results of deuterated compounds. Binding affinity was determined from binding displacement isotherms using [³H]-CP55940 as the labeled ligand and plasma membranes from CB₁R-expressing cells. K_i values were calculated using the Cheng-Prusoff equation. Data represent mean ± SEM from 3 independent experiments performed in triplicates.

Scheme 1.
Synthesis of [²H₈]-JD5037