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Synthesis, Radio-Synthesis and *in vitro* Evaluation of Terminally Fluorinated Derivatives of HU-210 and HU-211 as Novel Candidate PET Tracers

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We report the synthesis of terminally fluorinated **HU-210** and **HU-211** analogues (**HU-210F** and **HU-211F**, respectively) and their biological evaluation as ligands of cannabinoid receptors (CB_1 and CB_2) and *N*-methyl D-aspartate receptor (NMDAR). [^{18}F]-labelled **HU-210F** was radiosynthesised from the bromo-substituted precursor. *In vitro* assays showed that both **HU-210F** and **HU-211F** retain the potent pharmacological profile of **HU-210** and **HU-211**, suggesting that [^{18}F]-radiolabelled **HU-210F** and **HU-211F** could have potential as PET tracers for *in vivo* imaging.

Introduction

Cannabinoid receptors – CB_1 and CB_2 – are involved in psychiatric, neurological, and behavioural disorders, as well as in non-CNS pathologies such as liver fibrosis, metabolic disorders such as diabetes, and cancer. PET imaging has emerged as a powerful technology for the diagnosis and staging of CNS and non-CNS pathologies, as well as a tool in drug design and development. We and others have actively investigated novel cannabinoid-ligands as PET tracers,[1] however few [^{18}F]-labelled tracers have demonstrated true potential to image cannabinoid receptors *in vivo*.

Recently, Kassiou *et al*. have shown that synthetic cannabinoid ligands carrying a terminal fluorine atom on linear aliphatic chains have superior or equivalent pharmacological properties relative to non-fluorinated counter-parts.[2] This observation supported our idea to investigate novel selectively fluorinated cannabinoid analogues as candidate PET tracers, in particular **HU-210** (Fig. 1) which is a potent (–)-1,1-dimethylheptyl analogue of 11-hydroxy-Δ^8-tetrahydrocannabinol.[3] This cannabinoid receptor agonist was reported to have 0.061 and 0.52 nM K_i values at cloned human CB_1 and CB_2 receptors, respectively. **HU-210** also displays agonist activity at GPR55 (EC_{50} = 26 nM). Interestingly, its enantiomer **HU-211** (dexanabinol, Fig. 1) has very different pharmacological properties, being essentially inactive as a cannabinoid but active as *N*-methyl D-aspartate receptor (NMDAR) ligand (IC_{50} = 11 µM for inhibition of [^{3}H]MK-801 binding to rat forebrain membranes).[4] **HU-211** has been shown to have neuroprotective effects and is being clinically tested for treating traumatic and ischemic brain injury. **HU-211** is also an effective free radical scavenger, and is currently in phase-II clinical trials for the therapy of solid tumours.[5]

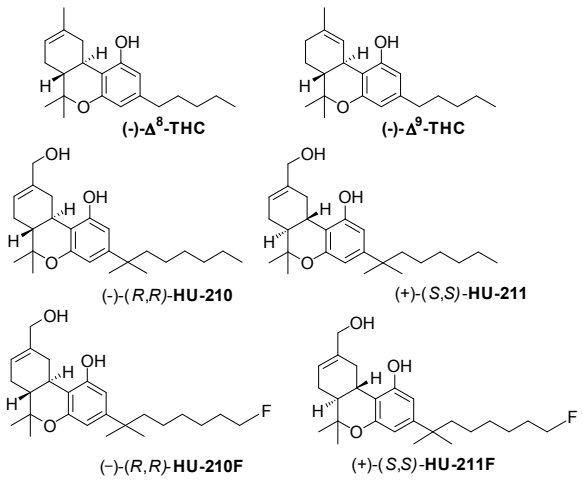

Figure 1 Natural cannabinols, analogues and radioactive derivative.

Despite the strong interest in **HU-210** and **HU-211**, to our knowledge the use of radiolabelled analogues for *in vivo* imaging has never been reported, and fluorinated analogues have not been described either.

We hereby report a synthesis of terminally fluorinated **HU-210** and **HU-211** analogues, namely **HU-210F** and **HU-211F** (Fig. 1), we demonstrate that both compounds maintain their original pharmacological properties *in vitro*. Finally we describe the radio-synthesis of **[^{18}F]HU-210**. These results will enable the

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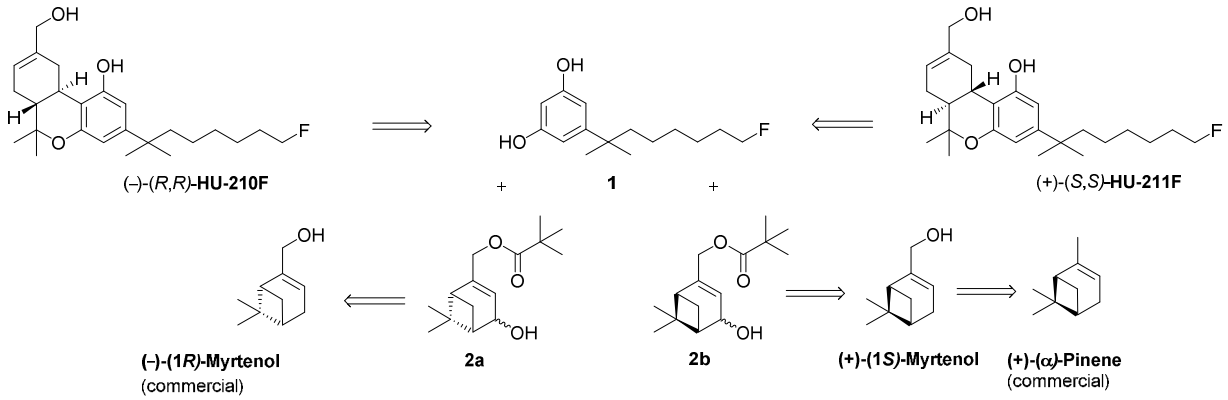
use of these compounds as PET tracers for *in vivo* imaging studies.

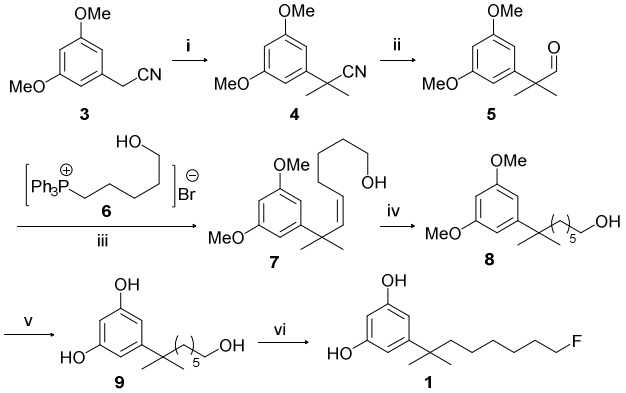


Scheme 1 Retrosynthetic approach to (–)-(*R*,*R*)-**HU-210F** and (+)-(*S*,*S*)-**HU-211F**.

Results and discussion

Synthesis of (–)-(*R*,*R*)-**HU-210F** and (+)-(*S*,*S*)-**HU-211F**

The retrosynthetic approach to the target compounds (–)-(*R*,*R*)-**HU-210F** and (+)-(*S*,*S*)-**HU-211F** (Scheme 1) was inspired by the chemistry published by Zahalka[6] and Huffman.[7] Fluorinated resorcinol **1** was identified as a key intermediate to be reacted with both enantiomers of 4-hydroxy-myrtenyl pivalate **2a**,**b** forming the desired THC-like structural frameworks. Synthesis of the key fluorinated building block **1** proved to be challenging. In fact, a number of unsuccessful approaches to **1** were attempted (more information is provided in the Electronic Supporting Information section) before identifying a successful synthetic strategy (Scheme 2). The synthesis of **1** started with the methylation[8] of commercially available (3,5-dimethoxyphenyl)acetonitrile (**3**), that afforded the α,α-dimethylnitrile **4** in excellent yield. Subsequent hydride reduction[9] of **4** afforded the aldehyde **5**, which was subjected to a Wittig olefination[9] with the ylide generated[10] from the phosphonium salt **6** that yielded the unsaturated alcohol **7** as a single isomer, presumably having (*Z*)-geometry. The C=C bond of **7** was then hydrogenated (Pd/C, H_2, in EtOAc)[9] to give compound **8** in quantitative yield. During a first *O*-demethylation attempt (BBr_3 in DCM from -78°C to -10 °C)[11] an undesired replacement of the primary OH of **8** with a Br atom occurred, leading to the corresponding alkyl bromide. However, treatment of **8** with MeMgI at 170°C[12] provided the demethylated alcohol **9** in very good yield. Next, **9** was dehydroxyfluorinated with DAST[13] (the use of DEOXOFLUOR provided lower yields) affording the target building block **1**. The synthesis of (–)-(*R*,*R*)-**HU-210F** (Scheme 3) was achieved from commercially available (–)-(1*R*)-Myrtenol (≥95% *ee*) that was transformed into 4-hydroxy-myrtenyl pivalate **2a** using the method of Zahalka and Huffman.[6,7] The synthesis of (+)-(*S*,*S*)-**HU-211F** (Scheme 3) was achieved from commercially available (+)-(α)-Pinene (≥99% *ee*), that was first transformed into the corresponding (+)-(1*S*)-Myrtenol by means of an allylic oxidation promoted by SeO_2,[14] and then converted into **2b**. The common intermediate **1** was condensed with **2a** or **2b** in the presence of $BF_3{\cdot}Et_2O$ at -20°C, affording the protected esters **10a** and **10b**, respectively.



Scheme 2 Synthesis of common intermediate **1**. *Reagents and conditions:* i) NaH, MeI, DMF, 0°C to rt, 2h (98%); ii) DIBALH, DCM, rt, 1h (93%); iii) LiHMDS, THF, 0°C to rt, on (92%); iv) H_2 (1 atm), 10% Pd/C, EtOAc, on (quantitative); v) MeMgI, Et_2O/THF, 0°C to 170°C, 1h (70%); vi) DAST, DCM, -78°C, 0°C, 15 min (50%).

Finally, reduction with $LiAlH_4$ gave the target fluorinated compounds (–)-(*R*,*R*)-**HU-210F** and (+)-(*S*,*S*)-**HU-211F**. Since the source of chirality in the syntheses are the two 4-hydroxy-myrtenyl pivalate enantiomers, **2a** and **2b**, the *ee* of the two final compounds reflected the *ee* of the commercially available starting materials (≥95% *ee* for **2a** and ≥99% *ee* for **2b**).The enantiomeric purity of the cannabinoids mimics was confirmed by analytical chiral HPLC. Subsequently (–)-(*R*,*R*)-**HU-210F** and (+)-(*S*,*S*)-**HU-211F** were purified by semi-preparative chiral HPLC in order to obtain high-purity (>99% *ee*) single enantiomers for the pharmacological assays (see the Experimental for details).

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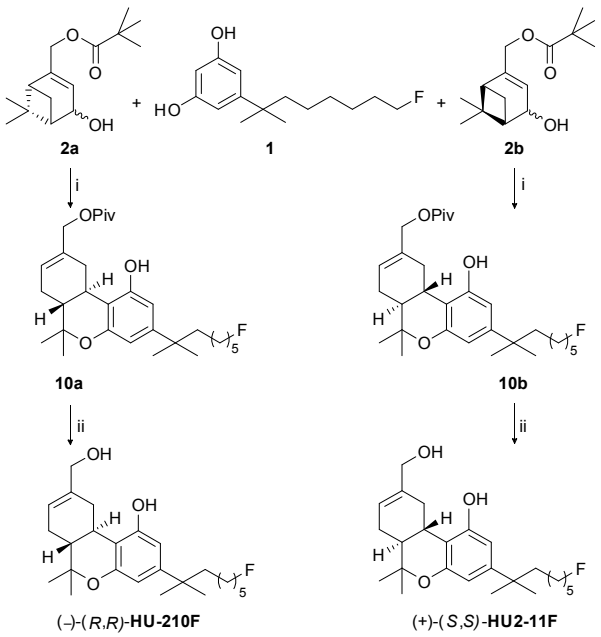

Scheme 3 Synthesis of (-)-(*R*,*R*)-**HU-210F** and (+)-(*S*,*S*)-**HU-211F**. *Reagents and conditions*: i) BF_3Et_2O, DCM, -20°C to rt, 2h (55%); ii) $LiAlH_4$, THF, 0°C, 2h (50%).

Biological tests on CB_1 and CB_2 receptors

We performed [^{3}H]CP55940 displacement binding assays with membranes obtained from hCB_1 and hCB_2 CHO cells using methods we have described previously.[15] (–)-(*R*,*R*)-**HU-210F** and (+)-(*S*,*S*)-**HU-211F** each shared the ability of the established CB_1/CB_2 receptor agonist CP55940 to induce a full displacement of [^{3}H]CP55940 from binding sites on both human CB_1 and human CB_2 CHO cell membranes (Figures 2 and 3). (–)-(*R*,*R*)-**HU-210F** displayed similar affinities for these CB_1 and CB_2 binding sites, as did (+)-(*S*,*S*)-**HU-211F**. It was also found, however, that (–)-(*R*,*R*)-**HU-210F** showed significantly greater potency than (+)-(*S*,*S*)-**HU-211F** at displacing [^{3}H]CP55940 both from CB_1 and from CB_2 binding sites (Table 1). As expected for such potency differences, the corresponding CB_1 and CB_2 K_i values of (–)-(*R*,*R*)-**HU-210F** were each found to be less than the CB_1 and CB_2 K_i values of (+)-(*S*,*S*)-**HU-211F** (Tables 1).

Biological tests on NMDA receptors

To assess the ability of (–)-(*R*,*R*)-**HU-210F** and (+)-(*S*,*S*)-**HU-211F** to antagonise NMDAR we made whole-cell patch-clamp recordings from primary rat cultured cortical neurons. Neurons were clamped at -60 mV and NMDA (50 μM) applied to evoke a current that was mediated predominantly by diheteromeric GluN1/GluN2B NMDARs.[16] Once a steady-state response was observed, we co-applied each compound of interest and assessed the percentage reduction in current (Fig. 4). To control for desensitisation of response during continued agonist exposure, we also applied NMDA with vehicle alone (1% DMSO) (Fig. 4A). We found that (–)-(*R*,*R*)-**HU-210F** showed no NMDAR antagonism when compared to vehicle alone (Fig. 4B, F). In contrast, (+)-(*S*,*S*)-**HU-211F** gave a maximal block of around 80% at the concentration used (Fig. 4C, F). In addition, we compared NMDAR antagonism of the parent compound, (+)-(*S*,*S*)-**HU-211**, which we found blocked NMDAR-mediated currents to an extent not significantly different from that seen with (+)-(*S*,*S*)-**HU-211F** (Fig. 4D, F). We noted a slow blocking on-rate with both (+)-(*S*,*S*)-**HU-211** and (+)-(*S*,*S*)-**HU-211F** which was not due to slow rates of perfusion as MK-801 (a NMDAR open channel blocker which binds at a similar site) blocked NMDAR-mediated responses rapidly (Fig. 4E).

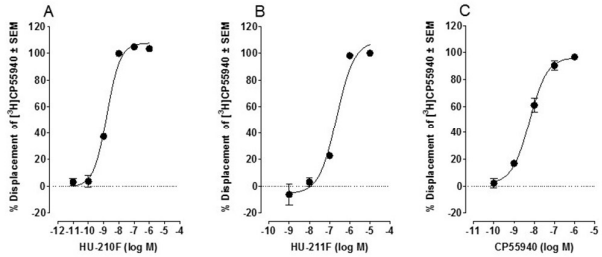

Figure 2 Effects of (A) **HU-210F** (n=5), (B) **HU-211F** (n=7) and (C) CP55940 (n=7) on [^{3}H]CP55940 binding to human CB_1 CHO cell membranes.

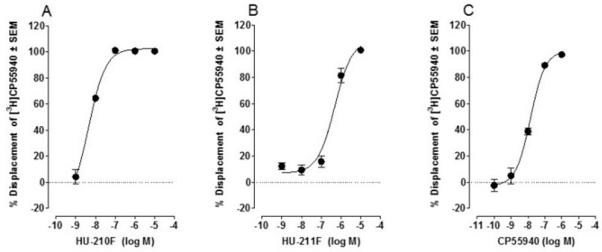

Figure 3 Effects of (A) **HU-210F** (n=5), (B) **HU-211F** (n=5) and (C) CP55940 (n=6) on [^{3}H]CP55940 binding to human CB_2 CHO cell membranes.

These experiments assessing NMDAR antagonism confirmed that (+)-(*S*,*S*)-**HU-211** is a NMDAR antagonist and that its potency is retained in its fluorinated form (+)-(*S*,*S*)-**HU-211F** (Table 2). The finding that (+)-(S,S)-**HU-211** is an NMDAR antagonist is consistent with previous work using radioligands, behavioural assays, excitotoxicity assays and calcium influx.[17] Based on the previously reported IC_{50} of 11 μM,[17b] we would have predicted a more complete block at the concentration used here (100 μM).

However, this IC_{50} was based on radioligand studies assessing displacement of MK-801 whereas our work is the first electrophysiological assessment of the effect of (+)-(*S*,*S*)-**HU-211**. Using electrophysiology to assess the effect of (+)-(*S*,*S*)-**HU-211** and its fluorinated analogue (+)-(*S*,*S*)-**HU-211F** has also allowed us to highlight their very slow on-rate, a property not identified previously.

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Compound	Membrane	Mean EC_{50} nM (95% CL)	Mean E_{max} % (95% CL)	Mean K_i nM (95% CL)
(–)-(*R*,*R*)-**HU-210F**	hCB_1 CHO cells	1.63 (1.25-2.14)	107.5 (103.5-111.5)	0.98 (0.76-1.31)
	hCB_2 CHO cells	4.46 (316-6.28)	102.3 (98.9-105.8)	3.83 (2.72-5.40)
(+)-(*S*,*S*)-**HU-211F**	hCB_1 CHO cells	222.7 (149.8-331.1)	108.8 (101.4-116.2)	136.1 (91.53-202.3)
	hCB_2 CHO cells	480.4 (281.2-820.7)	108.2 (97.25-119.2)	413.1 (241.8-705.8)
CP55940	hCB_1 CHO cells	5.87 (4.00-8.61)	96.39 (91.1-101.7)	3.59 (2.45-5.26)
	hCB_2 CHO cells	14.17 (9.83-20.42)	100.1 (93.71-106.5)	12.18 (8.45-17.56)

Table 1 Mean K_i, E_{max} and EC_{50} values of **HU-210F**, **HU-211F** and the control molecule CP55940 on hCB_1 CHO and hCB_2 CHO membranes.

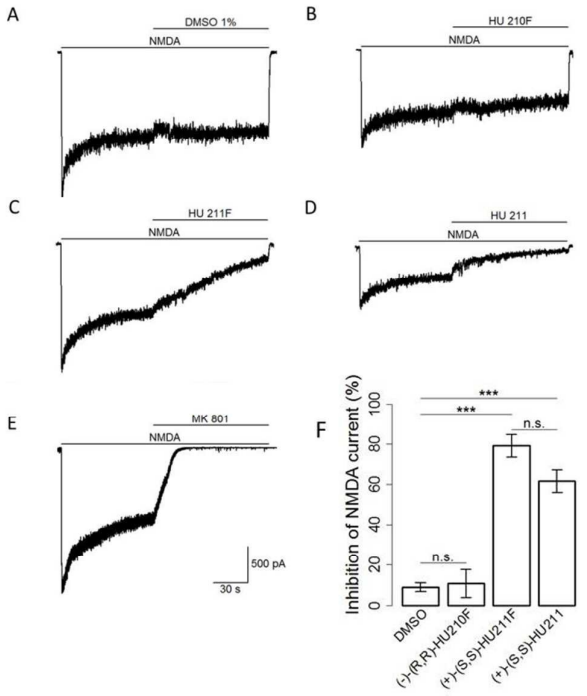

Figure 4 (+)-(*S*,*S*)-**HU-211F** and (+)-(*S*,*S*)-**HU-211** are both NMDAR antagonists. (A-E) Example whole-cell current recordings from rat cortical neurons. (A) NMDA (50 µM) with vehicle (1% DMSO), demonstrating desensitisation (mean percentage reduction in current compared to NMDA alone 8.7 ± 5.9 (n=7 cells)). (B) NMDA co-applied with (–)-(*R*,*R*)-**HU-210F** (100 µM), showing no additional block compared to vehicle (10.8 ± 17.5 (6), p =0.995). (C) NMDA co-applied with (+)-(*S*,*S*)-**HU-211F** (100 µM), showing significant block compared to vehicle (79.2 ± 15.8% (8), p<0.0001)). (D) NMDA co-applied with (+)-(S,S)-HU-211 (100 µM), showing significant block compared to vehicle (61.6 ± 13.9% (6), p<0.0001) but not significantly different to (+)-(*S*,*S*)-**HU-211F** (p=0.115). (E) NMDA co-applied with MK-801 (10 µM), demonstrating faster on rate and 100% block. Scale bar applies to all traces. (F) Summary data. Data represent mean ± SEM. Mean percentage inhibition of NMDAR-mediated currents for vehicle alone and the three compounds of interest were first compared using a one way ANOVA, which showed a significant effect of compound ($F(3,23)$ = 46.9, p<1E-9). We then compared individual means using post-hoc Tukey t-tests. *** p<0.001.

Compound	Inhibition of NMDAR current (%) [mean ± SD (n)]
(–)-(*R*,*R*)-**HU-210F**	10.8 ± 17.5 (6)
(+)-(*S*,*S*)-**HU-211F**	79.2 ± 15.8 (8)
DMSO	8.7 ± 5.9 (7)
(+)-(*S*,*S*)-**HU-211**	61.6 ± 13.9 (6)

Table 2 Inhibition of NMDAR current by (–)-(*R*,*R*)-**HU-210F**, (+)-(*S*,*S*)-**HU-211F**, DMSO and (+)-(*S*,*S*)-**HU-211**.

However, this IC_{50} was based on radioligand studies assessing displacement of MK-801 whereas our work is the first electrophysiological assessment of the effect of (+)-(*S*,*S*)-**HU-211**. Using electrophysiology to assess the effect of (+)-(*S*,*S*)-**HU-211** and its fluorinated analogue (+)-(*S*,*S*)-**HU-211F** has also allowed us to highlight their very slow on-rate, a property not identified previously.

Radio-Synthesis of (–)-(*R*,*R*)-[^{18}F]HU-210

The synthesis of radiofluorinated (–)-(*R*,*R*)-**[^{18}F]HU-210** was addressed next. As a radiosynthesis precursor, we opted for the bromo-analogue of **HU-210F**, which was synthesised both in di-acetylated form **14a** and hydroxyls-free form **14b** (Scheme 4).

The starting alcohol **8** was subjected to the Appel reaction leading to the corresponding alkyl bromide **11** that was first *O*-demethylated[11] to give the substituted resorcinol **12** and, then, reacted with myrtenyl acetate **2c** (obtained from commercially available (–)-(1*R*)-Myrtenol as described in the Electronic Supporting Information section) to give the mono-acetylated bromo-derivative **13**. From this common intermediate we synthesised both **14a** and **14b**, via O-acetylation and basic hydrolysis, respectively

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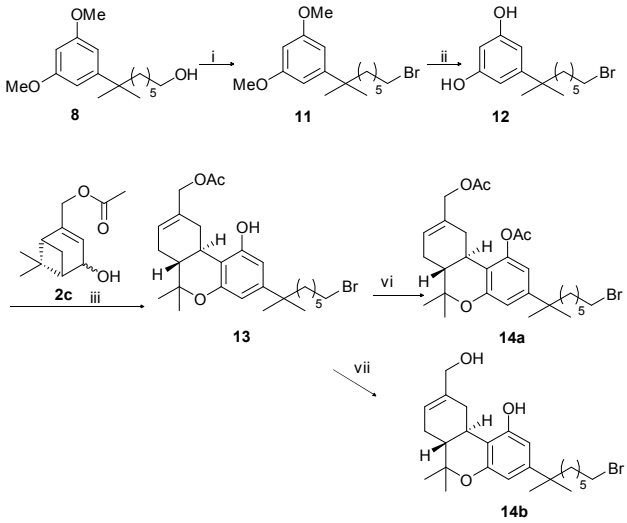
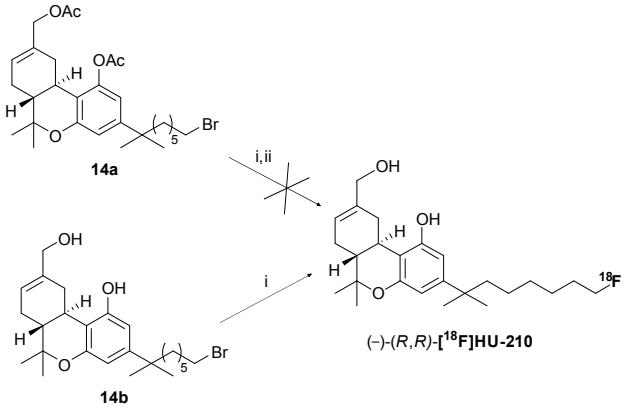


Scheme 4 Synthesis of precursors **14a** and **14b**. *Reagents and conditions:* i) CBr_4, PPh_3, CH_3CN, 0°C to rt, 1h (90%); ii) BBr_3, DCE, rt, 1.5h (82%); iii) $BF_3{\cdot}Et_2O$, DCM, -20°C to rt, 2h (20%); iv) Acetyl chloride, TEA, DCM, rt. 1h (quantitative); vii) NaOH, CH_3CN, 36°C, 4h (quantitative).

The radio-synthesis of (–)-(*R*,*R*)-**[^{18}F]HU-210** (Scheme 5) was then attempted from both **14a,b** by nucleophilic substitution of the bromine atom with the [^{18}F]KF 2.2.2 Kriptofix® complex.



Scheme 5 Radio-synthesis of **(–)-(*R*,*R*)-[^{18}F]HU-210**. *Reagents and conditions:* i) [^{19}F]KF 2.2.2 Kryptofix®, CH_3CN, 100 °C, 15 min.; ii) NaOH 4M, 50 °C, 10 min

Using the di-acetylated derivative **14a,** the radio-fluorination was not successful, since complete and rapid degradation of the starting material was observed. However, **14b** was successfully radio-fluorinated to (–)-(*R*,*R*)-**[^{18}F]HU-210** with a preliminary 6% radio-chemical conversion (evaluated by Radio-HPLC) using a non-optimised automated procedure in a remotely controlled modular reactor system. Identity of the tracer was confirmed by co-injection with the cold reference compound.

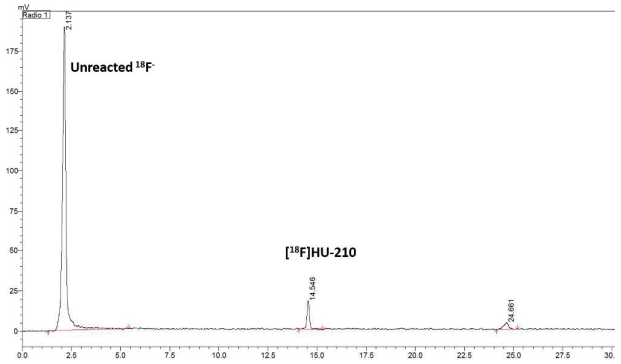


Figure 5 Radio-HPLC Chromatogram of the crude mixture after 15 min labelling.

The same method could be used to produce (+)-(*S*,*S*)-**[^{18}F]HU-211**.

Conclusions

Terminally fluorinated analogues of the potent cannabinoid receptor ligand (–)-(*R*,*R*)-**HU-210** and its enantiomer (+)-(*S*,*S*)-**HU-211**, which is an NMDAR antagonist, were efficiently synthesised. *In vitro* biological evaluations of (–)-(*R*,*R*)-**HU-210F** and (+)-(*S*,*S*)-**HU-211F** showed that both analogues retain the original pharmacological profiles. The non-optimised radio-synthesis of **[^{18}F]HU-210** from the bromo-precursor **14b** was achieved as a proof-of-concept for demonstrating the feasibility of this method for enabling *in vivo* PET imaging studies. Although **HU-210F** is a non-selective ligand for CB-receptors (K*i* = 0.98nM for CB_1 and 3.83nM for CB_2), its very high affinity is in the classical range for enabling PET imaging studies. Therefore (–)-(*R*,*R*)-**[^{18}F]HU-210** could be used either for overall mapping and observation of CB-receptors distribution and occupancy (mostly central for CB_1 and peripheral for CB_2), or for selective blocking and competition experiments by co-administration with either CB_1 or CB_2-specific ligands. The low CB and NMDA receptors affinity of (+)-(*S*,*S*)-**[^{18}F]HU-211** is likely to preclude its use for in vivo imaging of these receptors, but this tracer could still represent a useful tool for investigating bio-distribution and mechanism of action of the parent anticancer drug dexanabinol, which is currently in advanced clinical trials.

Experimental

Biological tests on CB_1 and CB_2 receptors

CHO cells

Chinese hamster ovary (CHO) cells transfected with cDNA encoding human cannabinoid CB_1 or CB_2 receptors were maintained at 37 °C in Dulbecco's modified Eagle's medium nutrient mixture F-12 HAM, supplemented with 1 mM L-glutamine, 10% fetal bovine serum, 0.6% penicillin–streptomycin and G418 (400 mg/mL). All cells were exposed to 5% CO_2 in their respective media, and were passaged twice a week using non-enzymatic cell dissociation solution. For

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membrane preparation, cells were removed from flasks by scraping, centrifuged, and then frozen as a pellet at -20 °C until required. Before use in a radioligand binding assay, cells were defrosted, diluted in Tris buffer (50 mM Tris–HCl and 50 mM Tris–base) and homogenized with a hand-held homogenizer.

Radioligand displacement assay

The assays were carried out with [^{3}H]CP55940 and Tris binding buffer (50 mM Tris–HCl, 50 mM Tris–base, 0.1% BSA, pH 7.4), total assay volume 500 µl, using the filtration procedure described previously.[15a,15b] Binding was initiated by the addition of transfected human CB_1 or CB_2 CHO cell membranes (50 µg protein per well). All assays were performed at 37 °C for 60 min before termination by the addition of ice-cold Tris binding buffer, followed by vacuum filtration using a 24-well sampling manifold (Brandel Cell Harvester; Brandel Inc, Gaithersburg, MD, USA) and Brandel GF/B filters that had been soaked in wash buffer at 4°C for at least 24 h. Each reaction well was washed six times with a 1.2 mL aliquot of Tris-binding buffer. The filters were oven-dried for 60 min and then placed in 3 mL of scintillation fluid (Ultima Gold XR, PerkinElmer, Seer Green, Buckinghamshire, UK). Radioactivity was quantified by liquid scintillation spectrometry. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 1 µM unlabelled CP55940. The concentration of [^{3}H]CP55940 used in our displacement assays was 0.7 nM. The compounds used in this investigation were stored as stock solutions of 10 mM in DMSO, the vehicle concentration in all assay wells being 0.1% DMSO.

Data Analysis

Results were calculated as percentage changes from a basal level (zero) of [^{3}H]CP55940 binding (in the presence of vehicle). GraphPad Prism 5.0 (GraphPad, San Diego, CA) was used to construct sigmoidal log concentration-response curves, and to calculate values of EC_{50}, E_{max}, S.E.M and 95% confidence intervals. K_i values were calculated as described previously.[15a,15b,15c] P values < 0.05 were considered to be significant.

Biological tests on NMDA receptors

Culturing of primary rat cortical neurons

Culturing was a modified version of the one reported by Furshpan and Potter.[18] Brains from E20.5 Sprague Dawley rats (sex not determined) were micro-dissected in medium containing (in mM): Na_2SO_4 81.8, K_2SO_4 30, $MgCl_2$ 5.84, $CaCl_2$ 0.252, HEPES 1, Phenol Red 0.001%, 2 D–glucose 20, kynurenic acid 1. Cortices were incubated for 40 minutes in papain enzyme (10U/mL) (Worthington Biochemical Corporation, Lakewood, NJ, USA) and L-cysteine (3.7 mM) then washed and triturated in NeuroBasal A medium (supplemented with 1% rat serum (Harlan laboratories, Indianapolis, IN, USA), 1 x B–27 supplement, 1% antibacterial/antimycotic and 1 mM glutamine). The cell suspension was diluted in opti–MEM (supplemented with 20 mM glucose and 1% antibacterial/antimycotic) to give an end concentration of 1 hemisphere per 14 mL, and 0.5 mL/coverslip plated onto 13 mm diameter coverslips precoated with poly–D–lysine (1.33% w/v in H_20) and laminin (0.5% w/v) (Roche, Basel, Switzerland) in 24 well plates. Plates were kept at 37 °C in a humidified 5% CO_2 incubator for 2.5 hours before replacement of the cell suspension with supplemented NeuroBasal A. On the fourth day *in vitro* (DIV 4) 1 ml/well of supplemented NeuroBasal A containing 9.6 µM cytosine β–D–arabinofuranoside hydrochloride was added to the cells.

Whole-cell voltage-clamp recordings

Recordings were made from DIV7 neurons at room temperature (18–21 °C) superfused (at a flow rate of 2 mL/min) with an external recording solution comprising (in mM) NaCl 150, KCl 2.8, HEPES 10, $CaCl_2$ 0.5, glucose 10, glycine 0.05, diethylene triaminepentaacetic acid 0.01, tetrodotoxin 0.0003; pH 7.35 using NaOH (300–330 mOsm). 50 µM NMDA was applied alone for 80 s (to allow response to stabilise), then 50 µM NMDA was applied along with the compound of interest or vehicle alone for 110 s (a lengthy application was required due to compound's slow rate of block). The average current during the final 10 s of response to NMDA alone or NMDA plus compound was used to calculate percentage inhibition. 100 µM of (–)-(*R*,*R*)-**HU-210F**, (+)-(*S*,*S*)-**HU-211F** and (+)-(S,S)-**HU-211** were used, 10 µM of MK-801 and vehicle was 1% dimethyl sulfoxide. Application of solutions was controlled manually. Patch–pipettes were made from thick–walled borosilicate glass (GC150F–7.5, Harvard Apparatus) using a P–87 puller (Sutter Instruments) to give a resistance of 2–4 MΩ when filled with an internal solution containing (in mM): K–gluconate 141, NaCl 2.5, HEPES 10, EGTA 11; pH 7.3 with KOH (300 mOsm). Currents were recorded using an Axopatch 200B amplifier (Molecular Devices). Data were filtered at 5 kHz and digitized at 20 kHz via a National Instruments BNC–2090A analogue–digital interface (National Instruments, Newbury, UK) using WinEDR software (Strathclyde Electrophysiology Software). Neurons were voltage– clamped at –60 mV, and recordings were rejected if the holding current was greater than 150 pA or if the series resistance was greater than 30 MΩ.

Synthetic procedures

2-(3,5-dimethoxyphenyl)-2-methylpropanenitrile (4)

To a stirred suspension of sodium hydride (60% in mineral oil, 677 mg, 16.9 mmol, 3 eq) in dry DMF (5.0 mL) at 0°C was added drop-wise a solution of commercially available 2-(3,5-dimethoxyphenyl)acetonitrile **3** (1.0 g, 5.64 mmol, 1 eq) and iodomethane (1.1 mL, 16.9 mmol, 3 eq) in dry DMF (5.0 mL). The reaction temperature rose to 25 °C over a 15 min period and stirring was continued for 2 h. The reaction mixture was quenched with a saturated aqueous NH_4Cl solution (5.0 mL) and diluted with diethyl ether (10 mL). The organic layer was separated and the aqueous layer was extracted with diethyl ether (3 x 10 mL). The combined organic layer was washed

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with water and brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 8:2) to give compound **4** (1.1 g, 98%) as a colorless oil.

R_f 0.35 (Hexane/EtOAc 8:2).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.73 (s, 6H), 3.84 (s, 6H), 6.43 (t, 1 H, *J* = 2.2 Hz), 6.63 (d, 2H, *J* = 2.2 Hz).

MS (ESI) for $C_{12}H_{15}NO_2$: *m/z* calculated 206.1 $[M^+H]^+$, 228.1 $[M^+Na]^+$; *m/z* found (relative intensity) 206.1 $[M^+H]^+$ (100), 228.1 $[M^+Na]^+$ (45).

2-(3,5-dimethoxyphenyl)-2-methylpropanal (5)

To a solution of **4** (1.21 g, 5.90 mmol, 1 eq) in dry DCM (50.0 mL) at -78°C was added DIBALH (1M solution in hexane, 14.75 mL, 14.75 mmol, 2.5 eq). The reaction mixture was stirred at the same temperature for 1 h and then quenched by drop-wise addition of potassium sodium tartrate (10% solution in water, 20 mL). The resulting mixture was warmed to room temperature, stirred vigorously for 1 h, and then diluted with EtOAc (20 mL). The organic phase was separated and the aqueous phase extracted with EtOAc (3 x 50mL). The combined organic layer was washed with water and brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 8:2) to give aldehyde **5** (1.14 g, 93%) as a colorless oil.

R_f 0.37 (Hexane/EtOAc 8:2).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.46 (s, 6H), 3.81 (s, 6H), 6.29-6.53 (m, 3 H), 9.49 (s, 1H).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.6 (x 2C), 50.6, 55.3(x 2C), 98.7, 105.2 (x 2C), 143.7, 161.2 (x 2C), 201.9.

MS (ESI) for $C_{12}H_{16}O_3$: *m/z* calculated 209.1 $[M^+H]^+$; *m/z* found (relative intensity) 209.1 $[M^+H]^+$ (100).

5-(bromotriphenyl-λ^5-phosphanyl)pentan-1-ol (6)

To a solution of commercially available 5-bromo-1-pentanol (2.0 mL, 16.6 mmol, 1 eq) in EtOH (35 mL) was added triphenylphosphine (4.35 g, 16.6 mmol, 1 eq) and K_2CO_3 (2.30 g, 16.6 mmol, 1 eq) and the mixture was heated at reflux overnight. The solvent was evaporated under reduced pressure, the crude product was dissolved in toluene (35 mL) and the mixture was stirred vigorously at 100 °C for 5 min. The mixture was allowed to cool down to r.t. and the crystallized phosphonium salt **6** (9.6 g, 74%) was collected by filtration as a white crystalline solid.

MS (ESI) for $C_{23}H_{25}OP$: *m/z* calculated 349.2 $[M^+H]^+$; *m/z* found (relative intensity) 349.1 $[M^+H]^+$ (100).

(5*Z*)-7-(3,5-dimethoxyphenyl)-7-methyloct-5-en-1-ol (7)

To a suspension of phosphonium salt **6** (11.7 g, 27.3 mmol, 5 eq) in dry THF (130 mL) at 0 °C was added drop-wise LiHMDS (1M in THF, 27.3 mL, 27.3 mmol, 5 eq). The mixture was warmed to 10 °C and stirred for 30 min to ensure complete formation of the orange ylide. A solution of aldehyde **5** (1.1 g, 5.46 mmol, 1 eq) in THF (15 mL) was added drop-wise to the resulting slurry at the same temperature. The reaction was stirred overnight at room temperature. The mixture was quenched by addition of saturated aqueous NH_4Cl (10 mL). The organic layer was separated and the aqueous phase was extracted with Et_2O (3 x 100). The combined organic layer was washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 7:3) to give the alkene **7** (1.4 g, 92%, single diastereoisomer) as a colorless oil.

R_f 0.35 (Hexane/EtOAc 6:4).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.18-1.26 (m, 2H), 1.32-1.40 (m, 2H), 1.41 (s, 6H), 1.68 (ddd, 2 H, *J* = 1.7, 7.4, 14.9 Hz), 3.46 (t, 2 H, *J* = 6.6 Hz), 3.80 (s, 6H), 5.29 (dt, 1H, *J* = 7.4, 11.4 Hz), 5.66 (dt, 1H, *J* = 1.7, 11.4 Hz), 6.31 (t, 1H, *J* = 2.3 Hz), 6.58 (d, 2H, *J* = 2.3 Hz).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.2, 27.9, 31.3 (x 2C), 32.0, 40.3, 55.3 (x 2C), 62.7, 96.9, 105.0 (x 2C), 131.0, 139.6, 153.3, 160.4 (x 2C).

MS (ESI) for $C_{17}H_{26}O_3$: *m/z* calculated 279.2 $[M^+H]^+$, 301.2 $[M^+Na]^+$; *m/z* found (relative intensity) 279.2 $[M^+H]^+$ (100), 301.2 $[M^+Na]^+$ (60).

7-(3,5-dimethoxyphenyl)-7-methyloctan-1-ol (8)

To a solution of **7** (1.39 g, 5.0 mmol, 1 eq) in EtOAc (200 mL) was added 10% Pd/C (139 mg), and the resulting suspension was stirred vigorously under hydrogen atmosphere overnight at room temperature. The catalyst was removed by filtration through Celite and the filtrate was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 6:4) to give the hydrogenated compound **8** (1.40 g, quantitative yield) as a colorless oil.

R_f 0.35 (Hexane/EtOAc 6:4).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.06-1.14 (m, 2H), 1.21-1.36 (m, 4H), 1.28 (s, 6H), 1.49-1.54 (m, 2H), 1.56-1.60 (m, 2H), 3.62 (t, 2 H, *J* = 6.6 Hz), 3.82 (s, 6H), 6.32 (t, 1H, *J* = 2.2 Hz), 6.51 (d, 2H, *J* = 2.2 Hz).

^{13}C NMR (100 MHz, $CDCl_3$) δ: 24.6, 25.6 (x 2C), 29.0, 30.0, 32.8, 38.0, 44.4, 55.2 (x 2C), 63.0, 96.6, 104.7 (x 2C), 152.5, 160.5 (x 2C).

MS (ESI) for $C_{17}H_{28}O_3$: *m/z* calculated 281.2 $[M^+H]^+$, 303.2 $[M^+Na]^+$; *m/z* found (relative intensity) 281.2 $[M^+H]^+$ (100), 303.2 $[M^+Na]^+$ (40).

5-(8-hydroxy-2-methyloctan-2-yl)benzene-1,3-diol (9)

To a solution of compound **8** (336 mg, 1.20 mmol, 1 eq) in dry Et_2O (5.0 mL) and dry THF (0.4 mL) MeMgI (3M in Et_2O, 8.0 mL, 24.0 mmol, 20 eq) was added at 0 °C. The slurry was heated to 100 °C under reduced pressure, then the residue was heated to 170 °C for 1 h under a flux of nitrogen. The cooled reaction mixture was quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (5 x 20 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 1:1) to give the alcohol **9** (212 mg, 70%) as a waxy white solid.

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R_f 0.20 (Hexane/EtOAc 6:4).
^{1}H NMR (400 MHz, CD_3OD) δ: 1.06-1.14 (m, 2H), 1.18-1.34 (m, 4H), 1.23 (s, 6H), 1.43-1.51 (m, 2H), 1.55-1.59 (m, 2H), 3.52 (t, 2 H, *J* = 6.6 Hz), 6.10 (t, 1H, *J* = 2.2 Hz), 6.31 (d, 2H, *J* = 2.2 Hz).
^{13}C NMR (100 MHz, CD_3OD) δ: 24.4, 25.4, 28.1 (x 2C), 29.9, 32.2, 37.1, 44.2, 61.6, 99.3, 104.4 (x 2C), 151.9, 157.6 (x 2C).
MS (ESI) for $C_{15}H_{24}O_3$: *m/z* calculated 253.2 $[M^+H]^+$; *m/z* found (relative intensity) 253.2 $[M^+H]^+$ (100).

5-(8-fluoro-2-methyloctan-2-yl)benzene-1,3-diol (1)

To a solution of alcohol **9** (140 mg, 0.55 mmol, 1.0 eq) in DCM (10 mL) at -78 °C, DAST (0.1 mL, 0.66 mmol, 1.2 eq) was added drop-wise. The reaction was stirred at 0 °C for 15 min, then a saturated aqueous solution of $NaHCO_3$ (10 mL) was added. The resulting mixture was extracted with DCM (2 x 100 mL), dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 6:4) to give the fluorinated compound **1** (70 mg, 50%) as waxy white solid.
R_f 0.35 (Hexane/EtOAc 6:4).
^{1}H NMR (400 MHz, CD_3OD) δ: 1.07-1.15 (m, 2H), 1.24 (s, 6 H), 1.24-1.37 (m, 4H), 1.55-1.68 (m, 4H), 4.38 (dt, 2H, J_{H-H} = 6.2, J_{H-F} = 47.6 Hz), 6.10 (t, 1H, *J* = 2.1 Hz), 6.31 (d, 2H, *J* = 2.1 Hz).
^{19}F NMR (376 MHz, CD_3OD) δ: -219.9 (tt, 1F, *J* = 24.7, 47.6 Hz).
^{13}C NMR (100 MHz, CD_3OD) δ: 24.3, 24.8 (d, J_{C-F} = 5.4 Hz), 28.1, (x 2C), 29.6, 30.1 (d, J_{C-F} = 19.5 Hz), 37.1, 44.1, 83.4, (d, J_{C-F} = 163.7 Hz), 99.3, 104.3, 151.9, 157.7 (x 2C).
MS (ESI) for $C_{15}H_{23}FO_2$: *m/z* calculated 255.2 $[M^+H]^+$, 277.2 $[M^+Na]^+$; *m/z* found (relative intensity) 255.3 $[M^+H]^+$ (100), 277.2 $[M^+Na]^+$ (60).

[(6a*R*,10a*R*)-3-(8-fluoro-2-methyloctan-2-yl)-1-hydroxy-6,6-dimethyl-6H,6aH,7H,10H,10aH-benzo[c]isochromen-9-yl]methyl 2,2-dimethylpropanoate (10a)

To a solution of resorcinol **1** (49 mg, 0.19 mmol, 1 eq) and pivalate ester **2a** (48 mg, 0.19 mmol, 1 eq) in dry DCM (65 mL) at -20 °C was added $BF_3{\cdot}OEt_2$ (0.12 mL, 1.0 mmol, 5.3 eq). The mixture was allowed to warm up to room temperature and then stirred for 2 h. The mixture was carefully washed with brine, over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 9:1) to give compound **10a** (51 mg, 55%) as a waxy white solid.
R_f 0.60 (Hexane/EtOAc 8:2).
$[\alpha]_D^{27}$: -112 (*c* = 0.8, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.08-1.15 (m, 2 H), 1.15 (s, 3H), 1.23 (s, 6 H), 1.23-1.28 (m, 2 H), 1.25 (s, 9 H), 1.32-1.38 (m, 2 H), 1.42 (s, 3 H), 1.51-1.56 (m, 2 H), 1.57-1.70 (m, 2 H), 1.83-1.96 (m, 3H), 2.20-2.32 (m, 1 H), 2.63-2.80 (m, 1 H), 3.38 (dd, 1H, *J* = 3.2, 16.6 Hz), 4.42 (dt, 2H, J_{H-H} = 6.2, J_{H-F} = 47.4 Hz), 4.53 (d, 1H, *J* = 4.1 Hz), 4.75 (bs, OH), 5.78 (d, 1H, *J* = 4.5 Hz), 6.25 (d, 1H, *J* = 1.6 Hz), 6.41 (d, 1H, *J* = 1.6 Hz).
^{19}F NMR (376 MHz, CDCl3) δ: -218.9 (tt, 1F, *J* = 25.0, 47.4 Hz).
^{13}C NMR (100M Hz, $CDCl_3$) δ: 18.5, 24.5, 25.0 (d, J_{c-F} = 5.5 Hz), 27.3, (x 4C), 27.6, 27.7, 28.7 (x 2C), 29.8, 30.4 (d, J_{c-F} = 19.3 Hz), 31.2, 31.6, 37.3, 38.9, 44.3, 44.8, 68.0, 82.2, (d, J_{C-F} = 163.9 Hz), 105.4, 108.0, 109.8, 123.3, 134.0, 150.0, 154.5, 154.6, 178.5.
MS (ESI) for $C_{30}H_{45}FO_4$: *m/z* calculated 489.3 $[M^+H]^+$; *m/z* found (relative intensity) 489.3 $[M^+H]^+$ (100).

(–)-(*R*,*R*)-HU-210F

A solution of the protected ester **10a** (47.9 mg, 0.098 mmol, 1 eq) in dry THF (1.0 ml) was added drop-wise to a suspension of $LiAlH_4$ (15.3 mg, 0.39 mmol, 4 eq) in THF (1.0 mL) at 0 °C. The reaction mixture was stirred for 2 h and allowed to warm to room temperature. The reaction was quenched with water (2 mL) and extracted with ether (2 x 5 mL).
The combined organic layer was washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 8:2) to give the final compound (–)-(*R*,*R*)-**HU-210F** (19.8 mg, 50%, *ee* 97%) as a hygroscopic white solid. The final product was subsequently purified by a chiral HPLC (see the Experimental for details) in order to obtain (–)-(*R*,*R*)-**HU-210F** enantiomerically pure (*ee* 100%).
R_f 0.30 (Hexane/EtOAc 8:2).
$[\alpha]_D^{27}$: -128 (*c* = 0.4, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.07-1.14 (m, 2 H), 1.12 (s, 3H), 1.22 (s, 6 H), 1.22-1.28 (m, 2 H), 1.31-1.37 (m, 2 H), 1.41 (s, 3 H), 1.50-1.54 (m, 2 H), 1.58-1.71 (m, 2 H), 1.82-1.93 (m, 3H), 2.25 (dd, 1H, *J* = 4.4, 11.0 Hz), 2.73 (td, 1H, *J* = 4.6, 10.9 Hz), 3.47 (dd, 1H, *J* = 4.0, 15.7 Hz), 4.09 (q, 2H, *J* = 12.7 Hz), 4.42 (dt, 2H, J_{H-H} = 6.2, J_{H-F} = 47.4 Hz), 5.54 (bs, OH), 5.77 (d, 1H, *J* = 4.7 Hz), 6.25 (d, 1H, *J* = 1.8 Hz), 6.40 (d, 1H, *J* = 1.8 Hz).
^{19}F NMR (376 MHz, $CDCl_3$) δ: -217.8 (tt, 1F, J = 25.0, 47.4 Hz).
^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.5, 24.5, 25.0 (d, J_{C-F} = 5.5 Hz), 27.6, 27.7, 28.7 (x 2C), 29.9, 30.4 (d, J_{C-F} = 19.3 Hz), 31.3, 31.5, 37.3, 44.2, 45.0, 67.1, 76.5, 84.3, (d, J_{C-F} = 163.8 Hz), 105.5, 107.8, 109.9, 121.5, 138.2, 149.9, 154.5, 154.7.
MS (ESI) for $C_{25}H_{37}FO_3$: *m/z* calculated 405.3 $[M^+H]^+$; *m/z* found (relative intensity) 405.3 $[M^+H]^+$ (100).
HRMS calculated for $C_{25}H_{38}F_1O_3$: 405.2799, found: 405.2800.

[(6a*S*,10a*S*)-3-(8-fluoro-2-methyloctan-2-yl)-1-hydroxy-6,6-dimethyl-6H,6aH,7H,10H,10aH-benzo[c]isochromen-9-yl]methyl 2,2-dimethylpropanoate (10b)

To a solution of resorcinol **1** (42 mg, 0.16 mmol, 1 eq) and pivalate ester **2b** (41 mg, 0.16 mmol, 1 eq) in dry DCM (60 mL) at -20 °C was added $BF_3{\cdot}OEt_2$ (0.10 mL, 0.85 mmol, 5.3 eq). The mixture was allowed to warm up to room temperature and then stirred for 2 h. The mixture was carefully washed with brine, over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 9:1) to give compound **10b** (43 mg, 55%) as a waxy white solid.
R_f 0.60 (Hexane/EtOAc 8:2).
$[\alpha]_D^{27}$: +114 (*c* = 0.8, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.07-1.17 (m, 2 H), 1.14 (s, 3H), 1.23 (s, 6 H), 1.23-1.28 (m, 2 H), 1.25 (s, 9 H), 1.31-1.38 (m, 2 H), 1.42 (s, 3 H), 1.51-1.55 (m, 2 H), 1.58-1.71 (m, 2 H), 1.83-

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1.95 (m, 3H), 2.25-2.39 (m, 1 H), 2.70-2.76 (m, 1 H), 3.38 (dd, 1H, *J* = 3.2, 16.6 Hz), 4.42 (dt, 2H, J_{H-H} = 6.2, J_{H-F} = 47.4 Hz), 4.53 (d, 1H, *J* = 4.1 Hz), 4.89 (bs, OH), 5.78 (d, 1H, *J* = 4.5 Hz), 6.25 (d, 1H, *J* = 1.6 Hz), 6.41 (d, 1H, *J* = 1.6 Hz).
^{19}F NMR (376 MHz, $CDCl_3$) δ: -218.9 (tt, 1F, *J* = 25.0, 47.4 Hz).
^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.5, 24.5, 25.0 (d, J_{C-F} = 5.5 Hz), 27.3, (x 4 C), 27.6, 27.7, 28.7 (x 2 C), 29.8, 30.4 (d, J_{C-F} = 19.3 Hz), 31.2, 31.6, 37.3, 38.9, 44.3, 44.8, 68.0, 82.2, (d, J_{C-F} = 163.9 Hz), 105.4, 108.0, 109.8, 123.3, 134.0, 150.0, 154.5, 154.6, 178.5.
MS (ESI) for $C_{30}H_{45}FO_4$: *m/z* calculated 489.3 $[M^+H]^+$; *m/z* found (relative intensity) 489.3 $[M^+H]^+$ (100).

(+)-(*S*,*S*)-HU-211F

A solution of the protected ester **10b** (40.0 mg, 0.082 mmol, 1 eq) in dry THF (1.0 ml) was added drop-wise to a suspension of $LiAlH_4$ (12.4 mg, 0.33 mmol, 4 eq) in THF (1.0 mL) at 0 °C. The reaction mixture was stirred for 2 h and allowed to warm to room temperature. The reaction was quenched with water (2 mL) and extracted with ether (2 x 5 mL).
The combined organic layer was washed with brine, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc 8:2) to give the final compound (+)-(*S*,*S*)-**HU-211F** (16.6 mg, 50%, *ee* 99%) as a hygroscopic white solid. The final product was subsequently purified by a chiral HPLC (see the Experimental for details) in order to obtain (+)-(*S*,*S*)-**HU-211F** enantiomerically pure (*ee* 100%).
R_f 0.30 (Hexane/EtOAc 8:2).
$[\alpha]_D^{27}$: +128 (*c* = 0.4, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.08-1.15 (m, 2 H), 1.12 (s, 3H), 1.22 (s, 6 H), 1.22-1.31 (m, 2 H), 1.31-1.37 (m, 2 H), 1.41 (s, 3 H), 1.50-1.54 (m, 2 H), 1.58-1.71 (m, 2 H), 1.85-1.94 (m, 3H), 2.26 (dd, 1H, *J* = 4.4, 11.0 Hz), 2.73 (td, 1H, *J* = 4.6, 10.9 Hz), 3.45 (dd, 1H, *J* = 4.0, 15.7 Hz), 3.92 (bs, OH), 4.12 (q, 2H, *J* = 12.7 Hz), 4.42 (dt, 2H, J_{H-H} = 6.2, J_{H-F} = 47.4 Hz), 5.77 (d, 1H, *J* = 4.7 Hz), 6.25 (d, 1H, *J* = 1.8 Hz), 6.40 (d, 1H, *J* = 1.8 Hz).
^{19}F NMR (376 MHz, $CDCl_3$) δ: -217.8 (tt, 1F, *J* = 25.0, 47.4 Hz).
^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.5, 24.5, 25.0 (d, J_{C-F} = 5.5 Hz), 27.6, 27.7, 28.7 (x 2C), 29.9, 30.4 (d, J_{C-F} = 19.3 Hz), 31.3, 31.5, 37.3, 44.2, 45.0, 67.1, 76.5, 84.3, (d, J_{C-F} = 163.8 Hz), 105.5, 107.8, 109.9, 121.5, 138.2, 149.9, 154.5, 154.7.
HRMS calculated for $C_{25}H_{38}F_1O_3$: 405.2799, found: 405.2800.

1-(8-bromo-2-methyloctan-2-yl)-3,5-dimethoxybenzene (11)

To an ice-cooled solution of **8** (303 mg, 1.08 mmol, 1 eq) and CBr_4 (717 mg, 2.16 mmol, 2 eq) in dry CH_3CN (12 mL), PPh_3 (482 mg, 1.84 mmol, 1.7 eq) was added in one portion. After 10 minutes the ice bath was removed and the reaction was stirred at room temperature for 1 h. A solution of TEA (0.25 mL) and MeOH (0.25 mL) was then added to quench the reaction. The solvent was evaporated under reduced pressure and the crude mixture purified by flash chromatography on silica gel (Hexane/EtOAc 98:2) to give **11** (333 mg, 90% yield) as a colourless oil.
Rf 0.52 (Hexane/EtOAc 9:1).
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.03-1.14 (m, 2H), 1.17-1.30 (m, 8H), 1.33-1.40 (m, 2H), 1.50-1.64 (m, 2H), 1.75-1.83 (m, 2H), 3.36 (t, 2H, *J* = 6.9 Hz), 3.80 (s, 6H), 6.31 (t, 1 H, *J* = 2.2 Hz), 6.49 (d, 2 H, *J* = 2.2 Hz).
^{13}C NMR (100 MHz, $CDCl_3$) δ: 24.5, 20.1, 29.0 (x 2C), 29.4, 32.8, 34.0, 38.0, 44.4, 55.2 (x 2C), 96.6, 104.7 (x 2C), 152.3, 160.5 (x 2C).
MS (ESI) for $C_{17}H_{28}BrO_2$: *m/z* calculated 343.1 $[M^+H]^+$; *m/z* found (relative intensity) 343.0 $[M^+H]^+$ (100).

5-(8-bromo-2-methyloctan-2-yl)benzene-1,3-diol (12)

To a solution of **11** (124 mg, 0.36 mmol, 1eq) in DCE (7 mL) at room temperature, BBr_3 (1.8 mL of a solution 1M in DCM, 1.8 mmol, 5 eq) was added and the resulting mixture was stirred at reflux for 1.5 h. Then the reaction was cooled to room temperature, quenched with water (1 mL) and the solvent evaporated. The crude was diluted in water, extracted with EtOAc (3 x 10 mL) and the combined organic extracts were dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The resulting deprotected compound **12**, as a dark yellow oil (94 mg, 82% yield), was used for the next synthetic step without further purification.
Rf 0.25 (Hexane/EtOAc 8:2).
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.01-1.12 (m, 2H), 1.18-1.27 (m, 8H), 1.31–1.40 (m, 2H), 1.50-1.56 (m, 2H), 1.71-1.85 (m, 2H), 3.37 (t, 2H, *J* = 6.8 Hz), 4.65 (s, 2H), 6.17 (t, 1H, *J* = 2.2 Hz), 6.37 (d, 2H, *J* = 2.2 Hz).
^{13}C NMR (100 MHz, $CDCl_3$) δ: 24.6, 28.2, 29.0 (x 2C), 29.5, 32.9, 34.2, 37.9, 44.4, 100.1, 106.0 (x 2C), 153.4, 156.5 (x 2C).
MS (ESI) for $C_{15}H_{24}BrO_2$: *m/z* calculated 315.1 $[M^+H]^+$; *m/z* found (relative intensity) 315.0 $[M^+H]^+$ (100).

(6a*R*,10a*R*)-3-(8-bromo-2-methyloctan-2-yl)-1-hydroxy-6,6-dimethyl-6H,6aH,7H,10H,10aH-benzo[c]isochromen-9-yl]methyl acetate (13)

To a solution of **12** (58.5 mg, 0.19 mmol, 1 eq) and **2c** (39 mg, 0.19 mmol, 1 eq) in dry DCM (65 mL) at -20 °C, $BF_3{\cdot}OEt_2$ was added (0.12 mL, 1.0 mmol, 5.3 eq). The mixture was allowed to warm up to room temperature and then stirred for 2 h. The mixture was carefully washed with brine, dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (Hexane/EtOAc 9:1) to give compound **13** (20 mg, 20% yield) as a dark yellow oil.
Rf 0.45 (Hexane/EtOAc 8:2).
$[\alpha]_D^{27}$: -76 (*c* = 1.0, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.02-1.15 (m, 5H), 1.18-1.27 (m, 8H), 1.31-1.41 (m, 5H), 1.47-1.54 (m, 2H), 1.74-1.98 (m, 5H), 2.07 (s, 3H), 2.19-2.29 (m, 1H), 2.67-2.77 (m, 1H), 3.23-3.45 (m, 3H), 4.49 (d, 1 H, *J* = 12.3 Hz), 4.53 (d,1 H, *J* = 12.3 Hz), 4.78 (s, 1H), 5.78 (d, 1 H, *J* = 4.9 Hz), 6.22 (d, 1 H, *J* = 1.8 Hz), 6.38 (d, 1 H, *J* = 1.8 Hz).
^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.4, 21.0, 24.4, 27.5, 27.6, 28.0, 28.7, 29.3 (x 2C), 31.1, 31.9, 32.7, 34.0, 37.3, 44.2, 44.6, 68.4,

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76.5, 105.4, 108.0, 109.7, 124.3, 133.6, 149.9, 154.49, 154.53, 171.1.
MS (ESI) for $C_{27}H_{40}BrO_4$: *m/z* calculated 507.2 $[M^+H]^+$; *m/z* found (relative intensity) 507.2 $[M^+H]^+$ (100).

(6a*R*,10a*R*)-3-(8-bromo-2-methyloctan-2-yl)-1-hydroxy-6,6-dimethyl-6H,6aH,7H,10H,10aH-benzo[c]isochromen-9-yl]methyl acetate (14a)

To a solution of **13** (21.4 mg, 0.042 mmol, 1 eq) and TEA (28 µL, 0.21 mmol, 5 eq) in DCM (1 mL) at room temperature, acetyl chloride (6 µL, 0.084 mmol, 2 eq) was added and the reaction was stirred for 30 minutes. Then the solvent was evaporated in vacuo and the crude partitioned between DCM (3 x 5 mL) and water (3 mL). The combined organic extracts were dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure to afford the desire product **14a** (23 mg, quantitative yield) as a dark yellow oil.
Rf 0.5 (Hexane/EtOAc 8:2).
$[\alpha]_D^{27}$: -75 (*c* = 1.0, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.04-1.14 (m, 5H), 1.17-1.29 (m, 8H), 1.30-1.41 (m, 5H), 1.49-1.54 (m, 2H), 1.71-1.99 (m, 5H), 2.07 (s, 3H), 2.19-2.36 (m, 4H), 2.58-2.66 (m, 1H), 2.93 (dd, 1H, *J* = 4.4, 16.5 Hz), 3.36 (t, 2H, *J* = 6.9 Hz), 4.42 (d, 1H, *J* = 12.1 Hz,), 4.54 (d, 1H, *J* = 12.1 Hz), 5.80 (d, 1H, *J* = 4.8 Hz), 6.52 (1H, d, *J* = 1.9 Hz), 6.68 (1H, d, *J* = 1.9 Hz).
^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.4, 20.9, 21.2, 24.3, 27.4, 27.6, 28.0, 28.5, 29.3 (x 2C), 31.4, 31.8, 32.7, 34.0, 37.4, 44.2, 44.4, 68.3, 77.2 (under solvent residual peak), 112.2, 113.1, 115.2, 125.2, 132.9, 149.6, 150.14, 154.17, 168.8, 170.8.
MS (ESI) for $C_29H_{42}BrO_5$: *m/z* calculated 571.2 $[M^+Na]^+$; *m/z* found (relative intensity) 571.2 $[M^+Na]^+$ (100).
RP-HPLC: Luna C18, 5 µm, 100 Å, 250 × 4.6 mm (L × ID); injected volume: 20 µL; flow rate: 1 mL/min; mobile phase: solvent A = H2O, solvent B = CH3CN; gradient: from 50% B to 100% B in 15 minutes, then 100% B for 30 minutes. Retention Time = 16.1 min. UV absorption was detected at 218 nm. Agilent 1200 HPLC system.

(6a*R*,10a*R*)-3-(8-bromo-2-methyloctan-2-yl)-9-(hydroxymethyl)-6,6-dimethyl-6H,6aH,7H,10H,10aH-benzo[c]isochromen-1-ol (14b)

To a solution of **13** (9.5 mg, 18.7 µmol, 1 eq) in CH_3CN (0.5 mL), NaOH (0.5 mL of a 4 M aqueous solution) was added. The mixture was stirred at 36 °C for 4 h. After this time the acetyl group was completely hydrolysed, as shown by TLC analysis, the reaction was stopped and the solvent was evaporated under pressure. The crude was diluted in water (2 mL), extracted with DCM (3 x 5 mL), the combined organic extracts were dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure to afford the desire product **14b** (8.7 mg, quantitative yield) as a dark yellow oil.
Rf 0.3 (Hexane/EtOAc 7:3).
$[\alpha]_D^{27}$: -70 (*c* = 0.8, $CHCl_3$).
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.02-1.14 (m, 5H), 1.17-1.29 (m, 9H), 1.31-1.42 (m, 5H), 1.48-1.52 (m, 2 H), 1.74-1.94 (m, 5H), 2.16-2.28 (m, 1H), 2.66-2.75(m, 1H), 3.29-3.49 (m, 3H), 3.96-4.15 (m, 2H), 4.95 (bs, 1H), 5.74 (d, 1H, *J* = 4.6 Hz), 6.23 (d, 1 H, *J* = 1.8 Hz), 6.38 (d, 1H, *J* = 1.8 Hz).
^{13}C NMR (100 MHz, $CDCl_3$) δ: 18.5, 24.4, 27.6, 28.0, 28.71, 28.74 (x 2C), 29.4, 29.7, 31.3, 31.6, 32.8, 34.1, 37.3, 44.3, 45.0, 67.1, 76.5, 105.4, 107.9, 109.9, 121.1, 138.2, 154.5, 154.6.
MS (ESI) for $C_{25}H_{38}BrO_3$: m/z calculated 465.2 $[M^+H]^+$; *m/z* found (relative intensity) 465.2 $[M^+H]^+$ (100).
RP-HPLC: Luna C18, 5 µm, 100 Å, 250 × 4.6 mm (L × ID); injected volume: 20 µL; flow rate: 1 mL/min; mobile phase: solvent A = H2O, solvent B = CH3CN; gradient: from 50% B to 100% B in 15 minutes, then 100% B for 30 minutes. Retention Time = 16.1 min. UV absorption was detected at 218 nm. Agilent 1200 HPLC system.

(–)-(*R*,*R*)-[^{18}F]HU-210

[^{18}F]-Fluoride was produced on a cyclotron by proton bombardment of 97% enriched [^{18}O]H_2O (Cambridge Isotope Laboratories, Inc.) by the $^{18}O(p,n)^{18}F$ nuclear reaction. The silver target (1.1ml) was pressurised to 600psi and irradiated with 11MeV protons produced by the CTI/SIEMENS RDS-111 cyclotron at the John Mallard Scottish PET Centre in Aberdeen. Irradiation with a beam current of 29 µA for 7 minutes was typically used (3.9 GBq). At the end of bombardment (EOB) the target was unloaded within 5 min using argon gas.

The radiosynthesis of [^{18}F]**HU210F** was carried out using a remotely controlled synthesis module Eckert&Ziegler ModularLab synthesizer. Prior to the EOB, vial PO4 was filled with KH_2PO4 aqueous solution (0.5 mL, 10 mg/mL), and vial K222 was filled with Kryptofix 2.2.2 (15 mg) in dry acetonitrile (1 mL). Vial ACN was filled with 1 mL of dry acetonitrile and vial PREC was filled with precursor 14b (7.7 mg) dissolved in 1 mL of dry acetonitrile.

After the EOB [^{18}F]-fluoride was unloaded and transferred with argon gas to the synthesis module, which was contained in a lead shielded hot cell. The [^{18}F]-fluoride was subsequently isolated using a CHROMAFIX anion exchange cartridge (Macherey Nagel, Germany) and then eluted into the reaction vessel with the dipotassium hydrogen orthophosphate solution (vial PO4). The solution in vial K222 was added to the reaction vessel and the azeotropic mixture of water and acetonitrile was evaporated under vacuum using a stream of helium. The content of the vial ACN was then added and the mixture was evaporated under vacuum using a stream of helium to ensure formation of the dried complex $[K/K_{222}]^{18}F$.

Precursor **14b** in vial PREC was then added and the reaction mixture was heated at 100˚C for 15 minutes to produce the fluorinated desired product [^{18}F]HU210 (Radiochemical conversion by RadioHPLC of 6%). Purification of the crude mixture was attempted; an aliquot (100 µL, 120 MBq) of the crude mixture was purified by semipreparative HPLC (Phenomenex Luna C18(2) column 10x250 mm 100Å 5 µm, 5 mL/min) affording 4.65 Mbq of [^{18}F]HU210; the collected peak was contaminated by approx. 20% of unreacted [^{18}F]fluoride. Identity of the product was confirmed by comparison with cold reference. (–)-(*R*,*R*)-[^{18}F]**HU210** production was achieved using a non-optimised procedure.

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