Synthesis, radio-synthesis and in vitro evaluation of terminally fluorinated derivatives of HU-210 and HU-211 as novel candidate PET tracers

Synthesis and *in vitro* Evaluation of Fluorinated Derivativesof HU210 and HU211 as Novel Candidate PET Tracers

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Abstract

Introduction
CB1 receptors are involved in psychiatric, neurological, and behavioral 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 CB1-targeted PET tracers, however few [18F]-tracers have demonstrated true potential to image CB1 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. 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. This cannabinoid receptor agonist was reported to have 0.061 and 0.52 nM K_i values at cloned human $CB_1$and CB_2receptors, respectively. HU-210 also displays agonist activity at GPR55 (EC_{50} = 26 nM). Interestingly, its enantiomer HU-211 (dexanabinol) has very different pharmacological properties, being essentially inactive as a cannabinoid but active as NMDA receptor ligand (IC_{50} = 11 µM for inhibition of [^{3}H]MK-801 binding to rat forebrain membranes). 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. 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, and demonstrate that both compounds maintain their native pharmacological properties in vitro, thus supporting the hypothesis that [18F]-labelled HU210 and HU-211 could be valuable PET tracers for in vivo imaging.
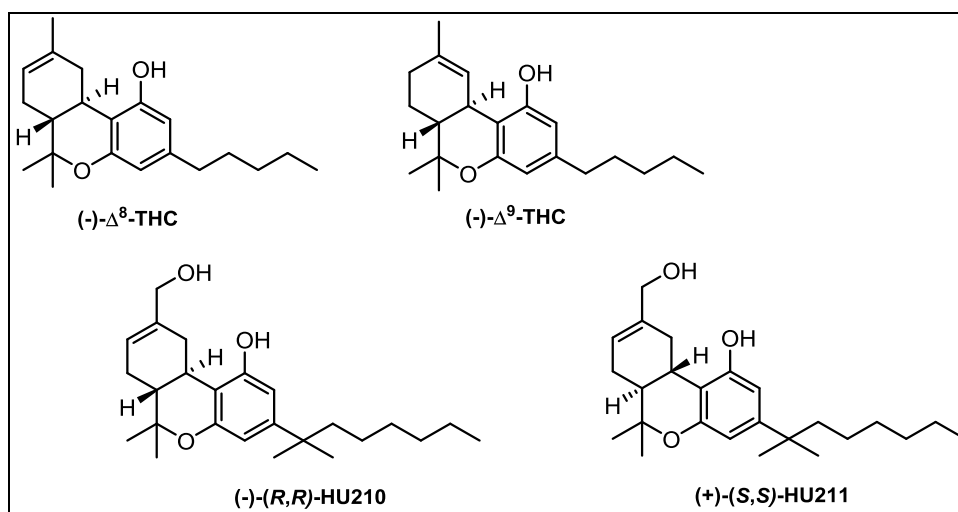
Figure 1.

Results

The synthesis of the two stereochemically pure fluorinated compounds (-)-(R,R)-HU210F and (+)-(S,S)-HU211F (Fig. 2), which are enantiomers, was undertaken.
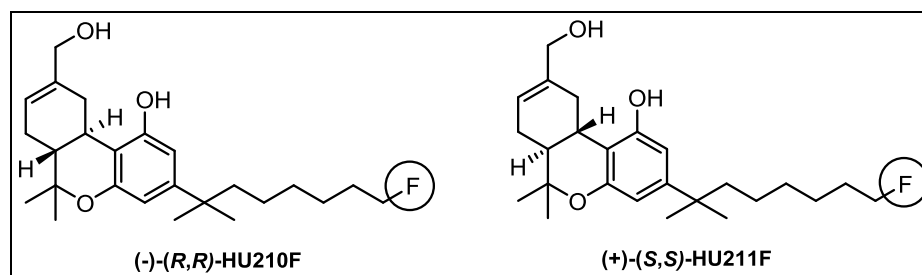

Figure 2.

The retrosynthetic approach to the target compounds HU-210F and HU-211F (**Scheme 1**)was designed taking advantage of the chemistry published by Mechoulam[1] and Huffman[2]. Fluorinated resorcinol **1** was identified as the key intermediate to be reacted with both enantiomers of 4-hydroxy-myrtenyl pivalate**2a,b** forming the desired THC-like structural frameworks.
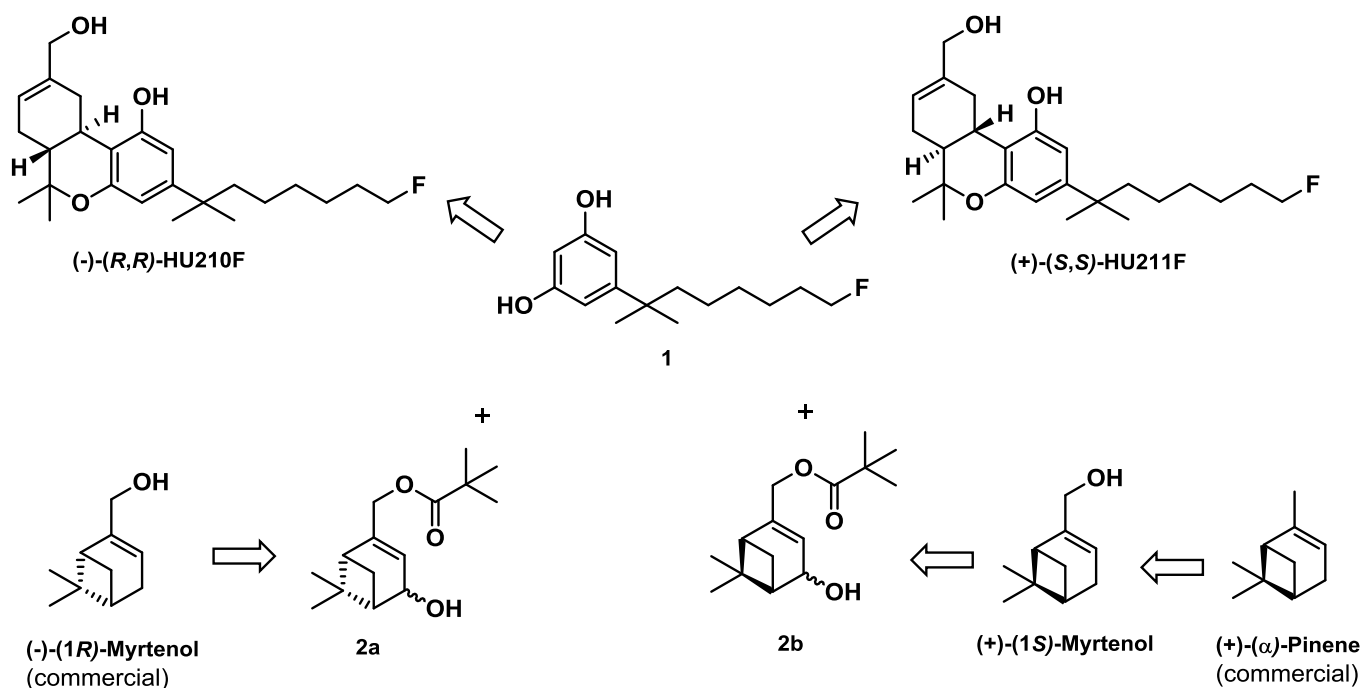
Scheme 1. Retrosynthetic approach to **(-)-(R,R)-HU210F** and **(+)-(S,S)-HU211F**

Synthesis of the key fluorinated building block **1** proved to be challenging. In fact, a number of unsuccessful approaches to **1** were explored (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 alkylation[3] of commercially available dimethoxyphenyl)acetonitrile (**3**), that afforded the α,α-dimethylnitrile**4** in excellent yield. Subsequent hydride reduction[4] afforded the aldehyde **5**that yielded the unsaturated alcohol **7** by means of a Wittig olefination[4]with freshly synthesised[5]phosphine **6**. The alkene bond was then hydrogenated (Pd/C, H_2,inEtOAc)[4]to give compound **8** in quantitative yield. During the first deprotection's attempt (BBr$_3$in DCM from -78°C to -10 °C)[6]the undesired nucleophilic substitution of the primary OH of **8**with a Br occurs, leading to the corresponding unwanted alkyl bromide. However, treatment of **8** with MeMgI at 170°C[7] provided the deprotected alcohol **9** in very good yield. Next, **9**wasdehydroxyfluorinatedwith DAST[8](the use of DEOXOFLUORprovided lower yields) affording the target compound**1**.
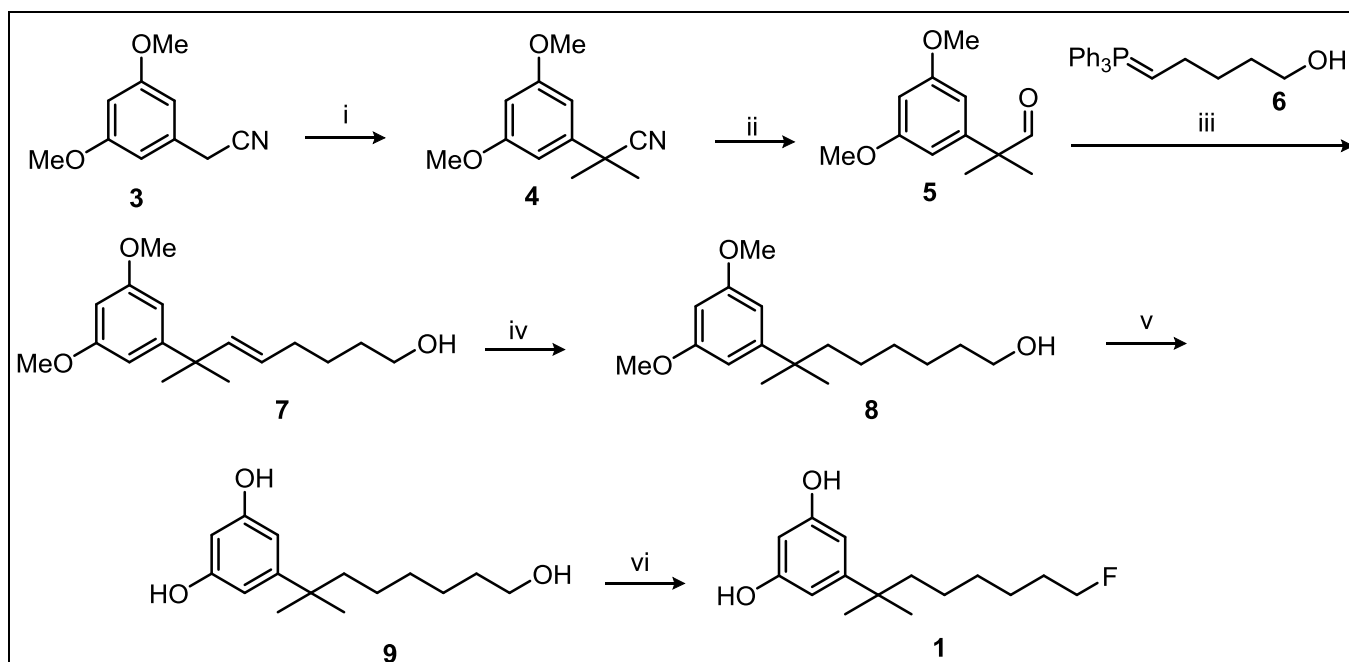
Scheme 2. Synthesis of common intermediate **1**
Reagents and conditions: i) NaH, MeI, DMF, 0°C to rt, 2h (98%); ii) DIBALH, DCM, rt, 1h (93%); iii) **6**, LiHMDS, THF, 0°C to rt, on (92%); iv) H$_2$ 1 atm, 10% Pd/C, EtOAc, on (quantitative); v) MeMgI, Et$_2$O/THF, 0°C to 170°C, 1h (70%); vi) DAST, DCM, -78°C, 0°C, 15 min (50%).

The synthesis of (-)-(R,R)-HU210F was achieved from commercially available (-)-(1*R*)-Myrtenol(≥95% ee) that was transformed into 4-hydroxy-myrtenyl pivalate**2a**(**Scheme 3**) using the method of Huffman (Ref. 1 and 2).The synthesis of (+)-(S,S)-HU211F was achieved from the 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$_2$,[9]and then converted into **2b**.In brief, the common intermediate **1**was condensed with **2a** or **2b**in the presence of BF$_3$·Et$_2$O at -20°C, affording the protected esters **10a** and **10b**. Finally, reduction with LiAlH$_4$gave the desired cannabinoids (-)-(R,R)-HU210F and (+)-(S,S)-HU211F.
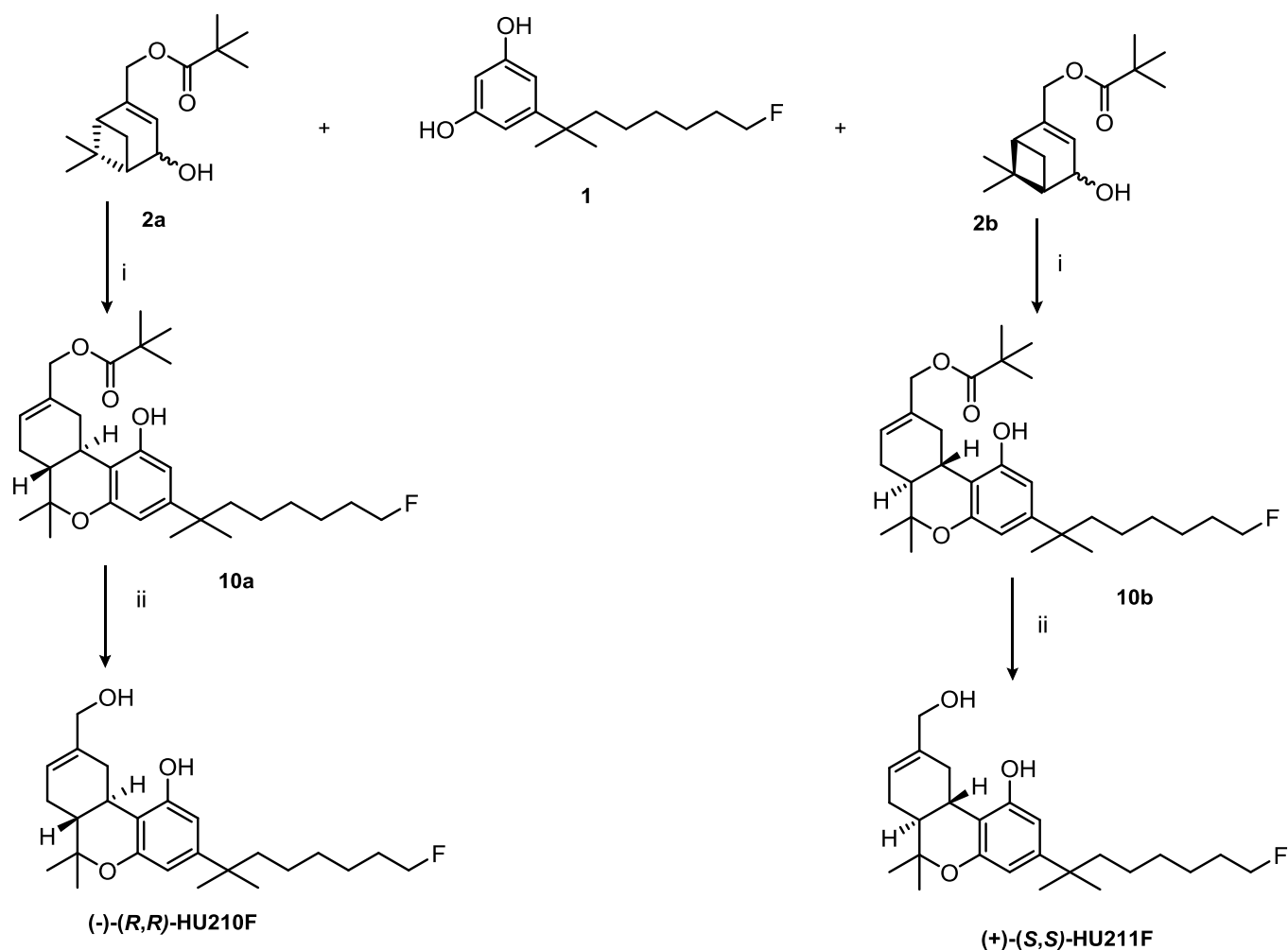
Scheme 3. Synthesis of **(-)-(*R,R*)-HU210F** and **(+)-(*S,S*)-HU211F**

Reagents and conditions: i) BF$_3$·Et$_2$O, DCM, -20°C to rt, 2h (55%); ii) LiAlH$_4$, THF, 0°C, 2h (50%).

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 ≥98% ee for **2b**). The enantiomeric purity of the cannabinoids mimics was confirmed by an analytical chiral HPLC analysis, subsequently **(-)-(*R,R*)-HU210F** and **(+)-(*S,S*)-HU211F** were purified in order to obtain pure (>99% e.e.) single enantiomers (see the experimental part for details).

Biological Tests

NMDAR antagonism

To assess the ability of (-)-(R,R)-HU210F and (+)-(S,S)-HU211F 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 NMDAR (McKay et al., 2012)). Once a steady-state response was observed, we co-applied each compound of interest and assessed the percentage reduction in current (Fig. 3). To control for desensitisation of response during continued agonist exposure, we also applied NMDA with vehicle alone (1% DMSO) (Fig. 3A). We found that (-)-(R,R)-HU210F showed no NMDAR antagonism when compared to vehicle alone (Fig. 3B, F). In contrast, (+)-(S,S)-HU211F gave a maximal block of around 80% at the concentration used (Fig. 3C, F).In addition, we compared NMDAR antagonism of the parent compound, (+)-(S,S)-HU211, which we found blocked NMDAR-mediated current to an extent not significantly different from that seen with (+)-(S,S)-HU211F (Fig. 3D, F).We noted a slow blocking on-rate with both (+)-(S,S)-

HU211 and (+)-(S,S)-HU211F 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. 3E).
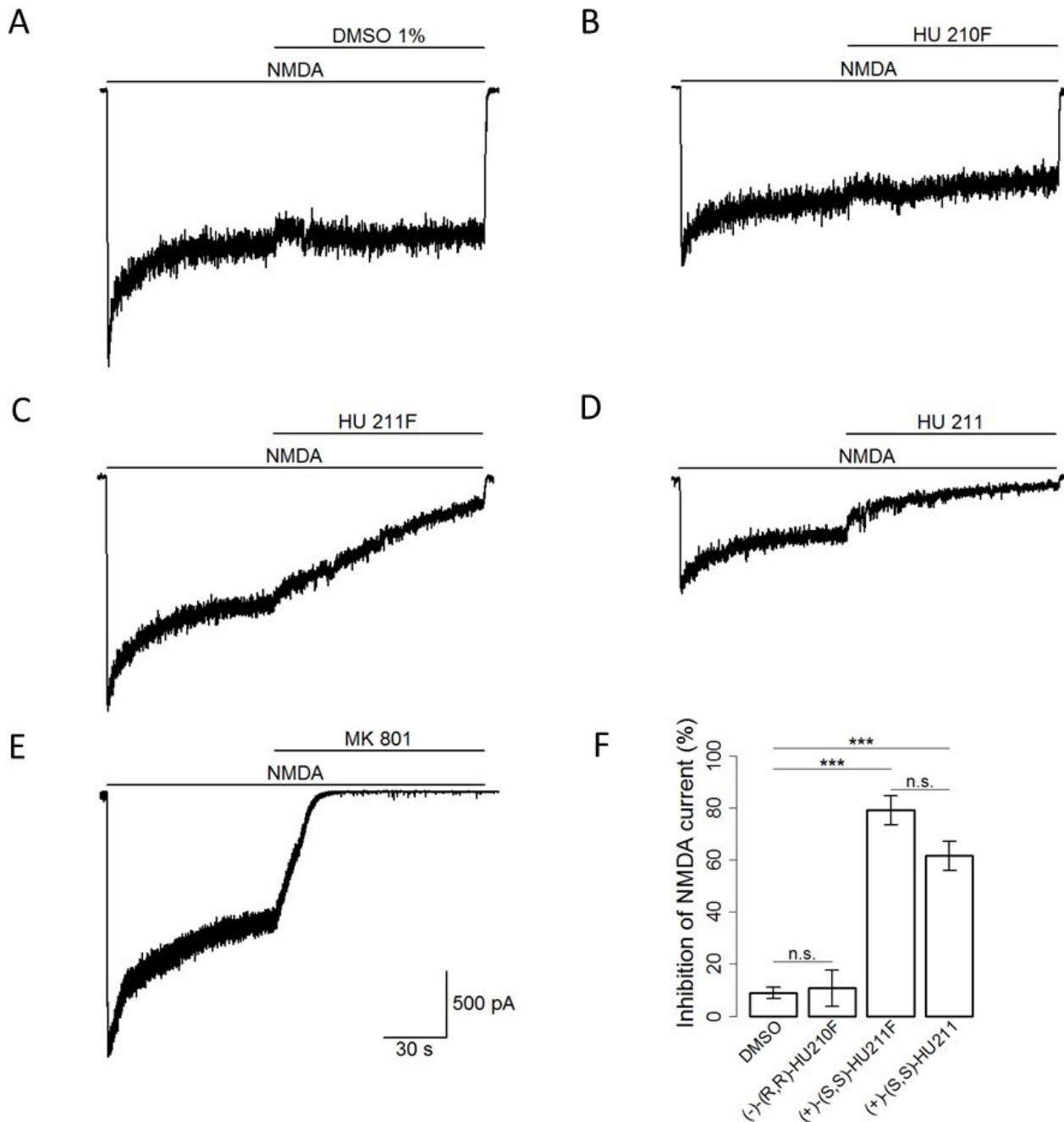
Figure 3: (+)-(S,S)-HU211F and (+)-(S,S)-HU211 are both NMDAR antagonists. (A-E) Example whole-cell current recordings from rat cortical neurons. **(A)** NMDA (50 µM) with vehicle (1% DMSO), demonstrating desensitisation (mean percentage reduction in current compared to NMDA alone 8.7 ± 5.9 (n=7 cells)). **(B)** NMDA co-applied with (-)-(R,R)-HU210F (100 µM), showing no additional blockade compared to vehicle (10.8 ± 17.5 (6), p =0.995). **(C)** NMDA co applied with (+)-(S,S)-HU211F (100 µM), showing significant block compared to vehicle (79.2 ± 15.8% (8), p<0.0001)). **(D)** NMDA co applied with (+)-(S,S)-HU211 (100 µM), showing significant block compared to vehicle (61.6 ± 13.9% (6), p<0.0001) but not significantly different to (+)-(S,S)-HU211F (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 NMDA 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.

Conclusions

Our experiments assessing NMDAR antagonism confirmed that (+)-(S,S)-HU211 is a NMDAR antagonist and that its potency is retained in its fluorinated form (+)-(S,S)-HU211F. The finding that (+)-(S,S)-HU211 is an NMDAR antagonist is consistent with previous work using radioligands, behavioural assays, excitotoxicity assays and calcium influx (Feigenbaum 1989, Esshar 1993, Biegon and Joseph 1995). Based on the previously reported IC50 of 11 μM (Esshar 1993), we would have predicted a more complete block at the concentration used here (100 μM). However, this IC50 was based on radioligand studies assessing displacement of MK-801 whereas our work is the first electrophysiological assessment of the effect of (+)-(S,S)-HU211. Using electrophysiology to assess the effect of (+)-(S,S)-HU211 and its fluorinated analogue has also allowed us to highlight their very slow on-rate, a property not identified previously.

ExperimentalSection

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

To a stirred suspension of sodium hydride (60% in mineral oil, 677 mg, 16.9 mmol, 3eq) in dry DMF (5.0 mL) at 0°C was added dropwise a solution of the commercial available 2-(3,5-dimethoxyphenyl)acetonitrile **3**(1.0 g, 5.64 mmol, 1eq) 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 saturated aqueous NH$_4$Cl 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 with water and brine, dried over Na$_2$SO$_4$ 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, 5.90mmol, 1eq) 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 thenquenched by dropwise addition of potassium sodium tartrate (10%solution in water, 20 mL). The resulting mixture was warmed to roomtemperature, stirred vigorously for 1 h, and then diluted withEtOAc (20 mL). The organic phase was separated and the aqueous phaseextracted with EtOAc (3 x 50mL). The combined organic layer was washed with water and brine, dried over Na$_2$SO$_4$ 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 (100MHz, CDCl$_3$) δ:22.6 (x 2 C), 50.6, 55.3(x 2 C), 98.7, 105.2 (x 2 C), 143.7, 161.2 (x 2 C), 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)

A solution the commercially available 5-bromo-1-pentanolo (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$_2$CO$_3$ (2.30 g, 16.6 mmol, 1 eq) and 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 cooled down and the phosphonium salt crystallized was collected by filtration. The resulting product **6** (9.6 g, 74%), obtained as with solid, was used crude, without further purification.

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).

(5E)-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 dropwise LiHMDS (1 M 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, 1eq) inTHF (15 mL) was added dropwise to the resulting slurry at the same temperature. The reaction was stirred overnight at room temperature. The mixture was quenched by the 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/ EtOAc7:3) to give alkene**7** (1.4 g, 92%, single diastereoisomer) as a colorless oil.

R_f 0.35 (Hexane/EtOAc6:4).

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

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 temper-ature. 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 saturated 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 (100MHz, $CDCl_3$) δ:24.6, 25.6 (x 2 C), 29.0, 30.0, 32.8, 38.0, 44.4, 55.2 (x 2 C), 63.0, 96.6, 104.7 (x 2 C), 152.5, 160.5 (x 2 C).

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** (336mg, 1.20mmol, 1 eq)in dry Et_2O (5.0 mL) and dry THF (0.4 mL) MeMgI (3 M in Et_2O, 8.0 mL, 24.0 mmol, 20 eq) was added at 0 °C After the mixture was heated to 100 °C in vacuum, the residue was then heated at 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/ EtOAc1:1) to give alcohol **9** (212mg, 70%) as a waxy white solid.

R_f 0.20 (Hexane/EtOAc 6:4).

[1H] NMR (400 MHz, CD₃OD) 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).

[13C] NMR (100MHz, CD₃OD) δ:24.4, 25.4, 28.1 (x 2 C), 29.9, 32.2, 37.1, 44.2, 61.6, 99.3, 104.4 (x 2 C), 151.9, 157.6 (x 2 C).

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** (140mg, 0.55mmol, 1.0 eq) in DCM (10 mL) at -78 °C, DAST (0.1 mL, 0.66mmol, 1.2eq) is added dropwise. The reaction was stirred at 0 °C for 15 min, then a saturated aqueous solution of NaHCO₃ (10 mL) was added. The resulting mixture was extracted with DCM (2 x 100 mL), dried over Na2SO4, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 6:4) to give fluorinated compound **1** (70mg, 50%) as waxy white solid.

R_f 0.35 (Hexane/EtOAc 6:4).

[1H] NMR (400 MHz, CD₃OD) 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).

[19F] NMR (376.45 MHz, CD₃OD) δ: -219.9 (tt, 1F, J = 24.7, 47.6 Hz).

[13C] NMR (100MHz, CD₃OD) δ:24.3, 24.8 (d, J_{C-F} = 5.4 Hz), 28.1, (x 2 C), 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 2 C).

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).

[(6aR,10aR)-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 borontrifluorideetherate(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 Na2SO4, filtered and the solvent was evaporated under reduced pressure. .The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc9:1) to give compound**10a** (51mg, 55%) as a waxy white solid.

R_f 0.60 (Hexane/EtOAc 8:2).

$[\alpha]_D^{27}$: -112 (c = 0.8, CHCl₃).

[1H] NMR (400 MHz, CDCl₃) 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).

[19F] NMR (376.45 MHz, CDCl₃) δ: -218.9 (tt, 1F, J = 25.0, 47.4 Hz).

[13C] NMR (100MHz, CDCl₃) 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).

(-)-(R,R)-HU210F

A solution of the protected ester **10a** (47.9mg, 0.098mmol, 1 eq) indry THF (1.0 ml) was added dropwise to a suspension ofLiAlH$_4$ (15.3mg, 0.39mmol, 4 eq) in THF (1.0 ml) at 0 °C. The reaction mixture was stirred for 2 h and allowedto warm to room temperature. The reaction was quenchedwith water (2 mL) and extracted with ether (2 x 5 mL).

The combined organic layer was washed with brine, dried over Na$_2$SO$_4$ and the solvent was evaporated under reduced pressure.The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc8:2) to give the final compound**(-)-(R,R)-HU210F** (19.8mg, 50%) as a hygroscopic white solid.

R_f 0.30 (Hexane/EtOAc 8:2).

$[\alpha]_D^{27}$: -128 (c = 0.6, 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 (376.45 MHz, CDCl$_3$) δ: -217.8 (tt, 1F, J = 25.0, 47.4 Hz).

^{13}C NMR (100MHz, CDCl$_3$) δ: 18.5, 24.5, 25.0 (d, J_{C-F} = 5.5 Hz), 27.6, 27.7, 28.7 (x 2 C), 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 calcd. for C$_{25}$H$_{38}$F$_1$O$_3$: 405.2799, found: 405.2800.

[(6aS,10aS)-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)[1]

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 borontrifluorideetherate(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$_2$SO$_4$, filtered and the solvent was evaporated under reduced pressure. .The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc9:1) to give compound**10b** (43mg, 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: 1.07-1.17 (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), 1.83-1.95 (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 (376.45 MHz, CDCl$_3$) δ: -218.9 (tt, 1F, J = 25.0, 47.4 Hz).

^{13}C NMR (100MHz, 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)-HU211F [1]

A solution of the protected ester **10b** (40.0mg, 0.082mmol, 1 eq) indry THF (1.0 ml) was added dropwise to a suspension ofLiAlH$_4$ (12.4mg, 0.33mmol, 4 eq) in THF (1.0 ml) at 0 °C. The reaction mixture was stirred for 2 h and allowedto warm to room temperature. The reaction was quenchedwith water (2 mL) and extracted with ether (2 x 5 mL).

The combined organic layer was washed with brine, dried over Na$_2$SO$_4$ and the solvent was evaporated under reduced pressure.The crude product was purified by flash chromatography on silica gel (Hexane/ EtOAc8:2) to give the final compound**(+)-(S,S)-HU211F** (16.6mg, 50%) as a hygroscopic white solid.

^{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 (376.45 MHz, CDCl$_3$) δ: -217.8 (tt, 1F, J = 25.0, 47.4 Hz).

^{13}C NMR (100MHz, CDCl$_3$) δ: 18.5, 24.5, 25.0 (d, J_{C-F} = 5.5 Hz), 27.6, 27.7, 28.7 (x 2 C), 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.

R_f 0.30 (Hexane/EtOAc 8:2).

$[\alpha]_D^{27}$: +128 (c = 0.6, CHCl$_3$).

HRMS calcd. for C$_{25}$H$_{38}$F$_1$O$_3$: 405.2799, found: 405.2800.

Culturing of primary rat cortical neurons

Culturing was a modified version of (Furshpan and Potter, 1989). Brains from E20.5 Sprague Dawley rats (sex not determined) were microdissected in medium containing (in mM): Na$_2$SO$_4$ 81.8, K$_2$SO$_4$ 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 (10 U / 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 13mm diameter coverslips precoated with poly– D–lysine (1.33% w/v in H$_2$0) and laminin (0.5% w/v) (Roche, Basel, Switzerland) in 24 well plates. Plates were kept at 37°C in a humidified 5% CO$_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 in neurons

Recordings were made on DIV7 at room temperature (18–21 °C) with neurons 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 (lengthy application 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)-HU210F, (+)-(S,S)-HU211F and (+)-(S,S)-HU211 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 MW 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.

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