Figure 1

a

11β–HSD1 ⇌ 11β–HSD2

R= H **Cortisone**
R= OH **11-Dehydrocorticosterone**

R= H **Cortisol**
R= OH **Corticosterone**

b

11β–HSD1 ⇌ 11β–HSD2

11-Dehydrodexamethasone

Dexamethasone

c

11β–HSD1 →

c1a

c1b

Figure 1: Metabolism of substrates by 11β-hydroxysteroid dehydrogenases

11β-Hydroxysteroid dehydrogenase (11β-HSD) 1 reduces 11-keto steroids and non-steroidal tracers. **a)** 11β-HSD1 converts inactive keto glucocorticoids cortisone or 11-dehydrocorticosterone into active hydroxy glucocorticoids, cortisol or corticosterone, respectively. 11β-HSD2 catalyses the reverse dehydrogenase reactions. **b**) 11β-HSD1 and 11β-HSD2 also interconvert synthetic fluorinated steroids, 11-dehydrodexamethasone and dexamethasone. **c**) The synthetic non-steroidal fluorinated compound **c1a** is also a substrate for 11β-HSD1, being converted to **c1b**.

Figure 2

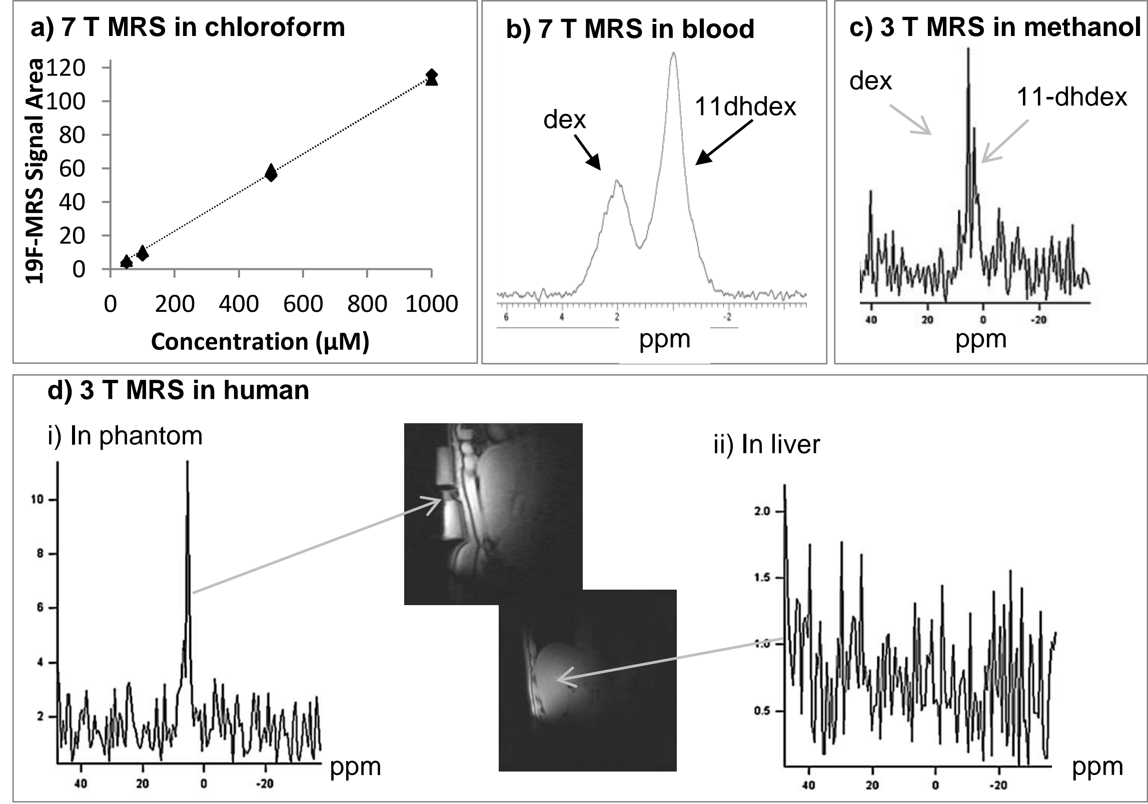

Figure 2: Evaluation of 11-dehydrodexamethasone as a tracer for 11β-HSD1 activity by ^{19}F-MRS

a) Linearity of ^{19}F-MRS signal vs. concentration (50 µM to 1000 µM) ketosteroid 11-dehydrodexamethasone (triangles) and hydroxysteroid dexamethasone (rhombi).
b) Broadening and overlap of peaks in whole blood biological matrix (5 mL) with 2.1 mg dex plus 4.2 mg 11-dhdex scanned on the Agilent 7 T pre-clinical MR scanner with 300 repetitions and total scan time of 5 minutes. The spectrum is centred on the 11-dhdex peak, and the dex peak is 2.2 ppm apart
c) Discrimination of dex and 11-dhdex is attenuated on the clinical Verio 3 T scanner (10 mg each in 20 mL methanol).
d) (i) In healthy men, dex was detected in a phantom containing 10 mg dex placed within the coil next to the patient. (ii) Dex was not detected in the liver after oral administration of dexamethasone; a representative image is shown from a volunteer 10 minutes after a 12 mg dex dose.

FIGURE 3

Compound	IC50 Rat	IC50 Human
c1a	0.34 µM	0.13 µM
c2a	>50 µM	>50 µM
c3a	>50 µM	1.8 µM
c4a	1.3 µM	0.48 µM
c5a	>50 µM	>50 µM
c6a	>50 µM	2.3 µM
c7a	>50 µM	>50 µM
c8a	>50 µM	>50 µM
c9a	>50 µM	>50 µM
c10a	>50 µM	1.9 µM
c11a	>50 µM	>50 µM
c12a	>50 µM	>50 µM

Figure 3: Screening putative polyfluorinated tracers for competition with cortisone for metabolism by 11β-HSD1

Inhibition of cortisone reductase activity by potential fluorinated keto tracers (**c1a-c12a**) thought likely to be substrates for 11β-hydroxysteroid dehydrogenase (11β-HSD) 1 was evaluated in HEK293 stably transfected with human or rat *Hsd11b1.* IC50C values represent the mean of two experiments.

Figure 4

b) 7T MRS in Methanol

19F-MRS Signal Area

350
300
250
200
150
100
50
0

0 200 400 600 800 1000

Concentration (µM)

b) 7T MRS in blood

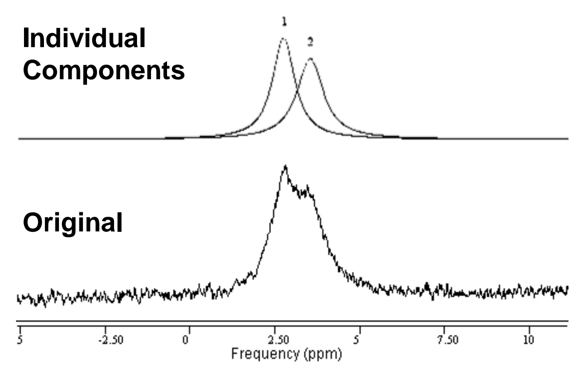

Figure 4: *In vitro* and *ex vivo* evaluation of compound c1a as a tracer for 11β-HSD1 activity by ^{19}F-MRS

a) Increased ^{19}F-MRS signal from trifluorinated **c1a** (circles) vs monofluorinated 11-dehydrodexamethasone (triangles), measured in chloroform solution on the 7 T pre-clinical scanner with T_R of 0.5s, 800 repetitions and 400s acquisition time. The ^{19}F-MRS signal was linearly correlated with tracer amount ($r^2 > 0.99$ for both c1a and 11-dehydrodexamethasone) and signal intensity increased linearly with numbers of fluorine atoms per molecule, as demonstrated by trifluorinated **c1a** giving a signal threefold higher than 11-dehydrodexamethasone.

b) Overlap of ^{19}F-MRS peaks (upper panel) for **c1a** and **c1b** in blood and their resolution (lower panel) by jMRUI software using predefined peak ppm values for **c1a** (1) and **c1b** (2).

Figure5

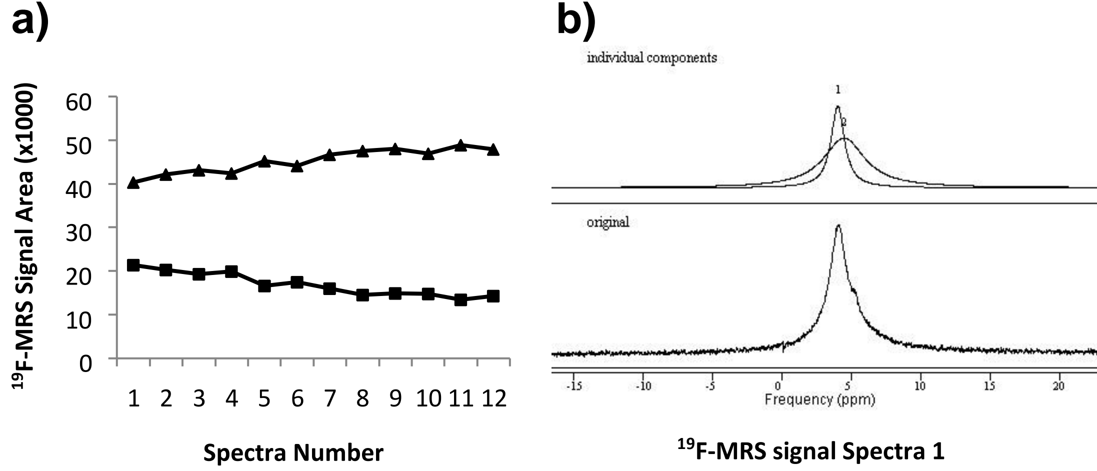

Figure 5: Ex-vivo (perfusion) and In-vivo conversion of keto compound c1a to hydroxy metabolite c1b detected by ^{19}F-MRS scan liver in rat.

(a) Ex-vivo scan of perfused liver. Signal areas vs spectrum on the serial scans of the pilot experiment, each for 400 s, beginning ~20 minutes after the end of a 30 minutes perfusion with **c1a** 100 µM solution. **c1b** was formed and accumulated rapidly during the perfusion. Generation of **c1b** from **c1a** and continued to happen on the excised liver during scanning.

(b) The 19F-MRS spectra reveals further broadening of peaks compared with scanning in blood (representative spectrum and signals deconvolution shown), with the overlap of wide signals from tracer and metabolite seen across all timepoints. Representative spectra shown.

Figure 6

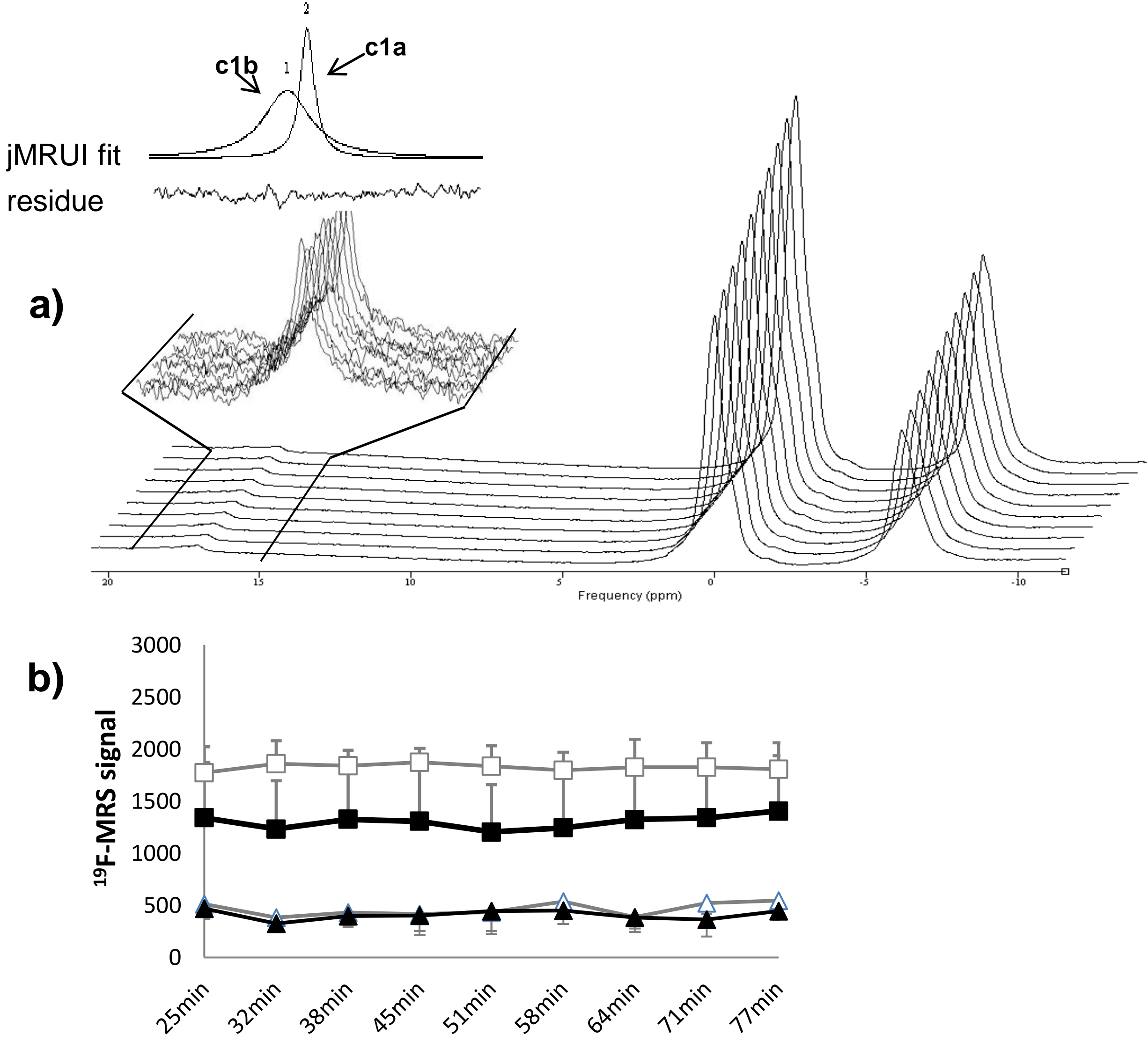

Figure 6 : Effects of 11β-HSD1 inhibition in rats on c1a and c1b in rat liver *in vivo*

a) Sequential (front to back) *in-vivo* in rat liver ^{19}F-MRS spectra after gavage of 15 mg/kg of **c1a** post administration of weight matched dose of vehicle. 800 repetitions (400 seconds) per spectrum were used to allow fine monitoring of signal change over time. ^{19}F-MRS signal from **c1a** and **c1b** were detected, in low intensity compared to isoflurane peaks

b) Peak areas for c1a and c1b measured by ^{19}F-MRS after oral gavage of c1a in rats pre-treated with Merck544 (MK544) selective 11β-HSD1 inhibitor (c1a black squares, c1b black triangles) or vehicle (c1a white squares, c1b white triangles). Data are mean ± SEM from n=3 per group. The time shown is between c1a gavage and the start of the scan for each spectrum (aligned±2 minutes). Scan time per spectrum was 400 s (6 minutes 40 s). There were no statistically significant differences in amount of metabolite formed or ratio of substrate to product. However, by repeated measures ANOVA ^{19}F-MRS signal of the substrate (c1a) on Merk544 group was lower than for the control group ($p<0.04$).