(–)-CHANA, a fluorogenic probe for detecting amyloid binding alcohol dehydrogenase HSD10 activity in living cells

Kirsty Muirhead, Mary Froemming, Xiaoguang Li, Kamil Musilek, Stuart J. Conway, Dalibor Sames & Frank J. Gunn-Moore

Supporting information

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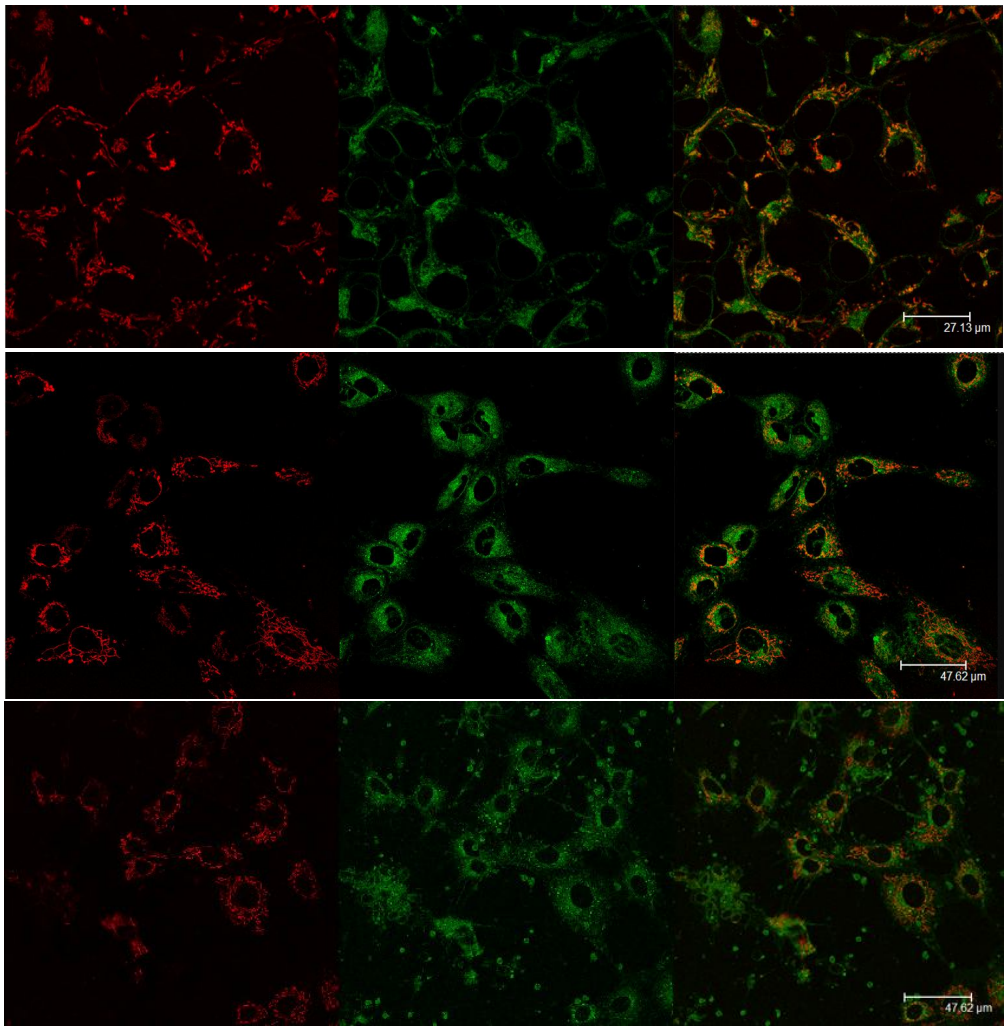


Figure S1: Localisation of (−)-CHANA metabolism product in HEK (top), SK-N-SH (middle) and mouse cortical neurons (bottom). Cells were stained with MitoTracker Deep Red (HEK/SK-N-SH 25 nM, neurons 100 nM, 40 min), then (−)-CHANA (2 µM, 1 h). Images of live cells were taken by confocal microscopy: MitoTracker (left), (−)-CHANA (centre) and merged image (right) (63 × oil objective, 200 Hz; MitoTracker: Ex 633 nm, em 640-700 nm; (−)-CHANA: Ex 405 nm, em 470-540 nm).

HSD10 knockdown in HEK cells

Method:

Transfection of siRNA was performed using GeneEraser (Stratagene) according to the manufacturer's instructions. siRNA oligomers AAGAAGUUAGGAAACAACUGCGUUU and AAACGCAGUUGUUUCCUAACUUCUU (Invitrogen, HSD17B10HSS179169), were used at a final concentration of 15 nM for 72 h. Non-targeted siRNA controls were performed using non-targeted siRNA (Thermoscientific non-targeting siRNA #2). Knockdown was confirmed by Western blotting.

Results:

In order to investigate the origin of the endogenous (–)-CHANA metabolism, ABAD expression was down-regulated in HEK cells before imaging with (–)-CHANA. Endogenous HSD10 expression was decreased in HEK293 cells using short interference RNA (siRNA) targeted to HSD10. After 72 h siRNA treatment, HSD10 expression was reduced by ~95%, as confirmed by Western blotting and Image J analysis (Figure S2a). However (−)-CHANA metabolism experiments did not reveal a decrease in fluorescence intensity and still displayed a similar level of fluorescence to the control cells (Figure S2b, c). Again the reasons for this may be complex as it is possible that the remaining ~5% expression of HSD10 could be responsible for the all of the observed activity seen; however, this is unlikely as the previous over-expression of HSD10 increased activity, implying that a maximum had not been reached. Therefore this led us to conclude that the low level of background activity seen is potentially as a result of other enzymes, such as alternative hydroxysteroid dehydrogenases. Additionally after 72 hours it is also possible that the cells may have responded to the knockdown of HSD10 by up-regulating enzymes that fulfil a similar metabolic role, thereby compensating for the loss of the enzyme.

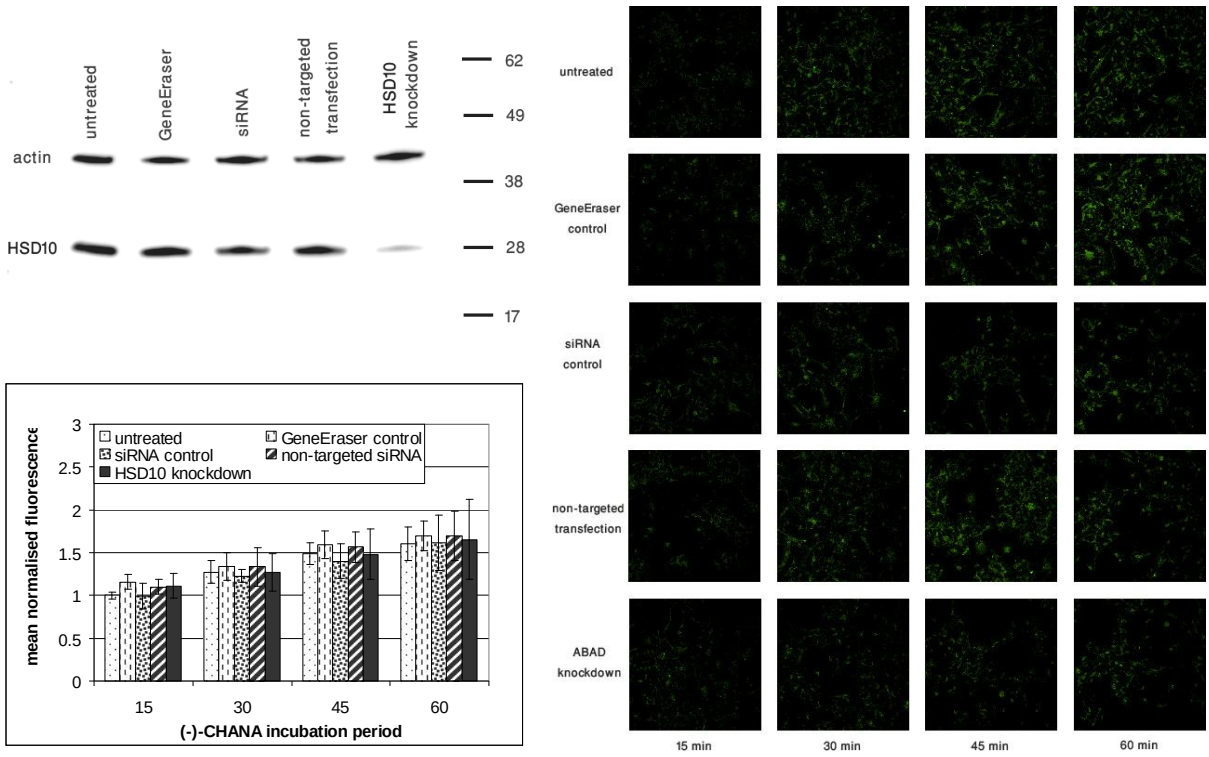
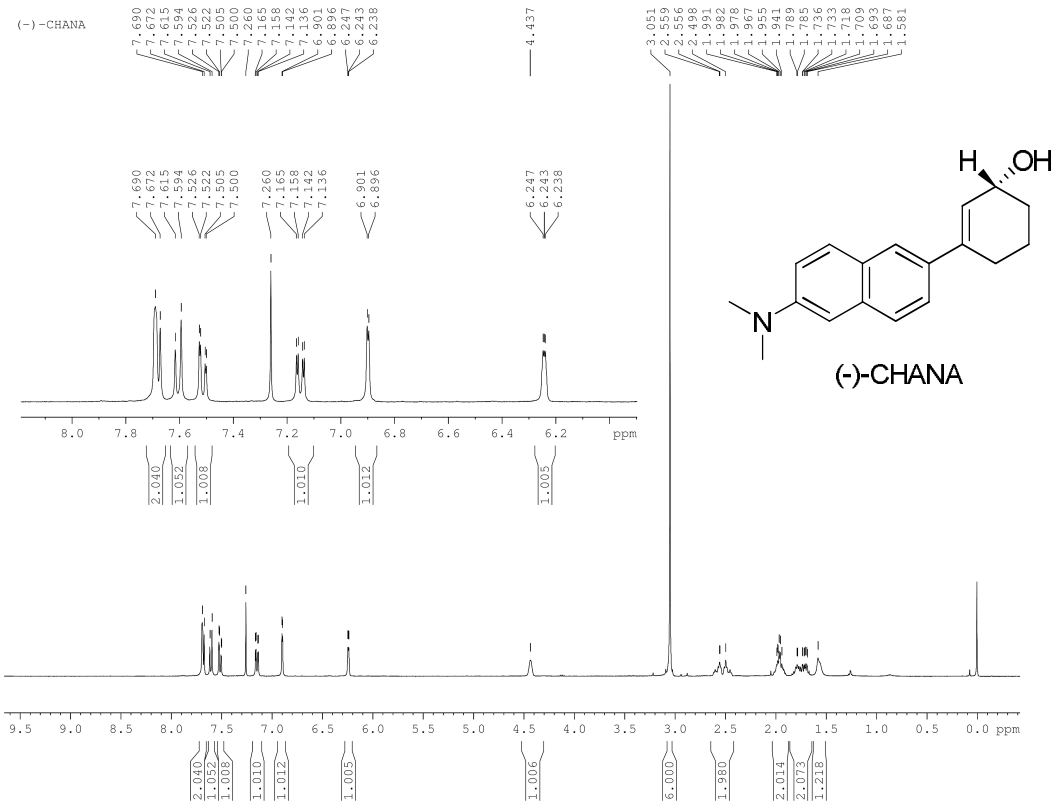


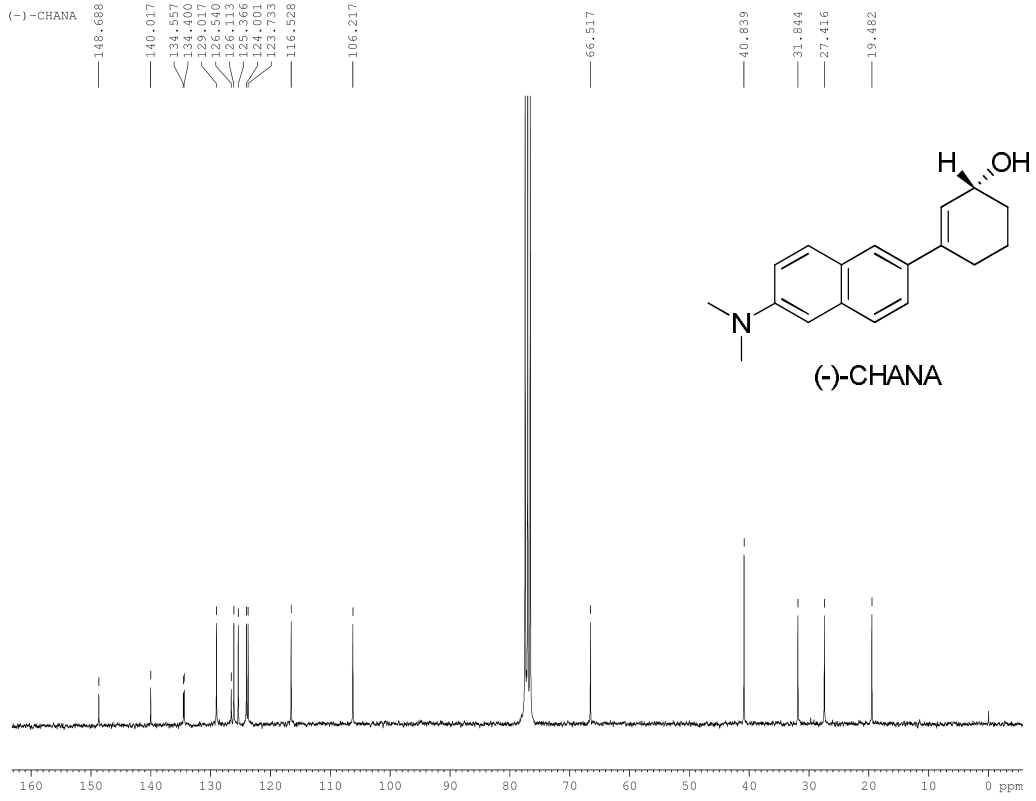
Figure S2: (−)-CHANA activity in HSD10-knockdown HEK293 cells. a) siRNA (15 nM, 72 h) was used to knock down HSD10 gene expression. Western blot analysis of HSD10 (lower band) and β-actin (upper band) showing HSD10 knockdown (lane 5) compared with controls (lane 1: untreated cells; lane 2: cells treated with GeneEraser [lipofection reagent] only; lane 3: siRNA only; lane 4: non-targeted siRNA transfection). b) (−)-CHANA activity over 1 h remains in HSD10-knockdown (row 5) compared with controls (rows 1-4). After HSD10 knockdown (15 nM siRNA or appropriate control, 72 h), HEK293 cells were incubated with 2 µM (−)-CHANA in phenol red-free DMEM and the fluorescence produced was monitored over 1 h by confocal microscopy. Ex. 405 nm, emission 500-520 nm, 40 × oil objective, 200 Hz. c) Quantification was performed using ImageJ. For each data point, three images per dish were taken and each dish was carried out in three separate experiments, giving a total of 9 intensity values, from which the mean and standard error was calculated. Errors are given as SEM. Statistical analysis was performed using the Student's t-test and showed no significant difference between samples.

Characterisation of (+)- and (–)-CHANA

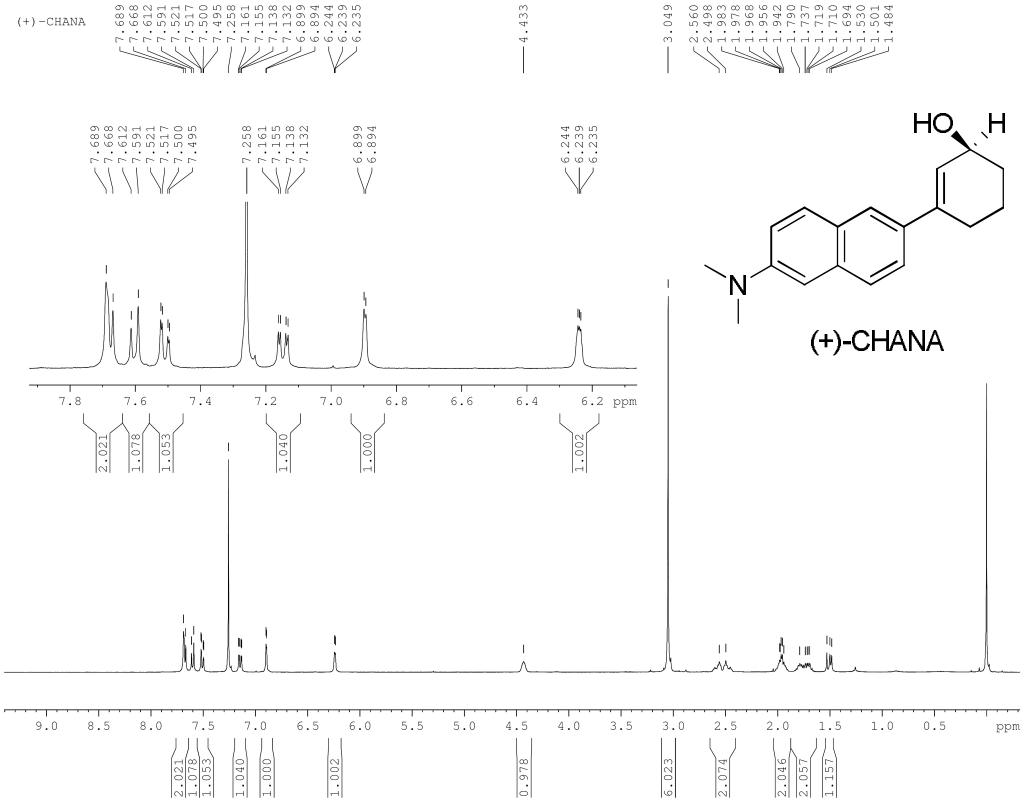
(–)-CHANA ^{1}H NMR spectrum (400 MHz, $CDCl_3$)



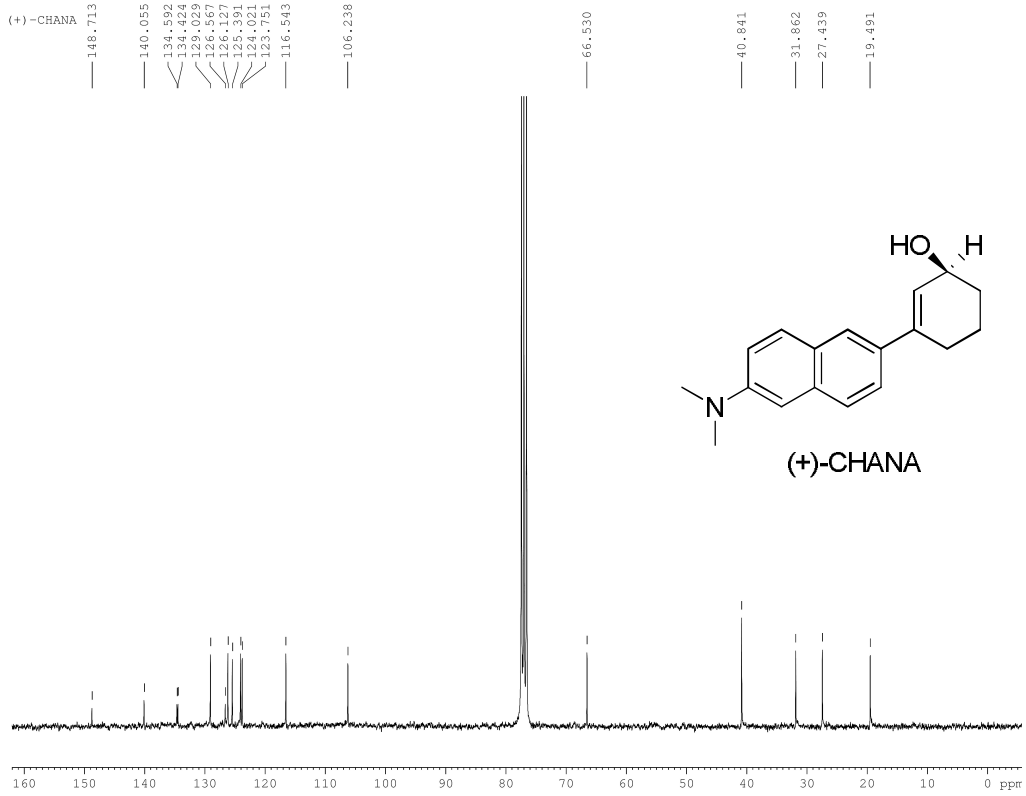
(–)-CHANA ^{13}C NMR (75 MHz, $CDCl_3$)



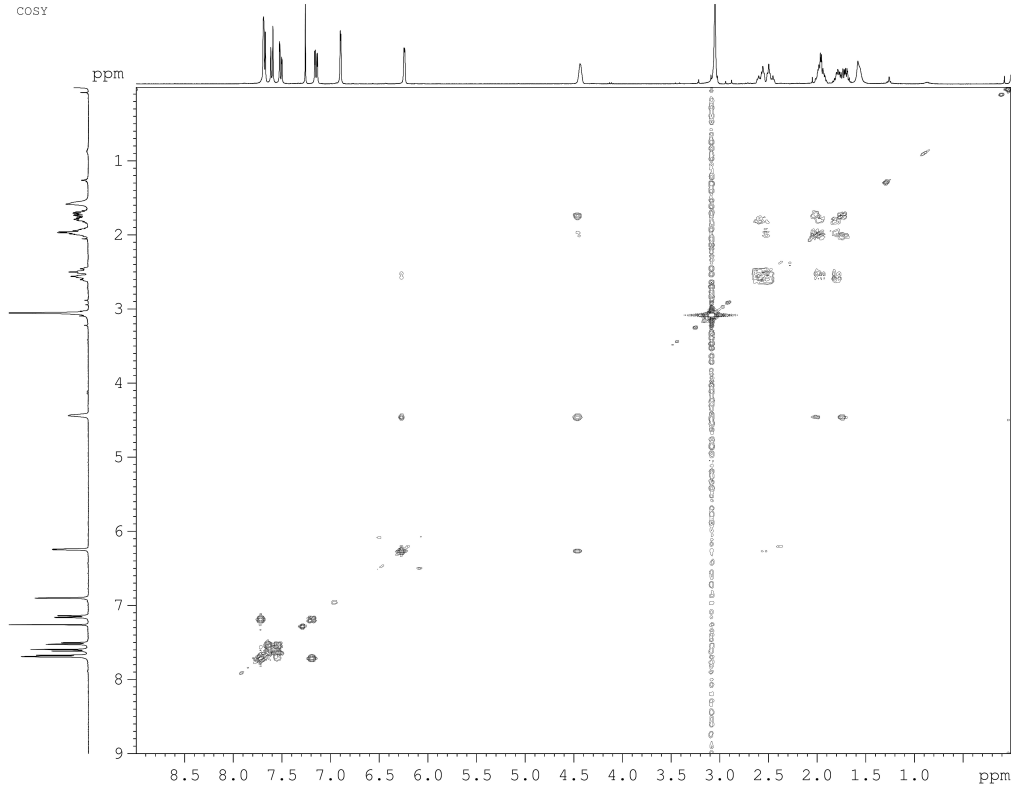
(+)-CHANA ^{1}H NMR (400 MHz, $CDCl_3$)



(+)-CHANA ^{13}C NMR (75 MHz, $CDCl_3$)



COSY (–)-CHANA



HSQC (–)-CHANA