

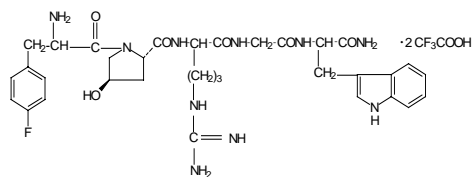
An Evaluation of the Induction Potential of INN 00835, a Pentapeptide Antidepressant, on Cytochrome P450 Isoforms 1A, 2C, 2E, and 3A in Rat and Human Hepatocytes

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INN 00835

4-Fluoro-L-phenylalanyl-L-trans-4-hydroxy-L-prolyl-L-arginylglycyl-L-tryptophanamide ditrifluoroacetate



Objective

CYP450 induction is one of the key mechanisms for pharmacokinetic drug–drug interactions (1). Freshly isolated human hepatocytes represent the most appropriate pre-clinical experimental system for the evaluation of CYP450 induction potential of xenobiotics in humans (2). This study was performed to evaluate the potential of INN 00835 to induce CYP450 isoforms 1A, 2C, 2D, 2E, and 3A in primary rat and human hepatocytes.

Methodology

Incubation Conditions and Sample Size

- All incubations conducted at 37 °C, 95% air/5% CO₂, and saturating humidity.
- Sample size was N = 3 replicates for samples incubated with INN 00835 and N = 6 for controls.

Test Article and Control Preparation

- INN 00835 solutions were prepared in incubation medium to achieve final concentrations of 1, 10, and 100 µM, each containing 1% methanol.

Positive Control (PC):

- 50 µM dexamethasone (for 3A in rat),
- 50 µM omeprazole (for 1A in human),
- 25 µM rifampin (for 2C and 3A in human),
- 1 mM 3-methylcholanthrene (for 1A in rat),

- Vehicle Control (VC): Hepatocytes incubated in 1% methanol without INN 00835.

Hepatocyte Isolation and Incubation

- Fresh hepatocytes were isolated using the two-step collagenase perfusion procedure of Li et al. (3).

- Viability, measured using Trypan blue exclusion, was 82% for rat and 92% for human.

- Isolated hepatocytes were transferred to collagen-coated 24-well plates, each well containing a cell density of 3.5 x 10⁶ viable cells in 0.5 mL of plating medium.

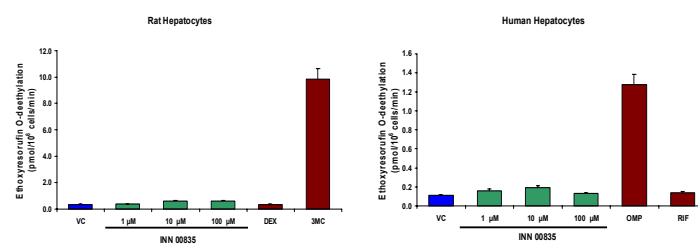
- Hepatocytes were incubated for 48 hrs then treated with INN 00835 at concentrations of 1, 10, and 100 µM or with known inducer for a total of 48 hrs.

- After 48 hrs of treatment, the dosing medium were replaced with 300 µL of Krebs-Henseleit buffer (KHB) containing the following substrates: 2 µM ethoxyresorufin with 3 mM salicylamide (CYP1A), 50 µM tolbutamide (CYP2C), 100 µM S-mephenytoin (CYP2C), 16 µM dextromethorphan (CYP2D), 300 µM chlorzoxazone (CYP2E), and 125 µM testosterone (CYP3A).

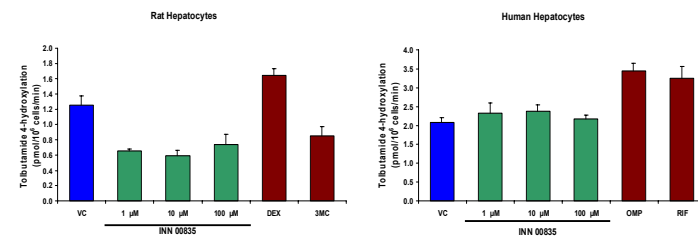
- Hepatocytes were then incubated for 2 hrs, except for the ethoxyresorufin and testosterone incubations, which were conducted for 1 hr.

Results

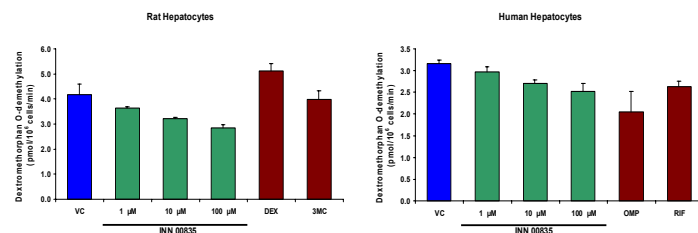
CYP 1A Induction



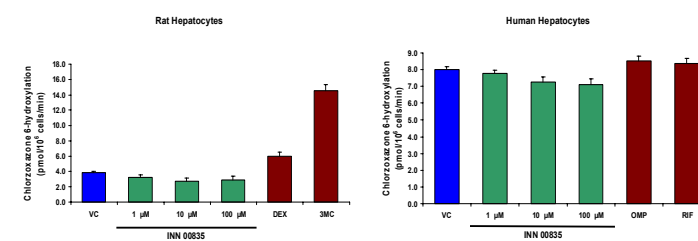
CYP 2C Induction



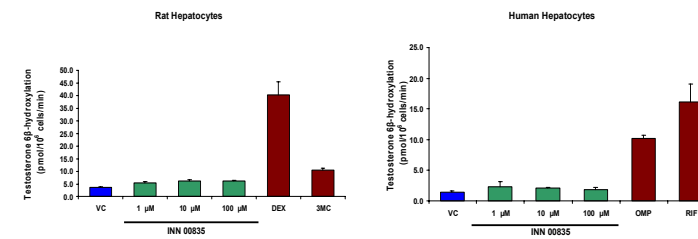
CYP 2D Induction



CYP 2E Induction



CYP 3A Induction



Abbreviations

VC — Vehicle Control

Rat Positive Controls:

DEX — Dexamethasone
3MC — 3-Methylcholanthrene

Human Positive Controls:

OMP — Omeprazole
RIF — Rifampin

Conclusions

- INN 00835 did not exhibit significant induction potential for the CYP activities studied.
- The positive controls showed the expected induction of the appropriate CYP activities.
- Freshly isolated human and rat hepatocytes represent a useful experimental system for the evaluation of CYP induction.

References

- Li, A. P. Primary hepatocyte cultures as an in vitro experimental model for the evaluation of pharmacokinetic drug–drug interactions. *Adv. Pharmacol.* **1997**, Nov, 43, 103–130.
- Li, A. P.; Maurel, P.; Gomez–Lechon, M. J.; Cheng, L. C.; Jurima–Romet, M. Preclinical evaluation of drug–drug interaction potential: Present status of the application of primary human hepatocytes in the evaluation of cytochrome P450 induction. *Chem. Biol. Interact.* **1997**, Nov, 107(1–2), 5–16.
- Li, A. P.; Roque, M. A.; Beck, D. J.; Kaminski, D. L. Isolation and culturing of hepatocytes from human liver. *J. Tiss. Culture Methods* **1992**, 14, 139–146.

INNAPHARMA

