Poster 278

In vitro studies investigating the mechanism of interaction between TMC435 and hepatic transporters

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1. Premise

- TMC435 is a potent, once-daily NS3/4A protease inhibitor in Phase IIb clinical development for the treatment of hepatitis C virus (HCV) infection in combination with pegylated interferon and ribavirin (Figure 1).

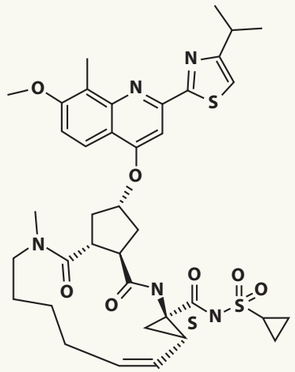


Figure 1. Structural formula of TMC435 (molecular weight, 749.96).

- In HCV-infected patients participating in two Phase IIa trials (TMC435-C201 and TMC435-C202), mild and transient increases in bilirubin (total, direct, and indirect) were observed with higher doses of TMC435.[1,2] No increases in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) were reported.
- Decreased clearance of bilirubin is the most likely cause of bilirubin elevations observed in some patients treated with TMC435. We investigated the *in vitro* effects of TMC435 on bilirubin clearance.

2. Methods

- Bilirubin clearance from the blood is a three-step process (Figure 2):
  - First, bilirubin and bilirubin conjugates are taken up by the liver cells, mainly by the organic anion transporter, OATP1B1. Next, unconjugated bilirubin is conjugated, primarily by glucuronyl transferase enzyme, UGT1A1, and then transported into the bile, mostly by the efflux transporter, MRP2.

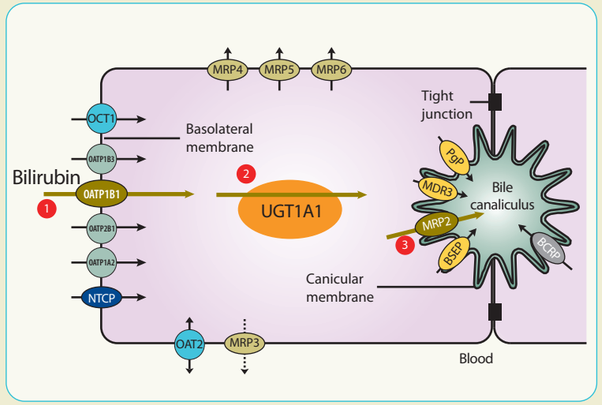
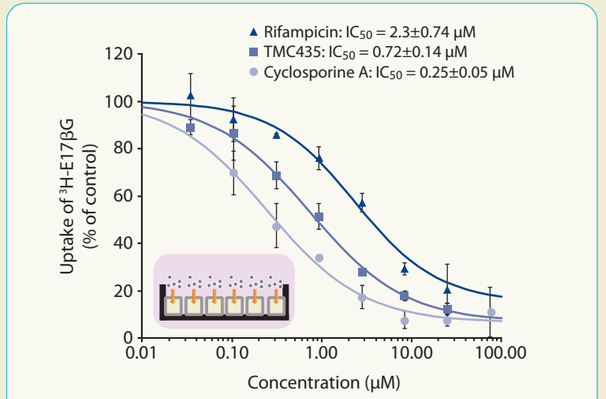


Figure 2. The three stages of bilirubin clearance from the blood (picture from Solvo and adjusted).

- The ability of TMC435 to inhibit each of these steps was investigated using mechanistic *in vitro* experiments:
  - The inhibition of OATP1B1 was tested in Chinese Hamster Ovary cells stably over-expressing this transporter. Due to the instability of bilirubin, 1 µM ^{3}H-estradiol-17β-glucuronide (^{3}H-E17βG) was chosen as the probe substrate. Two known inhibitors of OATP1B1, cyclosporine A and rifampicin, were used as reference compounds in this assay.
  - Inhibition of bilirubin conjugation was assessed using human liver microsomes.
  - The inhibition of MRP2 was tested using inside-out vesicles. Here, carboxydichlorofluorescein and ^{3}H-E17βG (data not shown) were used as probe substrates. The MRP2 inhibitor, benzbromarone, was used as a reference in this assay.

3. Results

- TMC435 inhibited OATP1B1 with a 50% inhibitory concentration (IC_{50}) of 0.72±0.14 µM (Figure 3). This compared with IC_{50} values of 0.25±0.05 µM and 2.3±0.74 µM for the known OATP1B1 inhibitors, cyclosporine A and rifampicin, respectively.



IC_{50}, 50% inhibitory concentration

Figure 3. Inhibition of OATP1B1-mediated ^{3}H-estradiol-17β-glucuronide (^{3}H-E17βG) uptake in Chinese Hamster Ovary cells.

- No inhibition of bilirubin conjugation was observed at TMC435 concentrations up to 750 µM (Figure 4). These data, which were collected at a bilirubin concentration of 0.75 µM, indicate that TMC435 does not inhibit UGT1A1.

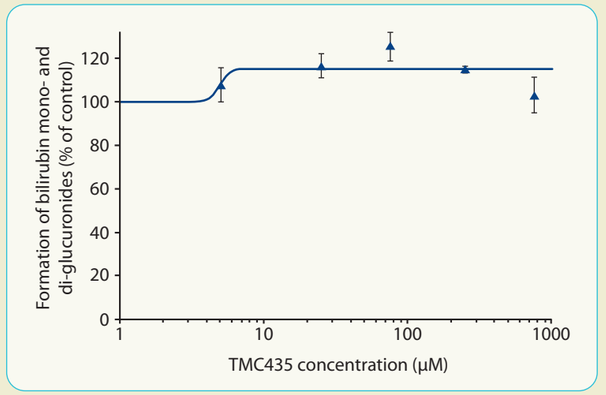
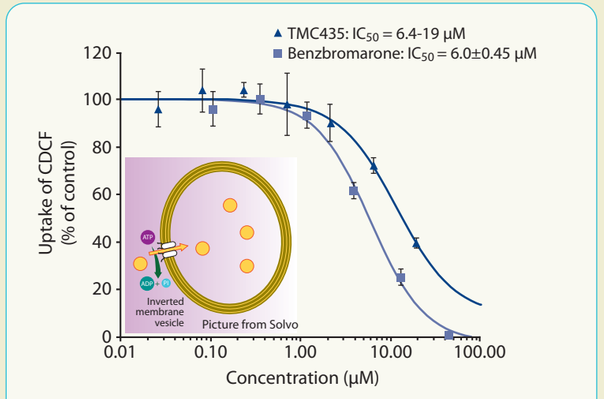


Figure 4. No inhibition of bilirubin glucuronidation in human liver microsomes.

- TMC435 inhibited MRP2 with an IC_{50} of 6.4–19.1 µM (Figure 5). This compared with an IC_{50} value of 6.0±0.45 µM for benzbromarone.



IC_{50}, 50% inhibitory concentration

Figure 5. Inhibition of MRP2-mediated carboxydichlorofluorescein (CDCF) transport.

4. Conclusions

- **TMC435 is an inhibitor of the transporters OATP1B1 (influx) and MRP2 (efflux), which may explain the mild and transient increases in bilirubin that were observed in Phase II trials, predominately with higher doses of TMC435.**
- **The affinity of TMC435 for these transporters is analogous to a drug–drug interaction with an endogenous substrate.**

5. Acknowledgments
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6. References

1. Sekar V et al. Poster presented at the 45th Annual Meeting of European Association for the Study of the Liver (EASL), Vienna, Austria, April 14-18, 2010.
2. Moreno C et al. Poster 895 at the 61st American Association for the Study of Liver Disease (AASLD) Meeting, Boston, MA, USA, October 29-November 2, 2010.