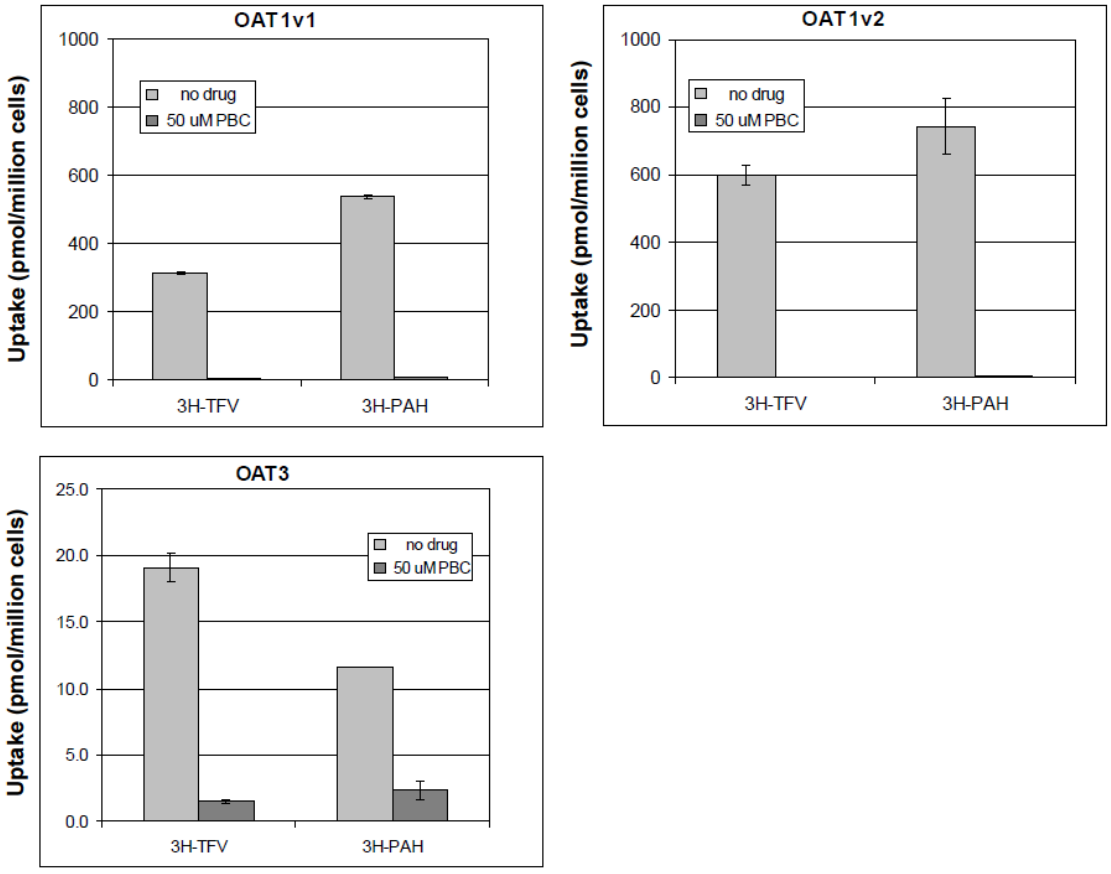
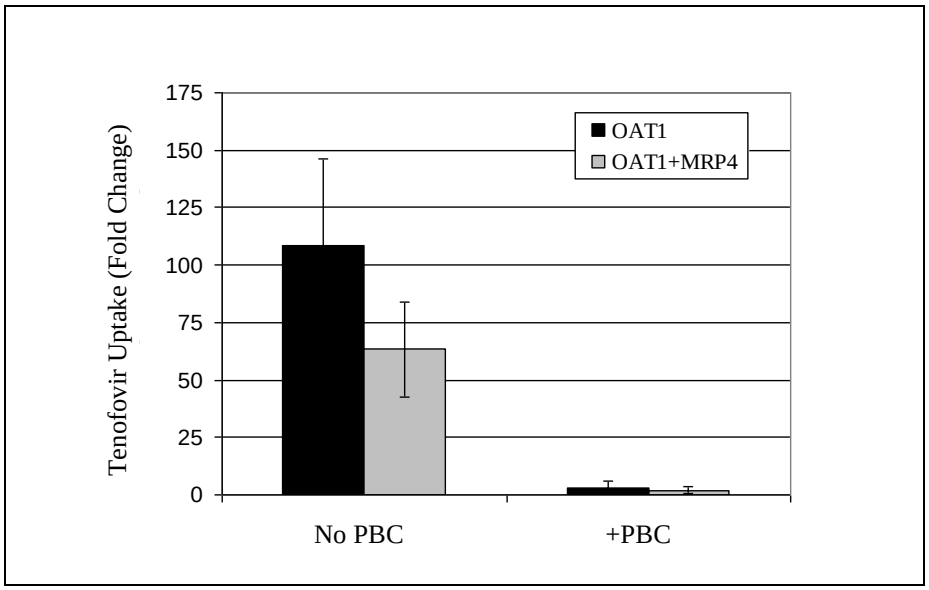
Supplementary Material

Supplemental Figure 1. Active uptake of TFV and PAH induced by transient transfection of OAT1v1, OAT1v2, and OAT3 into HEK293T cells. The accumulation of both TFV and PAH in HEK293T cells transiently transfected with empty expression vector was below the level of that in the presence of 50 μM probenecid, indicating a low endogenous anion transport activity in the parent cell line (data not shown).



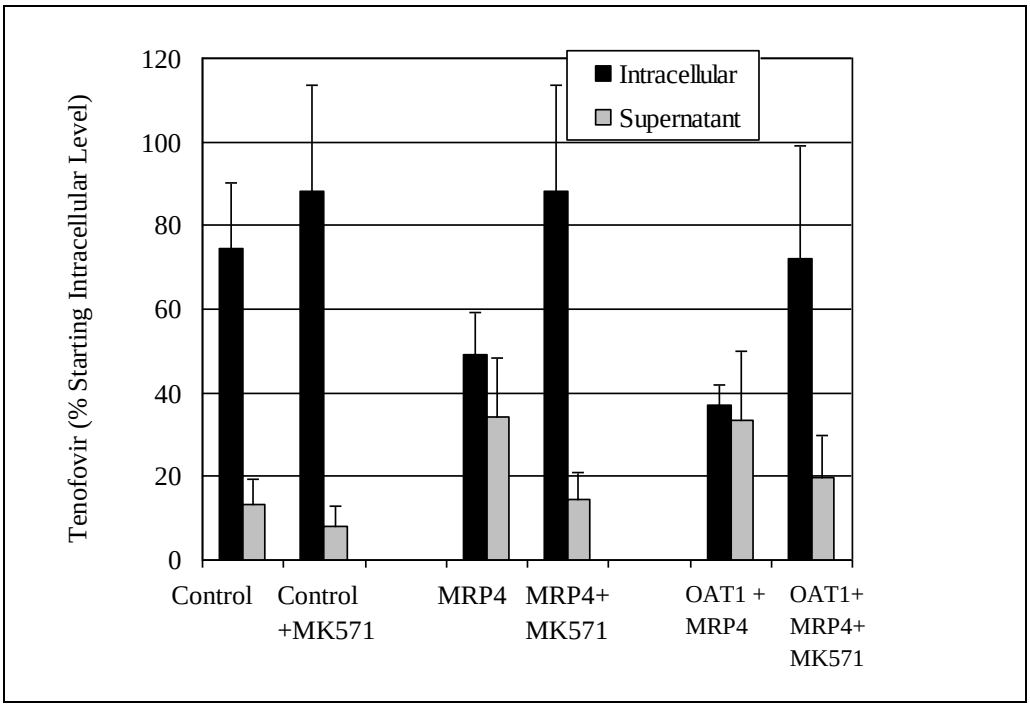
Results represent mean ± s.d. from at least 4 independent measurements. Probenecid (PBC, 50 μM) was used as inhibitor of the active uptake of TFV.

Supplemental Figure 2. OAT1-mediated uptake of TFV in HEK293T cells transiently expressing transporters. Functional expression of OAT1 in co-transfected cells was confirmed using the TFV uptake assay. The accumulation of TFV was significantly enhanced in cells expressing either OAT1 or OAT1 + MRP4 relative to corresponding control cells transformed with empty expression vector. The reduced accumulation of TFV in cells expressing both transporters as compared to OAT1 alone is attributed to the elimination of TFV from the cells via MRP4**.**



Results represent mean ± s.d. from at least 4 independent measurements. Probenecid (PBC, 50 μM) was used as inhibitor of the active uptake of TFV.

Supplemental Figure 3. MRP4-mediated efflux of TFV in HEK293T cells transiently expressing the renal anion transporters. To confirm the functional expression of MRP4, co-transfected HEK293T cells were preloaded with a prodrug [^{3}H]TFV-DF, which is known to convert to the parent nucleotide TFV intracellularly (28). Consistent with prior studies (20), MRP4 expression enhanced the elimination of TFV from cells transiently expressing MRP4 or OAT1+MRP4, as evidenced by a lower intracellular concentration and a higher extracellular level of the compound following the incubation of preloaded transporter-expressing cells relative to controls.



Results represent mean ± s.d. from at least 6 independent measurements. MK-571 (50 μM) was used as an inhibitor of the active efflux of TFV.

References

20. **Boffito M, Back DJ, Blaschke TF, Rowland M, Bertz RJ, Gerber JG, Miller V.** 2003. Protein binding in antiretroviral therapies. AIDS Res. Hum. Retroviruses **19:**825-835.

28. **van Acker BA, Koomen GC, Koopman MG, de Waart DR, Arisz L.** 1992. Creatinine clearance during cimetidine administration for measurement of glomerular filtration rate. Lancet **340:**1326-1329.