exhibited 93% of E_{max} (the response seen with 10 μM rifampicin was considered to be 100% of the E_{max}) and the corresponding EC_{50} was 1.9 μM. Ritonavir is thus confirmed as a potent PXR agonist with the potential to achieve significant activation in humans where the C_{max} is around 2 μM. Cobicistat was considerably weaker than ritonavir in activating PXR, with 15% of E_{max} detected at a concentration of 10 μM. The shapes of the curve also suggest that a 100% response with cobicistat would be difficult to achieve. Cobicistat is therefore much weaker than ritonavir as an activator of PXR and is potentially less likely to cause clinical drug–drug interactions through induction mechanisms.

Chronic treatment of HIV-infected patients with ritonavir is known to induce changes in body fat distribution (lipodystrophy), elevated cholesterol and triglycerides (hyperlipidemia) and insulin resistance, known collectively as metabolic syndrome.[8d] It is believed that some of these effects are due, at least in part, to the direct effects of ritonavir on adipocytes.[53] *In vitro*, ritonavir has been shown to affect adipocyte functions, such as lipid accumulation during differentiation and insulin-stimulated glucose uptake.[54] Therefore, cobicistat was evaluated for its effects on adipocytes in comparison with ritonavir. Atazanavir, the PI used in the clinic with the least pronounced metabolic syndrome,[55] was also evaluated as a comparator.

The lipid accumulation assay monitored normal lipid accumulation in cultured human adipocytes following induction of differentiation in the presence of tested PIs for 9 days. Ritonavir showed a clear effect, with an EC_{50} of 16 μM (Table 13.6). In contrast, both cobicistat and atazanavir exhibited no effect at concentrations up to 30 μM. The glucose uptake assay monitored insulin-stimulated glucose uptake in mouse adipocytes in the presence of 10 μM ritonavir, atazanavir or cobicistat. Ritonavir showed a pronounced effect at this concentration. In contrast, the effects on glucose uptake by cobicistat and atazanavir were significantly less. The minimal adverse effects of cobicistat in these assays suggest a lower potential for toxicity related to altered lipid metabolism compared with ritonavir.

Cobicistat greatly improved aqueous solubility compared with ritonavir under both neutral (pH 7.4: 75 *versus* $\sim 2.0\ \mu g\ mL^{-1}$) and acidic (pH 2.2: >6500 *versus* $\sim 3.1\ \mu g\ mL^{-1}$) conditions.

Both cobicistat and ritonavir exhibit poor metabolic stability in preclinical species *in vitro*. Their metabolic stability is concentration dependent and both can inhibit their own metabolism at high concentrations. The PK of cobicistat in preclinical animals is consistent with this, as high clearance is observed at low

Table 13.6 Inhibition of cell functions in adipocytes.

Compound	*Lipid accumulation: EC_{50} (μM)*	*Glucose uptake (% inhibition at 10 μM)*
Cobicistat	>30	9.5 ± 6.4
Ritonavir	16 ± 8	55 ± 10
Atazanavir	>30	0.4 ± 0.9