The bicyclohex-2-ol derivatives **57a,b**, **59a,b** and **63a,b** showed similar binding affinity for NS4B, which correlated well with the genotype 1b replicon potency (Table 5.4). However, much larger differences were observed *versus* the H94N virus. For example, three compounds (**59a**, **63a**, **63b**) were inactive against the H94N variant and (+)-**57a** exhibited low nanomolar activity. The antiviral potency of (+)-**57a** represents a vast improvement over the majority of compounds in the series, despite being about eightfold less potent than the unsubstituted analog **51**. The corresponding enantiomer (–)-**57b** also showed good replicon potency, although it was less active than (+)-isomer. As expected, removing the key H-bond acceptor (C=O) from the piperazinone ring (analogs **60a** and **60b**) resulted in a marked decrease in binding and replicon activity (EC_{50}s of 264 and 50 nM *versus* genotype 1a replicon, respectively).

The initial justification for pursuing the complex bicyclohexan-2-ol tail of was the hypothesis that introducing a hydroxyl group would improve metabolic stability. The PK profile of (+)-**57a** was examined and, consistent with the findings in the cyclohexyl series (*i.e.* **31**), the *in vivo* rat clearance of (+)-**57a** was about threefold lower than that of **51** (Cl_{rat} of 25 *versus* 70 mL min^{-1} kg^{-1}, respectively) (Table 5.5). Overall, (+)-**57a** exhibited low to moderate clearance across all four species and high bioavailability (>90%) in all species except mouse. In addition, higher doses of (+)-**57a** yielded plasma drug exposures that were sufficient to support animal safety studies without the need for a prodrug.

Using potency, metabolic stability and physicochemical properties to guide the medicinal chemistry direction, we successfully transformed our initial lead **1** into the highly optimized (+)-**57a**. The net result was a >1200-fold increase in replicon 1a and 1b activity and with only a modest inflation of MW (increase of 77 amu). Equally important was the net reduction in lipophilicity that accompanied the optimization of **1** into (+)-**57a** (cLog*P*s of 3.9 and 3.6, respectively). A number of synthetic challenges were overcome to enable the profiling of this molecule, including the development of a novel, scalable route to the pyrazolopyridine core and an asymmetric route to the cyclopentenol tail bearing four contiguous chiral centers. The high binding affinity for NS4B, potent antiviral activity against the HCV replicons, and favorable PK profile in multiple species supported the preclinical development of (+)-**57a**, the details of which will be published later.

5.5 *In Vivo* Proof of Concept

Several small molecules have previously been reported to interact directly with NS4B but no clinical or *in vivo* data have emerged to validate this target as a viable approach for treating HCV infection. Having identified a number of potent antiviral compounds with high affinity for NS4B, we aimed to demonstrate *in vivo* efficacy *via* this novel mechanism of action. The human specificity of the HCV virus has hindered the development of preclinical animal models of infection.[58] Chimpanzees are susceptible to HCV and have been used to study the disease and potential treatments;[59] however, studies in chimpanzees are undesirable for many reasons. Therefore, we focused on a model involving