Table 8.1 Comparative profiles of S- and C-linked dihydropyranone thumb pocket 2 inhibitors from Pfizer.

Property	***35*** *(X = S)*	***36*** *(X = CH_2)*
pK_a	4–5	5–7
IC_{50} (mm)	0.036	0.020
EC_{50} (mm)	3.25	0.33
Cl ($mL\,min^{-1}\,kg^{-1}$)	14.8 ± 2.6	8.3 ± 1.1
V_{SS} ($L\,kg^{-1}$)	2.0	0.3
Caco-2 AB/BA ($\times 10^6\,cm\,s^{-1}$)	3/2.3	10/6.8
F (%)	2	42

derivatives, were ascribed to a lack of permeability. These issues were resolved through modulation of the physicochemical properties and, in particular, through adjustment of the acidity of the enol moiety present in the molecules. Indeed, S-linked derivatives were found to be 10–100-fold more acidic than the corresponding C-linked analogs ($pK_a \sim 4$–5 *versus* ~ 5–7, respectively) resulting in reduced permeability and compromising *in vivo* profiles.[50] The comparative profiles of an S-linked (**35**) and corresponding C-linked (**36**) analog are shown in Table 8.1.

Simple S→C atom replacement increased cell permeability and provided a 10-fold improvement in cell-based potency while maintaining intrinsic potency in the nanomolar range, thus narrowing the IC_{50}/EC_{50} ratio. Furthermore, oral absorption of C-linked analogs was also improved to set the stage for further optimization. Introduction of *gem*-dimethylcyanomethyl and fluorine substituents on the aromatic ring, followed by evaluation of the two separate enantiomers, led to the identification of analog **37**, which was selected as the first compound from the class for preclinical evaluation.[50] Development of **37** was soon compromised, as a low-solubility crystal form that significantly reduced exposure in animal studies was identified during scale-up. Furthermore, **37** was identified as a strong CYP2D6 inhibitor ($IC_{50} = 0.3\,\mu M$), which generated concerns for potential drug–drug interaction issues during clinical development and led to a decision to resume optimization to resolve these liabilities.[51] Through a systematic SAR exploration, the culprit for CYP2D6 inhibition was soon identified as the aromatic ring, the substitution of which had a profound impact on the undesired off-target activity. In particular, it became apparent that the cyano group in **37** was likely involved in an interaction with the CYP isozyme and removal of this group or hindrance through steric interaction had a beneficial impact. Fortunately, modifications in this part of the molecule were well tolerated and compound potency was not affected. Leveraging structural information, key protein–inhibitor hydrophobic interactions were optimized. Replacement of the phenyl ring by ethyl-substituted pyridines was well tolerated and provided a basic center that decreased lipophilicity and improved solubility.

Compound **38** (filibuvir) is a potent inhibitor of HCV polymerase ($IC_{50} = 7\,nM$) and the replicon ($EC_{50} = 41\,nM$). It did not inhibit any CYP450