Abstract
As part of an ongoing effort to apply the Deuterated Chemical Entity Platform (DCE Platform)® to clinically validated drugs, several deuterated analogs of the CFTR potentiator ivacaftor (Kalydeco®) have been prepared. The devised synthetic routes allowed for site selective deuterium incorporation with high levels of isotopic purity. Due to the fact that ivacaftor was poorly metabolized in standard liver microsome assays under the conditions tested, human CYP3A4 Supersomes™ were used to assess and compare the in vitro metabolic stability of ivacaftor and the DCEs. In this manner, multiple deuterated analogs displaying marked levels of in vitro metabolic stabilization have been identified. One such analog, compound 106, exhibited a 55% increase in half life vs. ivacaftor. Synthetic routes to the individual isotopologs along with metabolic stabilization data using human CYP3A4 Supersomes™ will be presented.

Introduction
Ivacaftor undergoes extensive metabolism in humans as shown in Figure 1 (only 2.52% excreted as unchanged parent).1,2 The two major circulating metabolites, M1 and M6, exhibit significantly greater plasma exposure than ivacaftor: the ratios of AUCs for M1 and M6 to ivacaftor are 6 and 2, respectively. While M1 represents a pharmacologically active metabolite with 1/30 the potency of ivacaftor, M6 exhibits less than 1/50th the potency and is considered not active. In vitro studies have established that ivacaftor and M1 are both substrates for CYP3A4 in humans. The importance of metabolism by CYP3A4 in humans is shown by the marked drug interaction observed with ketoconazole, a strong CYP3A4 inhibitor. The approved standard dosing regimen for ivacaftor is 150 mg q12h. However, when taken concomitantly with a strong CYP3A4 inhibitor, a significant dose reduction to 150 mg twice-a-week is recommended. These metabolic properties made ivacaftor a promising candidate for optimization via our deuterium technology.

Figure 1: 14C-ivacaftor metabolism

Results

Table 1: Metabolic stability in CYP3A4 suprersomes

<table>
<thead>
<tr>
<th>Compound</th>
<th>incubant</th>
<th>t1/2 (hr)</th>
<th>t1/2 (hr)</th>
<th>AUClast (ng*hr/mL)</th>
<th>AUClast (ng*hr/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>150 mg</td>
<td>10.40 ± 1.75</td>
<td>13.24 ± 1.20</td>
<td>14.08 ± 1.32</td>
<td>14.46 ± 1.32</td>
</tr>
<tr>
<td>M6</td>
<td>150 mg</td>
<td>10.11 ± 1.21</td>
<td>13.07 ± 1.04</td>
<td>14.04 ± 1.12</td>
<td>14.40 ± 1.12</td>
</tr>
<tr>
<td>ivacaftor</td>
<td>150 mg</td>
<td>10.11 ± 1.21</td>
<td>13.07 ± 1.04</td>
<td>14.04 ± 1.12</td>
<td>14.40 ± 1.12</td>
</tr>
</tbody>
</table>

Figure 2: Crude product profile for 3b to 3c

Figure 3: Attempted synthesis of 2a

Figure 4: Metabolic stability in CYP3A4 suprersomes

Figure 5: Oral PK in rats

Figure 6: Oral PK in dogs

Conclusions
• Several deuterated analogs of the CFTR potentiator ivacaftor have been prepared via synthetic routes that allow for highly site-selective deuterium incorporation.
• The in vitro metabolic stability of ivacaftor and the DCEs was assessed in the presence of human CYP3A4-supersomes. Compounds 105, 106 and 110 exhibit significant stabilization to CYP3A4 metabolism (~50% increase in t1/2) whereas stabilization was not observed with compound 123.
• Ivacaftor, 105 and 106 were then dosed orally to both rats and dogs and plasma levels were measured out to 72 and 96 hours respectively. In rats and dogs, both DCEs were metabolically stabilized and achieved greater exposure levels than ivacaftor. In rats, compound 105 was stabilized to a greater extent and exhibited a 59% increase in AUC. Interestingly, compound 106 was stabilized to a greater extent in dogs and exhibited an 80% increase in AUC.
• Studies in rat, dog, and human hepatocytes are in progress to determine which compound holds promise for clinical development. One such compound, 106, was selected for further optimization.

Literature cited
2. Fda.gov/drugsatfda_docs/nda/2012/203188Orig1s000Ciplapp.pdf
5. Low-resolution mass spectra (LRMS) were collected on an Agilent 1100 Series LC/MSD (Column: 20mm C18-RP S-5% ACN, 0.1% HOAc or 0.1% HOAc in 5 min with a 5 min hold at 5% ACN, 0.1% HOAc or 0.1% HOAc, MSD: single quadrupole LC/MS (Agilent 6120) mass spectrometer using electrospray ionization (ESI) in positive or negative mode).
6. Using Chamber Assay performed at ChanTest

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