8  PREDICTING HUMAN ORAL ABSORPTION OF NEW CHEMICAL ENTITIES

8.1 Introduction

With the rise of combinatorial chemistry and the ability to produce large collections of individual compound sets, the number of new compounds to be characterized as potential drug candidates has increased. As a result drug design and discovery cannot have pharmacodynamic potency as the sole criterion of optimization but must also take pharmacokinetic behavior into account, absorption and distribution in particular. Many compound with good therapeutic potential fail to progress beyond the early developmental stages primarily because of insufficient oral bioavailability. Around one-third of development candidates are lost due to inappropriate pharmacokinetic properties (Prentis et al., 1988). The prediction of drug-membrane permeability is important during the lead optimization stage of drug discovery. Several factors viz. experimental difficulty, high cost and low throughput, involved in screening of lead compounds in animals for oral drug absorption have led to the development of various in vitro prediction model. The drug development process is therefore relied increasingly upon in vitro methods to screen a large number of potentially therapeutic compounds in terms of oral absorption in humans. This will be helpful in an effective lead candidate selection and its optimization in the development phase.

The BCS guidance provides recommendations for the drug of interest to be classified as either having high or low permeability. A rather high cutoff value of HOA or FA ≥ 90% is often chosen for such classification, as recommended in the biopharmaceutical classification system implemented by FDA (Amidon et al., 1995; FDA guidance, 2004). The guidance defines a drug to be highly permeable when the extent of absorption in humans is 90% or more based on a mass balance determination or in comparison to an intravenous reference dose. For example 90% absolute bioavailability or 90% administered drug recovered unchanged in urine indicate high permeability. The BCS implemented by FDA has been developed for drug development purposes and reflects a desire to identify well-absorbed compounds that can subject to a waiver in bioequivalence studies. In contrast, for permeability screening early on in the drug discovery process, it is often relevant to also identify compound with an FA significantly lower than 80-90%. Based on the literature survey,
it was observed that in many drug discovery programs FA around 30% is considered as an acceptable starting point for new chemical entities (NCEs) in early stage of drug discovery program. (Kansy et al., 1998; Matsson et al., 2005; Flaten et al., 2006).

In present work, it was aimed to generate HOA classification system to classify NCEs into low, moderate and high absorption category based on already screened known drugs on IAM column. Further to this, human oral absorption was predicted for some NCEs using established regression model based on IAM capacity factor and PSA. These predicted values were compared with respective rat oral bioavailability data in conjunction with \textit{in vitro} metabolism data generated using rat liver microsomes respectively.

8.2 Materials and methods

8.2.1 Materials

NCEs were selected from different discovery program running at Torrent Research Centre (TRC) (Gandhinagar, India). They were used in their available highest purity. Materials used to determine IAM capacity factors were as described section 4.2.1. \textit{In vitro} metabolism experiments were conducted using following reagents and materials;

- Liver microsomes – BD Biosciences, CA, USA.
- Na$_2$HPO$_4$, NaH$_2$PO$_4$ – Qualigen, Mumbai.
- MgCl$_2$ – SRL, Mumbai.
- Ethylene diamine tetra acetic acid disodium salt dehydrate (EDTA) – Sigma, Mumbai.
- Acetonitrile – Ranbaxy, New Delhi.
- Di-methyl sulphoxide (DMSO) – Sigma, Mumbai.
- Nicotinamide adenine dinucleotide phosphate reduced (NADPH) - SRL, Mumbai.

8.2.2 Isocratic determination of log $k'_{IAM}$\textsuperscript{4.5-7.4}

The log $k'_{IAM}$\textsuperscript{4.5-7.4} for each compound was determined as described in section 4.2.4 and 5.2.4 using chromatographic system described in section 4.2.2. Samples were prepared as described in section 4.2.3.
8.2.3 *In vitro* metabolism experiments

Metabolic stability studies were conducted with liver microsomes from human and rat (pooled). Data for these compounds were generated as follows. All substrates were incubated in assay buffer pH 7.4 at 37°C consisted of 100mM phosphate buffer, 5mM magnesium chloride and 1mM EDTA. The reaction mixture consisted of 1mg/ml microsomal protein, 1mg/ml NADPH and 10µM substrate containing 0.5% DMSO. Table 8.1 gives details about typical reaction set up for one reaction. Aliquots were (0.1 ml) removed from incubation at time points of 0, 5, 10, 20, 30 and 60 min. Similarly plain standards were prepared without microsomal protein (0, 30 and 60 min) and controls were prepared (0 and 60 min) without substrate (compound) to evaluate chemical stability of substrate in given condition and method specificity respectively. The reactions were terminated with 50µl of acetonitrile after each time point. After centrifugation, supernatants were analyzed using HPLC/UV to determine percent of parent drug remaining (%R) at different time points. %R was calculated as follows:

\[
%R = \left( \frac{C_t}{C_0} \right) \times 100
\]  

(8.1)

where \(C_0\) and \(C_t\) were the compound concentration in the microsomal incubation mixture at time points of 0 and 5, 10, 20, 30, 60 min respectively. The rate of compound disappearance was expressed as intrinsic clearance value (Clint, µl/min/mg of protein) using following equations:

\[
y = 100 \times \exp (-K \times x)
\]  

(8.2)

\[
T_{1/2} = \frac{0.693}{K}
\]  

(8.3)

\[
Clint = \left( \frac{0.693}{in \text{ vitro } T_{1/2}} \right) \times (\mu l \text{ incubation/mg protein})
\]  

(8.4)

Based on this Clint value compounds were classified into low, moderate and high clearance category. This classification system (Table 8.2) has been established by screening 20 known drugs from different therapeutic category at TRC. *In vitro* intrinsic clearance values for known drugs and NCEs are presented in Table 8.3.
Table 8.1. Reaction set up for in vitro metabolism study.

<table>
<thead>
<tr>
<th>Assay Component</th>
<th>Plain standard (µl)</th>
<th>Control sample (µl)</th>
<th>Test sample (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay buffer</td>
<td>80</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>NADPH</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Compound</td>
<td>10</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>5% DMSO</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Microsomes</td>
<td>-</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total volume</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 8.2. In vitro metabolism stability guide.

<table>
<thead>
<tr>
<th>Category</th>
<th>Intrinsic clearance values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human</td>
</tr>
<tr>
<td>Low clearance (Highly stable)</td>
<td>&lt;7</td>
</tr>
<tr>
<td>Moderate clearance (Moderately stable)</td>
<td>7-40</td>
</tr>
<tr>
<td>High clearance (Low stable)</td>
<td>&gt;40</td>
</tr>
</tbody>
</table>

8.2.4 In vivo studies

In vivo studies data were generated by ADME and bioanalytical laboratory of TRC. Absolute oral bioavailability of NCEs was determined following oral and intravenous administration of compounds to rats. Compounds were administered as a solution. Plasma concentrations of the parent compounds were quantitated by LC/MS/MS analysis.

8.2.5 Validation of human oral absorption predictive model for new chemical entities

When in vivo hepatic clearance is much greater than renal, biliary, and GI clearances then the rate of hepatic clearance (metabolism) is equal to systemic clearance. Under this special circumstance, one can approximate oral bioavailability with in vitro absorption and hepatic metabolism (Mandagere et al., 2002). In present work to check the reliability of HOA prediction for NCEs by established regression model, NCEs were categorized into low, moderate and high oral absorption in human by integrating the rat oral bioavailability and metabolic rate of disappearance (in vitro
intrinsic clearance). It has been reported that rat model can be used to predict oral drug absorption in the small intestine of human (Amidon et al., 1988), but not to predict drug metabolism or oral bioavailability in human (Cao et al., 2006). To check the reliability of HOA prediction model, known drugs were also screened for metabolic stability using human liver microsomes and were seen in conjunction with observed human oral bioavailability to approximate HOA and correlated with their predicted HOA from established regression model. The bioavailability data from rat and human are divided into three classes representing the low (<30%), medium (30-80%), and high (>80-100%).

Table 8.3. In vitro intrinsic clearance values for known drugs and new chemical entities.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Therapeutic area</th>
<th>Metabolic stability Clint (μl/min/mg protein)</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Indomethacin</td>
<td>Analgesic</td>
<td></td>
<td>1.94</td>
<td>1.01</td>
</tr>
<tr>
<td>2</td>
<td>Cimetidine</td>
<td>H2 antagonist</td>
<td></td>
<td>5.25</td>
<td>0.83</td>
</tr>
<tr>
<td>3</td>
<td>Verapamil</td>
<td>Calcium channel blocker</td>
<td></td>
<td>289.62</td>
<td>195.72</td>
</tr>
<tr>
<td>4</td>
<td>TRC4149</td>
<td>AGE Breaker</td>
<td></td>
<td>4.45</td>
<td>3.37</td>
</tr>
<tr>
<td>5</td>
<td>TRC8156</td>
<td>Anti-diabetic</td>
<td></td>
<td>50.67</td>
<td>24.59</td>
</tr>
<tr>
<td>6</td>
<td>TRC150094</td>
<td>Anti-Obesity</td>
<td></td>
<td>0.51</td>
<td>0.08</td>
</tr>
</tbody>
</table>

8.3 Results and Discussion

8.3.1 Human oral absorption classification

The sigmoidal relationship in Figure 6.5 (Chapter 6) was used to determine limiting log $k'_{IAM}^{4.5-7.4}$ values that divided the compounds into three classes: poorly, moderately or excellently absorbed. For the present model it could be stated that compounds with log $k'_{IAM}^{4.5-7.4}$ values < -0.3 are poorly absorbed (< 30% absorbed in vivo), compounds with log $k'_{IAM}^{4.5-7.4}$ values between -0.3 and 1.5 are moderately
absorbed (30-80% absorbed in vivo), while compounds with log $k'_{IAM}^{4.5-7.4}$ values of > 1.5 have excellent oral absorption (>80-100% in vivo absorption). Using these classification scheme 24 out of 28 drugs (86%) could be correctly predicted in their in vivo absorption ability. The four drugs for which the log $k'_{IAM}^{4.5-7.4}$ values did not lead to correct classification, were carbamazepine, furosemide, metolazone and timolol (Column No. 9, Table 6.1, Chapter 6). On the other hand drugs that are not correctly classified are found in the neighbouring class to the correct one (Figure 8.1), for instance carbamazepine and timolol those are reported to be well absorbed in vivo in human are classified as moderately absorbed according to the present model. Whereas furosemide and metolazone are reported to be moderately absorbed in vivo are classified as excellently absorbed according to the present model. In case of timolol possible reason for false classification could be diversity in intestinal absorption data in the literature ranging from 72-100% (Dollery, 1999; Sugano et al., 2001; Wohnsland and Faller 2001; Zhu et al., 2002). None of the four compounds was classified completely wrong, e.g., being poorly absorbed in humans and excellently absorbed according to our model or the opposite.

The log $k'_{IAM}$ values of three NCEs, TRC4186, TRC8156 and TRC150094 from different drug discovery projects dealing with metabolic disorders were determined at different pH values (4.5 – 7.4). Table 8.4 shows pH dependent $k'_{IAM}$ values for three NCEs. Overall TRC4186 and TRC8156 had highest retention at pH 6.5 and 7.4 respectively, whereas TRC150094 had highest retention at pH 4.5. TRC4186 is a quaternary ammonium compound, which remains ionized throughout GI tract pH gradient. The log $k'_{IAM}$ value of TRC4186 increased with increasing pH from 4.5 to 6.5, with highest log $k'_{IAM}$ obtained at pH 6.5, which is in agreement with its pKa value 5.9. The log $k'_{IAM}^{6.5}$ value -0.330 indicates low human oral absorption of TRC4186 (i.e. < 30%).

TRC8156 is a weak base, so ionization at low pH would prevent its crossing the lipid bilayer. Indeed, retention of TRC8156 on IAM column was lowest at pH 4.5. The log $k'_{IAM}$ values of TRC8156 increased with increasing pH from 4.5 – 7.4, with highest value at pH 7.4. The log $k'_{IAM}$ value at pH 6.5 is quite comparable to value obtained at pH 7.4, which is in agreement with its pKa value 6.1. The log $k'_{IAM}$ value at pH 7.4 i.e. 1.354 indicates moderate human oral absorption as per given classification scheme. TRC150094 is a weak acid, so ionization at high pH would
prevent its absorption. The retention data on IAM column suggests highest retention at lowest pH 4.5, which is expected as its pKa value is 4.3. The log $k'_{IAM}^{4.5}$ 2.224 suggests that TRC150094 is likely to be well absorbed following oral administration in humans. Indeed, the small intestine has been shown to be the primary site of absorption for TRC150094 in rats.

<table>
<thead>
<tr>
<th>Fraction absorption in humans after oral administration</th>
<th>Excellent</th>
<th>Moderately</th>
<th>Poorly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>Chlorpromazine Dexamethasone Diclofenac Diltiazem Imipramine Indomethacin Ketorolac Naproxen Propranolol Verapamil</td>
<td>Furosemide Metolazone</td>
<td></td>
</tr>
<tr>
<td>Moderately</td>
<td>Carbamazepine Timolol</td>
<td>Amiloride Atenolol Chlorothiazide Chlorthalidone Ciprofloxacin Famotidine Hydrochlorothiazide Norfloxacin Ranitidine Terbutaline</td>
<td></td>
</tr>
<tr>
<td>Poorly</td>
<td>Acyclovir Bretylium Ganciclovir Lisinopril</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 8.1.** Diagram showing the correlation of permeability classes according to percent absorbed in human and the log $k'_{IAM}^{4.5}$ values obtained from the present model. White boxes indicate perfect classification, light grey indicate classification into the neighbour class to what was expected from percent absorbed in humans while dark grey indicate complete incorrect classification.
Table 8.4. pH dependent partitioning of NCEs in IAM. PC. DD2 stationary phase.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>pH 4.5</th>
<th>pH 5.5</th>
<th>pH 6.5</th>
<th>pH 7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRC4149</td>
<td>0.292</td>
<td>0.427</td>
<td>0.468</td>
<td>0.356</td>
</tr>
<tr>
<td>TRC8156</td>
<td>3.89</td>
<td>6.47</td>
<td>17.26</td>
<td>22.58</td>
</tr>
<tr>
<td>TRC150094</td>
<td>167.42</td>
<td>90.74</td>
<td>38.08</td>
<td>25.21</td>
</tr>
</tbody>
</table>

8.3.2 Human oral absorption prediction

Using molecular size and hydrogen bonding, a fresh view can be taken on the BCS, providing a better insight into its physicochemical meaning (van de Waterbeemd, 1998). Multiple non-linear regression analysis results indicated that hydrogen bonding efficiency of a molecule in terms of PSA improved relationship between log $k'_{IAM}^{4.5-7.4}$ and HOA (Eq. 7.12, Table 7.3, Chapter 7). With this relationship, two out of four misclassified drugs (carbamazepine and furosemide) were correctly classified based on their back calculated HOA value. Nearly 96% of the drugs (from training set) could be correctly predicted in their in vivo absorption ability.

The HOA for NCEs was predicted using regression coefficients from Eq. 7.12. The physicochemical descriptors of three NCEs are mentioned in Table 8.5. Prediction model suggested 38%, 71% and 90% HOA for TRC4186, TRC8156 and TRC150094 respectively based on their log $k'_{IAM}^{4.5-7.4}$ and PSA. The observed (reported or experimental) HOA values are not available for NCEs to compare with predicted one. In absence of these data rat oral bioavailability data and microsomal stability data were used to classify compounds in low, moderate and high human oral absorption category for comparison. The estimated oral bioavailability in rat for these three NCEs is 16%, 28% and 92% respectively.

There is an obvious difference between predicted human oral absorption and rat oral bioavailability for TRC4186 and TRC8156. The metabolic stability data (Table 8.5) from rat liver microsomes suggests that TRC4186 is highly stable in microsomal environment. Despite its high stability to phase I metabolism, the reason for poor oral bioavailability in rat is the presence of the positively charged quaternary nitrogen (chemical structure is not shown under confidentiality agreement) which inhibits partitioning into the intestinal membranes. High stability in microsomal environment
and low oral bioavailability in rat suggest low absorption in rat. The metabolic stability
data from human liver microsome incubation and low oral absorption in rat implies
low absorption (<30%) in human also. The over prediction of HOA (>30 %, Table 8.6,
column 7) in case of TRC4186 could be because of low PSA. The PSA value of
TRC4186 is 100.2 Å², which suggests good oral absorption property. TRC8156 is
moderate stable in rat microsomal environment and it is having low oral
bioavailability; both these data predict moderate oral absorption in rat. The regression
model predicts moderate oral absorption in humans i.e. 71% and metabolic stability
data from human liver microsomes also supports this.

Table 8.5. Physicochemical properties of new chemical entities.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>MW a</th>
<th>pKa b</th>
<th>PSA c</th>
<th>log kIAM pH 4.5-7.4 d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HA</td>
<td>HB+</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>TRC4149</td>
<td>340</td>
<td>5.9</td>
<td>100.2</td>
<td>-0.330</td>
</tr>
<tr>
<td>2</td>
<td>TRC8156</td>
<td>438</td>
<td>6.1</td>
<td>118.2</td>
<td>1.354</td>
</tr>
<tr>
<td>4</td>
<td>TRC150094</td>
<td>328</td>
<td>4.3</td>
<td>75.5</td>
<td>2.224</td>
</tr>
</tbody>
</table>

a MW values were obtained from program Cerius² ADME module v4.10.
b pKa values were obtained from program ACD/Lab log D suite v10.0.
c PSA values were obtained from program Cerius² ADME module v4.10.
d Highest value of log kIAM among pH 4.5-7.4 determined by HPLC (n = 3, SD<0.03).

The compound TRC150094 with both the properties high partitioning value (IAM
capacity factor) and high metabolic stability, exhibits oral bioavailability of more than
90% in the rat. This may results in a similar way in humans as TRC150094 is stable
with human liver microsomes. Bioavailability is a method of obtaining absorption
data if bioavailability is high (>80%). It reflects that first pass metabolism is minimal
and almost all the absorbed drug can reach the systemic circulation. The rat oral
bioavailability data and microsomal stability data both in rat and human microsomes
indirectly confirms the reliability of oral absorption prediction in humans for all three
NCEs.
### Table 8.6. Microsomal stability, oral bioavailability and predicted %HOA of compounds in humans and rats.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>Intrinsic clearance category&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Observed oral bioavailability &lt;sup&gt;b&lt;/sup&gt; (Species)</th>
<th>Predicted %HOA&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Predicted %HOA&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Indomethacin</td>
<td>low low</td>
<td>98 (human)</td>
<td>high</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>Cimetidine</td>
<td>low low</td>
<td>71 (human)</td>
<td>moderate</td>
<td>78</td>
</tr>
<tr>
<td>3</td>
<td>Verapamil</td>
<td>high high</td>
<td>22 (human)</td>
<td>high</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>TRC4149</td>
<td>low low</td>
<td>16 (rat)</td>
<td>low</td>
<td>38</td>
</tr>
<tr>
<td>5</td>
<td>TRC8156</td>
<td>moderate moderate</td>
<td>28 (rat)</td>
<td>moderate</td>
<td>71</td>
</tr>
<tr>
<td>7</td>
<td>TRC150094</td>
<td>low low</td>
<td>92 (rat)</td>
<td>high</td>
<td>90</td>
</tr>
</tbody>
</table>

<sup>a</sup> Categorized based on stability guide mentioned in Table 8.2.

<sup>b</sup> Human oral bioavailability data were obtained from Goodman, 2001; rat oral bioavailability data were obtained from TRC.

<sup>c</sup> Predicted human oral absorption by integrating human/rat oral bioavailability and metabolic stability data from human/rat liver microsomal stability study.

<sup>d</sup> %HOA calculated using coefficients of Eq. 7.12 from Table 7.3 (Chapter 7.0).

Likewise, 95%, 78% and 95% HOA have been predicted for known drugs, indomethacin, cimetidine, and verapamil respectively (Table 8.5). The known drugs were also screened for in vitro metabolism and categorized as low, moderate and high hepatic clearance in same order. Good microsomal stability and high oral absorption in case of indomethacin results into high human oral bioavailability. While in case of cimetidine good microsomal stability and moderate oral absorption results into moderate bioavailability. Low oral bioavailability of verapamil despite its high oral absorption reflects low stability to phase I metabolism. These results confirm the hypothesis that when in vivo hepatic clearance is much greater then one can predict bioavailability with in vitro absorption and hepatic metabolism data. In present work it
has been shown that oral bioavailability data from rat with hepatic metabolism data from human & rat can predict in vivo absorption.

### 8.4 Conclusion

The results of this study using a validated IAM chromatography suggest that TRC4149 is likely to be low absorbed via transcellular passive diffusion across the human gastrointestinal tract following oral administration whereas, TRC8156 and TRC150094 are likely to be moderately and well absorbed respectively via same absorption route. The HOA prediction data for these three compounds after considering rat oral bioavailability data and liver microsomes data (rat and human) are consistent with HOA prediction values from developed regression model derived using experimental IAM index and computational value PSA. These in vitro findings demonstrate the potential predictive value of IAM for HOA prediction.

### 8.5 References


**FDA, 2004.** guidance for industry. Waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a biopharmaceutics classification system.


