The Evolution of Structure-Activity Relationships (SAR) Methodology in 21st Century Toxicity Prediction?

Falgun Shah, PhD

250th ACS National Meeting, Fall 2015, Boston, MA
role: Practice computational toxicology within WWMC

- Identify chemotypes safety risks early in the drug discovery process
- Generate potential hypothesis around specific toxicity phenotype
- Develop/validate predictive *in silico* and *in vitro* models of *in vivo* toxicity
OUTLINE

- Background: adverse safety profile and computational toxicology
- Traditional applications of structural alerts
  - Prediction of idiosyncratic toxicity
  - Prediction of mutagenicity
- Predicting complex *in vivo* organ toxicity
  - Challenges in modelling *in vivo* data
  - Role of early screening paradigm
  - Identifying *in silico* and *in vitro* knowledge gaps to improve *in vivo* risk assessment paradigm
  - Addressing knowledge gaps to improve in vivo safety prediction
- Summary
What is adverse safety profiles?

- PDE-4 inhibitors are linked to emesis and vasculitis

- hERG inhibition cause QT prolongation

Primary pharmacology

- Reactive metabolites
- Structural Alerts

Chemical structure

- Lipophilic basic compounds at risk of phospholipidosis and hepatotoxicity
- Lipophilic acidic compounds may cause uncoupling of oxidative phosphorylation

Secondary pharmacology

- Amiodarone

Origins of adverse safety profile

Physicochemical properties
ROLE OF COMPUTATIONAL TOXICOLOGIST IN TOXICITY PREDICTION

SAR/QSAR → Computational Toxicologist

In chemico
- Structural alerts
- Physicochemical Properties

In vitro
- Biochemical or cellular tox assays

Ex Vivo
- Dose
- Exposure
- Safety margin

In vivo
- Toxicity phenotype
  - e.g. Cardiotoxicity
  - Hepatotoxicity

R N
O
X
N  O
O
OUTLINE

- **Background**
- Traditional applications of structural alerts
  - Prediction of idiosyncratic toxicity
  - Prediction of mutagenicity
- Predicting complex *in vivo* organ toxicity
  - Challenges in modelling *in vivo* data
  - Role of the early screening paradigm
  - Identifying *in silico* and *in vitro* knowledge gaps to improve *in vivo* risk assessment paradigms
  - Addressing knowledge gaps
- **Summary**
STRUCTURAL ALERTS: THE STALWART OF TOXICITY PREDICTION

A common theme in drugs associated with idiosyncratic toxicity is the presence of alerts.

Some exceptions:

Stepan et al., Chem. Res. Toxicol. 2011, 24, 1345
Mitigating Structural Alerts in Drug Discovery

**Clozapine vs. Quetiapine**

Clozapine:
- **Hepatotoxicity**
- **Blood dyscrasias**
- **RM +ve**
- Daily dose = 300–900 mg

Quetiapine:
- **RM –ve**
- Daily Dose = 400 mg

**Alpidem vs. Zolpidem**

Alpidem:
- **Hepatotoxicity**
- **RM +ve**
- GSH depletor in hepatocytes
- Daily dose = 25–150 mg

Zolpidem (Ambien):
- **RM –ve**
- No GSH depletion
- Daily dose = 5–10 mg

*Stepan et al., Chem. Res. Toxicol. 2011, 24, 1345*
Should We Not Make Compounds With Structural Alerts In Drug Discovery?

Top 4 best selling drugs in USA in 2009 contains structural alerts

1. Atorvastatin (Lipitor®)
   Dose=10-80mg

2. Esomeprazole (Nexium®)
   Dose=20-40mg

3. Clopidogrel (Plavix®)
   Dose=75mg

4. Fluticasone (Adavir®)
   0.5mg (inhal.)

Amoxicillin
β-lactam intrinsically electrophilic
Covalent modification of target
Daily dose = 2000 mg

Things to keep in mind:
- In any given instance, although a reactive metabolite *may* not be a bad thing, it will not offer any benefit (unless the RM is required for the MAO, see e.g., Amoxicilllin).
- Other factors of influence, such as daily dose; chronic vs. acute use; target population; medical need. Need to act early, during drug design.
OUTLINE

- **Background**

- Traditional applications of structural alerts
  - Prediction of idiosyncratic toxicity
  - Prediction of mutagenicity

- Predicting complex *in vivo* organ toxicity
  - Challenges in modelling *in vivo* data
  - Role of early screening paradigm
  - Identifying *in silico* and *in vitro* knowledge gaps to improve *in vivo* risk assessment paradigms
  - Addressing knowledge gaps

- **Summary**
SIMPLE MECHANISTIC MODELS TO PREDICT MUTAGENICITY OF AROMATIC AMINES

- General mechanism involves formation of reactive intermediates:

  - **Phase I Metabolism**
    - Oxidation
  
  - **Phase II Metabolism**
    - Sulphonation
    - Acetylation

  - **Nitrenium ion formation**
WHAT FEATURES DRIVE AROMATIC AMINE MUTAGENICITY?

- Not all aromatic amines are mutagenic.
- The energy of theHighest Occupied Molecular Orbital (HOMO) reflects the ability of amine to be oxidized by Cytochrome P450s.
- Mutagenicity correlates strongly with increasing HOMO energy.

Electron donating groups (SR, OR, NR2) and Electron withdrawing groups (F, CF3, C=OR, SO3R) are indicated in the density plot.
MISPREDICTED COMPOUNDS

- Despite high predictive performance, HOMO energy does not describe mutagenic activity of all chemical space.
- These compounds require additional features within the model to describe their activity.
- Should be investigated if we are to fully understand the predictive performance of the model.
- Understanding the applicability of a model is essential to understand the relevance of external validation statistics.

![Chemical Structures](image)

1. Mutagenic\(^1\) HOMO = -9.27 eV
2. Mutagenic\(^2\) HOMO = -8.55 eV

The Truth Tables

<table>
<thead>
<tr>
<th>HOMO POS</th>
<th>Ames POS</th>
<th>Ames NEG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP</td>
<td>FP</td>
</tr>
<tr>
<td>HOMO NEG</td>
<td>FN</td>
<td>TN</td>
</tr>
</tbody>
</table>
The Real Value of Truth Tables

<table>
<thead>
<tr>
<th></th>
<th>Ames POS</th>
<th>Ames NEG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HOMO POS</strong></td>
<td>TP</td>
<td>FP</td>
</tr>
<tr>
<td>Correlative</td>
<td></td>
<td>1) Steric crowding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2) Cyp-deactivation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3) Diverted metabolism</td>
</tr>
<tr>
<td><strong>HOMO NEG</strong></td>
<td>FN</td>
<td>TN</td>
</tr>
<tr>
<td>1) Another mechanism</td>
<td></td>
<td>useful in SAR determination</td>
</tr>
<tr>
<td>2) Impurity?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Focused testing in the future must prioritize these areas.
OUTLINE

- Background
- Traditional applications of structural alerts
  - Prediction of idiosyncratic toxicity
  - Prediction of mutagenicity
- Predicting complex *in vivo* organ toxicity
  - Challenges in modelling *in vivo* data
  - Role of Early screening paradigm
  - Identifying *in silico* and *in vitro* knowledge gaps to improve *in vivo* risk assessment paradigms
  - Addressing knowledge gaps
- Summary
CAN WE PREDICT IN VIVO TOXICITY?

- Yes - all compounds are toxic
- Calculated human LD50 values:
  - Water – 6 liters
  - Caffeine – 118 coffees
  - Alcohol – 13 shots

- Focus on toxicity observed at therapeutically relevant levels

---

^1http://www.compoundchem.com/2014/07/27/lethaldoses/
What Drives *in vivo* Toxicity?

- **Exposure**
  - ABV\(^1\) 2-12%  9-16%  35-50%

- **Toxic Potential**

![agaritine molecule](image)

\(^1\)https://en.wikipedia.org/wiki/Alcohol_by_volume
EXAMPLE - NEFAZODONE

- Potent 5-HT$_{2A}$ receptor antagonist and antidepressant
- Withdrawn in 2003 owing to very rare, but severe, liver toxicity
- Has multiple safety liabilities
  - Contains structural alert (aniline)$^1$
  - Metabolic liabilities$^2$
  - Inhibitor of bile-salt export pump$^3$
  - Cytotoxic$^4$
  - Mitochondrial dysfunction$^4$
  - Max daily dose: 600mg/day

Refs
2. Kalgutkar et al., Drug Metab. Disp., 2005, 33, 243-253
WHAT ABOUT ARIPIPRAZOLE?

- Structurally similar, yet successfully marketed drug (D2 antagonist)
  - **No** reports of acute hepatotoxicity
- Has multiple liabilities
  - Contains structural alerts (aniline, acetanilide)\(^1\)
  - Metabolic liabilities\(^2\)
  - Cytotoxic\(^3\)
  - Mitochondrial dysfunction\(^4\)
  - Low dose: 10-20 mg/day
- Why is aripiprazole not hepatotoxic?
  - Related to different pharmacological profile?
  - Different metabolic profile?
  - Low dose?
  - Better ADME profile?

---

**Refs**
4. Internal data (unpublished)
Challenges of Modelling *In Vivo* Toxicology Data

- **Nefazodone**
  - Dose = 400-600 mg/day
  - ‘hepatotoxic’
  - Non-Hepatotoxic at 20mg/day?
  - Can we predict:

- **Aripiprazole**
  - Dose = 10-30 mg/day
  - ‘non-hepatotoxic’
  - Hepatotoxic at 400 mg/day?
  - Can we predict:
Challenges of Validating *In Silico/In Vitro* Models

- **Nefazodone**
  - Aniline structural alert for hepatotoxicity?
    - True positive ✓
  - Cytotoxicity assay
    - True positive ✓
  - Mitochondrial dysfunction
    - True positive ✓

- **Aripiprazole**
  - Aniline structural alert for hepatotoxicity?
    - False positive ✗
  - Cytotoxicity assay
    - False positive ✗
  - Mitochondrial dysfunction
    - False positive ✗
DO SIMILAR COMPOUNDS HAVE SIMILAR TOXICOLOGICAL PROFILES?

- Similar compounds with a similar *in vitro* assay profile may not express similar *in vivo* findings...

**Exposure?**

<table>
<thead>
<tr>
<th>Glycine transporter-1 inhibitors (Sanofi)</th>
<th>Matrix metalloprotease inhibitors (Pfizer)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Glycine transporter-1 inhibitor" /></td>
<td><img src="image" alt="Matrix metalloprotease inhibitor" /></td>
</tr>
<tr>
<td>TX006223</td>
<td>TX006158, MMP-2 inhibitor</td>
</tr>
<tr>
<td>in vivo findings: No Uterus malformation or Skeletal muscle myopathies</td>
<td>in vivo findings: No findings in testis</td>
</tr>
<tr>
<td>chem sim=0.77, biol sim=0.65</td>
<td>chem sim=0.86, biol sim=0.77</td>
</tr>
<tr>
<td><img src="image" alt="Uterus malformation, skeletal muscle myopathies" /></td>
<td><img src="image" alt="Severe testicular degeneration" /></td>
</tr>
</tbody>
</table>

**ADME?**

*Shah F et. al., Chem. Res. Tox., 2014, 27, 86-98*
n=125 known drugs; 70=most-DILI concern; 55=no-DILI-concern

 VALIDATING MODELS OF *IN VIVO* DATA

For *in vivo* endpoints, performance is dependent upon:
- Appropriate annotation of toxicological data
- Incorporation of exposure and ADME profile

<table>
<thead>
<tr>
<th>Without Plasma $C_{\text{max, total}}$ Exposure</th>
<th>With Plasma $C_{\text{max, total}}$ Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepatotoxic</strong></td>
<td><strong>non-hepatotoxic</strong></td>
</tr>
<tr>
<td>Cytotoxicity assay Positive (&lt;100 µM)</td>
<td>True POS</td>
</tr>
<tr>
<td>Cytotoxicity assay Negative (&gt;100 µM)</td>
<td>False NEG</td>
</tr>
</tbody>
</table>

Low Sensitivity (39%) and specificity (65%)

| Cytotoxicity assay Positive (>1 µM & <100 µM) | True POS | False POS | 19 | 2 |
| Diff. mech.? | True NEG | False NEG | 51 | 53 |

Low Sensitivity (27%) but high specificity (96%)
MULTIFACTORIAL NATURE OF HEPATOTOXICITY

- Platform of assays developed to identify risk of idiosyncratic drug reactions for 36 compounds with liver toxicity profiles.

Integrated risk score:
- Able to differentiate 27 idiosyncratic liver toxicants from 7 of the 9 clean compounds.

Thompson et al; Chem. Res. Toxicol., 2012, 25 (8), 1616
OUTLINE

- Background
- Traditional applications of structural alerts
  - Prediction of idiosyncratic toxicity
  - Prediction of mutagenicity
- Predicting complex *in vivo* organ toxicity
  - Challenges in modelling *in vivo* data
  - Role of Early screening paradigm
  - Identifying *in silico* and *in vitro* knowledge gaps to improve *in vivo* risk assessment paradigms
  - Addressing knowledge gaps
- Summary
The Role of Early Screening Paradigms

- *in vivo* Toxicology is complex to predict

- Identify potential safety risk early using *in silico* and *in vitro* models

- SAR-out in vitro/in silico liabilities early-on.

- Recognize that *in vitro-in vivo* translation may not be possible without in-depth, costly, exposure-related studies
IMPROVING *in vivo* TOXICITY PREDICTION

- Broad toxicity mechanisms
- Compound-specific pharmacology

**in vitro**/**in silico** models

Computational Toxicology

Investigative Toxicology

**in vivo** toxicology data
Improving in vivo toxicity prediction

1. Sizable dataset
2. Effective annotation
3. Identify knowledge gaps

- Broad toxicity mechanism
- Compound-specific pharmacology
- Computational Toxicology
- Investigative Toxicology
- in vitro/ in silico models
- in vivo toxicology data

Pfizer
Worldwide Research & Development
Medicinal Chemistry
OUTLINE

- Background
- Traditional applications of structural alerts
  - Prediction of idiosyncratic toxicity
  - Prediction of mutagenicity
- Predicting complex \textit{in vivo} organ toxicity
  - Challenges in modelling \textit{in vivo} data
  - Role of Early screening paradigm
  - Identifying \textit{in silico} and \textit{in vitro} knowledge gaps to improve \textit{in vivo} risk assessment paradigms
  - Addressing knowledge gaps
- Summary
FEATURES THAT ARE PREDICTIVE OF *IN VIVO* TOXICITY

- **Study 1: 207 preclinical candidates investigated**
  - Compounds were annotated against the observation of any *in vivo* toxicity findings at 10µM (total plasma exposure)
  - Odds of toxicity established for various physicochemical properties

<table>
<thead>
<tr>
<th>TPSA and ClogP are calculated measures of lipophilicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPSA@10µM</td>
</tr>
<tr>
<td>ClogP&lt;3</td>
</tr>
<tr>
<td>ClogP&gt;3</td>
</tr>
</tbody>
</table>

≥ 6-times

⇒ **Study conclusions: likelihood of toxicity increases with lipophilicity**

FEATURES THAT ARE PREDICTIVE OF IN VIVO TOXICITY

Study 2: Odds of failure in preclinical/Phase 1 studies

- Authors found that compounds were more likely to fail due to toxicity in non-lipophilic space:

![Graph showing toxicity odds for different ClogP and TPSA values for non-lipophilic and lipophilic compounds.]

Hughes training set is dominated by lipophilic basic drugs

Lipophilic basic drugs cause general toxicity, e.g. through lysosomal dysfunction and disruption of ion channels

What factors drive toxicity of neutral +acidic compounds?
All models are useful… but only for a portion compounds in the training set.

It is essential to understand in which chemical space the model works and where it doesn’t.

Ximelagatran

- ClogP = 2.0 ✓ - Hughes
- PSA = 146 ✗ - Muthas
OUTLINE

- Background
- Traditional applications of structural alerts
  - Prediction of idiosyncratic toxicity
  - Prediction of mutagenicity
- Predicting complex *in vivo* organ toxicity
  - Challenges in modelling *in vivo* data
  - Role of Early screening paradigm
  - Identifying *in silico* and *in vitro* knowledge gaps to improve *in vivo* risk assessment paradigms
  - Addressing knowledge gaps
- Summary
## Gaps for Predictive Tools for Acid/Neutral Space

<table>
<thead>
<tr>
<th>Toxic @10 µM</th>
<th>Acid</th>
<th>Base</th>
<th>Neutral</th>
<th>Total (n=446)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean: Toxic</td>
<td>30:26</td>
<td>33:144</td>
<td>60:153</td>
<td>123:323</td>
</tr>
</tbody>
</table>

Combined criteria worked well for basic space but not for neutrals.
Outline

- Background
- Traditional applications of structural alerts
  - Prediction of idiosyncratic toxicity
  - Prediction of mutagenicity
- Predicting complex *in vivo* organ toxicity
  - Challenges in modelling *in vivo* data
  - Role of Early screening paradigm
  - Identifying *in silico* and *in vitro* knowledge gaps to improve *in vivo* risk assessment paradigms
  - Addressing *In Vitro/In Silico* knowledge gaps
- Summary
TOXICITY AND ACIDIC COMPOUNDS

- Acidic compounds tend to have low cytotoxicity in cytotoxicity assays.
- Acids tend to be highly protein bound.
- Hypothesis: Is toxicity mitigated by high protein binding to assay serum?

- The impact of this result is not clear without an assessment of the toxicological and ADME profile of compounds in the dataset.

Kiyota et al., The Toxicologist (SOT), 2015
74% marketed CNS drugs and 60% Pfizer CNS clinical candidates have MPO score of 4 and above.

A relationship between an increasing CNS MPO score and alignment of key *in vitro* ADME and safety parameters was also noted.

Pfizer’s Neuroscience research unit utilizes CNS MPO score prospectively to accelerate the identification of CNS compounds with increased probability of clinical success.

WOULD WE NOMINATE THIS COMPOUND TODAY?

EC$_{50}$ = 0.72 μM (HepG2 transfected)

BSEP IC$_{50}$ = 9.1 μM

Cytotoxicity
THLE IC$_{50}$ : 78 μM

Mitochondrial Dysfunction
Uncoupler 22.9 nmol/mg
Inhibitor: 55 nmol/mg

Lipophilic
(clogP = 5.6)

Max Dose: 400-800 mg/day

- Withdrawn in UK after just 12 months on the market in December 1997 for severe liver toxicity, withdrawn in the USA in 2000.
OUTLINE

○ Background
○ Traditional applications of structural alerts
  ➢ Prediction of idiosyncratic toxicity
  ➢ Prediction of mutagenicity
○ Predicting complex in vivo organ toxicity
  ➢ Challenges in modelling in vivo data
  ➢ Role of Early screening paradigm
  ➢ Identifying in silico and in vitro knowledge gaps to improve in vivo risk assessment paradigms
  ➢ Addressing In Vitro/In Silico knowledge gaps
○ Summary
SUMMARY

- **Computational Toxicology**: essential to develop effective risk assessment strategies and models via SAR and QSAR.
- **Structural Alerts**: Avoid them!
- **Know FPs, and FNs**: understand applicability domain of model
- **in vivo toxicology** is complex: careful annotations of **in vivo** toxicology data, exposure assessments and ADME considerations are required.
- Recognize the utility and limitations of current predictive tools
  - **in silico**
  - Identify toxicological knowledge gaps
  - **in vitro**
- Resources needs to be focused on addressing **in silico tools/in vitro assays** gaps.
SUMMARY

Your TI (and dose!) is not a certainty

- Why risk a safety liability?
- Find *productive* chemistry space early
ACKNOWLEDGEMENTS

- Russell Naven
- Compound Safety Prediction colleagues at Pfizer WWMC
- Robert Stanton