Predicting the Toxic Potential of Drugs and Chemicals In Silico

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Summary
Based on the 3D structure of the target protein (ERαβ, AR, PPARγ, TRαβ, GR; CYP3A4) or a surrogate thereof (AhR), the Biographics Laboratory 3R has generated a series of virtual test kits and validated them against 693 compounds. In a pilot project (ToxDataBase), both existing and new drugs or environmental chemicals can be screened for their endocrine-disrupting potential or the probability to trigger drug-drug interactions in silico. After peer testing (2007–8), it is planned to make the database available on the Internet.

Keywords: in silico, QSAR, toxicity, receptor, REACH, ToxDataBase, Quasar, Raptor

Quantifying the binding of a drug or chemical to a target protein in silico
In the past decade, computer-based (in silico) concepts matured into powerful tools for simulating and quantifying biochemical processes at the molecular level. This became possible due to over 40,000 protein structures known at atomic resolution, a more detailed understanding of the forces governing molecular interactions and the nowadays available computing power. The philosophy of structure-based design is based on the lock-and-key analogon — recognized as early as 1894 by Emil Fischer (Nobel Laureate, 1902) — the three-dimensional complementarity of drug and its target receptor or enzyme. In absence of structural information on the target protein, receptor-mapping technologies were developed allowing to construct 3D surrogates of the binding pocket. In a QSAR context, those can act as substitutes for the structure of the true biological receptor.

Poor pharmacokinetics, side effects and compound toxicity are not only frequent causes of late-stage failures in drug development but also a source for unnecessary animal tests. In silico methods are nowadays routinely used in the early stages of drug development. In the context of the REACH (Registration, Evaluation and Authorization of Chemicals) initiative of the European Union, computer-based experiments have received additional attention as they can predict the toxic potential of existing and hypothetical compounds. In silico techniques are fast, reproducible, and are typically based on human bioregulators, making the question of data transferability between species obsolete.

Reception of chemicals at biological relevant structures
Nuclear receptors are an important protein class in living organisms. They comprise a family of ligand-dependent transcription factors that transform extra- and intracellular signals into cellular responses by triggering the transcription of target genes. In particular, they mediate the effects of hormones (ligand) and hormonally active compounds (endocrine disruptors). Nuclear receptors are specific for the various steroid hormones, e.g. the estrogens (ER), androgens (AR), progestones, and glucocorticoids. A number of receptor-mediated adverse effects by xenobiotics have been identified in the past. This includes toxicity mediated by the thyroid hormone receptor, the epidermal growth factor and aryl hydrocarbon receptor (AhR). The concern about chemicals which bind to these receptors and induce adverse, uncontrolled effects has created a need to both screen and monitor compounds before they are further developed as potential drugs or manufactured or released into our environment. At the Biographics Laboratory 3R, we have developed and validated a series of virtual test kits for the AhR, ERαβ, AR, PPARγ, TRαβ, and the GR. Models for the pregnan-X (PXR) and mineralocorticoid receptor are in preparation (Fig. 1).

Metabolic transformation
Competition of drugs for metabolism at Cytochrome P450 3A4 (CYP3A4) may result in undesired drug-drug interactions in patients. In addition CYP3A4 might transform chemicals into reactive metabolites. The development of a computational model to accurately predict the docking potential of a diverse set of ligands was based on the X-ray crystal structure of the human CYP3A4 enzyme and a total of 48 structurally diverse

www.forschung3r.ch/en/projects/pr_75_00.html
Fig. 1: Flowchart of the virtual laboratory with five receptor surrogates (estrogen, androgen, thyroid, and aryl hydrocarbon receptor and cytochrome P450 3A4) shown. The hypothetical test compound (a dibenzofuran) is submitted via the Internet to a central server, which schedules the processors with the various receptor surrogates. No significant binding affinities are calculated for the estrogen and androgen receptor and CYP3A4 (IC50 > 0.1 mM); against PPARγ, an IC50 of 58 µM is calculated. The interaction with the aryl hydrocarbon receptor (IC50 = 8 nM) indicates a high toxic potential of the compound; in this test example, the compound should consequently be removed from the evaluation pipeline at this point.

<table>
<thead>
<tr>
<th>System</th>
<th># of compounds</th>
<th>q²</th>
<th>rms training</th>
<th>max. training</th>
<th>p²</th>
<th>rms test</th>
<th>max. test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aryl hydrocarbon</td>
<td>105 + 35 = 140</td>
<td>0.782</td>
<td>2.2</td>
<td>15.8</td>
<td>0.766</td>
<td>2.2</td>
<td>12.5</td>
</tr>
<tr>
<td>Estrogen α</td>
<td>80 + 26 = 103</td>
<td>0.895</td>
<td>2.0</td>
<td>8.6</td>
<td>0.892</td>
<td>2.9</td>
<td>9.5</td>
</tr>
<tr>
<td>Estrogen α*</td>
<td>80 + 23 = 106</td>
<td>0.787</td>
<td>0.9</td>
<td>2.5</td>
<td>0.505</td>
<td>1.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Estrogen β</td>
<td>80 + 23 = 106</td>
<td>0.703</td>
<td>1.4</td>
<td>6.7</td>
<td>0.561</td>
<td>1.9</td>
<td>5.7</td>
</tr>
<tr>
<td>Androgen</td>
<td>88 + 26 = 114</td>
<td>0.858</td>
<td>1.7</td>
<td>7.8</td>
<td>0.792</td>
<td>1.6</td>
<td>13.9</td>
</tr>
<tr>
<td>Thyroid α</td>
<td>64 + 18 = 82</td>
<td>0.919</td>
<td>1.8</td>
<td>4.3</td>
<td>0.814</td>
<td>2.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Thyroid β</td>
<td></td>
<td>0.909</td>
<td>2.0</td>
<td>7.7</td>
<td>0.796</td>
<td>2.7</td>
<td>8.8</td>
</tr>
<tr>
<td>PPARγ</td>
<td>75 + 20 = 95</td>
<td>0.832</td>
<td>1.4</td>
<td>6.2</td>
<td>0.723</td>
<td>1.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Glucocorticoid**</td>
<td>80 + 30 = 110</td>
<td>0.743</td>
<td>1.2</td>
<td>6.1</td>
<td>0.623</td>
<td>2.3</td>
<td>6.1</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>38 + 10 = 48</td>
<td>0.825</td>
<td>2.7</td>
<td>7.0</td>
<td>0.659</td>
<td>3.8</td>
<td>7.1</td>
</tr>
</tbody>
</table>

q² = cross-validated r², p² = predictive r²; the rms and maximal deviation from the experimental binding affinity is given as factor in Ki or IC50.

* model under development (ongoing PhD thesis); different compounds than for the 80/26 model above
** model under development (ongoing PhD thesis)
molecules (39 training and 9 test compounds). The results are
given in Table 1 and were used to validate the predictability. A
model for CYP2A13 is in preparation.

**Construction of receptors**

Model construction and optimization was achieved by combin-
ing protein and receptor modeling. First, the binding mode of
each investigated compound was identified using the 3D struc-
ture of the target protein (for the Ah receptor, where no experi-
mental structure is available, we used 4D pharmacophore
generation instead) by automated, flexible docking combined
with dynamic solvation of the binding pocket. Typically, the four
lowest-energy orientations were composed in a 4D data set. In
contrast to a 3D representation, each compound can be repre-
sented as an ensemble of conformations, orientations, protona-
tion states, tautomers and stereoisomers (Tab. 2). This ligand
superposition (the binding hypothesis) is then used for our multi-
dimensional QSAR technologies named Quasar and Raptor. It is
based on a quasi-atomic model representation and explicitly
allows for induced fit – the ligand-induced adaptation of the
topology of the macromolecule (see, for example, www.
biograf.ch and www.modeling.unibas.ch). Next, the models are
validated using test compounds (ligands different from those in
the training set), scramble testing and consensus scoring.

**Validation of the virtual test kits**

Vedani et al. (2006) gives details of model construction and val-
ification. For generating a model a series of compounds (training
set) is needed for which experimental binding affinities (Ki or
IC\textsubscript{50} values are available). The quality of the reproduction
of these values is reflected by the $q^2$ value – the cross-validated $r^2$.
Next, a series of ligands different from those of the training set
(test set) is used to validate the model. The $p^2$ (the predictive $r^2$)
value indicates the predictive power of the model. The predic-
tivity can be given as $100 \times \sqrt{p^2}$ – e.g. 87.5% for the AhR.

**Testing via Internet**

The Biographics Laboratory 3R is presently implementing an
Internet database for the screening of adverse effects triggered
by drugs and chemicals in silico. The bioregulators described so
far in this account (AhR, ER\textsubscript{β}, AR, PPAR\textgamma, TR\textsubscript{β}, GR and
CYP3A4) represent the backbone of this Internet Database;
PXR, MCR and 2A13 are in preparation. Within this framework,
hypothetical or existing compounds can now be tested for their
activity towards the various virtual test kits (Fig. 1) and their
toxic potential may be estimated therefrom. Adverse effects
mediated by receptors other than those compiled in the database
can, of course, not be identified. Accordingly, the present
approach based on receptor modeling will result in the produc-
tion of false-negative results for classes of toxic chemicals
which do not interact via receptor or which interact via so far
unknown receptor-based pathways. Therefore, QSAR technolo-
gies may be used to identify the harmful potential of a drug or
chemical and no false positives are produced. However, they are
not (yet) suited to prove its harmlessness.

**Outlook**

Up to date, our concept has not produced any false-positive
results. At the current level, however, false-negative predictions
are still obtained, as a compound of interest cannot be tested
against all potential receptors it may bind to in vivo. Some
macromolecular targets will remain unknown, for others no
experimental structure exists or too few affinity data are avail-
able (prerequisites for a QSAR study). We are therefore extend-
ing the current concept by implementing a set of virtual filters,
which can recognize benign compounds. These filters are based
on criteria such as the molecular weight, drug-like properties,
and the presence or absence of characteristic structural motifs.
After successful completion of a peer testing, it is planned to
make the database – along with all supporting software – freely
available to universities, hospitals, governmental agencies and
regulatory bodies worldwide.

**3R relevance**

The envisioned Internet laboratory and the already functional
virtual test kits can contribute to a significant reduction in ani-
mal testing. In drug development, it allows for an early – even
before compound synthesis – recognition of potentially harmful
substances. By removing those candidate substances from the
evaluation pipeline, they will not be forwarded to any in vivo
toxicity tests. These expectations are supported by the fact that

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Method</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1D-QSAR</td>
<td>Affinity correlates with pKa, logP, electronic properties, etc.</td>
<td>no</td>
</tr>
<tr>
<td>2D-QSAR</td>
<td>Affinity correlates with structural patterns (connectivity, 2D pharmacophore)</td>
<td>no</td>
</tr>
<tr>
<td>3D-QSAR</td>
<td>Affinity correlates with the three-dimensional structure of the ligands</td>
<td>possible</td>
</tr>
<tr>
<td>4D-QSAR</td>
<td>Ligands are represented as an ensemble of conformers, orientations</td>
<td>typical</td>
</tr>
<tr>
<td>5D-QSAR</td>
<td>as 4D-QSAR + representation of different induced-fit models</td>
<td>yes</td>
</tr>
<tr>
<td>6D-QSAR</td>
<td>as 5D-QSAR + representation of different solvation scenarios</td>
<td>yes</td>
</tr>
</tbody>
</table>
our virtual experiments have so far not produced any false-positive results. In testing of industrial chemicals for toxicity – for example the 30,000 compounds that have to be retested within the REACH framework – and causing an estimated toll of 10 Million laboratory animals, our approach can be used to safely identify the most harmful compounds in silico and prevent their further testing in vivo.

Of course, with only a limited number of enzyme/receptor systems known to mediate adverse effects and even fewer accessible in a QSAR context (due to lacking experimental affinity data), false-negative results will always be present. It will selectively recognize potentially hazardous compounds associated with major mechanisms (e.g. metabolic degradation, endocrine disruption) and allow for discarding them early on. Second, a widely used database of this kind might reduce the number of otherwise doubly-conducted toxicity tests at research laboratories focusing on closely related biomedical targets. The main advantage of the proposed virtual laboratory is that it can be applied to hypothetical substances, produces reliable results and is fast and cheap.

References

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