Leadscope Enterprise model for human Thyroid hormone Receptor alpha (hTRa) binding *in vitro*

1. QSAR identifier

1.1 QSAR identifier (title)

Leadscope Enterprise model for human Thyroid hormone Receptor alpha (hTRa) binding *in vitro*, Danish QSAR Group at DTU Food.

1.2 Other related models

MultiCASE CASE Ultra model for human Thyroid hormone Receptor alpha (hTRa) binding *in vitro*, Danish QSAR Group at DTU Food.

SciMatics SciQSAR model for human Thyroid hormone Receptor alpha (hTRa) binding *in vitro*, Danish QSAR Group at DTU Food.

1.3 Software coding the model

Leadscope Predictive Data Miner, a component of Leadscope Enterprise version 3.1.1-10.

2. General information

2.1 Date of QMRF

January 2015.

2.2 QMRF author(s) and contact details

QSAR Group at DTU Food;

Danish National Food Institute at the Technical University of Denmark;

http://qsar.food.dtu.dk/;

qsar@food.dtu.dk

Eva Bay Wedebye;

National Food Institute at the Technical University of Denmark;

Nikolai Georgiev Nikolov;

National Food Institute at the Technical University of Denmark;

Marianne Dybdahl;

National Food Institute at the Technical University of Denmark;

Sine Abildgaard Rosenberg;

National Food Institute at the Technical University of Denmark;

2.3 Date of QMRF update(s)

2.4 QMRF update(s)

2.5 Model developer(s) and contact details

Sine Abildgaard Rosenberg;

National Food Institute at the Technical University of Denmark;

Marianne Dybdahl;

National Food Institute at the Technical University of Denmark;

Jay Russel Niemelä;

National Food Institute at the Technical University of Denmark;

Nikolai Georgiev Nikolov;

National Food Institute at the Technical University of Denmark;

Eva Bay Wedebye;

National Food Institute at the Technical University of Denmark;

Danish QSAR Group at DTU Food;

National Food Institute at the Technical University of Denmark;

http://qsar.food.dtu.dk/;

qsar@food.dtu.dk

2.6 Date of model development and/or publication

January 2014.

2.7 Reference(s) to main scientific papers and/or software package

Roberts, G., Myatt, G. J., Johnson, W. P., Cross, K. P., and Blower, P. E. J. (2000) LeadScope: Software for Exploring Large Sets of Screening Data. *Chem. Inf. Comput. Sci.*, 40, 1302-1314.

Cross, K.P., Myatt, G., Yang, C., Fligner, M.A., Verducci, J.S., and Blower, P.E. Jr. (2003) Finding Discriminating Structural Features by Reassembling Common Building Blocks. *J. Med. Chem.*, *46,* 4770-4775.

Valerio, L. G., Yang, C., Arvidson, K. B., and Kruhlak, N. L. (2010) A structural feature-based computational approach for toxicology predictions. *Expert Opin. Drug Metab. Toxicol.*, 6:4, 505-518.

2.8 Availability of information about the model

The training set is non-proprietary and was compiled from the published literature (see 6.6 and 9.2 for more details). The model algorithm is proprietary from commercial software.

2.9 Availability of another QMRF for exactly the same model

3. Defining the endpoint

3.1 Species

Human (cell-free assay containing the human Thyroid hormone Receptor alpha, hTRa).

3.2 Endpoint

QMRF 4. Human Health Effects

QMRF 4.18.a. Endocrine Activity. Receptor-binding (human Thyroid Receptor alpha)

3.3 Comment on endpoint

Endocrine disruption not only involves the sex hormone system, but also includes disruption of the thyroid hormone (TH) system. Exposure to chemicals can potentially disrupt the TH system by a number of different mechanisms, one of them being binding to the thyroid hormone receptor (TR). Thyroid disrupting chemicals (TDCs) can affect important physiological processes such as metabolism, growth and development, including development of the brain, and are therefore of high concern (OECD 2006; Murk *et al.*2013).

Thyroid hormones are produced in the thyroid gland and exert a wide array of effects through binding of the active hormone triiodothyronine (T3) to TR. The TR is a member of the nuclear receptor superfamily and two isoforms of the receptor exist in humans, TR alpha (TRa) and beta (TRb). The TR usually heterodimerize with the retinoid X receptor (RXR), another nuclear receptor, and binds to a thyroid response element (TRE) on the DNA. At low levels of T3, a nuclear receptor transcriptional co-repressor is bound to the activation function 2 (AF-2) domain in the Ligand Binding Domain (LBD) of TR and represses the basal transcription through chromatin deacetylase activity. When the thyroid hormone level is high, T3 binds to the TRs LBD causing conformational changes in the LBD that leads to release of the co-repressor from the AF-2 domain and the basal transcriptional activity is restored. Subsequently a nuclear receptor transcriptional co-activator (SRC-1, SRC-2 and others) bind to the AF-2 domain. This binding causes a destabilization of the chromatin and enhances the transcriptional activity through histone acetylation and contacts with the basal transcriptional machinery. Together the binding of T3 and a co-activator to TR leads to an increased transcription of the genes downstream the TRE.

Multiple assays have been established for different mechanisms in the TH system in order to identify TDCs. For this (Q)SAR model data compiled from *in vitro* assays for binding to the human TR alpha (hTRa) have been used to make a model that within the defined applicability domain (see 5.) can predict if a chemical binds to the LBD of hTRa. The *in vitro* binding assay is a cell free assay that detects a compounds affinity to hTRa by determining its ability to compete with radioactive [$_{125}$I]triiodothyronine ([^{125}I]T3) for binding to hTRa. Using the concentration response curve an IC50 value can be determined for each compound as the concentration of compound measured in µM required to inhibit 50% of the binding of [^{125}I]T3 to hTRa. The assay does not say anything about if the binding induces transcription of the target genes (i.e. if the chemical is an hTRa agonist or antagonist).

3.4 Endpoint units

-log(IC50), where IC50 is in µM.

3.5 Dependent variable

Binding affinity to human Thyroid Receptor alpha *in vitro*, –log(IC50) (µM).

3.6 Experimental protocol

Data for the training set was obtained from studies using a hTRa-binding *in vitro* assay to measure affinity of a compound to hTRa. Currently this assay does not have an internationally agreed guideline. The assay has been described in Greenidge et al. (1998) The assay is known to have a potential for a high rate of false negatives (Murk *et al.*2013).

3.7 Endpoint data quality and variability

All data in the final training sets originated from the same laboratory (Karo Bio). From the publications it is expected that the tests were performed by the same protocol, possibly with minor justifications, however it is not explicitly stated. The overall data variability is assumed to be low due to the fact that all data points originate from the same laboratory.

4. Defining the algorithm

4.1 Type of model

A continuous (Q)SAR model based on structural features and numeric descriptors.

4.2 Explicit algorithm

This is a continuous (Q)SAR model made by use of Partial Least Squares (PLS) regression method. The specific implementation is proprietary within the Leadscope software.

4.3 Descriptors in the model

- structural features,
- aLogP,
- polar surface area,
- number of hydrogen bond donors,
- Lipinski score,
- number of rotational bonds,
- parent atom count,
- parent molecular weight,
- number of hydrogen bond acceptors

4.4 Descriptor selection

Leadscope Predictive Data Miner is a software program for systematic substructural analysis of a compound using predefined structural features stored in a template library. The feature library contains approximately 27,000 structural features and the structural features chosen for the library are motivated by those typically found in small molecules: aromatics, heterocycles, spacer groups, simple substituents. Additionally, Leadscope also calculates eight molecular descriptors for each structure: the octanol/water partition coefficient (alogP), hydrogen bond acceptors, hydrogen bond donors, Lipinski score, atom count, parent compound molecular weight, polar surface area and rotatable bonds. It is further possible to generate training set-dependent structural features (scaffold generation) and use these features in the model building process. Redundant features are removed and the remaining features are used in the model building. The automatic feature selection process in Leadscope selects the top 30% of the features according to X^2-test for a binary variable, or the top and bottom 15% according to *t*-test for a continuous variable. LeadScope treats numeric property data as ordinal categorical data. If the input data is continuous such as IC_{50} or cLogP data, the user can determine how values are assigned to categories: the number of categories and the cutoff values between categories. (Roberts *et al.*2000).

For this model scaffolds were generated for the training set and added to the library features. Features were first automatically selected. The number of features was reduced further using the built-in filter to remove similar features (both the “more similar” and the “less similar”). This gave a total of 111 descriptors (structural features and molecular descriptors).

4.5 Algorithm and descriptor generation

For descriptor generation see 4.4.

After selection of features the Leadscope Predictive Data Miner program performs partial least squares (PLS) regression for a continuous response variable, or partial logistic regression (PLR) for a binary response variable, to build a predictive model. By default the Predictive Data Miner performs leave-one-out or leave-groups-out (in the latter case, the user can specify any number of repetitions and percentage of structures left out) cross validation on the training set depending on the size of the training set.

In this model because of the continuous outcome in the response variable PLS regression was used to build the predictive model.

4.6 Software name and version for descriptor generation

Leadscope Predictive Data Miner, a component of Leadscope Enterprise version 3.1.1-10.

4.7 Descriptors/chemicals ratio

The model system uses molecular descriptors and structural features specific to a group of structurally related chemicals from the global training set. Therefore estimations of the number of used descriptors may be difficult. In general, we estimate that the models effectively use an order of magnitude less descriptors than numbers of chemicals in the training set when we set our domain definition where we weed out low probability active and inactive predictions (see 5.1).

For this model 111 descriptors were selected to build the model. These includes 8 Leadscope calculated molecular descriptors, 50 hierarchy features and 53 dynamic features. The 111 descriptors were distributed on 4 PLS factors.

5. Defining Applicability Domain

5.1 Description of the applicability domain of the model

For assessing if a test compound is within the applicability domain of a given model Leadscope examines whether the test compound bears enough resemblance to the training compounds used in the model. This is done by calculating the distance between the test compound and all compounds in the training set (distance equals 1 - similarity). The similarity score is based on the Tanimoto method. The numbers of neighbors is defined as the numbers of compounds in the training set that have a distance ≤ 0.7 with respect to the test compound. The higher the number of neighbors the more reliable the prediction of the test compound. Statistics of the distances are also calculated. Effectively no predictions are made for test compounds which are not in the structural domain of the model or for which the molecular descriptors could not be generated.

In addition to the general Leadscope applicability domain definition the Danish QSAR group has applied the further requirement that only predictions within the –logIC50 interval of the training set are considered in domain.

5.2 Method used to assess the applicability domain

The system does not generate predictions for test compounds which are not in the structural domain or for which the molecular descriptors could not be generated. Predictions outside the $-\log IC_{50}$ interval [-5.2;1.39] are considered out of domain.

5.3 Software name and version for applicability domain assessment

Leadscope Predictive Data Miner, a component of Leadscope Enterprise version 3.1.1-10.

5.4 Limits of applicability

The Danish QSAR group applies an overall definition of structures acceptable for QSAR processing which is applicable for all the in-house QSAR software, i.e. not only Leadscope. According to this definition accepted structures are organic substances with an unambiguous structure, i.e. so-called discrete organics defined as: organic compounds with a defined two dimensional (2D) structure containing at least two carbon atoms, only certain atoms (H, Li, B, C, N, O, F, Na, Mg, Si, P, S, Cl, K, Ca, Br, and I), and not mixtures with two or more ‘big components’ when analyzed for ionic bonds (for a number of small known organic ions assumed not to affect toxicity the ‘parent molecule’ is accepted). Calculation 2D structures (SMILES and/or SDF) are generated by stripping off ions (of the accepted list given above). Thus, all the training set chemicals are used in their non-ionized form. See 5.1 for further applicability domain definition.

6. Internal validation

6.1 Availability of the training set

Yes

6.2 Available information for the training set

SMILES

6.3 Data for each descriptor variable for the training set

No

6.4 Data for the dependent variable for the training set

All

6.5 Other information about the training set

118 compounds are in the training set.

6.6 Pre-processing of data before modeling

Data used to develop the (Q)SAR model was compiled from two public databases, ChEMBL and BindingDB, on the internet . Data in the two databases originate from published literature, and in this case all the data were made by Karo Bio (Karo Bio AB, Novum, Huddinge S-141 57, Sweden) (Carlsson et al. 2002, Ye et al. 2003, Hangeland et al. 2004, 2005, Hedfors et al. 2005, Collazo et al. 2006, Koehler et al. 2006, Li et al. 2006, Garg et al. 2007, Malm et al. 2007). The initial data sets consisted of results from various *in vitro* assays measuring different endpoints related to the TH system. Therefore a thorough manual review of the data sources was performed, and data originating from other assays than the hTRa binding assay were removed.

Only structures acceptable for the commercial software were used in the training set, that is only discrete organic chemicals as described in 5.4 were used. Subsequently, duplicates were identified and removed according to defined criteria: IC50 values for duplicates were compared and in case the difference between the IC50 values were more than 10 fold both data points were removed. If the difference was less than 10 fold the data point with the lowest IC50 value was kept (conservative approach). For this model no duplicates had a 10 fold or more difference in the IC50 values. IC50 values were transformed into –logIC50.

6.7 Statistics for goodness-of-fit

The R-squared (internal performance) was calculated by Leadscope and gave 0.829.

6.8 Robustness – Statistics obtained by leave-one-out cross-validation

Not performed. (It is not a preferred measurement for evaluating large models).

6.9 Robustness – Statistics obtained by leave-many-out cross-validation

A 20 times 20% out cross-validation was performed in Leadscope. Leadscope removes 20% from the full training set and builds a model on the remaining 80%. This model is then used to predict the removed 20%. Then another 20% is removed from the training set, a model is built on the remaining 80% and used for predicting the removed 20%. This is done 5 times in total, each time removing new 20% from the training set. The procedure was done 20 times and the statistics averaged and a Q-square is returned by Leadscope. The Q-squared (predictive performance) was 0.68.

6.10 Robustness - Statistics obtained by Y-scrambling

Not performed.

6.11 Robustness - Statistics obtained by bootstrap

Not performed.

6.12 Robustness - Statistics obtained by other methods

Not performed.

7. External validation

7.1 Availability of the external validation set

7.2 Available information for the external validation set

7.3 Data for each descriptor variable for the external validation set

7.4 Data for the dependent variable for the external validation set

7.5 Other information about the training set

7.6 Experimental design of test set

7.7 Predictivity – Statistics obtained by external validation

7.8 Predictivity – Assessment of the external validation set

7.9 Comments on the external validation of the model

External validation has not been performed for this model.

8. Mechanistic interpretation

8.1 Mechanistic basis of the model

The global model identifies structural features and molecular descriptors which in the model development was found to be statistically significant associated with effect. Many predictions may indicate modes of action that are obvious for persons with expert knowledge for the endpoint.

8.2 A priori or posteriori mechanistic interpretation

A posteriori mechanistic interpretation. The identified structural features and molecular descriptors may provide basis for mechanistic interpretation.

8.3 Other information about the mechanistic interpretation

9. Miscellaneous information

9.1 Comments

The model can be used to predict a chemicals binding affinity to the human Thyroid hormone Receptor alpha *in vitro*. The outcome from the prediction is –logIC50 and this can be transformed to make an estimate for the IC50 (µM) value for the chemical.

9.2 Bibliography

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9.3 Supporting information