QSAR modeling of non-steroidal Farnesoid X Receptor activators

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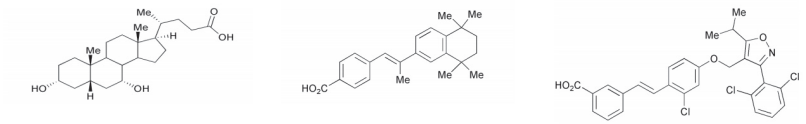
Abstract: *Recent advances in Farnesoid X Receptor (FXR) biology demonstrate that FXR may represent a valuable target for the identification of novel drugs to treat variety of biological disorders. However, for therapeutic purposes the advanced development of selective FXR modulators requires preliminary in silico evaluation of potential ligand candidates. In the current study the capabilities for structure-activity modeling incorporated in the non commercial computational tool have been employed for investigation the activating effect of various non-steroidal FXR ligands. A total of 97 molecules, representing three chemical classes – n-benzylamine, Diarylethene and Cycloalkyl amide have been analyzed. The ultimate models associated to each chemical class provide knowledge about molecular descriptors that may influence the activation of FXR.*

Key words: *Farnesoid X Receptor (FXR), REACH, QSAR, Toxicology, Computational tools*

INTRODUCTION

Farnesoid X receptor (FXR) is a member of nuclear hormone receptor family of ligand activated transcription factors that function as key sensors for bile acid by regulation of gene expression [1]. Experimental studies in animal models and cell lines have shown the high expression level of FXR in liver, intestine, adrenal gland and particularly in the kidney [2]. FXR is activated by the hydrophobic bile acids, including chenodeoxycholic acid, cholic acid and deoxycholic acid, which are the most potent endogenous agonists [3]. FXR can also be activated by a number of nonsteroidal and steroidal compounds not structurally related to bile acids [4]. The most potent endogenous FXR activator, chenodeoxycholic acid (CDCA), along with steroidal and non steroidal ligands are shown in Fig. 1.



Chenodeoxycholic acid (CDCA) TTNPB* GW4064**

* Benzoic acid, 4-(2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl)-, (E)

**3-[2-[2-Chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4-isoxazolyl]methoxy]phenyl]ethenyl]BA

Figure. 1 Natural and synthetic agonists of FXR (farnesoid X receptor).

The characterization of the physiological role of FXR has suggested several important therapeutic applications associated with its selective modulation. For example, several bile acid analogs have been developed as hypocholesterolemic agents using a combination of structure and ligand-based approaches [5]. GW4064 is the first synthetic non-steroidal FXR agonist described in the literature [6]. It demonstrates potent FXR binding and activation in biochemical and cellular in vitro assays as well as pharmacological effects in different rodent models of metabolic and other diseases. However, its suitability as a drug for FXR targeted pharmacotherapy is questionable given that this compound harbors a trans stilbene moiety as a potential toxicophore and given its photolability [7]. Several groups have tried to overcome the liabilities associated with GW4064 and have generated various type of derivative compounds [8, 9].

The ligand-based approaches used in structure-activity correlations have become a vital component of modern drug design [10]. Computational methods such as quantitative structure-activity relationships (QSARs) have been widely used to prioritize chemicals for in vitro or in vivo testing [11]. Based on the available collections of experimentally tested FXR ligands, several groups have reported QSAR models for identification of new FXR activators. Although these models were reported to have significant predictive power, all of

them were created using relatively small datasets with limited chemical diversity and consequently limited application coverage.

The aim of this study is development of model for identification of non-steroidal FXR ligands based on QSAR evaluation of large chemical set with experimental data for FXR activity. The model can be used as an external profiling scheme in the most popular freely available in silico tool for risk assessment of chemicals.

EXPERIMENTAL

Farnesoid X receptor activity data

The data set of 97 compounds used for the QSAR analyses was taken from the literature [12]. The chemical structures and biological properties for the complete set of compounds could be obtained upon request. The EC_{50} values vary from 0.019 to 1.47 μM and were measured under the same experimental conditions.

OECD QSAR Toolbox

This is a unified and reliable platform for chemical risk assessment [13]. A key part of ToolBox is so called categorization of chemicals. The categorization is ability of the system to group chemical substances to chemical categories. The chemical category is such a group of substances possessing similar physicochemical, toxicological and ecotoxicological properties or their fate in environmental and occupational surrounding or they behave using the common pattern as a result of chemical similarity.

Another advantage of the system is the large number of built-in toxicological profilers. Each profile consist a set of rules related to specific or general structural requirements. As an example a general profile encoding rules for organic functional groups can be applied for identification of specific functional groups in the chemicals under study.

The availability of all features in the system can be used successfully in QSAR studies regarding variety of biological/toxic endpoints. It should be mentioned that another important advantage is the possibility of independent reproducibility of the obtained results which is one of the main requirements for validity of new models.

RESULTS AND DISCUSSION

Because the data set is constituted by FXR activators only, the aim of the present study was to define a specific structural features or molecular descriptors which can explain the potency in predefined activity ranges. Thus, the data set was initially divided into three categories: strong activators $EC_{50} \geq 0.9$ μM; moderate activators $0.1 < EC_{50} \leq 0.9$ μM and low activators $EC_{50} < 0.1$ μM. Consequently three subsets have been defined on the basis of common structural moieties which were further investigated.

The group with largest number of representative chemicals contains sixty six compounds sharing n-benzylamine moiety. It was found that the distribution in respect to the EC_{50} values is - 25 strong; 30 moderate and 11 low activators. Due to the limited number of low activators they were combined with moderate activators in order to form one “general low activators” set. Such grouping does not change the modeling strategy since the aim is to find those chemical characteristics which discriminate strong activators from those with lower activating effect. In order to be modeled, all chemicals coded as SMILES notations have been transferred in the QSAR Toolbox. Next, they were optimized by application of MOPAC by semi-empirical molecular orbital calculations using the method AM1 [14] a module implemented in the system.

By contrasting both groups (strong and low activators) in respect to various molecular descriptors a specific range of van der Waals partial positive area (28.7÷33.7a.u./$Å^2$) was identified which discriminate correctly 70% of strong and 71% of low activators. From mechanistic point of view the discriminating parameter could be associated with the interaction forces – considered to be purely electrostatic between ligands and the FXR macromolecule. In respect to the “active” range of this parameter it can be concluded that

structural variations which lead to higher values than the upper border may result in inactivation of the ligands toward FXR.

The second group was constructed on the basis of a presence of Diarylethene structural moiety. This structural element was found in twenty compounds. In respect to their FXR activating potential it was found that there are no ligands with potency greater than 0.9 μM. Thus, in agreement with the predefined activity ranges all chemicals were defined as moderate activators ($0.1<EC_{50}\leq0.9$ μM) and low activators ($EC_{50}<0.1$ μM). Both groups were combined and technically processed in the Toolbox. Then as a result from analysis based on calculated parameters it was found that maximum discrimination could be obtained by making use of the Electronegativity as suitable molecular descriptor. The identified range of -4.8÷-4.7 eV allow correct predictions for 12 out of all 15 moderate activators which correspond to sensitivity of 80%. Regarding low activators the specificity was found to be also 80%, reached as a result of correct predictions for 4 out of 5 low activators.

The role of electronegativity as discriminating parameter is expected due to the fact that it can be associated with the nature of the interaction between ligands and FXR. In a recent study Honorio et al. [12] discussed the activating affect of series activators for liver X receptor. They have found that the activating effect depends from electronegativity of molecular regions associated with specific substituents. This finding gives the idea for further analysis of the investigated FXR ligands by application of algorithm(s) for estimation of electronegativity distribution in the molecules. Such a study will bring more clear mechanistic rational which may contribute in investigations for the sake of drug design.

The third set of chemicals was constructed as a result of identified Cycloalkyl amide feature in eleven compounds. According to their observed activating effect they were splitted identically with the second group - moderate activators - seven chemicals ($0.1<EC_{50}\leq0.9$ μM) and low activators - four chemicals ($EC_{50}<0.1$ μM). It was found that the most suitable parameter which discriminate both groups is again electronegativity. However, the active range was found to be slightly extended in comparison with the group of Diarylethenes. In the range of -4.9÷-4.6 eV falls 6 out of 7 activators which correspond to 86% sensitivity. The presence of one false positive leads to satisfactory specificity of 75%.

CONCLUSIONS

In this work, a QSAR model for identification of FXR activators has been described. It is based on preliminary segregation of a set of non-steroidal FXR activators in sub groups on structural basis. Each group has been additionally investigated and specific molecular descriptors are associated with activators. The effect of Diarylethene and Cycloalkyl amideis associated with molecular descriptor electronegativity along with specific activity ranges for both groups. The activating effect of n-benzylamins is considered to be dependent from the positive charge of the ligand surfaces quantified by using range of the descriptor van der Waals partial positive area. The obtained results in terms of sensitivity and specificity higher than 70% for all models confirm that the identified descriptors and their specific ranges can be used as preliminary in silico evaluation tool for in identification of potential FXR activators.

REFERENCES

[1] Kalaany N.Y., D.J. Mangelsdorf, LXRs and FXR: the Yin and Yang of cholesterol and fat metabolism, Ann. Rev. Physiol. 68, 2006, 159–191.

[2] Epifano F. Genovese, E.. The active principle from Ligularia nelumbifolia, is a novel farnesoid X receptor agonist. Bioorg Med Chem Lett ,2012, 22, 3130–5.

[3] Lin HR. Triterpenes from Alisma orientalis act as farnesoid X receptor agonists. Bioorg Med Chem Lett ,2012, 22, 4787–92.

[4] Duran-Sandoval D., B.Cariou, J.Fruchart, B. Staels, Potential regulatory role of the farnesoid X receptor in the metabolic syndrome, Biochimie 87 ,2005, 93–98.

[5] Pellicciari R., G. Costantino, S. Fiorucci, Farnesoid X receptor: from structure to potential clinical applications, J. Med. Chem. 48, (2005), 5383–5403.

[6] Maloney P.R., D.J. Parks, C.D. Haffner, A.M. Fivush, G. Chandra, K.D. Plunket, K.L. Creech, L.B. Moore, Wilson J.G., Lewis M.C., Jones S.A., Willson T.M. J. Med. Chem., 43 (2000), p. 2971.

[7] Bass J.Y., Caldwell R.D., Caravella J.A., Chen L., Creech K.L., Deaton D.N., Madauss K.P., Marr H.B., McFadyen R.B., Miller A.B., Parks D.J., Todd D., Williams S.P., Wisely G.B. Bioorg. Med. Chem. Lett., 19 ,2009, p. 2969.

[8] A. Akwabi-Ameyaw, J.Y. Bass, R.D. Caldwell, J.A. Caravella, L. Chen, K.L. Creech, D.N. Deaton, S.A. Jones, I. Kaldor, Y. Liu, K.P. Madauss, H.B. Marr, R.B. McFadyen, A.B. Miller, F.N. Iii, D.J. Parks, P.K. Spearing, D. Todd, S.P. Williams, G.B. Wisely Bioorg. Med. Chem. Lett., 18 ,2008, p. 4339.

[9] S. Feng, M. Yang, Z. Zhang, Z. Wang, D. Hong, H. Richter, G.M. Benson, K. Bleicher, U. Grether, R.E. Martin, J.M. Plancher, B. Kuhn, M.G. Rudolph, L. Chen Bioorg. Med. Chem. Lett., 19 ,2009, p. 2595.

[10] Salum, L.B.; Polikarpov, I. Andricopulo, A.D. Structural and chemical basis for enhanced affinity and potency for a large series of estrogen receptor ligands: 2D and 3D QSAR studies. J. Mol. Graph. Model., 2007, 26, 434-42.

[11] E. Lo Piparo, A. Worth Tools for predicting developmental and reproductive toxicity. Institute for health and consumer protection JointResearch Centre, European Commission,Luxembourg,2010,http://ihcp.jrc.ec.europa.eu/our_labs/predictive_ toxicology/doc/EUR_24522_EN.pdf

[12] Kathia M., L. Salum, R. Garratt, I. Polikarpov, A. Andricopulo. Two- and Three-Dimensional Quantitative Structure-Activity Relationships Studies on a Series of Liver X Receptor Ligands. Open Med Chem J. 2008; 2: 87–96.

[13] OECD QSAR Tollbox http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm (last accessed 20.08.2015).

[14] Stewart, J.J., 1990. MOPAC: a semiempirical molecular orbital program.J. Comput. Aided Mol. Des., 1990, 4, 1.

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