A QSAR evaluation of glucocorticoid receptor binding

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Abstract: *Exposures to environmental concentrations of endocrine disrupting compounds are now a known threat to both human and ecological health. In the current study capabilities for structure-activity modeling incorporated in the platform QSAR Toolbox were employed for investigation the binding effect of set of chemicals toward glucocorticoid receptor. A total of 39 steroidal ligands were split in categories, representing strong, moderate and weak binders. As a result of comparative analysis a mechanistic reasonable molecular descriptors were found to be useful for prediction of strong and moderate receptor binders. It was found that the important feature related to strong binders is their surface which is assessed by specific range of van der Waals surface area. Regarding moderate binders it was found that the interaction can be assessed by using more specific descriptor van der Waals partial negative surface area. The obtained results suggest that identified descriptors and their specific ranges are reliable and can be used as preliminary in silico evaluation in identification of potential glucocorticoid binders.*

Key words: *Glucocorticoid receptor, REACH, QSAR, Toxicology, Computational tools*

INTRODUCTION

Endocrine disrupting chemicals (EDCs) are considered to be a serious health threat by contributing to variety diseases [1]. EDCs, such as some environmental chemicals, food preservatives, dyes, and chemicals used in cosmetics, can interfere with endocrine functions, either by directly activating or inactivating endocrine target receptors or by disrupting the synthesis of hormones or the local control of active to inactive hormones by inhibiting or activating their metabolizing enzymes. Endocrine disruption affects various body functions, depending on the pathway that is disrupted. It has been proposed that exposure to xenoestrogens and xenoandrogens led to the increased prevalence of breast cancer, prostate cancer and testicular cancer [2, 3]. Xenobiotics may disturb also glucocorticoid and mineralocorticoid actions, contributing to cardiovascular complications, disturbances in energy metabolism, immune responses, as well as impairment of cognitive functions and the regulation of cell proliferation and differentiation [4].

Because of their ability to exert intense biological effects in almost any organ, corticosteroids are one of the most widely used drug classes [5]. These steroids exert their main effect by binding to glucocorticoid receptors (GRs), a member of the steroid–thyroid–retinoid receptor super-family [6, 7]. GRs are predominantly localized to the cytoplasm of target cells and move into the nuclear compartment only on binding of the glucocorticoid.

Unfortunately, because of the intrinsic multiple activities of steroids and structural similar xenobiotics and because of the ubiquitous distribution of the corticosteroid receptors, unwanted side-effects such as osteoporosis, hypertension, insulin resistance, weight gain, fat redistribution, growth inhibition, and others [8], can be initiated.

A huge variety of chemicals exist in the environment, and their potential for binding to the glucocorticoid receptor has not been evaluated. Since June 2007, the REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) legislation has been implicated in the European Union [9]. The main goal of REACH is to protect human health and environment from hazardous chemicals. Testing the actions of all used chemicals – possible EDCs – against all the potential targets related to endocrine disruption is an important but also expensive and difficult, if not impossible, task. Therefore reliable *in silico* alternatives such as quantitative structure–activity relationship (QSAR) models are becoming important tools for rapid and cost-effective prediction of biological activities [10]. Such models may have a great potential for use in the early identification of large numbers of potential glucocorticoid ligands.

The aim of this study is development of model for identification of glucocorticoid ligands based on QSAR evaluation of chemicals with experimental data for relative receptor-binding affinity. 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

Glucocorticoid receptor binding data

Experimental data for 39 chemicals with glucocorticoid relative receptor-binding affinity data (rRBA), was taken from literature [11] determined using standard methodology. Experimental GR-binding affinities are obtained with rat cytosol preparations by determining the concentration (IC_{50}) necessary to inhibit by 50% the binding of a given concentration of 3H-dexamethasone as radioligand.

OECD QSAR Toolbox

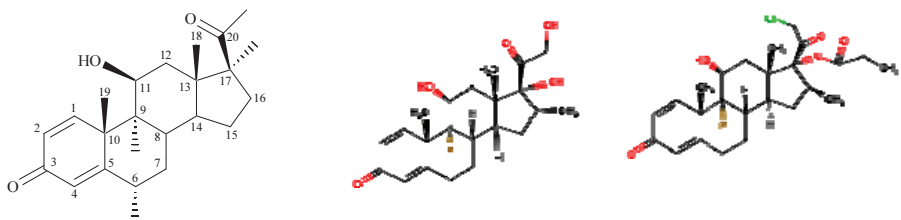
This is a unified and reliable platform for chemical risk assessment [12]. A key part of ToolBox is so called categorization of chemicals. The categorization is ability of the system to group chemical substances to chemical categories. The chemical category is such a group of substances possessing similar 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 the 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

A number of structural requirements for glucocorticoid activities are now commonly accepted [13]. Some of the more important ones are summarized in Fig. 1 together with the common numbering and notation system of representative structures.



(a) General structure (b) Dexamethasone (c) Clobetasol propionate

Fig. 1. Numbering and notation system of steroid structures (a), representative GR binders – a reference chemical (b) and most potent binder used in this study (c).

Along with general structural requirement for steroidal skeleton (Fig 1.) it has been known that RBA is strongly dependent by halogenation at 6α or 9α position (mainly fluorination) [14] or by introduction of cyclic 16,17-acetal moieties. These findings were used as initial rules which were further expanded by addition of other appropriate requirements. There have been also a few studies in which the binding effect is explained mainly by using lipophilicity (e.g., log Ko/w) as descriptor [15]. The influence of logKo/w was examined however it was found that it is not suitable for discrimination purpose in this study. One has to point out that the reason is related to the similar structural variation (and size) of the investigated chemicals which explains their correlation of lipophilicity. On the

other hand a general range of logKo/w is defined (0.4÷5.4) as e prescreening rule extracted from all training set chemicals.

Because the training data set is constituted by GR binders only, the aim of the present study was to define a specific structural features or molecular descriptors (or combination of both) which may explain the binding potency in predefined GR binding ranges. In this sense, the data set was divided into three categories: a set of strong GR binders with RBA≥200; a set of weak binders RBA≤70 and a set of moderate binders 70<RBA<200 which include Dexamethasone as referential ligand (RBA=100).

In order to discriminate strong from moderate binders both groups were compared and analyzed. Initially, all molecular structures represented as SMILES notations were transferred in the Toolbox. Next, they were individually optimized by application of MOPAC by semi-empirical molecular orbital calculations using the method AM1 [16] implemented in the system. To explain the difference between strong binders (12 chemicals) and moderate binders (14 chemicals) additional investigation was performed to distinguish both groups. As a result a specific range of the descriptor *van der Waals surface* area was identified (453÷483 $Å^2$) which discriminate 10 from all 12 strong binders (sensitivity 83%). The specificity which is used in this case as measure for predictions of moderate binders is found to be 65% as a result of correct predicted 9 out off 14 chemicals. These results are presented in Table 1.

Table 1. Prediction results based on specified range of *van der Waals surface.*

#	NAME	RBA [%]	Prediction*	#	NAME	RBA [%]	Prediction*
1	Clobetasol propionate	6300	STRONG	14	Flunisolide	165	MODERATE
2	Mometasone furoate	1833	STRONG	15	Hydrocortisone	162	STRONG
3	Fluticasone propionate	1796	STRONG	16	Betamethasone	151	MODERATE
4	Mifepristone (RU 486)	1528	STRONG	17	Loteprednol	150	STRONG
5	Beclomethasone	1440	STRONG	18	Dichlorisone	148	MODERATE
6	Budesonide, 22R	1120	STRONG	19	Beclomethasone	140	MODERATE
7	Betamethasone	1048	STRONG	20	Fluocinolone	107	MODERATE
8	Itrocinonide	800	MODERATE	21	Dexamethasone	100	MODERATE
9	Budesonide, 22S	420	STRONG	22	Hydrocortisone	95	STRONG
10	Paramethasone	257	MODERATE	23	Mometasone	88	MODERATE
11	Fluocinolone acetonide	234	STRONG	24	Betamethasone	79	MODERATE
12	Etiprednol dicloacetate	200	STRONG	25	Beclomethasone	76	MODERATE
13	Flurandrenolide	186	STRONG	26	Prednicarbate	75	STRONG

*STRONG - RBA≥200; MODERATE - 70<RBA<200

From mechanistic point of view the discriminating parameter could be associated with the interacting areas of ligands and the macromolecule of the receptor. In respect to the "active" range of this parameter it is showed that strong binders exhibit large values in comparison with moderate binders. It can be concluded that structural variations which lead to decreasing of the *van der Waals surface* area may result in inactivation of the ligands as strong binders toward GR.

The investigation related to discrimination of moderate from weak binders was performed following the same sequence of steps. 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 molecular descriptor *van der Waals partial negative surface area* (VWPNSA). A specific range -44.9÷-36.1 $Å^2$a.u is defined in which 12 out of 14 moderate binders fall in. This corresponds to 86% sensitivity. Regarding weak binders a specificity of 91% was reached as a result of correct predictions for 10 out of total 11 weak binders. The results are presented in Table 2.

Table 2. Prediction results based on specified range of *VWPNSA.*

#	NAME	RBA [%]	Prediction	#	NAME	RBA [%]	Prediction
1	Flurandrenolide	186	MODERATE	15	Desoximetasone	68	WEAK
2	(ED) (ED) Flunisolide	165	MODERATE	16	Fluocortolone	67	WEAK
3	Hydrocortisone	162	MODERATE	17	Medroxyprogesterone	44	WEAK
4	Betamethasone	151	MODERATE	18	Corticosterone	35	WEAK
5	Loteprednol	150	MODERATE	19	Methylprednisolone	33	MODERATE
6	Dichlorisone	148	WEAK	20	Prednisolone	19	WEAK
7	Beclomethasone	140	MODERATE	21	Methylprednisolone	17	WEAK
8	Fluocinolone	107	MODERATE	22	Onapristone	12	WEAK
9	Dexamethasone	100	MODERATE	23	Aldosterone	10	WEAK
10	Hydrocortisone	95	MODERATE	24	Hydrocortisone	10	WEAK
11	Mometasone	88	WEAK	25	Desoxycorticosterone	9	WEAK
12	Betamethasone	79	MODERATE				
13	Beclomethasone	76	MODERATE				
14	Prednicarbate	75	MODERATE				

*MODERATE –70< RBA<200; WEAK - RBA<70

The role of *VWPNSA* as discriminating parameter is expected due to the fact that the nature of the interaction between ligands and the GR is electrostatic. In contrast to strong binders where the effect can be related to the ligand surface in this study moderate binders can be distinguished by using more specific descriptor which accounts local charges of the interacting areas of ligands and the receptor. Regarding the range of variation of the *VWPNSA* it becomes evident that moderate binders more negatively charged. Hence it is expected that variation in the structures of ligands which decrease the negative charge will decrease the binding effect to GR.

CONCLUSION

The current study presents an evaluation of the prediction results for binding to glucocorticoid receptor obtained for a set of chemicals with experimentally measured effect. The investigated chemicals forming the training set were categorized according to their binding potency as strong, moderate and weak binders. The binding effect was analyzed in the QSAR Toolbox by contrasting the groups of Strong-Moderate as well as Moderate-Weak binders regarding large number of molecular descriptors. It was found that measurement of ligands surface could be used successfully in discrimination of the binders in both groups. The parameter *van der Waals surface* area is used as discriminating parameter between strong and moderate binders with overall statistical performance of 73% correct predicted binders. More specific descriptor *van der Waals partial negative surface area* was found to discriminate moderate from weak binders with performance of 88% correct results. The results suggest that identified descriptors and their specific ranges are reliable and can be used as preliminary *in silico* evaluation in identification of potential glucocorticoid binders.

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