Chemosphere 135 (2015) 325–334

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Computational study of binding affinity to nuclear receptors for some cosmetic ingredients

Alja Plošnik, Marjan Vračko, Janez Mavri *

National Institute of Chemistry, Hajdrihova 19, SI-1000 Ljubljana, Slovenia

HIGHLIGHTS

- We examined the endocrine disrupting potential of ingredients in cosmetics products.
- Predictions were performed with direct binding of molecules on nuclear receptors.
- 122 compounds, out of 558, were estimated to be possible endocrine disruptors.
- This tool can be useful for industry on decision for further *in vitro/in vivo* testing.

ARTICLE INFO

Article history:
Received 15 December 2014
Received in revised form 22 April 2015
Accepted 24 April 2015

Keywords:
Endocrine disruption
Cosmetic ingredients
Nuclear receptors
In silico
Molecular docking

ABSTRACT

We studied the ingredients of cosmetic products as potential endocrine disruptors (ED) by *in silico* methods (docking). The structures of 14 human nuclear receptors have been retrieved from the protein data bank (PDB). We only considered the mechanism linked with direct binding to nuclear receptors with well-defined crystal structures. Predictions were performed using the Endocrine Disruptome docking program http://endocrinedisruptome.ki.si/ (Kolšek et al., 2013). 122 compounds were estimated to be possible endocrine disruptors bind to at least one of the receptors, 21 of them which are predicted to be probable toxicants for endocrine disruption as they bind to more than five receptors simultaneously. According to the literature survey and lack of experimental data it remains a challenge to prove or disprove the *in silico* results experimentally also for other potential endocrine disruptors.

1. Introduction

Endocrinology is the branch of medicine dealing with the endocrine system. Hormones are endogenic signaling molecules that play pivotal roles in the embryological development and physiology of the organism. However certain xenobiotics interfere with the endocrine system and change its function. However, interference with the endocrine system is a broad term and different agencies have different definitions of what this interference can mean. The definition of endocrine disruptor (ED) as defined by European Union is follows: "*An ED is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function*" (EU, 1996) European Workshop, 1996. The US Environmental Protection Agency has been chosen a slightly more detailed definition: "*Endocrine disrupting chemicals (EDCs) have been defined as exogenous agents that interfere with the production, release, transport, metabolism, binding action, or elimination of the natural blood-borne hormones in the body responsible for the maintenance of homeostasis, reproduction and regulation of developmental processes*" (EPA, 2012) Diamanti-Kandarakis et al., 2009. In recent years endocrine disruptors, both synthetic and natural, have become an important environmental concern, mainly because of their interference with various nuclear hormone receptors. Receptors that belong to superfamily of ligand-dependent transcription factors (androgen-AR, estrogen-ER, glucocorticoid-GR, liver X-LXR, mineralocorticoid-MR, peroxisome proliferator-PPAR, progesterone-PR, retinoid X-RXR and thyroid -TR) are therefore the most exposed. These receptors cover the fields of reproduction, behavior, development, and the immune system (Diamanti-Kandarakis et al., 2009). Therefore understanding of endocrine disruption on molecular level for the entire AOP (Adverse Outcome Pathway) is essential. According to this concept a xenobiotic triggers a chain of causally linked events, which at the end lead to adverse effect (in our case malformation due the disruption of hormone system). The chain of events is gives as: molecular initiating event, cell organelle event, cellular event, tissue

* Corresponding author at: Laboratory for Biocomputing and Bioinformatics, National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia.
E-mail address: janez.mavri@ki.si (J. Mavri).

http://dx.doi.org/10.1016/j.chemosphere.2015.04.075
0045-6535/

event, organ event, organism event, population event (Tollefsen et al., 2014). Our study is focused mainly to the first event in the chain, i.e., to the molecular level. Nuclear receptors are composed of three essential functional domains: the N-terminal transactivation domain participates in co-activator interactions, the central DNA binding domain (DBD) and ligand binding domain (LBD), which is involved in interactions with heat-shock protein and receptor homo- or hetero-dimerization (Rüegg et al., 2009). The nuclear receptor superfamily of proteins regulates gene expression for various physiological processes. Transcription is regulated by changes in receptor conformation and is modulated by ligand binding. The classes of endocrine disruptors that bind to nuclear receptors act by the same mechanism. They mimic the action of natural ligands as agonists or antagonists and typically they have similar chemical characteristics (Rüegg et al., 2009; Marques-Pinto and Carvalho, 2013; Ingerslev et al., 2003). ED can be classified into two major subgroups: natural, such as plant constituents, and synthetic, which are often included as a component of various industrial products that we are exposed to on a daily basis (pesticides, drugs, cleaning products, cosmetic products, etc.) (Ingerslev et al., 2003). In its final stage, identification of ED involves experimental work that includes the use of *in vitro* tests and experimental animals in addition to epidemiological studies. There are efforts to minimize the former as much as possible. The REACH legislation, adopted in 2007, and Cosmetic regulation, adopted in 2009 has forced industry and research institutions to search for alternative methods to detecting potentially toxic compounds as standard *in vivo* screenings are, besides being ethically questionable, also expensive and time-consuming (REACH, 2014; REGULATION, 2009). Computational studies that consider the full dimensionality of the receptor structure and ligand offer attractive alternatives and complementarity to the study of endocrine disrupting potential (Piparo and Worth, 2010; Novič and Vračko, 2010; Vuorinen et al., 2013; Muster et al., 2008; Vedani et al., 2009; Endocrine Disruptome, 2014; PubChem system, 2014).

In this study we used molecular docking to study the binding affinity to both the inactive conformations of 14 nuclear receptors and activated conformations of 4 nuclear receptors. The Endocrine Disruptome program package that works as a server (http://endocrinedisruptome.ki.si/) and was developed in our laboratory was used to predict the endocrine disrupting potential (Kolšek et al., 2013; Endocrine Disruptome, 2014). We considered a set of 558 compounds found in traces in cosmetic products and we predicted that 122 compounds would act on at least one receptor, while 21 compounds has showed the potential to bind to five or more receptors. It is to emphasize that this study was limited only to direct binding of the compounds to the nuclear receptors, while other steps in AOP or more complex mechanisms such as receptor interactions with metabolites and enzyme inhibition were not considered.

2. Studied compounds

Production of cosmetics is associated with a large number of compounds that are present in the final products. The cosmetic ingredients are on the EU market regulated over Cosmetic Product Regulation (earlier European Cosmetic Directive from 1976) adopted in 2009 (REGULATION, 2009). The set of 558 structurally diverse compounds was randomly selected from the CosIng (Cosmetic ingredients and substances) Inventory database (REGULATION, 2009; Inventory CosIng Database, 2014; Anzali et al., 2012). The CosIng database includes data of about 20,000 compounds, which are ingredients in cosmetic products (in some cases also in medical products). The main purpose of this directive is to ensure the safety of use of cosmetic products for consumers (Anzali et al., 2012). For each entry the CosIng database contains chemical names, CAS and/or EC inventory numbers, use category of compounds, and eventual regulation or links to opinions of the Scientific Committee on Consumer Safety (SCCS). The chemicals belong to 23 different chemical categories, which are described in details in Anzali et al. (2012). For our exercise we randomly selected 558 compounds, which cover almost the entire CosIng chemical space. From the selected compounds, 200 were aliphatic, 238 aromatic and 120 cyclic or polyclic. For technical reasons we omitted salts, metals and mixtures and considered only compounds with defined chemical structure. In the end we constructed a data set of 558 compounds and we added SMILES (Simplified

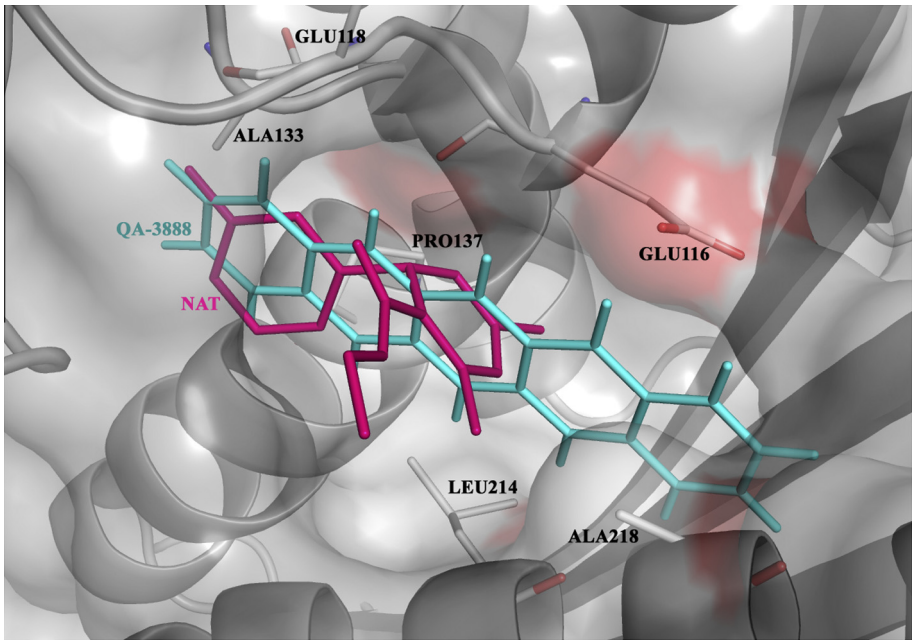

Fig. 1. A snapshot from the docking simulation of the studied ligand Quinacridone (CAS No. 1047-16-1) bound thyroid receptor superimposed to the experimental structure of RCSB PDB.