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A high-throughput, computational system to predict if environmental contaminants can bind to human nuclear receptors

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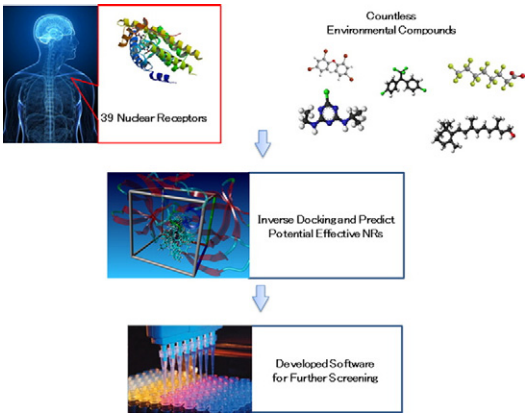
HIGHLIGHTS

- A database covering all existing crystal structures of 39 nuclear receptors was built.
- An inverse docking method was developed to predict highly vulnerable NRs.
- Some rarely reported targets (e.g. LRH-1) are suggested to be vulnerable NRs.

GRAPHICAL ABSTRACT



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ABSTRACT

Some pollutants can bind to nuclear receptors (NRs) and modulate their activities. Predicting interactions of NRs with chemicals is required by various jurisdictions because these molecular initiating events can result in adverse, apical outcomes, such as survival, growth or reproduction. The goal of this study was to develop a high-throughput, computational method to predict potential agonists of NRs, especially for contaminants in the environment or to which people or wildlife are expected to be exposed, including both persistent and pseudo-persistent chemicals. A 3D-structure database containing 39 human NRs was developed. The database was then combined with AutoDock Vina to develop a System for Predicting Potential Effective Nuclear Receptors (SPEN), based on inverse docking of chemicals. The SPEN was further validated and evaluated by experimental results for a subset of 10 chemicals. Finally, to assess the robustness of SPEN, its ability to predict potentials of 40 chemicals to bind to some of the most studied receptors was evaluated. SPEN is rapid, cost effective and powerful for predicting binding of chemicals to NRs. SPEN was determined to be useful for screening chemicals so that

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pollutants in the environment can be prioritized for regulators or when considering alternative compounds to replace known or suspected contaminants with poor environmental profiles.

1. Introduction

The adverse outcome pathway (AOP) is a framework proposed recently for use in toxicology and risk assessment and links exposures to chemicals to series of adverse outcomes (Vinken, 2013). AOPs are initiated by interactions of chemicals with biomolecules (Allen et al., 2014). The AOP framework is being applied to link burgeoning information at the molecular level of organization to adverse outcomes that can be used to make regulatory decisions. As a particular type of interaction, chemicals bind to nuclear receptors (NRs) and by mimicking natural ligands can lead to pleiotropic, adverse effects, such as modulation of the endocrine system (Grun and Blumberg, 2006) and immunodeficiency (Adorini et al., 2006). Contaminants that bind to ligand binding domains of NRs can be either agonists or antagonists. Disruption of endocrine function by contaminants through hormone NRs is one of the most significant issues of concern in environmental toxicology and ecotoxicology (Hopkins and Groom, 2002; Grun and Blumberg, 2006). Various NRs are linked to pathways and outcomes that can be adversely affected by small concentrations of environmental agonists or antagonists. Thus, understanding initiating effects, modulated via NRs, is important for prediction of outcomes of various contaminants. While determining whether a chemical binds to a NR as an agonist or an antagonist was beyond the scope of this study. The assessment of potential effects is a multi-step process, the first step of which is to determine if a chemical has potential to bind with a NR.

Efforts have been made to detect interactions between emerging pollutants and some NRs including androgen receptors (AR) or estrogen receptors (ER) (Fang et al., 2003; Blair et al., 2000), while it is far from enough to assess the effects of countless compounds on human through variety types of NRs. Thus, high throughput methods to determine the binding potential of NRs with chemicals of concern to humans or wildlife are needed for timely assessment. Since the initial event determining such interactions is binding to NRs, if binding affinities can be predicted it would allow an initial prioritization of which chemicals are likely to cause adverse effects via NR-mediated pathways. And it will also give some insight into what in vitro transactivation assays would be appropriate or what endpoints would be appropriate to monitor during in vivo exposures. This information would also be useful to determine potential cross-talk between or among pathways and to determine consistent effects among chemicals, thus informing categorization during assessments of hazard or risk. Finally, this information would be useful to determine which chemicals would be most likely to cause the same or similar effects such that they should be considered together during assessments of hazard or risk. Integrative experimental approaches, such as *High Information Content Toxicity Screening* (USEPA) and *Molecular Screening and Toxicogenomics* (Toxicogenomic), have been proposed to solve this problem. However, all of these strategies are time-consuming and expensive and some require use of live animals.

Developments in computational chemistry have demonstrated potential to supplement experimental testing for chemical hazard assessment. Several computational tools have been developed to predict potential targets of chemicals based on inverse docking (Chen and Zhi, 2001; Kumar et al., 2014), whereby a small molecule is docked into a panel containing multiple receptors (Fig. 1). Recently, an online tool was developed based on this method and used to investigate cosmetic ingredients (Kolšek et al., 2014; Plošnik et al., 2015). In this study, a database containing 39 human NRs was constructed and a software program, based on inverse docking, was developed to predict effective NRs for several chemicals of emerging concern. The System for Predicting Potential Effective Nuclear Receptors (SPEN) was used to predict the most probable effective NRs of 40 emerging environmental contaminants (Fig. 2).

2. Methods

2.1. Target database and SPEN based on AutoDock Vina

The target database for inverse docking contained 39 NRs (Table 1). There are 48 types of NRs in humans, but only 39 of these NRs have known structures (Zhao et al., 2015). The 3D crystal structures of LBDs (agonist conformation) of NRs can be obtained from the Protein Data Bank (PBD; http://www.rcsb.org/pdb/). After obtaining most of the necessary 3D structures from the PDB, additional residues were added with Swiss-PdbViewer 4.0 (Guex and Peitsch, 1997). Further information which included removing water molecules and buffers because the existing water in crystal structure could affect the binding, assigning charges and adding polar hydrogens, was obtained by use of AutoDock

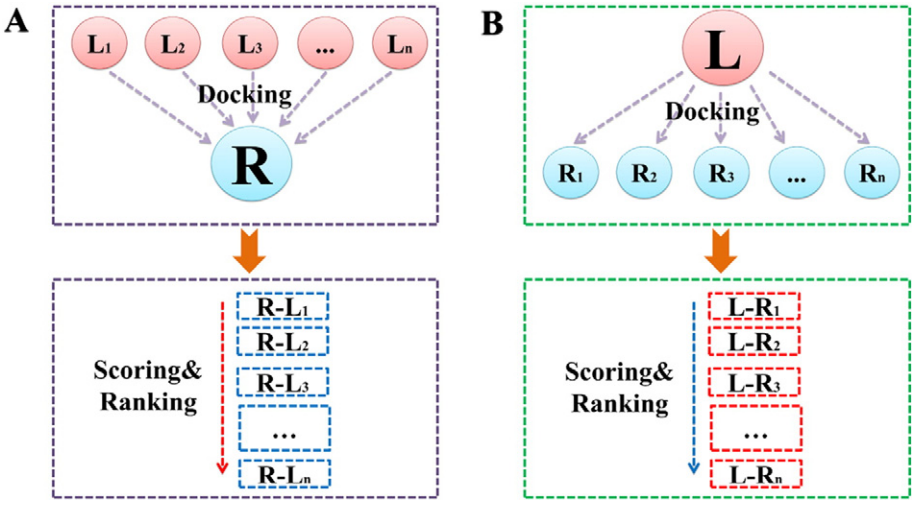

Fig. 1. Schematic representations of docking (A) and inverse docking (B). The term "L" and "R" represent ligand and receptor, respectively.

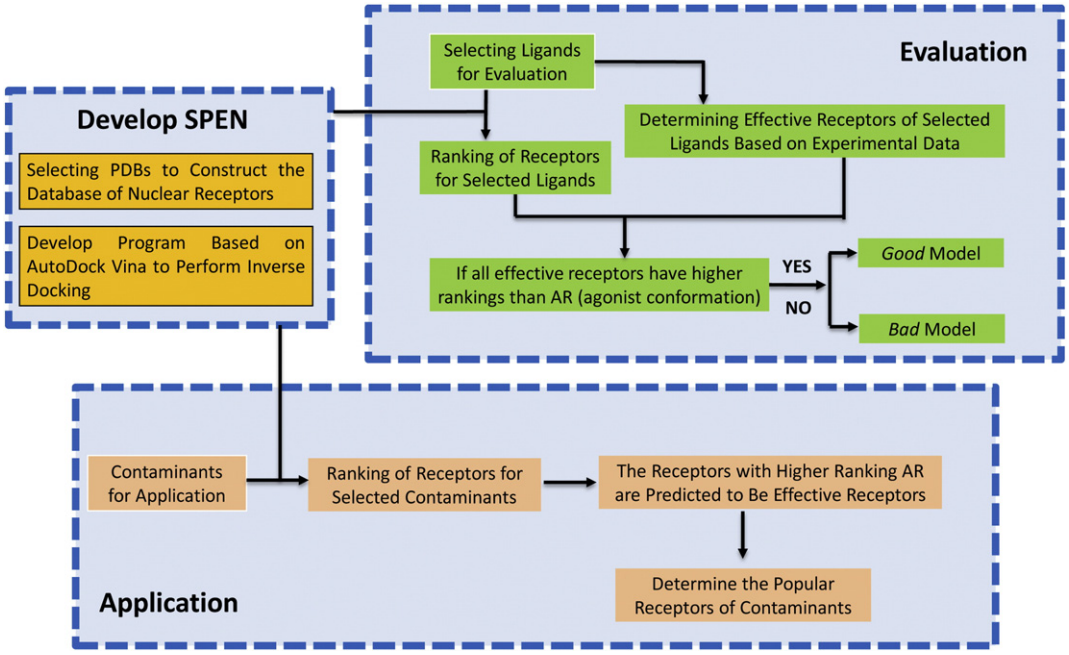

Fig. 2. Schematic representation of this study. Development, evaluation and application of SPEN are shown in three separate boxes. PDBs represent Protein Data Bank files.

Table 1
Information for selected human nuclear receptors (NRs) in database. PDB represents Protein Data Bank file.

NO.	Name of NRs	Abbreviation	PDB entry
1	Androgen receptor	AR	1T73
2	Constitutive androstane receptor	CAR	1XNX
3	COUP transcription factor 2	COUP-*TFII*	3CJW
4	DAX1		3F5C
5	Estrogen receptor α	ERα	1QKU
6	Estrogen receptor β	ERβ	3OLL
7	Estrogen-related receptor α	ERRα	3D24
8	Estrogen-related receptor γ	ERRγ	1KV6
9	Farnesoid X receptor	FXR	3BEJ
10	Glucocorticoid receptor	GR	3MNE
11	Hepatocyte nuclear factor 4α	HNF4α	1M7W
12	Hepatocyte nuclear factor 4γ	HNF4γ	1LV2
13	Liver receptor homolog-1	LRH-1	4DOS
14	Liver X receptor α	LXRα	3KFC
15	Liver X receptor β	LXRβ	1PQ6
16	Mineralocorticoid receptor	MR	2A3I
17	Nerve growth factor I B	NGFI B	2QW4
18	Peroxisome proliferator-activated receptor α	PPARα	1K7L
19	Peroxisome proliferator-activated receptor γ	PPARγ	2PRG
20	Peroxisome proliferator-activated receptor Δ	PPARΔ	1GWX
21	Photoreceptor-specific nuclear receptor	PNR	4LOG
22	Pregnane X receptor	PXR	1NRL
23	Progesterone receptor	PR	1A28
24	RAR-related orphan receptor α	RORα	1N83
25	RAR-related orphan receptor β	RORβ	1K4W
26	RAR-related orphan receptor γ	RORγ	3L0L
27	Retinoic acid receptor α	RARα	1DKF
28	Retinoic acid receptor β	RARβ	1XAP
29	Retinoic acid receptor γ	RARγ	3LBD
30	Retinoid-X receptor α	RXRα	1LBD
31	Retinoid-X receptor β	RXRβ	1UHL
32	Retinoid-X receptor γ	RXRγ	2GL8
33	Rev-ErbAβ	EAR1	2V0V
34	Small heterodimer partner	SHP	4NUF
35	Steroidogenic factor 1	SF1	1YP0
36	Testicular receptor 4	TR4	3P0U
37	Thyroid hormone receptor α	TRα	4LNX
38	Thyroid hormone receptor β	TRβ	1NAX
39	Vitamin D receptor	VDR	1DB1

Tools (http://autodock.scripps.edu/resources/adt). All prepared protein structures were saved in pdbqt (Protein Data Bank, Partial Charge) format in SPEN.

AutoDock Vina (Trott and Olson, 2010) was adopted for inverse molecular docking. The database of target chemicals was compiled by use of the C/C++ language. The protocol used by SPEN can be briefly described as follows: First, molecular structures of each chemical were prepared. Then AutoDock Vina was used to dock a given ligand to the LBD of each target NR. Confirmation of each protein-ligand complex was generated and free energies of binding were calculated. NRs were ranked based on calculated energies of binding of the chemical with each NR.

2.2. Identifying potential agonist-effective and ineffective NRs

In principle, a cutoff value of binding energy should be determined to identify if a ligand can activate a receptor or not. However, we failed to find a specified value that could be used as a cutoff that was consistent with experimental results (data not shown) so another strategy was proposed. The majority of chemicals that could bind to the AR were anti-androgenic, but not androgenic. At the same time, the contained conformation of AR in SPEN is based on the agonist-state. Thus NRs can be classified as ineffective NRs if their binding energies for the tested chemical are less than the energy of binding between the AR and the same chemical. NRs for which binding energies were greater than the energy between the agonist-state AR and a chemical could be classified as being *potential agonist-effective NRs*. Here, we use the word of potential because the binding energy between chemicals and ineffective NRs can also be greater than the one between chemicals and AR. However, this approach cannot be used to predict antagonist-effective NRs of tested chemicals.

2.3. Evaluation of SPEN

Ten chemicals representing various types of pollutants were selected for testing (Table 2) and potential effective NRs of these 10 chemicals were predicted by SPEN. The objective of this study was to determine potential agonist-effective NRs and exclude ineffective NRs of

Table 2
Information about the chemicals for initial evaluation and the prediction from SPEN.

Chemical name	CAS number	Proved agonist-effective receptors[a]	Demonstrated Ineffective receptors	Ranking of AR (agonist conformation)	Binding score within AR
Fluoranthene	206-44-0	RARα (2), RXRβ (3), LXRα (8), PPARΔ (10), ERα (11) RXRα (12), PXR (30)	TRα (13), FXR (14), VDR (18), ERRγ (19) ERRα (24), GR (26), PPARγ (29), HNF4α (34)	15	−9.4
Linuron	330-55-2	TRβ (1), VDR (2), PXR (31)	LXRα (7), FXR (9), ERRα (11), RXRβ (15), GR (19), PPARγ (22), ERRγ (29), HNF4α (30)	12	−6.8
Triclosan	3380-34-5	RARα (1), LXRα (6), TRβ (7), ERα (16), PXR (20), RXRα (23), PPARγ (28)	ERRα (13), PPARα (17), ERRγ (29), HNF4α (33)	25	−7.4
p,p'-DDT	50-29-3	LXRα (3), RARα (10), ERα (11), PPARΔ (15), TRβ (18), GR (21), PXR (36)	FXR (9), HNF4α (20), TRα (24) ERRα (27), ERRγ (30)	23	−7.3
Benzo[*A*]pyrene	50-32-8	VDR (5), TRβ (6), HNF4α (8), ERα (10)	NONE	12	−11.3
Vinclozolin	50471-44-8	RARα (4), ERα (20), RXRα (26) PXR (39)	RXRβ (16), ERRγ (25), HNF4α (38)	30	−6.6
Retinal	514-85-2	RORγ (23), PPARγ (25), ERα (15)	NONE	28	−6.9
BDE-47	5436-43-1	TRβ (5), PPARγ (23)	LXRα (3) FXR (13)	25	−6.7
Bisphenol A	80-05-7	ERα (2), NGFIB (6), LXRα (9), DAX1 (11), PXR (33)	FXR (5), RXRβ (14), ERRγ (30), HNF4α (32), ERRα (34)	12	−8.2
Phenanthrene	85-01-8	LXRα (7), PPARΔ (13), PXR (14), ERα (19), GR (23), MR (24), ERβ (25)	FXR (11), TRα (17), ERRγ (19), ERRα (29), HNF4α (33),	27	−7.6

[a] The ranking of target NRs based on SPEN predictions is shown in brackets.

chemicals. Thus, a small rate of false negatives was acceptable in a robust SPEN. Thus, SPEN was determined to be an acceptable predictive model and had sufficient power to be applied to screen environmental contaminants provided that we can get high true positive and true negative. Agonist-effective receptors of chemicals that have been confirmed by use of in vitro or in vivo tests can be found in the ToxCast database (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data) and ChemProt database (http://potentia.cbs.dtu.dk/ChemProt/). The latter set of values assembles chemical–protein connections from multiple sources, such as ChEMBL, DrugBank, BindingDB, STITCH, PharmGKB, IUPHAR, Ki Database, CTD and WOMBAT.

A classical method to evaluate the power of inverse docking is the "receiver operating characteristic" (ROC), which is a plot in which the ordinate is sensitivity (SE) and abscissa is 1-minus specificity (1-SP). Comparison to the line of SE = 1-SP provides an estimation of accuracy of predictions. A more detailed description of the ROC and it's calculation is presented elsewhere (Triballeau et al., 2005). In principle, SE and SP can be calculated (Eqs. 1 and 2, Fig. 3A).

$$SE = TP/(TP + FN) \quad (1)$$

$$SP = TN/(TN + FP) \quad (2)$$

Here, TP, TN, FP and FN represent true positives, true negatives, false positives and false negatives, respectively. For this study, data on effects of chemicals on several receptors were not available. When tested for 10 chemicals, NRs for which no data were available were not considered (Fig. 3B). When results for the same chemical-receptor pair were contradictory, the receptor was classified as undetermined and the results were not considered in the overall assessment of accuracy. For example, benzo[*a*]pyrene cannot activate PPARΔ in the test of *TOX21_PPARd_BLA_agonist_ch1*, but it can activate PPARΔ in *TOX21_PPARd_BLA_agonist_ch2*. In this study PPARΔ was neither considered to be an agonist-effective receptor nor proved to be an ineffective receptor binding to benzo[*a*]pyrene.

2.4. Application to environmental contaminants

SPEN was applied to 40 environmental contaminants (Table 3). Molecular structures of these chemicals were prepared according to the above method. Then NRs that were predicted to interact with the

A

	Experimental Results	
In *Silico*	Actives	Inactives
Selected	True Positives (TP)	False Positives (FP)
Discarded	False Negatives (FN)	True Negatives (TN)
	SE=TP/(TP+FN)	SP=TN/(TN+FP)

B

	Experimental Results		
In *Silico*	Actives	Undetermined/ Untested	Inactives
Selected	True Positives (TP)	Not Counted	False Positives (FP)
Discarded	False Negatives (FN)		True Negatives (TN)

Fig. 3. Overview of receiver operating characteristic (ROC). (A) Typical method for calculating sensitivity and specificity. (B) The undetermined or untested targets are neglected in this study, and then we can calculate the sensitivity and specificity. SE and SP calculations the same in panel A and B except that undetermined/untested targets are excluded.

Table 3
Information on 40 chemicals.

No.	Name of ligands	CAS number	NO.	Name of ligands	CAS number
1	Diethylhexyl phthalate	117-81-7	21	BDE-28	41318-75-6
2	Hexachlorobenzene	118-74-1	22	Linolelaidic acid	506-21-8
3	Fipronil	120068-37-3	23	Arachidonic acid	506-32-1
4	Anthracene	120-12-7	24	Serotonin	50-67-9
5	Pyrene	129-00-0	25	Norepinephrine	51-41-2
6	Vaccenic acid	143-25-9	26	o,p'-DDD	53-19-0
7	Kepone	143-50-0	27	Dibenz[*a*,*h*]anthracene	53-70-3
8	TCDD	1746-01-6	28	Myristoleic acid	544-64-9
9	PFOS	1763-23-1	29	Benz[*a*]anthracene	56-55-3
10	BDE-75	189084-63-7	30	Chlordane	57-74-9
11	Atrazine	1912-24-9	31	Apomorphine	5817-39-0
12	4,4′-dichlorobiphenyl	2050-68-2	32	BDE-99	60348-60-9
13	Benzo[*b*]fluoranthene	205-99-2	33	Dieldrin	60-57-1
14	Benzo[*k*]fluoranthene	207-08-9	34	Ketoconazole	67747-09-5
15	Acenaphthylene	208-96-8	35	Endrin	72-20-8
16	Chrysene	218-01-9	36	p,p'-DDD	72-54-8
17	2,2′,4,4′-tetrachlorobiphenyl	2437-79-8	37	o,p'-DDT	789-02-6
18	Tretinoin	302-79-4	38	Cholic acid	81-25-4
19	Aldrin	309-00-2	39	Acenaphthene	83-32-9
20	PFOA	335-67-1	40	Dibutylphthalate	84-74-2

most chemicals were determined by using hierarchical clustering with Euclidean distance.

3. Results and discussion

3.1. Software development, evaluation

After preparing 3D structures of all 39 target NRs, their ligand binding domains (LBDs) of target NRs were defined as a rectangular box covering all the amino acid residues in active sites. The size (length, width and height) and center of the box were entered into configuration files. Users can change parameters according to their specific objectives. No graphical interfaces were developed for this software, and it can only be used in CMD.exe or Linux terminal. A brief description of SPEN is shown in the supplementary material, but a complete manual for operation can be obtained from the authors. A copy of the code for the algorithm can also be obtained from the authors.

Ten chemicals were selected for initial evaluation included those from several classes including polycyclic aromatic hydrocarbon (PAH), chlorinated pesticides, flame retardant and organic acids (Table 2). Affinities of binding between these 10 selected chemicals and all 39 NRs in the database were calculated and NRs were ranked based on binding affinity predicted by SPEN (Table S1 and Table S2) and potential agonist-effective NRs were identified (red in Table S1). For comparison, experimentally determined agonist-effective and ineffective NRs of these 10 chemicals are summarized in Table 2. Almost all NRs that were predicted to be agonist-effective by SPEN were also demonstrated to be agonist-effective empirically. This result suggests that SPEN can be used to exclude ineffective NRs and select potentially agonist-effective NRs for further experimental screening. However, based on ROCs for the 10 chemicals there were some false positive and false negative assignments of agonist-effective chemicals (Fig. 4). In Fig. 4, each point represents one target NR. All of the points were above the line of Sensitivity = 1-Specificity, which suggests that the SPEN approach described herein was better than random classification and that SPEN has acceptable power of prediction.

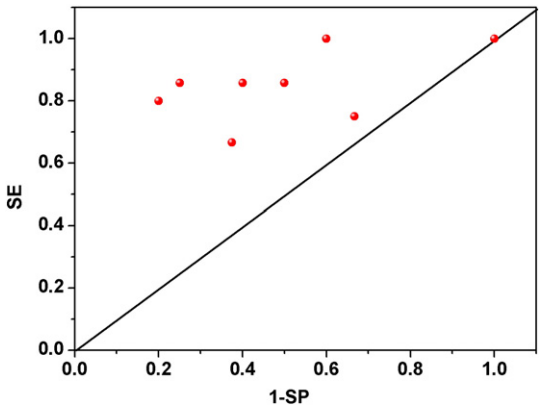

Fig. 4. ROC for evaluation. Red circles represent the receiver operating characteristic of 10 selected chemicals and the black solid line represents the receiver operating characteristic of a random prediction. The result demonstrates our prediction is much better than the random prediction. SE and SP represent sensitivity and specificity, respectively.

The alpha conformation of the estrogen receptor (ERα) is the most studied NR (David et al., 1995) and many chemicals have been shown to bind to it. According to our predictions, ERα was predicted to be agonist-effective for 9 chemicals (Table S1). It was predicted by SPEN to be ineffective for linuron, which is consistent with empirical measurements of binding of ERα by linuron in the cell-free ER binding assay (Agency, 2015).

Results for Bisphenol A (BPA), which is a widely studied chemical, were analyzed in more detail. BPA is a weak ERα agonist (Nunez et al., 2001; Ge et al., 2014b; Zhang et al., 2001). However, BPA can have a number of effects in vitro and in vivo that might be caused by pathways modulated by other NRs (Chen et al., 2015; Xi et al., 2012; Ge et al., 2014a). Small heterodimer partner (SHP), ERα, TRs, CAR, farnesoid X receptor (FXR), nerve growth factor I B (NGFIB), liver X receptors (LXRs), retinoic acid receptor α (RARα) and DAX1 were identified as potential agonist-effective NRs of BPA by SPEN. Of these NRs, ERα, NGFIB and DAX1 have been classified as effective NRs by experiments. However, NGFIB and DAX1 have not yet been paid attentions in the previous theoretical study. Therefore, the former tool cannot predict that NGFIB and DAX1 are effective NRs of BPA. HNF4α can bind with benzo[*A*] pyrene, and SPEN also predicted that HNF4α is a potential agonist-effective NR of benzo[*A*]pyrene (Table 2), but this target has not been contained in the Kolšek model. These comparisons demonstrate the advantages of SPEN. In this study, binding scores (= − log[Equilibrium Constant], expressed as kcal/mol) were calculated for binding of NRs with chemicals. Values for AR are shown (Table 2). Binding affinities between NRs and chemicals are inversely proportional to binding scores. All of the binding scores for interactions between chemicals and the AR were less than −*6.5*. Thus, it is proposed that −*6.5* be used as a threshold to classify chemicals as agonist-effective NRs or -ineffective NRs.

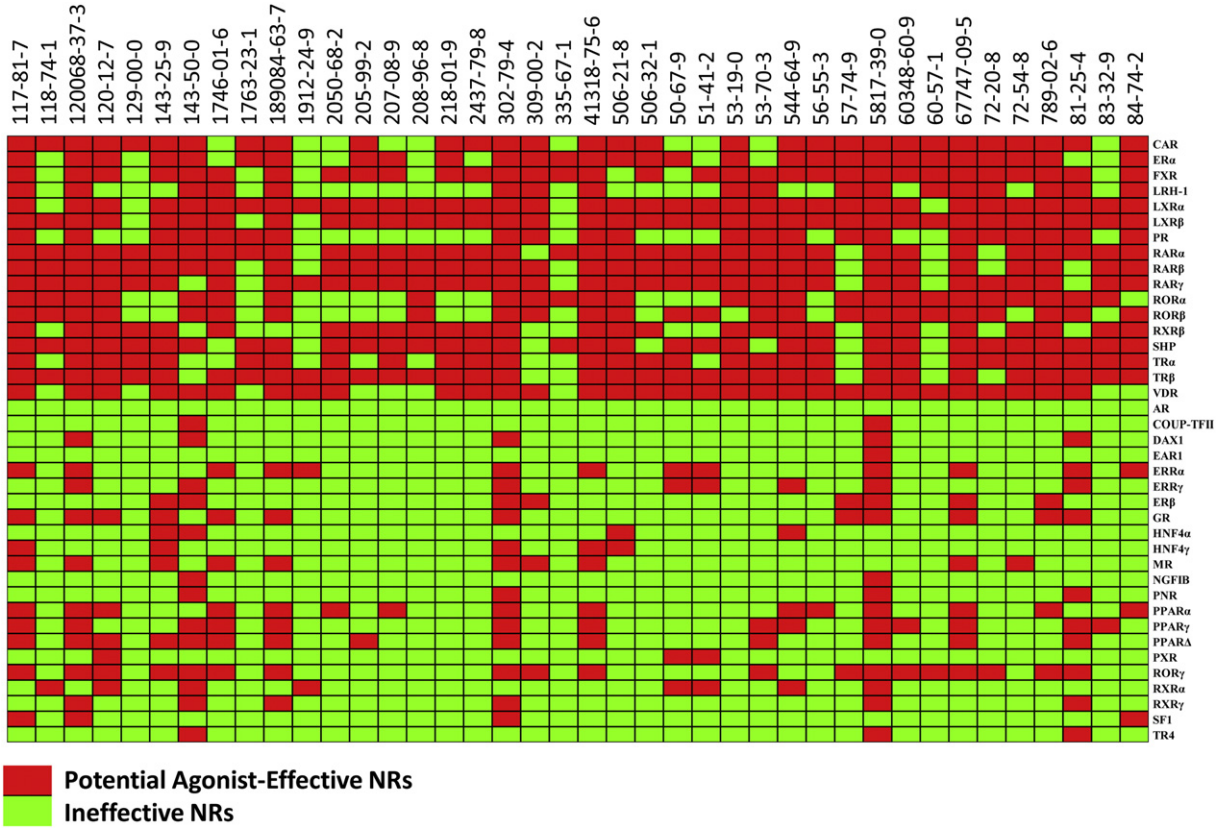

Fig. 5. Heat map of interactions of 40 chemicals with 39 NRs. Red cells represent NRs predicted to be effective, while green cells represent NRs predicted to be ineffective. Horizontal axis and vertical axis present chemicals (CAS number) and target NRs, respectively.

3.2. Application to pollutants

The 40 chemicals studied for application have diverse structures and include both traditional and emerging pollutants (Table 3). Thus, results based on this set of chemicals represent a range of types of potential pollutants. When classifications based on results of SPEN are summarized in a heat plot (Fig. 5 and Table S3), red cells represent potential agonist-effective NRs whose ranking is greater than that of the AR and green cells represent ineffective NRs. A large area of red cells covers the zone of CAR, ERα, FXR, liver receptor homolog-1 (LRH-1), LXRs, progesterone receptor (PR), RARs, RAR-related orphan receptors (RORα and RORβ), retinoid X receptor β (RXRβ), SHP, TRα, TRβ and vitamin D receptor (VDR). Therefore, these NRs were suggested to be more vulnerable targets for contaminants. The widely studied ERα, PR and TRs are vulnerable to potential interactions by most of the contaminants studied. This suggests that assessment of endocrine disrupting activities of chemicals mediated by ERα, PR or TRs should be priorities for assessment. However, many potential NR targets, which are presented by a large area of red cells in Fig. 5, haven't been investigated. It is suggested that these NRs should be included in experimental screening. LRH-1 is important for embryonic development (Gu et al., 2005) and SHP is a heterodimer partner of other NRs, that could be activated by various contaminants as prediction from SPEN show. However, there is little research into effects of chemicals on humans through LRH-1 and SHP. As type II nuclear receptors, in the absence of ligand, RARs and RXRβ can become heterodimers with each other. Thus, the probability of dissociation of heterodimers by exogenous contaminants is quite high. Because these targets are important for germ cell differentiation, adverse effects of chemicals mediated by RARs and RXRβ should be priorities for assessment. CAR, FXR, LXR and VDR are responsible for detoxification of foreign substances, regulation of hepatic triglyceride levels, metabolism of physiological lipid/cholesterol and mineral metabolism (Adorini et al., 2006; Peet et al., 1998; Jiao et al., 2015; Wada et al., 2009), respectively. The RORs are important for many biological processes, such as lipid metabolism and maintenance of bone and other functions (Jetten, 2004). Some chemicals, which have been studied for other reasons, were found to also interact with NRs. For instance, a binding score of -6.5 from SPEN was suggested to be a criterion to identify the effective NRs and ineffective NRs. While it was found that scores for PAHs, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, dibenz[*a*,*h*]anthracene and benz[*a*]anthracene, were all less than -6.5 (Table S4), they have been widely studied due to their persistence and toxic potencies. Results of the SPEN analysis emphasize the potential for adverse effects of PAHs in the environment that are mediated by NRs.

3.3. Limitations and perspective

While chemicals can have adverse effects via other pathways (Ma et al., 2011; Huang et al., 2001; Ma et al., 2012; Nusrin et al., 2014; Hecker et al., 2011) or initiate adverse effect through pathways other than binding to NRs, those effects are not predicted by use of SPEN. For this reason, use of SPEN in an overall assessment of risks posed by chemicals is only one aspect of the assessment that can help guide design of additional screening. Most of the pollutants studied had anti-androgenic activity but not androgenic (Kelce et al., 1997; Orton et al., 2014). This is due, in part, because the conformation of AR specified in SPEN was based on the agonist-state. To solve this problem, conformations of the antagonist-states of NRs will be included in future versions of SPEN. External factors can affect the binding of ligands to NRs (i.e. increase or decrease strengths). For example, water molecules in the binding pocket can result in hydrogen bonding with ligands that can stabilize the ligand in the pocket. This phenomenon is not currently included in the SPEN prediction of docking between ligands and NRs (Yi and Zhang, 2012). More knowledge of these factors can improve

simulations of 3D-structures of targets for docking. And of course, other computational methods should be combined. It is still impossible to distinguish by molecular simulation between agonists and antagonist of G protein-coupled receptors (GPCRs), while quantitative structure-activity relationship (QSAR) is the method of choice. We think QSAR will also be useful for NRs (Don and Riniker, 2014).

While only the "pocket mode" was considered here, chemicals can interfere with behaviors of targets in direct and indirect non-binding modes. For example, ligands could interact with activation function 1 (AF1) regions of NRs to prevent proper folding (Freedman, 1999). Chemicals can also affect pathways that are not mediated by NRs. For instance concentrations of hormones that act upon specific receptors can be directly or indirectly affected by exposure to chemicals (Liu et al., 2010; Hecker and Giesy, 2008). Also, crosstalk between receptors was a common phenomenon in the organisms. The potential for crosstalk between receptors must also be taken into consideration in terms of future directions of this approach. The protonation states of the ionizable residues can also make effect on the affinity between ligands and targets, and we will adopt this phenomenon in our study (Czerwinski et al., 2001; Stivers et al., 1996). Therefore, a more complete, detailed mode of interaction should be incorporated to optimize our system approach moving forward.

Author contributions

X. X. W., W. S. and H. X.Y. designed the study; X. X. W., J. J. Z., Y. T. W. and R. Z. conducted the study and collected data; X. X. W., X. W.Z. and P. X. performed analyses; X. X.W. wrote the first draft of the manuscript, J.P.G. rewrote the draft manuscript and edited the final version, while X. W.Z., W.S. and H. X. Y. contributed to revisions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2016.10.093.

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A high-throughput, computational system to predict if environmental contaminants can bind to human nuclear receptors

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Brief Manual

Users put the prepared pdbqt file of the ligand to be screened (chemical of interest) into the folder of ligands, and then use the command “spen.exe ..\ligands\name(ligand) ..\name (result)” or “spen.exe ../ligands/name(ligand) ../name (result)” to start the program. When the simulations and comparisons are completed the file of result will generate in the parent folder. PDB entries, name of targets and scores were listed in file of result based on ranking of score. Complexes of ligand and all receptors were generated in the folder of results. Notably, the complexes will be overwritten by the next job.

Table S5. The resolutions of obtained PDBs of nuclear receptors.

PDB Entry	Resolution (Å)	PDB Entry	Resolution (Å)
1T73	2.2	4LOG	2.7
1XNX	2.9	1NRL	2
3CJW	1.48	1A28	1.8
3F5C	3	1N83	1.63
1QKU	3.2	1K4W	1.9
3OLL	1.5	3L0L	1.74
3D24	2.11	1DKF	2.5
1KV6	2.7	1XAP	2.1
3BEJ	1.9	3LBD	2.4
3MNE	1.96	1LBD	2.7
1M7W	2.8	1UHL	2.9
1LV2	2.7	2GL8	2.4
4DOS	2	2V0V	2.4
3KFC	2.4	4NUF	2.8
1PQ6	2.4	1YP0	1.5
2A3I	1.95	3P0U	3
2QW4	2.8	4LNX	2.05
1K7L	2.5	1NAX	2.7
2PRG	2.3	1DB1	1.8
1GWX	2.5		

potential effective NRs
ineffective NRs

	NAME	Fluoranthe	Linuron	Triclosan	p,p'-DDT	Benzo[a]p	Vinclozolin	Retinal	BDE-47	Bisphenol	Phenanthrene
	CAS	206-44-0	330-55-2	3380-34-5	50-29-3	50-32-8	50471-44-8	514-85-2	5436-43-1	80-5-7	85-1-8
1		SHP	TRβ	RARα	LXRβ	RARβ	LXRα	RARγ	LXRβ	SHP	LXRβ
2		RARα	VDR	LXRβ	RARβ	RARγ	ERRα	RARβ	RARβ	ERα	SHP
3		RXRβ	RARα	RARγ	LXRα	RXRβ	SHP	RARα	LXRα	TRα	RXRβ
4		TRβ	RARγ	VDR	CAR	RARα	RARα	RXRβ	CAR	CAR	RARγ
5		RARβ	LXRβ	RARβ	SHP	VDR	RARβ	RORα	TRβ	FXR	RARα
6		CAR	RARβ	LXRα	RARγ	TRβ	VDR	RORβ	RXRβ	NGFIB	TRβ
7		RARγ	LXRα	TRβ	RXRβ	FXR	PR	VDR	RARγ	LXRβ	LXRα
8		LXRα	TRα	RXRβ	PR	HNF4α	LXRβ	CAR	VDR	TRβ	CAR
9		RORα	FXR	CAR	FXR	CAR	CAR	FXR	TRα	LXRα	RORα
10		PPARΔ	PPARα	TRα	RARα	ERα	RARγ	LXRα	RARα	RARα	RARβ
11		ERα	ERRα	FXR	ERα	LXRα	RORβ	LXRβ	RORβ	DAX1	FXR
12		RXRα	AR	PPARΔ	VDR	AR	RORα	TRα	RORγ	AR	PPARΔ
13		TRα	PR	ERRα	RORα	TRα	TRβ	SHP	FXR	RORβ	PXR
14		FXR	SHP	RORβ	RORβ	LXRβ	FXR	TRβ	PPARΔ	RXRβ	RXRα
15		AR	RXRβ	SHP	PPARΔ	SHP	MR	ERα	RORα	RORα	VDR
16		LXRβ	CAR	ERα	RORγ	PR	RXRβ	PPARΔ	PR	RARγ	ERα
17		RORβ	RORα	PPARα	MR	PPARΔ	TRα	PPARα	SHP	RARβ	TRα
18		VDR	ERα	PR	TRβ	RORβ	RORγ	LRH-1	MR	VDR	RORβ
19		ERRγ	GR	MR	LRH-1	GR	PPARΔ	RXRγ	LRH-1	PR	ERRγ
20		PR	RORβ	PXR	HNF4γ	RORγ	ERα	GR	ERRα	MR	RORγ
21		RORγ	MR	RORα	GR	RORα	GR	ERβ	ERα	GR	PR
22		LRH-1	PPARγ	GR	PPARγ	LRH-1	PPARα	HNF4γ	GR	ERβ	LRH-1
23		PPARα	PPARΔ	RXRα	AR	ERβ	LRH-1	RORγ	PPARγ	HNF4γ	GR
24		ERRα	HNF4γ	HNF4γ	TRα	MR	PPARγ	ERRα	ERRγ	RORγ	MR
25		ERβ	RORγ	AR	ERβ	ERRα	ERRγ	PPARγ	AR	PPARΔ	ERβ
26		GR	LRH-1	LRH-1	PPARα	PPARα	RXRα	SF1	RXRα	PPARα	PPARα
27		PNR	ERβ	RORγ	ERRα	PNR	DAX1	HNF4α	PPARα	PPARγ	AR
28		MR	SF1	PPARγ	HNF4α	PPARγ	NGFIB	AR	DAX1	RXRα	HNF4γ
29		PPARγ	ERRγ	ERRγ	RXRγ	RXRα	ERβ	RXRα	PNR	LRH-1	ERRα
30		PXR	HNF4α	ERβ	ERRγ	HNF4γ	AR	PR	RXRγ	ERRγ	PNR
31		HNF4γ	PXR	SF1	DAX1	RXRγ	RXRγ	DAX1	HNF4γ	RXRγ	PPARγ
32		RXRγ	RXRγ	RXRγ	TR4	ERRγ	HNF4γ	COUP-TFI	SF1	HNF4α	DAX1
33		DAX1	RXRα	HNF4α	PNR	DAX1	PNR	EAR1	TR4	PXR	HNF4α
34		NGFIB	DAX1	DAX1	COUP-TFI	SF1	SF1	ERRγ	COUP-TFII	ERRα	RXRγ
35		COUP-TFI	COUP-TFI	PNR	RXRα	COUP-TFI	COUP-TFII	PXR	NGFIB	PNR	TR4
36		HNF4α	TR4	TR4	PXR	TR4	TR4	PNR	ERβ	SF1	NGFIB
37		SF1	PNR	COUP-TFI	NGFIB	NGFIB	EAR1	MR	EAR1	COUP-TFI	SF1
38		TR4	NGFIB	NGFIB	EAR1	EAR1	HNF4α	TR4	HNF4α	TR4	COUP-TFII
39		EAR1	EAR1	EAR1	SF1	PXR	PXR	NGFIB	PXR	EAR1	EAR1

NAME	p,p'-DDT	Benzo[a]py	Bisphenol	Phenanthre	Fluoranthe	Linuron	Retinal	Triclosan	BDE-47	Vinclozolin
CAS	50-29-3	50-32-8	80-5-7	85-1-8	206-44-0	330-55-2	514-85-2	3380-34-5	5436-43-1	50471-44-8
AR	-7.3	-11.3	-8.2	-7.6	-9.4	-6.8	-6.9	-7.4	-6.7	-6.6
CAR	-9	-11.4	-8.8	-9.1	-10	-6.7	-9.4	-8	-8	-8.2
COUP-TFI	-5.9	-8	-6.3	-6.2	-7	-5.5	-6.6	-5.9	-5.6	-6.4
DAX1	-6.4	-9	-8.3	-7	-7.8	-5.5	-6.7	-6.6	-6.4	-6.7
EAR1	-5.4	-6.9	-5.2	-5.1	-5.7	-4.8	-6.2	-5.5	-5.2	-6.2
ERRα	-7.1	-10.1	-6.4	-7.6	-8.7	-6.9	-7.5	-7.8	-7.1	-8.7
ERRγ	-6.4	-9	-7	-8.6	-9.2	-5.8	-6.1	-7.3	-6.7	-7.2
ERα	-8.3	-11.4	-9	-8.6	-9.6	-6.7	-8.6	-7.7	-7	-7.8
ERβ	-7.3	-10.3	-7.6	-7.9	-8.6	-6.2	-8	-7.1	-5.3	-6.7
FXR	-8.6	-11.6	-8.7	-8.9	-9.4	-7	-9.2	-7.9	-7.6	-8
GR	-7.5	-10.6	-7.8	-8	-8.5	-6.7	-8	-7.5	-7	-7.7
HNF4α	-6.9	-11.5	-6.8	-7	-6.9	-5.8	-7.1	-6.7	-5.1	-6.2
HNF4γ	-7.6	-9.3	-7.4	-7.6	-7.9	-6.4	-7.9	-7.4	-6.1	-6.6
LRH-1	-7.6	-10.3	-7	-8.2	-8.8	-6.3	-8.2	-7.3	-7.2	-7.4
LXRα	-9.1	-11.3	-8.5	-9.3	-9.9	-7.2	-9.1	-8.2	-8.3	-8.9
LXRβ	-9.2	-11.2	-8.6	-9.9	-9.3	-7.5	-9	-8.7	-8.9	-8.2
MR	-7.8	-10.1	-7.8	-7.9	-8.3	-6.6	-5.8	-7.6	-7.3	-8
NGFIB	-5.7	-7.8	-8.7	-6.3	-7	-5.4	-5.7	-5.7	-5.4	-6.7
PNR	-5.9	-9.6	-6.3	-7.6	-8.4	-5.4	-5.9	-6.2	-6.4	-6.5
PPARα	-7.2	-9.6	-7.2	-7.7	-8.7	-7	-8.4	-7.7	-6.4	-7.7
PPARγ	-7.4	-9.5	-7.2	-7.1	-8.3	-6.6	-7.2	-7.3	-6.8	-7.2
PPARΔ	-8	-10.9	-7.2	-8.9	-9.7	-6.5	-8.5	-7.8	-7.6	-7.8
PR	-8.8	-11.1	-7.8	-8.3	-9.1	-6.8	-6.7	-7.6	-7.5	-8.3
PXR	-5.8	-6.8	-6.8	-8.9	-8.2	-5.7	-6.1	-7.6	-4.7	-5.6
RARα	-8.4	-12	-8.5	-9.4	-10.5	-7.6	-10.2	-8.7	-7.7	-8.5
RARβ	-9.2	-12.4	-7.9	-9	-10.1	-7.4	-10.4	-8.3	-8.8	-8.4
RARγ	-9	-12.4	-8	-9.5	-10	-7.5	-10.8	-8.5	-7.8	-8.2
RORα	-8.1	-10.3	-8.1	-9	-9.7	-6.7	-9.7	-7.5	-7.6	-8.2
RORβ	-8.1	-10.7	-8.2	-8.6	-9.3	-6.6	-9.5	-7.7	-7.7	-8.2
RORγ	-7.8	-10.4	-7.2	-8.4	-8.9	-6.3	-7.7	-7.3	-7.7	-7.8
RXRα	-5.8	-9.5	-7.1	-8.8	-9.6	-5.5	-6.8	-7.5	-6.5	-6.7
RXRβ	-8.8	-12.3	-8.2	-9.5	-10.2	-6.7	-10	-8	-7.9	-7.9
RXRγ	-6.6	-9.2	-6.8	-7	-7.9	-5.6	-8.1	-6.7	-6.3	-6.6
SF1	-4.9	-8.2	-6.3	-6.3	-6.9	-6	-7.2	-7	-5.8	-6.4
SHP	-9	-11.1	-9.2	-9.5	-10.8	-6.7	-8.8	-7.7	-7.4	-8.6
TR4	-6	-7.9	-6.2	-6.5	-6.8	-5.5	-5.8	-6.2	-5.6	-6.3
TRα	-7.3	-11.3	-8.9	-8.6	-9.5	-7.1	-8.9	-8	-7.8	-7.8
TRβ	-7.6	-11.8	-8.6	-9.3	-10.1	-7.7	-8.7	-8.2	-7.9	-8.1
VDR	-8.2	-12	-7.8	-8.7	-9.3	-7.7	-9.4	-8.5	-7.8	-8.4

Gold Standard of Ligands Name of Targets	117-81-7	118-74-1	120068-37	120-12-7	129-00-0	143-25-9	143-50-0	1746-01-6	1763-23-1	189084-6	1912-24-9	2050-68-2	205-99-2	207-08-9	208-96-8	218-01-9	2437-79-8	302-79-4	309-00-2	335-67-1	41318-75-	506-21-8	506-32-1	50-67-9	51-41-2	53-19-0	53-70-3	544-64-9	56-55-3	57-74-9	5817-39-0	60348-60-	60-57-1	67747-09-	72-20-8	72-54-8	789-02-6	81-25-4	83-32-9	84-74-2
AR	27	11	29	22	9	22	27	21	9	25	7	13	13	13	11	14	13	32	16	6	25	18	13	16	15	17	18	22	15	14	34	18	10	26	15	17	22	24	12	19
CAR	6	2	24	12	4	10	1	27	7	10	16	14	7	15	14	13	5	13	1	19	13	8	8	27	28	4	24	9	14	1	32	4	1	4	1	4	4	20	14	2
COUP-TFII	33	37	33	38	35	32	25	34	37	33	38	38	32	34	37	34	37	33	29	38	35	31	35	39	39	36	36	35	35	32	29	36	32	38	34	34	32	26	36	32
DAX1	32	33	28	33	32	30	18	29	31	28	35	32	28	29	33	31	30	30	25	34	28	34	32	37	37	31	30	31	33	26	23	27	23	31	23	33	27	18	33	31
EAR1	38	38	36	39	39	39	31	38	38	37	37	39	39	39	39	39	39	38	37	39	39	39	39	39	38	38	39	39	39	39	34	33	37	36	36	37	39	39	32	39	38
ERRα	26	18	19	30	27	24	35	7	23	24	4	28	25	20	19	26	22	25	30	26	24	28	22	9	10	30	19	24	25	36	20	23	34	21	29	24	36	7	19	17
ERRγ	31	28	23	25	26	35	14	23	33	27	21	31	31	30	18	27	28	31	21	33	27	30	30	3	14	28	31	15	26	22	25	24	21	29	32	31	28	22	22	26
ERα	13	13	27	15	11	12	3	22	5	11	24	19	8	9	13	12	14	21	5	3	22	12	9	14	17	11	21	8	13	2	31	16	3	19	3	14	8	36	13	5
ERβ	28	21	30	29	21	17	5	32	19	29	27	26	24	21	27	21	25	27	15	17	30	19	20	31	32	20	29	25	24	12	30	29	15	25	17	25	17	35	26	25
FXR	9	19	13	7	16	18	10	6	15	7	12	12	11	7	23	7	12	8	6	2	11	21	12	17	12	10	8	13	6	9	11	7	5	3	9	5	5	1	16	6
GR	24	12	22	11	19	16	28	14	17	15	26	25	22	23	28	22	21	22	18	16	26	24	17	21	23	22	26	32	20	13	27	25	27	20	20	23	21	23	28	29
HNF4α	30	35	37	34	36	20	23	37	25	36	33	33	38	36	36	36	35	37	36	23	33	13	15	26	34	29	38	18	29	35	37	35	35	39	36	29	38	34	37	34
HNF4γ	16	34	35	26	31	19	29	26	24	31	32	23	34	31	29	24	29	24	31	22	23	16	14	28	36	26	22	28	27	37	38	32	31	27	33	20	25	37	32	23
LBH-1	20	22	15	23	24	23	6	18	14	22	29	17	15	25	26	20	23	16	13	25	20	25	25	29	31	14	12	27	18	6	16	19	9	17	14	18	16	13	23	16
LXRα	5	20	4	8	18	6	21	2	3	3	6	5	6	3	8	5	1	3	10	11	6	6	7	4	1	3	1	6	3	5	7	2	12	10	2	8	7	11	9	4
LXRβ	1	6	12	1	10	3	11	4	20	1	15	8	9	10	1	1	10	5	2	15	2	3	6	6	4	1	6	5	5	8	17	1	4	6	7	2	1	17	4	3
MR	25	27	14	27	25	14	30	17	11	20	19	16	23	19	25	18	16	23	11	20	12	20	18	22	20	18	25	29	19	16	35	28	22	24	19	13	24	25	27	20
NGFIB	36	29	34	37	33	37	22	30	36	35	39	37	35	33	34	35	34	35	24	36	36	36	37	32	33	38	32	37	37	25	26	34	26	35	27	36	34	27	34	39
PNR	34	31	31	28	20	36	17	31	35	30	30	36	30	32	31	29	31	29	22	35	31	37	34	35	29	33	28	36	31	19	24	30	20	33	30	37	30	21	30	36
PPARα	19	15	21	18	30	26	34	9	30	17	11	11	20	12	32	25	20	20	32	31	8	22	29	19	22	25	20	21	12	28	15	22	25	22	16	26	19	31	29	13
PPARγ	18	25	18	31	29	28	13	20	29	16	23	29	29	26	17	30	24	17	17	28	21	27	27	30	25	24	16	19	28	17	10	15	18	23	22	27	26	10	11	33
PPARδ	12	23	20	20	23	13	20	16	16	19	20	22	10	16	15	16	26	18	27	24	19	23	24	25	24	23	14	23	17	21	12	20	24	11	28	22	23	16	24	21
PR	14	17	2	24	15	7	7	13	6	18	13	15	19	18	16	15	18	19	12	12	16	15	16	20	16	13	10	12	16	4	22	21	13	16	4	15	12	12	17	15
PXR	39	36	39	21	38	33	37	36	39	38	31	35	37	37	20	38	38	39	39	29	38	35	33	13	8	32	33	33	38	38	36	39	39	37	38	30	35	39	25	27
RARα	3	1	10	4	2	2	12	11	8	13	14	4	3	4	4	8	4	2	19	4	1	2	3	15	5	7	17	1	7	20	5	5	17	9	26	11	14	15	2	12
RARβ	2	7	3	2	6	5	15	3	18	9	8	1	1	1	6	3	9	1	14	9	3	4	4	1	3	5	3	2	2	18	2	12	11	1	18	7	3	28	6	9
RARγ	7	3	6	3	7	4	33	1	10	21	3	2	2	2	3	4	11	4	7	10	4	5	1	5	9	2	2	3	1	24	19	14	14	5	8	9	2	33	5	8
RORα	17	8	7	17	14	29	2	12	22	6	25	18	16	17	9	19	17	7	3	5	14	14	23	24	18	15	15	11	21	3	9	9	2	14	5	16	10	2	8	22
RORβ	8	10	5	16	13	25	9	10	12	12	17	21	14	22	10	23	19	11	4	21	17	11	21	8	11	19	13	16	22	7	4	10	7	12	11	21	13	4	15	18
RORγ	21	24	16	19	17	21	8	15	26	14	28	20	21	24	22	17	15	9	9	30	18	29	26	36	30	21	11	30	23	11	14	13	6	18	10	19	20	3	21	28
RXRα	37	5	38	13	12	34	24	35	28	34	1	30	26	27	24	28	33	36	23	18	37	33	36	2	7	37	37	20	30	31	28	31	16	34	31	38	29	29	20	30
RXRβ	4	16	11	6	5	8	32	8	21	26	22	7	5	6	5	2	7	14	33	14	10	7	5	18	21	9	4	10	8	29	1	6	33	13	35	1	6	30	7	10
RXRγ	29	32	26	32	28	31	26	28	32	23	36	24	27	28	30	32	27	26	34	32	29	32	31	33	35	27	23	34	32	27	21	26	29	28	25	28	31	14	31	35
SF1	22	39	25	36	37	27	39	39	27	39	9	34	36	38	35	33	36	28	38	27	32	26	28	23	26	34	35	26	34	39	39	38	38	30	39	32	37	38	35	7
SHP	23	4	17	5	1	15	16	25	4	5	18	9	4	8	2	11	8	15	20	1	15	17	19	7	2	12	27	14	9	15	6	11	19	7	6	12	9	8	1	1
TR4	35	30	32	35	34	38	19	33	34	32	34	27	33	35	38	37	32	34	28	37	34	38	38	34	27	35	34	38	36	23	18	33	28	32	24	35	33	19	38	37
TRα	15	14	8	14	8	11	36	24	1	2	10	10	17	11	12	10	6	12	26	8	5	9	10	10	19	8	9	17	10	30	13	3	30	2	13	6	18	6	10	14
TRβ	10	9	1	9	3	1	38	19	2	4	5	6	12	5	7	6	3	10	35	7	7	1	2	11	6	6	7	4	4	33	8	17	37	15	21	3	11	5	3	11
VDR	11	26	9	10	22	9	4	5	13	8	2	3	18	14	21	9	2	6	8	13	9	10	11	12	13	16	5	7	11	10	3	8	8	8	12	10	15	9	18	24

	117-81-7	118-74-1	120068-37-3	120-12-7	129-00-0	143-25-9	143-50-0	1746-01-6	1763-23-1	189084-64-0	1912-24-9	2050-68-2	205-99-2	207-08-9	208-96-8	218-01-9	2437-79-8	302-79-4	309-00-2	335-67-1	41318-75-6	506-21-8	506-32-1	50-67-9	51-41-2	53-19-0	53-70-3	544-64-9	56-55-3	57-74-9	5817-39-6	60348-60-8	68-57-1	67747-09-4	72-20-8	72-54-8	789-02-6	81-25-4	83-32-9	84-74-2
AR	-6.4	-5.9	-7.1	-8.3	-9.5	-6.3	-4.8	-7.4	-9.7	-6.3	-6.4	-7.7	-10.8	-11.3	-7.7	-10.2	-8	-6.6	-7.2	-8.8	-6.9	-6.6	-7.4	-6.5	-6	-7.9	-10.6	-6.1	-10.4	-7.5	-5.1	-7.2	-7.5	-6.9	-7.7	-7.8	-7.7	-6.8	-7.6	-6.8
CAR	-8	-6.6	-7.8	-8.7	-9.9	-6.8	-9.2	-6.8	-9.8	-7.5	-6.2	-7.6	-11.5	-11.1	-7.6	-10.3	-8.7	-9	-9.4	-8.4	-7.7	-7.1	-7.8	-6.1	-5.5	-9.1	-9.9	-6.6	-10.5	-9.1	-5.5	-8.2	-8.5	-8.1	-9	-8.7	-9.2	-7.3	-7.5	-7.5
COUP-TFII	-5.9	-4	-6.6	-5.9	-6.8	-5.3	-5.3	-5.9	-6.8	-5.2	-4.8	-5.3	-8.4	-8	-5.6	-7.3	-5.7	-6.6	-5.6	-6.1	-5.6	-5.6	-5.2	-5.1	-4.9	-5.6	-7.5	-5	-7.5	-5.5	-6	-5.2	-5.3	-5.3	-5.4	-5.8	-6.1	-6.7	-5.6	-5.8
DAX1	-6	-4.6	-7.2	-6.9	-7.4	-5.5	-5.9	-6.6	-8.2	-6	-5.1	-6.6	-9.2	-9	-6.3	-8.4	-6.7	-7	-6.2	-6.9	-6.6	-5.3	-5.7	-5.5	-5.1	-6.5	-9.1	-5.5	-8.4	-5.9	-6.5	-6.3	-6	-6.2	-6.8	-6	-6.6	-7.6	-6.2	-5.8
EAR1	-4.6	-3.9	-6.1	-5.3	-5.4	-4	-4.5	-5.1	-6.7	-4.6	-5	-4.7	-6.8	-6.5	-4.7	-6.4	-5.1	-5.5	-4.6	-6	-5.1	-4.1	-4.2	-5.3	-5	-4.8	-6.5	-4.3	-6.4	-5	-5.3	-5.1	-4.7	-5.7	-5.2	-5.5	-4.7	-5.2	-4.6	-4.9
ERRα	-6.6	-5.6	-8.3	-7.5	-8.2	-6.1	-4.1	-8.6	-9.2	-6.5	-6.6	-6.8	-9.3	-10.5	-7.5	-9	-7.6	-7.8	-5.6	-7.8	-7	-5.9	-6.8	-6.7	-6.2	-6.5	-10.4	-5.9	-9.3	-4.7	-6.9	-6.9	-5	-7.1	-6.2	-7.3	-5.6	-9	-7.4	-6.9
ERRγ	-6.1	-4.8	-8	-8.1	-8.3	-4.9	-6.6	-7.2	-8	-6.2	-6.1	-6.6	-8.9	-8.9	-7.6	-8.8	-6.8	-6.8	-6.4	-7.2	-6.6	-5.7	-6.2	-7.1	-6.1	-6.6	-9.1	-6.3	-9.1	-6.2	-6.5	-6.7	-6.2	-6.5	-5.7	-6.4	-6.5	-7	-7.2	-6.4
ERα	-7.6	-5.9	-7.5	-8.6	-9.4	-6.8	-8.6	-7.3	-10.1	-7.3	-6	-7.3	-11.4	-11.7	-7.7	-10.4	-8	-8.1	-8.3	-8.9	-7.1	-6.9	-7.7	-6.5	-5.9	-8.6	-10.1	-6.6	-10.6	-8.5	-5.6	-7.3	-8.2	-7.6	-8.8	-8.1	-8.8	-4	-7.6	-7.3
ERβ	-6.2	-5.4	-7.1	-7.7	-8.6	-6.5	-7.8	-6.1	-9.3	-5.8	-5.8	-6.9	-9.9	-10.3	-7	-9.5	-7.2	-7.4	-7.2	-8.4	-6.2	-6.5	-6.9	-5.8	-5.3	-7.7	-9.2	-5.9	-9.4	-7.7	-5.7	-6.1	-6.8	-7	-7.4	-7.2	-8	-4.8	-6.9	-6.5
ERR	-7.9	-5.6	-9	-9	-9	-6.5	-7	-8.7	-9.6	-7.6	-6.3	-7.7	-10.9	-11.9	-7.4	-10.8	-8.1	-9.5	-8	-9.2	-7.8	-6.4	-7.5	-6.5	-6.1	-8.6	-11.8	-6.4	-11.1	-7.9	-7.6	-7.9	-8	-8.2	-8.2	-8.7	-9	-10.4	-7.5	-7.3
GR	-6.7	-5.9	-8.1	-8.8	-8.7	-6.5	-4.8	-7.9	-9.3	-6.9	-5.9	-6.9	-10.2	-10.3	-7	-9.5	-7.7	-7.9	-6.7	-8.4	-6.8	-6.3	-7.2	-6.3	-5.7	-7.6	-8.7	-5.5	-9.8	-7.5	-6.1	-6.6	-5.6	-7.2	-7	-7.3	-7.7	-6.8	-6.9	-6
HNF4α	-6.1	-4.1	-5.9	-6.7	-6.7	-6.4	-5.4	-5.1	-9.2	-5.1	-5.3	-6.2	-7	-7.4	-5.8	-7.2	-6.1	-5.5	-5.1	-8.2	-5.9	-6.8	-7.3	-6.1	-5.2	-6.5	-7.1	-6.2	-8.8	-4.8	-5.4	-4.9	-5	-5.2	-6.9	-4.9	-5	-5.5	-5.7
HNF4γ	-7.4	-4.5	-6.2	-8	-7.8	-6.4	-4.7	-6.9	-9.2	-5.4	-5.4	-7.2	-8.1	-8.6	-6.8	-9.4	-6.8	-7.8	-5.5	-8.3	-7	-6.7	-7.4	-6	-5.1	-7	-10	-5.8	-9	-4.6	-4.6	-5.7	-5.3	-6.6	-5.5	-7.6	-7.5	-3.7	-6.5	-6.5
LRH-1	-7.3	-5.4	-8.6	-8.2	-8.5	-6.2	-7.8	-7.8	-9.6	-6.6	-5.6	-7.3	-10.8	-10.2	-7.2	-9.6	-7.6	-8.6	-7.5	-8.1	-7.2	-6.2	-6.7	-5.9	-5.4	-8.1	-11.3	-5.8	-9.8	-8	-7.4	-7.1	-7.6	-7.7	-7.7	-7.7	-8.1	-8.1	-7.1	-6.9
LXRα	-8.2	-5.6	-9.5	-8.9	-8.8	-7.3	-5.6	-9.4	-10.3	-7.9	-6.4	-8.4	-11.6	-12.3	-7.8	-11	-9	-10.2	-7.5	-8.6	-8.2	-7.6	-8	-7.1	-6.9	-9.1	-13.6	-6.8	-11.4	-8	-7.8	-8.5	-7.3	-7.9	-9	-8.6	-8.8	-8.4	-7.8	-7.4
LXRβ	-8.7	-6.1	-9	-9.8	-9.5	-7.7	-7	-8.9	-9.3	-8.6	-6.2	-8.2	-11.3	-11.6	-8.6	-11.4	-8.3	-9.7	-9	-8.4	-8.8	-8	-8.1	-7	-6.5	-9.7	-12.3	-7	-11.3	-7.9	-7.4	-8.9	-8.1	-8.1	-8.2	-8.8	-10	-7.7	-7.9	-7.4
MR	-6.6	-5.1	-8.8	-7.7	-8.3	-6.6	-4.6	-7.8	-9.7	-6.7	-6.1	-7.4	-10.1	-10.8	-7.3	-9.7	-8	-7.8	-7.5	-8.4	-7.8	-6.5	-7.2	-6.2	-5.8	-7.9	-9.9	-5.7	-9.8	-7.1	-4.9	-6.1	-6.2	-7.1	-7.2	-8.1	-7.6	-6.7	-6.9	-6.7
NGFIB	-4.9	-4.8	-6.5	-6.1	-7	-4.5	-5.6	-6.4	-7	-5.1	-4.7	-5.4	-7.5	-8.1	-6.2	-7.2	-6.1	-6.5	-6.3	-6.7	-5.4	-4.7	-5	-5.7	-5.3	-5.4	-8.1	-4.6	-7.3	-5.9	-6.2	-5.5	-5.8	-5.8	-6.4	-5.7	-5.8	-6.7	-6.1	-4.6
PNR	-5.6	-4.8	-7	-7.7	-8.7	-4.6	-6.1	-6.4	-7.2	-5.6	-5.5	-5.9	-9.1	-8.3	-6.6	-8.6	-6.2	-7	-6.4	-6.9	-6.1	-4.6	-5.3	-5.6	-5.4	-6	-9.2	-4.8	-8.6	-6.4	-6.5	-6	-6.3	-5.9	-6	-5.7	-6.3	-7.3	-6.6	-5.3
PPARα	-7.3	-5.7	-8.2	-8.5	-7.8	-6	-4.1	-8.6	-8.4	-6.8	-6.3	-7.9	-10.5	-11.5	-6.6	-9.2	-7.7	-8.2	-5.5	-7.5	-8.1	-6.4	-6.4	-6.4	-5.7	-7	-10.2	-6.1	-10.7	-5.8	-7.4	-7	-5.8	-7.1	-7.5	-7.1	-7.8	-5.8	-6.7	-7.1
PPARγ	-7.4	-5.1	-8.4	-7.3	-7.8	-5.8	-6.7	-7.6	-8.6	-6.9	-6.1	-6.8	-9.1	-10.1	-7.6	-8.5	-7.5	-8.4	-7.1	-7.8	-7.2	-6	-6.6	-5.8	-5.6	-7.2	-10.8	-6.2	-8.9	-7	-7.6	-7.4	-6.5	-7.1	-6.9	-7	-7.4	-8.4	-7.6	-5.8
PPARΔ	-7.8	-5.4	-8.3	-8.4	-8.6	-6.7	-5.7	-7.9	-9.3	-6.7	-6.1	-7.2	-11.2	-11.1	-7.6	-10	-7.2	-8.3	-5.9	-8.1	-7.3	-6.4	-6.8	-6.1	-5.7	-7.5	-11.1	-6	-10.2	-6.2	-7.6	-7.1	-5.8	-7.8	-6.2	-7.4	-7.7	-7.8	-7.1	-6.6
PR	-7.5	-5.7	-9.5	-8.2	-9	-7.1	-7.8	-8	-9.9	-6.7	-6.3	-7.5	-10.7	-10.9	-7.6	-10	-7.9	-8.3	-7.5	-8.5	-7.4	-6.7	-7.3	-6.3	-6	-8.1	-11.4	-6.4	-10.3	-8.2	-6.6	-7.1	-7.3	-7.7	-8.7	-8.1	-8.4	-8.3	-7.4	-7
PXR	-4.6	-4.1	-3.7	-8.4	-6.3	-5.2	-3	-5.7	-5.8	-4.2	-5.5	-5.9	-7.1	-7.1	-7.5	-6.5	-5.6	-4.5	-3.6	-7.7	-5.2	-5.3	-5.7	-6.5	-6.4	-6.4	-7.9	-5.3	-7.2	-4.2	-4.9	-4.3	-2.3	-5.5	-4.4	-6.6	-5.6	7.9	-7.1	-6.3
RARα	-8.2	-6.7	-9.1	-9.3	-10.5	-7.9	-6.9	-8.4	-9.8	-7.3	-6.3	-8.7	-12.1	-12	-8.2	-10.8	-8.8	-10.2	-6.7	-8.9	-8.9	-8	-8.7	-6.5	-6.5	-9	-10.8	-7.6	-11.1	-6.4	-8.1	-8	-6.5	-7.9	-6.5	-8.4	-8.3	-7.8	-8.1	-7.1
RARβ	-8.6	-6	-9.5	-9.8	-9.8	-7.6	-6.5	-9.2	-9.3	-7.5	-6.3	-9.3	-12.3	-12.5	-8.1	-11.3	-8.5	-10.5	-7.3	-8.7	-8.4	-7.8	-8.4	-7.3	-6.5	-9	-13.1	-7.3	-11.5	-6.6	-8.3	-7.4	-7.4	-8.4	-7.4	-8.6	-9.3	-6.2	-7.9	-7.2
RARγ	-8	-6.6	-9.2	-9.8	-9.7	-7.7	-4.1	-9.5	-9.7	-6.6	-6.7	-9.3	-12.2	-12.4	-8.2	-11.2	-8.2	-10.2	-7.6	-8.7	-8.4	-7.8	-8.9	-7.1	-6.4	-9.4	-13.4	-7.3	-12.1	-6.1	-7	-7.4	-7.2	-8.1	-8.2	-8.6	-9.5	-5.2	-7.9	-7.2
RORα	-7.4	-6	-9.2	-8.5	-9.1	-5.8	-8.7	-8.1	-9.2	-7.7	-6	-7.3	-10.8	-11	-7.8	-9.6	-8	-9.5	-9	-8.8	-7.6	-6.8	-6.8	-6.1	-5.8	-8.1	-11	-6.5	-9.8	-8.4	-7.6	-7.7	-8.2	-7.8	-8.6	-8	-8.5	-10.2	-7.8	-6.6
RORβ	-7.9	-6	-9.2	-8.5	-9.3	-6	-7.3	-8.5	-9.7	-7.3	-6.2	-7.2	-10.8	-10.3	-7.7	-9.5	-7.8	-9.2	-8.4	-8.3	-7.4	-7	-6.8	-6.7	-6.2	-7.9	-11.2	-6.2	-9.6	-7.9	-8.2	-7.6	-7.8	-7.8	-7.9	-7.5	-8.3	-9.8	-7.5	-6.8
RORγ	-7.1	-5.4	-8.6	-8.4	-8.9	-6.3	-7.7	-7.9	-9	-7.1	-5.7	-7.2	-10.4	-10.2	-7.5	-9.7	-8	-9.4	-7.6	-7.6	-7.3	-5.9	-6.6	-5.6	-5.4	-7.6	-11.4	-5.6	-9.5	-7.7	-7.4	-7.4	-7.8	-7.7	-7.9	-7.6	-7.8	-9.9	-7.3	-6.1
RXRα	-4.7	-6.4	-5.7	-8.6	-9.3	-5	-5.3	-5.9	-8.8	-5.2	-6.8	-6.7	-9.2	-9.5	-7.3	-8.7	-6.2	-6.2	-6.4	-8.4	-5.4	-5.4	-5.1	-7.2	-6.4	-5.6	-7.5	-6.1	-8.8	-5.6	-6.1	-5.9	-6.7	-5.8	-5.8	-5.7	-6.4	-6.2	-7.3	-5.8
RXRβ	-8.2	-5.7	-9	-9.2	-9.8	-7	-4.2	-8.6	-9.3	-6.3	-6.1	-8.3	-11.9	-11.9	-8.1	-11.3	-8.6	-9	-5.4	-8.5	-7.9	-7.3	-8.1	-6.5	-5.8	-8.8	-12.8	-6.5	-11.1	-5.7	-8.4	-8	-5	-7.8	-5.4	-9	-8.8	-5.9	-7.8	-7.2
RXRγ	-6.2	-4.8	-7.8	-7.1	-8	-5.3	-4.9	-6.8	-8	-6.6	-5	-7	-9.2	-9.3	-6.6	-8.4	-6.9	-7.6	-5.4	-7.3	-6.2	-5.6	-6.1	-5.7	-5.1	-6.8	-8.9	-5.2	-8.5	-5.8	-6.8	-6.5	-5.4	-6.6	-6.5	-6.9	-6.2	-7.9	-6.6	-5.6
SF1	-6.9	-3.5	-7.8	-6.5	-6.4	-5.9	7.5	-4.7	-8.8	-4.1	-6.3	-6.1	-7.3	-7	-5.9	-7.7	-6	-7.1	-3.6	-7.8	-6.1	-6.1	-6.5	-6.2	-5.6	-5.9	-7.6	-5.8	-7.6	-3.2	-4	-5	-3.4	-6.2	-4.2	-6.1	-5.6	-3.2	-5.7	-7.2
SHP	-6.9	-6.6	-8.6	-9.3	-10.8	-6.6	-6.2	-7.2	-10.2	-7.8	-6.2	-8.2	-12.1	-11.8	-8.3	-10.6	-8.6	-9	-6.5	-9.3	-7.5	-6.7	-7.2	-6.9	-6.7	-8.5	-9.7	-6.4	-10.9	-7.5	-8.1	-7.6	-6.4	-8	-8.3	-8.2	-8.6	-8.9	-8.3	-7.6
TR4	-5.3	-4.8	-6.8	-6.7	-6.9	-4.2	-5.8	-6	-7.6	-5.3	-5.2	-6.9	-8.1	-7.5	-5.5	-7.1	-6.2	-6.5	-5.8	-6.7	-5.7	-4.5	-5	-5.6	-5.5	-5.8	-7.9	-4.6	-7.5	-6.1	-7	-5.6	-5.6	-6.1	-6.6	-5.7	-5.9	-7.5	-5.5	-5.2
TRα	-7.5	-5.8	-9.2	-8.6	-9.7	-6.8	-3.6	-7.2	-10.4	-8.1	-6.3	-7.9	-10.7	-11.5	-7.7	-10.7	-8.7	-9.1	-5.9	-8.8	-8.2	-7	-7.6	-6.6	-5.8	-8.9	-11.6	-6.2	-10.8	-5.7	-7.5	-8.3	-5.3	-8.2	-7.7	-8.6	-7.8	-9.1	-7.7	-7
TRβ	-7.9	-6	-9.6	-8.9	-10.4	-8.2	-1.4	-7.7	-10.3	-7.8	-6.6	-8.3	-10.9	-12	-8	-10.9	-8.9	-9.4	-5.3	-8.8	-8.2	-8.2	-8.8	-6.6	-6.5	-9	-12	-7.2	-11.4	-5.1	-7.7	-7.2	-4.3	-7.7	-6.9	-8.8	-8.4	-8.8	-8	-7.1
VDR	-7.9	-5.1	-9.1	-8.9	-8.6	-6.9	-8.1	-8.7	-9.6	-7.5	-6.7	-8.7	-10.7	-11.1	-7.5	-10.8	-8.9	-9.6	-7.6	-8.5	-7.9	-7	-7.5	-6.6	-6.1	-8	-12.4	-6.7	-10.8	-7.7	-8.2		-7.8	-7.9	-7.7	-8.5	-8.2	-8.4	-7.4	-6.5
	26	4	33	35	36	15	14	29	38	23	5	32	39	38	32	37	30	33	19	37	28	18	27	12	2	28	38	9	38	18	22	24	16	28	24	30	27	27	31	22

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