Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

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Abstract

Widespread exposure to bisphenol A (BPA) is suspected to affect a variety of physiological functions. There is accumulating evidence showing the role of BPA as an endocrine disrupting chemical mainly due to its estrogenic activity. Although BPA has been substituted by its analogues, effects of BPA and its analogues on metabolic pathways have been poorly investigated. Additionally, BPA has been also shown to bind and activate other receptors including peroxisome proliferator-activated receptors (PPARs). PPARs act as transcription factors by regulating genes involved in adipogenesis, and glucose, lipid, and cholesterol metabolism. An *in silico* study looking into the binding efficiency of BPA and its halogenated analogues with PPARα of mouse was undertaken. The idea was to understand the mechanism of interaction of bisphenols with PPARα. In the absence of a crystal structure for mouse PPARs, homology modelling was performed to generate the crystal structure of PPARα using Modeller 9 v10. The desired protein template was identified through BLASTp search against the protein database. For docking studies, grid boxes were created at the hinge of PPARα and each ligand was individually docked using the AutoDock-4.2. Docking analysis was done by PyMOL and results prepared on Chimara v1.8. Upon interaction with mouse PPARα BPA and its halogenated analogues showed lowest binding energy scores (Kcal/Mol) were in the order: TBBPA (-8.75) >BPZ (-8.09) > BPC = TCBPA (-7.77) > BPE (-7.24) > BPB (-7.15) > BPF (7.02) > BPS (6.02) > BPA (5.60) > BPAF (-5.39). Lowest binding energy score of positive control GW 7647 was -7.89. Certain BPA analogues showed higher binding efficiency with PPARα than BPA. Overall, the results suggest that some of the halogenated analogues of BPA have strong binding affinity. Further *in vivo* and *in vitro* studies are required for determining their actual safety profiling.

Keywords: *Endocrine Disrupting Chemical; Bisphenol A; Bisphenol A Analogues; Nuclear Receptors; Peroxisome Proliferator-Activated Receptors; Molecular Docking*

Abbreviations

BPA: Bisphenol A; EDC: Endocrine Disrupting Chemical; NR: Nuclear Receptor; PPAR: Peroxisome Proliferator-Activated Receptor; RXR: Retinoid X Receptor

Introduction

In the early 1990s the study of a group of xenobiotics acting as peroxisome proliferators triggered discovery of a novel subfamily of nuclear receptors (NR) [1,2]. The NR subfamily of peroxisome proliferator-activated receptors (PPARs) includes three members, PPARα (also called NR1C1), PPARβ/δ (NR1C2), and PPARγ (NR1C3) [2,3]. These receptors bind to PPAR-responsive DNA regulatory elements in

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

216

the form of heterodimers with the retinoid X receptor (RXR). They control the expression of genes involved in adipogenesis, and glucose, lipid, and cholesterol metabolism [3,4]. PPARs bind and respond to dietary fatty acids and diverse lipid metabolites, including prostaglandins, eicosanoids and oxidized phospholipids [3,5]. These receptors have different tissue distribution and physiological roles [6]. PPARα is expressed predominantly in the liver, heart, and brown adipose tissue, whereas PPARβ/δ is ubiquitously expressed [6]. Both play a major role as activators of fatty acid oxidation pathways, and therefore, have a role in the regulation of energy homeostasis. In addition, PPARα stimulates heme synthesis, cholesterol catabolism, and participates in the control of amino acid metabolism and urea synthesis. PPARβ/δ has a role in the control of cell proliferation and differentiation, and is necessary for placental and gut development. PPARγ is highly expressed in adipose tissues and plays key roles in regulating adipogenesis, and glucose homeostasis through improving insulin sensitivity [7,8]. Additionally, PPARγ is required for the function and survival of mature adipocytes [7,8].

Endocrine disrupting chemicals (EDCs) are widely distributed in our environment and food chain. They are known to induce various toxic effects in mammals and species [9-13]. A privileged mechanism for interference of EDCs with metabolic pathways is their direct or indirect activity on nuclear receptors e.g., PPARs. PPARs act as adaptive molecules for EDCs and mediate their pathways in the mechanism of endocrine disruption [2,14,15]. The "obesogen hypothesis" states that in addition to the imbalance between caloric intake and expenditure, the rapidly growing obesity epidemic could implicate environmental risk factors, such as an increased exposure to chemicals that interfere with any aspects of metabolism including EDCs [16,17]. Accordingly, compounds that have the potential to disrupt any metabolic signalling pathways and lead to increased fat accumulation and obesity are referred to as "obesogens" [18]. Evidence points to EDCs that interfere with the body's adipose tissue biology, endocrine hormone systems or central hypothalamic-pituitary-adrenal axis as suspects in derailing the homeostatic mechanisms important to weight control [19]. EDCs are of great concern because of their ubiquitous presence in our environment and food [20-22]. EDCs can alter endocrine functions and cause infertility, malformations, metabolic disorders or increased incidence of cancers [20,23]. A better understanding of the differential activities, binding affinities, and specificities of environmental NR ligands, and their structural aspects at a near-atomic level may rationalize guidelines to design safer chemicals characterized by fewer NR-mediated side effects.

3D-structure based computational tools aimed at study of NR-EDC interaction may predict endocrine disrupting action of xenobiotics acting through receptor interaction [24]. Moreover, studies aimed at identifying and characterizing EDCs may aid in finding new ligands for NRs including orphan receptors and may reveal unforeseen binding modes. The weak structural relationships between EDCs and natural hormones render their interaction with these cellular targets poorly understood and barely predictable. Because of these reasons, it is necessary to characterize the interactions between NRs and environmental compounds, at both structural and functional levels, and develop robust *in silico, in vitro*, and *in vivo* screening methods. *In silico* approach has been helpful in identifying mechanism of action of EDCs and their biological interactions [24].

The group of PPAR ligands is heterogeneous and contains a structurally and chemically disparate ensemble of molecules with very few shared molecular features, suggesting that each of them possibly interacts with the receptor through a specific mechanism [2,14,15]. This set of compounds includes phthalates, plasticizers, certain herbicides, organotins, biocides, and pharmaceuticals. Recent data have revealed that some natural compounds derived from plants can also bind to PPARγ and modulate its activity [25,26].

Numerous BPA analogues and derivatives including bisphenol B (BPB), bisphenol C (BPF), bisphenol F (BPF), brominated derivative bisphenols [e.g., tetrabromobisphenol A (TBBPA)], and chlorinated bisphenols [e.g., tetrachlorobisphenol A (TCBPA)] are used in commodity products [2,15,27]. BPB, TBBPA, and TCBPA have been detected in human serum and tissues. Studies have shown that BPA can alter the expression of PPARs isoforms in some species but much information is not available on the effects of BPA analogues on PPARs [15,27]. In view of their increasing importance as alternative to BPA, an *in silic*o docking study of BPA and its analogues with PPARα has been performed.

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

217

Materials and Methods

Ligands

Bisphenol A (4-[2-(4-hydroxyphenyl)propan-2-yl]phenol) and its nine halogenated analogues viz., bisphenol B (4-[2-(4-hydroxyphenyl)butan-2-yl]phenol), bisphenol C (4-[2,2-dichloro-1-(4-hydroxyphenyl)ethenyl]phenol), bisphenol E (4-[1-(4-hydroxyphenyl)ethyl]phenol), bisphenol F (2-[(2-hydroxyphenyl)methyl]phenol), bisphenol S (4-(4-hydroxyphenyl)sulfonylphenol), bisphenol Z (4-[1-(4-hydroxyphenyl)cyclohexyl]phenol), bisphenol AF (4-[1,1,1,3,3,3-hexafluoro-2-(4-hydroxyphenyl)propan-2-yl]phenol), tetrabromobisphenol A (2,6-dibromo-4-[2-(3,5-dibromo-4-hydroxyphenyl)propan-2-yl]phenol) and tetrachlorobisphenol A (2,6-dichloro-4-[2-(3,5-dichloro-4-hydroxyphenyl)propan-2-yl]phenol). GW7647 (2-[4-[2-[4-cyclohexylbutyl(cyclohexylcarbamoyl)amino]ethyl]phenyl]sulfanyl-2-methylpropanoic acid), a potent, selective agonist of human and murine PPARα) was used as a positive control ligand (Figure 1).
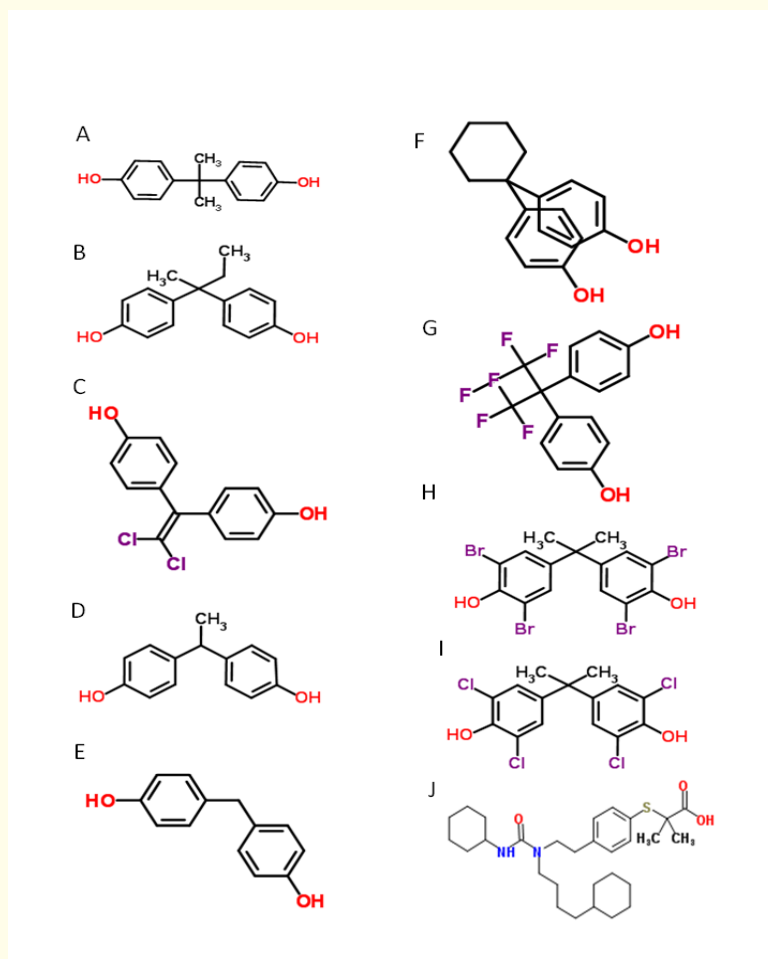
Figure 1: *Chemical structures of ligands (A) bisphenol A, (B) bisphenol B, (C) bisphenol C, (D) bisphenol E, (E) bisphenol F, (F) bisphenol Z, (G) bisphenol AF, (H) tetrabromobisphenol A, (I) tetrachlorobisphenol A, (J) and reference compound, GW7647.*

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

218

Sequence retrieval of PPAR receptors

Primary amino acid sequence of three different isoforms of PPAR from mouse (*Mus musculus*), α, β and γ, were retrieved from the Protein Data Bank (PDB). The unit protein identifiers (unitprot ID) were: PPARα P23204, 52 kDa; PPAR δP35396, 49.715 kDa; and PPARγ P37238, 57.598 kDa, respectively.

Multiple sequence alignment

In order to identify the similarities between the different forms of PPAR proteins, a similarity search has been performed using Multiple Sequence Alignment (MSA) approach [28]. The output for sequence similarity search was determined using the tool 'MultAlign'.

Homology modeling

The basic concept of homology modeling is to build the probable secondary structure of proteins based on the most similar protein template [29]. Since there is no crystal structure available for mouse (*Mus musculus*), PPARs, molecular structure of PPARα was developed through homology modeling approach using the tool Modeller9 v10 [30]. In this study, homology modeling was performed to generate the crystal structure of PPARα protein by identifying the most similar protein template. The desired protein template was identified through BLASTp (Basic Local Search Alignment Tool for Protein) search against the PDB database. 3-D docking models were verified using 'The Structure Analysis and Verification Server' (SAVeS) for the Ramachandran Plot, Verify3D and ERRAT values to check overall quality of the modeled structure.

Molecular docking

Molecular docking of BPA and BPA analogues with PPARα was performed using Auto Dock 4.2 docking tool [31]. Auto Dock is an automated procedure for predicting the interaction of ligands with macromolecular targets. Auto Dock has been the most preferred tool for docking studies and its results are reliable and accepted widely [32].

Results and Discussion

Multiple sequence alignment output

Based on the multiple sequence alignment of PPARα, PPARδ(=β) and PPARγ, a consensus sequence was identified and used for docking studies. Figure 2 displays the output for sequence similarity search using the tool 'MultAlign' [28]. Sequence alignment studies shows that α, β,and γ forms of PPAR proteins share more than 80% sequence similarity. Based on the assumption that similar protein sequences would also exhibit the similar 3D structure and folds, alpha-form of PPAR protein has been selected and modeled to evaluate the interaction pattern and binding efficiency with BPA and its analogues.
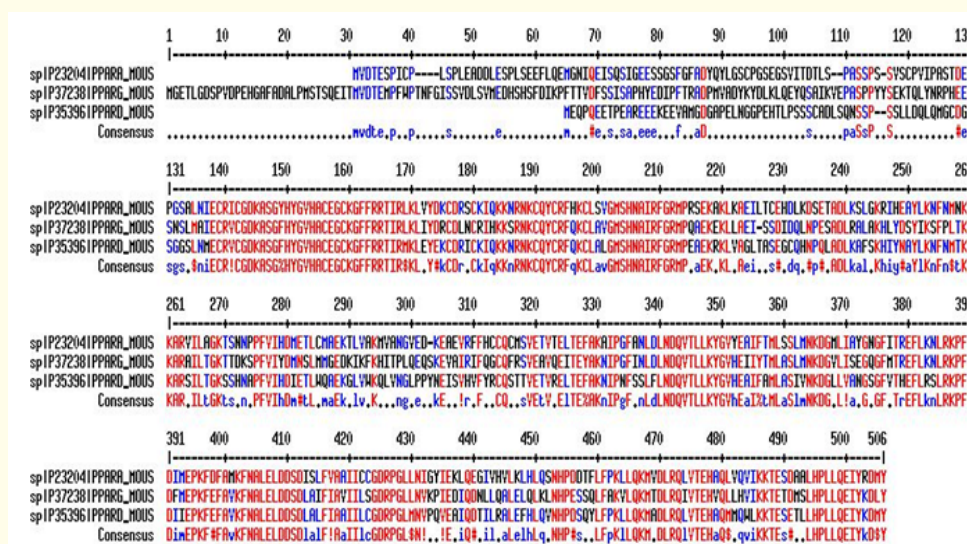
Figure 2: *Figure 2: Multiple sequence alignment with similarity consensus of mouse PPAR α, β, γ. Complete and high similarity sequences are shown in blue and red colour..*

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study
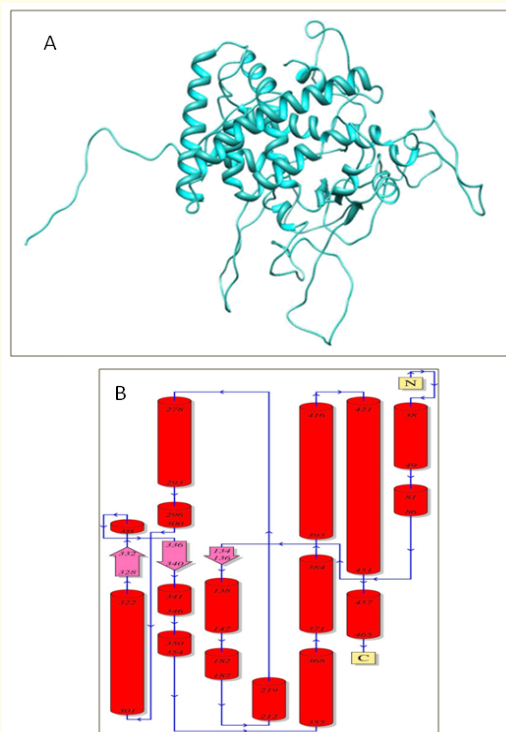
219

Homology modeling outcome

The search exhibits '1k7l chain A' as the most similar template for PPARα protein with e-value score of zero (0), query coverage of 58% and sequence identity of 92% which is a convincing score to select 1k7l as the most suitable template protein for the query template. Five structures of PPARα were modeled, for each of which DOPE and other scores were determined. Subsequently, model was verified using 'The Structure Analysis and Verification Server' (SAVeS) for the Ramachandran Plot, Verify3D and ERRAT values to check overall quality of the modeled structure. Table 1 and 2 shows the template values and model verification parameters for the five different structures of PPARα based on homology modeling. SAVeS analysis performed showed that the modeled structure is reliable and suitable for further docking studies. Based on homology modeling results, it is clear that the Model 3 has best DOPE score and shows considerable results on verification analysis. Hence, Model 3 was selected for further docking studies. The three dimensional structure generated for PPARα, and its secondary structure topology is shown in figure 3. The structure contains one each of β-sheet, β-hairpin and ψ-loop, three strands, 17 helices, 21 Helix-Helix interacts, 39 β-turns, and7 γ-turns.

Model Number	Molpdf	DOPE Score	GA341 Score
Model 1	2943.55396	-40751.02734	1.00000
Model 2	3540.32129	-40660.81250	1.00000
Model 3	3343.01660	-41254.80078	1.00000
Model 4	2914.83618	-40902.41797	1.00000
Model 5	5618.63184	-38941.36719	1.00000

Table 1: *Homology modeling to derive the 3D structure of PPARα (Mus musculus).*

Models	Ramachandran plot	Verify-3D (%)	ERRAT (%)
Model 1	Most favored region: 340 Additional allowed region: 70 Disallowed region: – 6	51.28	49.40
Model 2	Most favored region: 342 Additional allowed region: 62 Disallowed region: 7	47.01	38.795
Model 3	Most favored region: 356 Additional allowed region: 51 Disallowed region: 4	57.91	36.712
Model 4	Most favored region: 347 Additional allowed region: 57 Disallowed region: 7	50.21	46.991
Model 5	Most Favoured region: 352 Additional allowed region: 65 Disallowed region: 6	47.65	42.995

Table 2: *Model verification scores of PPARα (Mus musculus).*

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

220

Figure 3: *(A) Modeled three dimensional secondary structure of PPARα protein. (B) Secondary structure topology of PPARα protein. The structure contains one each of β-sheet, β-hairpin and ψ-loop, three strands, 17 helices, 21 Helix-Helix interacts, 39 β-turns, and 7 γ-turns.*
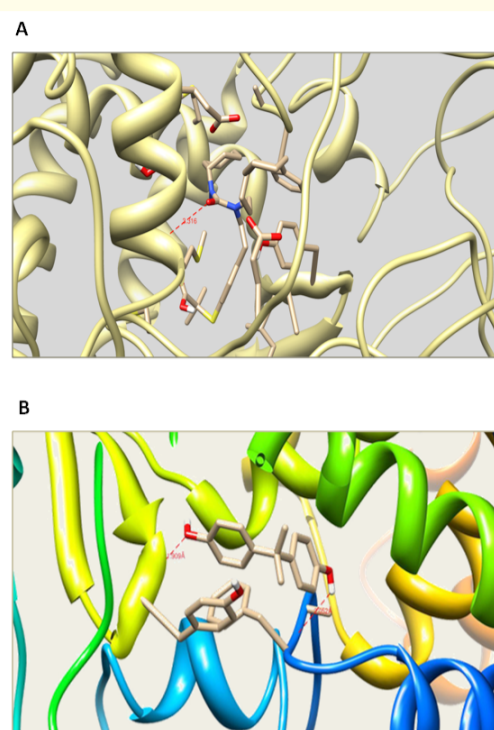
Molecular docking of BPA analogues with PPARα

The list of ligands with their PUBCHEM IDs are shown in table 3. The structures of these compounds were retrieved and used for docking with PPARα. GW7647 was used as a reference compound and the docking parameters obtained for GW7647 and other BPA analogues are also shown in table 3. It is important for a molecule to have hydrogen atoms to perform docking studies, hence, polar hydrogen atoms were added to the PPARα. For each analogue, the torsion angles were specified and gasterious charges were added to calculate the binding interactions. Binding pose of reference compound GW7647 in the binding pocket of PPAR α is shown in Figure 4A. Reference compound GW7647 has shown lowest binding energy of -7.89 kcal/mol and mean binding energy of -5.02 kcal/mol. In the present study, docking score of reference molecule was considered as a threshold value and the neighboring residues of the binding residues were targeted for the docking of BPA and its analogues. BPA has a strong binding affinity towards the PPARα with a docking score of -6.91 kcal/mol (Figure 4B). Most of the analogues exhibit docking characteristics comparable to the reference molecule. Tetrabromobisphenol A and bisphenol Z exhibit clearly stronger binding (Figure 5A and 5B, respectively) while bisphenol C and tetrachlorobiphenol A shown a good binding affinity with the same binding score (Figure 6A and 6B, respectively). Bisphenol S and bisphenol AF showed comparatively weaker binding to PPARα than the reference compound.

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

221

Ligands	Lowest Binding Energy (kcal/mol)	Mean Binding Energy (kcal/mol)	H_Don::H_Acc* Bond Length(Å)	Inhibition constant
GW7647 (CID_3392731)	-7.89	-5.02	MET279:O::LIG/O (3.316)	1.66 µM
Bisphenol A (CID_6623)	-6.91	-6.78	Phe51:O::Lig1:H (2.052) Lig1:O::Tyr334:HN (1.90)	8.60 µM
Bisphenol B (CID_66166)	-7.15	-6.97	LEU331/O:: LIG1/H (2.204) PHE51/O::LIG1/H (2.147)	5.78 µM
Bisphenol C (CID_84677)	-7.77	-7.64	PHE51/O :: LIG1/O (2.070) GLY335/N:: LIG1/O (2.948)	2.02 µM
Bisphenol E (CID_608116)	-7.24	-7.12	GLY50/N:: LIG1/O (2.772) PHE51/N:: LIG1/O (2.062) GLU286/OE2:: LIG1/H (1.90) GLY335/N:: LIG1/O (2.982)	4.97 µM
Bisphenol F (CID_75575)	-7.02	-6.94	GLU286/OE2:: LIG1/H (2.125) GLU286/OE2:: LIG1/H (2.233) GLY50/N:: LIG1/O (3.115) PHE51/N:: LIG1/O (3.017)	7.09 µM
Bisphenol S (CID_6626)	-6.74	-6.70	GLY335/N :: LIG1/O (3.290) PHE51/O:: LIG1/H (2.124)	11.51 µM
Bisphenol Z (CID_232446)	-8.09	-8.07	GLY335/N:: LIG1/O (3.173)	1.18 µM
Bisphenol AF (CID_73864)	-5.39	-5.20	GLU245/OE2:: LIG1/H (2.112)	112.77 µM
Tetrabromo Bisphenol A (CID_6618)	-8.75	-8.75	GLU286/OE2:: LIG1/H (1.675) GLY50/N:: LIG1/O (2.463) PHE51/N:: LIG1/O (2.814) LEU331/O:: LIG1/H (2.421) ALA333/N:: LIG1/O (3.002)	384.81 µM
Tetrachloro bisphenol A (CID_6619)	-7.77	-7.67	ALA333/N:: LIG1/O (2.945) LEU331/O:: LIG1/O (2.238)	2.00 µM

Table 3: Docking parameters and scores for BPA and its analogues with PPARα.

*H_Don: Hydrogen bond donor, H_Acc: Hydrogen bond acceptor

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study
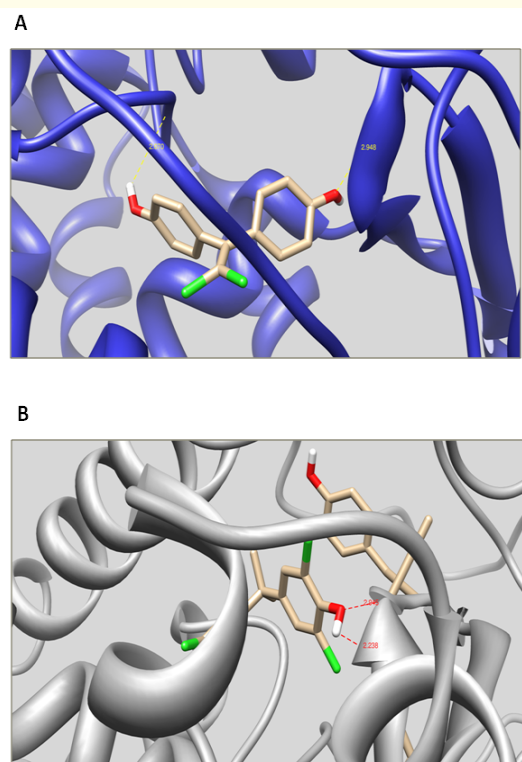
222

Figure 4: *Binding pose of (A) Reference compound GW7647 in binding pocket of PPARα showed hydrogen bond interaction between MET279:O::LIG/O with the hydrogen bond length of 3.316 Å. (B) The binding pose of BPA at binding site of PPARα showed hydrogen bond interaction between Phe51 and Try334 with hydrogen bond lengths of 2.052 Å and 1.90 Å, respectively. Ligands are shown in stick format while hydrogen bonds are shown as red dotted line.*
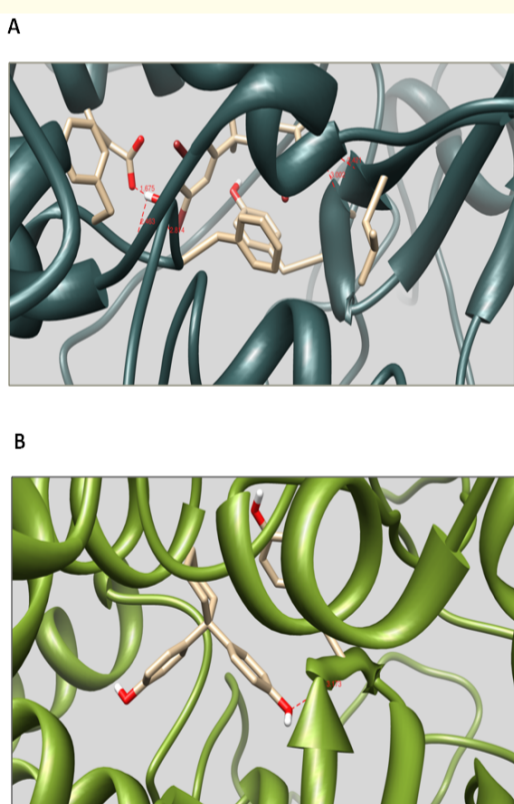
Figure 5: *The binding pose of (A) Tetrabromobisphenol A showed the highest binding efficacy with PPARα and the hydrogen bond interaction was shown between GLU286, GLY50, PHE51, LEU331 and ALA33 with hydrogen bond lengths of 1.675 Å , 2.463 Å , 2.814 Å , 2.421 Å and 3.002 Å. (B) Bisphenol Z showed the second highest binding efficacy with PPARα and hydrogen bond interaction was shown between GLY335/N:: LIG1/O with the hydrogen bond length of 3.173 Å. Ligands are shown in stick format while hydrogen bonds are shown as red dotted line.*

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

223

Figure 6: *Figure 6: Binding pose of (A) Bisphenol C shows the interaction between Phe51 and Gly335 with the hydrogen bond lengths of 2.070 Å and 2.948 Å whereas, (B) Tetrachlorobisphenol A shown the interaction between ALA333 and LEU331 with the hydrogen bond lengths of 2.945 Å and 2.238 Å. Ligands are shown in stick format while hydrogen bonds are shown as red dotted line.*

Discussion

In this docking study of BPA and PPARα it is observed that BPA has a strong binding affinity towards the PPARα with a docking score of -6.91 kcal/mol. The study also showed hydrogen bond interactions between Phe51 and Try334 with hydrogen bond lengths of 2.052 Å and 1.90 Å. Moreover, it has given satisfactory inhibition binding constants of 8.60 μM. Therefore, it is strongly recommended to evaluate its inhibition efficacy through experimental studies. Earlier, using molecular dynamics simulations Li., *et al.* [33] have reported that binding affinities of BPA with three selected nuclear receptors were slightly lower than that of 17 β-estradiol (E2). Li., *et al.* [33] also proposed that that the binding process was mainly driven by direct hydrogen bond and hydrophobic interactions and structural analysis revealed that BPA interacted with the NRs by mimicking the action of natural hormone and keeping the nuclear receptors in active conformations.

We studied molecular docking of PPARα with a series of BPA analogues and analyzed their binding efficacy. Docking parameters suggested that BPA analogues have an appreciable binding ability towards PPARα protein (Table 3). It was observed that of all the analogues

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

224

studied tetrabromobisphenol A, has the highest binding efficacy in the hinge region of PPARα protein which was greater that BPA as well as reference ligand (GW7647). It exhibited a mean binding energy of -8.75 kcal/mol indicating a docking interaction stronger than that of the reference compound which had mean energy of -5.02 kcal/mol. The binding site residues Glu286 (1.675), Gly50 (2.463), Phe51 (2.814), Leu331 (2.421) and Ala333 (3.002) actively takes parts in the hydrogen bond interactions with the tetrabromobisphenol A. These residues play a very significant role to strengthening this complex.

Bisphenol Z showed the second highest binding energy in the binding pocket of PPARα. It exhibited -8.09 kcal/mol energy as the lowest binding energy and -8.07 kcal/mol energy as the mean binding energy. It was also noted that that bisphenol Z had 20 clusters during the molecular docking experiment which indicating that it has strong binding affinity toward the PPARα in various conformations of the protein and the ligand. Therefore, the binding of bisphenol Z to PPARα is expected to be strong.

Bisphenol C and tetrachlorobisphenol A showed the same docking energy of -7.077 kcal/mol indicating reasonable interaction. However, it is below the cut-off value set based upon the binding of the reference compound. Bisphenol C interacted with the Phe51 (2.070) and Gly335 (2.948) whereas tetrachlorobisphenol A interacted with the Ala333 (2.945) and Leu331 (2.238) to make strong hydrogen bond interactions.

The *in silico* studies thus reveal that although major focus of research has been BPA, there are other strong candidates of interaction with PPARs. These analogues of BPA have been scarcely been investigated *in vitro* or *in vivo*. In this context *in silico* and crystallographic studies are unraveling unanticipated mechanisms by which EDCs interact with the ligand-binding domain of NRs [2,α24].

In most of the bisphenols and PPARα docking complexes it is observed that among the nearest amino acid residues of PPARα capable of forming hydrogen bond with the ligand, Leu331, Phe51, Gly335 were the most common. These three residues interact with BPA and all of the analogues studied, except for bisphenol AF which has weakest binding with PPARα. Of the three residues, Phe51 was the most common, followed by Gly335. Leu331 forms hydrogen bonding, through it oxygen atom, with bisphenol B, tetrachlorobisphenol A and tetrabromo- bisphenol A.

Halogenated derivatives of BPA, tetrabromobisphenol A and tetrachlorobisphenol A, are known to be present in the environment and human samples [34-36], as they are used in the manufacture of computer motherboards and other electronic products. Recent studies have indicated that halogenated BPA can disrupt the activity of PPARs from different species [37]. A crystal structure of PPARγ in complex with tetrabromo and tetrachlorobisphenol A revealed identical binding mode where the ligands occupy the β-sheet sub pocket. One of the phenol rings shows an indirect link to H12 through a water-mediated hydrogen bond to Tyr473. In contrast, the present docking of halogenated BPA analogues with PPARα did reveal the formation of water mediated hydrogen bonds, but shows only direct hydrogen bonding with Ala333, Leu331, Phe51, Glu286 and Gly50 residues.

The present study highlights role of various BPA analogues for their efficiency to bind with PPARα. Besides, PPARα, the other PPAR which has been in focus is PPARγ mainly due to suspected role of interaction of EDCs with it [16,38]. Furthermore, it has been suggested by Vuorinen., *et al*. [24] that PPARs should be taken into consideration when exploring the mechanisms of endocrine disruption and *in silico* approaches may be quite helpful in the primary screening and decision making. Recently, Wang and Zhang., *et al*. [39] have used these approaches to study interaction of BPA and its analogues with DNA strengthening relevance of *in silico* studies in risk assessment and critical first hand screening.

Conclusion

EDCs are known to cause changes in endocrine activity, infertility, metabolic disorders and even cancers. Therefore, it is very important to understand the interactions between EDCs such as BPA and its analogues and the NRs at structural as well as functional levels.

Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

225

The present *in silico* docking study of BPA and its halogenated analogues with PPARα indicated strong binding of tetrabromobisphenol A, bisphenol Z, and bisphenol C, among the analogues studied. Phe51 has been identified as the residue with maximum interaction with the BPA family of ligands. A limitation of the study is the lack of a crystal structure, and the reliance on a three dimensional structure of PPARα derived by homology modeling. Nevertheless, the results of the docking study are promising and warrant further experimental study.

Acknowledgements

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

226

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Differential Targeting of Peroxisome Proliferator-Activated Receptor-α by Bisphenol A and its Halogenated Analogues: An *In Silico* Study

227

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