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In Silico Study Highlighting the Interactions Between the Active Sites of Steroidogenic Enzymes and Endocrine Disruptors Found in Daily Use Products

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Abstract: Endocrine-disrupting chemicals (EDC) mimic steroid hormones; they can get easily bonded with enzymes even if their affinity is somewhat similar to natural reactants of the respective enzyme, forming subsequent hormones. This study aims to show the interactions between the active sites of key steroidogenic enzymes and endocrine disruptors. The main method used to reach the objective was molecular docking to study the relationship between the receptors and ligands and the binding affinity between EDC and Steroidogenic enzymes' active site amino acids. Molecular docking was also performed between the natural reactants of steroidogenesis and their respective steroidogenic enzymes so that a comparison could be made between the binding affinities of endocrine-disrupting chemicals and natural reactants with the respective steroidogenic enzymes. All the EDCs showed an affinity for the active site amino acids of key steroidogenic enzymes. Triclosan showed the highest affinity with the lowest binding energy required for all three key steroidogenic enzymes, while nitrate had the lowest affinity with the highest binding energy required. In comparison with the affinities showed by the reference reactants, all the EDCs studied show low affinities. Still, since EDCs tend to accumulate in the tissues, as their amount increases, it would surpass the reference reactants and would bind more efficiently, leading to endocrine disruption.

Keywords: endocrine disruptors; steroidogenic enzymes; active site amino acids; molecular docking.

1. Introduction

Any external chemical compound that alters the normal process of steroidogenesis, i.e., production, metabolism, transport, or affects the formation and functioning of hormones, thereby hindering the process of homeostasis, reproduction, and development, is known as an endocrine disruptor [1].

Since we are getting so modernized, our whole lives have changed so far, and now we are inclining much more towards synthetic things, such as food or personal care products. We are exposed to these endocrine-disrupting chemicals randomly and do not even recognize them. These endocrine-disrupting chemicals bind to the active site of enzymes involved in the synthesis of steroid hormones (Fig 2.), thus possibly causing a disturbance in the normal steroidogenesis process by mimicking the actual reactants of the process upon exposure.

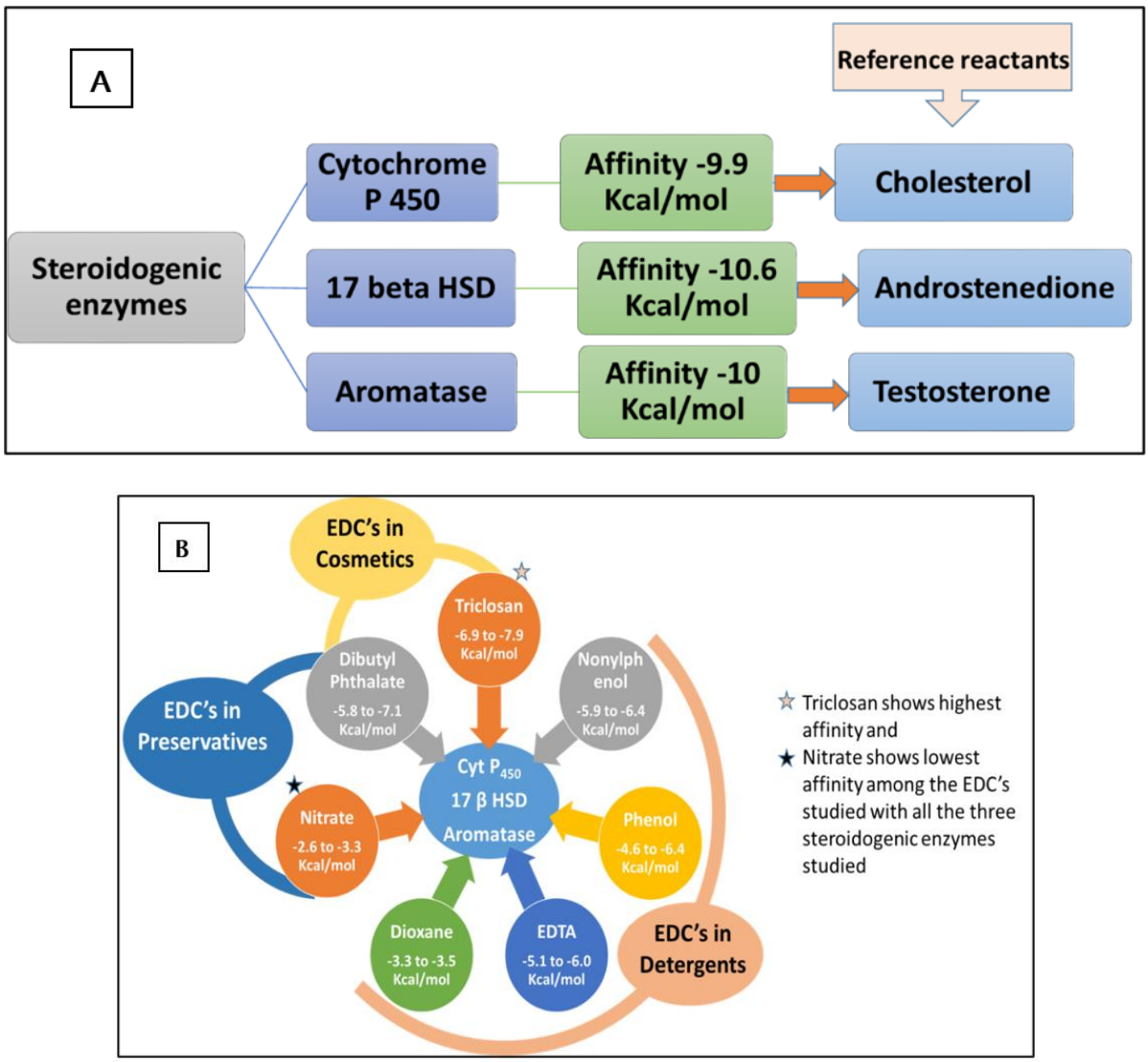


Figure 1. The graphical abstract, (A) Interaction between reference reactants of steroidogenesis with its steroidogenic enzyme; **(B)** Interaction of EDCs with active site amino acids of steroidogenic enzymes.

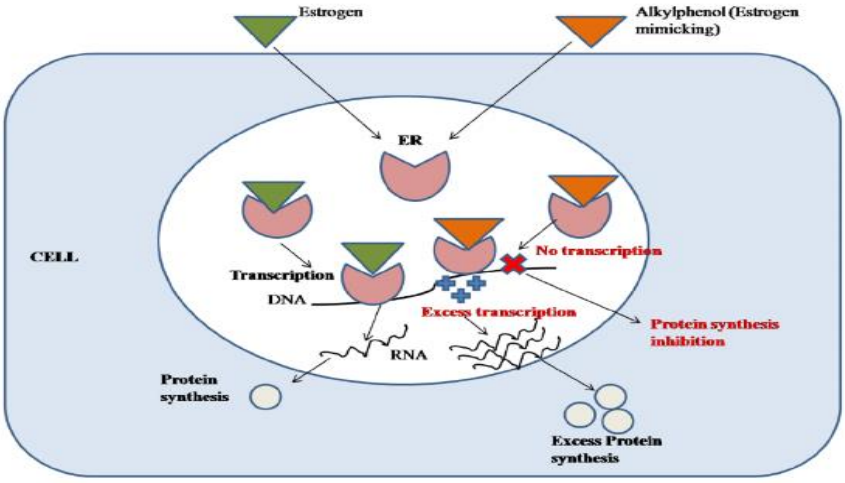


Figure 2. Endocrine disruptive role of estrogen mimicking alkylphenols [2].

The process gets imbalanced, as shown in Figure 2. Alkylphenol, one of the endocrine disruptors, can act as an antagonist and block the further synthesis of hormones by binding the enzyme's active site, or it could act as an agonist and accelerate the rate of formation of steroids [3]. In both ways, normal standard conditions are disturbed.

Steroidogenesis is basically the process of the formation of active steroids from cholesterol [4, 5]. An endocrine disruptor acts as a key when it binds with a specific steroidogenic enzyme, while the latter acts as a lock for a particular key [6]. Formation of steroid hormones takes place with the help of several enzymes at each subsequent step. The steroid-forming enzymes belong to two major groups: Cytochrome P450 and hydroxyl steroid dehydrogenase [7]. The first step in steroid formation is the conversion of cholesterol, a 21-carbon compound in Pregnenolone, by Cytochrome P450 side chain cleavage enzyme. The gene CYP11A1 encodes Cytochrome P450 scc, a mitochondrial protein. Meanwhile, an endoplasmic reticulum-related protein-encoding gene CYP19A1 catalyzes hydroxylation [8].

In this study, we have mainly considered three sources commonly used as household products and have certain endocrine-disrupting chemicals incorporated in them [9, 10]. These groups or sources are detergents, preservatives, and cosmetics. The common chemicals found in them are Triclosan, Nonylphenol, Phenol, Ethylene diamine tetra acetic acid (EDTA), Dioxane, Nitrate, and Dibutyl Phthalate.

Triclosan is an endocrine-disrupting chemical found in cosmetics. A recent study found that several household accessories like lotion, deodorants, and toothpaste contain 10 mM triclosan [11]. Nonylphenol is one of the most harmful endocrine-disrupting chemicals belonging to the alkylphenol family and can disrupt the function of endocrine glands of humans and animals [12].

Phthalate is another endocrine disruptor that is added to plastic ware and the materials used to wrap food. When these compounds are subjected to high temperatures or changes in pH, they are released and, along with food material, get ingested into the gastrointestinal tract [13].

In order to find the affinity of these EDCs with the respective enzymes, molecular docking is considered one of the best methods so far. Using molecular docking, different confirmations of small molecules (in this case, endocrine disruptors) along with their respective affinity associated with each docking pose with a receptor (in this case, enzymes) were studied, and inferences were obtained about the most prone amino acids of enzymes and which EDCs are more harmful to the steroidogenesis process. We specifically targeted the active sites of these enzymes, as it is quite evident that active-site amino acids are the most important key players in their function. Molecular docking is one of the most efficient ways to analyse the receptor-ligand interaction [14].

2. Material and method

2.1. Molecular docking.

We did molecular docking to know the strength of the bonding interaction of endocrine disruptors under study with respective enzymes involved in steroid synthesis.

2.1.1. Software used.

The following software was used during the study: Open Babel software, which converts one file format to another and prepares ligands [15]. It is the first step of ligand preparation. Pymol - to find the active site amino acids within the steroidogenic enzymes from co-crystallized enzymes with ligands taking ligands as a reference. The active site amino acids were detected by fetching the receptor protein co-crystallized with certain ligands to them using PDB ID. The sequence was read in Pymol using the software's options. Ligand was selected

by clicking on the provided sequence. Using the code ‘show sticks byres all within 5 of “here providing the name of ligand,” we can get all the amino acid residues which lie within the range of 5 angstroms to this ligand will appear like sticks. Next, hiding the cartoon will cause the whole protein structure to disappear except the ligand and the interacting active site amino acids. We can see the residue name too. Receptor preparation: Autodock 4.2.6 was used for receptor preparation. The heteroatoms and water molecules need to be removed from 3-D protein structures, which may otherwise interrupt the interaction. A receptor could be prepared by removing water molecules, adding polar hydrogen, adding Kollman charges, etc. [16] Virtual screening: Since Auto dock vina [version 1.1.2] allows the docking of multiple ligands with a single receptor at a time, therefore it was used for the purpose of virtual screening along with Perl - software.

The grid dimension for all three enzymes was:

For cytochrome P450, it was 34.819 X -1.929 X 55.254

For 17 beta HSD, it was 12.047 X -1.441 X -2.148

For aromatase it was 83.884 X 49.323 X 49.323

The docked complex was visualized with Biovia Discovery Studio. It was also used to generate 2-D interactions of EDCs with the respective key enzymes [16].

2.1.2. Endocrine disruptors.

Three important sources of EDCs for maximum exposure are detergents, preservatives, and cosmetic products. The EDCs that are mainly present in these sources are Triclosan, Nonylphenol, Phenol, Ethylene diamine tetra acetic acid (EDTA), Dioxane, Nitrate, and Dibutyl Phthalate. These EDCs were grouped into three groups based on their sources as follows. Group A (detergent based): nonylphenol, phenol, ethylene diamine tetra acetic acid (EDTA), dioxane; Group B (preservative based): dibutyl phthalate, nitrate; Group C (cosmetics based): dibutyl phthalate, triclosan.

2.1.3. Steroidogenic enzymes.

The key enzymes considered for this study were: Cytochrome P450 scc; 17 beta-hydroxysteroid dehydrogenase; Aromatase.

The steroidogenesis process starts with the cleavage of the side chain of cholesterol, leading to conversion into Pregnenolone by the action of cytochrome P450 side chain cleavage enzyme in the inner mitochondrial membrane [17]. The first enzyme, i.e., Cytochrome P450 scc, used in this study converts cholesterol to Pregnenolone, the precursor of all other steroids. The next one is 17, beta-HSD, which converts Androstenedione to Testosterone. Testosterone is produced by androstenedione by taking action with 17 beta hydroxyl steroid dehydrogenase [18, 19]. Third, the aromatase enzyme encoded by gene CYP19A1 is the key enzyme required for the conversion of testosterone to estradiol, one of the naturally occurring estrogen [20, 21].

2.1.4. Data collection.

For docking purposes, a three-dimensional structure of protein and ligand is required. PDB was used to get the 3D structure of proteins, and for the Ligand collection, the ZINC database and PubChem were considered.

Enzymes involved in the pathway of steroid formation are listed (Table 1.) below:

Table 1. Proteins (enzymes) are involved in the process of steroidogenesis.

S.No.	Protein involved in steroid formation pathway	PDB ID
1.	P450scc (Endoplasmic Reticulum)	3NA1, 3NA0
2.	17 alpha-hydroxylase	3RUK
3.	3-beta hydroxy steroid dehydrogenase	2FVL
4.	17 beta- HSD	1A27
5.	5alpha-reductase	7BW1
6.	21-hydroxylase	4Y8W
7.	Aromatase	5JL9

All three violet-colored enzymes (proteins) were used in this study to reveal the extent of binding shown by different groups of endocrine disruptors.

3. Result and Discussion

3.1. Identification of active site amino acids in key steroidogenic enzymes.

Before proceeding to the docking study, knowledge of the active site amino acids of the protein of interest was required. In this study, we have mainly focused on the three enzymes cytochrome P450 (PDB ID: 3NA0), 17 beta-hydroxysteroid dehydrogenase (PDB ID: 1A27) and aromatase (PDB ID: 5JL9). Pymol is one of the GUI software used to find the active site of protein along with the amino acid that binds with different ligands. For that purpose, 3D structures of proteins and co-crystalized ligands were used. Using Pymol, we got the result shown in Table 2. Different color codes indicate the interactions of active site amino acids with the different EDCs used in the study. Amino acids shown in red are the ones that interacted with the EDCs belonging to a detergent group, the blue amino acids interacted with EDC's belonging to a preservative group, while the green color interacted with the EDCs belonging to the cosmetics group. A few amino acids shown in mixed color combinations show interaction with two or all three groups of EDCs used in the study.

Table 2. The active site amino acids of corresponding enzymes.

S.No.	Enzymes	PDB ID	Active Site Amino Acid
1.	**CYP P450 scc**	**3NA0**	GLN 416, GLN 395, ARG 120, PHE 121, LEU 140, LEU 394, THR 393, VAL 139, LEU 140, ILE 123, PHE 455, VAL 392, SER 391 LEU 248, TRP 126, ILE 390, GLU 322, LEU 499, ILE 500, PHE 497, THR 330, ALA 325, THR 334, THR 331, PHE 241, MET 240, TRP 270, GLY 326, VAL 392, ILE 390, GLY 464, GLY 327, GLY 326, ARG 460, VAL 139, GLY 454, CYS 462, ALA 120, ARG 151, TRP 147, GLN 461, MET 472, ILE 467, LEU 463, MET 323, ILE 210, ARG 396, TRP 457, GLY 456, LEU 453, LEU 385
2.	**17-beta hydroxy steroid dehydro genase**	**1A27**	SER 222, ALA 191, VAL 225, VAL 143, PHE 192, THR 190, VAL 188, PHE 226, PHE 226 CYS 185, MET 193, MET 193 SER 142, GLY 141, LYS 195, VAL 66, TYR 155, LEU 93, ILE 14, GLY 15, ALA 191, CYS 10, THR 41, ASN 90, GLY 9, LEU 36, ALA 91, GLY 92, LEU 93, ASP 38, **ARG 37**, LEU 64, ASP 65, VAL 113
3.	**CYP19 (Aromat ase)**	**5JL9**	Pro 429, GLY 439, ALA 438, MET 311, ALA 307, SER 314, PHE 134, SER 478, PHE 148, LEU 152, ARG 145, ALA 306, ALA 443, ARG 435, MET 447, ASP 309, THR 310, GLY 431, PHE 221, ARG 115, PRO 429, VAL 373, MET 364, ILE 398, MET 107, ARG 145, LEU 152, PHE 148, ARG 375, MET 374, LEU 372, TRP 141, LEU 447, ILE 133, ILE 132, PHE 432, GLY 436, TRP 224, VAL 370, PHE 430, CYS 437, MET 303, ILE 305, PHE 203

Red amino acids interact with the endocrine disruptors of detergents, while blue amino acids interact with those of preservatives. Similarly, amino acids with a green color interact

with endocrine disruptors in cosmetics, while those with a combination of colors are either interacting with two or all three groups.

3.2. Docking between endocrine disruptors and steroidogenic enzymes.

The results of molecular docking between endocrine disruptors and proteins (Enzyme, i.e., Cytochrome P450, 17 beta HSD, and aromatase) are shown in Tables 3, 4, and 5. Low binding energy corresponds to higher stability of the docking complex.

In Group A: Nonylphenol has more affinity with the lowest binding energy interaction for all three cytochrome P450, 17 beta-hydroxysteroid dehydrogenase and aromatase with -5.9, -6.4 and -6.0 Kcal/mol, respectively (Table 3).

Table 3. Docking of endocrine disruptors found in detergents (Group A) with that of three prominent enzymes involved in steroidogenesis.

S.No.	Enzymes	Endocrine Disrupting chemicals found in Detergents	Affinity
1	Cytochrome P450scc	Nonylphenol	-5.9 Kcal/mol
		Phenol	-4.6 Kcal/mol
		Ethylene diamine tetra acetic acid	-5.1 Kcal/mol
		Dioxane	-3.5 Kcal/mol
2	17beta HSD	Nonylphenol	-6.4 Kcal/mol
		Phenol	-4.8 Kcal/mol
		Ethylene diamine tetra acetic acid	-6.0Kcal/mol
		Dioxane	-3.4 Kcal/mol
3	Aromatase	Nonylphenol	-6.0 Kcal/mol
		Phenol	-4.9 Kcal/mol
		Ethylene diamine tetra acetic acid	-5.6 Kcal/mol
		Dioxane	-3.3 Kcal/mol

In Group B: Dibutyl phthalate has much more affinity with the lowest binding energy for all three enzymes, i.e., Cytochrome P450, 17 beta-hydroxysteroid dehydrogenases, and aromatase with -6.7 Kcal/mol, -7.1 Kcal/mol, and -5.8 Kcal/mol, respectively (Table 4).

Table 4. Docking of endocrine disruptors found in preservatives (Group B) with that of three prominent enzymes involved in steroidogenesis.

S.No.	Enzymes	Endocrine Disrupting chemicals found in Detergents	Affinity
1	Cytochrome P450scc	Dibutyl phthalate	-6.7 Kcal/mol
		Nitrate	-3.1 Kcal/mol
2	17beta HSD	Dibutyl phthalate	-7.1 Kcal/mol
		Nitrate	-3.3 Kcal/mol
3	Aromatase	Dibutyl phthalate	-5.8 Kcal/mol
		Nitrate	-2.6 Kcal/mol

While in Group C, triclosan has a higher affinity with the lowest binding energy for cytochrome P450, 17 beta-hydroxysteroid dehydrogenase, and aromatase, i.e. 7.1Kcal/mol, -7.9Kcal/mol and -6.8Kcal/mol respectively (Table 5).

Table 5. Docking of endocrine disruptors found in cosmetics (Group C) with that of three prominent enzymes involved in steroidogenesis.

S.No.	Enzymes	Endocrine Disrupting chemicals found in Detergents	Affinity
1	Cytochrome P450scc	Dibutyl phthalate	-6.7 Kcal/mol
		Triclosan	-7.1 Kcal/mol
2	17beta HSD	Dibutyl phthalate	-7.1 Kcal/mol
		Triclosan	-7.9 Kcal/mol
3	Aromatase	Dibutyl phthalate	-5.8 Kcal/mol
		Triclosan	-6.8 Kcal/mol

3.3. Docking between reference reactants and their respective Steroidogenic enzymes.

In order to compare the binding affinities of EDCs with steroidogenic enzymes with their actual reactants, the docking study was performed with reference reactants of respective enzymes, and their affinities were obtained, as shown in Table 6. Cholesterol showed affinities of about -9.9 Kcal/mol in terms of binding energy for cytochrome P450, androstenedione has -10.6 Kcal/mol affinities for 17beta HSD, and testosterone has -10.0 Kcal/mol affinity for aromatase.

Table 6. Showing the affinity of each reference reactant during the process of steroid formation.

S.No.	Enzymes	Corresponding reactants in steroidogenesis	Affinity (Kcal/mol)
1.	Cytochrome P450scc	Cholesterol	-9.9
2.	17beta HSD	Androstenedione	-10.6
3.	Aromatase	Testosterone	-10

Graphical representation was used to show the comparison of affinities with reference reactants (Figures 3, 4, and 5). It is seen that triclosan has a somewhat nearest affinity for all three enzymes, i.e., cytochrome P450, 17 beta HSD, and aromatase with respect to the original reactants, i.e., cholesterol, androstenedione, and aromatase with -7.1 Kcal/mol, -7.9 Kcal/mol, and -6.8 Kcal/mol, respectively, followed by dibutylphthalate and nonylphenol which shows highest affinity for Cytochrome P450, 17beta HSD and Aromatase, respectively.

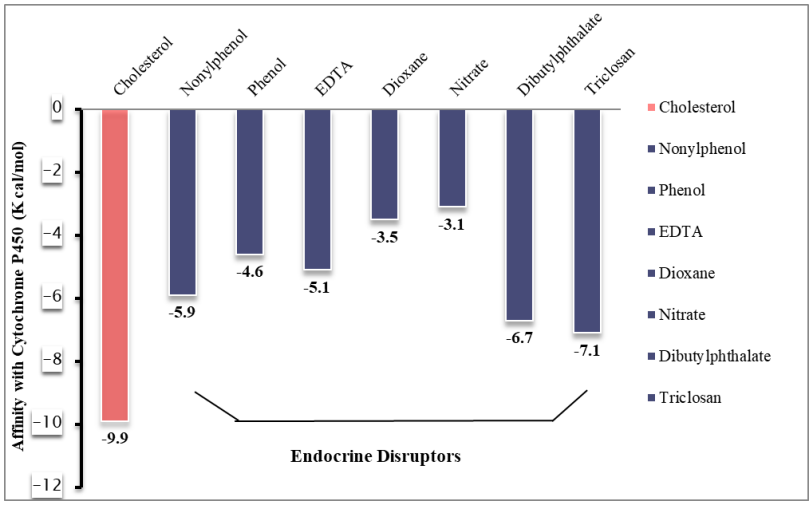


Figure 3. Graphical representation of affinity Cholesterol v/s EDCs for cytochrome P450.

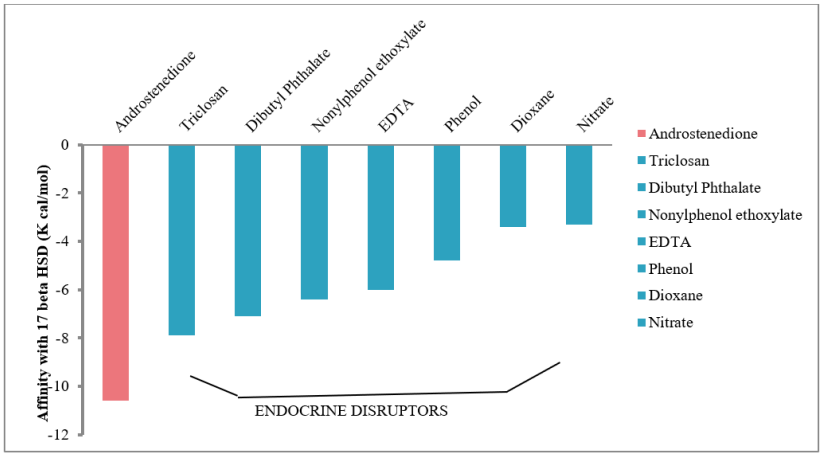


Figure 4. Graphical representation of affinity of androstenedione v/s EDCs for 17beta HSD.

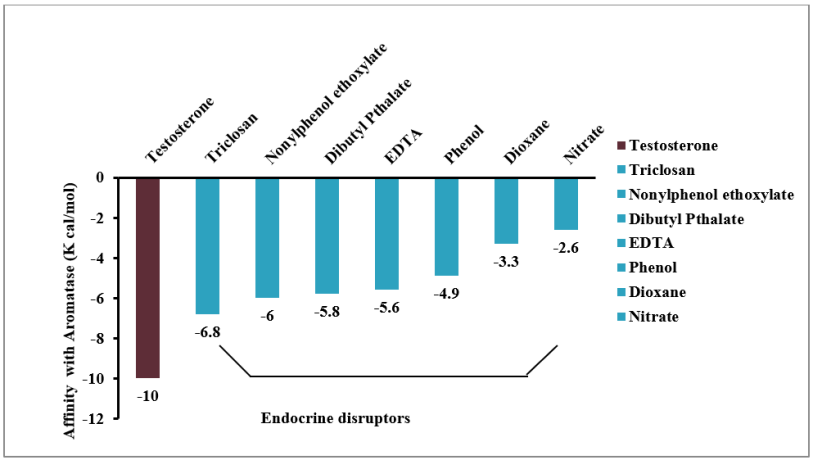


Figure 5. Graphical representation of affinity testosterone v/s EDCs for aromatase.

3.4. Interaction of active site amino acids with reference reactants.

In order to find out which amino acids are binding with different endocrine disruptors, it's obvious to know first the amino acids involved in forming the active sites of receptors of cytochrome P450scc, 17 beta-hydroxysteroid dehydrogenase, and aromatase.

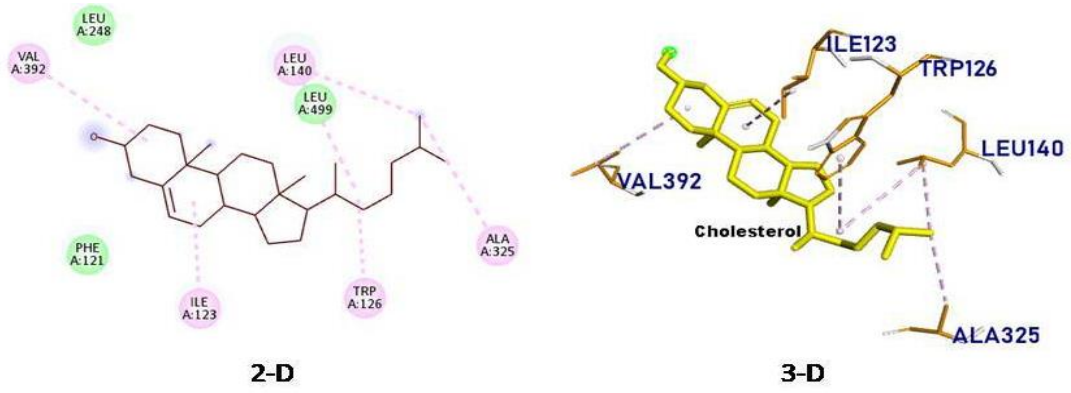
Since we did docking, we have some interacting amino acids with the corresponding Endocrine disruptors. Next, we aimed to find whether these amino acids are of interest. That means whether these are active site amino acids or not. So basically, we compared all the interacting amino acids with that of active site amino acids shown in Table 2.

Why bother about the active site amino acid only, and what about the other amino acids that interact with these EDCs? It is evident that the biocatalytic action of the enzyme does not occur at any random places; in contrast, it occurs at a specific site known as the enzyme's active site [22]. There is a difference between the enzyme's active site and the binding site. The former is present in a single copy, whereas, in enzymes, the latter may be present in multiple copies [23]. Amino acids that are not part of the active site but are interacting with that of enzymes or ligands along with active site amino acids belong to the binding sites inside which the active sites of enzymes reside; in fact, the binding site provides the phenomenon of induced-fit for making the suitable cleft for the substrate inside the active region.

We already know that the active site of any protein plays a crucial role in substrate formation as it is similar to lock and key. If the right key is placed, the lock will be opened; otherwise, it will not be. Here, the active site amino acids act as the conformational partners that help the specific reactant or the ligand attach to the protein's pocket. In the process of steroid formation, specific products of each step bind with enzymes for normal functioning. If any chemicals that have similar structures or functions with nearly the same affinity for the respective enzyme compete and, if by chance, bind to the enzyme, they would hamper normal functioning and thereby disturb hormone formation.

For comparison, molecular docking for all three enzymes under study, along with the actual reactants of steroidogenesis, has also been done. For cytochrome P450scc, cholesterol acts as the primary reactant that, upon action, gets converted into Pregnenolone and shows the following 2-D and 3-D interactions (Figure 6).

All the 3-D and 2-D images have been analyzed using bio via Discovery Studio, PyMOL, and Protein Plus server.

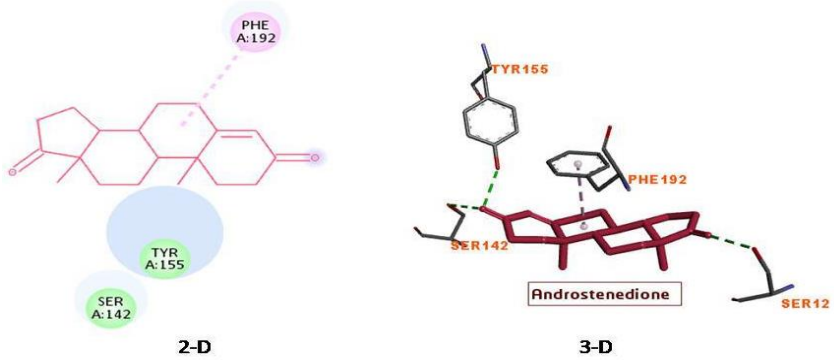


(a) **(b)**

Lowest affinity= -9.9 Kcal/mol with RMSD value = 0

Figure 6. Interaction of cholesterol with cytochrome P450 scc **(a)** 2D diagram; **(b)** 3D diagram.

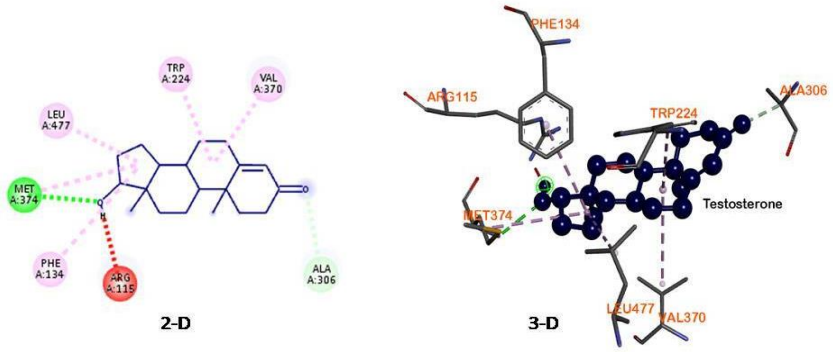
Here in Figure 6, the binding of cholesterol by alkyl interactions with valine 392, leucine 140, alanine 325, tryptophan 126, and isoleucine 123 is clearly visible, whereas other amino acids like leucine 248, leucine 499, and phenylalanine are showing vander waal interactions. All these are active site amino acids of cytochrome P450. For 17 beta-hydroxysteroid dehydrogenase, androstenedione acts as the reactant that gets converted into testosterone upon the action of the enzyme. In the 2D interaction of the enzyme and the androstenedione, the precursor for testosterone binds with phenylalanine 192, tyrosine 155, and serine 142 of 17beta HSD in which phenylalanine 192 showed pi-alkyl interaction, whereas serine 142 and tyrosine 155 showed vander waal interactions (Figure 7)



Lowest affinity= -10.6 Kcal/mol with RMSD value = 0

Figure 7. Interaction of 17beta HSD with Androstenedione.

For the third enzyme, i.e., aromatase, testosterone acts as a reactant, which, on subsequent reaction, converts into estradiol, a form of naturally occurring estrogen. 2d interaction shows binding of testosterone with tryptophan 224, valine 370, alanine 306, arginine 115, phenylalanine 134, methionine 374, and leucine 447 of aromatase of which alanine 306 shows a carbon-hydrogen bond. Methionine shows a conventional hydrogen bond, phenylalanine, valine, leucine, and tryptophan show alkyl or pi-alkyl interactions, and one unfavorable bond is seen in arginine 115 via donor-donor interaction towards the aromatase (Figure 8).



Highest affinity=-10 Kcal/mol RMSD value = 0
Figure 8. Interaction of testosterone with aromatase.

3.5. Interaction of active site amino acids with the EDCs.

In this study, endocrine disruptors have been divided into three groups: group A for endocrine disruptors found in detergents, group B for endocrine disruptors found in preservatives, and at last group C for endocrine disruptors found in cosmetics**.**

3.5.1. In group A (Endocrine disruptors of detergents).

3.5.1.1. Interactions of nonylphenol, EDTA, phenol, and dioxane with the cytochrome P450.

Nonylphenol has the highest affinity for cytochrome P450 (Figure 9) with about -6.6 Kcal/mol among all the endocrine disruptors found in detergents, while EDTA has the highest affinity for 17 beta HSD with about -3.8 Kcal/mol and for aromatase too with -4.7 Kcal/mol.

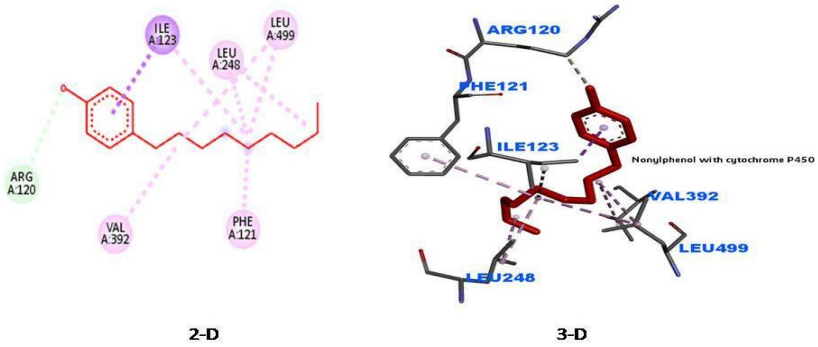


Figure 9. Interaction of nonyl phenol with amino acids of cytochrome P450.

The docking between cytochrome P450 and nonylphenol as receptor and ligand, respectively, revealed the interaction of nonylphenol with active site amino acids of cytochrome P450 like arginine 120, isoleucine 123, phenylalanine 121, leucine 248, leucine 499 and valine 392 (Figure 9).

The interaction between phenol and dioxane with cytochrome P450 clearly shows the affinity of the former for the active site amino acid viz. isoleucine 123, serine 391, and glycine 395, out of which isoleucine shows pi-alkyl interaction, glycine shows pi-donor H-bond and serine shows conventional H-bond. Dioxane, however, binds with threonine 330 and shows conventional H-bonding (Figure 10).

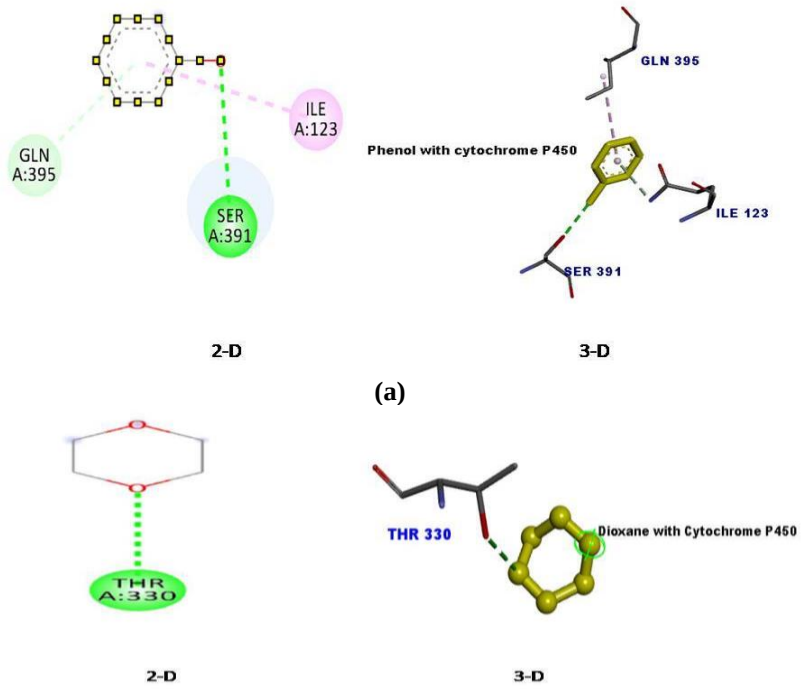


Figure 10. Interaction of **(a)** Phenol; **(b)** Dioxane with cytochrome P450.

The interaction between Ethylene diamine tetra acetic acid, cytochrome P450 (Figure 11) shows binding with glutamic acid 322, arginine 151, arginine 120, arginine 460, cysteine 462, glycine 464 and methionine 323, the active site amino acid of cytochrome P450.In these all, only glycine shows carbon-hydrogen bonding, while others show conventional hydrogen bonding.

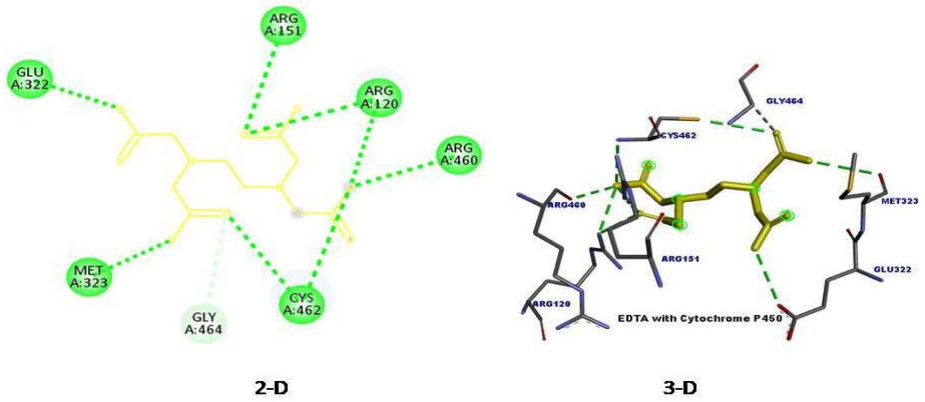


Figure 11. Interaction of EDTA with amino acids of cytochrome P450.

3.5.1.2. Interaction of nonylphenol, EDTA, phenol, and dioxane with the enzyme 17 beta-hydroxysteroid dehydrogenase.

The interaction between nonylphenol and 17 beta HSD (Figure 12 (a)) shows alkyl and pi-alkyl bonding with cysteine 185, isoleucine 14, valine 188, phenylalanine 192, threonine 190 (all are active site amino acids except for serine 12) while serine 12 shows conventional H-bonding.

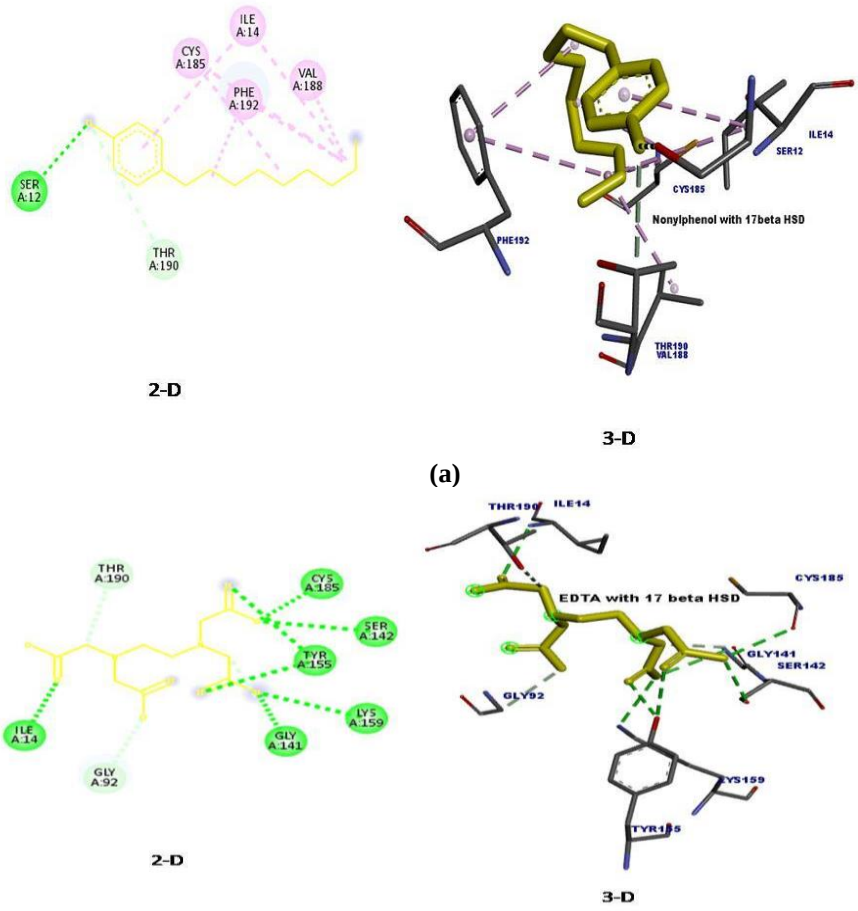
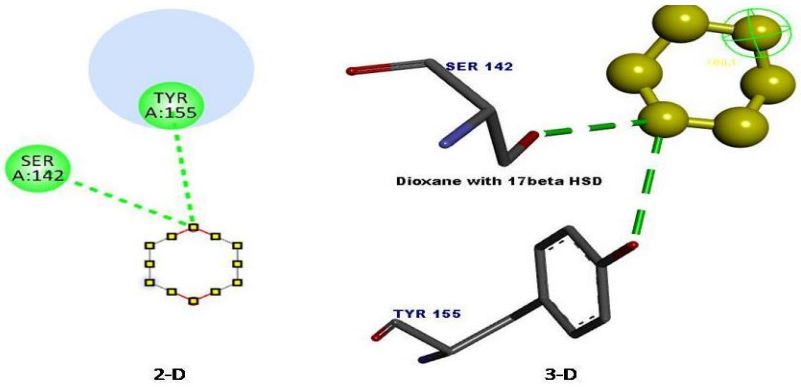


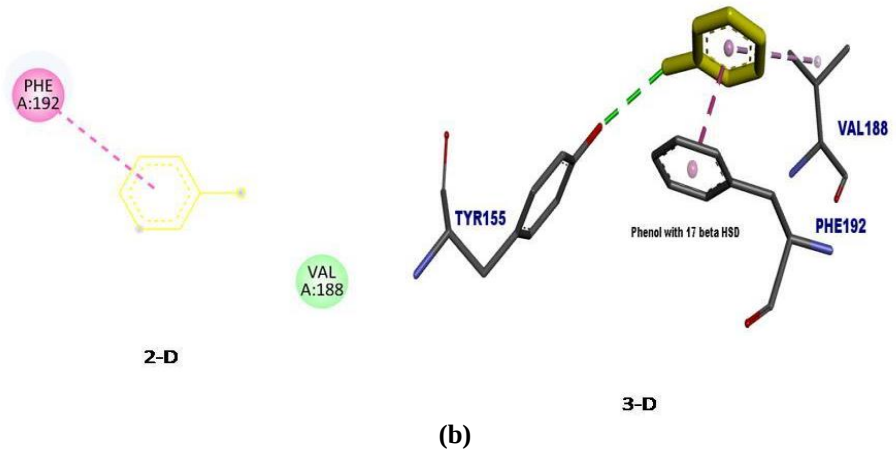
Figure 12. Interaction of **(a)** Nonylphenol; **(b)** EDTA with amino acids of 17 beta HSD.

EDTA binds with active site amino acids of 17 beta HSD that are cysteine 185, serine 142, tyrosine 155, isoleucine 14, glycine 141, glycine 92, threonine 190, and lysine 159; all are active site amino acids except lysine 159 (Figure 12 (b)).

It is also observed that glycine 92 and threonine 190 had carbon-hydrogen bonds, while all other amino acids showed conventional H-bonding with 17 beta HSD.

The interaction between the amino acids of 17 beta HSD with Dioxane and Phenol is shown in Figure 13. Dioxane interacts with tyrosine 155 and serine 142 by conventional H-bond with both active site amino acids. Phenylalanine 192 showed pi-pi stacked bonding, whereas valine 188 and tyrosine 155 exhibited vander waal interaction with phenol.





(b)

Figure 13. Interaction of **(a)** Dioxane; **(b)** Phenol with amino acids of 17 beta HSD.

3.5.1.3. Interaction of nonylphenol, EDTA, phenol, and dioxane with the enzyme aromatase.

Nonylphenol shows binding with lysine 440, tyrosine 441, phenylalanine 430, arginine 115, Met 374, leucine 447, isoleucine 133, tryptophan 224, and valine 370, all active site amino acids, except for leucine 477 (Figure 14).

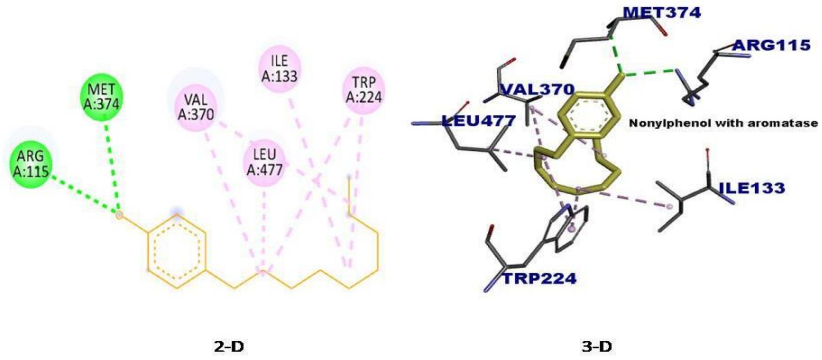


Figure 14. Interaction of nonylphenol with amino acids of aromatase.

Meanwhile, phenol shows interaction with serine 478, which is an active site amino acid, while other interacting amino acids are not active site amino acids (Figure 15).

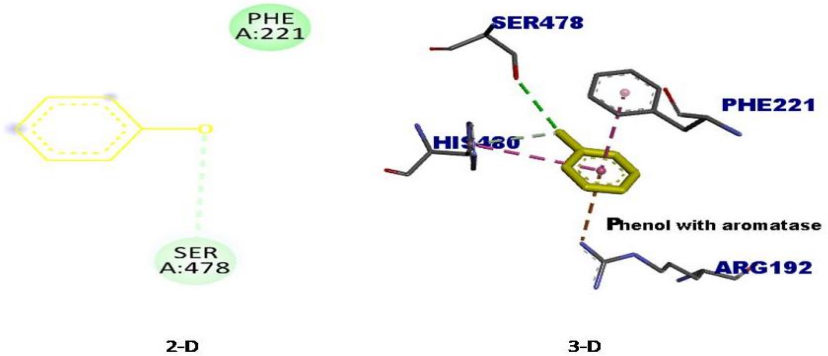


Figure 15. Interaction of phenol with amino acids of aromatase.

Dioxane shows interaction (Figure 16) with methionine 374 and arginine 315 of aromatase, where methionine 374 is an active site amino acid (Figure 16).

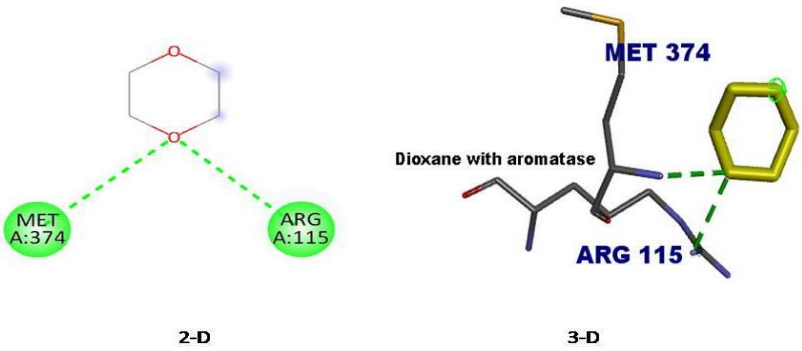


Figure 16. Interaction of dioxane with amino acids of aromatase.

EDTA is bound with arginine 115, methionine 374, and tryptophan 224, which are active site amino acids of aromatase. Tryptophan 224 showed carbon-hydrogen bonding, while arginine 115 and methionine 374 exhibited conventional hydrogen bond formation with ethylene diamine tetra acetic acid (Figure 17).

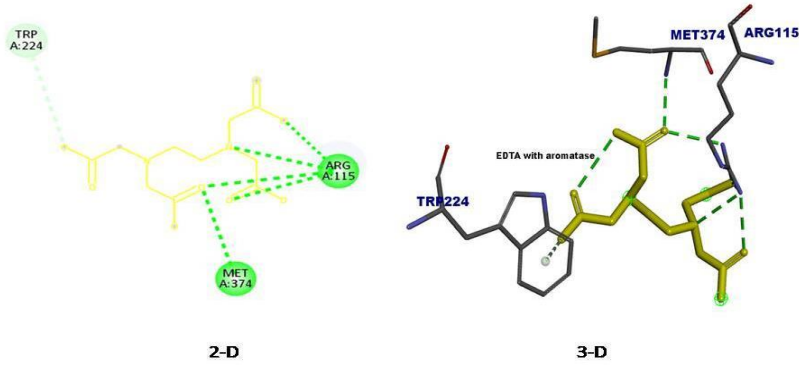


Figure 17. Interaction of EDTA with amino acids of aromatase.

3.5.2. In group B (Endocrine disruptors of preservatives): Commonly found endocrine disruptors in food preservatives are dibutyl phthalate and nitrate.

3.5.2.1. Interaction of dibutyl phthalate and nitrate with the enzyme cytochrome P450.

From the interaction (Figure 18), it is visible that dibutyl phthalate binds with many amino acids of cytochrome P450, out of which only leucine 122 and leucine 141 are not active site amino acids, while all others are active site amino acids of cytochrome P450.

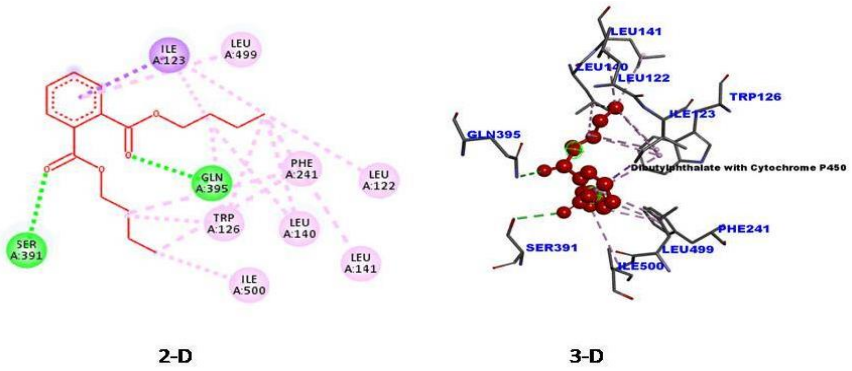


Figure 18. Interaction of dibutyl phthalate with cytochrome P450.

Nitrate binds with lysine 382, asparagine 452, glycine 454, and leucine 453, of which Leucine 453 and Glycine 454 are active site amino acids of cytochrome P450 (Figure 19;

Figure 23). Since the ligand preference is not fulfilled by nitrate over bio via Discovery Studio, these images have been generated by different tools like 2D interaction extracted using an online protein plus server [24] and 3D image by PyMOL software [25].

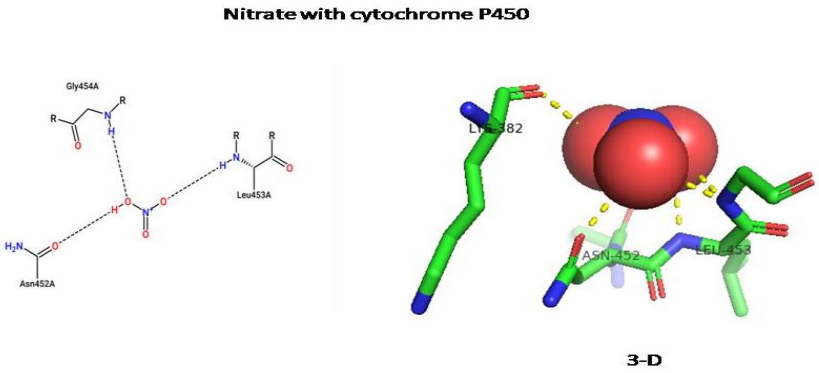


Figure 19. interaction of nitrate with cytochrome P450.

3.5.2.2. Interaction of nitrate and dibutyl phthalate with the enzyme 17 beta-hydroxysteroid dehydrogenase.

Dibutyl phthalate interacted with 17 beta HSD (Figure 20) active site amino acids viz. valine 188, methionine 193, phenylalanine 192, and tyrosine 155.

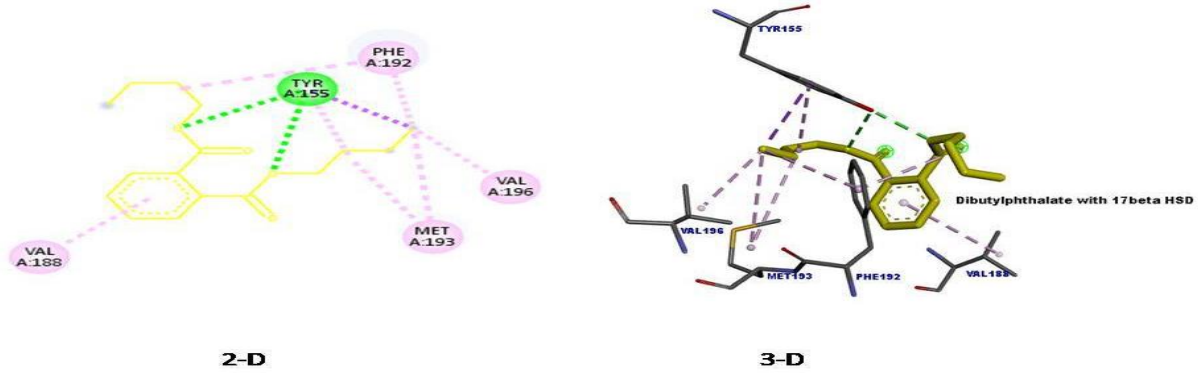


Figure 20. Interaction of dibutyl phthalate 17 beta HSD.

Nitrate interacts with17 beta HSD (Figure 21) active site amino acids viz. asparagine 153, phenylalanine 151, valine 154, tyrosine 155, and leucine 95, of which tyrosine 155 is an active site amino acid of 17 beta HSD.

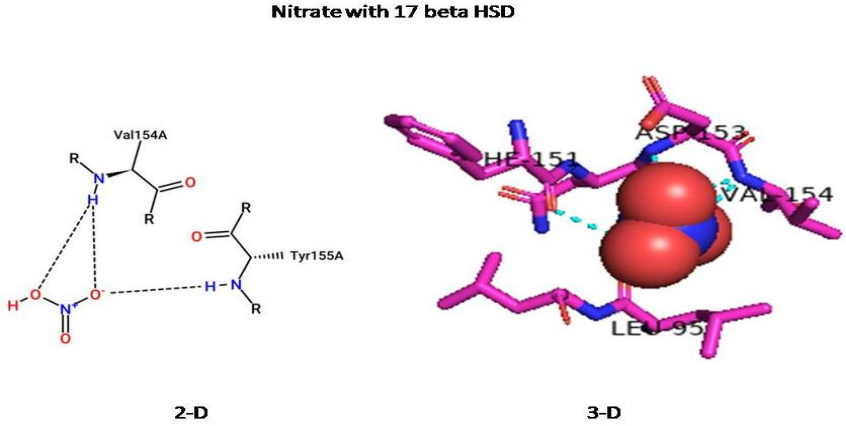


Figure 21. Nitrate with 17beta HSD.

3.5.2.3. Interaction of dibutyl phthalate and nitrate with the enzyme aromatase.

In the interaction of dibutyl phthalate and nitrate (Figure 22) with aromatase, Phenylalanine 148, Alanine 438, Cysteine 437, Valine 370, Isoleucine 133, and Serine 314 are the active site amino acids of respective enzyme. Arginine, alanine, and cysteine showed conventional H-bond formation with dibutyl phthalate, while isoleucine, valine, alanine, and phenylalanine showed alkyl-alkyl interaction. The binding of nitrate with aromatase is mentioned earlier in group B EDC interactions (Figure 23).

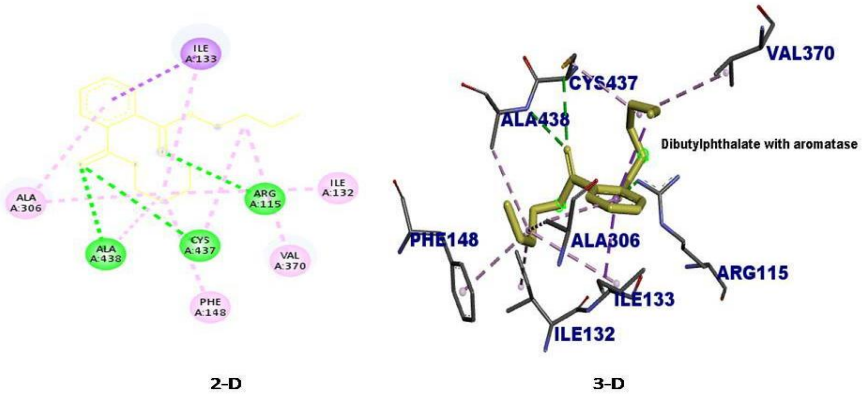


Figure 22. Interaction of dibutyl phthalate with aromatase.

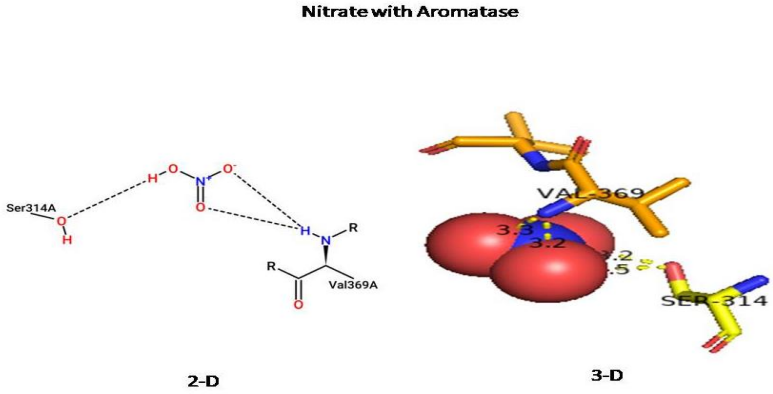


Figure 23. Interaction of nitrate with aromatase.

3.5.3. In group C (endocrine disruptors of cosmetics).

Triclosan has the lowest binding energy in terms of affinity towards all three enzymes, i.e., cytochrome P450, 17 beta HSD, and aromatase with -7.1 Kcal/mol, -7.9 Kcal/mol, and -6.8 Kcal/mol, respectively. The interaction of dibutyl phthalate with all three steroidogenic enzymes has already been studied in group B.

3.5.3.1. Interaction of triclosan with the enzyme cytochrome P450.

Triclosan has the lowest binding energy of -6.8 Kcal/mol for aromatase (Figure 24), followed by dibutyl phthalate with -5.8 Kcal/mol. Phthalates are used in cosmetics and in plastic wares to preserve foods.

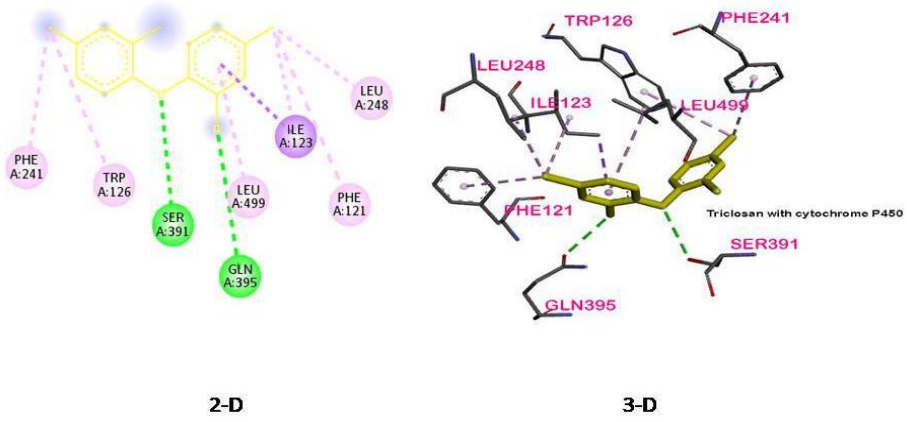


Figure 24. Interaction of triclosan with cytochrome P450.

Triclosan showed binding with Leu 248, isoleucine 123, phenylalanine 121, leucine 499, serine 391, glycine 395, phenylalanine 241, and tryptophan 126, out of which tryptophan 126 is not an active site amino acid of cytochrome P450 (Figure 24).

3.5.3.2. Interaction of triclosan with the enzyme 17 beta-hydroxysteroid dehydrogenase.

Triclosan showed binding with valine 188, phenylalanine 192, isoleucine 14, glycine 15, asparagines 90, and serine 12, all are active site amino acids of 17 beta hydroxyl steroid dehydrogenase (Figure 25).

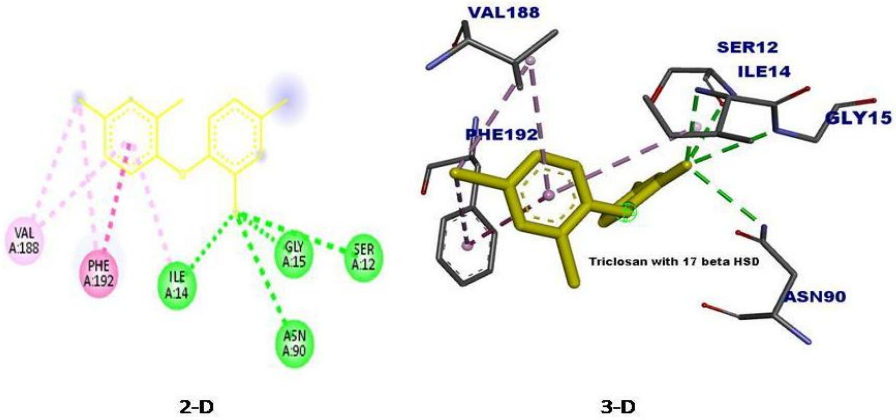


Figure 25. Interaction of Triclosan with 17 beta HSD.

3.5.3.3. Interaction of triclosan with the enzyme aromatase.

Triclosan showed interaction with active sites as amino acids of aromatase such as isoleucine 133, tryptophan 224, cysteine 437, alanine 306, threonine 310, methionine 446, alanine 307, and phenylalanine 203 (Figure 26). H bonding was shown by threonine and cysteine. Alanine and methionine, however, had a pi-sigma bond and pi-sulfur bonding, respectively. The others are bound with alkyl and pi-alkyl bonds.

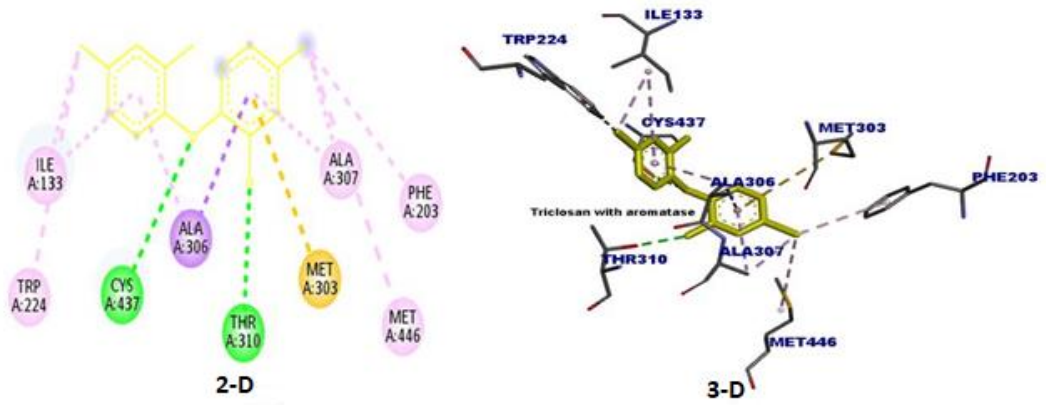


Figure 26. Interaction of triclosan with aromatase.

4. Conclusion

From the results obtained, it is clear that EDCs belonging to all three groups had an affinity for the amino acids present in the active sites of the three steroidogenic enzymes studied. Compared to the affinities shown by the natural reference reactants, with their respective steroidogenic enzymes, the affinity of all the EDCs studied is less, but as it is already known that EDCs are lipophilic in nature [26] and they tend to bioaccumulate in the tissues [27, 28]. Hence, it is quite clear that if the amount of these EDCs exceeds a threshold, it will certainly surpass the natural reactant and bind to the active site amino acids of key steroidogenic enzymes, thereby causing endocrine disruption. Bioaccumulation tends to occur only if some product is used longer, thereby providing ample time for continuous entry into the body. The study shows that the product containing these EDCs should be properly screened, and the same product should not be used continuously for a prolonged time. However, the wet lab validation of this study is desirous.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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