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Analysis of human health risk assessment for stereoisomers of propiconazole fungicide by cheminformatics study

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Abstract
Propiconazole is a broad-spectrum systemic triazole class of fungicides. This fungicide is used on many fruits, vegetables, cereal grains, turf grass and ornamental plants. It is used to combat powdery mildew, rusts, and leaf spot diseases. Propiconazole is a demethylase inhibitor (DMI) fungicide and it inhibits the activity of lanosterol-14-*a*-demethylase enzyme. This enzyme synthesises ergosterol which is key component of fungal cell wall. Propiconazole inhibits the growth of fungi by blocking synthesis of ergosterol and thus, belongs to the class of ergosterol biosynthesis inhibiting fungicides (EBIFs). Fungicides are indiscriminately used in large amounts and pose a huge threat to environment and living organisms. The use of fungicides may cause accumulation of their residue in agricultural products. It can result in various types of toxicities and may lead to human health risk. It has moderate acute oral toxicity in rats and mice and has been reported to be liver hepatotoxicant and a hepatocarcinogen having adverse reproductive and developmental toxicities. Exposure to propiconazole has been documented for behavioural disturbances like anxiety, depression and cognitive impairment. Propiconazole has two chiral centres and exist as four stereoisomers. In this paper predictive study of the impact of stereochemistry on toxicological aspects of propiconazole has been carried out using Chem Axon-chemicalize (licenced) and ADMETlab 2.0 cheminformatic tools.

Keywords: Propiconazole, stereoisomers, demethylase inhibitor, ergosterol, toxicity, health risk, cheminformatic tools

Introduction
Fungicides are a class of pesticides responsible for killing or destruction of fungi. Fungicides are an integral part of agriculture for prevention and increasing crop production. Propiconazole is a systemic triazole fungicide and is chemically 1-{[2-(2, 4-dichlorophenyl)-4-propyl-1, 3-dioxolan-2-yl] methyl}-1*H*-1, 2, 4-triazole. It is used on cereal grains, coffee, fruits, lemons, vegetables, wheat, tree nuts, turfgrass and ornamental plants. Propiconazole, is widely used to combat powdery mildew, rusts, and leaf spot diseases. It is also known as DMI, or demethylation inhibiting fungicide. It inhibits the CYP51 enzyme lanosterol-14-*a*-demethylase (EC 1.14.13.70) from demethylating [1, 2]. Demethylation results in biosynthesis of ergosterols that are essential in the formation of cell walls of fungi. In absence of this demethylation step (Figure 1), ergosterol depletion occurs and C-14 methylsterols get accumulated in fungal cells. This impairs the bio membrane function and inhibits the cellular growth.
Fungicides are indiscriminately used in large amounts and pose a huge threat to environment and living organisms [3]. Propiconazole is moderately persistent in soil with DT_{50} (typical) 71.8 days. The use of fungicides may cause accumulation of their residue in agricultural products [4]. This result in various types of toxicities and may lead to human health risk [5]. Propiconazole is moderately toxic with an oral acute LD50 of 1500 mg/kg. It is a skin sensitizer and may cause an allergic reaction [6, 7].
Propiconazole has moderate acute oral toxicity in rats and mice. The rodent oral LD_{50} for propiconazole ranges from 1211 to 2233 mg/kg, depending on the animal species. It has been reported to be liver hepatotoxicant and a hepatocarcinogen having adverse reproductive and developmental toxicities [8. 9, 10, 11].
According to the World Health Organization, propiconazole is classified as a Group 2B carcinogen, which means it is possibly carcinogenic to humans [4]. The United States Environmental Protection Agency (EPA) has classified propiconazole as a Group C

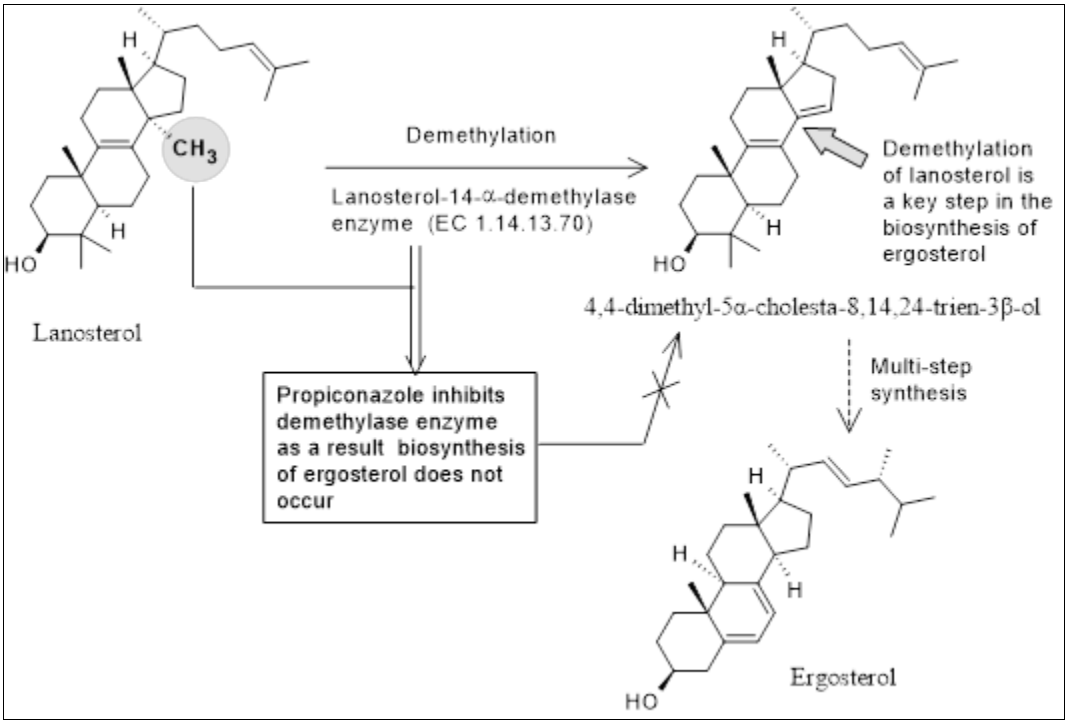


Fig 1: Depicting the Inhibitory effect of propiconazole on biosynthesis of ergosterol from lanosterol.

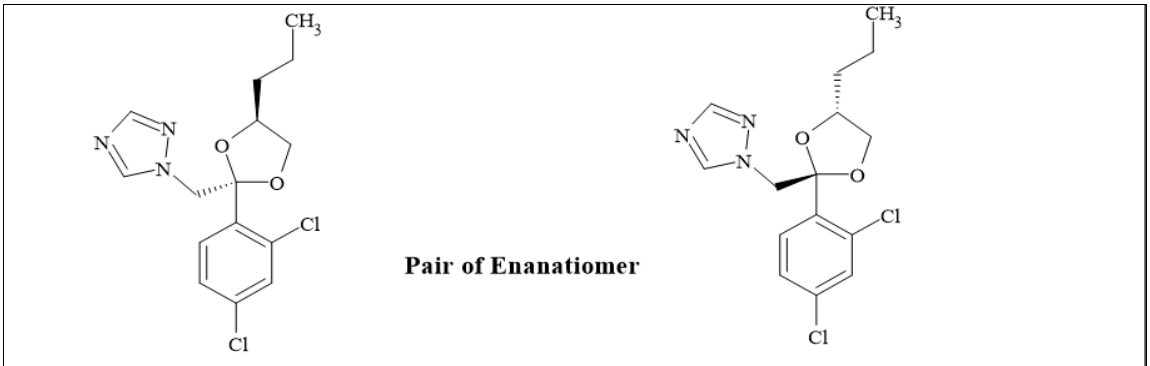
chemical, which means it is a possible human carcinogen based on animal studies. Exposure to propiconazole has been documented for behavioural disturbances like anxiety, depression and cognitive impairment. Symptoms of ingestion may include abdominal pain, nausea, vomiting and diarrhoea [12, 13].

The organic compounds having chiral centre exist as stereoisomers. The number of stereoisomers possible for an organic compound is related to the number of chiral centre(s) present in it [14]. Propiconazole, a chiral fungicide, has two chiral centres at 2- and 4-positions of the dioxolane ring and exist as four stereoisomers (Figure 2). The pair of enantiomers (2R,4S) and (2S,4R) are referred as *cis* isomers and pair of enantiomers (2R,4R) and 2S,4S) are referred as *trans* isomers. The stereoisomers and their relationship as pair of enantiomers, pair of diastereomers [15] and as cis/trans isomers is summarised in Table 1. Due to their distinct molecular configurations, these stereoisomers may interact differently with molecular targets.

In present work predictive study has been carried out for stereoisomers of propiconazole (Figure 2) to correlate the impact of stereochemistry on their bioactivity and toxicity profile. The cheminformatics study has been carried out using ADMET lab 2.0 online web server.

Materials and Methods

In present study all four stereoisomers of the fungicide propiconazole, (2*S*, 4*S*)-, (2*R*, 4*R*)-, (2*S*, 4*R*)-, and (2*R*, 4*S*)-, are considered as shown in Figure 2. The PubChem database [6] has been used in order to extract the IUPAC (International Union of Pure and Applied Chemistry) name, the SMILES (Simplified Molecular Input Line Entry System) formula, Further structures of all stereoisomers (Structures 1-4, Figure 2) were drawn using Chemsketch software 2D formula. For cheminformatics study ChemAxon-chemicalize (licenced) [16] and ADMET lab2.0 online webserver [17] has been used.



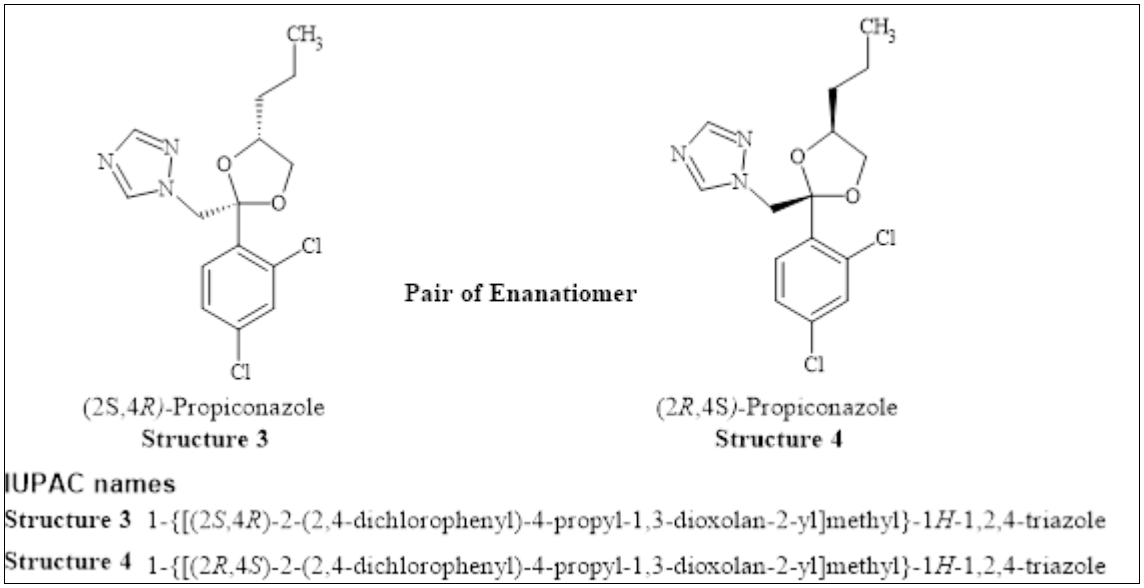


Fig 2: Stereoisomers of propiconazole and their IUPAC name (Structures 1-4)

Table 1: Stereoisomers (1-4) of propiconazole - Relationship as enantiomers, diastereomers and Cis/trans isomers

Propiconazole		Stereoisomers and Relationship		
Structure No.	**Stereoisomers**	**Pair of enantiomers**	**Pair of diastereomers**	**Cis/trans**
1 and 2	(*2S,4S*) and (*2R,4R*)	Yes	No	*s*-trans and s-trans
3 and 4	*(2S,4R)* and (*2R,4S*)	Yes	No	*s*-cis and s-cis
1 and 3	(*2S,4S*) and *(2S,4R)*	No	Yes	*s*-trans and s-cis
1 and 4	(*2S,4S*) and (*2R,4S*)-	No	Yes	*s*-trans and s-cis
2 and 3	(*2R,4R*) and *(2S,4R)*	No	Yes	*s*-trans and s-cis
2 and 4	(*2R,4R*) and (*2R,4S*)	No	Yes	*s*-trans and s-cis

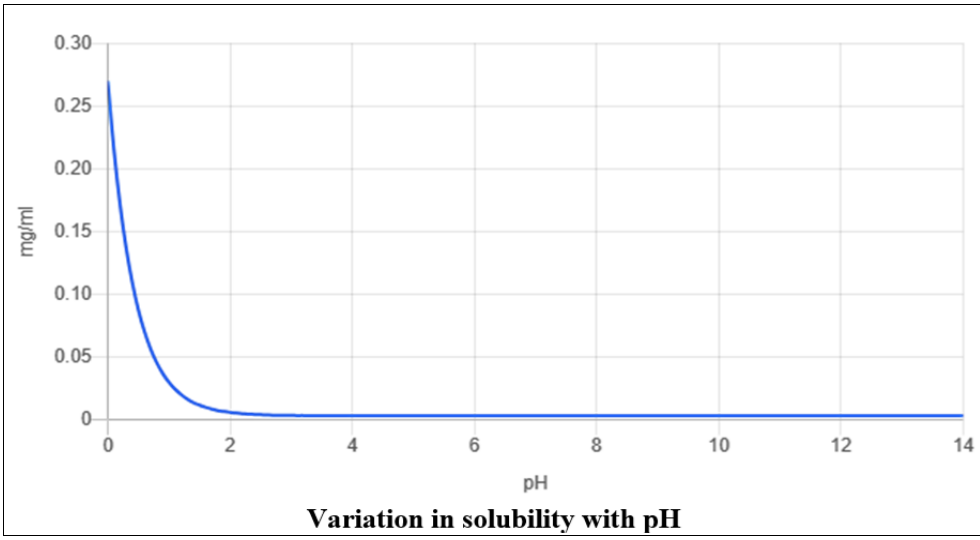
Results and Discussion

Physicochemical properties

The physicochemical properties for all four stereoisomers of propiconazole (Figure 2) are found to be same, as evaluated computationally, using ChemAxon-chemicalize (licenced) on line webserver. The values for physicochemical properties namely TPSA, strongest basic pKa, logP, log S and HLB are summarized in Table 2 and their interpretation is incorporated therein.

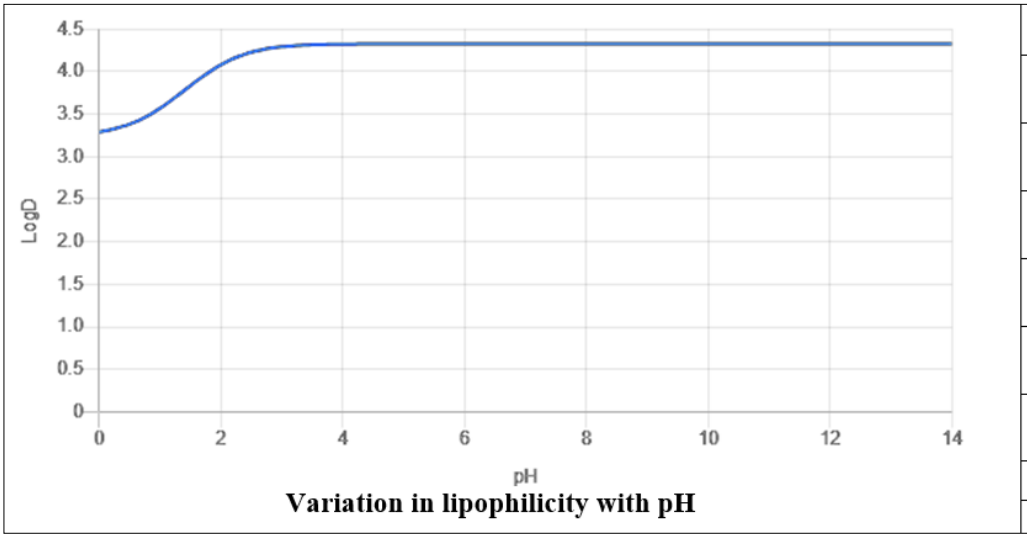
Table 2: Physicochemical Properties of stereoisomers of Propiconazole

All four Stereoisomers of propiconazole have same physicochemical properties					
Formula (Molar mass)	**TPSA Å²**	**Strongest basic pKa**	**log P**	**log S mg/mL**	**HLB**
$C_{15}H_{17}Cl_2N_3O_2$ (342.22)	49.17	1.95	4.326	-5.056	6.563
Interpretation		Strong base	High Lipophilicity	Low solubility	Indicates High Lipophilic
TPSA: Topological polar surface area; log P: Logarithm of n-Octanol-Water distribution coefficient; log S: Intrinsic solubility; HLB: Hydrophilic-lipophilic balance					



pH	mg/mL
1.0	0.0297
1.7	0.0083
2.0	0.0057
3.0	0.0033
4.6	0.003
7.4	0.003
8.0	0.003
14.0	0.003

Fig 3: Change in solubility (mg/mL) with variation in pH (0-14) for stereoisomers of propiconazole. Data tabulated for selected pH



pH	logD
1.0	3.5695
1.7	3.9419
2.0	4.0802
3.0	4.2917
4.6	4.3246
7.4	4.3255
8.0	4.3255
14.0	4.3255

Fig 4: Change in distribution coefficient -an indicator of lipophilicity, with variation in pH (0-14) for stereoisomers of propiconazole. Data tabulated for selected pH

All stereoisomers show poor solubility and high lipophilicity. The logarithm of the partition coefficient (log P) is a measure of the lipophilicity and is the ability of a compound to dissolve in lipids or fats.

pH dependent variation in Solubility and Lipophilicity

All four Stereoisomers of propiconazole show consistency in solubility over a long range of pH (Figure 3). These stereoisomers have poor solubility in water. The plot of logD v/s pH show an initial increase in lipophilicity (Figure 4). At pH higher than 2, all four stereoisomers show consistency in lipophilicity over a long range of pH. High lipophilicity of stereoisomers indicates an increased adsorption to soil and decreased solubility in water.

ADMET Profiles of stereoisomers of propiconazole Absorption, Distribution, Metabolism, Excretion

The predictive medicinal chemistry data using ADMETlab 2.0 online tool for stereoisomers of propiconazole showed that these compounds fulfilled the Lipinski Rule of Five and thus considered to have good bioavailability. The stereoisomers satisfied GSK rules and considered to have favourable ADMET profile. However, they did not satisfy the Pfizer rule and thus considered to be potentially toxic compounds (Table 3).

Table 3: Lipinski rules governing the bioavailability, and Pfizer and GSK rules governing safety of the stereoisomers of propiconazole.

Stereoisomer of Propiconazole	Rule					
	LogS	LogD	LogP	Lipinski	Pfizer	GSK
(2S, 4S)-	-4.671	3.816	3.835	Accepted	Rejected	Accepted
(2R, 4R)-	-4.507	3.733	3.696	Accepted	Rejected	Accepted
(2S, 4R)-	-4.666	3.856	3.813	Accepted	Rejected	Accepted
(2R, 4S)-	-4.398	3.754	3.735	Accepted	Rejected	Accepted

Predictions using ADMETlab 2.0 server, at https://admetmesh.scbdd.com/ for absorption, distribution and excretion for stereoisomers of propiconazole are summarized in Table 4.

Table 4: Predictive activity data for absorption, distribution, and excretion for stereoisomers of propiconazole**.**

Stereoisomer of Propiconazole	Activity: Absorption, Distribution and Excretion					
	HIA < 30%	P-gp s	P-gp i	BBBP	PPB (%)	CL (mL/min/kg
(2S, 4S)-	0.002	0.001	0.015	0.054	96.30%	13.009
(2R, 4R)-	0.002	0.064	0.008	0.025	96.77%	12.249
(2S, 4R)-	0.002	0.002	0.004	0.062	97.07%	12.867
(2R, 4S)-	0.002	0.014	0.022	0.019	96.21%	12.465

HIA—human intestinal absorption, P—gp-permeability glycoprotein, s—substrate, i—inhibitor, BBBP—blood– brain barrier permeation, PPB—plasma protein binding, CL—clearance.

All the stereoisomers of propiconazole had high intestinal absorption. This prediction correlates well with the fulfilment of Lipinski's rule. Permeability glycoprotein is a cell membrane protein which pumps out foreign substances from cell. None of the stereoisomers showed the probability of being an inhibitor or substrate of the permeability glycoprotein. The stereoisomers of propiconazole did not show blood–brain barrier penetration. All four stereoisomers showed high Plasma Protein binding and moderate clearance. The highest value for the clearance being registered for (2*S*, 4*S*)-propiconazole (Structure 1; Figure 2). The predictive metabolism data and toxicological end point data for stereoisomers of propiconazole has been summarized in Table 5 and Table 6 respectively.

Table 5: Predictive metabolism data for stereoisomers of propiconazole as inhibitors and substrates of cytochromes

Stereoisomer of Propiconazole	Metabolism data (human cytochromes)									
	CYP1A2 (Inhibitor)	CYP1A2 (Substrate)	CYP2C19 (Inhibitor)	CYP2C19 (Substrate)	CYP2C9 (Inhibitor)	CYP2C9 (Substrate)	CYP2D6 (Inhibitor)	CYP2D6 (Substrate)	CYP3A4 (Inhibitor)	CYP3A4 (Substrate)
(2S, 4S)-	0.706	0.928	0.866	0.644	0.438	0.056	0.136	0.101	0.891	0.834
(2R, 4R)-	0.753	0.817	0.89	0.585	0.657	0.078	0.169	0.13	0.925	0.869
(2S, 4R)-	0.737	0.916	0.863	0.633	0.492	0.049	0.165	0.1	0.902	0.875
(2R, 4S)-	0.731	0.828	0.878	0.559	0.596	0.082	0.126	0.137	0.915	0.815

The metabolism data in Table 5 shows the probabilities of interaction of propiconazole stereoisomers with human cytochromes and their ability to be inhibitors / substrates of cytochromes. All the stereoisomers of propiconazole showed high probabilities of being a substrate for CYP1A2, and CYP3A4 and reasonable of being a substrate for CYP2C19. The stereoisomers are not considered to be substrate for CYP2D6 and CYP2C9. All stereoisomers showed high probabilities of inhibiting CYP2C19 and CYP3A4.They showed reasonable probabilities of inhibiting CYP1A2 and low probabilities of inhibiting CYP2C9. The stereoisomers are not considered to be CYP2D6 inhibitors.

Toxicological end points

The predictive toxicological endpoints data indicates that all stereoisomers of propiconazole showed high probabilities of causing induced liver injuries (DILI), mutagenicity (AMES toxicity) and skin sensitization. The stereoisomers showed very low risk probability for cardiotoxicity by inhibiting the hERG potassium channel. These stereoisomers are predicted to show extremely low respiratory toxicity and eye corrosion. The stereoisomers are predicted to have low to moderate eye irritation. The (*2S*, 4S)-propiconazole showed highest carcinogenicity.

Table 6: Predictive toxicological endpoints for stereoisomers of propiconazole

Stereoisomer of Propiconazole	Toxicological Endpoint							
	hERG	DILI	AMES Toxicity	Skin Sensibilization	Carcinogenicity	Eye Corrosion	Eye Irritation	Respiratory Toxicity
(2S, 4S)-	0.318	0.951	0.98	0.891	0.726	0.005	0.176	0.027
(2R, 4R)-	0.299	0.928	0.989	0.917	0.482	0.004	0.447	0.021
(2S, 4R)-	0.307	0.904	0.985	0.909	0.576	0.004	0.24	0.029
(2R, 4S)-	0.327	0.944	0.985	0.9	0.682	0.004	0.48	0.025

hERG— cardiotoxicity, DILI—drug-induced liver injury

Predictive probabilities of interaction with Nuclear Receptors and Stress Response Hormones

Androgen receptor (AR) is a nuclear hormone receptor also known as steroid hormone receptor. Interaction of Endocrine disrupting chemicals (EDC) with androgen receptors (AR) causes disruption of normal endocrine function and also interfere with metabolic homeostasis, reproduction, behavioural and developmental functions.

The predictive ADMET Tox 21 pathways results are summarised in Table 7 and Table 8.

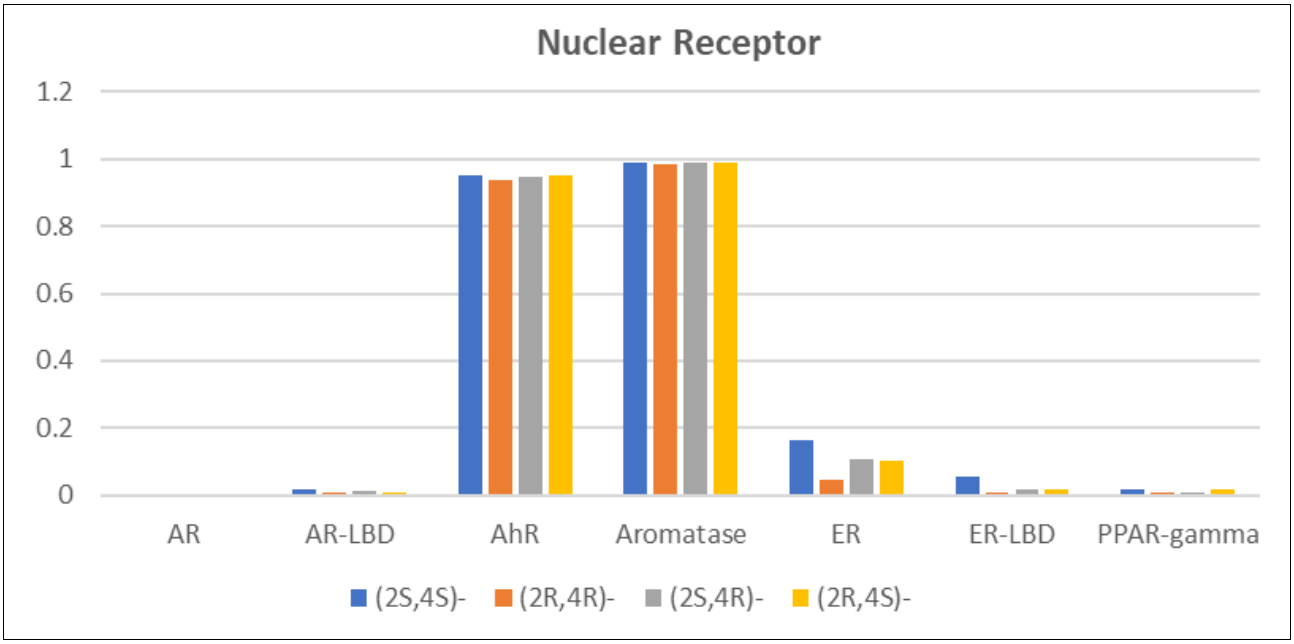


Fig 5: Graphical representation of interaction of stereoisomers of propiconazole with nuclear receptors.

Table 7: Predictive Tox 21 pathway for Nuclear Receptors indicating Human Health Hazards for Propiconazole

Stereoisomer of Propiconazole	Nuclear Receptors						
	NR-AR	NR-AR-LBD	NR-AhR	NR-Aromatase	NR-ER	NR-ER-LBD	NR-PPAR-gamma
(2S, 4S)-	0.001	0.019	0.953	0.99	0.164	0.053	0.019
(2R, 4R)-	0.001	0.009	0.937	0.987	0.044	0.006	0.009
(2S, 4R)-	0.001	0.014	0.945	0.989	0.105	0.017	0.009
(2R, 4S)-	0.001	0.01	0.953	0.989	0.101	0.017	0.015

A broad view of predictive relative study of four stereoisomers for Nuclear Receptors interactions has been given as graphical representation (Figure 5).

Stereoisomers of Propiconazole have negligible effect on Androgen receptors (AR and AR-LBD), Estrogen receptor (ER and ER-LBD) and peroxisome proliferator-activated receptors (PPAR-γ). However alarming and crucial observations are predicted for interaction of all stereoisomers (Structure 1-4; Figure 2) with two nuclear receptors namely Aryl hydrocarbon Receptor (AhR) and Aromatase.

The Aryl hydrocarbon Receptor (AhR), is crucial to adaptive responses to environmental changes. Nuclear receptor Aromatase is responsible for biosynthesis and normal functions of steroid hormones including estrogen and androgen in the body. The propiconazole stereoisomers act as endocrine disruptors and inhibit the activities of these two nuclear receptors.

Table 8: Predictive Tox 21 pathway for Stress Response Hormones indicating Human Health Hazards for Propiconazole

Stereoisomer of Propiconazole	Stress Response Hormones				
	SR-ARE	SR-ATAD5	SR-HSE	SR-MMP	SR-p53
(2S,4S)-	0.798	0.111	0.183	0.60	0.175
(2R,4R)-	0.788	0.014	0.327	0.651	0.123
(2S,4R)-	0.662	0.012	0.173	0.646	0.054
(2R,4S)-	0.852	0.057	0.244	0.622	0.285

A broad view of predictive relative study of four stereoisomers for Stress Response Hormones activation has been given as graphical representation (Figure 6).

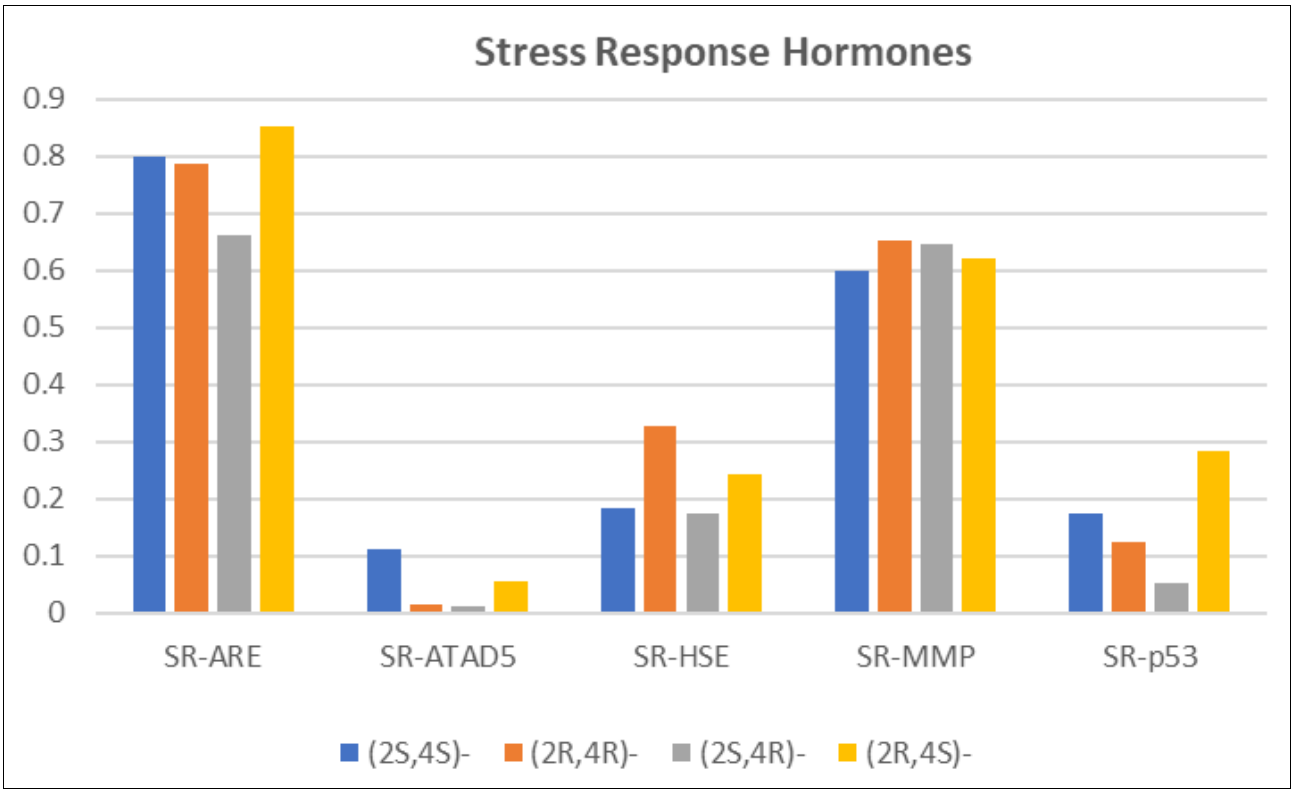


Fig 6: Graphical representation of interaction of stereoisomers of propiconazole with Stress response hormones.

Propiconazole stereoisomers are predicted to be very active for stress response hormones SR-ARE. Oxidative stress has been responsible for a variety of diseases ranging from cancer to neurodegeneration. The antioxidant response element (ARE) signalling pathway has an important role in dealing with oxidative stress. Mitochondrial membrane potential (SR-MMP) is one of the parameters for mitochondrial function. MMP is generated by mitochondrial electron transport chain that creates an electrochemical gradient by a series of redox reactions. The effect of chemicals on mitochondrial function is assessed by change in MMP. Propiconazole show high activation of SR-MMP. These stereoisomers are considered to be hazardous and pose high risk alert to human health.

A tumour suppressor protein SR-p53 is activated due to DNA damage and cellular stresses. Stereoisomers of propiconazole show poor activation of SR-p53. These stereoisomers show poor to low activation for SR-ATAD5 (ATPase family AAA domain-containing protein 5) and SR-HSE (Heat shock factor response element). An increase in ATAD5 occurs as a response to various types of DNA damage. Various chemicals, environmental and physiological stress lead to the activation of heat shock response.

Conclusions

Relative study of stereoisomers of propiconazole was carried out in view of the impact of stereochemistry on their physicochemical properties, biological activities and toxicity profile using Chem Axon-chemicalize (Licenced) and ADMETlab2.0 online webservers. A broad view of predictive relative study has also been given as graphical representations. The predictive physicochemical properties of all four stereoisomers were found to be same. They exhibit poor solubility and high lipophilicity over a long range of pH.

The ADMET prediction showed these stereoisomers to have good bioavailability, high intestinal absorption, high plasma protein binding and moderate clearance. These also showed good probabilities of being a substrate and inhibitor for CYP3A4 and CYP2C19. None of them was substrate or inhibitor for CYP2D6.

All stereoisomers were predicted to cause liver injury, mutagenicity, and skin sensitization. Highest carcinogenicity and highest clearance predicted for (2S, 4S) stereoisomer. All four stereoisomers were predicted to be endocrine disruptor (ED) and inhibit the activities of Aryl hydrocarbon receptor (AhR) and Aromatase nuclear receptor. These were very active for stress response hormones (SR) namely SR-ARE and SR-MMP.

These stereoisomers of propiconazole are considered to be hazardous and are high risk alert to human health.

Predictive studies revealed all four stereoisomers to exhibit consistent pattern for various physicochemical properties, bioactivities and toxicity profile.

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