International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

In Silico, in Vitro and in vivo Ecotoxicology and Biodegradability Evaluations of Bioactive Schiff Base Ligand

Kamel Mokhnache[a*], Soraya Madoui[b], Noureddine Charef[c]

[a,b,c] *Laboratory of Applied Biochemistry, University Ferhat Abbas Setif 1,19000, Algeria*

[a]*Email: kamelmokhnacke@yahoo.com*

Abstract

Bioactive Schiff base ligand, 2-[(1*E*)-*N*-{2-[(2-{(*Z*)-[1-(2 hydroxyphenyl)ethylidene]amino} ethyl)amino]ethyl}ethanimidoyl]phenol was selected for *in silico*, *in vitro* and *in vivo* ecotoxicological and biodegradability studies. *In vivo* and *in vitro* ecotoxicological evaluations were estimated by the use of snails (*Eobania vermiculata)* and microorganisms' bacteria and fungus respectively. *In silico* ecotoxicological and biodegradability predictions were carried out online computer software programs such as Pro Tox, Pred-skin, Endocrine Disruptor Knowledge Base (EDKB) and UM-BBD. The obtained results from *in vivo* bioassays showed moderate toxicity of the ligand at the high concentration (1000 µg/mL) with mortality percent value of 35%. For *in vitro* evaluations, results showed negative effect against bacteria and fungus. *In silico* predictions, results showed low toxicity with high LD50 of 4340 mg/kg, no toxic targets and low probability to bind with the majority of endocrine receptors with docking ranging between -7.4 and -8.9. In addition, the results from **Human skin sensitization and Murine local lymph node assay** indicate sensitizer effect of the ligand. For biodegradability prediction, the results indicate the ability of microorganism to degrade the ligand with no-toxic resultant products. We conclude the possibility to using the ligand without risks from environment and human health.

Keywords: eco-toxicological; biodegradability; microorganisms; snails; online software programs.

---

* Corresponding author.

1. Introduction

The development of civilization led to the introduction of improved technologies and the production of new chemical compounds [1]; which led to high contact between human and chemical compounds through the environment, nutrition, cosmetics, and drugs [2]. These chemicals mostly interact with the endocrine or hormone systems [3]. This interaction can caused several effects [4]. These substances are named endocrine disrupting chemicals which alter the endocrine system and caused undesirable effects to living organism, and pose important risks to human health, society, and the environment [5]. For the protection of environmental and human health, be obliged to pass all chemical compounds to acceptable toxicity test, which is the measure of any undesirable or bad effect of chemicals. Specific types of these adverse effects are called toxicity endpoints, such as carcinogenicity or genotoxicity [6]. *In silico* toxicology (computational toxicology) is one type of toxicity assessment that uses computational resources (i.e., methods, algorithms, software, data, etc.) [7]; which can lead to screening a large numbers of substances in a short time and with low expenses [8]. In addition, these efficient approaches have an exclusive advantage of being able to estimate the toxicity of substances before they are synthesized [9]. On the other hand, the great numbers of chemicals (more than 67 million organic and inorganic substances in 2012 [10] pose the question: how to reduce or to eliminate pollutant substances from the environment and protect living organism?

Several studies showed that the biodegradation of chemicals as the major solution." If microorganisms are used to biodegrade sustained, toxic pollutants to live organisms" [11]. In addition, Microbes are considered as the tools of nature that help overcome this problem of recalcitrance [12]. Several researches indicate that biodegrading microbes degrade toxic chemicals via either mineralization or co-metabolism [13]. In this study, the Schiff base ligand 2-[(1*E*)-*N*-{2-[(2-{(*Z*)-[1-(2hydroxyphenyl)ethylidene]amino} ethyl) amino]ethyl} ethanimidoyl]phenol (**Figure 1**) was selected for *in silico* and *in vitro* studies such as toxicity, eco-toxicology and biodegradability.

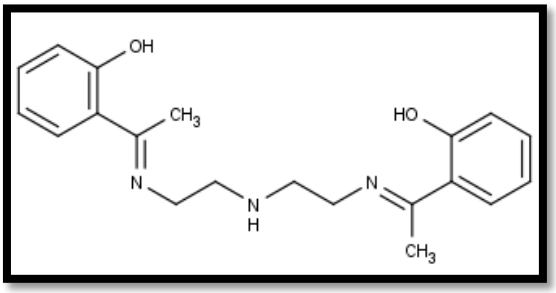


Figure 8

In silico by the use of online computer software programs such as ProTox and Endocrine and EAWAG-PPS. *In vitro and in vivo* the tests were realized on microorganisms (bacteria and fungi) and on snails respectively.

2. Methods

2.1. In silico online software programs

ProTox web server was used for toxicity prediction method [14]. Pred-Skin web app (http:// labmol.com.br/predskin/) was employed for skin sensitization prediction [15]. Endocrine Disruptor Knowledge Base (EDKB) database (US FDA) http://www.fda.gov/ScienceResearch/BioinformaticsTools/EndocrineDisruptorKnowledgebase/default.htm was used to predict the binding of chemicals to the estrogen and androgen nuclear receptor proteins [16]. The online mcule 1-click docking server was used for docking study [17]. The University of Minnesota Biocatalysis/Biodegradation Database (UM-BBD, http://umbbd.msi.umn.edu/) was used for predict microbial biocatalytic reactions and biodegradation pathways [18].

2.2. In vivo and vitro assay

To explore in vitro, the toxicity effect of the Schiff base ligand**,** we use the antimicrobial, and molluscicidal activities [19].

2.3. Toxicity evaluation to snails

Snails (*Eobania vermiculata)* from the region of Setif (east of Algeria), were devised in groups, each group containing 10 snails, were placed in glass containers with 250 ml of water at 26.7 ºC, containing the test compounds the Schiff base ligand or Chloracetic acid (was used as positive control) . Snails were prevented from crawling out of the containers by a fine stainless steel mesh suspended just above the water surface. After 24 h of incubation the snails were transferred to distilled water and maintained for another 48 h. Mortality of the snails was determined by absent of any reaction to irritation of the foot with a needle [20].

2.4. Toxicity evaluation to bacteria and fungi

The Schiff base ligand was examined against bacteria, such as *Lysteria monocytogenes* ATCC 15313, *Bacillus cereus* ATCC 10876 and against fungi such as *Fusarium oxysporum*, *Aspargilus flavus* NRRL 391, *Aspargilus niger* 2AC 936, *Penicillium sp.* The studies were carried out using the standardized disc agar diffusion method [13]. According to this method, the bacterial and fungal cultures were sub cultured on nutrient agar and potato dextrose agar medium, respectively. Gentamicin (10 µg/disk) and Econazole (750 µg/mL) were used as reference antibacterial and antifungal drug, respectively. The tested compound was dissolved in dimethyl solfoxide (DMSO). The test was performed on Mueller Hinton Agar for antibacterial activity and on potato dextrose agar (PDA) which contains infusion of 200 g potatoes, 6 g dextrose, and 15 g agar for antifungal activity. Uniform size Whatman filter paper disks of 6 mm-diameter sterilized in an autoclave were saturated with 15 µL of the tested compound dissolved in DMSO (3 disks per compound) and were carefully placed on an incubated agar surface. After an incubation period of 24 h at 37 ^{o}C for bacteria and 72 h at 27 ºC for fungi.

3. Results and Discussions

3.1. In vitro eco-toxicological evaluations

Determination and quantification of toxic or contaminant agent in the environment necessitate the use of the

bioindicators, which are organisms or groups of organisms [21]. In our study, the eco-toxicity effect of the Schiff base ligand was estimated by the mortality test against snails (*Eobania vermiculata).* The results represented in **Table 1**, indicate moderate toxic effect, with mortality percent of 35% at the high concentration (1000 µg/mL). While Chloracetic acid at 1000 µg/mL (positive control) represent 100% of snails' mortality. We can explain the low toxicity of our ligand by the absence of Chlorine atoms in the structure. In the literature [22], Niclosamide (2',5-dichloro-4'nitrosalicylanilide) was used as a molluscicid agent which contain tow Chlorine atom and represent 100% of snails' mortality.

Table 1: Effect of the Schiff base ligand and chloracetic acid on snails

Tested compounds	**Negative control**	**The Schiff base ligand**	**Positive control, Chloracetic acid**
	(0 µg/mL)	(1000 µg/mL)	(1000 µg/mL)
Snails mortality (%)	0.00%	35%	100%

In the case to confirm low or no-toxicity of the Schiff base ligand, which assessed against microorganisms, bacteria and fungus. Results of the bioassay represented in **Table 2,** indicate a negative effect of the ligand against all the tested microorganisms, with the increase in the concentrations (0 µg/ mL to 1000 µg/ mL), this result show the absence of any risks of the substance to microorganisms and the impossibility to be used as antimicrobial agent. These results are in good agreement with previous studies [23, 24] which indicate low or negative effect of Schiff bases ligand against bacteria and fungus; which are coordinated with different metal ions for formed a potential antimicrobial agent, for the reason that metal ions are adsorbed in microbe cells and kills the microorganisms.

Table 2: Effect of the Schiff base, Gentamicine and Econazol on bacteria and fungi strains

	Zone of inhibition (mm)				
	Concentration of the ligand (µg/mL)			**Control positive**	
	0	**500**	**1000**	**Gentamicine**	**Econazol**
Microorganisms				**(10 µg/disc)**	**(750 µg/mL)**
Lysteria monocytogenes	0.00	0.00	0.00	13.5	-
Bacillus cereus	0.00	0.00	0.00	0.00	-
Aspergillus niger	0.00	0.00	0.00	-	35.00
Penicillium sp.	0.00	0.00	0.00	-	31.00
Aspergillus flavus	0.00	0.00	0.00	-	29.00
Fusarium oxysporum.	0.00	0.00	0.00	-	36.00

3.2. In silico eco-toxicological predictions

The development and the progress in theoretical chemistry led to developed numerous *in silico* models for chemicals toxicity prediction. In our study, LD_{50} value and the predicted toxicity class of the Schiff base ligand were predicted by Pro Tox software in rodent with oral administration. The results in **Figure 1** showed the low toxicity with LD_{50} value of 4340 mg/kg, which classified in the class 5, with average similarity and prediction accuracy of 68.44 and 68.07% respectively.

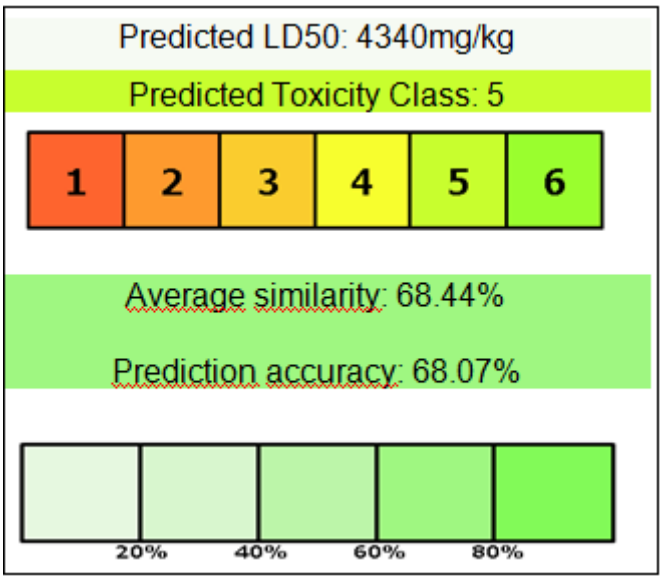


Figure 1: Predicted LD_{50} and toxicity class of the ligand

For molecular weight and dose (LD50) values distribution of the ligand, **Figure 2** represent the above parameters and indicates in black color the value of 339.43 g/mol from ligand molecular weight and LD50 of 2319.9 mg/kg from the dataset with red color. The high value of LD50 from the ligand which no apparent in dose diagram distribution, validate the low toxicity of the ligand

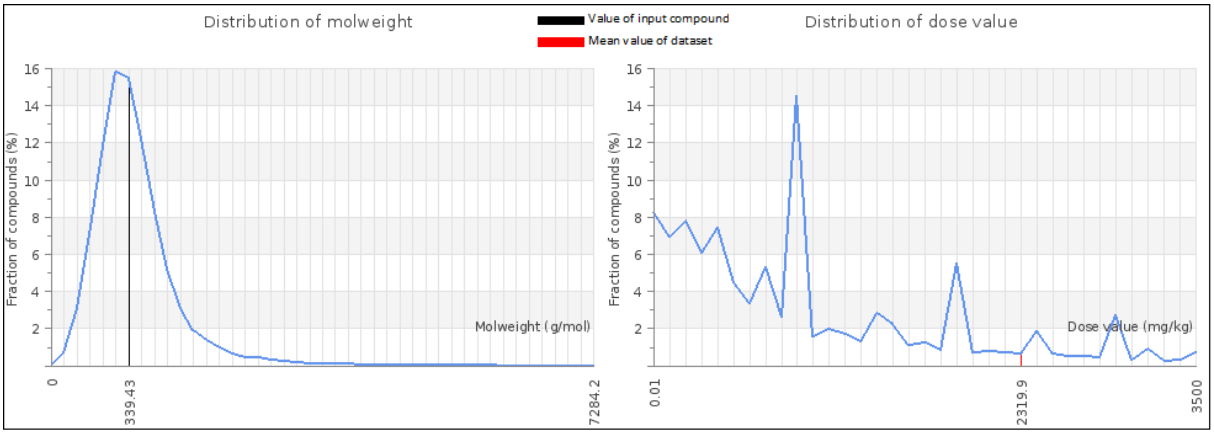


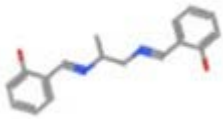
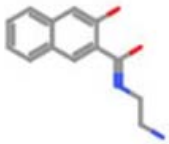
Figure 2: Molecular weight and dose distribution prediction of the ligand

In addition, the results displayed in **Table 3** showed the majority similar compounds from dataset of Pro Tox software and our Schiff base ligand (the input compound), these results indicate the high similarity in the

structure, which led to classified in the same predicted toxic class **5**. The similarity in structure was explained by the presence of: tow aromatic ring, tow hydroxyl groups and the presence of Nitrogen atom.

On the other hand, our results from the presence of toxic fragment in our ligand indicate the absence of any toxic fragment, which confirmed the absence of risks to environment and human health.

Table 3: Similar compounds of the Schiff base ligand

Similar compound	**1**	**2**
Structure		
Formula	C17H18N2O2	C13H14N2O2
molweight	282.34	230.26
endpoint	LD50	LD50
tox class, avg	5	5

Furthermore, the prediction of possible toxicity target was also evaluated by Pro Tox software. Toxicity targets were defined as all targets belonging to the Novartis *in vitro* safety-panel of protein targets associated with adverse drug reactions [25]. Among of these targets:

Adenosine A2A receptor (**AA2R**), Adrenergic beta 2 receptor (**ADRB2**), Androgen receptor (**ANDR**), Amine oxidase (**AOFA**), Dopamine D3 receptor (**DRD3**), Estrogen receptor 1 and 2 (**ESR1, ESR2**), Glucocorticoid receptor (**GCR**), Histamine H1 receptor (**HRH1**), Nuclear receptor subfamily 1 group I member 2 (**NR1I2**), Opioid receptor kappa (**OPRK**), Opioid receptor mu (**OPRM**), cAMP-specific 3',5'-cyclic phosphodiesterase 4D (**PDE4D**), Prostaglandin G/H synthase 1 (**PGH1**) and Progesterone receptor (**PRGR**), our results displayed in **Figure 3**, indicate the binding possibility between the ligand and Prostaglandin G/H synthase 1 (yellow color) with average pharmacophore of 35.75%. In addition, there is no possibility to bind with the other targets (black color).

In addition, the major undesirable effects of Endocrine disrupting chemicals are alteration of endocrine regulation and modification of physiological functions [26]. Therefore an effort is continuing to expand substitute *in vitro* and *in silico* methods to evaluate reproductive toxicity [27]. In this study, we evaluate *in silico* the effects of the Schiff base ligand on endocrine system which led to classify our ligand in endocrine disrupting chemicals or in no-toxic chemicals. Binding probabilities are classified: red color with sensitivity SE < 0.25 indicates high probability, orange color (0.25 < SE < 0.50) indicates intermediate probability, yellow (0.50 < SE < 0.75) indicates medium probability and green (SE > 0.75) indicates low probability. The obtained results in

Figure 4 showed the low probability to bind the Schiff base ligand with the majority of hormone receptors, with docking score comprise between -7.4 and -8.9, which colored with the green and medium probability with score ranging between -7.5 and 8.5. These results confirmed low or no interaction between the ligand and the endocrine receptors, which led to conclude that our ligand cannot induce alteration or damage in living endocrine system. Among of these receptors: **AR** androgen receptors, **ER** estrogen receptor (α and β), **GR** glucocorticoid receptor, **TR** thyroid hormone receptors (α and β), **LXR** Liver X receptors (α and β), **PPAR** peroxisome proliferator activated receptors (α, β/δ and γ).

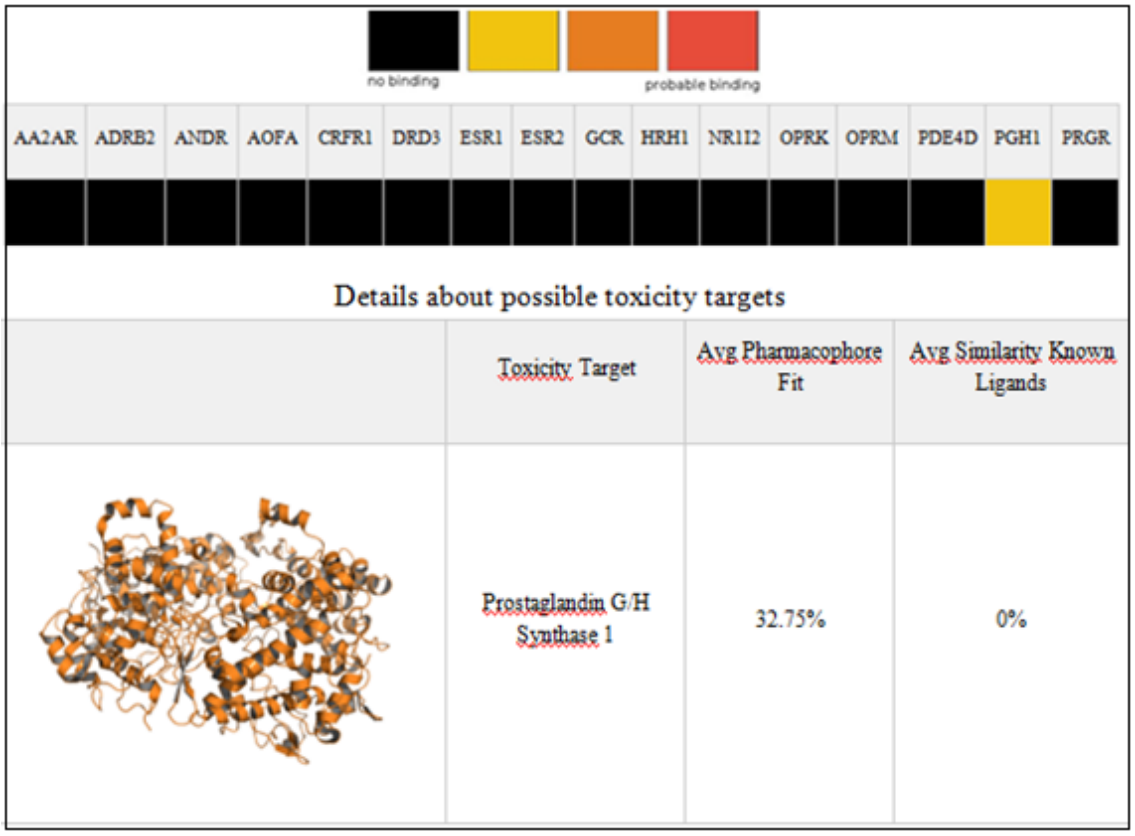


Figure 3: Possibility to bind to toxicity targets predictions

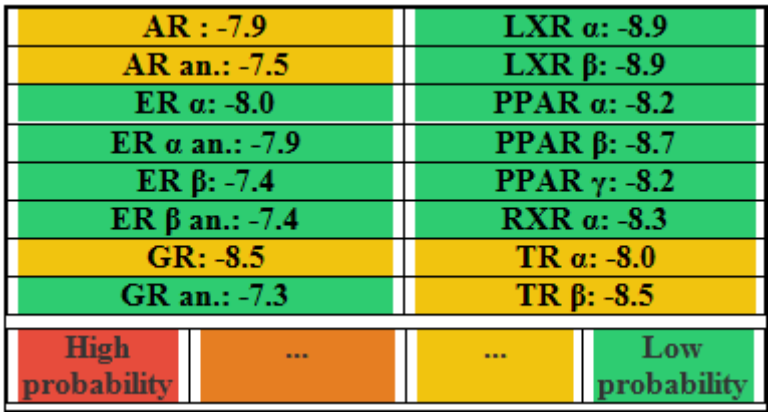


Figure 4: Effect of Schiff base ligand on endogen receptors

For confirmed the results in **Figure 4**, peroxisome proliferator-activated receptor gamma (PPAR)γ with Pdb (1i7i) was selected for study the interaction using online **mcule 1-click docking**, the results displayed in **Figure 5**, showed the low binding between ligand and the receptor with best docking score value of -8.2.

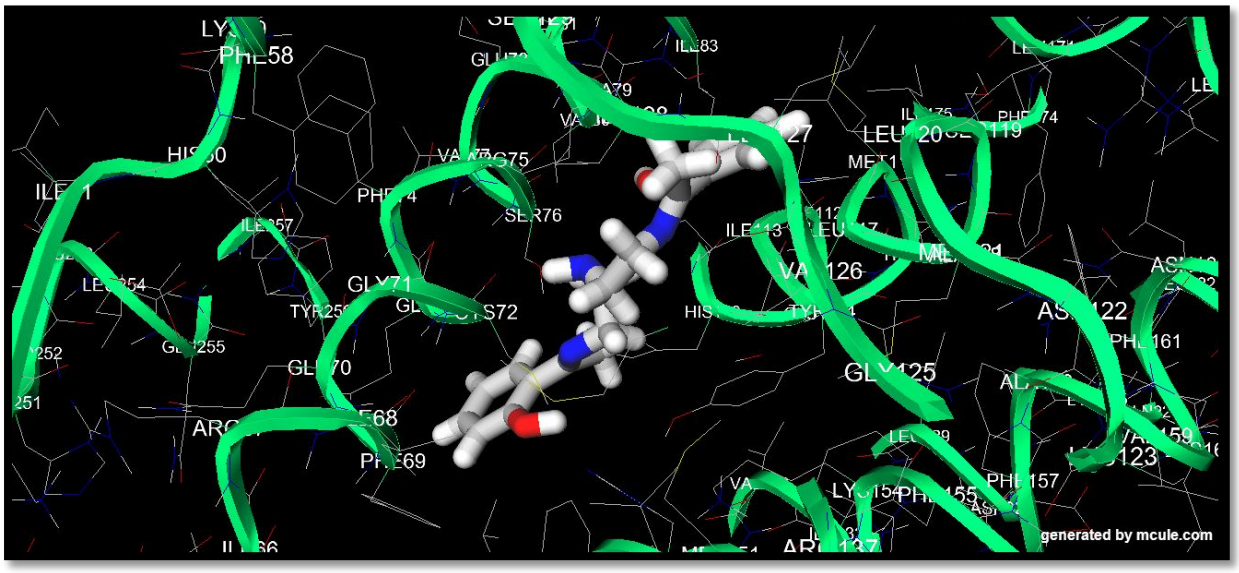


Figure 5: Binding of the ligand and Peroxisome proliferator-activated receptor gamma Docking Score: - 8.2.

In the other hand, the effect of the Schiff base ligand on human skin sensation was predicted. The reactivity profile of chemical substances plays an important role in its tendency to be an allergen agent [28]. Skin sensitization is the most steps in the inflammation process in allergic contact dermatitis [29]. The approaches of Quantitative structure activity relationships represented an important substitute for the evaluation of chemicals toxicity, including skin sensitization [30]. For our molecule, the results represented in **Table 4**, showed the sensitizer effect on human skin and in murine local lymph node assay, with probability of 58 and 66% respectively.

Table 4: Prediction of skin sensitization

Test	Prediction	Probability	Multiclass Prediction	Probability
Human skin sensitization	**Sensitizer**	58.00	-	-
Murine local lymph node assay (LLNA)	**Sensitizer**	66.00	**Weak/Moderate**	65.00

In addition the results displayed in **Figure 6**, confirm the precedent results in **Table 4**, which indicate the atoms or the fragments contributed in sensation, green atom or fragment represent an increase in skin sensitization potential; whereas, pink fragments represent a decrease in skin sensitization potential, and gray fragments do not

contribute to skin sensitization potential.

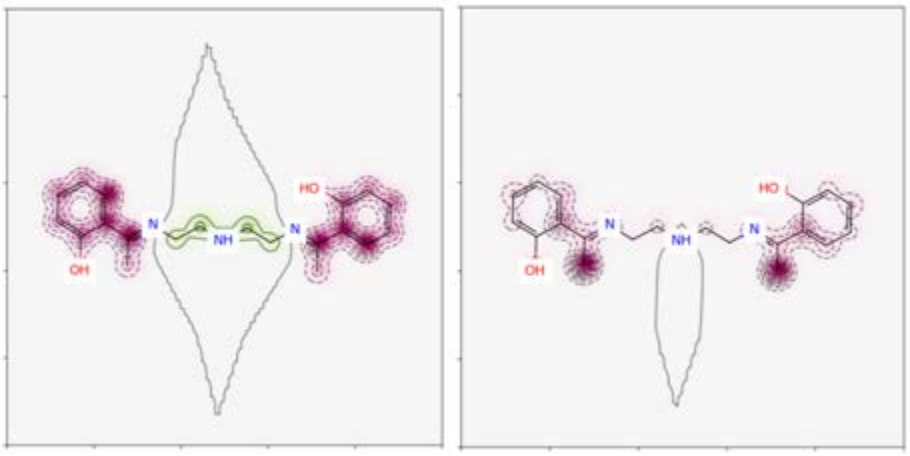


Figure 6: Human skin sensitization Murine local lymph node assay (LLNA)

In silico biodegradability predictions

3.3. In silico biodegradability prediction

Biodegradability is a significant property of chemical industry. The adverse effects resultant from pollutants accumulation in the environment, caused lethal interaction between biological system and chemicals, which obliged to establish processes for reduce the pollutants and protect living organism, among of these processes; the biodegradability [31]. Therefore, the ability of soil bacteria to decomposed organic matter by extracellular enzymes production, led to facilitate and accelerate the mineralization process [32]. Several studies, confirmed the accompaniment of both increasing of microorganisms and the rate of mineralization when the organic matter introduced in the soil [33]. Recently, the progress in computational studies led to developed different rules of biotransformation of organic groups from UM-BBD data [18]. In this study, we evaluate *in silico* the biodegradability of the Schiff base ligand. The results displayed in **Figure 7**; which represents various pathways of the ligand biodegradation and the resultants products. Biodegradation of the ligand was predicted with the following transformation rules:

bt0014: 1-Hydroxy-2-unsubstituted aromatic → 1,2-Dihydroxyaromatic

4-Hydroxypyridine derivative →3,4-Dihydroxypyridine derivative

bt0063: primary Amine →Aldehyde or Ketone

secondary Amine→Amine + Aldehyde or Ketone

tertiary Amine → secondary Amine + Aldehyde or Ketone (bt0063)

Methylammonium derivative → Trimethylamine + Aldehyde or Ketone

bt0064: 1-Hydroxy-4-unsubstituted benzenoid → 1,4-Dihydroxybenzenoid derivative

bt0351: 1-Amino-2-hydroxybenzenoid → 2-Amino-2,4-dienoate derivate + Carboxylate

vic-Dihydroxybenzenoid → 2-Oxopent-4-enoate derivative + Carboxylate

bt0357: 1,4-Dihydroxybenzenoid → Maleylacetate derivative

bt0003: Aldehyde → Carboxylate

The results indicate the likely of all transformations (green color), with no-toxic resultant products.

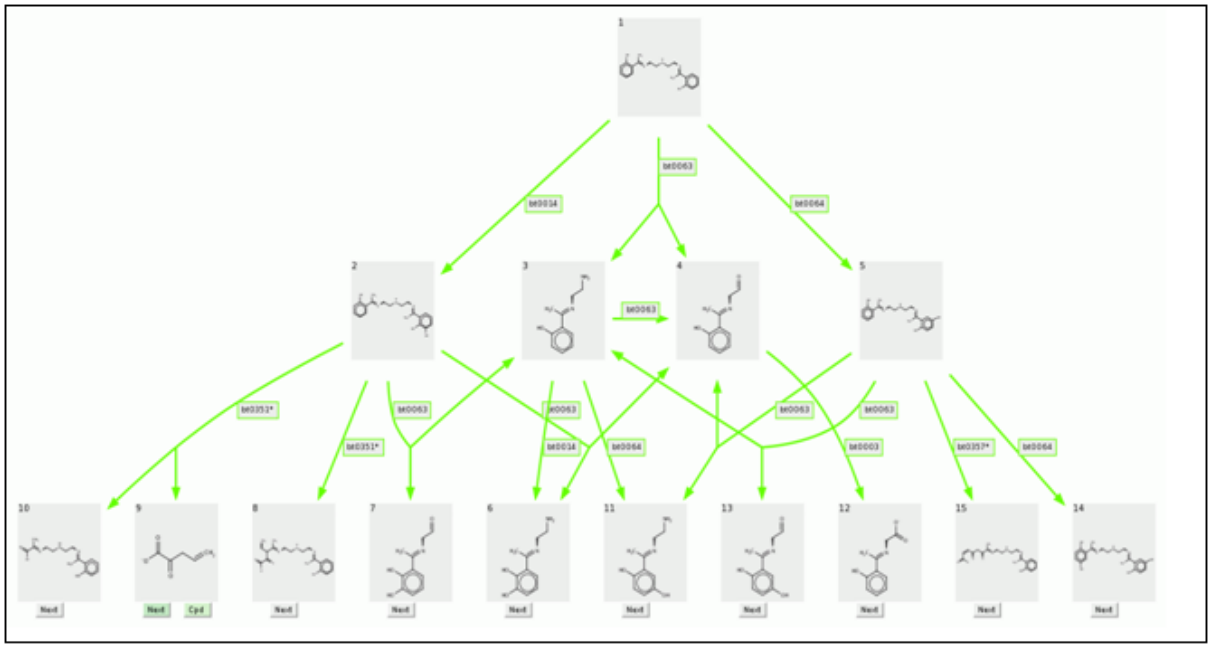


Figure7: Biodegradation pathway of the Schiff base ligand

4. Conclusion

Eco-toxicological and biodegradability of the Schiff base ligand 2-[(1*E*)-*N*-{2-[(2-{(*Z*)-[1-(2 hydroxyphenyl)ethylidene]amino}ethyl)amino]ethyl}ethanimidoyl]phenol were evaluated by *in vitro* bioassays and *in silico* predictions. Results showed that the ligand has a negative effect against bacteria and fungus, moderate toxicity on snails, high LD50, low probability to bind to endocrine receptors and able to degrade by microorganisms with different biotransformation reactions. The obtained results indicate the possibility to used ligand without risks from environment and human health.

Acknowledgments

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (MESRS), the Thematic Agency for the Research in Health Sciences (ATRSS) and by the University of Jordan.

5. Compliance with ethical standards

Ethical Approval

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Public Health of Algeria (INSP). The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Ferhat Abbas–Setif 1.

6. Conflict of Interest

Authors have declared that no competing interests exist.

References

[1] A.P. Paulina, W. Mirosław. Environmental contamination with phthalates and its impact on living organisms. Ecological Chemistry and Engineering. Vol 23, pp. 347-356, 2016.

[2] M. Andreas, K. Günter, U. Thomas, H. Sepp. DeepTox:Toxicity Prediction using Deep Learning. Front Environ Sc.Vol 3,pp.1-15, 2016.

[3] Y. Oneyeol, L.K. Hye, W. Jong-Il, R.S. Young. Review of Toxicological Mechanisms Using Molecular Pathway Analysis. J Cancer Prev. Vol 20,pp. 12-24, 2015.

[4] T.T. Schug , A. Janesick , B. Blumberg , J.J. Heindel. Endocrine disrupting chemicals and disease susceptibility. J Steroid Biochem Mol Biol. Vol 127, pp. 204-215, 2011.

[5] F.M. McRobb, I. Kufareva, R. Abagyan. In Silico Identification and Pharmacological Evaluation of Novel Endocrine Disrupting Chemicals That Act via the Ligand-Binding Domain of the Estrogen Receptor. Toxicol Sci. Vol 141,pp. 188-197, 2014.

[6] B.R. Arwa, B.B. Vladimir. In silico toxicology: computational methods for the prediction of chemical toxicity. WIREs Comput Mol Sci. Vol 6, pp. 147-172, 2016.

[7] L.G. Valerio. In silico toxicology for the pharmaceutical sciences. Toxicol Appl Pharmacol. Vol 241,pp. 356-370, 2009.

[8] I. Rusyn, G.P. Daston. Computational Toxicology: Realizing the Promise of the Toxicity Testing in the 21st Century. Environ Health Perspect. Vol 118, pp.1047-1050, 2010.

[9] A.K. Madan, S. Bajaj, H. Dureja. Classification models for safe drug molecules. In: Reisfeld B, Mayeno AN, eds. Computational Toxicology. New York: Humana Press, 2013

[10] E.C. Richard, G. Juergen, W. Inge. Effect-Based Tools for Monitoring and Predicting the

Ecotoxicological Effects of Chemicals in the Aquatic Environment. Sensors. Vol 12,pp. 12741-12771, 2012.

[11] B. Magdalena, P. Robert, G. Paulina. Screening of Microorganisms for Biodegradation of Simazine Pollution (Obsolete Pesticide Azotop 50 WP). Water Air Soil Pollut. Vol 220, pp. 373-385, 2011.

[12] P.J. Strong, J.E. Burgress. Treatment Methods for Wine-Related and Distillery Wastewaters: A Review. Bioremediat J. Vol 12, pp. 70-87, 2008.

[13] P.K. Arora, C. Sasikala, C.V. Ramana. Degradation of chlorinated nitroaromatic compounds. Appl Microbiol Biotechnol . Vol 93, pp. 2265-2277, 2012.

[14] M.N. Drwal, P. Banerjee, M. Dunkel, M.R. Wettig, R. Preissner. ProTox: a web server for the in silico prediction of rodent oral toxicity. Nucleic Acids Research . Vol 42, pp. 53-58, 2014.

[15] R.C. Braga, V.M. Alves**,** E.N. Muratov, J. Strickland**,** N**.** Kleinstreuer, A. Trospsha**,** C.H. Andrade. Pred-Skin: A Fast and Reliable Web Application to Assess Skin Sensitization Effect of Chemicals. J Chem Inf Mode.l Vol 57, pp. 1013-1017, 2017.

[16] K. Kolšek, J. Mavri, D.M. Sollner, S. Gobec, S. Turk. Endocrine disruptome—An open source prediction tool for assessing endocrine disruption potential through nuclear receptor binding. J Chem Inf Model. Vol 54, pp. 1254-1267, 2014.

[17] R. Jayaprakash, K.S. Saroj, S. Hemalatha, D. Easwaramoorthy. Synthesis, characterization, quantitative structure-activity relationship, docking, antibacterial activity, and brine shrimp lethal studies on l-phenylalanine schiff bases. Asian J Pharm Clin Res. Vol 9, pp. 203-208, 2016.

[18] L.B. Ellis, D. Roe, L.P. Wackett. The University of Minnesota Biocatalysis/ Biodegradation Database: the first decade. Nucleic Acids Res.Vol 34, pp. 517-521, 2006.

[19] R.K. Devappa, S.K. Rajesh, V. Kumar, H.P. Makkar, K. Becker. Activities of Jatropha curcas phorbol esters in various bioassays. Ecotox Environ Safe. Vol 8, pp. 57-62, 2012.

[20] G.T. El-Sherbini, R.A. Zayed, E.T. El-Sherbini. Molluscicidal Activity of Some Solanum Species Extracts against the Snail Biomphalaria alexandrina. J Parasitol Res. pp. 3-5, 2009.

[21] A. Boukachabia, M. Soucha, O. Maamcha. Impact of environmental and chemical stress on the activity of the acetylcholinesterase and glutathione S-transferase during reproduction of Scolopendra morsitans (Myriapoda Chilopoda) in the north-east of Algeria . Journal of Entomology and Zoology Studies. Vol 3, pp. 433-439, 2015.

[22] P. Andrews, J. Thyssen, D. Lorke. The biology and toxicology of molluscicides, Bayluscide. Pharmacol Ther. Vol 19, pp. 245-295, 1983.

[23] SA. Patil, C.T. Prabhakara, B.M. Halasangi, S.S. Toragalmath, P.S. Badami. DNA cleavage, antibacterial, antifungal and anthelmintic studies of Co(II), Ni(II) and Cu(II) complexes of coumarin Schiff bases: synthesis and spectral approach. Spectrochimica Acta Part A. Vol 137, pp. 641-651, 2015.

[24] N. Charef, F. Sebti, L. Arrar, M. Djarmouni, N. Boussoualim et al. Synthesis, characterization, X-ray structures, and biological activity of some metal complexes of the Schiff base 2,2′-(((azanediylbis(propane-3,1-diyl)) bis(azanylylidene)) bis(methanylylidene)) diphenol. Polyhedron. Vol 85, pp. 450-456, 2015.

[25] E. Lounkine, M.J. Keiser, S. Whitebread, D. Mikhailov et al. Large-scale prediction and testing of drug activity on side-effect targets. Nature. Vol 486, pp. 361-367, 2012.

[26] M. Morales, P. Martinez-Paz, R. Martin, R. Planello et al. Transcriptional changes induced by in vivo exposure to pentachlorophenol (PCP) in Chironomus riparius (Diptera) aquatic larvae. Aquatic Toxicol. Vol 157, pp. 1-9, 2014.

[27] N. Marjana, V.Marjan. QSAR Models for Reproductive Toxicity and Endocrine Disruption Activity. Molecules. Vol 15, pp. 1987-1999, 2010.

[28] L. Jianzhong, S. Petra, G. Kern, G. Frank, A. Osvaldo, X.E. Emilio et al. Categorical QSAR models for skin sensitization based on local lymph node assay measures and both ground and excited state 4D-fingerprint descriptors. J Comput Aided Mol Des. Vol 22, pp. 345-366, 2008.

[29] J. Macan, D. Rimac, S. Kezić, V.M. Varnai. Occupational and Non-Occupational Allergic Contact Dermatitis: A Follow-Up Study. Dermatology. Vol 227, pp. 321-329, 2013.

[30] D. Pan, M. Iyer, J. Liu , Y. Li, A.J. Hopfinger. Constructing optimum blood brain barrier QSAR models using a combination of 4D-molecular similarity measures and cluster analysis. J Chem Inf Comput Sci . Vol 44, pp. 2083-2098 , 2004.

[31] S. Vorberg, IV. Tetko. Modeling the Biodegradability of Chemical Compounds Using the Online CHEmical Modeling Environment (OCHEM). Mol Inform . Vol 33, pp 73- 85, 2014.

[32] D. Vlădoiu, M.N. Filimon, V. Ostaf, A. Isvoran. Effects of herbicides and fungicides on the soil chitinolytic activity. A molecular docking approach. Ecological Chemistry and Engineering. Vol 22, pp. 439-450, 2015.

[33] A. Księżopolska, J. Joniec. Activity of Microorganisms Participating In Organic Matter Transformation In Haplic Luvisol Soil. Ecological Chemistry and Engineering. Vol 21, pp. 499-514, 2014.