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Toxicological and Mutagenicity Study of Some Marketable Insomnia Medications

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ABSTRACT

Insomnia, a habitual yet vicious disorder characterized by depriving of sleep resulting in patients to consume medications for relieving their minds. Of these medications, a few may possess the aptitude to harm the biochemical receptors of the human body, resulting in mutagenicity, and the body may manifest acute toxicological symptoms. Execution of quantitative analysis by QSAR (quantitative structure-activity relationship) methods forms a reliable platform for interpreting the toxicological status of insomnia medicines. Further molecular docking software was utilized for determining the extent binding affinity of the molecules on the protein structures.

Keywords: Insomnia medications, Toxicity analysis, Molecular Docking, Molecular complexes, Lethal Dose**.**

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1. INTRODUCTION

Various medical ailments, substance abuse, genetic disorders, and psychosocial issues concluded the genesis of insomnia, where the victims tend to relieve themselves through the application of pills[1]. Though initially providing solace to the body, repeated exposure may convert the drugs into toxic substances capable of damaging the organ system of the body[2][3]. These drugs could be mutagenic, carcinogenic, and toxic, which, as a silent killer may cause indirect alternations capable of fatal consequences. In the market, the most effective insomnia placebos are trazodone, zolpidem, temazepam, amitriptyline, estazolam, etc.[4] Many of these got harmful pharmacophore components.[5] Many of these can bind effectively to protein receptors like Amine Oxidase A, Prostaglandin G/H Synthase I, Progesterone Receptor, etc. provoking idiosyncratic manifestations. [6][7][8][9] In this paper, we are detecting the mutagenicity and the LD50 test manifestation of the insomnia medicinal compounds on *Rattus sp.* Further docking analysis is being done to detect the extent of molecular binding to the receptors.

2. LITERATURE REVIEWS

In 1967,(Bernstein et al.) detected the presence of psychosis from the sleeping agents containing scopolamine.[10] In 1983, (Chu et al,) detected liver toxicity in the presence of Trazodone, an important facet of the insomnia medicine.[11] In 2008. Dykens et al found that Trazodone inhibits mitochondrial membrane potential and imposed oxidative stress of rat liver.[12]In 2000, Toner et al. investigated the side effects of Zolpidem and found that the patients are suffering from delirium, nausea, and hallucinations. [13]In 2012, Darke et al of the University of New South Wales stunned everyone when he reported that Zolpidem was a prime drug abuser, taking away 91 victims, and in the drug abuse, 83.5% of the users got a detected level of Zolpidem. Temazepam.[14] Robinson et al., in 1984 made a landmark discovery when he found that Temazepam had deteriorated the mean body weights of rats and mice after 39 weeks of treatment. He also observed a surge in liver weight and rat mortality.[15]

3. MATERIALS AND METHODS

3.1. Toxicity Analysis

Eleven marketable drugs of insomnia were collected according to their popularity, and the CAS no. is noted from the respective drug class. The CAS (Chemical Abstract Services) NO. were retrieved from the PubChem database. The drug classes are Trazodone, Zolpidem, Temazepam, Lorazepam, Amitriptyline, Estazolam, Clonazepam, Diphenhydramine, Gabapentin, Flurazepam, and Zonalon. [16]The LD50 experimental oral merit for rat LD0 (mg/kg) was obtained from ChemID Plus.[17]

The QSAR modeling was executed with a sophisticated software created by USEPA (T.E.S.T. Version 4.1) and it is made on the virtue of doing the experimental run on every test species regulated by the drug laws and animal ethical regulations and was fabricated on two dimensional (2D) molecular moiety.[18] The acute toxicity divination of rat LD50 through oral ingestion was differentiated with the bioassay results retrieved from ChemID Plus. The data obtained was based on a consensus method of averaging all the values of tests obtained by virtue hierarchical clustering, FDA MDL, nearest neighbor protocols. [19]The structure of the chemical of interest was visualized after tabulating the CAS no. into the software. Toxicity prediction was later done with the virtue of the LD50 rat test on consensus method and mutagenicity test by the FDA method.

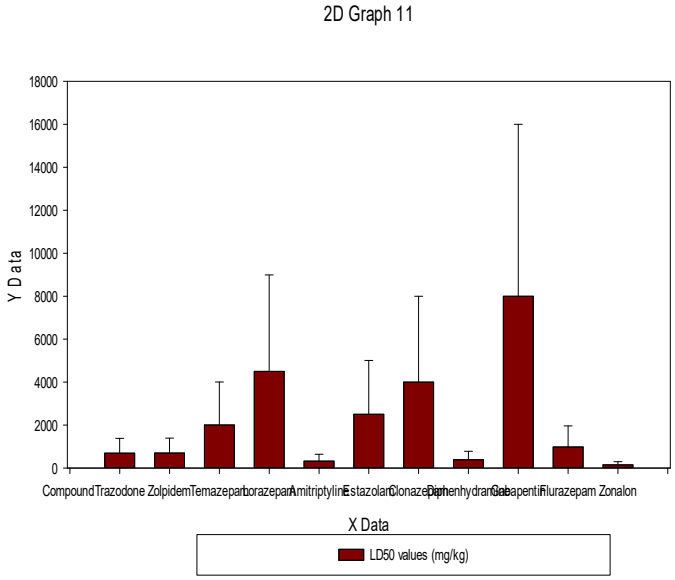
3.2. Molecular Docking

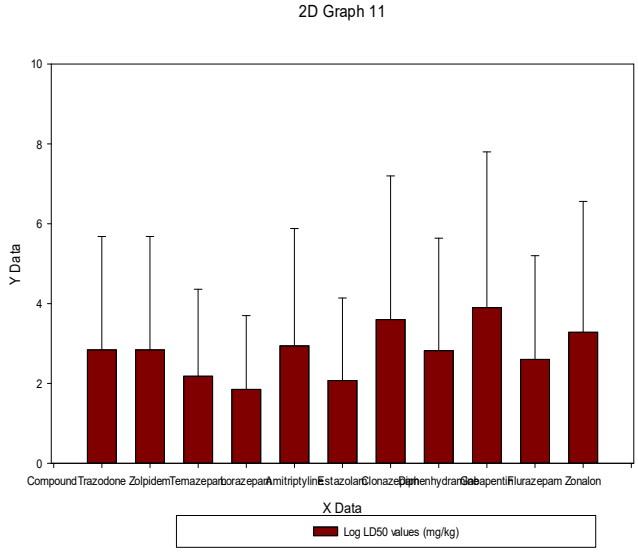
The crystalline three-dimensional (3-D) fabrication of ligands (Insomnia Placebo) and Macromolecules (Receptors under inhibitory effect) were captured from the website of PubChem (https://pubchem.ncbi.nlm.nih.gov/) and protein data bank (http://www.rcsb.org).[20] The structures were captured maintaining the ententes of the wwPDB validation report. This structure was provided based on the X-ray Diffraction method of 2.0 Å. Docking analysis was done with the help of Mcule 1-Click docking[21]

4. RESULTS

4.1. Toxicity Analysis

The values of 11 panaceas obtained from the T.E.S.T. software run are systematized in Table 1. The oral experimental LD50 values are inscribed in mg/kg as Trazodone (690), Zolpidem (695), Temazepam (2000.88), Lorazepam (4495.05), Amitriptyline (320.01), Estazolam (2503.04), Clonazepam (4000), Diphenhydramine (3901.13), Gabapentin (8000), Flurazepam (981.17) and Zonalon (146.97). (Fig 1) (Table 1) In the issue of predictive toxic analysis, the results were shown in mg/kg as Trazodone (598.91), Zolpidem (919.72),Temazepam(1929.39),Lorazepam(2785.68),Amitriptyline(309.08),Estazolam(1450.63),Clonazepam (831.44),Diphenhydramine (1841.52), Gabapentin (1687.57), Flurazepam (2061.77) and Zonalon (451.47). The estimation was based on the T.E.S.T. software consensus protocol. (Fig 1) (Table 1) Each of the LD50 merits (both experimental and predicted) were calculated in logLD50 for all the 11 chemical moieties. The R2 values of the predicted figures of the compounds from the FDA cluster model fit the upshot for the compounds that were indexed along with the residual values (Fig 7).





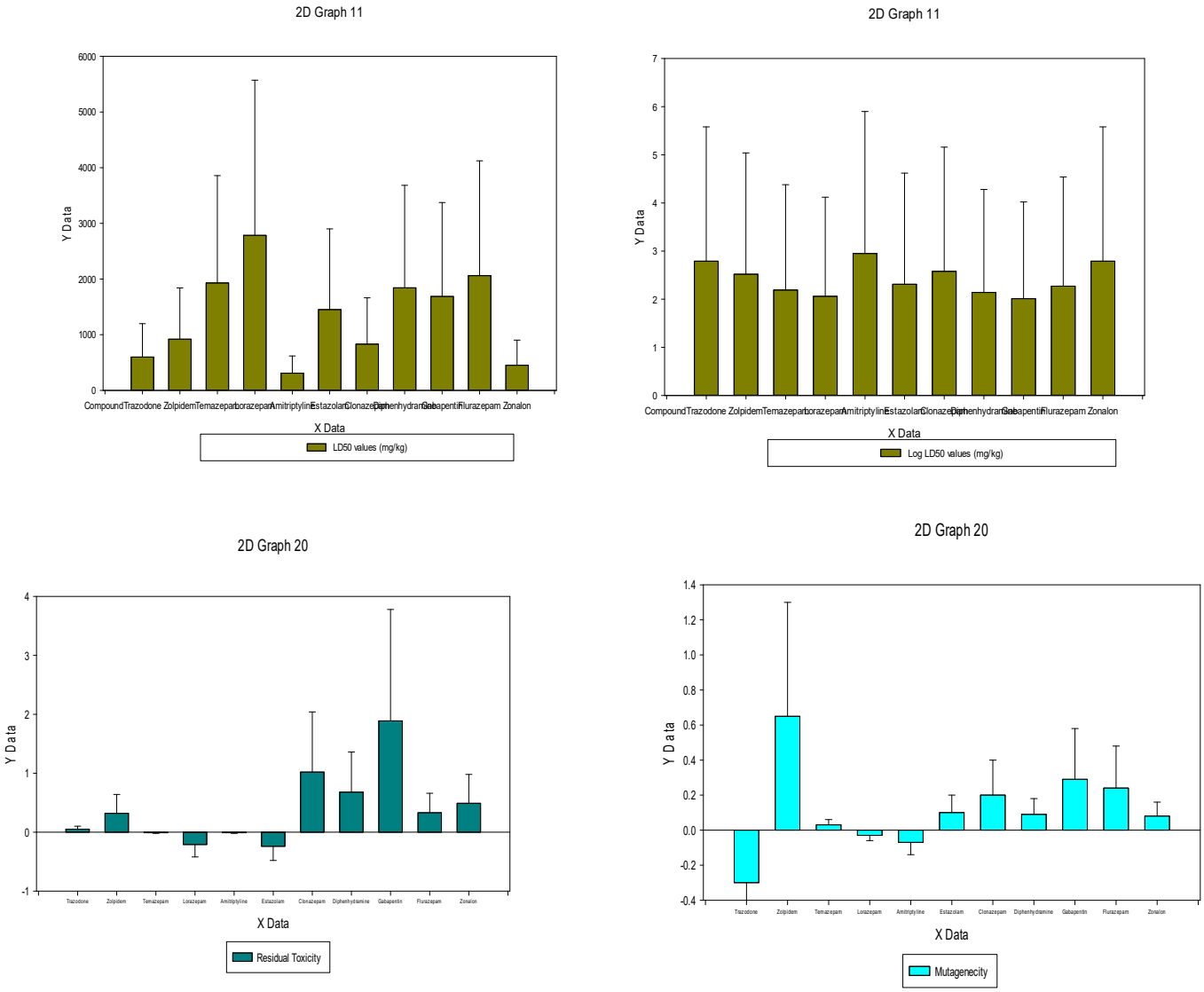


Figure 1. Graphs denoting the toxicological values of different insomnia medicines in different studies. X data denotes the medicines under consideration, while Y data denotes the toxicology value in mg/kg.

4.2. Molecular Docking

The docking probabilities of different receptors and the chemical agents were dome with the help of Pro-Tox II . It is observed that Trazodone binds effectively with Amine Oxidase A at MET426 ,SER193, GLY 60 and VAL59 residues at axisses X:21.2714, Y: 1.9225 and Z:5.8306 with the binding affinity peak at -10.2 followed by -10.0 ,-9.6 and ultimately stoping at -9.4 at the four scenario run of the in silico test .(Fig 2)(Fig 3)Analysis of Trazodone and Prostagladin G/H synthase 1 at X:40.614, Y: 66.304 and Z:45.584 with the docking scres at -5.2,-5.0,-5.0 and -4.9 at VAL50 CYS37 TYR55 ASP58 residues.(Fig 3)Analysis of Zolpidem-Amine Oxidase A structure reveals that the binding axisses are X:21.2714 , Y:1.9225 and Z: 5.8306 with the docking scores at -10.5, -8.1 and -7.7 along the residues of VAL59 GLY 60 TRP58 PRO61 . (Fig 3)Interaction of Zolpidem with Prostagladin G/H synthase 1 have shown the binding axisses at X:23.196 ,Y:32.109 and Z:29.398 at residues GLY223 GLY 226 GLU 140 LEU 145. (Fig 4)Docking score got a minimum result at -6.4, -5.5,-5.1 and -5.0. (Fig 2)Temazepam-Amine Oxidase A complex binds at X:21.2714, Y:1.9225 and Z:5.8306 giving a binding affinity score of -8.9,-8.4,-8.2 and -8.0 at residues GLY 60 VAL59 ILE66 MET 426. (Fig 4) Temazepam-Prostagladin G/H synthase 1 binding axisses are at X:16.482, Y:33.876 and Z:50.604 with docking scores of -8.4 and -6.5.(Fig 4)

The residues are GLY227 HIS226 GLY225 with the main binding axis at VAL228_C1571.Amitripyline-Dopamine Receptor D3 complexes at X:-0.0305, Y:-15.3792 and Z: 10.5439 with scores at -7.4, -7.3,-7.2 and -6.7 at residues GLY33, VAL49 and SER51(Fig 5).Amitriptyline-Histamine Receptor H1 binds at X:26.258, Y:27.477 and Z:58.714 with a near homogenous docking scores of -6.0,-6.0,-5.9 and -5.8 with the main binding axis at LEU405_C2812 and residues TRY1158 and ALA1160. (Fig 5)Flurazepam binds Amine Oxidase A at X:21.2714, Y:1.9225 and Z:5.8306 with docking poses at -8.2 and -5.3 at residues GLY60, LN 196 and VAL59 .(Fig 5)Flurazepam also binds with opoid Receptor MU with main axis at PRO1143_C2659 at X:-11.332, Y:39.454 and Z:2.099 giving docking scores -6.1 ,-6.0,-5.9 and -5.5.Resideus are ASN1020 and LYS1002.(Fig 2)(Fig 6)

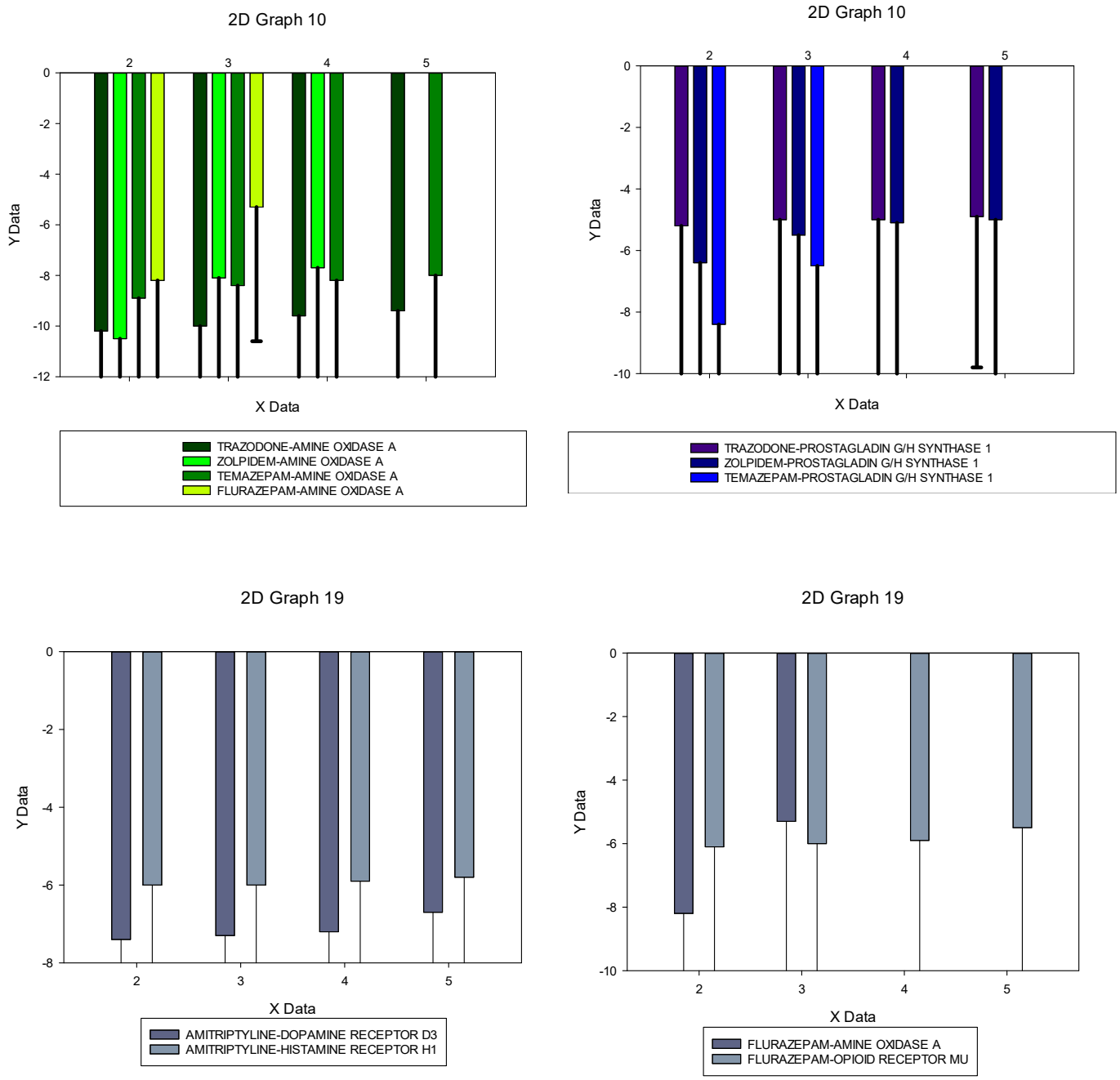


Figure 2. Binding Affinities of different compounds along with the binding score. X axis denotes the number of binding modes, and Y axis denotes the binding score values.

Table 1. Different Toxicological Assay Analysis of Insomnia Medicines.

Sl no.	Generic name	CAS no.	Bioassay Test Prediction		Predicted Acute Toxicity Value in T.E.S.T. software (consensus method)		Statistical Data Estimation by T.E.S.T.		Mutagenecity Estimation	Additional Remarks and Target Organs	Target Organs
			LD_{50} values (mg/kg)	Log LD_{50} values (mg/kg)	Predicted LD_{50} values (mg/kg)	Log LD_{50} value (mg/kg)	R^2 value	Residual			
1	Trazodone	19794-93-5	690	2.84	598.91	2.79	0.85	0.05	-0.30 (-)	Immunotoxic (0.78)	Amine Oxidase A Prostaglandin G/H Synthase 1
2	Zolpidem	82626-48-0	695	2.84	919.72	2.52	0.837	0.32	0.65 (+)	Mutagenic	Amine Oxidase A Prostaglandin G/H Synthase 1
3	Temazepam	846-50-4	2000.88	2.18	1929.39	2.19	0.740	-0.01	0.03 (-)		Amine Oxidase A Prostaglandin G/H Synthase 1 Progesterone Receptor
4	Lorazepam	846-49-1	4495.05	1.85	2785.68	2.06	0.789	-0.21	-0.03 (-)		Amine Oxidase A Prostaglandin G/H Synthase 1 Progesterone Receptor
5	Amitriptyline	50-48-6	320.01	2.94	309.08	2.95	0.770	-0.01	-0.07 (-)	Hepatotoxic (85 %)	Dopamine Receptor D3 Histamine Receptor H1
6	Estazolam	29975-16-4	2503.04	2.07	1450.63	2.31	0.815	-0.24	0.10 (-)		Amine Oxidase A Prostaglandin G/H Synthase 1

7	Clonazepam	1622-61-3	4000	3.60	831.44	2.58	0.830	1.02	0.20 (-)	Hepatotoxic (85 %)	Amine Oxidase A Prostaglandin G/H Synthase 1
8	Diphenhydramine	58-73-1	390.13	2.82	1841.52	2.14	0.779	0.68	0.09 (-)		Histanamine Receptor H1
9	Gabapentin	60142-96-3	8000	3.90	1687.57	2.01	0.775	1.89	0.29 (-)		Androgen Receptor Prostaglandin G/H Synthase 1
10	Flurazepam	17617-23-1	981.17	2.60	2061.77	2.27	0.777	0.33	0.24 (-)		Amine Oxidase A Opioid Receptor Mu Prostaglandin G/H Synthase 1 Progesterone Receptor
11	Zonalon	1668-19-5	146.97	3.28	451.47	2.79	0.760	0.49	0.08 (-)		Histamine Receptor H1

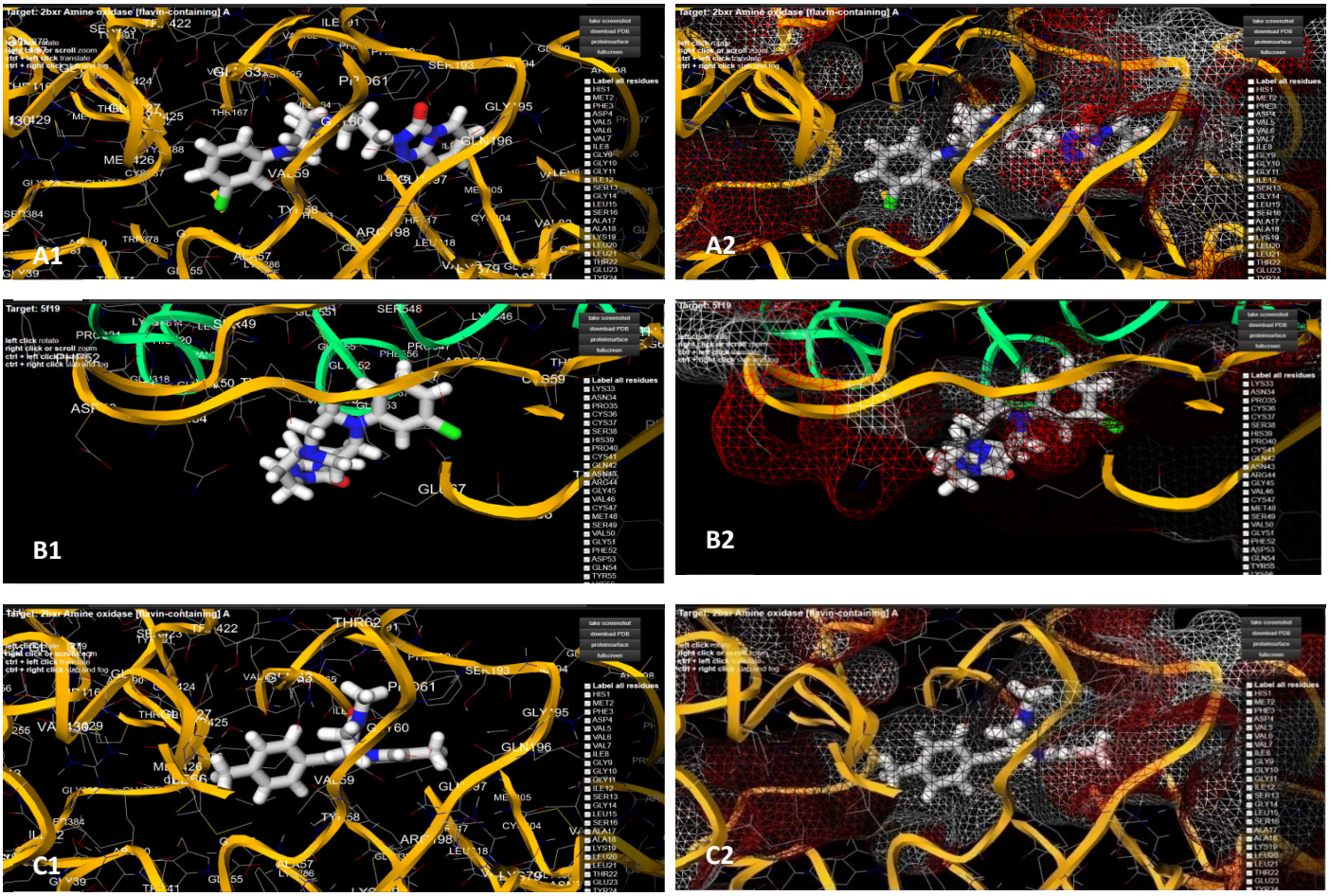


Figure 3. A series: Trazodone -Amine Oxidase A, B series: Trazodone Prostaglandin G/H Synthase 1, C series: Zolpidem -Amine Oxidase A. Red meshwork on right side denotes local protein complexes.

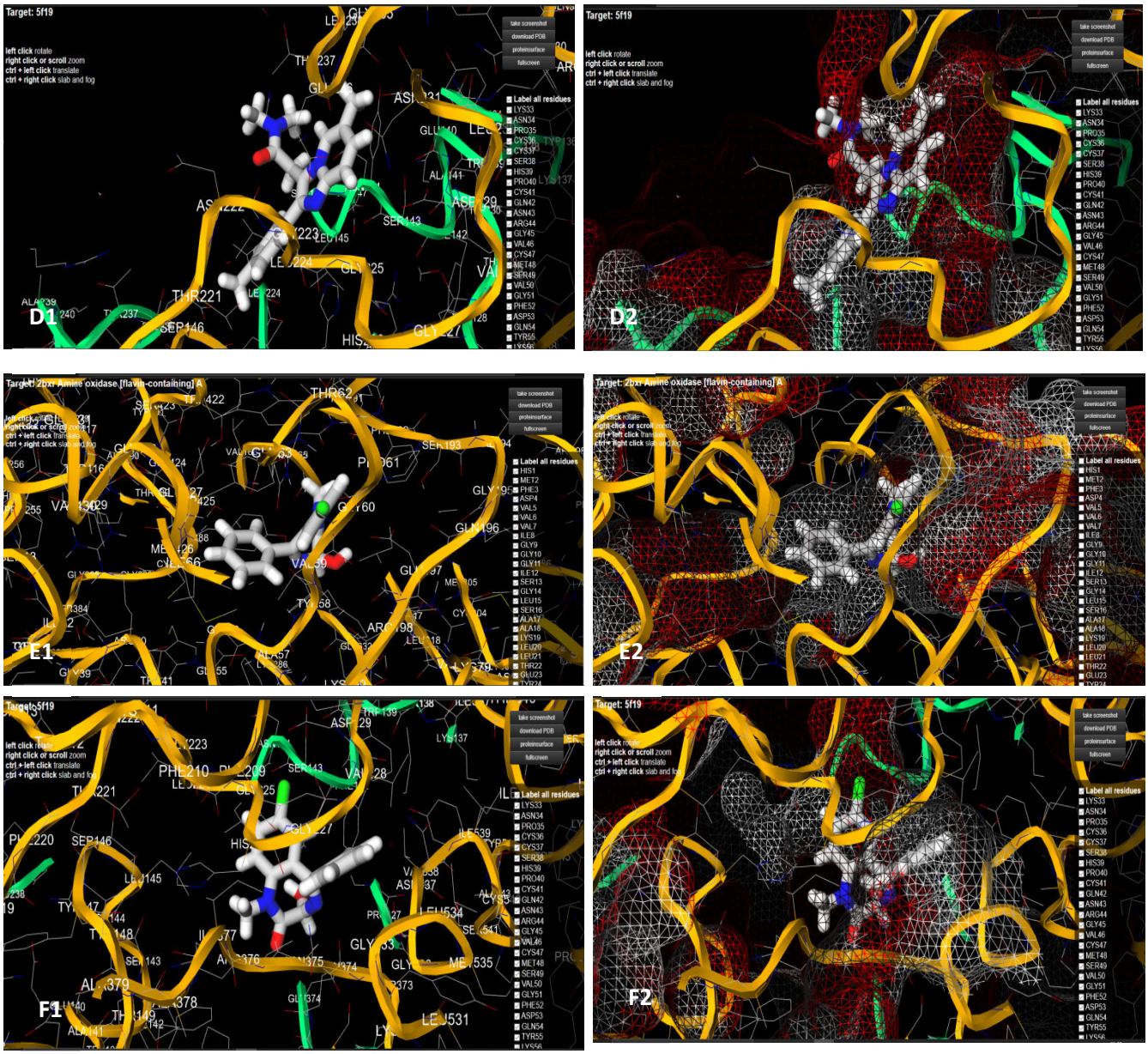


Figure 4. D series:Zolpidem-Prostaglandin G/H Synthase 1, E series: Temazepam-Amine Oxidase A, F series: Temazepam-Prostaglandin G/H Synthase 1. Red meshwork on right side denotes local protein complexes.

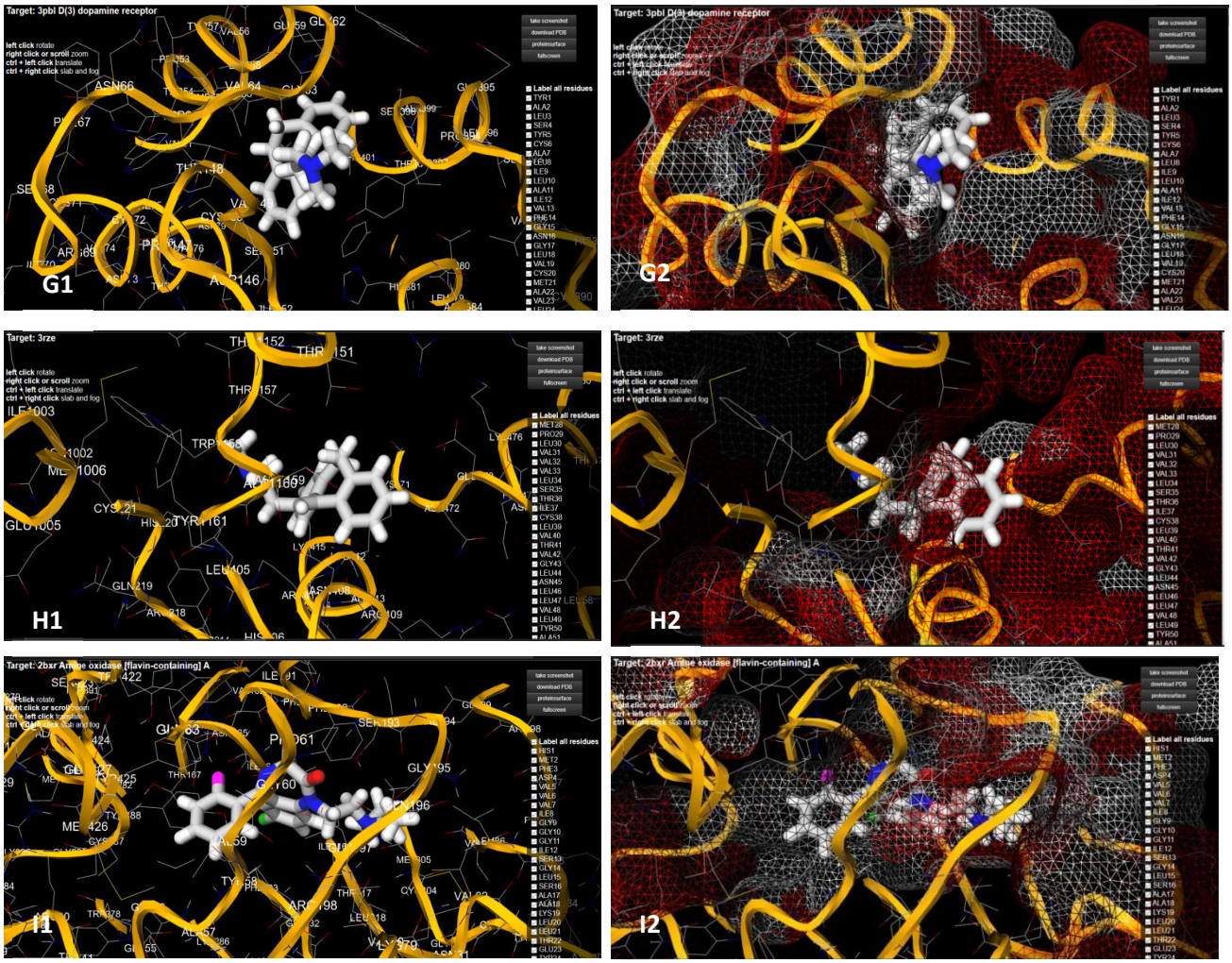


Figure 5. G series: Amitriptyline-Dopamine receptor D3, H series: Amitriptyline-Histamine Receptor H1 ,I series: Flurazepam-Amine Oxidase A. Red meshwork on right side denotes local protein complexes.

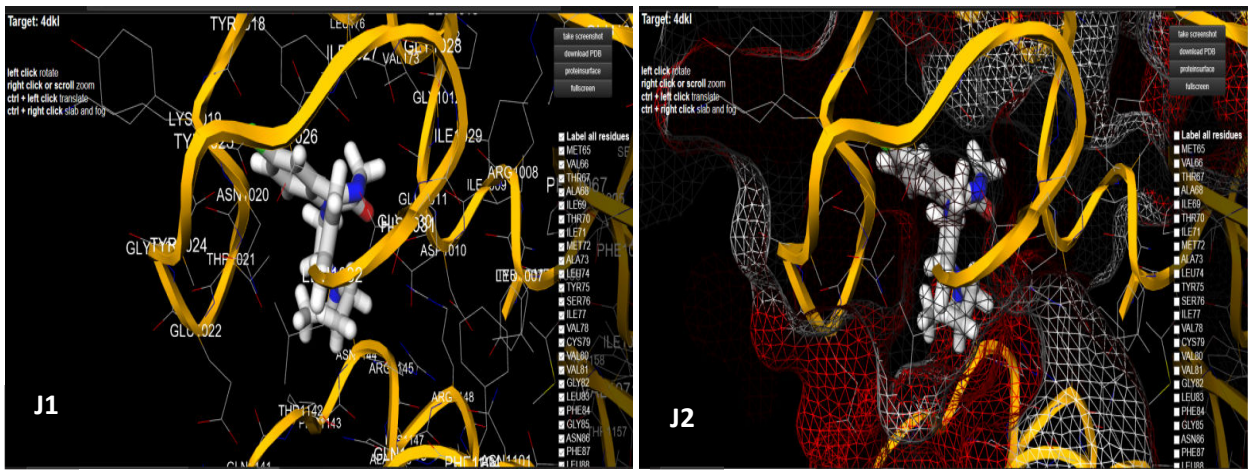


Figure 6. Flurazepam- Opioid Receptor Mu. Red meshwork on right side denotes local protein complexes.

5. DISCUSSION

Palliative drugs consist of a wide spectrum of pharmacophore which through various studies, have been proved to be malevolent to the biological life and got a high amount of potential to disrupt the known biochemical pathways.[22] In the present rostrum, we have employed the T.E.S.T. software to detect the consequences of harmful medications on different oraganismal systems, particularly in rats, with the utilization of analogous 2D molecular descriptors. After a thorough in silico analysis and molecular docking, it is seen that insomnia medicines are indeed quite harmful to the human body as they can inhibit the functions of a broad spectrum of receptors like Amine Oxidase A , Prostaglandin G/H Synthase 1,Progesterone Receptor.Inhibition of them can cause disorders in cycloogenase and hydroxiperoxidase activity.[23] Experimental study had seen that mice with knockout Progesterone Receptor has abnormal mammary gland development and delayed maturation of mammary duct.[24]The analysis of the modelling software is been stated in Table 1 has been employed with the help of the molecular descriptors. r^2 and q^2 values, i.e., correlation of determination of different mutagenecity test are found to be quite high for all the compounds (Fig 7). And mutagenicity test of zolpidem is found to be positive, which denotes that zolpidem is actually a mutagenic agent capable of producing carcinoma. (Table 1)

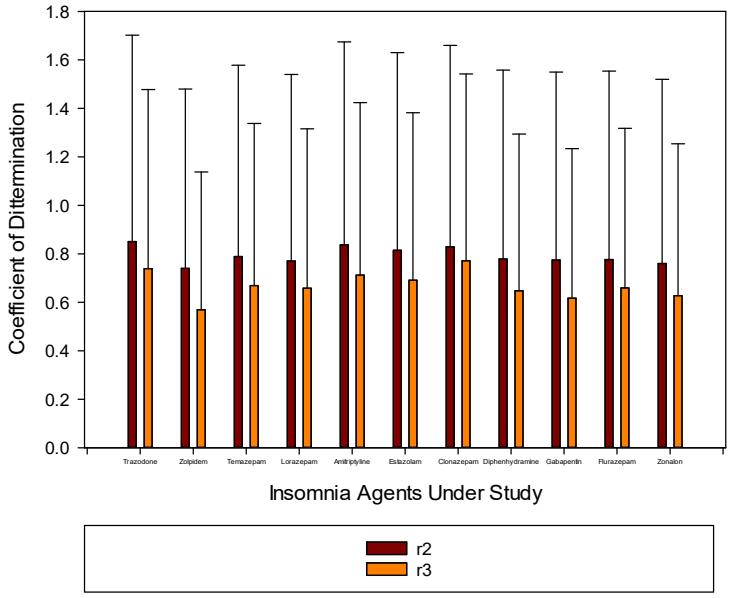


Figure 7. The coefficient of determination values for different insomnia compounds.

Studies have seen that the toxicity ranges as value of <5 mg/kg, 5–50 mg/kg, 50–500 mg/kg, 500–5,000 mg/kg, 5,000–15,000 mg/kg and>15,000 mg/kg are super toxic, extremely toxic, very toxic, moderately toxic, slightly toxic, and practically non-toxic, respectively.[25][26] LD_{50} study of the present paper has catalogued the compounds in following order with respect to their toxicity: Zolpidem > Gabapentin > Flurazepam > Clonazepam > Estazolam > Diphenhydramine > Zonalon > Temazepam > Lorazepam > Amitriptyline > Trazodone.Studies on aquatic organisms have been done to assess the effect of these medicines in waste water and aquatic systems.

6. CONCLUSION

The toxicity study using T.E.S.T. software have placed the insomnia medications in three categories, with seven in moderately toxic category, one in slightly toxic category, and three in extremely toxic category. This figure (Fig 8) is based on the categorization of the toxicity classes of the insomnia medications under our considerations showing the toxic input of chemical substances done by nearly 50% of the world's population, which in turn got disastrous consequences. The study also proved the effectiveness of T.E.S.T. software in detecting the initial toxic manifestations, and a further work to be done utilizing the help of 3 D molecular descriptors.

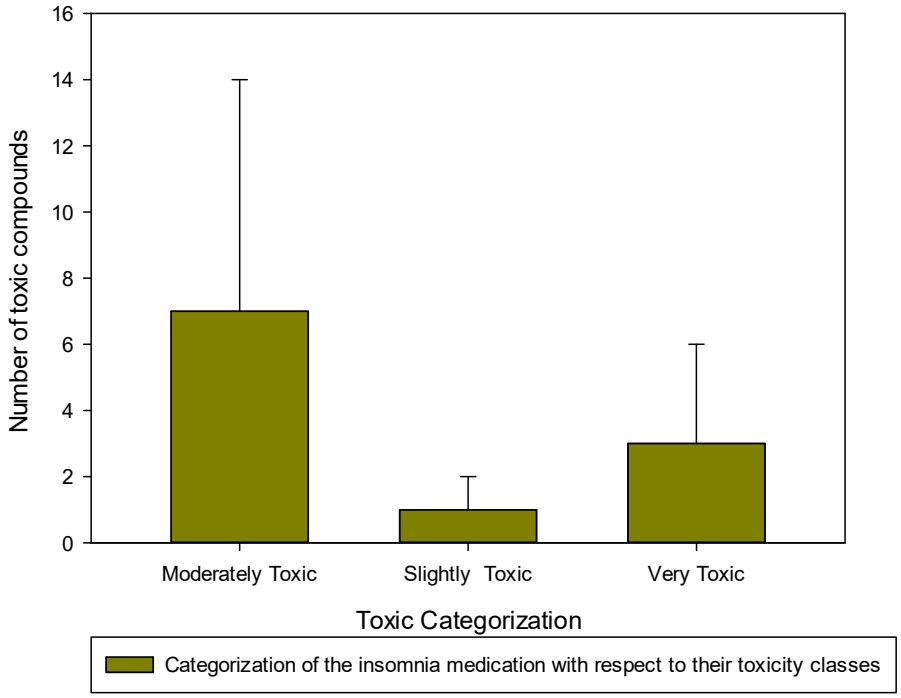


Figure 8. Categorization of the insomnia medications with respect to their toxicity classes.

References:

[1] Solomon, F., White, C. C., Parron, D. L., & Mendelson, W. B. (1979). Sleeping pills, insomnia and medical practice. *New England Journal of Medicine*, *300*(14), 803-808.

[2] Kang, D. Y., Park, S., Rhee, C. W., Kim, Y. J., Choi, N. K., Lee, J., & Park, B. J. (2012). Zolpidem use and risk of fracture in elderly insomnia patients. *Journal of Preventive Medicine and Public Health*, *45*(4), 219.

[3] Kripke, D. F. (2000). Chronic hypnotic use: deadly risks, doubtful benefit. *Sleep medicine reviews*, *4*(1), 5-20.

[4] Lucire, Y., & Crotty, C. (2011). Antidepressant-induced akathisia-related homicides associated with diminishing mutations in metabolizing genes of the CYP450 family. *Pharmacogenomics and personalized medicine*, *4*, 65.

[5] Chung, K. F., & Lee, C. K. (2002). Over-the-counter sleeping pills: a survey of use in Hong Kong and a review of their constituents. *General hospital psychiatry*, *24*(6), 430-435.

[6] Akindele, M. O., Evans, J. I., & Oswald, I. (1970). Mono-amine oxidase inhibitors, sleep and mood. *Electroencephalography and clinical neurophysiology*, *29*(1), 47-56.

[7] Obal Jr, F., & Krueger, J. M. (2003). Biochemical regulation of non-rapid-eye-movement sleep. *Front Biosci*, *8*(1), d520-550.

[8] Winkler, A., Auer, C., Doering, B. K., & Rief, W. (2014). Drug treatment of primary insomnia: a meta-analysis of polysomnographic randomized controlled trials. *CNS drugs*, *28*(9), 799-816.

[9] Trumic, E., Pranjic, N., Begic, L., Becic, F., & Asceric, M. (2012). Idiosyncratic adverse reactions of most frequent drug combinations longterm use among hospitalized patients with polypharmacy. *Medical Archives*, *66*(4), 243.

[10] Bernstein, S., & Leff, R. (1967). Toxic psychosis from sleeping medicines containing scopolamine. *New England Journal of Medicine*, *277*(12), 638-639.

[11] CHU, A. G., GUNSOLLY, B. L., SUMMERS, R. W., ALEXANDER, B., MCCHESNEY, C., & Tanna, V. L. (1983). Trazodone and liver toxicity. *Annals of Internal Medicine*, *99*(1), 128-129.

[12] Dykens, J. A., Jamieson, J. D., Marroquin, L. D., Nadanaciva, S., Xu, J. J., Dunn, M. C., ... & Will, Y. (2008). In vitro assessment of mitochondrial dysfunction and cytotoxicity of nefazodone, trazodone, and buspirone. *Toxicological Sciences*, *103*(2), 335-345.

[13] Toner, L. C., Tsambiras, B. M., Catalano, G., Catalano, M. C., & Cooper, D. S. (2000). Central nervous system side effects associated with zolpidem treatment. *Clinical neuropharmacology*, *23*(1), 54-58.

[14] Darke, S., Deady, M., & Duflou, J. (2012). Toxicology and characteristics of deaths involving zolpidem in New South Wales, Australia 2001–2010. *Journal of forensic sciences*, *57*(5), 1259-1262.

[15] Robison, R. L., Van Ryzin, R. J., Stoll, R. E., Jensen, R. D., & Bagdon, R. E. (1984). Chronic toxicity/carcinogenesis study of temazepam in mice and rats. *Toxicological Sciences*, *4*(3part1), 394-405.

[16] Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., ... & Wang, J. (2016). PubChem substance and compound databases. *Nucleic acids research*, *44*(D1), D1202-D1213.

[17] Tomasulo, P. (2002). ChemIDplus-super source for chemical and drug information. *Medical reference services quarterly*, *21*(1), 53-59.

[18] Valerio Jr, L. G. (2009). In silico toxicology for the pharmaceutical sciences. *Toxicology and applied pharmacology*, *241*(3), 356-370.

[19] Martin, T. (2016). User's guide for TEST (version 4.2)(Toxicity Estimation Software Tool): A program to estimate toxicity from molecular structure.

[20] Bank, P. D. (1971). Protein data bank. *Nature New Biol*, *233*, 223.

[21] Odhar, H. A., Rayshan, A. M., Ahjel, S. W., Hashim, A. A., & Albeer, A. A. M. A. (2019). Molecular docking enabled updated screening of the matrix protein VP40 from Ebola virus with millions of compounds in the MCULE database for potential inhibitors. *Bioinformation*, *15*(9), 627.

[22] Maguire, G., Lee, P., Manheim, D., & Boros, L. (2006). SiDMAP: a metabolomics approach to assess the effects of drug candidates on the dynamic properties of biochemical pathways. *Expert opinion on drug discovery*, *1*(4), 351-359.

[23] Kargman, S., Wong, E., Greig, G. M., Falgueyret, J. P., Cromlish, W., Ethier, D., ... & Tagari, P. (1996). Mechanism of selective inhibition of human prostaglandin G/H synthase-1 and-2 in intact cells. *Biochemical pharmacology*, *52*(7), 1113-1125.

[24] Mulac-Jericevic, B., Lydon, J. P., DeMayo, F. J., & Conneely, O. M. (2003). Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. *Proceedings of the National Academy of Sciences*, *100*(17), 9744-9749.

[25] P. Ruiz, G. Begluitti, T. Tincher, J. Wheeler, M. Mumtaz, Prediction of acute mammalian toxicity using QSAR methods: A case study of sulfur mustard and its breakdown products, Molecules 17 (2012) 8982-9001.

[26] Stellman, J. M. (1998). *Encyclopaedia of occupational health and safety* (Vol. 2). International Labour Organization.