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Determination of Mutagenic Potentials of Diarylmethylamine Based Imine Compounds By Ames Test And Computational Molecular Docking

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Abstract

In recent years, studies that investigate the effects of chemical compounds on organisms have increased in direct proportion to their widespread use. In this study, four different bidentate imine ligands and bidentate imine ligands+Cu(II) complexes were synthesized from the bioactive synthetic diarylmethylamine compound. After the ligands and metal complexes obtained were purified using chromatographic and analytical methods, their mutagenic effects were investigated with the Ames/Salmonella test system. In addition, interactions of four different Cu(II) complexes with B-DNA were evaluated with molecular docking analysis.

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Accordingly, the results indicated a significant increase in the colonies formed in the presence (+S9) and absence (-S9) of the metabolic activation system, meaning a mutagenic effect against strain TA98 and TA100 strains in general.

Key words: *Diarylmethylamine, Imine Compounds, Salicylaldehyde, Cu (II) Complexes, Salmonella/Microsome Test, Molecular Docking.*

1. Introduction

While the Industrial Revolution provided technological development, it also caused chemical pollution in the world. All living organisms are dealing with these pollutants in a slightly greater amount every day. There are many chemicals taken directly or indirectly in food consumed in daily life, inhaled air, clothes worn and drugs used for survival purposes (1). Living organisms are also exposed to these chemical pollutants, either directly or indirectly. Some of the chemicals do not cause any changes in the DNA structure of the living organism, or the resulting damage can be repaired through DNA repair enzymes, while some damage cannot be overcome. These damages in the living organism can cause mutations, recombinational changes, or structural chromosome errors. If these changes occur in reproductive attacks, they are passed on to subsequent generations, while if they occur in somatic attacks, they cause cancer (2). Various mutagenicity, toxicity, carcinogenicity and teratogenicity determination test systems have been developed to determine the effects of chemicals on living organisms. The studies, devoted to understanding the effects of chemical products on ecosystem and human health, are safety tests. For this purpose, back mutation tests are performed first (eg Ames). Additionally, medicines containing natural and synthetic chemical active substances are subjected to many safety tests considering the effects on human health and ecosystem before they are placed on the market (2, 3, 4, 5).

The Ames test system is a commonly used test to determine point mutations in DNA, such as modification, addition, or deletion of one or more base pairs. This test system uses *Salmonella typhimurium* strains (TA98 and TA100) derived by *in vitro* mutation from the LT2 ancestral strain (6).

It was first improved by Ames in 1975 (7, 8, 9, 10). TA100 has base change mutation, while TA98 has frameshift mutation. Because of these mutations of TA98 and TA100 strains, both strains cannot synthesize histidine (his-). The principle of the test system is based on the ability of strains TA98 and TA100 to re-synthesize histidine after exposure to various chemicals or test substances. Point mutations and frameshift mutations have been reported to induce tumor formation in both humans and animals due to base change mutations in tumor suppressor genes or oncogenes, and these mutations are also known to underlie many genetic diseases (6).

Schiff bases containing an azomethine group (-CH=N-) have been studied for a long time due to their high biological activity profiles (11). Numerous information on the properties of synthetic Schiff bases of potential biological interest suggests that some of these compounds have been identified and used as models for a number of systems **(**12, 13, 14, 15, 16).

Metal complexes are widely used in the treatment of cancer, arthritis and diabetes (17). Copper (II) complexes, for example, are known to be effective against joint inflammations and also to exhibit anti-ulcer activity (18, 19). This is significant because gastrointestinal irritation often hinders the treatment of other antiarthritic drugs. This suggests that copper's acidic anti-inflammatory agents play a role in preventing gastrointestinal damage (19).
The purpose of this study is to determine the mutagenic potential of four new Schiff base derivatives-Cu(II) complexes with the Ames/*Salmonella* test system. In this study, molecular docking analysis was performed using Autodock 4.2 for the purpose of the deeper perspective on the binding poses of four different bidentate imine ligands-Cu(II) complexes with B-DNA.

2. Materials and methods

All chemicals and solvents were of high quality and purchased from commercial suppliers (Aldrich or Merck). Elemental analyses (C, H, N) were carried out using Costech ECS 4010 (CHN). Infrared spectra were obtained using KBr disc (4000-400 cm^{-1}) on a PerkinElmer Spectrum 100 FT-IR. ^{1}H and ^{13}C NMR (Nuclear Magnetic Resonance) spectra were recorded on a Bruker 400 MHz instrument and TMS was used as an internal standard. Below presents the methodological concerns in detail:

2.1. Phenyl(p-tolyl)methanamine

The amine 2 was synthesized from benzoic acid following a detailed set of literature procedures (20, 21, 22). ^{1}H-NMR and ^{13}C-NMR data are in compromise with the literature data (22).

2.2. The method for the synthesis of imine compounds (3a-d).

The imine compounds were successfully prepared by following the well-described literature method. The ^{1}H-NMR data and ^{13}C-NMR data are in compatibility with literature (22).

2.3. The method for the synthesis of metal complexes (3a-d-Cu)

The complexes were prepared according to a known procedure. The Schiff base ligand (0.21 g; 0.0006 mol) was dissolved in MeOH (20 mL) solution and the metal salts [$CuCl_2.2H_20$ (0.107 g; 0.0006 mol) were added to the mixture of MeOH (20 mL) and the solution was refluxed for about 48 h. After confirming that the reaction was completed by thin layer chromatography, it was cooled to room temperature. The reaction mixture was evaporated; it was purified by filtration in a 3:1 hexane / ethyl acetate solvent mixture. The purity of the complexes was checked by TLC (Thin Layer Chromatography) studies.

3a-Cu: Color: Brown. melting point: 198-200°C. FTIR: (υ_{max} cm^{-1}): 3445, 3344, 3164, 1605, 1534, 1408, 1251, 995, 845, 696, 558 cm^{-1}. Anal. Calcd for $C_{22}H_{20}ClCuNO_2$: C, 61.54; H, 4.69; N, 3.26. Found C, 61.58; H, 4.71; N, 3.28.

3b-Cu: Color: Light brown. melting point: 98-102°C. FTIR: (υ_{max} cm^{-1}): 3410, 3153, 3010, 2920, 2577, 1592, 1506, 1374, 1279, 1194, 1096, 830, 777, 705, 549 cm^{-1}. Anal. Calcd for $C_{22}H_{20}ClCuNO_2$: C, 61.54; H, 4.69; N, 3.26. Found C, 61.52; H, 4.67; N, 3.27.

3c-Cu: Color: Brown. melting point: 150-152°C. FTIR: (υ_{max} cm^{-1}): 3421, 3159, 3038, 2923, 1606, 1522, 1453, 1242, 1185, 1094, 1028, 812, 703, 550 cm^{-1}. Anal. Calcd for $C_{21}H_{18}ClCuNO_2$: C, 60.72; H, 4.37; N, 3.37. Found C, 60.69; H, 4.38; N, 3.36.

3d-Cu: Color: Brick red. melting point: >250°C. FTIR: (υ_{max} cm^{-1}): 3452, 3351, 2922, 2224, 1608, 1542, 1509, 1447, 1385, 1260, 1230, 1036, 847, 806, 701, 555 cm^{-1}. Anal. Calcd for $C_{21}H_{18}ClCuNO_2$: C, 60.72; H, 4.37; N, 3.37. Found C, 60.71; H, 4.38; N, 3.36.

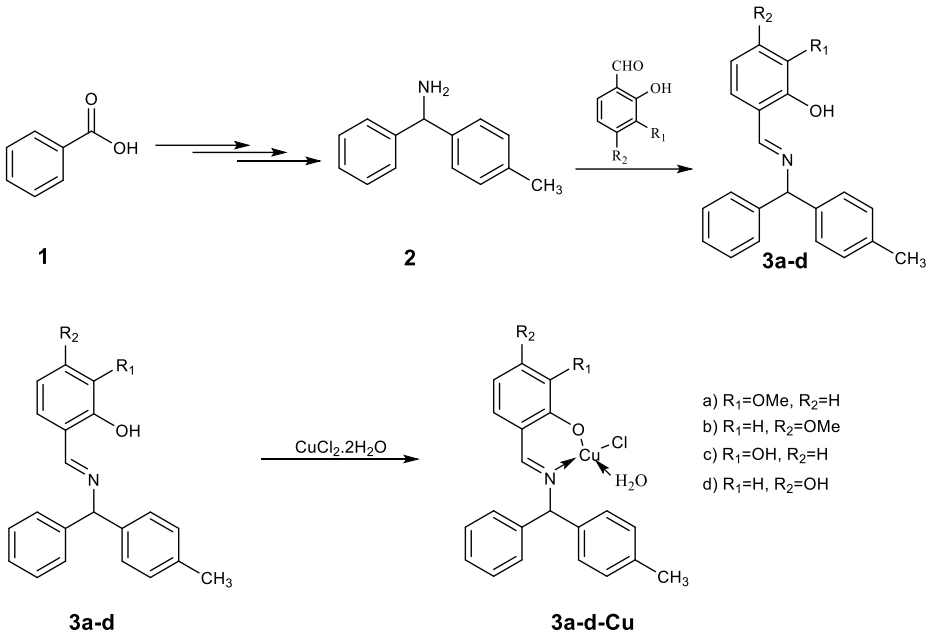


Figure 1. Synthesis scheme of imine compounds and metal complexes.

2.4. The culture of the bacterial strains TA98 and TA100

2.4.1. Salmonella/Microsome test (Ames)

The Salmonella microsome test system is one of the most preferred short-term genotoxic tests to detect the mutagenic potential of chemicals due to its rapid results and low cost. In the experiment TA98 and TA100 strains of *Salmonella*

typhimurium LT2 ancestral strain developed with *in vitro* mutations were used. Prior to experimentation, both strains were checked for the presence of strain-specific markers as suggested by Maron and Ames. The standard plate insertion test was investigated both in the presence and absence of a mixture of *Salmonella typhimurium* TA98 and TA100. The same experiment was performed in the S9 mixture as suggested by Maron and Ames Ames (8).

2.4.2. Bacterial strains

TA98 and TA100 were purchased commercially (J.L. Swezey, Curator, ARS Patent Culture Collection, Microbial Genomics, and Bioprocessing Research Unit, North University Street, Peoria, Illinois 61604, USA.). To the detection of frameshift mutagens and base-pair substitution mutagens, TA100 and TA98 strain are used respectively. Before the bacterial strains were used in the experiment, the strains were checked for the

presence of strain-specific markers as suggested by Maron and Ames Ames (8).

2.4.3. Mutagenicity assay

In order to determine the mutagenic effects of the test substances, TA98 and TA100 strains were examined in environments with and without S9 Ames (8). The S9 factor mix was used as 1/10 of the total volume for each plate. Test substances were prepared at concentrations of 0.80, 0.40, 0.20, 0.10, and 0.05 µg/plate (Solvent distilled water). For medium with S9, 2-AF (2-amino fluorene) was used as a positive mutagen (20 µg/plate) (in strains TA98 and TA100). Without the S9 medium, 4-NPD (4-nitro phenylenediamine) was used as the positive mutagen (200 µg/plate). All experiments were performed at two different times and in five replicates for each sample.

2.4.4. Preparation of S9

The fraction with and without S9 was prepared according to the literature Ames (8). S9 tablets were purchased commercially (Roche, Cat. no: 1.745.425). Freshly prepared S9 fraction was stored at -35ºC. The S9 mix was freshly prepared before each experiment.

2.5. *In silico* molecular docking analysis

Molecular docking analyses were performed, using AutoDock 4.2 (23), to predict possible binding sites on the B-DNA (PDB code: 1BNA) crystal structure of four different bidante imine ligands and their Cu(II) complexes synthesized from the bioactive synthetic diarylmethylamine compound). The crystal structure of the 1.9 Å resolution B-DNA molecule was chosen as the target (receptor) molecule. AutoDockTools (ADT) was used to prepare the parameters before starting the docking analysis of the receptor and ligand molecules. Nonpolar hydrogen atoms of B-DNA and ligand molecules were combined and the non-polar hydrogen atoms were removed. Gasteiger charge were detected according to Ricci and Netz (24, 25). B-DNA and ligand structures were saved in PDBQT format. The grid box and grid spacing were set 60 × 60 × 60 Å and 0.375 Å, respectively. Dockings were constructed from 50 GA (Genetic Alghorithm) runs using an initial population of up to 150 individuals, $5x10^5$ energy evaluation counts, a maximum of 27,000 generation. Mutation and transmission rates performed at 0.02 and 0.8, respectively. 4 different ligand molecules' 100 docking results were examined. Autodock was clustered and ranked for receptor/ligand all possible binding modes and according to the free energy of binding (kcal/mol) of the conformation with the lowest binding free energy and the best docking pose. The best docking pose between ligands and B-DNA using the AutoDock 4.2 output file was analyzed in BIOVIA Discovery Studio Visualizer 2016 (26).

2.6. Statistical significance

To determined the significance of the between control revertants and revertants of treated groups, used t-test in SPSS.

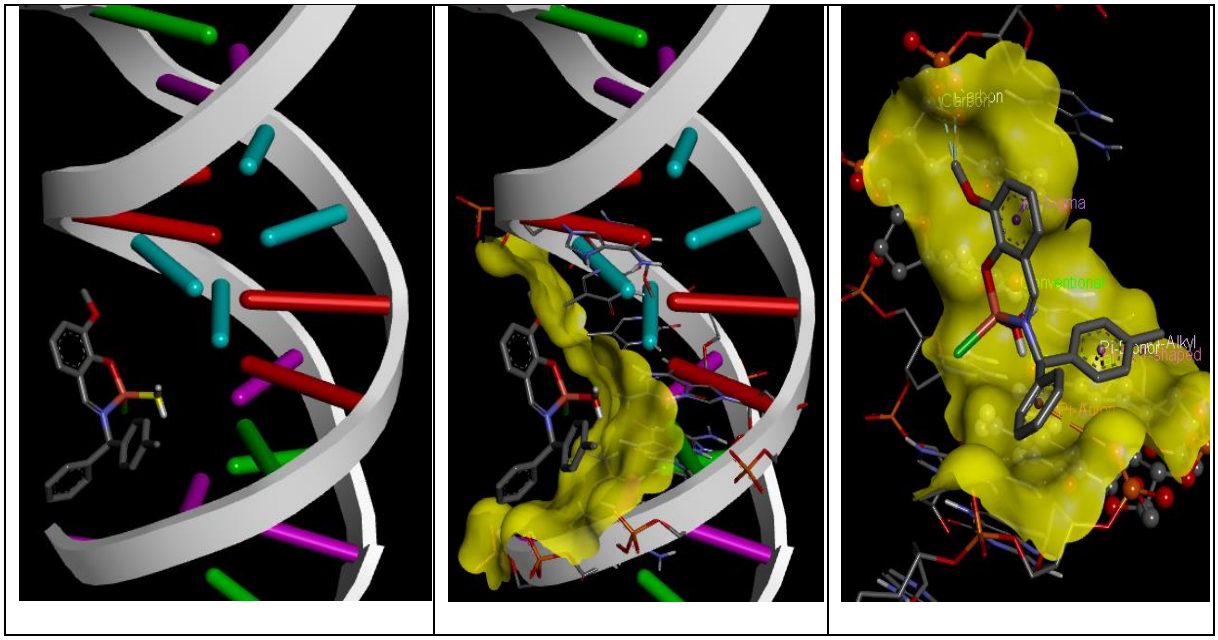
3. Results

Viability test results suggested that the highest concentration was 0.8 µg/plate. For that reason, other concentrations of 0.05, 0.10, 0.20, 0.40, and 0.80 µg/plate were chosen for each test compound in the mutagenicity assay, respectively.

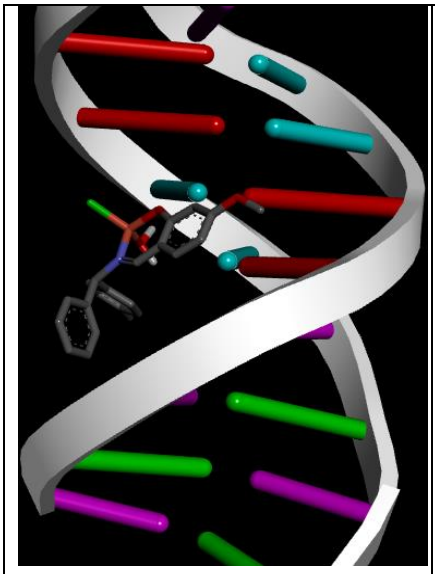
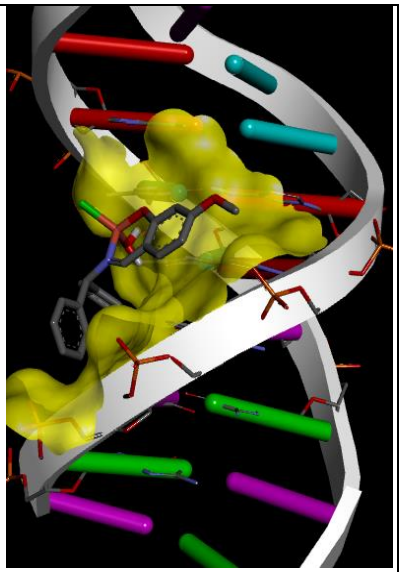
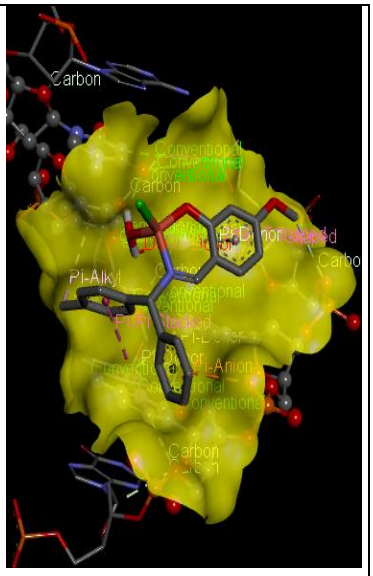
In our study, all test substances were showed significant mutagenic effects on TA98 and TA100 strains. Mutagenicity test results were given in Table 1.

The interaction of between target molecule (crystallographic B-DNA) and the 3a-Cu, 3b-Cu and 3c-Cu ligands based on gibbs free binding energies (kcal/mol) is shown in the B-DNA major groove (Fig. 1., 1a, 2a and 3a) while 3d-Cu ligand was found to bind to the B-DNA' minor groove (Fig. 2., 4a). Linkages with a mean distance measure (RMSD value) between the atoms of the B-DNA molecule of less than 2 Å were evaluated. At the same time, the lowest negative free binding energy (ΔG binding) was calculated for 3a-Cu, 3b-Cu, 3c-Cu and 3d-Cu ligands. Since the results were -5.09, -5.34, -4.46 and -6.73 kcal/mol, the interaction of ligands with B-DNA was thought to be significant. The best docking poses, including H-bonds of ligands and B-DNA, were shown in different poses (Figure 2).

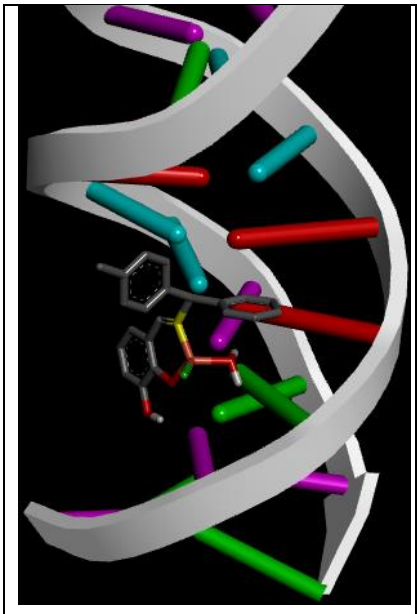
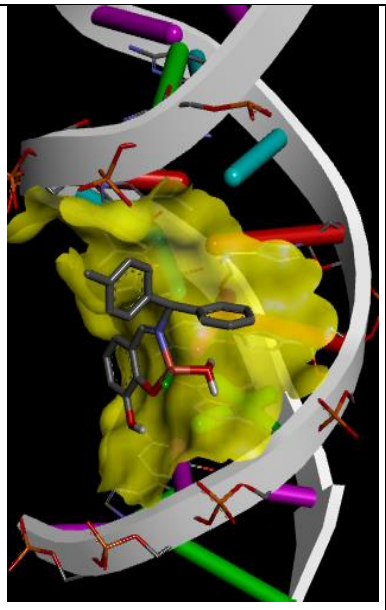
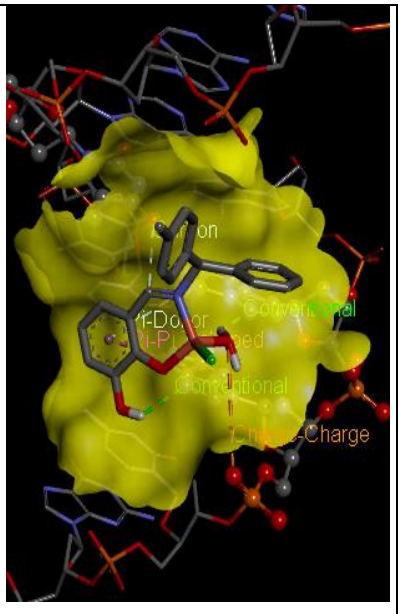
1-3a-Cu



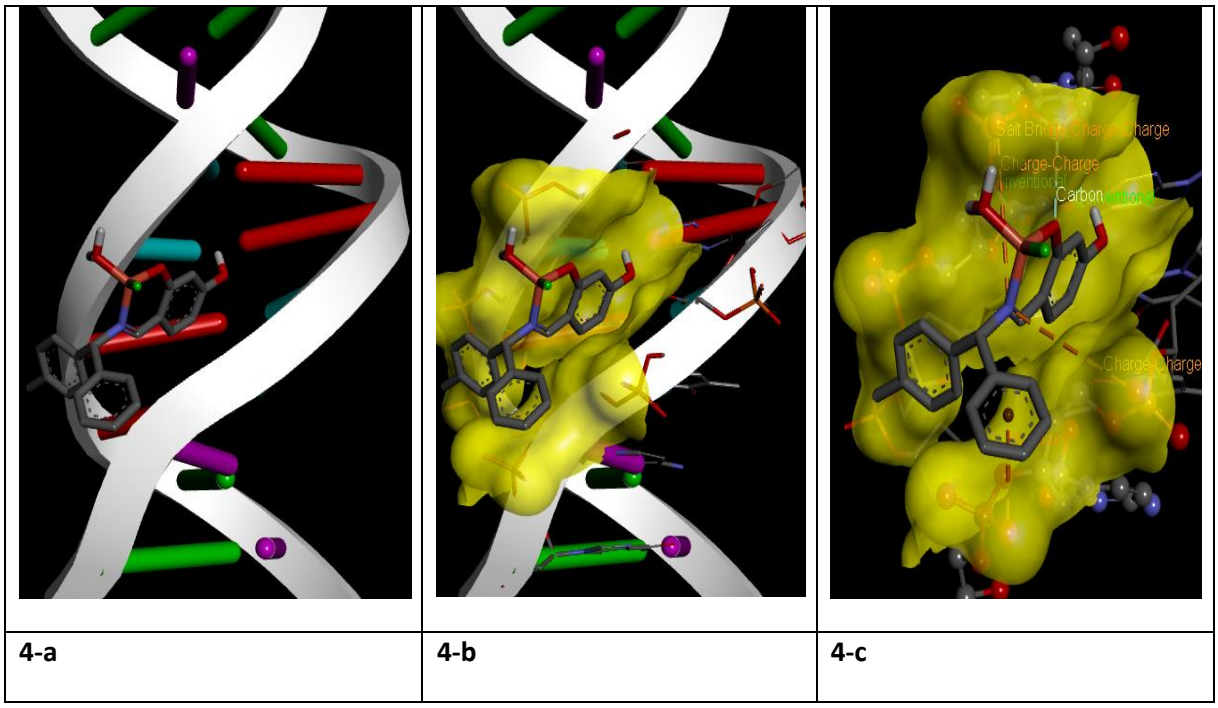
2-3b-Cu

		
2-a	2-b	2-c

3-3c-Cu

		
3-a	**3-b**	**3-c**

4-3d-Cu



4-a	4-b	4-c

Figure 2: Docking data display the interaction between four different bidentate imine ligands and their Cu(II) complexes and DNA (Pdb Code: 1BNA). 1:**3a-Cu**, 2: **3b-Cu**, 3: **3c-Cu** 4: **3d-Cu**, a: Best docking pose, b: Receptor ligand intraction with H-Bond surface, c: Solvent hydrogen bond donor/acceptor surface with other bond types.

Table 1. The mutagenicity of 3a-Cu, 3b-Cu, 3c-Cu and 3d-Cu on *S. Typhimurium* TA98 and TA100 strains in the presence or absence of S9 mix.

Test substances	**Cont.**	**TA 98**		**TA 100**	
	µg/plt.	**– S9**	**+ S9**	**– S9**	**+ S9**
Spontaneous Control	-	9.67±2.27	11.49±1.87	103.2±10.9	100.00±9.59
NPD		3001±172			
2-AF			3409±239		739.2±39.8
SA				644.8±50.2	
	0.80	26.34±3.40**	15.56±2.78	131.00±4.15**	52.14±4.86***
	0.40	31.19±1.84***	15.16±1.98	137.00±7.84*	47.26±7.09***
3a-Cu	0.20	22.40±1.86**	13.77±3.39	120.08±10.4	37.69±5.10***
	0.10	13.90±3.40	8.11±1.33*	110.32±9.12	31.70±2.74***
	0.05	13.40±1.67	7.59±3.40*	81.89±7.22**	41.22±15.23**

	0.80	26.51±2.48**	170.20±16.07***	28.73±3.00**	477.2±95.0***
	0.40	30.87±2.48***	144.00±12.34***	32.43±4.10**	489.5±92.3***
3b-Cu	0.20	28.17±2.77**	131.42±17.70**	29.18±2.91**	248.02±32.9**
	0.10	21.97±2.26**	85.0±12.89**	23.02±2.00**	200.6±22.67*
	0.05	21.00±2.58*	65.83±5.41***	18.13±1.87*	131.5±13.9
	0.80	35.37±1.78***	36.83±2.33***	121.17±2.32**	60.00±2.35
	0.40	28.33±2.17***	31.67±4.60**	90.33±3.99**	95.17±7.11
3c-Cu	0.20	27.33±1.23***	23.33±2.60**	93.50±3.98**	93.17±5.51
	0.10	29.33±2.54***	19.00±1.81**	74.0±4.03**	76.50±5.79*
	0.05	14.33±1.38**	15.00±1.37*	59.00±5.99***	67.2±10.9*
	0.80	29.17±3.63***	26.33±1.98***	138.70±3.10***	132.50±4.50***
	0.40	31.17±3.46***	29.33±3.65**	131.80±5.70**	126.00±2.30***
3d-Cu	0.20	27.00±2.44**	28.50±1.13***	124.70±4.10***	110.17±7.82*
	0.10	26.67±3.01*	27.33±1.00**	89.00±4.02*	98.33±9.75*
	0.05	16.67±1.94	18.67±1.93*	83.33±8.92	84.33±8.49***

*: P<0.05; **: P<0.01; ***: P<0.001

NPD: 4-nitro-o-phenylenediamine, 2AF: 2-Aminoflourene, SA: Sodium aside

4. Discussion

In our study, the 3a-Cu, 3b-Cu, 3c-Cu and 3d-Cu test substances caused a significant increase in the number of colonies returning in the presence (+S9) and absence (-S9) of the metabolic activation system in strains TA 98 and TA100.

The gibbs free binding energies of 3a-Cu, 3b-Cu, 3c-Cu and 3d-Cu chemicals, which were selected as ligands in molecular docking analysis and used as test substances in the study, were found to be -5.09, -5.34, -4.46 and -6.73 kcal/mol, respectively. These values are compared with the standard threshold binding free energy (-6.0 kcal/mol) due to the importance of this procedure (27). Accordingly, it was seen that the 3d-Cu ligand was higher than the threshold energy level (Figure 2., 1a).

3a-Cu ligand caused a significant increase in concentrations of 0.40 µg/plt (P<0.001) and 0.05 µg/plt (P<0.05) in –S9 and +S9 media, respectively (strain TA98). This suggests that concentrations higher than 0.10 µg/plt in +S9 environment are toxic. The 3a-Cu ligand caused a significant increase in concentrations of 0.05 µg/plt (P<0.01) and 0.10 µg/plt (P<0.001) in –S9 and +S9 medium (strain TA100), respectively. The gibss free binding energy of the same ligand to the major groove of B-DNA was calculated as -5.09 kcal/mol (Fig. 2., 1a).

The 3b-Cu ligand caused a significant increase in concentrations of 0.40 µg/plt (P<0.001) and 0.05 µg/plt (P<0.001) in –S9

and +S9 media, respectively (strain TA98). The 3b-Cu ligand caused a significant increase in concentrations of 0.10 µg/plt ($P<0.01$) and 0.40 µg/plt ($P<0.001$) in –S9 and +S9 medium, respectively (in strain TA100). The gibss free binding energy of the same ligand to the major groove of B-DNA was calculated as -5.34 kcal/mol (Figure 2., 2a).

The 3c-Cu ligand caused a significant increase in concentrations of 0.10 µg/plt ($P<0.001$) and 0.80 µg/plt ($P<0.001$) in –S9 and +S9 media, respectively (strain TA98). The 3c-Cu ligand caused a significant increase in concentrations of 0.05 µg/plt ($P<0.001$) and 0.05 µg/plt ($P<0.05$) in –S9 and +S9 media, respectively (strain TA100). The gibss free binding energy of the same ligand to the major groove of B-DNA was calculated as -4.46 kcal/mol (Fig. 2., 3a).

The 3d-Cu ligand caused a significant increase in concentrations of 0.40 µg/plt ($P<0.001$) and 0.20 µg/plt ($P<0.001$) in –S9 and +S9 medium, respectively (strain TA98). The 3d-Cu ligand was 0.20 µg/plt ($P<0.001$) and 0.05 µg/plt in –S9 and +S9 medium, respectively (strain TA100). ($P<0.001$) caused a significant increase in concentrations. The gibss free binding energy of the same ligand to the minor groove of B-DNA was calculated as -6.73 kcal/mol (Figure 2., 4a).

The molecular docking results are evaluated together with the reverse mutation test (Ames), it is seen that 3a-Cu, 3b-Cu and 3c-Cu ligands bind to the major groove of B-DNA and dock with a binding energy close to the threshold standard binding free energy, whereas the 3d-Cu ligand bound to the minor groove of B-DNA and was found to be clamped with a gibss free binding energy stronger than the threshold standard binding free energy (27). Our molecular docking results were consistent with the Ames test results.

Literature studies show that imidazole derivatives exhibit various pharmacological activities such as antiviral, anti-inflammatory and analgesic, antidepressant, antifungal and antibacterial, anticancer, antituberculosis and antileishmanial activity (28).

Onur S. et al. (2020) to determine antimicrobial activity, investigated the effects of imine compounds on bacterial (E. coli, S. typhimurium, S. aureus, B. cereus) and fungal (C. albicans) microorganisms. Some Imine compounds have been reported to show high activity against bacteria and fungi (22). In another study investigating insecticide properties, it was determined that a series of newly designed 4-(N, N-diarylmethylamine)furan-2(5H)-one derivatives showed strong toxic effects (34).

Schiff bases (Ni(II) complex) may show significant antimicrobial activity, and they significant activity against cancer cell lines. At the same time, these bases can exhibit strong DNA interactions (29). Metal complexes generally exhibit higher activity against microorganisms than Schiff base. This can be explained on the basis of the chelation effect, which can inhibit the role of metal-dependent proteins disrupting microbial cell homeostasis and blocking microbial nutrition, growth, and development (30). Our experimental results (significant mutagenic effects) showed differences when compared with other studies investigating the mutagenic (Ames Test) effects of Schiff bases (31, 32, 33).

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