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Evaluation of Bis-biphenyl Salicylaldehyde Schiff Base Derivatives for Alpha-Glucosidase Inhibition: Anticancerous Activity and Molecular Modelling Studies

Taher S. Ababneh[1,*], Taghreed M. Jazzazi[1], Mazhar Al Zoubi[2], Tareq. M. Alshboul[3], Walhan Alshaer[4], Abd Al-Aziz A. Abu-Saleh[5], Mansour H. Almatarneh[6], Mohammed-Jamal A. Shammout[7], Iyad Y. Natsheh[8], Majd M. Alsaleh[8]

[1]*Department of Chemistry, Yarmouk University, Irbid 21163, Jordan, email: tababneh@yu.edu.jo;* [2]*Department of Basic Medical Sciences, Faculty of Medicine, Yarmouk University, Irbid 21163, Jordan;* [3]*Department Of Scientific Service Courses, Faculty of Science,Yarmouk University, Irbid 21163, Jordan;* [4]*Cell Therapy Center, The University of Jordan, Amman 11942, Jordan;* [5]*Department of Chemistry and Biochemistry, University of Windsor, ON, N9B 3P4, Canada;* [6]*Department of Chemistry, The University of Jordan, Amman 11942, Jordan;* [7]*Chemistry Department, Faculty of Science, Applied Science Private University, P.O. Box 166, Amman 11931, Jordan;* [8]*Department of Medical Applied Sciences, Zarqa University College, Al-Balqa Applied University, Salt, Jordan*

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Abstract

In this study, we have successfully synthesized four symmetrical Schiff base ligands (L1, L2, L3 and L4), through the condensation reaction of 2,2'-diamino-4,4'-dimethyl-1,1'-biphenyl with 2-hydroxy-3-methoxy-5-nitrobenzaldehyde, 3-bromo-5-chloro-2-hydroxybenzaldehyde, 2-hydroxy-5-methoxy-3-nitrobenzaldehyde and 3-bromo-2-hydroxy-5-nitrobenzaldehyde, respectively. Characterization of the ligands was accomplished using elemental analysis, and infrared spectroscopy. To elucidate their potential biological activity, the binding affinities of the synthesized compounds towards alpha-glucosidase inhibition were assessed employing induced-fit molecular docking calculations. The docking analysis revealed diverse interactions, such as salt-bridge, hydrogen bonding, π-stacking, and hydrophobic interactions, facilitating a proper fit of these compounds into the binding pocket of α-glucosidase. Additionally, the prepared ligands' anti-cancer potential was evaluated against various cell lines. In the initial investigation, the IC_{50} of the synthesized compounds against three cancer cell lines (MDA-MB-231, MCF-7, and A549) and a normal fibroblast cell line (HDF) as a control was determined using the MTT assay. The results demonstrated a decreasing selective cytotoxic effect of compounds L1, L3, and L2 against the MDA-MB-231 breast cancer cell line. Additionally, compound L1 exhibited the lowest IC_{50} value against the A549 lung cancer cell line. Subsequently, the cytotoxic impact of the bis-biphenyl salicylaldehyde Schiff base derivatives were further explored on the MDA-MB-231 breast cancer cell line using the MTT assay. The results revealed that the derivatives demonstrated noteworthy cytotoxic activity against the cancer cell line, with compound (L3) exhibiting the highest toxic effect at a concentration of 25 µg/ml, resulting in a reduction of cell viability by 34.23% compared to the control group. These findings highlight the promising anti-cancer properties of the synthesized ligands and warrant further exploration of their potential applications.

Keywords: Schiff bases, molecular docking, α-glucosidase, anti-cancer

1. Introduction

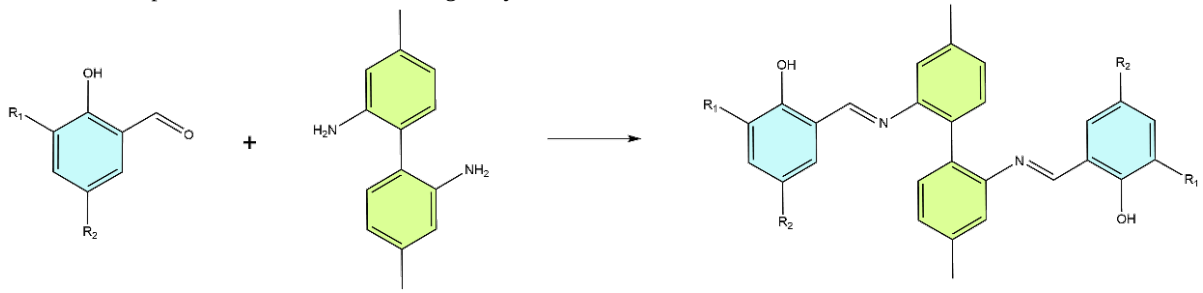
Diamine Schiff bases are a type of organic compound consisting of a Schiff base linkage (-N=CH-) formed from the reaction of a diamine and an aldehyde or ketone. The diamine portion of the molecule consists of two amino groups ($-NH_2$) attached to a biphenyl or naphthalene core, while the aldehyde or ketone component is usually an aromatic or heteroaromatic derivative such as salicylaldehyde or pyridine-2-carboxaldehyde (Dalia *et al*., 2018). Schiff bases have received significant attention in recent years due to their wide array of pharmacological properties including but not confined to antimicrobial (Chambhare *et al*., 2003), antifungal (Chohan *et al*., 2010), anticancer (Gupta*et al*., 2023), and anti-inflammatory (Przybylski *et al*., 2009) activities.

Recently, we have reported the synthesis, catalytic applications and antiproliferative effects of Schiff base derivatives of 2,2'-diamino-1,1'-biphenyl-salicylaldehyde (Ababneh *et al*., 2021; Jazzazi, *et al*., 2019; Al-Shboul *et al*., 2018). Herein, we present the preparation and characterization of four novel symmetrical Schiff base ligands (L1, L2, L3 and L4) via the condensation reaction of 2,2'-diamino-4,4'-dimethyl-1,1'-biphenyl with salicylaldehyde derivatives, as demonstrated in **Scheme 1**.

The analysis of bis-biphenyl salicylaldehyde Schiff base derivatives as possible inhibitors of alpha-glucosidase by the incorporation of molecular dynamics (MD) simulations, represents a significant step in the search for

* Corresponding author. e-mail: tababneh@yu.edu.jo.

effective anti-diabetic medications. These Schiff base compounds have demonstrated significant promise for inhibiting alpha-glucosidase, a key enzyme in the breakdown of carbohydrates (Daoud, *et al*., 2021). In this work, we have performed a molecular docking analysis on the human α-glucosidase enzyme to gain insights into the interactions between the ligands and the enzyme and to attain an understanding of the stability and conformational dynamics exhibited by ligand-enzyme complexes.



Scheme 1. Synthesis of L1 (R_1=OCH_3, R_2=NO_2), L2 (R_1=Br, R_2=Cl), L3 (R_1= NO_2, R_2= OCH_3) and L4 (R_1=Br, R_2=NO_2) Schiff base ligands via the reaction of 2,2'-diamino-4,4'-dimethyl-1,1'-biphenyl with 2-hydroxy-3-methoxy-5-nitrobenzaldehyde,3-bromo-5-chloro-2-hydroxybenzaldehyde, 2-hydroxy-5-methoxy-3-nitrobenzaldehyde and 3-bromo-2-hydroxy-5-nitrobenzaldehyde in a 1:2 molar ratio, respectively.

Building upon our analysis of the interaction dynamics between Schiff base compounds and alpha-glucosidase through molecular docking, our investigation extends to evaluate the cytotoxic impact of the newly synthesized bis-biphenyl-salicylaldehyde Schiff base derivatives (L1, L2, L3, and L4) against three cancer cell lines (MDA-MB-231, MCF-7, and A549) along with a normal fibroblast cell line (HDF) serving as a control. Triple-negative breast cancer (TNBC) has a greater morbidity than other breast cancer (BC) types because of its high molecular heterogeneity, propensity for metastatic spread, and poor prognosis. Because TNBC lacks the ER, PR, and HER2 receptors for estrogen, progesterone, and human epidermal growth factor, there are currently no viable treatments for TNBC (Chang-Qing*et al*., 2020). TNBC is not susceptible to endocrine treatment or molecular targeted therapy because of its unique molecular profile. As a result, chemotherapy is the primary systemic treatment, although postoperative adjuvant chemoradiotherapy is ineffective. Tumor recurrence will eventually result from the remaining metastatic lesions. In certain nations, bevacizumab has been used in conjunction with chemotherapeutic medications to treat TNBC; however, this has not appreciably increased patient survival time (Yin *et al*., 2020; Shawish *et al*., 2023). Therefore, the creation of novel treatment plans and objectives is essential.

2. Materials and Methods

2.1. Synthesis and Characterization of Schiff Bases

The elemental analyses (C, H and N) were carried out on a Perkin Elmer 240 elemental analyzer. ^{1}H-NMR spectra were recorded on a Bruker AC 400 spectrometer in $CDCl_3$. Infrared spectra were recorded using KBr on Bruker FT-IR-4100 spectrometer over the range 4000–400 cm^{-1}. All commercially available substrates were used without further purification. The target amine reactant, 2,2'-diamino-4,4'-dimethyl-1,1'-biphenyl, was synthesized as a yellow solid with a yield of 89% following a procedure described in a previously published work (Al-Shboul *et al*., 2018).

The synthetic protocol for Schiff bases (L1, L2, L3 and L4) involved the combination of 0.471 mmol of 2,2'-diamino-4,4'-dimethyl-1,1'-biphenyl with 0.942 mmol of a salicylaldehyde derivative in 20 ml of absolute methanol. The resulting mixture was refluxed overnight, during which time the corresponding Schiff base precipitated as a colored solid. The solid was subsequently collected through filtration, washed with cold methanol, and dried to yield the desired product, as depicted in **Scheme 1**.

Infrared spectra of the prepared ligands exhibit a characteristic peak at (1620-1637) cm^{-1} attributed to the imine (C=N) moiety, where the broad bands observed at (3048-3070) cm^{-1} are due to the presence of OH groups.

The results of the elemental analysis, which align with the calculated values and physical properties of the obtained ligands (L1, L2, L3 and L4) are reported in **Table 1**.

Table 1. Elemental analysis results and other physical properties of the isolated ligands

Ligand	Formula	F.Wt (g/mol)	Color	Yield (%)	Elemental Analyses Calculated (Found) (%) C H N
L1	$C_{30}H_{26}N_4O_8$	570.55	Red	49.2	63.15, 4.59, 9.82 (63.08), (4.45), (9.81)
L2	$C_{28}H_{20}Br_2Cl_2N_2O_2$	647.18	yellow	54.32	51.96, 3.12, 4.33 (51.89), (3.10), (4.31)
L3	$C_{30}H_{26}N_4O_8$	570.55	Red	60.58	63.15, 4.59, 9.82 (63.12), (4.52), (9.79)
L4	$C_{28}H_{20}Br_2N_4O_6$	668.29	orange	53.06	50.32, 3.02, 8.38 (50.29), (2.99), (8.35)

The spectral data obtained from the ^{1}H NMR analysis of the isolated Schiff bases have been selectively presented in **Table 2**.

Table 2. Selected ^{1}H-NMR parameters of the Schiff bases

	L1	L2	L3	L4
^{1}H NMR				
δ(OH)	14.08	13.29	13.52	15.06
δ(N=CH)	8.38	8.15	8.36	8.46
δ(CH_3)	2.41	2.39	2.39	2.43
δ(OCH_3)	3.88	-	3.71	-

2.2. Alpha-glucosidase docking study methodology

The initial coordinates of the human lysosomal acid α-glucosidase protein were retrieved from the RCSB [PDB entry 5NN8] determined at 2.45 Å resolution (Roig-Zamboni*et al*., 2017). We prepared the protein using the Protein Preparation Wizard (Sastry *et al*., 2013) of Schrodinger software to resolve clashes and missing atoms. This procedure addresses steric hindrance and unfavorable interactions among atoms, enhancing the accuracy of the protein structure by optimizing side-chain conformations and resolving clashes resulting from overlapping atoms. Protonation states of titratable residues were assigned using PROPKA (Bas *et al*., 2008). To conduct the molecular docking, we removed the co-crystalized ligand (acarbose) and waters from the binding pocket. The grid cell is located at the active site of the protein and centroid to the co-crystallized ligand. LigPrep tool of Schrodinger software (Schrödinger, LLC, New York, NY, 2021) was used to generate and minimize conformations (i.e., ring conformations and stereoisomers) of the compounds. Epik software (Shelley *et al*., 2007) was used for proper treatment of compound protonation states at a pH of 7.4 (±1). Finally, the prepared compounds were docked into the binding pocket of the preprocessed protein pocket by utilizing the induced fit docking (IFD) protocol (Sherman *et al*., 2006). IFD accounts for both ligand and protein flexibility, which results in an optimal protein-ligand binding mode. The glide extra precision (XP) docking score was chosen as the measure of the binding affinity of the compounds with the best-docked poses. To validate our docking experiment, we compared the docked poses generated by the induced-fit docking with the crystallographic pose of acarbose complexed within the α-glucosidase enzyme. The RMSD value of 1.48 Å (**Figure 1**) indicated that the docking method successfully reproduced the crystallographic pose of acarbose compound.

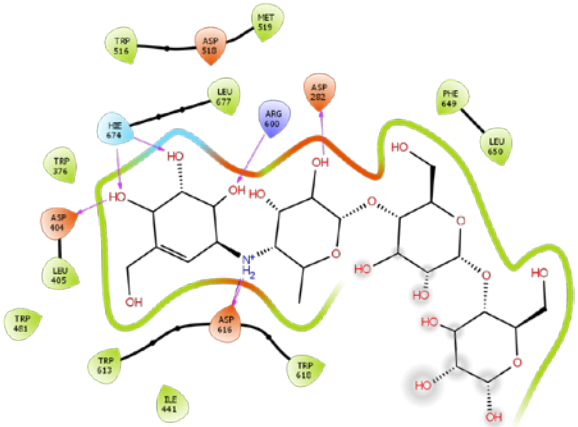


Figure 1. The 2D interaction map of acarbose with the binding site residues of the α-glucosidase enzyme. Only protein residues that are within 3 Å of the bound compound are shown. The docking method successfully reproduced the crystallographic pose of acarbose compound with an RMSD value of 1.48 Å.

2.3. Preliminary research

2.3.1. Cell culture

The MCF7, A549, and MDA-231 were obtained from (ATCC), and the human dermal fibroblast cell line (HDF) was isolated in the (Cell Therapy Center/University of Jordan/Amman-Jordan). MCF7 and A549 were cultured in RPMI 1640 Medium, while MDA-MB-231 and HDF were cultured in EMEM (Eagle's Minimum Essential Medium) and DMEM (Dulbecco's Modified Eagle Medium), respectively. A 5% penicillin-streptomycin (100 IU/mL–100 g/mL) and a 10% fetal bovine serum were added to all media, along with 1% L-glutamine for RPMI and EMEM and 2% L-glutamine for DMEM. We incubated all cell lines at 37 °C with 5% CO_2. Cells were subcultured every 3-5 days with 0.05% trypsin-EDTA, depending on their confluency.

2.3.2. Cell viability (MTT)

For calculating the IC_{50} of our compounds, MCF7, A549, MDA-231, and HDF were cultured in 96 well plates with 9000 cells/well, except for A549, we seeded 6500 cells/well, for 24 hours to allow attachment of the cells. Then, cells were treated with different concentrations of L1, L2, L3 and L4 (0 to 500 µM) dissolved in DMSO for 72 hours. 15 µl of MTT salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added per well with 100 µl of new media, for 3 hours. To stop the reaction and dissolve the formazan, 50 µl of DMSO was added to each well. The Glomax microplate reader (Promega, Madison, WI, USA) was used to measure absorbance at 560 nm.

2.4. 2.4 Main research process optimization

For experiment optimization, normal fibroblast cell lines were used to evaluate the safety profile, while triple negative breast cancer cell line (MDA-MB-231) was used for anticancer activity. In this study, different concentrations of the chemical compounds (50, 25, 12.5 µg/ml) were used for safety profile evaluation. However, only the concentration of 25 µg/ml was chosen to evaluate cytotoxic effect against cancer cell lines due to its high safety compared to 50 µg/ml concentration and its high anticancer activity compared to 12.5 µg/ml concentration.

2.4.1. MTT assay

An MTT colorimetric test (3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay) was used to assess cell viability in both normal and cancer cell lines following the described procedure (Juengel *et al*., 2019). In brief, cells (50 µL, 1X 10^5 cells/mL) were planted onto 96-well plates. After 24, 48, and 72 hours, MTT reagent was added for 4 hours. The plates were incubated overnight at 37 °C and 5% CO_2. Each well's absorbance at 550 nm was measured with a microplate enzyme-linked immunosorbent assay (ELISA) reader. The data were presented as mean cell numbers after eliminating background absorbance.

3. Results and Discussion

3.1. Alpha-glucosidase docking study

The outcomes of the induced fit docking computations are presented below. While the co-crystallized ligand produced a docking score of -14.9, the proposed compounds exhibited moderate docking scores of -5.7, -5.8, -6.2, and -7.6 for L1, L2, L3, and L4, respectively. This difference in the docking score is due to the fact that the anchoring mechanism of acrabose compound relies heavily on interactions involving hydrogen bonds. In contrast, the synthesized compounds consist of aromatic rings and hydrophobic substituents, which are primarily involved in hydrophobic interactions (**Figure 2**). These compounds tend to have a lower dehydration enthalpic cost (desolvation effects) than hydrophilic molecules such as acarbose (Bodnarchuk, 2016). Therefore, although these compounds have lower docking scores compared to acarbose, their total binding affinities could potentially be increased due to their low dehydration enthalpic costs and low conformational entropies, since they have a small number of rotatable bonds (Klebe, 2015). Furthermore. the synthesized compounds may have their binding affinities increased by carefully adding certain functional groups, especially OH- groups, which are known to improve hydrogen bond interactions. This strategy offers encouraging directions for future study in this area.

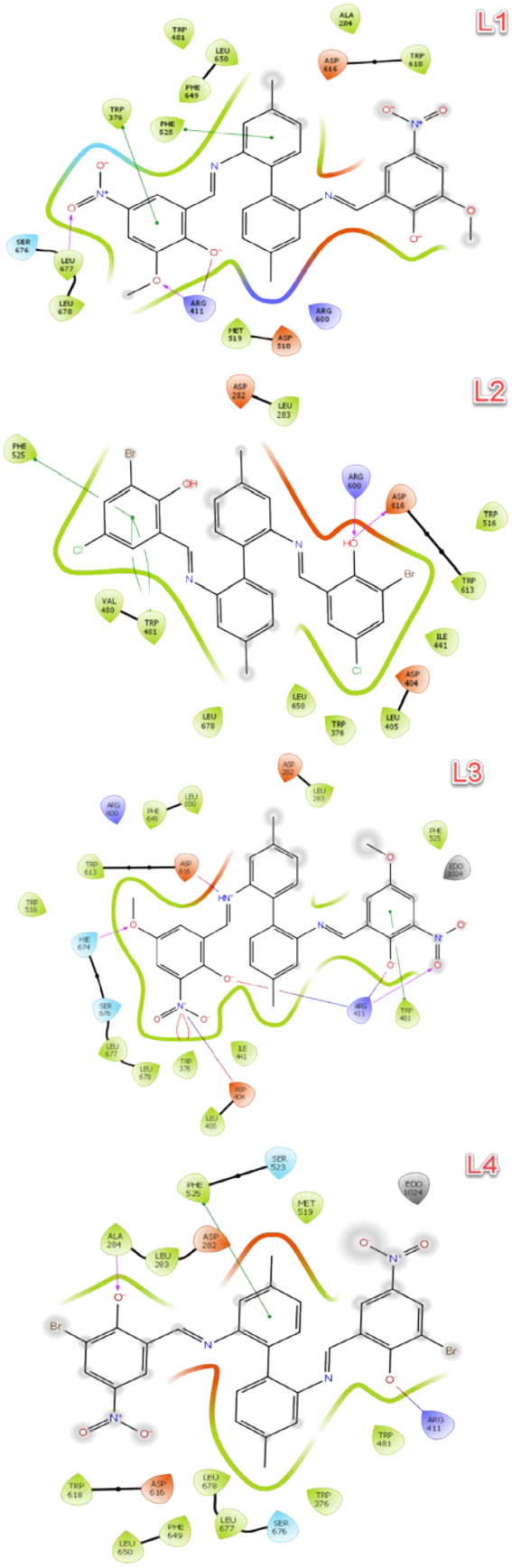


Figure 2. The 2D interaction map of the compounds (L1, L2, L3 and L4, respectively) with the binding site residues of the α-glucosidase enzyme. Only protein residues that are within 3 Å of the bound compounds are shown.

3.2. Preliminary research

The tested compounds showed cytotoxic activity against MDA-MB-231, MVF-7, and A549 cancer cell lines with variable IC_{50}. Compounds L4 and L1 showed the lowest IC_{50} against the MDA-MB-231 breast cancer cell line, 20.7 µM, and 37.9 µM, respectively. In addition, L1 showed the lowest IC_{50} against the A549 lung cancer cell line (36.4 µM). Generally, all tested compounds showed a selective cytotoxic effect against the MDA-MB-231 cancer cell line compared to the other cell lines; however, L4 showed low IC_{50} against all cell line types including the normal fibroblasts (**Table 3**). In summary, the results suggest that the tested compounds (L1, L2, and L3) are selective against MDA-MB-231 compared to other cancer cell lines and normal fibroblasts. However, the L4 compound showed the lowest IC_{50} against all tested cell lines, especially the MDA-MB-231 breast cancer cell line (**Figure 3**).

Table 3. Cytotoxic activity results against MDA-MB-231, MVF-7, and A549 cancer cell lines with variable IC_{50}

Compound	IC_{50} ±SD (µM)			
	MDA-MB-231	MCF-7	A549	Fibroblast
L1	37.9±1.8	123.1±8.4	36.4±2.8	65.4±12.1
L2	66.3±4.9	146.8±22.7	135.5±7.8	102.3±10.5
L3	54.5±4.8	64.7±13.9	91.6±4.9	64.9±8.8
L4	20.7±6.3	34.4±5.0	41.6±4.5	34.8±7.8

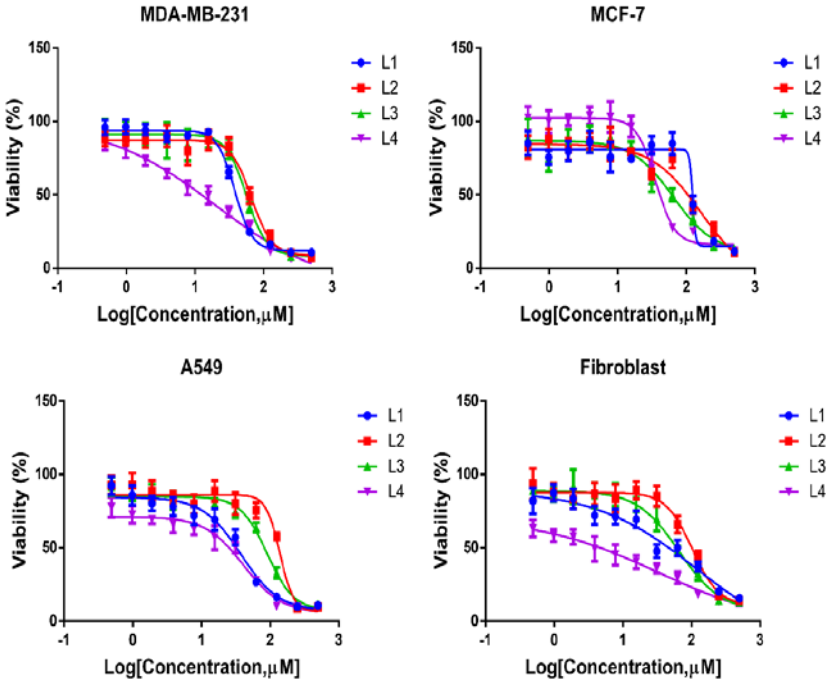


Figure 3. Graphical views of the Cytotoxic activity results against MDA-MB-231, MVF-7, and A549 cancer cell lines

3.3. Main research process optimization

3.3.1. Safety Profile

The Results revealed that (25 µg/ml) concentration of bis-biphenyl salicylaldehyde Schiff base derivatives against the normal cell lines showed (83.06, 80.4, 71.22, 72.85 % cells survival) for (L1, L2, L3, L4), respectively, as shown in **Figure 4**. These findings suggest that these compounds are considered relatively safe, given their cell survival rates exceeding 50% against normal cells. Moreover, the stability of these compounds was evaluated by measuring the survival rate unit within 72 hr and the results revealed that the survival rate remained constant with 72.04 % survival rate compared to their control.

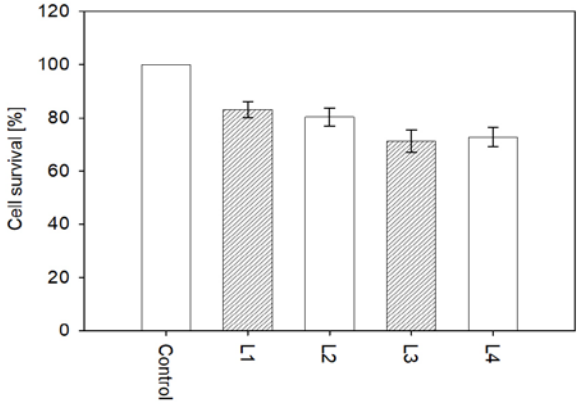


Figure 4. Survival rate of fibroblast normal cells at 25 µg/ml of bis-biphenyl salicylaldehyde Schiff base derivatives

3.3.2. Cytotoxic activity

Bis-biphenyl salicylaldehyde Schiff base derivatives showed selective cancer cell line cytotoxicity against (MDA-MB-231) breast cancer cell line, with inhibition rates of (25.97, 24.43, 34.23, 33.08 %) for (L1, L2, L3, L4), respectively compared to their controls at 25 µg/ml concentration as shown in **Figure 5**. Even though we choose this concentration as the ideal concentration for cancer inhibition after weighing the safety and activity of each concentration, our results revealed the stable effect of these derivative upon time intervals and their dose dependent relation as shown in **Figures 5** and **6**.

Our results revealed that the effect of these derivatives is stable with time, which means that the cells showed mild growth after the application of these chemicals particularly in L1 and L4 and showed no growth within 48 hr and mild elevation in growth in sample L2 after 72 hr; however, the most inhibition stability was revealed by L3 sample as illustrate in **Figure 5**. As detailed in a research report, certain 1,3,4-thiadiazole derivatives bearing Schiff base moiety demonstrated antitumor activity against the MCF-7 breast cancer cell line, showcasing IC_{50} values of 4.56 mM and 4.25 mM. which were associated with the presence of functional groups R=4-OCH_3 and R=4-NO_2 (Zhang *et al*., 2014). In another study, the highest inhibitory activity against cancer cells was detected for a Schiff base derived from 1,3,4-thiadiazole compound bearing three O-CH_3 groups (Gür *et al*., 2020). These outcomes can be ascribed to the presence of distinct functional groups, specifically for L3 (R_1= NO_2, R_2= OCH_3) underscoring the significance of the structure-activity relationship.

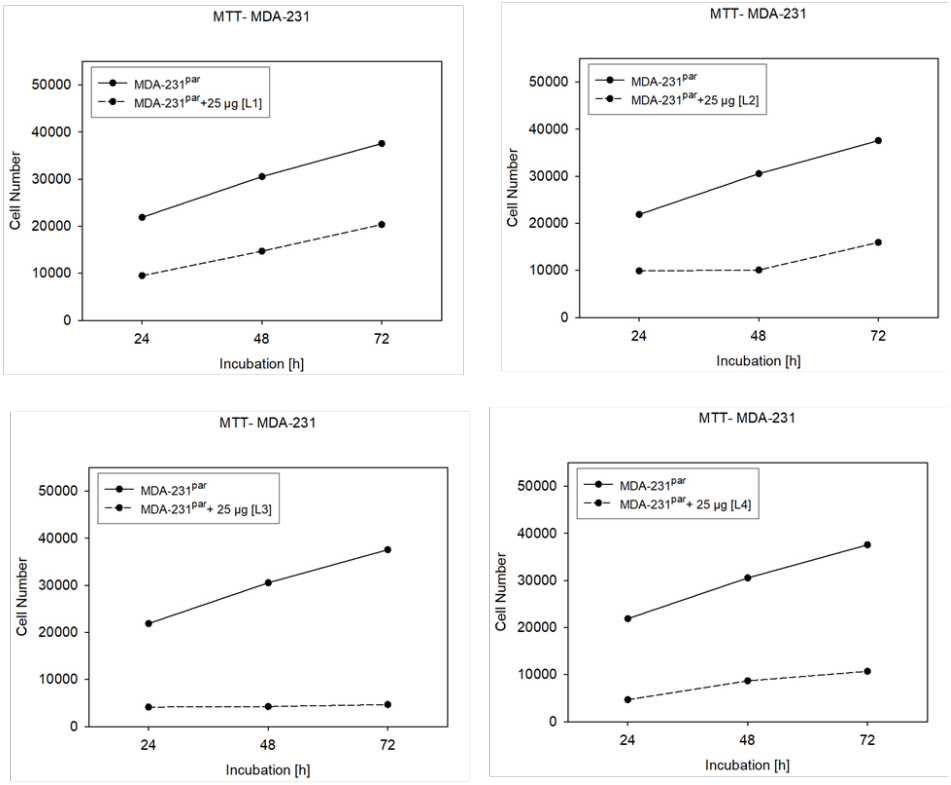


Figure 5. Cytotoxic activity of bis-biphenyl salicylaldehyde Schiff base derivatives against (MDA-MB-231) breast cancer cell line.

Our results demonstrated that there is a direct relationship between dose and activity, as **Figure 6** illustrates; however, when applying these chemicals *in vivo* or in proceeding clinical trials, the safety for each dose should be weighted with its activity before its usage. Hence, these findings provide groundwork for enhancing both the effectiveness and the specificity of this series of compounds. In summary, the tested bis-biphenyl salicylaldehyde Schiff base derivatives displayed significant cytotoxicity against the MDA-MB-231 breast cancer cell line, with ligand L3 exhibiting the highest toxicity at 25 µg/ml, reducing cell viability by 34.23% compared to the control group.

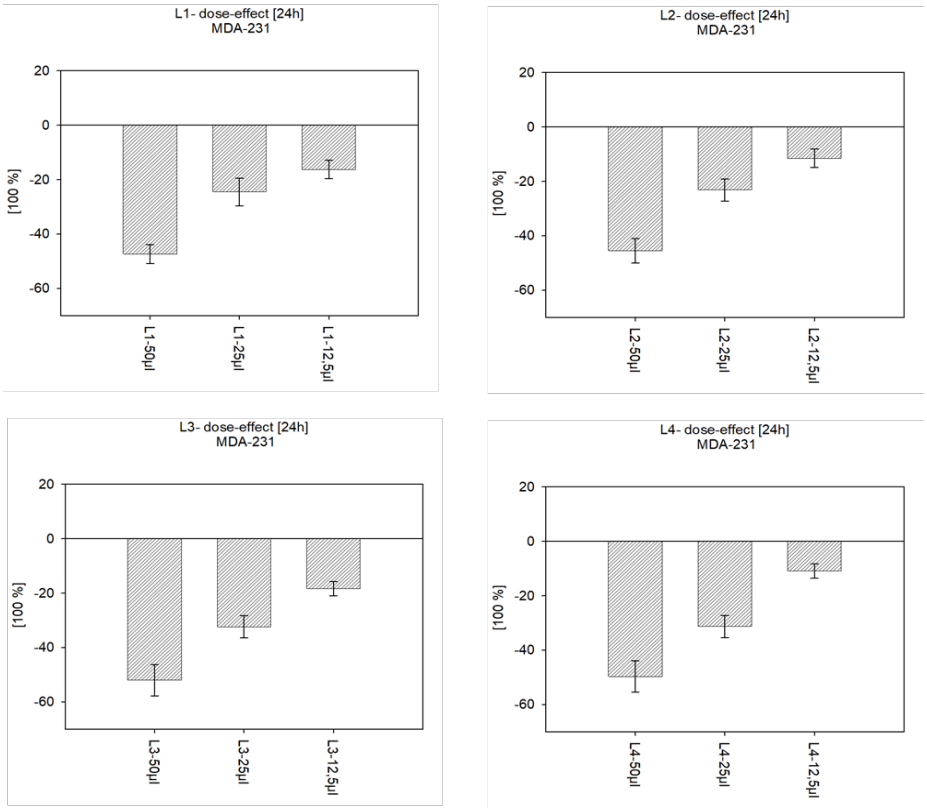


Figure 6. The relationship between dose and cytotoxicity of bis-biphenyl salicylaldehyde Schiff base derivatives against (MDA-MB-231) breast cancer cell line.

4. Conclusion

Four new symmetrical Schiff base ligands have been prepared and characterized by different spectroscopic techniques and elemental analysis. The induced-fit docking computations showed that the proposed compounds had moderate docking scores compared to the co-crystallized ligand acarbose, while the docking scores suggest that the proposed compounds may not be as effective as acarbose in binding to the target site; their unique physiochemical properties (*i.e.*, hydrophobicity and rigidity) could still make them viable candidates for further study and optimization. Compounds L4, L1, and L3 showed selective anticancer activity against the MDA-MB-231 breast cancer cell line and L1 showed a potential selectivity against the A549 lung cancer cell line, while L4 showed a pan-cytotoxic effect against all types of cells. The viability percentages of the normal fibroblast cell-line were calculated at (83.06, 80.4, 71.22, 72.85 %) for (L1, L2, L3, L4), respectively, at the concentration 25.0 µg/ml. Results revealed that normal fiber cell lines are not affected by the derivatives, indicating their potential selectivity towards cancer cells. These findings suggest that the derivatives have promising anti-cancer properties and warrant further investigation to determine the mechanism of action and in vivo efficacy of these compounds.

Acknowledgments

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