Malaysian Journal of Chemistry, 2026, Vol. 28(1), 296-312
(Special Issue: *12th International Conference of the College of Science & 3rd Conference of Chemistry Department 2025*)

Synthesis, Molecular Docking, ADMET, and Biological Evaluation of New Azodye-Curcumin Derivatives as Antibacterial Agents

Jawad Kadhim Abaies, Athra G. Sager* and Zeena R. Katoof
Department of Chemistry, College of Science, University of Wasit, Wasit, Iraq
*Corresponding author (e-mail: asaker@uowasit.edu.iq)

Design and synthesis of medicinal compounds from plant extract and investigating their biological characteristics using experimental and computational methods is the aim of this work. A plant extract, curcumin, was used to synthesize four azodye-curcumin compounds (C1-C4). The prepared compounds were identified by FT-IR and 1H NMR spectrometric methods. The biological activity of the created compounds against four kinds of bacteria, including Salmonella, Escherichia coli, Klebsiella pneumonia, and Staphylococcus aureus. Was tested. The findings revealed that all the synthesized compounds (C1-C4) exhibited a high activity against Klebsiella pneumoniae and E. coli, while they exhibited a weak antibacterial activity against Staphylococcus aureus. Furthermore, a molecular docking study of the synthesized compounds against two enzymes, 4H2M and then IKZN, was performed using the MOE.2015 program. The attained data disclosed that all compounds exhibit activity against 4H2M and IKZN enzymes and play a notable role in regulating DNA gyrase and D-Alanyl-D-Alanine Carboxypeptidase enzymes. The produced compounds also showed good results with the 4H2M enzyme, especially compound C1, which exhibited the lowest binding affinity (-9.9121 kcal/mol). The compound C4 showed the lowest binding affinity (-8.26745 kcal/mol) towards the 1KZN enzyme. Moreover, ADME studies revealed that not all the created compounds adhere to Lipinski's rule. Toxicity analysis data disclosed that the synthesized compounds have shown organ toxicity (Hepatotoxicity) and immunotoxicity upon consumption, except compound C2, which did not exhibit any toxicity after being consumed. Hence, compound C2 is a candidate medicine as an antibacterial agent.

Keywords: Antibacterial, curcumin, docking, 4H2M, 1KZN

Received: September 2025; Accepted: October 2025

Bacteria are the source of most of the infections that cause deadly diseases. They are also considered microbial pathogens. Bacterial pathogens have developed multiple strategies to evade the effects of bactericidal antiseptics and antibiotics. Hence, preserving human and animal health requires ready, effective antibiotics [1]. Owing to pathogens developing resistance to antibiotics and anti-cancer drugs, the effectiveness of these medications is reduced. To tackle this challenge, different approaches have been experimented with in recent years. Binding certain compounds with inefficient drugs to produce new medicinal compounds is a promising way, as it reestablishes the desired activity of anti-tumor and anti-microbial drugs. Natural products, in particular, have been extensively exploited as a drug to treat a variety of diseases that severely affect the health of human beings. These naturally occurring materials have a wide range of biological and therapeutic features, including their activity against hypertension, tumors, inflammation, and infectious diseases. The term "natural products" refers to any materials obtained from living organism sources [2, 3]. One of the most plants that widely studied plants is Turmeric, which is the main source of curcumin. Due to the distinctive features and various therapeutic applications of this plant, it has been extensively used as a drug by the Chinese and Indians (Ayurveda) for treating diverse health problems [4]. Curcumin is a derivative of the rhizome of Curcuma longa that belongs to the ginger family called Zingiberaceae, which also comprises curcuminoids, desmethoxy-curcumin, and bis-desmethoxy curcumin. Curcumin is easily distinguished by its bright yellow color. It is poorly soluble in water, but it has a high solubility in conventional organic solvents such as acetone, methanol, ethanol, and dimethyl sulfoxide [5, 6]. Curcumin is a symmetric compound and commonly called diferuloyl methane, but "(1E, 6E) 1, 7-bis (4-hydroxy-3-methoxy phenyl)-1, 6-heptadiene-3, 5-dione" is the IUPAC name. Since curcumin has been an essential ingredient for people in several societies for a long time, its safety profile is still postulated. In addition, it has been utilized by different societies as medicine for curing various diseases, including rheumatoid diseases, Alzheimer's, and cancer [7]. It was stated that consuming curcumin supplements contributes to curing many health issues due to its

†Paper presented at the 12th International Conference of the College of Science & 3rd Conference of Chemistry Dept. 2025

significant anti-inflammatory and antioxidant properties [8]. However, the use of the experimental applications of curcumin in drug synthesis is still limited because of its slight bioavailability [9]. This is attributed to the poor solubility in water, fast rate of metabolism, and slight oral absorbability. In order to overcome these problems, various methods have been employed, including an adjunct (which interferes with the glucuronidation), arrangement adaptation, and curcumin nano-encapsulation [10]. Azodyes are a very important sort of chromophores and have many applications in several fields such as scientific research, industry, and pharmaceutical chemistry. In addition to the synthesis of the azodyes, scientists have made significant efforts to discover simple procedures for preparing their derivatives with significant properties and various potential applications. As a class of colorant organic compounds, 1, 2-azo chromophores are identified by the presence of azo moieties within their core structure. Also, more than one azo group could be present in the compound structure, e.g., 6-hydroxy-1,4-dimethyl-2-oxo-5-((E)-(4-((E)-phenyldiazenyl)phenyl)diazenyl)-1,2-dihydropyridine-3- carbonitrile with two azo groups (dis-azo), three azo groups (tris-azo), four azo groups (tetrakis-azo), or even more than four groups (poly-azo). Azodyes have been utilized in numerous applications, including inkjet printing, photography, dye impart, bioscience (and related fields), light-controlled macromolecules, liquid crystals production, and colorants in more than half of marketable dyes [11-13]. The presence of (–N=N–) in their structure is a common characteristic of azodyes. This arrangement has several properties in textile manufacturing. In this regard, heterocycles comprising N, O, or S atoms are necessary to be present in azodyes in order to improve the dye color [14]. Although the pharmaceutical and drug industries have been developed as a result of the extensive contribution of heterocycle-containing azodye derivatives, the outcomes are still not entirely satisfactory. Nowadays, because of their magnificent bioactivities, including antimicrobial [15], antifungal, anti-convulsant [16], antidiabetic, anti-tuberculosis [17], DNA-binding antitumor [18], and chemosensing activities, azodyes incorporating heterocyclic and their derivatives have drawn a lot of attention [19, 20].

Sketching and creating new compounds with possible therapeutic characteristics based on plant-derived materials and testing their antibacterial activity via experimental and computational approaches is the main target of the study. Insilico studies, which comprise molecular docking via (MOE 2015) software, were employed to evaluate the pharmacological features of the hybrid compounds (curcumin derivatives). Molecular docking study aids in understanding the activity against bacteria through the binding between the prepared curcumin derivatives and the required moieties (targets). For the purpose of examining the toxicity and drug-relevant characteristics of compounds, the optimized compounds were exposed to absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis, aiming to determine the drug-likeness, which is governed by the properties of the designed compounds.

EXPERIMENTAL

Chemicals and Materials

All chemicals and solvents were purchased from Fluka and Sigma-Aldrich Companies. Folic acid was provided by Samara Company for the drug industries and medical appliances (SDI). All chemicals were directly utilized without any extra purification.

Characterization Methods

^{1}HNMR spectra were carried out in DMSO-d6 using a Bruker DRX system AL 400 MHz spectrometer, with Tetramethylsilane (TMS) as an internal reference at the University of Basra. The infrared spectra (FT-IR) were recorded utilizing a Shimadzu FT-IR 8400. All theoretical studies were conducted using a computer Intel Core i7/HP, RAM (64).

Curcumin Extraction Procedure

The Soxhlet apparatus was employed to extract curcumin from the roots of the curcumin plant according to the previously described method [21-22]. Briefly, 50 g of curcumin plant roots was mixed with 250 mL of ethanol as an extraction solvent. The extraction process was carried out using a Soxhlet extractor for 4 hours. The mixture was then subjected to a vacuum to eliminate the solvent. The obtained product was kept in a cool place at 40 ^{o}C. Yield was 5 g.

Procedure for Synthesis of Azo-curcumin (C1-C4)

Folic acid or (4-aminoantipyrine, 2, 6-diamino pyridine, and o-toluidine) (2 mmol) was dissolved in 3 mL of hydrochloric acid (12M) [23]. The solution was cooled in an ice bath at (0–5) ^{o}C. Then, a fresh solution of $NaNO_2$ (0.2 g, 2 mmol) in 4 mL of distilled water was gradually added at (0–5) ^{o}C. The mixture was left stirring for 10 min to produce the diazonium salt. The diazonium salts were separately added to fresh solutions of curcumin (0.5 g, 2 mmol) in 10 mL of 10% sodium hydroxide at 0-5 °C with stirring, then the reaction mixtures were left stirring for 10 minutes. After the completion of the reactions, the produced mixture was poured into crushed ice. The yielded precipitates were washed with cold distilled water. Then, they were left to dry using an oven at 50 ^{o}C. The products were purified via recrystallization using ethyl alcohol as a solvent.

Anti-Bacterial Activity.

The agar diffusion test was used in a diagnostic laboratory and utilized for determining the vulnerability of four bacterial strains, including *Salmonella, E. coli,*

Klebsiella pneumonia, and Staphylococcus aureus, which were obtained from a patient's infection. The prepared azodye-curcumin was dissolved in DMSO to prepare a solution with a concentration of (100µg/mL). All bacterial strains were grown and incubated for one day at 37° C. The formed solutions activities of the created compounds were compared with the activity of the standard drug, amoxicillin. When the region surrounding the disk appears visible, this indicates that the growth of bacteria is very weak or stopped due to the presence of the compounds (C1-C4). Inhibitory zone (ZI, mm) was measured to demonstrate the compound activity towards the examined bacterial strains after 24 hours [24].

Molecular Docking Study

The inhibition ability of the produced compounds C1-C4 and curcumin was identified hypothetically via molecular docking utilizing MOE-2015 software. Enzyme DNA gyrase (PDB ID: 1KZN) and Undecaprenyl pyrophosphate synthase (PDB ID: 4H2M) were saved as pdb. Crystal proteins were downloaded from the web. (Protein Data Bank). The resolution was greater than 2.0Å and comprised the gene code of the same bacteria. A molecule of H_2O was introduced into the active sites to guarantee the production of H- H-bonds between the ligands and the target. This is attributed to the critical role of the H_2O molecule located in the active site of the targeted enzyme. The structures of compounds C1-C4 and curcumin were drawn utilizing the ChemDraw program (Professional 15.0). The energy minimization of compounds was conducted via Chem3D Ultra 15.0 with MMFF94 force, and then saved as a (Mol) file. Files of pdb proteins were imported into (MOE-2015 software to prepare for the docking. The four molecules (C1-C4) were sequentially docked on-site1 of (4H2M and 1KZN). The perfect RMSD value should be close to 2Å and the energy score value = -7 Kcal/mol or less [25-27]. These values were considered standard.

Theoretical Studies of Absorption, Distribution, Metabolism, Excretion, and Toxicity

In the drug innovation field, numerous drugs are unapproved by clinical trials and ensuing improvement practices due to the undesired features. In the present study, the optimized molecules were examined using the online web tool Swiss ADME. Whereas, the online server ProTox-II was utilized to assess their toxicity. The ADME study findings disclosed that molecules (C1-C4) possess only hepatotoxicity and immunotoxicity.

RESULTS AND DISCUSSION

Synthesis and Characterization

In this study, new azodyes based on curcumin C1-C4 (as curcumin derivatives) were synthesized via the electrophilic aromatic substitution reaction of diazonium salts with sodium-curcumate in Scheme 1.

(4-((2-(-1,7-bis(4-hydroxy-3-methoxyphenyl)-3,5-dioxohepta-1,6-dien-4-yl)diazenyl)-4-oxo-3,4-dihydropteridin-6-yl)methyl)amino)benzoyl)-L-glutamic acid (C1)

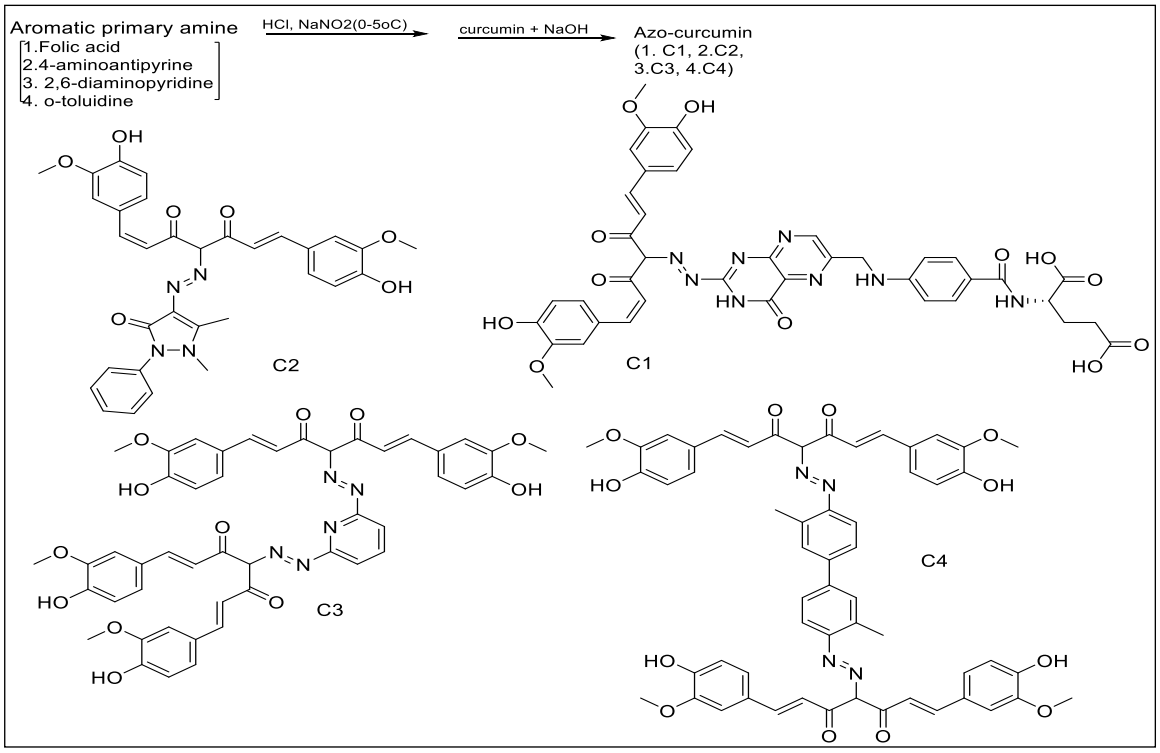
Color: Red; Yelid: %70; M.P.=210-212^{o}C;

Solvent: Ethanol, DMSO, and DMF;

Smiles: COC1=C(O)C=CC(/C=C\C(C(/N=N/C(N2)=NC3=NC=C(CNC4=CC=C(C=C4)C(N[C@@H](CCC(O)=O)C(O)=O)=O)N=C3C2=O)C(/C=C/C5=CC=C(O)C(OC)=C5)=O)=O)=C1;

FTIR (KBrν, cm^{-1}):3147-2940 stretching vibration of (COOH) carboxylic acid; 3501 stretching vibration of (OH) phenol, 1688 stretching vibration of (C=O) amide, 1505 of (C=C) benzene ring and alkene, 1266 stretching vibration of carbonyl group of (C-O), 1538(N=N) in heterocyclic. (see Figure 1).

^{1}HNMR (400 MHz, DMSO-d_6, δ, ppm): 9.98 (s, 1H, COOH), 10.09 (s, 1H, COOH), 9.75 and 9.8 (s, J = 2.1 Hz,1H, OH, phenol), 9.08 (s,1H, hetrocyclic), 8.75 (s,1H,NH), 8.17-7.02(H-aromatic), 6.65 (s,1H,vinyl), 6.82 (t, J = 7.6 Hz, 1H, diene), 4.57 (s, H, CH_2NH), 4.37 – 2.57 (m, 4H, CH_2), 2.41 (s, 6H, methoxy), 1.10 (m, 4H,CH_2).



Scheme 1. Synthesis of azodye-curcumin compounds C1-C4.

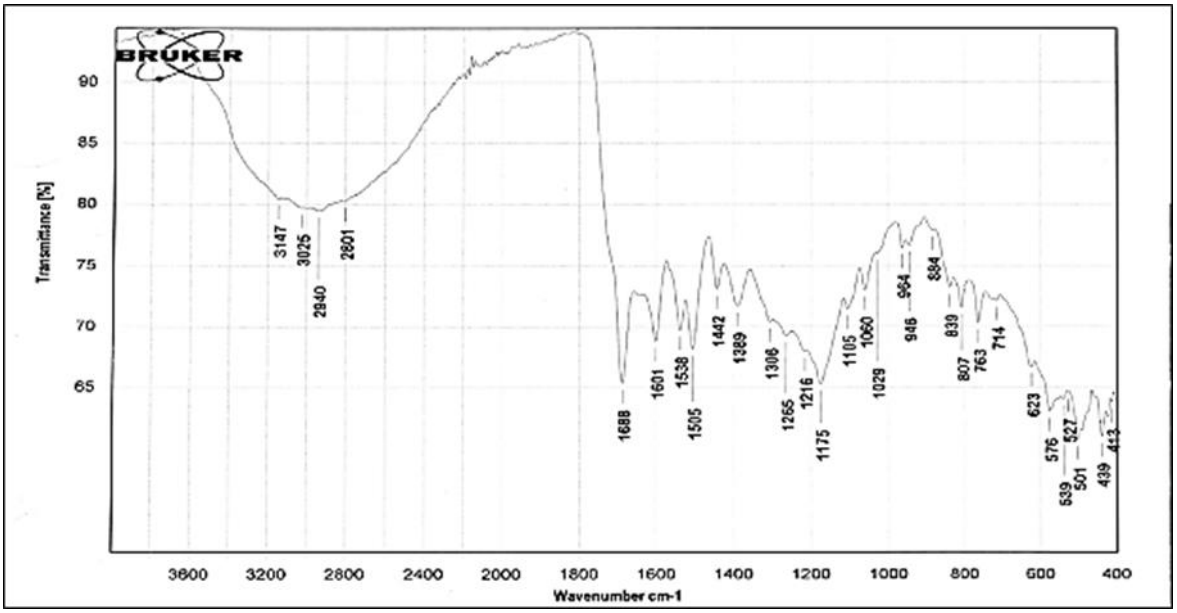


Figure 1. FT-IR spectrum of compound C1.

4-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)diazenyl)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione. (C2).

Color: Red; Yield: % 91%; M.P. = 189-191 °C; Solvent: Ethanol, DMSO, and DMF.

Smiles: O=C(C(/N=N/C1=C(C)N(C)N(C2=CC=CC=C2)C1=O)C(/C=C/C3=CC(OC)=C(O)C=C3)=O)/C=C\C4=CC(OC)=C(O)C=C4;

FTIR (KBr, cm[1]): 1658 stretching vibration of (C=O) carbonyl, 1513 stretching vibration of (C=C) diene, 1427(CH_2), 1275 stretching vibration of (C-O) carbonyl, 1597 (N=N)(heterocyclic) in Figure 2.

[1]HNMR (400MHz, DMSO-d_6, δ, ppm): (2.54, s, 3H, CH_3, Methyl), (3.28, s, CH_3, methyl), (3.81, s, CH_3, methyl), 6.81, and 6.79(H, ethylene), 6.39 (s, 2H, Ar-H), (7.37-7.58, m, 12H, ArH). (9.0, s, OH, phenol) in Figure.3.

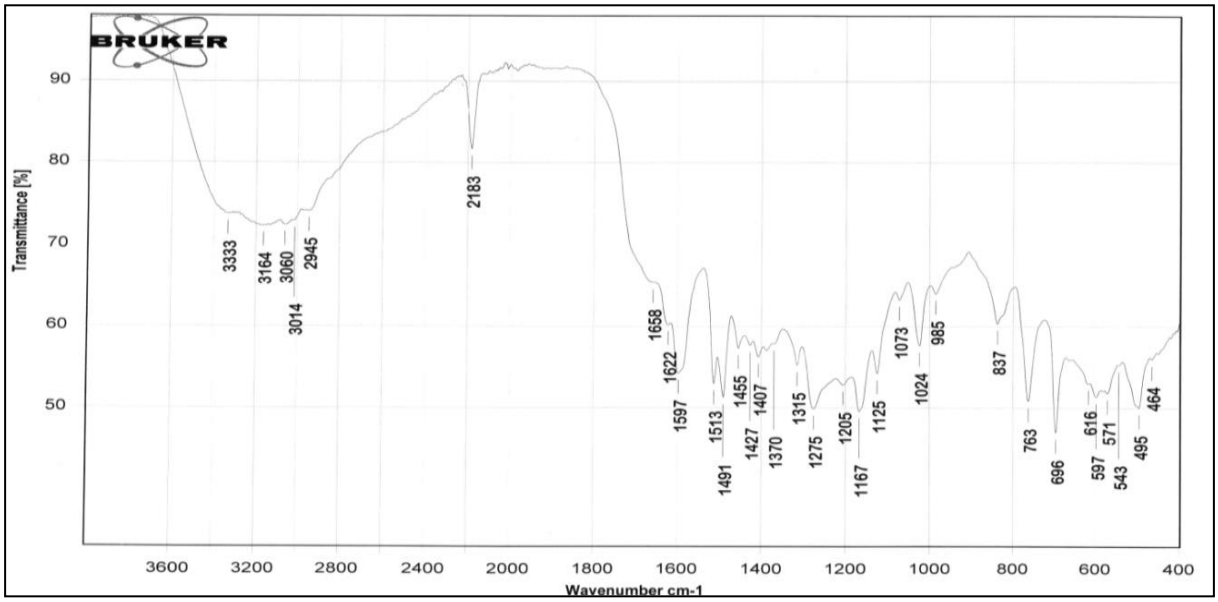


Figure 2. FT-IR spectrum of compound C2.

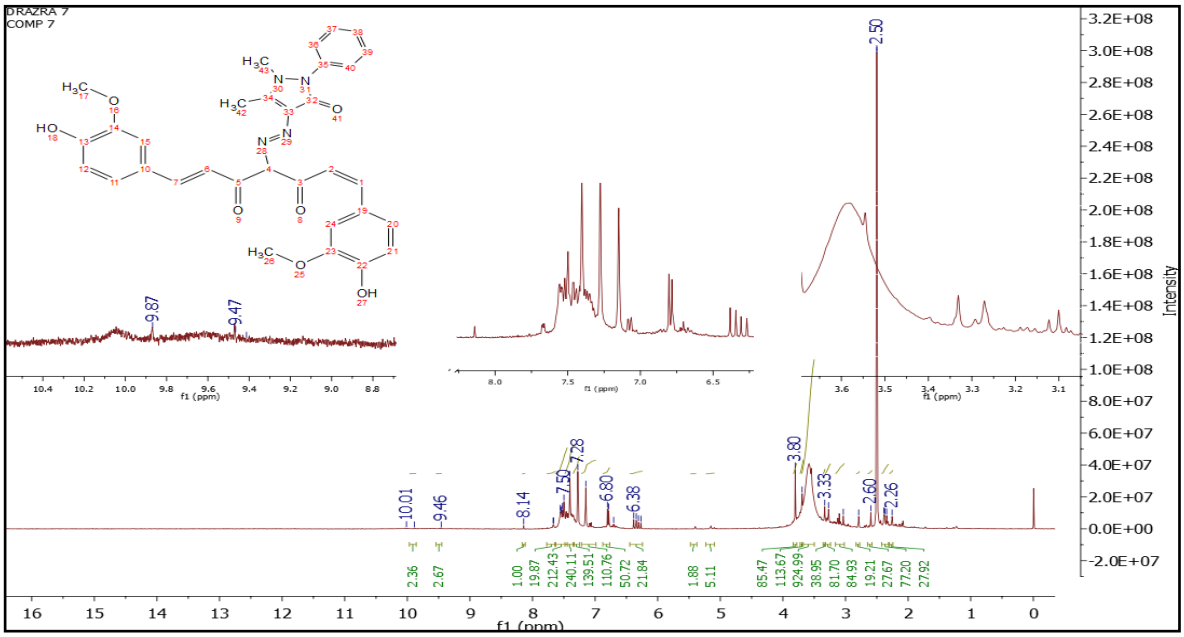


Figure 3. [1]HNMR spectrum of compound C2 in DMSO-d*6*.

4,4'-(-pyridine-2,6-diylbis(diazene-2,1-diyl))bis(1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione). (C3)

Color: Deep orange; yield: %71; M.P.= 231-233°C;

Solvent: Ethanol, DMSO and DMF;

Smiles: COC1=C(O)C=CC(/C=C\C(C(/N=N/C2=NC(/N=N/C(C(/C=C/C3=CC=C(O)C(OC)=C3)=O)C(/C=C\C4=CC(OC)=C(O)C=C4)=O)=CC=C2)C(/C=C/C5=CC=C(O)C(OC)=C5)=O)=O)=C1.

FTIR (KBrν,cm^{-1}):3567 stretching vibration of (OH) phenol, 3142-3042 stretching vibration of (C-H) aromatic ring; 1638 stretching vibration of (C=O) carbonyl, 1511 stretching vibration of (C=C) aromatic, 1272(C-O) carbonyl,1598 (N=N) in Figure 4.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 9.98(s, 1H, OH, phenol), δ 8.03-7.4 (15H, pyridine and aromatic ring), 6.7-7.1 (8H, C=C, alkene), 4.06 (s, H) in Figure 5.

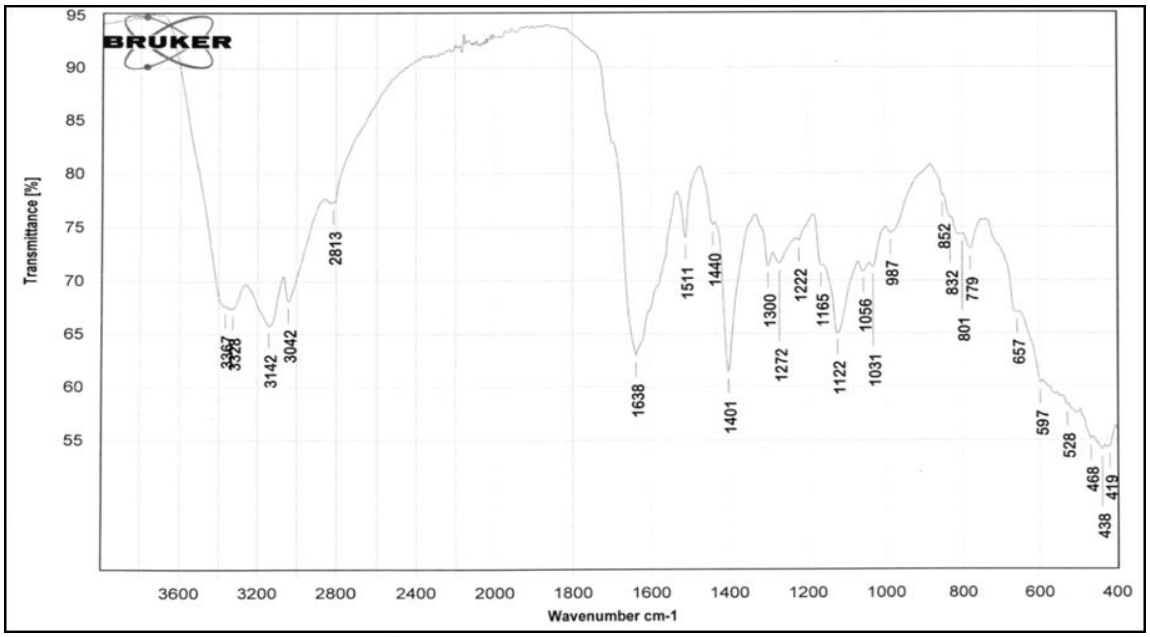


Figure 4. FT-IR spectrum of compound C3.

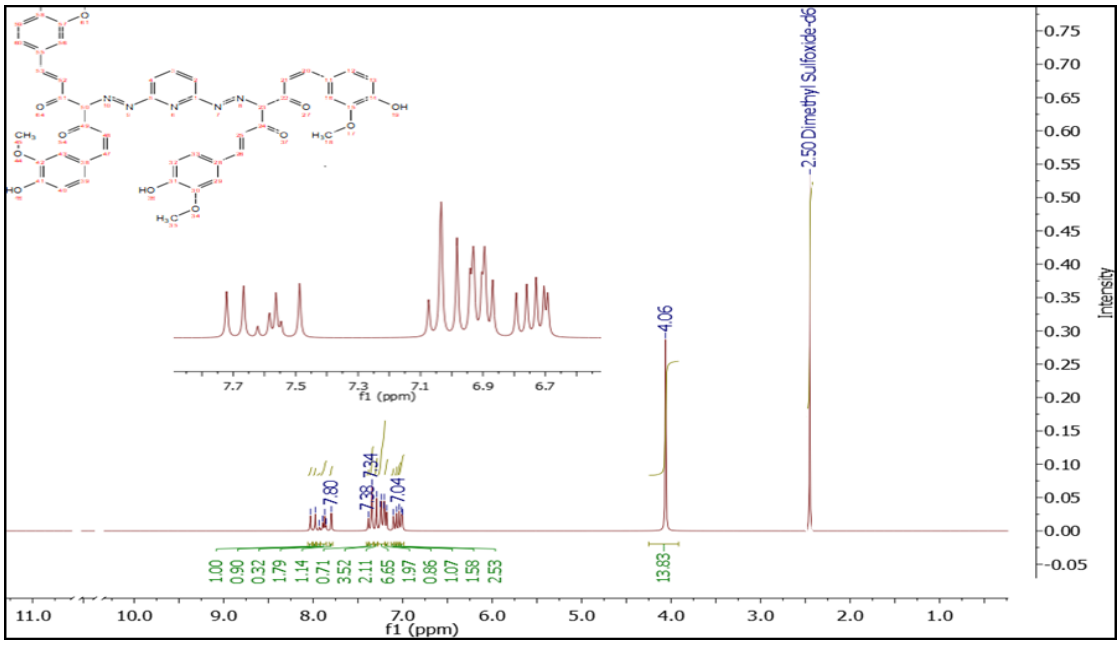


Figure 5. ^{1}HNMR spectrum of compound C3 in DMSO-d6.

4,4'-(3,3'-dimethyl-[1,1'-biphenyl]-4,4'-diyl)bis (diazene-2,1-diyl))bis(1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione) (C4)

Color: Orange; yield: % 89%; M.P. 244-246;

Solvent: Ethanol, DMSO.

Smiles: CC1=CC(C2=CC=C(/N=N/C(C(/C=C/C3=CC=C(O)C(OC)=C3)=O)C(/C=C/C4=CC(OC)=C(O)C=C4)=O)C(C)=C2)=CC=C1/N=N/C(C(/C=C/C5=CC=C(O)C(OC)=C5)=O)C(/C=C/C6=CC(OC)=C(O)C=C6)=O.

FT-IR(KBrν, cm^{-1}): 3417 stretching vibration of (OH) phenol, 3266,3018 stretching vibration of (C-H) aromatic, 1620 stretching vibration of (C=O) carbonyl, 1515 stretching vibration of (C=C) alkene, 1235 of (C-O) carbonyl, 1565(N=N) in Figure 6.

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 10.18 and 9.77 (s, 4H, OH, phenol) 7.62 (s, 3H, ArH), 7.56 (dd, J=8.2, 1.8 Hz, 3H, ArH), 7.48 (d, J=8.2 Hz, 3H, ArH), 7.44 (s, 1H, ArH), 7.3(s, 1H, Ar), 7.25 (d, J = 1.6 Hz, 1H, vinyl), 7.17 (d, J = 13.4 Hz, 1H, vinyl), 6.80 (d, J = 8.3 Hz, 1H, vinyl), 2.41 (s, 9H, methoxy), 2.15 (s, 1H, methyl), 1.2(s, 1H, methyl) in Figure 7.

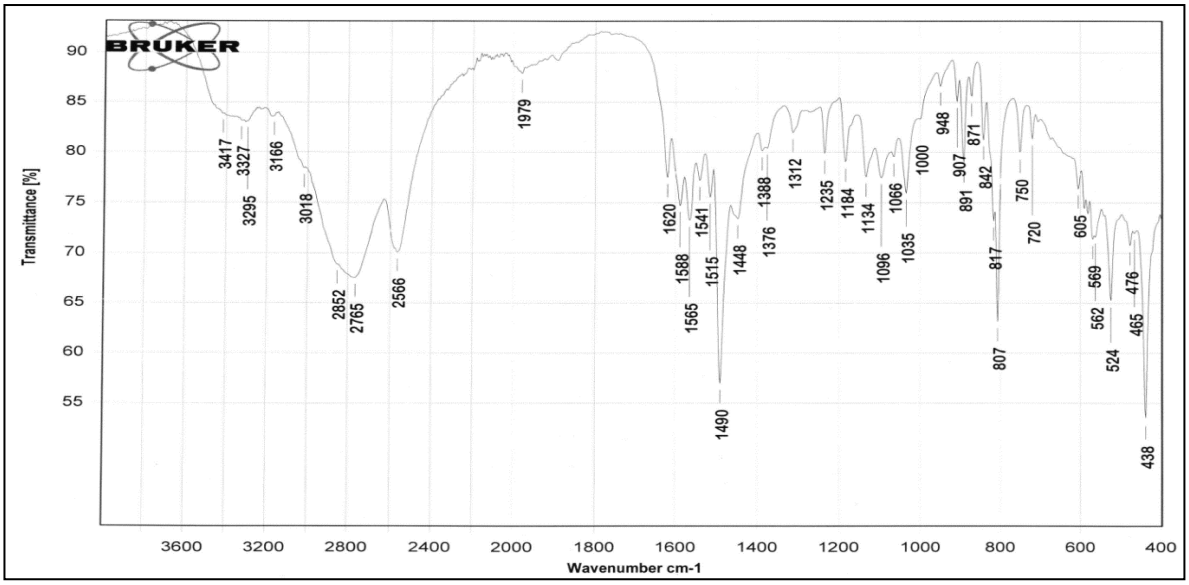


Figure 6. FT-IR spectrum of compound C4.

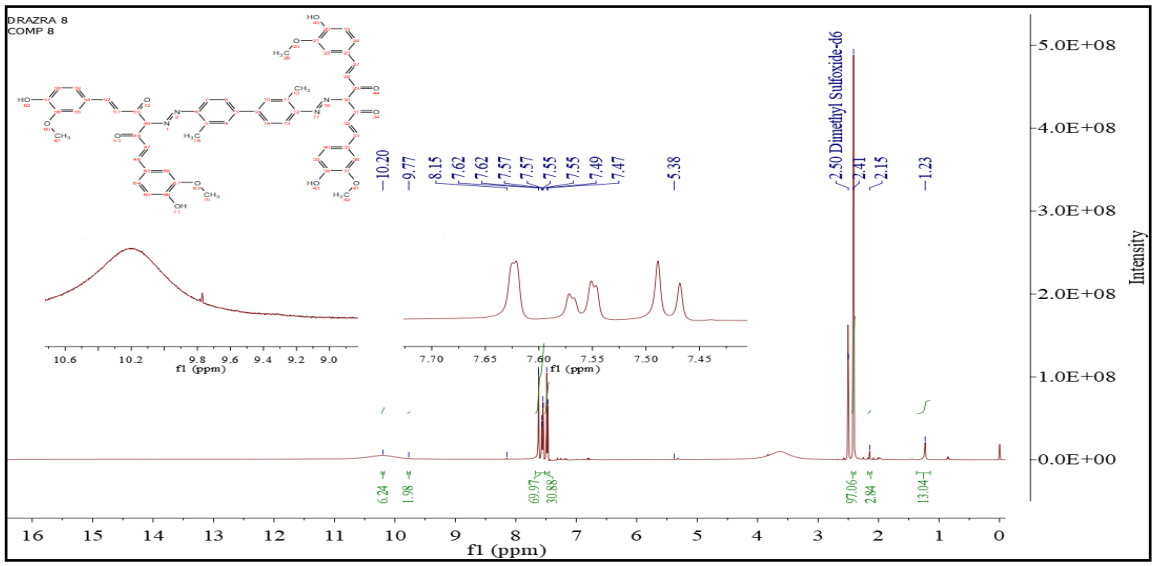


Figure 7. ^{1}HNMR spectrum of compound C4 in DMSO.

Molecular Docking Study

Molecular docking studies evaluated the binding affinity of curcumin derivatives with enzyme 4H2M (of *E. Coli*) and enzyme DNA gyrase (1KZN) in bacteria, aiming to find out the mechanism of dyes as antibacterial [31, 32]. The enzyme of DNA gyrase is an essential bacterial enzyme that is interested in replication and transcription and catalyzes the passive supercoiling of bacterial circular DNA. DNA gyrase is a well-known target for antibacterial drugs. Inhibiting this target causes bacterial mortality. Curcumin derivatives were designed to inhibit DNA gyrase. The selection of this enzyme (4H2M) was based on previous studies, which declared that the mono-carbonyl analogs of curcumin inhibited bacteria via obstruction of the cell wall biosynthesis of bacteria. The primary molecular docking step was determining one of the chains in the desired enzyme. The polar hydrogen bonds were then added, and the active site enzyme (in the chosen chain) was isolated and re-saved with Discover Studio Visualizer 4.0 software. The enzymes and synthesized compounds will be ready for the docking process of the active site after the preparation is completed. Table 1 shows the enzyme types and amino acid residues of the active site. Table 2 shows the docking scores and types of binding with 4H2M. All the synthesized compounds (C1-C4) revealed appropriate energy values with the best binding affinity for C1 (-9.912). Also, Figure 8 shows the compounds (C1-C4 and 2,2'-{benzene-1,3-diylbis[ethyne-2,1-diyl(5bromobenzene-3,1diyl)]} diethanamine)(Ref) binding with the active site in 4H2M. The compounds with the greatest antibacterial activity were curcumin, C1, C2, and C3. Compound C1 performed interaction bonds with TYR: 145 and LEU: 93, as shown in Figure 8.

Table 1. Binding site residues as input for receptor grid generation throughout docking.

Receptor	Site(A)	Residues
1KZN	1	(GLU42 VAL43 ASN46 ALA47 ASP49 GLU50 VAL71 GLN72 ASP73 GLY75 ARG76 GLY77 ILE78 PRO79 ILE90 MET91 VAL93 LEU94 HIS95 ALA96 GLY117 VAL118 GLY119 VAL120 SER121 ARG136 GLY164 THR165 MET166 VAL167)
4H2M	1	MET25 ASN28 GLY42 HIS43 GLY46 ALA47 VAL50 ARG51 VAL54 SER55 TYR68 ALA69 PHE70 SER71 LEU85 MET86 LEU88 PHE89 ALA92 LEU93 GLU96 SER99 LEU100 HIS103 ILE141 ALA142 ALA143 ASN144 TYR145 TRP221)

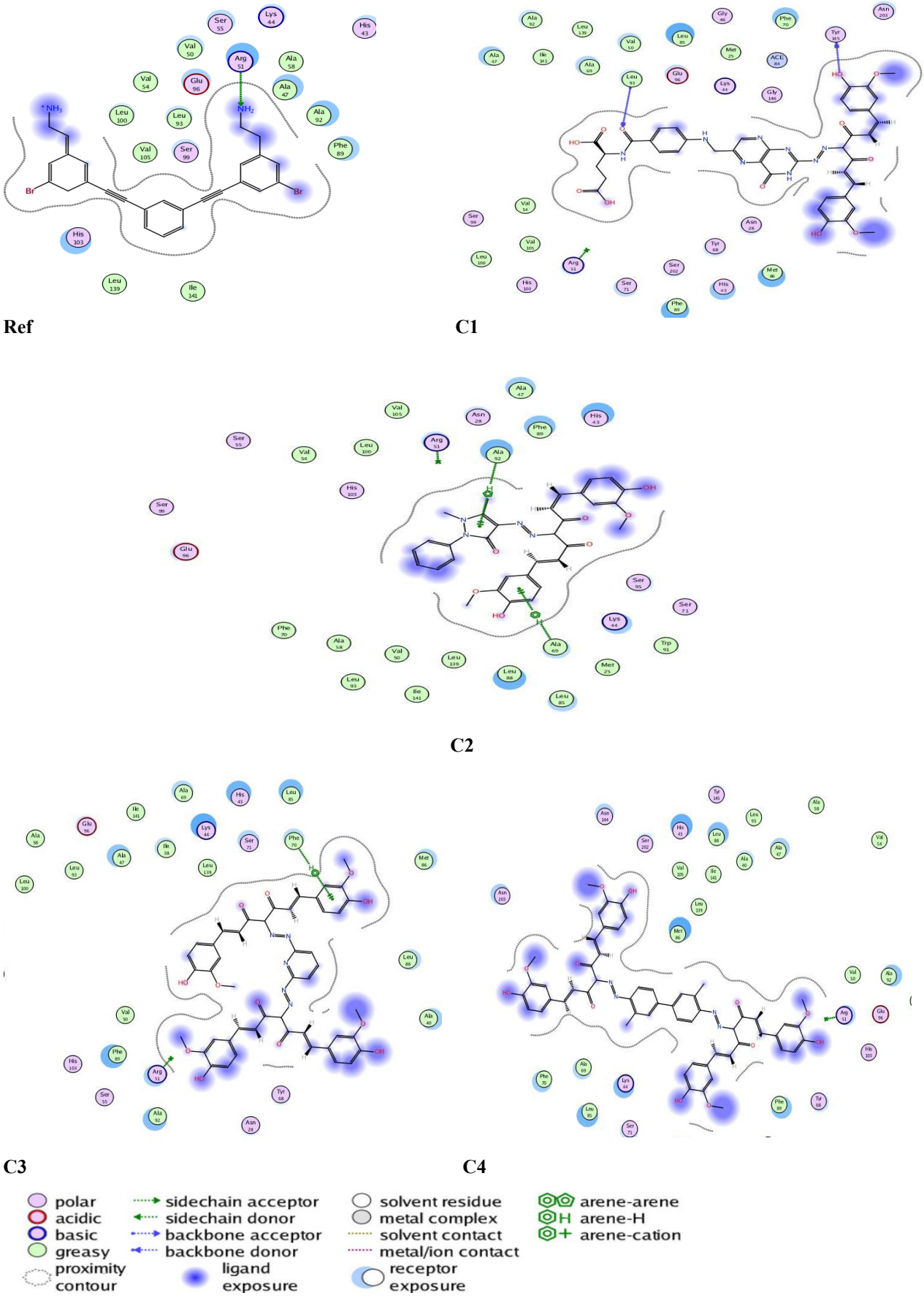


Figure 8. Binding of compounds C1-C4(2D) with 4H2M enzyme in site1(A).

Table 2. Docking findings of curcumin and azo-dyes-curcumin compounds C1-C4 with 4H2M enzyme in site 1.

code	S score (kcal/mol)	RMS D (Å)	Bonding between atoms of compounds and residues of the active site 1 in 1KZN					
			Atom of compound	Atom of receptor	Involved recept. residues	Interaction bond	Dist (Å)	E(Kcal/ mol)
C1	-9.9121	2.288	O 60 O 18	O CA	TYR 145 LEU 93	H-D H-A	3.06 3.21	-1.9 -0.8
C2	-8.1535	1.740	6-ring 5-ring	CB CB	ALA 69 ALA 92	pi-H pi-H	4.33 3.85	-0.6 -0.8
C3	-8.4232	1.814	6-ring	CA	PHE 70	pi-H	4.16	-0.6
C4	-9.8613	1.838	-	-	-	-	-	-
Ref	-7.0975	2.39	NAB51	NH_2	ARG 51	H-A	3.05	-5.2

Only compounds (C2–C4) showed acceptable energy values with the best binding affinity for the ligand (Clorobiocin). Furthermore, Figure 9 shows the bindings of compounds C1-C4 and Clorobiocin with the active site in the 1KZN enzyme. The compounds that showed the greatest antibacterial activity were compounds C2-C4. Compound C1 did not exhibit interaction bonds with the active site in the 1KZN enzyme, as illustrated in Table 3. On the other hand, compounds C2 and C3 showed one bond interaction with ASN 46(A) and H_2O 1065 (A), respectively. Compound C2 revealed three interaction bonds with HOH 1065, ASN 46, and ASN 46, as shown in Figure 9. Clorobiocin (ligand) was involved in two bond interactions with GLY 77 and ASP 73.

Table 3. Docking findings of curcumin and azodye-curcumin compounds (C1-C4) with 1KZN enzyme in site 1.

Code	S Score (kcal/mol)	RSMD (Å)	Bonding between atoms of compounds and residues of the active site 1 in 1KZN					
			Atom of Compounds	*Atom of Preceptor*	*Involved Preceptor residues*	*Interaction bond*	*Dist. (Å)*	*E(Kcal/ mol)*
C1	-5.04453	2.4996	-	-	-	-	-	-
C2	-7.61129	2.4822	O 9 5-ring 5-ring	O CB ND2	HOH 1065 ASN46 ASN46	H-A. pi-H pi-H	3.28 4.06 4.03	-0.7 -0.9 -0.8
C3	-8.11823	2.4464	6-ring	CB	ASN46	pi-H	3.72	-0.6
C4	-8.26745	2.5958	6-ring	O	H_2O1065	pi-H	3.92	-1.1
clorobiocin	-5.75173	2.4976	CL1 N2	22 O 76 OD1	GLY77 ASP73	H-D. H-D.	3.21 3.32	-0.8 -1.7

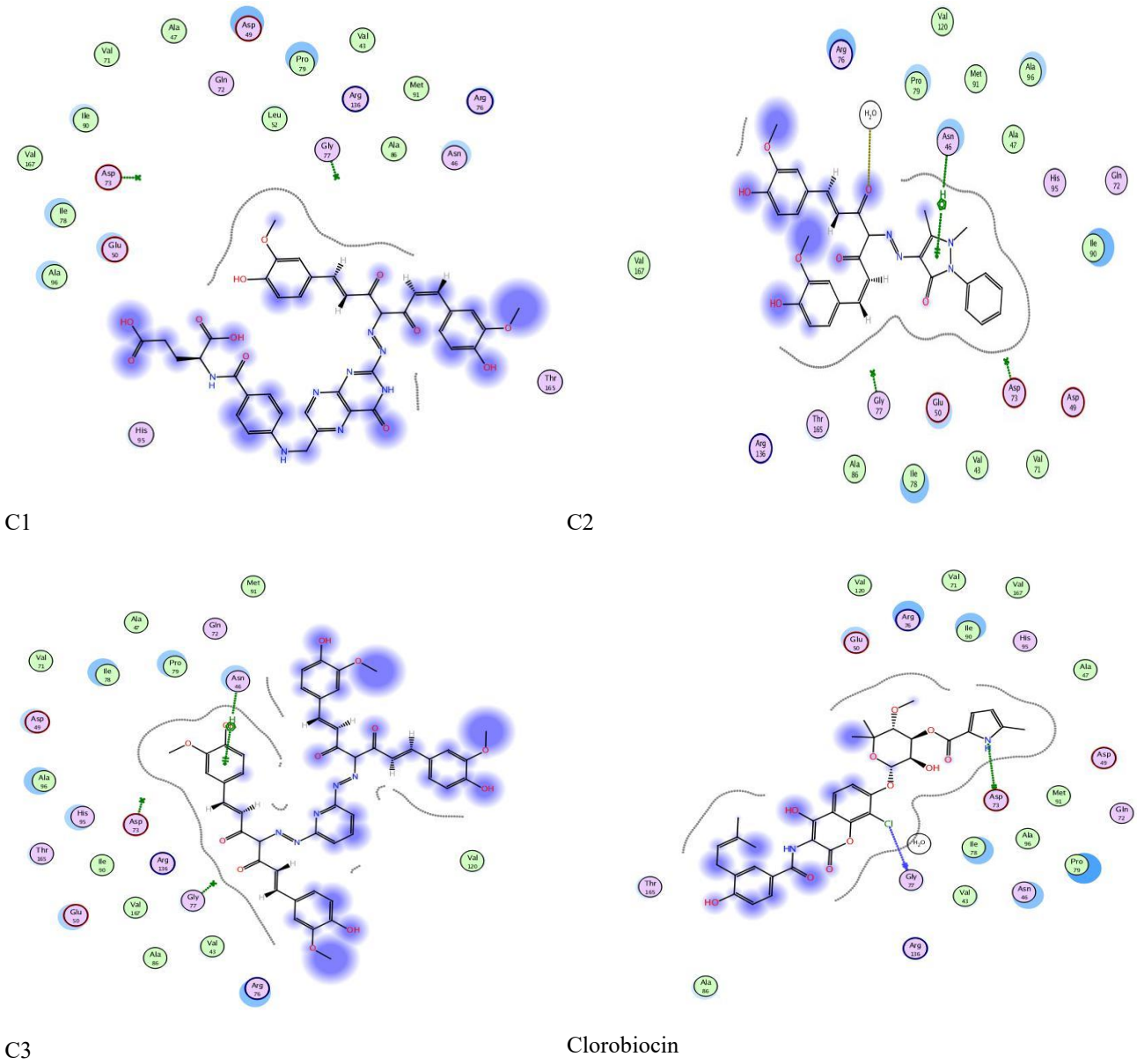


Figure 9. Binding of compound C1(D3) and curcumin with 1KZN in site 1.

Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) Study

In the sector of generation and improvement of innovative molecules for drug manufacturing, physicochemical properties have a vital impact and must be considered. The physicochemical parameters are: Molecular weight (MW), number of heavy atoms, H-bond acceptors (HBA), H-bond donors (HBD), Rotatable bonds, Molar refractivity, and Topological polar surface areas (TPSA). The compound's drug-likeness profile is evaluated via these parameters. Calculations of azodyes-based curcumin (C1-C4) and curcumin parameters were achieved and summarized in Table 4**.** The calculation of Drug-likeness was performed utilizing the Lipinski's rule [MW≤ 500, HBA ≤ 10 and HBD ≤ 5] and Goose's rule [160≤ MW≤ 480; 40≤ MR≤130 and 20 ≤ atoms ≤70], Veber's rule [rotatable bonds≤10 and TPSA ≤ 140], Egan (TPSA ≤ 131.6) and Muggers' rule (200≤ MW≤600 aromatic rings number ≤ 7; various rotatable bonds ≤15; HBA ≤ 10 and HBD ≤ 5) [33, 34].

The rule-based score defines the compound into four probable classes: 11%, 17%, 55%, and 85%. It turned out that compounds C1-C4 did not obey all five rules in Table 5 and Figure 10. The adequate probability score is 55% because it indicates that it obeys the rules. Aiming to determine the solubility medium of the compound (aqueous or not), the calculation and assessment of probable lipophilicity means were achieved with respect to the assent value of log Po/w in Table 6. Protein (P-gp protein) is crucial to appraise the active efflux through biological

membranes and cytochrome P450(CYP) enzymes in Table 6. Furthermore, it was observed that C1 and Curcumin molecules were non-substrates of the CYP2C19 inhibitor. Whereas, compounds (C3, C4, and curcumin) behaved as substrates of CYP3A4. Although all compounds were observed to act as substrates of CYP2C9 and CYP3A4 inhibitors, they did not act as substrates of the inhibitor CYP1A2, as illustrated in Table 6. Azodyes-curcumin (C1-C4) toxicity was estimated utilizing ProTox-II services online. C-compounds (C1-C4) were anticipated to be hepatotoxic based on Organ toxicity findings. Whereas, all compounds (C1-C4) with non-Carcinogenicity and non-Cytotoxicity, as it was designated by toxicological endpoint results. It was predicted that the prepared compounds (C1- C4) and Curcumin would have immunotoxicity and were non-mutagenic. The (C1-C4) and curcumin (C2, C3, and C4) toxicity category is 4 in Table 7. It is stated that the quantity at which half of the studied objects die when they are subjected to the examined compounds, referred to as LD_{50}. According to GHS (global harmonized classification system of chemical substances labelling), the following categories describe the compound toxicity: category 1: lethal if consumed ($LD_{50}\leq5$); category 2: fatal if consumed ($5< LD_{50}\leq 50$); category 3: poisonous if consumed ($50< LD_{50} \leq 300$); category 4: harsh if consumed ($300< LD_{50}\leq2000$); category 5: can harsh if consumed ($2000< LD_{50}\leq 5000$), and category 6: non-toxic ($LD_{50}>5000$) [35-27]. The findings exhibited that the compounds (C2) were non-toxic, but the compounds (C1) were toxic. Toxicity study data revealed that the produced compounds exhibited organ toxicity (Hepatotoxicity) and immunotoxicity upon consumption, except for compound C2, which did not show any toxicity after consumption.

Table 4. The physicochemical properties of compounds (C1-C4) and curcumin.

Code	Physicochemical properties						
	MWt. (g/mol)	Num. Heavy atoms	HBA	HBD	Rotatable Bonds	Molar Refractivity	TPSA ($Å^2$)
C1	820.76	28	17	7	20	213.04	305.04
C2	582.6	43	9	2	11	162.71	144.71
C3	867.85	64	17	4	20	236.76	248.45
C4	971.02	72	16	4	19	277.56	241.88
Curcumin	368.38	27	6	2	8	102.80	93.06

Table 5. Drug-likeness evaluation of compounds (C1-C4) and curcumin.

Code	Lipinski	Ghose	Veber	Egan	Muegge	Synthetic accessibility
C1	No;3violations: MW>500,NorO >10,NHorOH>5	No;3viol.:MW> 480,MR>130,#a toms>70	No;2violations :Rotors>10,TP SA>140	No;1violation: TPSA>131.6	No;5violations:M W>600,TPSA>15 0,Rotors>15,HA> 10,H-D>5	5.96
C2	No;2violations: MW>500,NorO >10	No;3violations: MW>480,MR> 130,#atoms>70	No;2violations :Rotors>10,TP SA>140	No;1violation: TPSA>131.6	No;1violation: XLOGP3>5	4.86
C3	No;2viol.:MW> 500,NorO>10	No;3viol.:MW> 480,MR>130,#a toms>70	No;2violations :Rotors>10,TP SA>140	No;1violation: TPSA>131.6	No;1violation: XLOGP3>5	4.21
C4	No;2violations: MW>500,NorO >10	No;4violations: MW>480,WLO GP>5.6,MR>13 0,#atoms>70	No;2violations :Rotors>10,TP SA>140	No;2violations :WLOGP>5.88 ,TPSA>131.6	No;5violations:M W>600,XLOGP3 >5,TPSA>150,Ro tors>15,H-acc>10	4.86
Cur.	No;5violations	No;4violations	Yes	Yes	Yes	7.14

Table 6. The pharmacokinetics of compounds (C1-C4) and curcumin.

Code	Lipophilicity	pharmacokinetics						Water Solubility Log S (ESOL)	Bioavailability Score
	logP ($P_{o/w}$)	GI Asb.	BBB	LogKp (cm/s)	CYP1A2	CYP2C19	P-gp		
C1	2.78	Low	No	-9.00	No	No	Yes	-6.00(/)	0.11
C2	4.22	Low	No	-5.93	No	No	No	-6.60(/)	0.11
C3	4.22	Low	No	-5.93	No	No	No	-6.60(/)	0.17
C4	8.34	Low	No	-4.46	No	No	Yes	-11.73(-)	0.17
curcumin	2.87	High	No	-6.28	No	No	No	-3.94(+)	0.5

Soluble (+)**,** insoluble **(-)**, Poorly Soluble**(/)**

Table 7. Theoretical toxicity evaluation of compounds (C1-C4) and curcumin.

Code	Organ Toxicity	Toxicity - endpoints				LD50 (mg/kg)
	Hepato toxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity	
C1	+	_	+	_	_	135 [3]
C_2	+	_	+	_	_	5600[6]
C3	+	_	+	_	_	1000[4]
C4	+	_	+	_	_	2000[4]
Curcumin	_	_	+	_	_	2000[4]

Inactive(-);active(+)

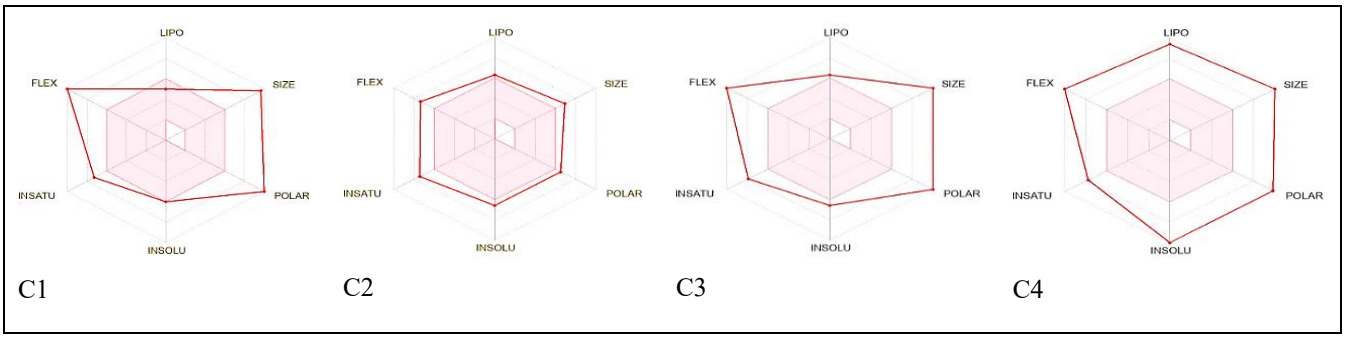


Figure 10. A spider shape of the designed compounds C1 and C4.

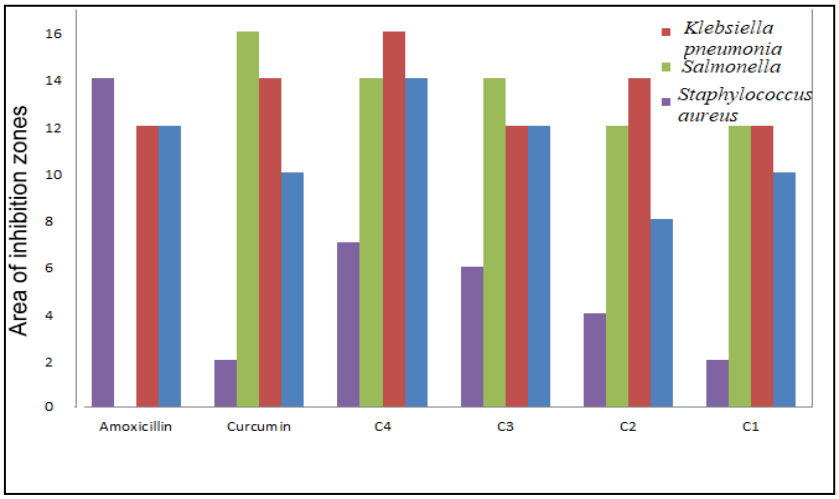


Figure 11. The effect of compounds (C1-C4), curcumin, and amoxicillin on the inhibition of *E.coli, Klebsiella pneumonia, Staphylococcus aureus, and Salmonella abony*.

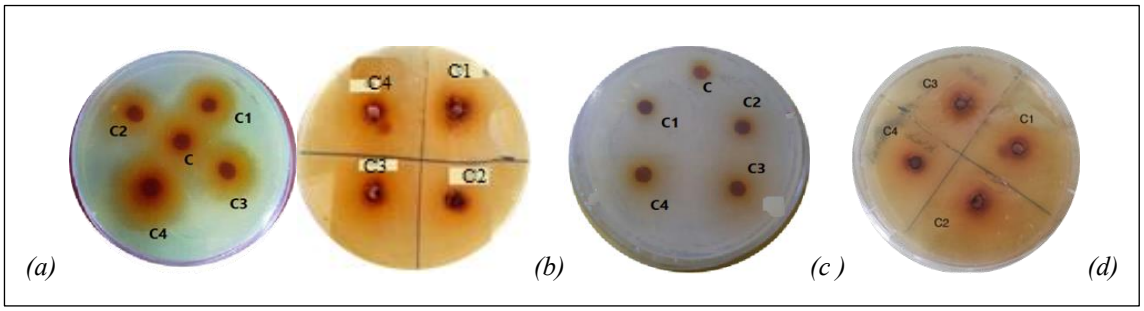


Figure 12. Zone of inhibition of compound C1, compound C2, compound C, 3 compound C4, and curcumin C against *E. Coli* (a), *Klebsiella pneumonia* (b), *Staphylococcus aureus* (c), and *Salmonella*(d).

Pharmacological Activity

The biological activities assessment of the synthesized compounds (C1-C4) was achieved, as it is necessary for the medical applications. The antibacterial activities of curcumin derivatives, azodyes-curcumin (C1-C4), towards both Gram-positive bacterial strains, including *Staphylococcus aureus,* and Gram-negative bacterial strains, including *Escherichia coli, Salmonella, and Klebsiella pneumonia,* were examined. Amoxicillin, as a standard antibiotic medicine, was utilized. The evaluation of the inhibition zone values, which reflect the inhibition activities towards the tested bacteria, was assessed and listed in Figure 11. The obtained data revealed that the biological activity of the molecules (C1-C4) towards *Klebsiella pneumonia and E. coli* was high, but their activity towards *Staphylococcus aureus* was low in Figure 11. The reason behind that is the presence of (OH) as an electron-donating group, which provides the resonance stability. Consequently, it plays a significant role in raising the activity against bacteria. In addition to delocalization of positive and negative charge, azene mesoionic properties (such as the existence of N atom in heterocyclic molecules, ethylene moieties), which highly affect the molecule's lipophilicity, and therefore affect pharmacokinetic features. These azene groups have a substantial role in improving the penetration of the cell membrane of bacteria by the molecules and subsequently interacting with diverse biological objects. Moreover, the formation manner of H-bonds with the targeted protein by the electron-donating moieties is better than that of those formed by electron-withdrawing moieties. Hence, the electron-donating moieties could have a remarkable influence on enhancing the biological activity [38-39].

CONCLUSION

Computational methods such as molecular docking and ADMET are powerful tools for studying newly designed medicinal compounds. New azodyes-based curcumin compounds C1-C4 (as curcumin analogs) were successfully produced and characterized using spectroscopic and classical methods. The experimental and computational methods were employed to study

the formed molecules. The synthesized azodyes-curcumin (C1-C4) exhibited biological activity against four harmful bacterial species (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, and Salmonella*). The results indicated that all the synthesized compounds have high activity against *Klebsiella pneumoniae, Salmonella, and E. coli*, while they show a weak antibacterial activity against *Staphylococcus aureus*. On the other hand, the Insilco study of compounds C1-C4 with Enzyme 4H2M and 1KZN revealed an appropriate binding interaction with the 4H2M enzyme, except for compound C4. Suitable binding interactions were observed between the compounds (C2-C4) and the 1KZN enzyme, but no interactions were detected between compound C1 and with 1KZN enzyme. Also, ADME studies disclosed that not all the prepared compounds adhere to Lipinski's rule. Toxicity study data revealed that the produced compounds have shown organ toxicity (Hepatotoxicity) and immunotoxicity upon consumption, except compound C2, which did not show any toxicity after being consumed. Consequently, compound C2 is a promising candidate to be used as an antibacterial drug.

ACKNOWLEDGEMENTS

The authors thank the College of Science/ University of Wasit for providing the facilities to carry out this study.

REFERENCES

1. Albanna, A. M. J., Al-Layl, A. A. H. (2020) Using real-time PCR to investigate some of antibiotic resistance genes from streptococcus agalactiae isolates from ewe mastitis cases in nineveh province. *Baghdad Science Journal*, **17(3),** 931–937. https://bsj.uobaghdad.edu.iq/index.php/BSJ/article/ view/3123.

2. Kamoon, R. A., Al-Mudhafar, M. M. J. and Omar, T. N -A. (2020) Synthesis, characterization and antimicrobial evaluation new azo compounds derived from sulfonamides and isatin schiff Base. *Infection and Drug Resistance*, **10**, 150–155. https://doi.org/10.25258/ijddt.10.1.22.

3. Benkhaya, S., M'rabet, S. and El-Harfi, A. (2020) Classifications, properties, recent synthesis and applications of azo dyes. *Heliyon,* **6**, 03271. https://doi.org/10.1016/j.heliyon.2020.e03271.

4. Mezgebe, K. and Mulugeta, E. (2022) Synthesis and pharmacological activities of azo dye derivatives incorporating heterocyclic scaffolds: a review. *RSC Advances*, **12**, 25932–25946. https://doi.org/10.1039/d2ra04934a.

5. Tahir, T., Ashfaq, M., Saleem, M., Rafiq, M., Shahzad, M. I., Kotwica-Mojzych, K. and Mojzych, M. (2021) Pyridine scaffolds, phenols and derivatives of azo Moiety: current therapeutic perspectives. *Molecules*, **26**, 4872. https://doi.org/10.3390/molecules26164872

6. Mezher, M. A., Toma, M., Dala, A. and Kaain, H. (2021) Synthesis and characterization of new azo dyes based on thiazole and assess the biological and l Efficacy for them and study their dyeing application. *Egyptian Journal of Chemistry*, **64**, 2903–2911. https://doi.org/10.21608/ejchem.2021.55296.3163.

7. Kmal, R. Q., Mohamed, A. M., Aljeboree, A. M., Jasim, L. S. and Alkaim, A. F. (2021) Synthesis and identification of azo diperse dye derived from 4-aminoantipyrine and their applications to determine some drugs. *International Journal of Drug Delivery Technology*, **11**, 1040–1044. https://doi.org/10.25258/ijddt.11.3.67.

8. Zobeidi, A., Neghmouche Nacer, S., Atia, S., Kribaa, L., Kerassa, A. and Kamarchou, A., (2023) Corrosion Inhibition of Azo Compounds Derived from Schiff Bases on Mild Steel (XC70) in (HCl, 1 M DMSO) Medium: An Experimental and Theoretical Study. *ACS Omega*. **8**, 21571–21584. https://doi.org/10.1021/acsomega.3c00741.

9. Sharifi-Rad, J., Rayess, Y. E., Rizk, A. A., Sadaka, C., Zgheib, R., Zam, W., Sestito, S., Rapposelli, S., Neffe-Skocińska, K., Zielińska, D., Salehi, B., Setzer, W. N., Dosoky, N. S., Taheri, Y., Beyrouthy, M. E., Martorell, M., Ostrander, E. A., Suleria, H. A. R., Cho, W. C., Maroyi, A. and Martins, N. (2020) Turmeric and its major compound Curcumin on health: bioac tive effects and safety profiles for food, pharmaceutical, biotechnological and medicinal applications. *Journal Frontiers in Pharmacology*, **11**, 01021. https://doi.org/10.3389/fphar.2020.01021.

10. Al-Abadi, F. F. S. (2022) Synthesis and characterization of azo dyes and study of the equilibrium and thermodynamics of adsorption of dyes on activated charcoal. *Materials Today: Proceedings*, **49**, 2699. https://doi.org/10.1016/j.matpr.2021.09.059.

11. Al-Hamdani, A. A. S. and Hasan, Z. A. (2018) Spectroscopic Studies and Thermal Analysis of New Azo Dyes Ligands and their Complexes with some Transition of Metal Ions. *Baghdad Science Journal*, **13(3)**, 0511. https://bsj.uobaghdad.edu.iq/index.php/BSJ/article/view/2938.

12. Pérez, S., Eichhorn, P. and Aga, D. S. (2005) Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. *Environmental Toxicology and Chemistry*, **24**, 1361–1368. https://doi.org/10.1897/04-211r.1.

13. Zhang, G. F., Liu, X., Zhang, S., Pan, B. and Liu, M. L. (2018) Ciprofloxacin derivatives and their antibacterial activities. *European Journal of Medicinal Chemistry*, **146**, 599–612. https://doi.org/10.1016/j.ejmech.2018.01.078.

14. Berglund, J., Gjondrekaj, R., Verney, E. and Maupin-Furlow, J. A. (2020) Edelmann. modification of the host ubiquinone by bacterial enzymes. *Microbiology Research*, **235**, 126429. https://doi.org/10.1016/j.micres.2020.126429.

15. Alsahib, S. A. (2020) Characterization and Biological Activity of Some New Derivatives Derived from Sulfamethoxazole Compound. *Baghdad Science Journal*, **17(2)**, 471–480. https://doi.org/10.21123/bsj.2020.17.2.0471

16. Castelli, M., Baggio, G., Ruberto, A. I., Rossi, T., Provvisionato, A. and Malagoli, M. (1995) In vitro activity of trimethoprim in association with sulfimidazole against aerobic gram-negative and gram-positive microorganisms and Clostridia. *Chemotherapy*, **141**, 337–344. https://doi.org/10.1159/000239365.

17. Fernandez, D., Restrepo-Acevedo, A., Rocha-Roa, C., Le Lagadec, R., Abonia, R, Zacchino, S. A. and Gómez Castaño, J. A. (2021) Synthesis, structural characterization, and in vitro and in Silico anti-fungal evaluation of azo-azomethine pyrazoles ($PhN_2(PhOH)CHN(C_3N_2(CH_3)_3)PhR$, R = H or NO_2). *Molecules*, **26**, 7435. https://doi.org/10.3390/molecules26247435.

18. Kaboudin, B., Daliri, P., Faghih, S. and Esfandiari, H. (2022) Hydroxy and amino-phosphonates and bisphosphonates: synthetic methods and their biological applications. *Frontiers in Chemistry*, **10**, 890696. https://doi.org/10.3389/fchem.2022.890696.

19. Kadhim, M. A., Zangana, E. K. M. and Jawad, A. H. (2024) Design, synthesis and characterization of a new series of 2, 3-dihydroquinazolin-4(1H)-one (DHQZ-1) derivatives and evaluation of antitumor resistant (by Molecule Docking). *Baghdad Science Journal*, **21(7)**, 2263. https://bsj.uobaghdad.edu.iq/index.php/BSJ/article/view/8232.

20. Mezgebe, K. and Mulugeta, E. (2022) Synthesis and pharmacological activities of azo dye derivatives incorporating heterocyclic scaffolds: a review. *RSC Advances*, **12**, 259–32. https://doi.org/10.1039/d2ra04934a.

21. Sai Lakshmi Manasa, P., Kamble, A. D. and Chilakamarthi, U. (2023) Various extraction techniques of curcumin─a comprehensive review. *ACS Omega,* **8(38)**, 34868–34878. https://doi.org/10.1021/acsomega.3c04205.

22. Jiang, T., Ghosh, R. and Charcosset, C. (2021) Extraction, purification and applications of curcumin from plant materials-A comprehensive review. *Trends in Food Science & Technology*, **112**, 419–430. https://doi.org/10.1016/j.tifs.2021.04.015.

23. Abdullah, H. M. and Tawfiq, M. T. (2013) Synthesis and characterization of some new azo dyes derivatives via chalcone and study some of their biological activity. *Baghdad Science Journal*, **13(1)**, 0122.

24. Al-Jeilawi, O. H. R. and Oleiwi, A. Q. (2023) Preparation, characterization, antioxidant activity of 1-(2-furoyl) thiourea derivatives and study the molecular docking of them as potent inhibitors of Urease enzyme. *Baghdad Science Journal*, **20(3)**, 994–1011. https://doi.org/10.21123/bsj.2023.7745.

25. Espinoza-Torres, S., López, Sotomayor, M. D. P. T., Tuesta, J. C., Picasso, G. and Khan, S. (2023) Synthesis, Characterization, and evaluation of a novel molecularly imprinted polymer (MIP) for selective quantification of curcumin in real food sample by UV-Vis spectrophotometry. *Polymers*, **15(16)**, 3332. https://doi.org/10.3390/polym15163332.

26. Al-Thubaiti, E. H. (2023) Antibacterial and antioxidant activities of curcumin/Zn metal complex with its chemical characterization and spectroscopic studies. *Heliyon*, **9(6)**, e17468. https://doi.org/10.1016/j.heliyon.2023.e17468.

27. Lee, H. S., Jo, S., Lim, H. S. and Im, W. (2012) Application of binding free energy calculations to prediction of binding modes and affinities of MDM2 and MDMX inhibitors. *Journal of Chemical Information and Modelling*, **52(7)**, 1821–1832. https://doi.org/10.1021/ci3000997.

28. Sager, A. G., Abaies, J. K. and Katoof, Z. R. (2024) Synthesis of dyes sulfamidazole: characterization, evaluation, molecular docking and global descriptors by density functional theory (DFT). *Karbala International Journal of Modern Science*, **10**, 286–296. https://doi.org/10.33640/2405-609X.3353.

29. Zhu, W. Zhang, Y., Sinko, W., Hensler, M. E., Olson, J., Molohon, K. J., Lindert, S., Cao, R., Li, K., Wang, K., Wang, Y., Liu, Y. L., Sankovsky, A., de Oliveira, C. A., Mitchell, D. A., Nizet, V., Mc Cammon, J. A., and Oldfield, E. (2013) Anti-bacterial drug leads targeting isoprenoid biosynthesis. *Proceedings of the National Academy of Sciences (PNAS)*, **110(1)**, 123–128. https://doi.org/10.1073/pnas.1219899110.

30. Rabeaa**,** M. M. and Khalaf, M. I. (2024) Modification and characterization of some new levofloxacin heterocyclic derivatives as COX-2 enzyme inhibitors

and evaluate their efficacy pharmacokinetics by molecular docking and the swiss ADME studies. *Baghdad Science Journal*, **21(4)**,1255. https://bsj.uobaghdad.edu.iq/index.php/BSJ/article/view/8295.

31. Zhang, L., Zhang, H., Ai, H., HuLi, H., S., Zhao, J. and Liu, H. (2023) Applications of machine learning methods in drug toxicity prediction. *Current Topics in Medicinal Chemistry*, **18(12)**, 987–997. https://doi.org/10.2174/1568026618666180727152557.

32. Abdulridha, M. Q., Al-hamdani, A. A. S. and Hussein, I. A. (2023) Synthesis characterization and antioxidant activity of new azo ligand and some metal complexes of tryptamine derivatives. *Baghdad Science Journal*, **20**, 1046–1063. https://dx.doi.org/10.21123/bsj. 2023.8227.

33. Sager, A. G., Abaies, J. K. and Katoof, Z. R. (2025) Design, Molecular Docking, ADMET studies, synthesis, characterization, and antimicrobial of nicotinamide derivatives via mannich reaction. *Iraqi Journal of Science*, **66(3)**, 1–17. https://dx.doi.org/10.24996/ijs.2025.66.3.2.

34. Pathania, S. and Singh, P. K. (2021) Analysing FDA-approved drugs for compliance of pharmacokinetic principles: should there be a critical screening parameter in drug designing protocols. *Expert Opinion on Drug Metabolism & Toxicology*, **17(4)**, 351–354. https://doi.org/10.1080/17425255.2021.1865309

35. Singh, R. Kumar, P. Sindhu, J., Kumar, A. and Lal, S. (2024) CORAL: probing the structural requirements for α-amylase inhibition activity of 5-(3-arylallylidene)-2-(arylimino)thiazolidin-4-one derivatives based on QSAR with correlation intensity index, molecular docking, molecular dynamics, and ADMET studies. *Journal of Biomolecular Structure and Dynamics*, **42(21)**, 11861–11878. https://doi.org/10.1080/07391102.2023.2265490.

36. Sager, A. G., Abais, J. K. and Katoof, Z. R. (2024) Molecular docking, synthesis and evaluation for antioxidant and antibacterial activity of new oxazepane and Benzoxazepine Derivatives. *Baghdad Science Journal*, **21(7)**, 2289–2307. https://doi.org/10.21123/bsj.2023.8553.

37. Sager, A. G., Abbood, A. F., Abais, J. K. and Khazal, F. A. (2025) A design, molecular docking, ADMET studies, synthesis, characterization, and in vitro pharmacological evaluation of tetrazole derivatives. *Iraqi Journal of Pharmaceutical Sciences*, **34(1)**, 10– 25. https://doi.org/10.31351/vol34iss1pp10-25.

38. Labby, K. J., Xue, F., Kraus, J. M., Mataka, J., Li, H., Martásek, P., Roman, L. J., Poulos, T. L. and Silverman, R. B.(2012) Intramolecular hydrogen bonding: a potential strategy for more bioavailable inhibitors of neuronal nitric oxide synthase. *Bioorganic & Medicinal Chemistry*, **20(7)**, 2435–2443. https://doi.org/10.1016/j.bmc.2012.01.037.

39. Christian, L., Ken, A., Brameld, J. G., Jean-Yves, L. Q. and Eric, R. (2009) The pK_{BHX} database: toward a better understanding of hydrogen-bond basicity for medicinal chemists. *Journal of Medicinal Chemistry*, **52(14)**, 4073–4086. https://doi.org/10.1021/jm801331y.