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Preparation, Antimicrobial Evaluation and Molecular docking of New 2,3-Substituted [1,3] Benzooxazin-4-one derivatives

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ABSTRACT

New 2, 3-substituted benzooxazin-4-one derivatives were prepared by means of a altered step by step proceedings in which Schiff bases were substituted with salicylic acid for a ring forming reaction. The compositions of the synthesized compounds were certain via methods spectrometry as elemental analysis, FT-IR, ^{13}C-NMR & ^{1}H-NMR spectral analysis. The bio-activities for the prepared compounds in-vitro as antibacterial and antifungal were estimated as opposed to two races of gram-positive & two races of gram-negative bacteria as parallel to Cefotaxime sodium as regular drug and assessed versus two types of fungi. The prepared compounds were got to have antimicrobial activities spreading from middling to perfect against of the bacteria strains with good percentage mycelial growth inhibition activity against fungi. Molecular docking displays the critical part while effect of variety of substituents on biological activity while mark the disadvantageous constitutional parameters in drawing medication: A different substitution does ensure additional efficiency in bioactivity.

1. INTRODUCTION

A research of present literatures has implied that series of compounds including six-membered ring (1, 3-oxazine) systems exhibit a broad-range of antimicrobial activities and pharmacological action [1,2]. Therefore, these type of compounds are researched widespread for the composition of biological activity compounds spreading from fungicides while herbicides to curative effect applicable drugs [3, 4]. Bioactivities showed by means of these heterocyclic compounds implicate antibacterial [5], anticancer [6], antifungal [7], antitumor [8], antiviral [9], anti-inflammatory [10] and Insect Growth Regulatory (IGR) activity. Also these compounds are perfect stock stability in detergent components consisting per-oxygen bleach and some of the benzoxazine compounds were notified to be possessing thorough implementation in the domain of macromolecule flag as the oxazine compounds were utilized in the output of polymeric items during the ring-opening reaction of thermal polymerization [11, 12]. In vision of the hopeful bioactivities of these ones heterocyclic rings connected through our explore for the study of powerful antimicrobial agents. We communicate the preparation of novel compounds utilizing an altered stepwise technicality in which salicylic acid which Schiff base for the cyclization reaction. The in-vitro antibacterial and antifungal bioactivities of these heterocyclic compounds were estimated while the outcome current in this working.

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2. EXPERIMENTAL PROCEDURE

The solvents, chemical materials and reagents utilized during this research were available bought from Romil, Sigma Aldrich and BDH are utilized as be given. Recorded infrared spectra were by using Shimadzu Infrared Spectrophotometer FT-IR model 8400s series spectrophotometer (KBr Pellet) in the region 400-4000 cm^{-1}. ^{1}H and ^{13}C-NMR spectral analysis were collected on NMR spectrometer 400 MHz, Bruker Biospin GmbH 400 MHz using DMSO-d_6 as the NMR solvent. Chemical shifts (δ) are expressed in ppm. The elemental CHNS analyses were complete on Vario-EL III (CHNS) GmbH Elemental Analyzer. Melting degrees were recorded on Sturat Scientific instrument SMPLU-K Model are uncorrected for the prepared compounds.

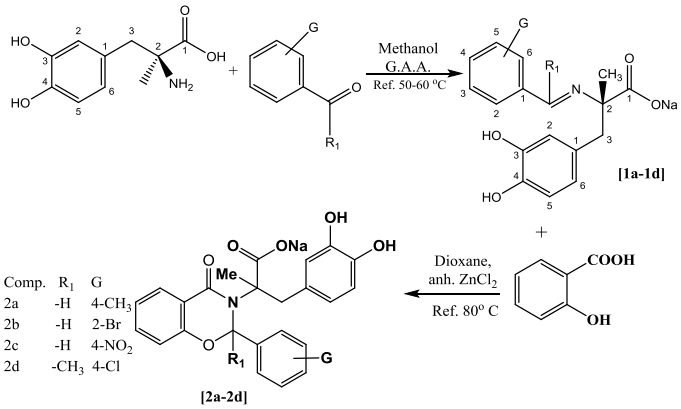
2.1. Procedure for the Synthesis of Schiff Bases Compounds 1(a-d)

Schiff base derivatives under research were designed according to the method formerly qualified in literature with

some modifications[13]. A solution of *(S)*-2-amino-3-(3,4-dihydroxyphenyl)-2-methylpropanoic acid (0.0142 mol) prepare a separate solution by dissolving (3g) with (0.0142mol, 0.58g) sodium hydroxide in (30ml) methanol, was added to 100 mL round bottom flask two-necks containing substituted aromatic aldehyde or ketone solution (0.0142mol) dissolved in (30mL) methanol with 5 drops glacial acetic acid was added drop wise to mixture reaction in flask. The colored mixture was heated under reflux with stirred in water bath at (50-60^{o}c) for an appropriate period. After that, the obtained mixture reaction was cooling and the colored solution was filtered and evaporated under reduced pressure until an oily like syrup the oily crude product was dissolved in minimum amount of absolute ethanol and precipitated by the addition of acetone the final precipitated product was filtered while washed thoroughly by ether and acetone, and leaved to dry at room temperature. The end reaction of the synthesized compounds was monitored by TLC using silica gel as stationary phase and ethanol cyclohexane (8:2) mobile phase (Scheme 1).

2.2. Procedure for the Synthesis 1,3-Oxazin-4-one 2(a-d)

1,3-oxazin-4-one derivatives under research were designed according to the method formerly qualified in literature [14], with some modifications. A mixture solution of salicylic acid (0.01 mol, 1.4 g) in hot dry (30 mL) 1,4-dioxane in presence of (0.01 mol, 1.363 g) anhydrous zinc chloride ($ZnCl_2$) (0.01 mol) was placed two-neck round bottom flask with condenser and stirred with heat for 15 minutes to completely dissolve the reactants, gradually add the solution of Schiff bases [1(a-d)] dissolved in dry hot (30 mL) 1,4-dioxane to the mixture for another 15 minutes. The reaction mixture was refluxed in bath water at 80^{o}c for an appropriate period, then allowed to cool to room temperature with stirred for overnight. The end of the reaction was checked by TLC (EtOAc:Toluene, 2:3). The solvent was evaporated under reduced pressure for one-third of solvent and separated precipitation was filtered and re-crystallized from ethanol (Scheme 1).



Scheme 1: Step by step method for the preparation [1,3]-benzoxazine-4-one compounds

Sodium (2*R*)-3-(3,4-dihydroxyphenyl)-2-methyl-2-(4-oxo-2-(p-tolyl)-2*H*-benzo[e][1,3] oxazin-3(4*H*)-yl)propanoate [2a]

Yield 70%, brown solid, mp = 230°C; R_f = 0.85 (EtOAc:Toluene 2:3); FT-IR (KBr) υ_{max}: 3464.8 (O-H), 3065.7 (C-H ar.), 2966.5, 28 69.7 (C-H al.), 1673.39 (C=O_{lactam}), 1610.4, 1369.5 ($COO^-_{as\ \&\ sy}$), 1589.9, 1513.5 (C=C ar.), 1313.43 (C-O-C), 1246.1 (C-N), 1088.56 (C-O), 727.12, 830.4 (C-$H_{oop.}$ ar.) cm^{-1}. ^{1}H-NMR (ppm) δ_H: 1.37 (s, 3H, -CH_3), 2.32 (s, 3H, Ar-CH_3), 3.11-3.29 (dd, *J*=12.9, 1.7Hz1H) (2H, -CH_2-), 6.47 (s, 1H, -N-CH-Ar), 5.95- 7.37 (m, 11H, Ar-H), 8.62 (s, 2H, Ar-OH). 13C NMR (ppm) δ_C : 22.43 (Ar-CH_3), 25.95 (-CH_3), 43.35 (-CH_2-), 69.75 (N-C-Me), 88.86 (N-C-O), 115.48, 116.22, 121.56, 128.81, 116.56, 118.06, 124.01, 128.63, 133.52, 157.21, 126.43, 130.73, 134.11, 138.78 (C-Ar), 144.63, 145.49 (Ar-OH), 168.54 (-CO_{lactam}), 181.33 (-$CO_{carboxylate}$). Elemental analysis: $C_{25}H_{22}NNaO_6$ (455.44). Calculated (%): C, 65.93; H, 4.87; N, 3.08. Experimental (%):C, 66.07; H, 4.83; N, 3.11.

Sodium(2*R*)-2-(2-(2-bromophenyl)-4-oxo-2*H*-1,3Benzooxazin-3(4*H*)-yl)-3-(3,4-dihydroxyphenyl)-2-Methylpropanoate [2b]

Yield 68%, gray solid, mp = 120°C; R_f = 0.72 (EtOAc:Toluene 2:3); FT-IR (KBr) υ_{max}: 3438.39 (O-H), 3071.7 (C-H ar.), 2924.8, 2854.3 (C-H al.), 1686.5 (C=O_{lactam}), 1592.8, 1375.4 ($COO^-_{as\ \&\ sy}$),1566.4, 1528.2 (C=C ar.), 1302.51 (C-O-C), 1240.3 (C-N), 1081.6 (C-O), 1031.7 (=C-Br), 831.91, 705.59 (C-$H_{oop.}$ ar.) cm^{-1}. ^{1}H-NMR (ppm) δ_H : 1.44 (s, 3H, -CH_3), 2.81-2.99 (dd, *J*=12.5, 1.8Hz, 1H) (2H, -CH_2-), 6.63 (s, 1H, -N-CH-Ar), 6.79- 7.64 (m, 11H, Ar-H), 9.00 (s, 2H, Ar-OH). ^{13}C-NMR (ppm) δ_C: 23.06 (-CH_3), 42.73 (-CH_2-), 66.49 (N-C-Me), 84.41(N-C-O), 127.88 (C-Br), 115.56, 116.34, 122.55, 129.44, 117.25, 119.32, 124.52, 128.79, 133.50, 158.00, 128.20, 129.92, 131.96, 132.90, 133.99 (C-Ar), 144.55, 146.88 (Ar-OH), 164.80 (-CO_{lactam}), 181.07 (-$CO_{carboxylate}$). Elemental analysis: $C_{24}H_{19}BrNNaO_6$

(520.31). Calculated (%): C, 55.40; H, 3.68; N, 2.69. Experimental (%):C, 55.26; H, 3.50; N, 2.61.

Sodium (2R)-2-(2-(4-chlorophenyl)-2-methyl-4-oxo-2H-1,3-benzooxazin-3(4H)-yl)-3-(3,4-dihydroxyphenyl)-2-Methylpropanoate [2c]

Yield 60%, black solid, mp = 110°C Dec.; *Rf* = 0.83 (EtOAc:Toluene 2:3); FT-IR (KBr) υ_{max}: 3489.4 (O-H), 3065.98 (C-H ar.), 2926.0, 2855.6 (C-H al.), 1676.6 (C=O_{lactam}), 1612.0, 1356.7 ($COO^-_{as\ \&\ sy}$), 1542.7, 1519.2 (C=C ar.), 1306.3 (C-O-C), 1230.6 (C-N), 1080.9 (C-O), 1052.4 (=C-Cl), 837.34, 719.97 (C-$H_{oop.}$ ar.) cm^{-1}. ^{1}H-NMR (ppm) δ_H : 1.39 (s, 3H, -CH_3), 2.67-2.73 (dd, *J*= 12.1, 2.0Hz, 1H) (2H, -CH_2-), 6.56 (s, 1H, -N-CH-Ar), 5.73- 7.56 (m, 11H, Ar-H), 8.71 (s, 2H, Ar-OH). ^{13}C NMR (ppm) δ_C: 24.56 (-CH_3), 28.69(O-C-CH_3), 44.89 (-CH_2-), 66.20 (N-C-Me), 95.24 (N-C-O), 135.20 (C-Cl), 115.40, 116.57, 121.64, 129.49, 117.35, 118.80, 123.47, 128.79, 132.93, 156.32, 127.28, 129.80, 140.02(C-Ar), 144.51, 145.24 (Ar-OH), 163.01 (-CO_{lactam}), 183.53 (-$CO_{carboxylate}$). Elemental analysis: $C_{25}H_{21}ClNNaO_6$ (489.88). Calculated (%): C, 61.30; H, 4.32; N, 2.86. Experimental (%):C, 61.09; H, 4.46; N, 2.67.

Sodium (2R)-3-(3,4-dihydroxyphenyl)-2-methyl-2-(2-(4-nitrophenyl)-4-oxo-2H-1,3-benzooxazin-3(4H)-yl) propanoate [2d]

Yield 72%, brown solid, mp = 170°C Dec.; R_f = 0.66 (EtOAc:Toluene 2:3); FT-IR (KBr) υ_{max}: 3441.8 (O-H), 3089.3 (C-H ar.), 2924.8, 2860.1 (C-H al.), 1673.4 (C=O_{lactam}), 1601.6, 1354.8 ($COO^-_{as\ \&\ sy}$), 1513.5, 1466.5 (C=C ar.), 1440.0, 1319.6 (=C-$NO_{2\ as\ \&\ sy}$), 1296.0 (C-O-C), 1252.0 (C-N), 1084.6 (C-O), 875.98, 787.85 (C-$H_{oop.}$ ar.) cm^{-1}. ^{1}H-NMR (ppm) δ_H : 1.36 (s, 3H, -CH_3), 1.93(s, 3H, CH_3- C-N-) 2.99-3.21(dd, *J*=13.0, 1.9Hz, 1H) (2H, -CH_2-), 6.43-7.39 (m, 11H, Ar-H), 8.60 (s,2H,Ar-OH). ^{13}C-NMR (ppm) δ_C : 23.29(-CH_3), 41.60(-CH_2-), 65.60(N-C-Me), 85.85(N-C-O), 149.23 (C-NO_2), 115.49, 116.16, 122.32, 128.68, 116.76, 118.46, 123.51, 128.07,133.28,157.66, 124.48,128.91,140.28 (C-Ar), 144.59,145.36(Ar-OH), 164.89(-CO_{lactam}), 177.88(-$CO_{carboxylate}$). Elemental analysis: $C_{24}H_{19}N_2NaO_8$ (486.41). Calculated (%): C, 59.26; H, 3.94; N, 5.76. Experimental (%):C, 59.1; H, 3.88; N, 5. 67.

2.3. Biological Evaluation

2.3.1. Antibacterial Activity Test:

Nutrient agar plates were prepared using standard procedures with commercially available Nutrient agar (Acumedia/LAB).

In order to assess the effectiveness of the compounds prepared as antibacterial, four types of bacteria were used in this study to study the effect of bacterial growth inhibition rates (*Klebsielsa pneumoniae*), (*Escherichia coli*) as gram-negative and (*Staphylococcus aureus*), (*Pseudomonas aeroginosa*) as gram-positive were used, and the method of (Kirby-Bauer) [15] in the test of sensitivity was transferred (4) colonies of the previous types of bacteria to the nutrient medium (Nutrient Agar) and incubate the medium (37°C) for (15-16 hours) and dilute the saline solution (Normal saline) and then transferred (0.1 mL) from the bacterial suspension to the nourishing media (Nutrient Agar) and spread on the surface of the dish, the dishes left about 30 minutes.

To measure the inhibitory effect of the prepared chemical compounds, tablets were taken from the filter flask and then immersed in solutions of different concentrations of the compounds whose antibacterial activity is to be studied. A solvent (dimethyl sulfoxide) was used to prepare the solutions of the chemical compounds studied and then the tablets saturated with the solutions were distributed on the surface of the agar at suitable distances and incubated for (20-24 hours). The antibiotic (Cefotaxime) was used as a reference to inhibit the bacteria of the four previous species based on what is used in the public health laboratory and approved by WHO tests. The inhibition area was measured and the experiment was repeated three times and then the measurement rate was taken.

2.3.2. Antifungal Activity Test:

The agar dilution method described by the literature for antifungal activity estimation with slight modification. The method was utilized for determining the inhibition of mycelial radial growth of the test organisms by the prepared compounds. Both types are pathological for fungal testing were cultured on Potato-Dextrose-Agar (PDA), prepared according to the specifications of the manufacturers by dissolved 39g from PDA in 1L distilled water then sterilize the solution by autoclave for 20 minutes at 121°C and press 15 lb/ang^2 and cooling to 45°C in water bath, then let the flask to cool and add the ampicillin antibiotic 42 mg/L to prevent bacterial growth. The prepared compounds were dissolved in DMSO and sterile filter papers are saturated with these different concentrated solutions. Working in a laminar flow cabinet, the medium was poured into 90mm sterile plastic Petri dish, at a temperature of 40-45°C and allowed to set. The center of each test plate was subsequently inoculated with a 6mm size plug of 5 days old cultures, for each of the pathogens separately. A plate containing only the 6mm size plug of fungi the basal medium served as control. Plates were incubated for 5 days at 25 °C in a growth cabinet. Each assay was replicated two times and the screening procedure repeated twice. Radial mycelial growth was evaluated each day during 5 days by calculating the mean of two perpendicular colony diameters for each replicate. The values were expressed in

millimeters diameters day and was calculated as percentage mycelial growth inhibition according to the formula [16]:

$$\% \text{ mycelial growth inhibition} = \frac{G_{Control} - G_{Test}}{G_{Control}} * 100$$

Where:

$G_{Control}$ = Average diameter of the fungal colony of the control.

G_{Test} = Average diameter of the fungal colony treated with the prepared compounds.

2.3.3. Docking Study

The docking is a method whose supplies to confirm bound safety with interaction confuses of ligand within the aim protein [17]. To support with assign the aim of antibacterial activity of new prepared benzo-[1,3]-oxazin-4-one derivatives, two various aim proteins is PDB ID 3FYV (dihydrofolate reductase) from *Staphylococcus aureus* and PDB ID 4H2M (undecaprenyl diphosphate synthase) from *E. coli* were got of the PDB [18]. The prepared compounds appeared interaction and binding affinities with target proteins in emulation via MOE 2009 [19]. Before simulation, constructions of prepared derivatives were structure and save as the 3D conformation via Discovery Studio 4.0 Client. The geometry optimization of synthesized compounds execute utilizing density functional theory (DFT) in the B3LYP hybrid selected the criterion 6-31G+(d,p) basis set by utilizing Gaussian 09W program package [20] , with the MMFF94 force-field minimization was employed for prepared compounds. Furthermore, for aim protein was protonated, charged and minimized energy via MOE 2009. The single chain of 3FYV and a single chain of 4H2M without H_2O molecules were chosen for the valuation of novel prepared derivatives. For simulation, default MOE parameters any derivatives. As an outcome, produce folder as mdb were created including simulation docking outcomes and scoring with various modification of ligands. Finally, outcomes were searched to locate the maximum powerful with active antibacterial inhibitor through visualizing different interaction of ligands with active side.

3. RESULTS AND DISCUSSION

Scheme 1 appears the synthetic path for the new benzo-1, 3-oxazine-4-one compounds. Stage 1and 2 are as notified in the step by step made method and includes condensation reaction of the substituted aromatic aldehydes or ketone with the amines to result azomethine compounds 1(a-d) follow by next reaction with solute salicylic acid during 1,4-dioxane in presence of anhydrous zinc chloride to the corresponding wanted compounds 2(a-d). The reaction methods in first and second steps were achieved utilizing FT- infrared spectroscopy. Manifestation of the band in the field 1596.09-1646.24 cm^{-1} in the infrared spectrum of the Schiff bases 1 (a-c) is suitable to C=N indicates condensation, the manifestation for region 1673.39-1686.5 cm^{-1} in the infrared spectrum of the prepared compound 2 (a-c) is due to $C=O_{lactam}$ with disappear the band of C=N bonds which is due results of ring-closure reaction leading to the aim 1,3-benzooxazine compounds.

The confirmed structures of the prepared compounds were determined depending on the bases of their spectroscopy datum (FT- infrared, ^{1}H-NMR, & ^{13}C-NMR) with analysis of the elements. The prepared compounds appeared suitable special signals needful to prove structures. FT- infrared spectrum presented absorption bands proper to mono- and di-substituted benzene rings in the area 705.59-875.98 cm^{-1} and 1252.0-1230.6 cm^{-1} are special absorptions of C-N in oxazine derivatives. As well as bans observed comprise that stretching for aromatic hydrogen asymmetric vibration during the area 3065.7-3089.3cm^{-1}, in the area 1296.0-1313.43$cm^{-1}$for C-O-C asymmetric stretching and aliphatic stretching bands through 2854.3 and 2966.5 cm^{-1}.

^{1}H-NMR spectrum for the prepared oxazine compounds presented appropriate for the aromatic proton with the proton shifts proper of the (O-CH-N) group are needful to emphasize the structure for oxazine membered system. ^{13}C-NMR for the prepared compounds proves the existence for chemical shifts of carbon identical the group O-CH-N as included in the spectral data set.

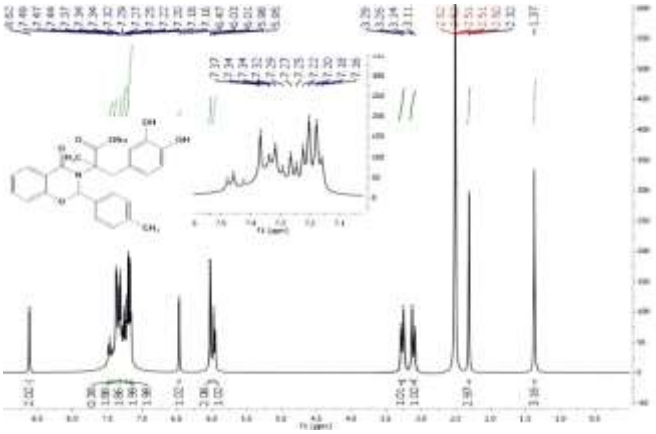


Fig:1: ^{1}H-NMR spectrum of (2a).

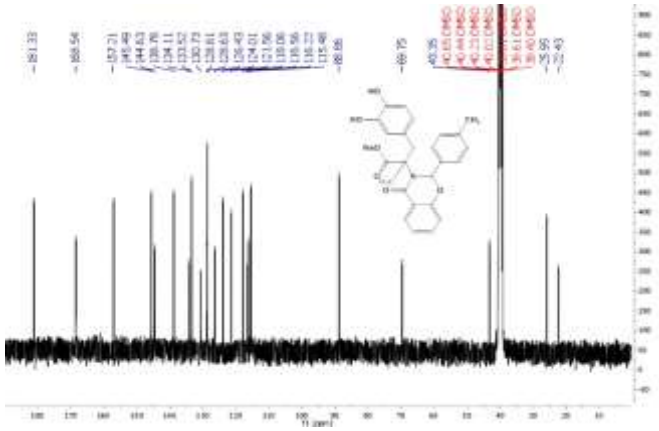


Fig:2: ^{13}C-NMR spectrum of (2a).

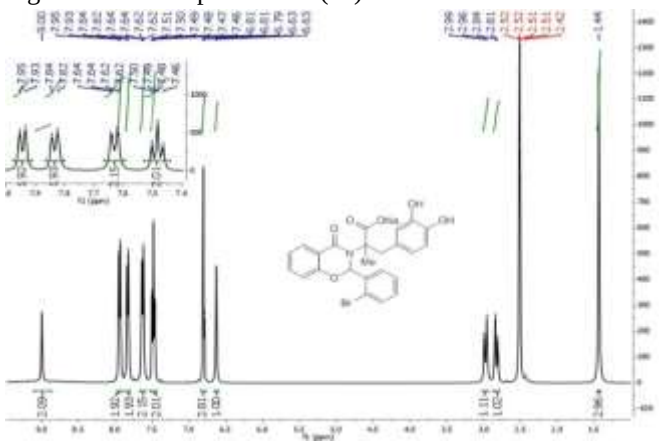


Fig:3: ^{1}H-NMR spectrum of (2b).

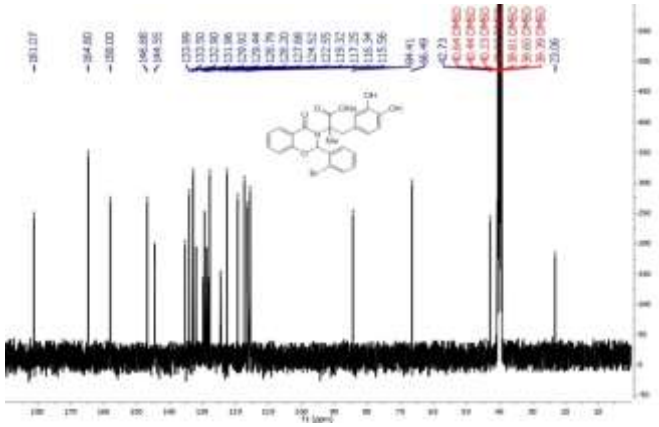


Fig:4: ^{13}C-NMR spectrum of (2b).

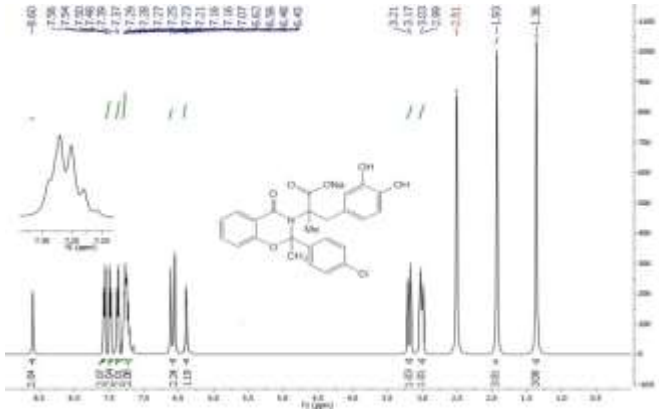


Fig:5: ^{1}H-NMR spectrum of (2c).

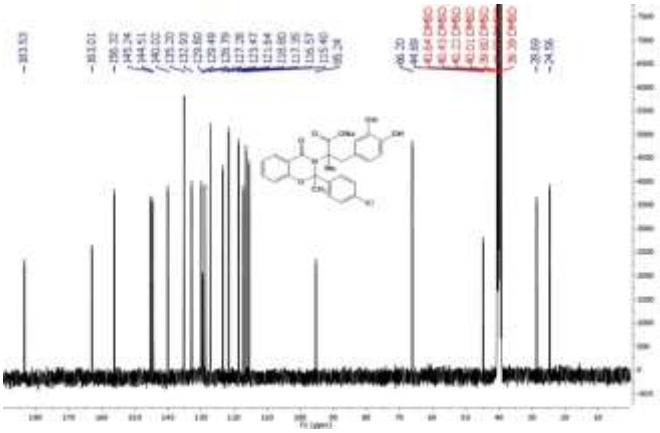


Fig:6: ^{13}C-NMR spectrum of (2c).

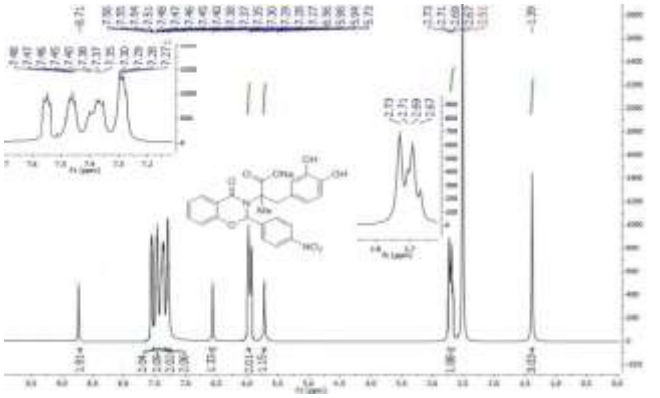


Fig:7: ^{1}H-NMR spectrum of (2d).

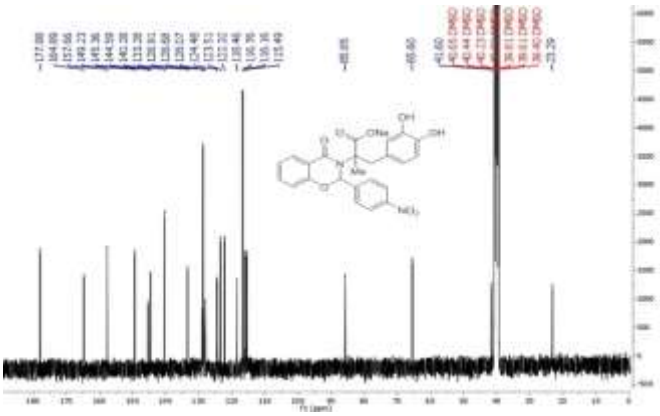


Fig:8: ^{13}C-NMR spectrum of (2d).

3.1. Antibacterial Activity

Tables 1 & 2 give the outcome of the antimicrobial activity versus two strains of Gram positive bacteria and two strains of Gram negative bacteria as confronted to Cefotaxime sodium was as the norm. The outcomes compound 2d was show excellent bioactivity against *Escherichia coli*, *Pseudomonas aeruginosa* (Gram negative), also give excellent activity against *Staphylococcus aureus* and *Streptococcus spp.* (Gram positive). The compound 2c give perfect action opposed to for GNB under study with also perfect bioactivity for GPB. As well as the other both compounds show a moderate activity

against of all types under study, while the Compound 2a presented weak activity against *Staphylococcus aureus* and *Streptococcus spp.* when the concentration 1.25 mg/mL.

3.2. Antifungal Activity

The prepared compounds show good and varying inhibition activity against fungal growth. Tables 3 give the result of the antifungal activity against two types of fungi. From the outcome, the prepared compounds were found to exhibit moderate activity against *Nattrassia mangiferae* when concentration 25 mg/mL except compound 2a was weak activity. Also, the synthesized compounds show acceptable Inhibition against *Nattrassia mangiferae* when concentration 12.5 mg/mL except compound 2a was weak activity. While, the all compounds show weak inhibition against *Nattrassia mangiferae* when concentration 5 mg/mL.

The prepared compounds were found to exhibit moderate activity against *Aspergillus niger* when concentration 25 mg/mL except compound 2a was acceptable activity. Also, the both 2c & 2d compounds show acceptable Inhibition against *Aspergillus niger* when concentration 12.5 mg/mL whilst, the both 2a & 2b compounds were weak activity. While, the all compounds show weak inhibition against *Aspergillus niger* when concentration 5 mg/mL. The control DMSO was however exhibtit no activity against all the fungi under study.

Table 1: Antibacterial action data against GNB

	Gram negative (GNB)*					
	Escherichia coli			*Pseudomonas aeruginosa*		
	Concentration (mg/mL)			**Concentration** (mg/mL)		
compound	1.25	2.5	12.5	1.25	2.5	12.5
2a	20	26	**	18	23	**
2b	23	28	**	20	24	**
2c	22	30	**	21	28	**
2d	27	31	**	25	30	**
Standard***	32	36	**	29	34	**

Table 2: Antibacterial action data against GPB

	Gram positive (GPB)*					
	Staphylococcus aureus			*Streptococcus spp.*		
	Concentration (mg/mL)			**Concentration** (mg/mL)		
compound	1.25	2.5	12.5	1.25	2.5	12.5
2a	9	14	**	11	17	**
2b	14	19	**	12	18	**
2c	13	18	**	13	20	**
2d	16	20	**	16	22	**
Standard***	18	23	**	20	25	**

*All results are in millimeter (mm). ** No bacterial growth was observed.
*** Cefotaxime sodium.

Table 3: Percentage mycelial growth inhibition data of prepared compounds

	Nattrassia mangiferae			*Aspergillus niger*		
	Concentration (mg/mL)			**Concentration** (mg/mL)		
Compound	5	12.5	25	5	12.5	25
2a	31	39	46	35	42	54
2b	39	51.5	62	38	43	60.5
2c	40.5	52	66.5	42	55	68.5
2d	43.5	55.3	65	39	52.7	67
Control DMSO	*	*	*	*	*	*

* No effect on the growth of the fungus was observed.

3.3. Result of Docking Study

In silico studies were completed for all prepared benzo-[1,3]-oxazin-4-one compounds so as to foretell their closeness to bacterial protein 4H2M from *E. coli* and 3FYV from *S. aureus*. Analysis of the docking demonstrates for all prepared [1,3] benzooxazin-4-one derivatives displayed that they inhabit the different domains of 4H2M & 3FYV binding pocket with perfect in docking interaction scores (Table 4). In addition, explained in the empirical outcomes, docking studies of benzo-[1,3]-oxazin-4-one derivatives uncovered their ability as antibacterial restraints. All recently prepared derivatives consist aromatic rings, showing remarkable hydrophobic interactions along with the crucial residues. However, docking outcomes signalized that lately prepared derivatives, principally 2d and 2c, showed hydrophobic interactions with 4H2M (E. coli) in the residues [(arene-arene His43, arene-cation Leu85, metal contact Phe70, backbone acceptor Ala69, backbone acceptor Tyr145) in 2d and (arene-arene His43, arene-cation Arg39, backbone donor Met25) in 2c] for inhibitor activity of this protein because the existence of electronegative operations. Unluckily, the two derivatives 2b and 2a were weak inhibitor, display interaction because require of appropriate in the bore of the aim protein, consequent to the turnout of bulky group (R-) that make steric hindering. The 2b compound connected with target protein in junctions (arene-arene His43, sidechain acceptor His43 & arene-cation Arg39), while the 2a compound binded in spots (arene-arene His43 & back bone acceptor Tyr145) as shown in the figures (9-12).

In general, the docking outcomes study indicates that the synthesis of the prepared compounds with 3FYV is less than 4H2M and this demonstrates the weak effectiveness towards Gram positive bacteria. The 2d compound is associated with 3FYV at the sites (sidechain donor Asn18, sidechain acceptor Ser49 & backbone acceptor Leu20), the 2c compound is associated at (arene-arene Phe92, metal contact Thr46 & backbone acceptor Ile50). While, the 2b compound is binding

(sidechain acceptor Ser49, metal contact Thr46 & backbone acceptor Ala7), the 2a compound is contacting at (sidechain acceptor Lys45, sidechain donor Asn18 & Ser49) as shown in the figures (13-16). The prepared compounds appeared good interaction and binding affinities with target proteins in emulation via MOE 2009.

Table 4: Binding free energy (ΔG_{bind}) with bacteria protein and hydrophobic contacts (from molecular docking) in ligands 2a, 2b, 2c & 2d

Com.	B. P.	ΔG_{bind} (kcal/mol)	Hydrophobic contacts
2a	4H2M (*E. coli*)	-7.41	His43, Tyr145
2b	4H2M (*E. coli*)	-9.17	His43, Arg39
2c	4H2M (*E. coli*)	-11.77	His43, Arg39, Met25
2d	4H2M (*E. coli*)	-15.56	His43, Leu85, Phe70, Ala69, Tyr45
2a	3FYV (*S. aureus*)	-6.70	Lys45, Ans18, Ser49
2b	3FYV (*S. aureus*)	-7.39	Ser49, Thr46, Ala7
2c	3FYV (*S. aureus*)	-10.17	Phe92, Thr46, Ile50
2d	3FYV (*S. aureus*)	-12.83	Asn18, Ser49, Leu20

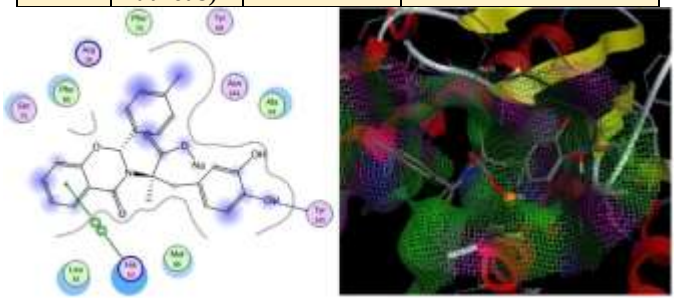


Fig:9: Interaction model for the compound 2a with *E. coli* (PDB ID.4H2M).

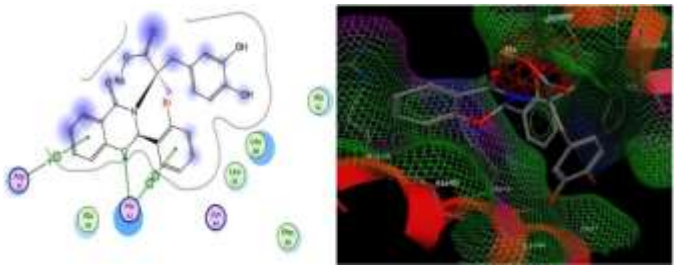


Fig:10: Interaction model for the compound 2b with E. coli (PDB ID.4H2M).

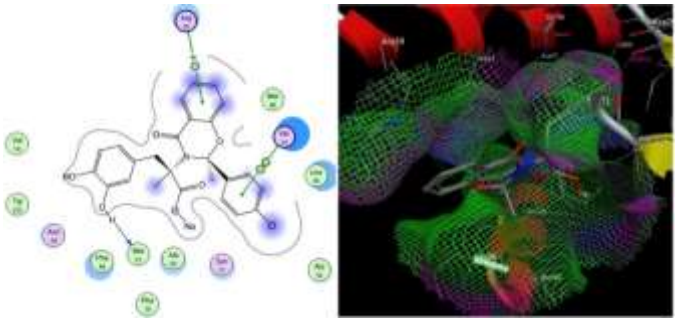


Fig:11: Interaction model for the compound 2c with E. coli (PDB ID.4H2M).

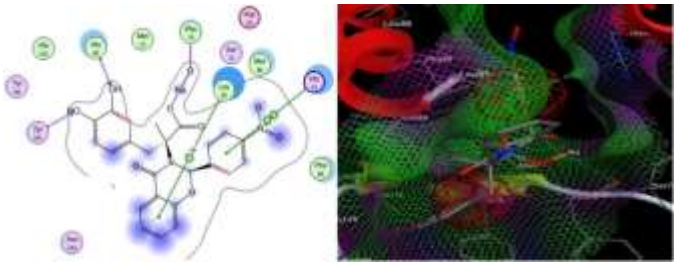


Fig:12: Interaction model for the compound 2d with E. coli (PDB ID.4H2M).

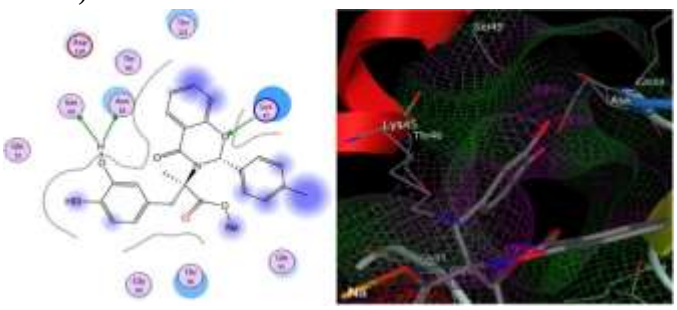


Fig:13: Interaction model for the compound 2a with S. aureus (PDB ID.3FYV).

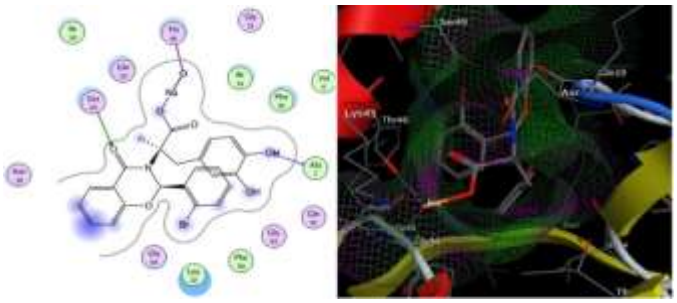


Fig:14: Interaction model for the compound 2b with S. aureus (PDB ID.3FYV).

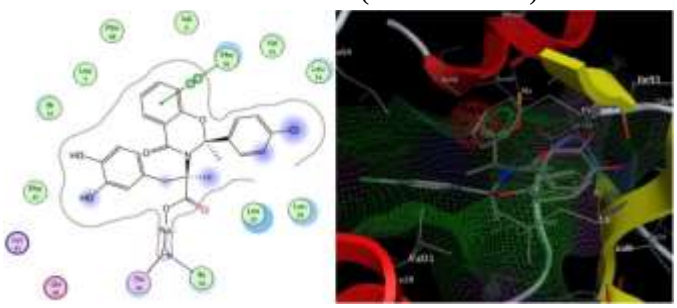


Fig:15: Interaction model for the compound 2c with S. aureus

(PDB ID.3FYV).

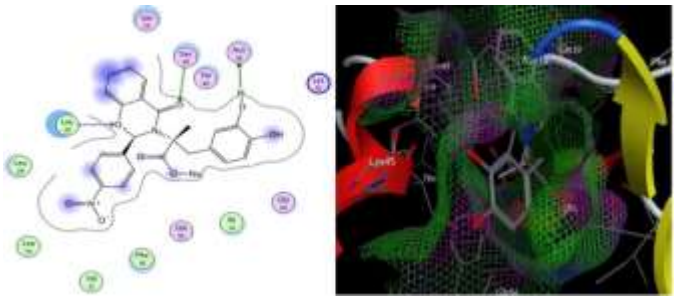


Fig:16: Interaction model for the compound 2d with S. aureus (PDB I D.3FYV).

4. CONCLUSIONS

Successfully, novel 1,3-benz-oxazine-4-one compounds were prepared and characterized utilizing a modified method in which salicylic acid was in presence of anhydrous zinc chloride ($ZnCl_2$) for cyclization reaction. The prepared derivatives were subsequently estimated for against bacterial action for two strains of GPB and two strains GNB. Generality of the prepared derivatives display hopeful against bacterial action of the strains under study. Also, the prepared compounds were evaluated for antifungal activity against for two type's fungi. Most of the prepared compounds display good antifungal activity against of *Nattrassia mangiferae* and *Aspergillus niger*. While some compounds were gradually from moderate to weak activity against of the fungi under exam. The docking study detects that, the only two compounds under study (2d and 2c) have a higher binding affinity with target proteins (3FYV & 4H2M). Whereas, the compounds (2b and 2a) were shown leas binding scores affinity with these proteins. This was further assured through its in vitro biological activity data.

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تحضير وتقييم الفعالية المضاد للميكروبات والالتحام الجزيئي لمشتقات [1,3] بنزووكسازاين-4- ون المستبدلة في الموقع 2,3 الجديدة

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الخلاصة:

تم تحضير مشتقات جديدة من [1,3] بنزووكسازاين–4– ون المستبدلة في الموقع 2,3 عن طريق إجراءات التفاعلات المباشرة لحامض الساليسيليك مع قواعد شيف المستبدلة المحضرة مسبقاً لغرض تكوين الحلقة السداسية المدمجة. شخصت تراكيب المركبات المحضرة وتم تأكيدها من خلال طرق التشخيص الطيفي كالتحليل الدقيق للعناصر وطيف الاشعة تحت الحمراء (FT–IR) وطيف الرنين النووي المغناطيسي للبروتون (^{1}H–NMR) وطيف الرنين النووي المغناطيسي للكاربون (^{13}C–NMR). تم تقدير الفعالية الحيوية للمركبات المحضرة في المختبر كمضادات للنمو البكتيري وكمضادات لنمو الفطريات وتمت الدراسة باستخدام اثنين من الأجناس الموجبة لصبغة جرام واثنين من الأجناس السالبة لصبغة جرام بالمقارنة مع سيفوتاكسيم الصوديوم كدواء مرجعي للمقارنة، كما تم تقييم الفعالية الحيوية للنمو الفطري باستخدام نوعين من الفطريات. أظهرت المركبات المحضرة فعالية كمضادات للميكروبات تراوح مدى تثبيطها من المتوسط إلى الممتاز ضد سلالات البكتيريا مع نسبة جيدة من النشاط التثبيطي لنمو الفطريات. تم دراسة الالتحام الجزيئي للمركبات المحضرة واظهرت الدراسة تأثير المجموعات المتنوعة من المستبدلات على النشاط البيولوجي في الموقع الفعال للبروتينات قيد الدراسة مع ظهور المعلمات الاساسية دلائل غير ملائمة عند رسم الليكاند مع الجزء الفعال في البروتين ان المستبدلات المختلفة قد اعطت كفاءة إضافية في النشاط الحيوي للمركبات المحضرة.

الكلمات المفتاحية: [1,3] بنزووكسازاين ، قواعد شيف ، حامض الساليسيليك ، الالتحام الجزيئي ، المثيل دوبا، Undecaprenyldiphosphate synthase ,

Dihydrofolate reductasa,