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DESIGN, SYNTHESIS AND MOLECULAR DOCKING STUDY OF NOVEL BIS-OXAZOLONE DERIVATIVES AS POTENT ANTIOXIDANT AND ANTIBACTERIAL AGENTS

Jalal Abdulla Haji[1,2*], Lana Hadi Chawishli[2] and Mohammed Kareem Samad[2]

[1]Department of Chemistry, College of Education, Salahaddin University-Erbil, Erbil, Kurdistan, Iraq
[2]Department of Chemistry, College of Science, Garmian University-Kalar, Kurdistan, Iraq

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ABSTRACT. In response to the challenge of antibiotic-resistant microorganisms, oxazolone analogs are frequently used for bacterial, antiviral, and anti-inflammatory treatments. However, few studies have shown bis-oxazolone analogs possess antibacterial activities. In this study, we modified bis-oxazolone molecules with various aromatic amines to create new bis-benzamide and bis-imidazolone derivatives. These derivatives were analyzed using FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy. Molecular docking revealed favorable interactions with DNA gyrase, with compounds 3, 4a, and 5e showing higher binding affinities than penicillin G and ampicillin. These findings suggest their potential as future antimicrobial agents. The tested compounds demonstrated efficacy against bacterial strains, particularly *E. coli* and *S. aureus*, with significant activity observed in compounds 4a, 4e, 5d, and 5e. Antioxidant activity, assessed using the DPPH method, showed bis-compounds with excellent results comparable to ascorbic acid. This encourages further studies to explore their potential. Overall, the synthesized bis-oxazolone derivatives demonstrated increased medicinal activity and high potential as future antimicrobial and antioxidant agents.

KEY WORDS: Bis-oxazolone, Bis-benzamide, Bis-imidazolone, Docking study, Antibacterial, Antioxidant

INTRODUCTION

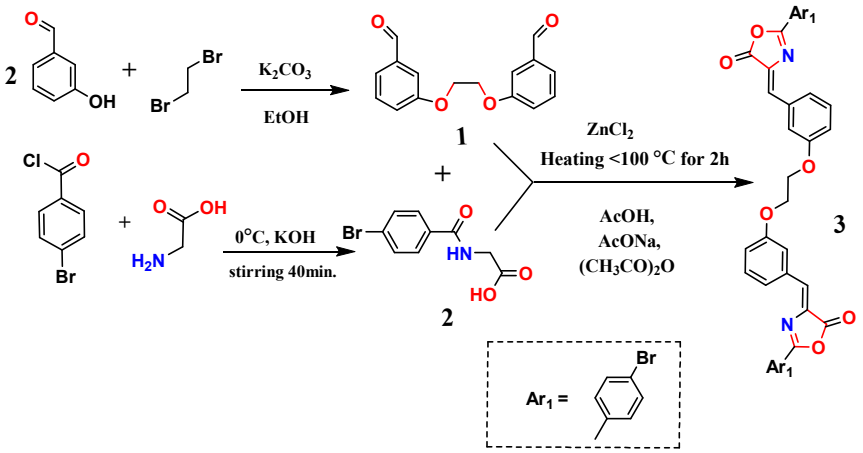
The rise in multidrug-resistant bacterial infections demands new antibacterial agents [1-3]. Oxazolone derivatives, including benzamides [4] and imidazolones [5], are versatile in medicinal chemistry [6-16], offering antimicrobial [15, 17], antiviral, anti-inflammatory [18], and anticancer activities [19, 20]. Benzamide, used in gut treatments [21], exhibits antimicrobial [22], antioxidant [23], anticonvulsant [24], antitumor [25], antifungal [26], and antiviral properties [27], acting as a histone deacetylase inhibitor [28]. Imidazolone compounds surpass conventional agents with diverse pharmacological benefits, serving as antimicrobial [29, 30], anticancer [31], anti-diabetic [8], antioxidant [23], anti-HIV [32], and anti-proliferative agents [33]. Additionally, imidazolones contribute to microfabrication, particularly in producing green fluorescent protein (GFP) [34].

RESULTS AND DISCUSSION

Chemistry

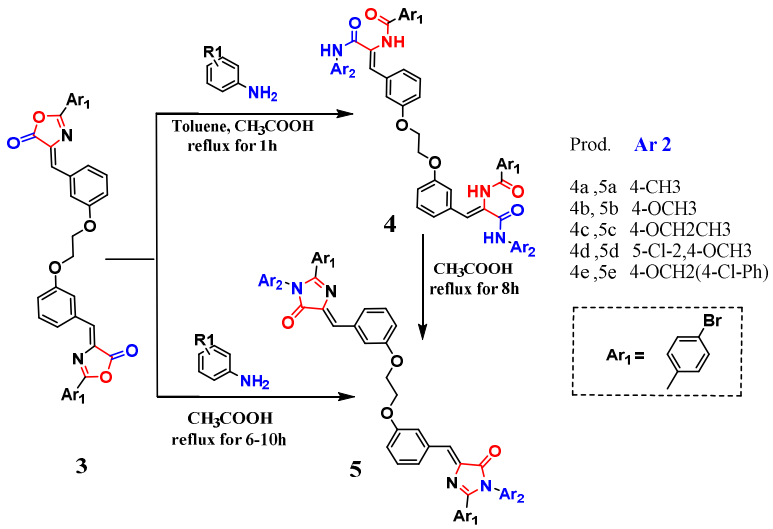
In this study, we synthesized and modified novel compounds from basic precursors to enhance their biological activity and potential as biomolecules and drug candidates. Molecular docking predicted the biological activities of ten oxazolone derivatives with different groups. Among these, compounds **3**, **4a**, **4e**, **5d**, and **5e** displayed higher antimicrobial activity against *E. coli* compared to *S. aureus* at 800 µg. Additionally, DPPH measured antioxidant scavenging, showing increased activity over time across various concentrations, comparable to ascorbic acid.

*Corresponding authors. E-mail: jalalchem80@gmail.com



Scheme 1. Synthesis of bis-oxazolones (**3**).

The Erlenmeyer reaction is commonly used for azlactone synthesis. Our modification efficiently yielded bisoxazolones (97%), offering advantages such as higher yield, shorter reaction time, and simpler workup, as shown in Scheme 1. Initially, bis-benzaldehyde (**1**) was obtained via the Williamson reaction between two moles of 3-hydroxybenzaldehyde and di-bromoethane [35], while 4-bromohippuric acid (**2**) resulted from the reaction of glycine with 4-bromobenzoylchloride [36]. Subsequently, intermediates (**1**) and (**2**) were reacted to yield amidated intermediate (**3**) [37].



Scheme 2. Synthesis of bis-benzamide (**4**) and bis-imidazolones (**5**).

Bis-oxazolone (**3**) quickly reacted with glacial acetic acid and various aromatic amines in toluene, yielding bis-benzamides (**4a-e**) [38]. Prolonged reactions with acetic acid and sodium

acetate formed bis-imidazolones (**5a-e**) [39]. FT-IR, ^{1}H NMR, and ^{13}C-NMR characterization confirmed successful synthesis (Figure 1).

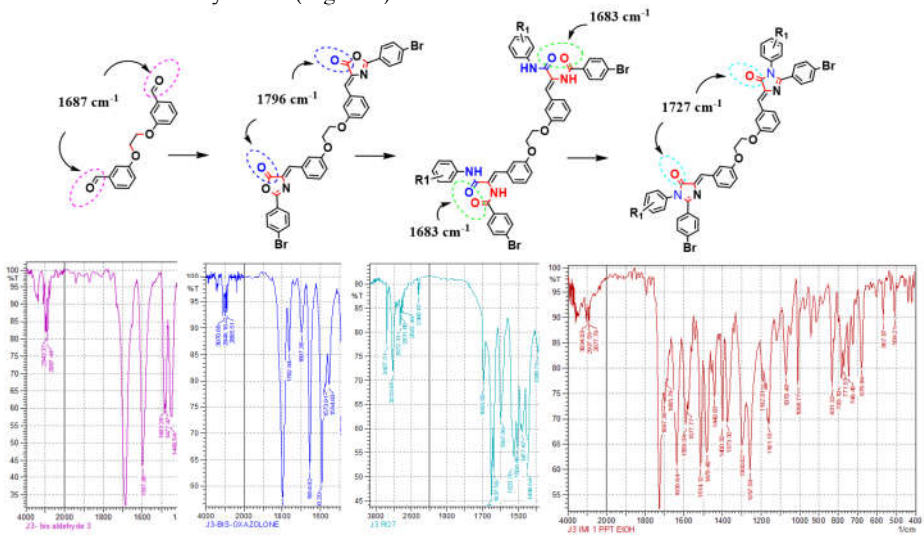


Figure 1. FT-IR spectrum charts of C=O str. shifting from bis-aldehyde compounds (**1**) to (**3**), (**4a-e**) and (**5a-e**) compounds.

Anti-bacterial activity

The compounds were tested for antimicrobial activity against *E. coli* and *S. aureus* using a dilution method [40]. Results in Figure 2 showed greater effectiveness against *E. coli* than *S. aureus* for all compounds. The strong antimicrobial effect, possibly due to increased surface area interaction with the bacteria, led to significant growth inhibition. The potency was concentration-dependent, with the highest inhibition observed at 800 µg. Notably **3**, **4a**, **4e**, **5d**, and **5e** exhibited the most significant activity, while others had varying efficacy against the bacterial strains.

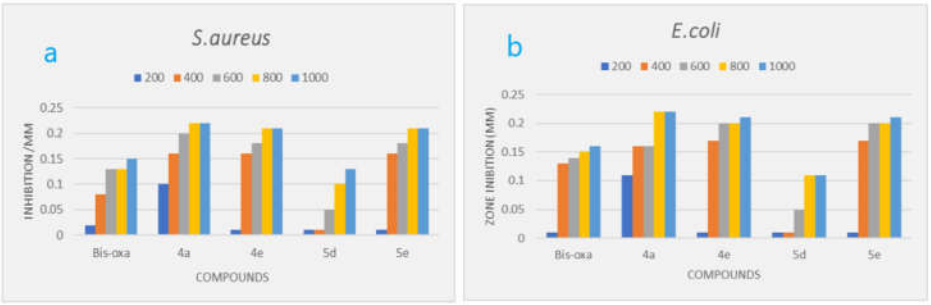


Figure 2. Antimicrobial activity of screened compounds (**3**, **4a**,**e** and **5d**,**e**) against: a. *S. aureas* and b. *E. coli*.

DPPH radical scavenging ability

DPPH, a stable organic nitrogen radical, assesses antioxidant scavenging capabilities. The deep violet color of the DPPH radical signifies its electron impairment, with radical scavenging

monitored spectrophotometrically by the decline in absorbance at 517 nm. The resulting pale yellow nonradical form is expressed as an inhibition percentage (%). Experimental results of synthesized compounds were compared with ascorbic acid. Five concentrations (200, 400, 600, 800, and 1000 µg) were tested against DPPH radicals for 30 min (Figures (3a and 3b) to illustrate radical-quenching abilities. Activity increased with concentration and reaction time up to 60 min, with no significant differences observed among tested compounds, highlighting the rapid response of OH, NH, and C=C functionalities in radical inhibition.

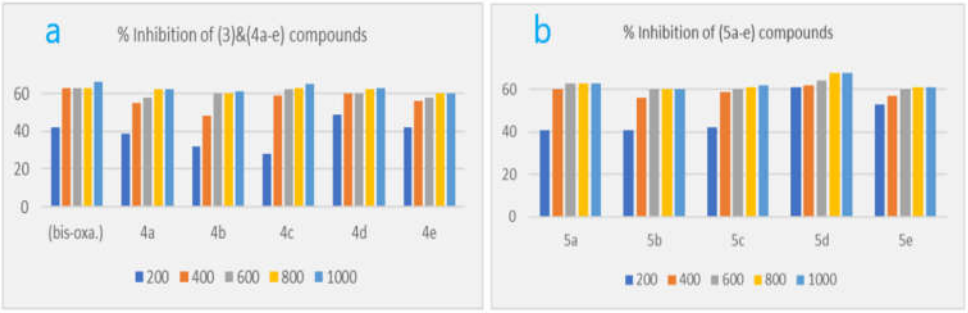


Figure 3. Antioxidant activity of: a. (**3**) and (**4a-e**) compounds, b. (**5a-e**) compounds in 30 min.

Molecular docking

Molecular docking is one of the most common methods used in structure-based drug design to analyze the interaction between a small molecule and a protein at the atomic level. Molecular docking was performed with optimized compounds targeting DNA gyrase, an ATPase vital for bacterial growth [41, 42]. It controls supercoiling processes necessary for DNA replication, chromosomal condensation, and gene expression, making it an excellent drug target [14, 43, 44].

Table 1. The compounds binding affinity.

Ligand code	Compound	Binding affinity (kcal/mol)	Ligand code	Compound	Binding affinity (kcal/mol)
3	Bis-oxazolone	-9.2	**5a**	IM1	-9.8
4a	RO1	-9.3	**5b**	IM2	-9.4
4b	RO2	-7.4	**5c**	IM3	-8.8
4c	RO3	-6.8	**5d**	IM4	-9.0
4d	RO4	-8.9	**5e**	IM5	-9.4
4e	RO5	-9.6			
Reference		-7.8			
Penicillin G		-7.7			
Ampicillin		-7.9			

The binding affinity of titled compounds was compared with ampicillin which was used as a positive drug for comparison purposes which was recommended for clinical therapeutic [45, 46]. The binding affinities of the synthesized compounds bis-oxazolone (**3**), **4a** and **5e** (-9.2, -9.3 and -9.4 kcal/mol, respectively) were higher than the binding affinity of the positive drug penicillin G and ampicillin (-7.7 and -7.9 kcal/mol, respectively) (Table 1). Thus, these three compounds could be effectively inhibiting the (DNAG) and (PBP1a), which are essential targets for the development of antibacterial medicines (Table 2, Figures 4, 5 and 6).

Table 2. Molecular docking scores and interactions of (**3**, **4a**, and **5e**) compounds.

Compound	Binding affinity (kcal/mol)	Interactions				
		H-bond	π-π T-shaped	Amide-π stacked	π-anion	π-sulfur
3	-9.2	Ser403 Ser462 Asn464 Tyr519 Lys597 Ser598	Tyr446	Arg445 Ser461	Glu602	Met641
4a	-9.3	Lys430 Asn446 Gln521 Ser598 Glu602 Ser643	Tyr446	Tyr446 His583	Tyr441 Met641 Ala642	His583
5e	-9.4	Tyr441 Asn464 Gln521 His583 Glu602	Tyr446	Met641	Tyr446 His583	Glu602

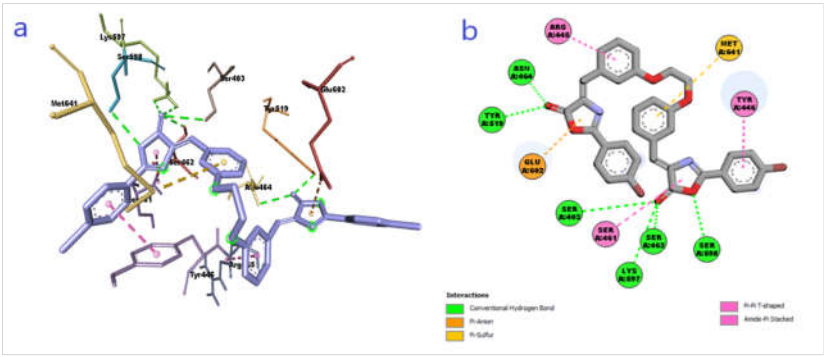


Figure 4. Interactions of compound (**3**) with active site of AChE (PBD ID: 4EY7) (a) 3D and (b) 2D.1

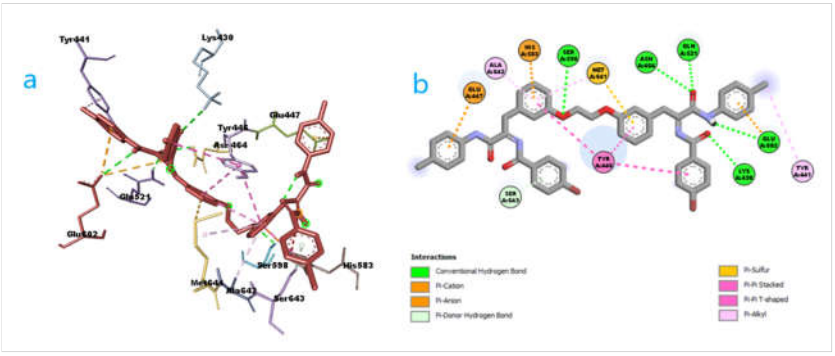


Figure 5. Interactions of compound (**4a**) with active site of AChE (PBD ID: 4EY7) (a) 3D and (b) 2D.

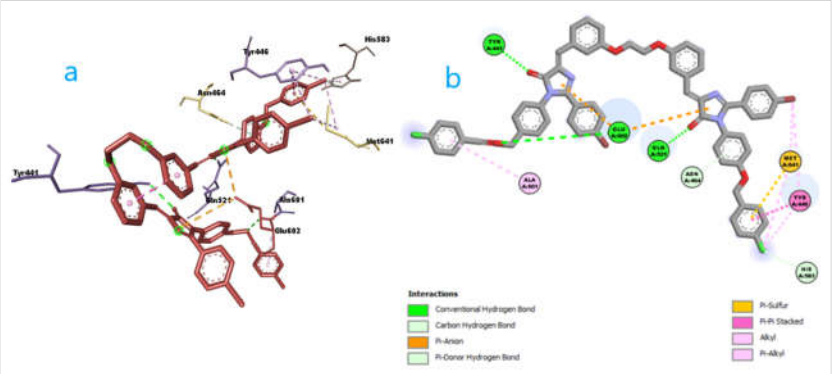


Figure 6. Interactions of compound (**5e**) with active site of AChE (PBD ID: 4EY7) (a) 3D and (b) 2D.

EXPERIMENTAL

Materials and methods

Chemicals, sourced from reputable brands such as Scharlau, Fluka, and Riedel-de Haen, were of analytical grade. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were acquired using a Bruker spectrometer at Esfahan University, Iran, with $CDCl_3$ and DMSO as solvents. Chemical shifts (δ) were referenced to TMS. Fourier transform infrared (FT-IR) spectra were recorded using an FT-IR-4100 (Shimadzu) spectrometer. Melting points were determined using an Electro-thermal melting point device 9100. TLC, employing Merck silica gel 60 F254 plates and n-hexane/ethyl acetate as a mobile phase, was utilized for purity checks with UV visualization.

*Synthesis of 3,3'-(ethane-1,2 diylbis(oxy)) dibenzaldehyde (**1**)*

In a round-bottom flask, *m*-hydroxybenzaldehyde (3.05 g, 0.025 mol) and anhydrous potassium carbonate (7.59 g, 0.055 mol) in 98% ethanol (100 mL) were refluxed for 20 min. 1,2-Dibromoethane was added and stirred, followed by refluxing for 6 hours. Reaction completion was monitored via TLC (2:8 EtOAc:hexane). The mixture was then poured into cold distilled water (150 mL) to yield a bright brown precipitate. After filtration and washing with a 1:1 cold water-ethanol mixture, recrystallization from a 1:4 toluene-ethanol mixture produced a milky-brownish precipitate of bis-aldehyde [35].

$C_{16}H_{14}O_4$: yield: 90.19%, m.p. found (155-157) °C, IR (KBr, cm^{-1}): 1687 (C=O, aldehydic), 2762, 2887 (C-H aldehyde). ^{1}H-NMR (δ, ppm): 4.46 (s, 4H, $C_{1,1'}$, -OCH_2), 7.29 (s, 2H, $C_{3,3'}$), 7.3 (s, 2H, $C_{7,7'}$), 7.49 (d, 2H, $C_{5,5'}$), 7.53 (t, 2H, $C_{4,4'}$), 10.02 (s, 2H, $C_{8,8'}$). ^{13}C-NMR (δ, ppm): 66.65:$C_{1,1'}$, 112.67:$C_{7,7'}$, 122.22:$C_{3,3'}$, 124.14:$C_{5,5'}$, 130.22:$C_{4,4'}$, 137.84:$C_{6,6'}$, 159.14:$C_{2,2'}$, 192.03:$C_{8,8'}$.

*Synthesis of 4-bromohippuric acid (**2**)*

In a 250-mL round-bottom flask, a mixture of glycine (8.25 g, 0.11 mol) and potassium hydroxide (11.22 g, 0.2 mol) in 50 mL water was cooled to 0 °C. 4-Bromobenzoylchlororide (21.95 g, 0.10 mol) was added in three portions with shaking. The reaction mixture was stirred for 1.0 hour. Then, the solution was acidified with conc. HCl, and the product (24 g) was collected and recrystallized from ethanol [36].

$C_9H_8BrNO_3$: yield: 96.89%, m.p. found (195-197) °C, IR (KBr, cm^{-1}): 3296 (NH-amide), (2550-3296 cm^{-1}, OH carboxylic), (1703 cm^{-1}, acid C=O), (1639.49 cm^{-1}, amide C=O).

*Synthesis of 4,4'-(((ethane-1,2-diylbis(oxy)) bis(3,1-phenylene)) bis(methaneylylidene)) bis(2-(4-bromophenyl) oxazol-5(4H)-one) (**3**)*

Bis-oxazolone according to modified procedure [37, 47] and simple techniques were applied, to a stirring mixture of (4-bromobenzoyl) glycine (25.807 g, 0.1mol), acetic acid (10 mL), fused sodium acetate (3.6 g, 0.032 mol), compound (**1**) 3,3'-(ethane-1,2-diylbis(oxy)) dibenzaldehyde (13.52 g, 0.05 mol), acetic anhydride (15.3 g, 0.15 mol), and a catalytic amount of $ZnCl_2$ was added then refluxed with stirring for 2.0 h in water bath, left overnight. The bright yellow product was collected and washed with aqueous sodium carbonate to afford the bright-yellow precipitate, compounds (**3**).

$C_{34}H_{22}Br_2N_2O_6$, bright yellow solid, yield: (94.5%), m.p. 183-184.1 °C. IR (KBr, cm^{-1}): 1796 (C=O) with shoulder at 1762, 1656 (C=N), 1592 (C=C), 1552 (N=N). ^{1}H-NMR (δ, ppm): 4.4 (s, 4H, $C_{1,1'}$, -OCH_2), 7.1 (d, 2H, $C_{3,3'}$),7.3 (s, 2H, $C_{7,7'}$), 7.47 (d, 2H, $C_{5,5'}$), 7.5 (t, 2H, $C_{4,4'}$), 7.8 (d, 4H, $C_{14,14',16,16'}$), 8.1 (d, 4H, $C_{13,13',17,17'}$),10.00 (s, 2H, $C_{8,8'}$). ^{13}C-NMR (δ, ppm): 66.65:$C_{1,1'}$, 112.67:$C_{7,7'}$, 122.22:$C_{3,3'}$, 124.14:$C_{5,5'}$, 130.22:$C_{4,4'}$, 132.35:C=C ring 137.84:$C_{6,6'}$, 159.14:$C_{2,2'}$, 192.03:$C_{8,8'}$, 193.43:C=O.

*Synthesis of bis benzamide (**4**)*

A mixture of bis-oxazolone (**3**) (714 mg, 1 mmol), substituted aromatic amine (3 mmol) in toluene (20 mL) in the presence trace of acetic acid was refluxed with stirring for 1.0 hour. The reaction mixture was monitored by changing of bright-yellow solution to pale yellow solution. The described product was filtered off, washed by ethanol, dried and re-washed by hot toluene then recrystallized in DMF to obtain pale yellow crystals of compound (**4**), Table 1, simple techniques and modified procedures were applied, resulting in good yields [38].

*N,N'-(-((Ethane-1,2-diylbis(oxy)) bis(3,1-phenylene)) bis(3-oxo-3-(substituted-phenyl) amino) prop-1-ene-1,2-diyl)) bis(4-bromobenzamide) (**4a**).* $C_{48}H_{40}Br_2N_4O_6$: yield: 96.89%, m.p. (237-238) °C, IR (KBr, cm^{-1}): 1687-1693 (amide C=O) 3223 and 3427 (NH_a and NH_b). ^{1}H-NMR (δ, ppm): 2.26 (s, 6H, 2Ar-CH_3), 4.15 (s, 4H, $C_{1,1'}$, -OCH_2), 6.88(s, 2H, $C_{8,8'}$), 6.91 (s, 2H, $C_{3,3'}$), 7.11 (s, 2H, $C_{7,7'}$), 7.21 (d, 2H, $C_{5,5'}$), 7.29 (t, 2H, $C_{4,4'}$), 7.72 (d, 4H, $C_{19,19',23,23'}$), 7.74 (d, 4H, $C_{20,20',22,22'}$), 7.95 (d, 4H, $C_{13,13',17,17'}$), 7.97 (d,4H, $C_{14,14',16,16'}$), 10.07 (s,1H, HN-C_{10}),10.17(s,1H, HN-C_9). ^{13}C-NMR (δ, ppm): 20.47:$C_{24,24'}$, 66.04:$C_{1,1'}$, 115.07:$C_{7,7'}$, 115.2:$C_{3,3'}$, 120.17:$C_{8,8'}$ 125.68:$C_{5,5'}$, 128.87:$C_{19,19',23,23'}$, 129.61:$C_{15,15'}$, 130.00:$C_{9,9'}$, 130.97:$C_{4,4',1313',17,17'}$, 131.38:$C_{20,20',22,22'}$, 132.36:C $_{12,12',14,14',16,16'}$, 132.57:$C_{18,18'}$, 135.51:$C_{6,6'}$, 136.64:$C_{21,21'}$, 158.12:$C_{2,2'}$, 163.85:$C_{11,11'}$,165.09:$C_{10,10'}$.

*N,N'-((1Z,1'Z)-((Ethane-1,2-diylbis(oxy)) bis(3,1-phenylene)) bis(3-((4-methoxyphenyl) amino)-3-oxoprop-1-ene-1,2-diyl)) bis(4-bromobenzamide) (**4b**).* $C_{48}H_{40}Br_2N_4O_8$: yield: 90%, m.p. (252-253) °C, ^{1}H-NMR (δ, ppm): 3.83 (s, 6H,$C_{24,24'}$, O-CH_3), 4.15 (s, 4H, $C_{1,1'}$, -OCH_2), 6.99 (s, 2H, $C_{8,8'}$), 7.27 (s, 2H, $C_{3,3'}$), 7.30 (s, 2H, $C_{7,7'}$), 7.39 (d, 2H, $C_{5,5'}$), 7.72 (t, 2H, $C_{4,4'}$), 7.82 (d, 4H, $C_{19,19',23,23'}$), 7.84 (d, 4H, $C_{20,20',22,22'}$), 8.05 (d, 4H, $C_{13,13',17,17'}$), 8.07 (d, 4H, $C_{14,14',16,16'}$), 10.12 (s, 1H, HN-C_{10}),10.25 (s, 1H, HN-C_9). ^{13}C-NMR (δ, ppm): 55.12:$C_{24,24'}$, 66.03:$C_{1,1'}$, 113.6:$C_{7,7'}$, 115.18:$C_{3,3'}$, 121.75:$C_{8,8'}$ 125.66:$C_{5,5'}$, 128.40:$C_{19,19',23,23'}$, 129.82:$C_{15,15'}$, 130.00:$C_{9,9'}$, 130.95:$C_{4,4',1313',17,17'}$, 131.36:$C_{20,20',22,22'}$, 132.22:C $_{12,12',14,14',16,16'}$, 132.61:$C_{18,18'}$, 135.54:$C_{6,6'}$, 155.38:$C_{21,21'}$, 158.11:$C_{2,2'}$, 163.61:$C_{11,11'}$,165.08:$C_{10,10'}$.

*N,N'-((1Z,1'Z)-((Ethane-1,2-diylbis(oxy)) bis(3,1-phenylene)) bis(3-((4-ethoxyphenyl) amino) -3-oxoprop-1-ene-1,2-diyl)) bis(4-bromobenzamide) (**4c**).* $C_{50}H_{44}Br_2N_4O_8$: yield: 85%, m.p. (257-

259) °C, ^{1}H-NMR (δ, ppm): 1.36 (t, 6H, $C_{25,25'}$, -CH_3), 4.04 (q, 4H, $C_{24,24'}$,-OCH_2), 4.2 (s, 4H, $C_{1,1'}$, -OCH_2), 6.92(s, 2H, $C_{8,8'}$), 7.21 (s, 2H, $C_{3,3'}$), 7.26 (s, 2H, $C_{7,7'}$), 7.34 (d, 2H, $C_{5,5'}$), 7.66 (t, 2H, $C_{4,4'}$), 7.77 (d, 4H, $C_{19,19',23,23'}$), 7.79 (d, 4H, $C_{20,20',22,22'}$),8.00 (d, 4H, $C_{13,13',17,17'}$), 8.03 (d,4H, $C_{14,14',16,16'}$), 10.07 (s, 1H, HN-C_{10}), 10.21 (s,1H, HN-C_9). ^{13}C-NMR (δ, ppm): 14.67 :$C_{25,25'}$, 63.02:$C_{24,24'}$, 66.02:$C_{1,1'}$, 114.11:$C_{7,7'}$, 115.03:$C_{3,3'}$, 121.72:$C_{8,8'}$ 125.66:$C_{5,5'}$, 128.34:$C_{19,19',23,23'}$, 129.82:$C_{15,15'}$, 130.00:$C_{9,9'}$, 130.98:$C_{4,4',1313',17,17'}$, 131.36:$C_{20,20',22,22'}$, 132.12:C $_{12,12',14,14',16,16'}$, 132.60:$C_{18,18'}$, 135.54:$C_{6,6'}$, 154.63:$C_{21,21'}$, 158.11:$C_{2,2'}$, 163.61:$C_{11,11'}$,165.08:$C_{10,10'}$.

*N,N'-((1Z,1'Z)-((Ethane-1,2-diylbis(oxy)) bis(3,1-phenylene)) bis (3-((5-chloro-2,4 dimethoxyphenyl) amino)-3-oxoprop-1-ene-1,2-diyl)) bis(4-bromobenzamide) (**4d**).* $C_{50}H_{42}Br_2Cl_2N_4O_{10}$: yield: 88%, m.p. (265-266) °C, ^{1}H-NMR (δ, ppm): 3.80(s, 6H, $C_{24,24'}$,-OCH_3), 3.92(s, 6H, $C_{25,25'}$,-OCH_3), 4.13(s, 4H, $C_{1,1'}$,-OCH_2), 6.87(s, 2H, $C_{8,8'}$), 7.22 (s, 2H, $C_{3,3'}$), 7.23 (s, 2H, $C_{22,22'}$), 7.26 (s, 2H, $C_{7,7'}$), 7.34 (d, 2H, $C_{5,5'}$), 7.66 (t, 2H, $C_{4,4'}$), 8.05 (d, 4H, $C_{13,13',17,17'}$), 8.21(d, 4H, $C_{14,14',16,16'}$) , 9.46 (s, 2H, $C_{19,19'}$), 10.20 (s, 1H, HN-C_{10}), 10.44 (s, 1H, HN-C_9). ^{13}C-NMR (δ, ppm): 56.35:$C_{24,24'}$, 56.39 :$C_{25,25'}$, 65.88:$C_{1,1'}$, 97.89:$C_{22,22'}$, 111.19:$C_{20,20'}$ 114.61:$C_{3,3',7,7'}$, 122.14:$C_{8,8'}$, 124.50:$C_{5,5'}$, 129.22 :$C_{19,19'}$, 129.82:$C_{15,15'}$, 129.71:$C_{9,9'}$, 129.94:$C_{4,4',1313',17,17'}$, 131.00 :$C_{12,12',14,14',16,16'}$, 131.67:$C_{18,18'}$, 136.58:$C_{6,6'}$, 148.98:$C_{23,23'}$, 151.00:$C_{21,21'}$, 157.97:$C_{2,2'}$, 163.91:$C_{11,11'}$,165.23:$C_{10,10'}$.

*Synthesis of bis-imidazolone (**5**)*

Bis imidazolone (**5a-j**) derivatives were synthesized in one step by mixing of bis-oxazolones (**3**) (1 mmol) with substituted aromatic amine (3 mmol) in acetic acid (10 mL) [39]. The reaction was refluxed with stirring for (6-10) h. it was monitored by color changing to pear-green soluble solution, or in two steps by refluxing bis-benzamide (**4**) in glacial acetic acid for 8 hours, Scheme 5. The reaction mixtures were poured into water, filtered off, dried, and crystallized in a mixture of toluene and ethanol to obtain bright pear-green crystals of (**5a-e**).

*(5,5'-(((Ethane-1,2-diylbis(oxy)) bis(3,1-phenylene)) bis (methaneylylidene)) bis(2-(4-bromophenyl)-3-(substitutedphenyl)-3,5-dihydro-4H-imidazol-4one) (**5a**).* $C_{48}H_{36}Br_2N_4O_4$: yield: 93%, m.p. (237-238) °C, IR (KBr, cm^{-1}): 1727 (C=O). ^{1}H-NMR (δ, ppm): 2.42 (s, 6H, 2Ar-$\underline{CH_3}$), 4.5 (s, 4H, $C_{1,1'}$, -OCH_2), 7.06 (s, 2H, $C_{3,3'}$), 7.08 (s, 2H, $C_{7,7'}$), 7.25 (d, 2H, $C_{5,5'}$), 7.27 (t, 2H, $C_{4,4'}$), 7.47 (d, 4H, $C_{19,19',23,23'}$), 7.76 (d, 4H, $C_{20,20',22,22'}$), 7.78 (d, 4H, $C_{14,14',16,16'}$), 8.14 (d, 4H, $C_{13,13',17,17'}$), 10.01(s, 2H, $C_{8,8'}$), ^{13}C-NMR (δ, ppm): 21.26:$C_{24,24'}$, 66.55:$C_{1,1'}$, 112.76:$C_{7,7'}$, 117.45:$C_{3,3'}$, 117.86:$C_{8,8'}$ 122.24:$C_{5,5'}$, 128.87:$C_{13,13',17,17'}$, 129.61:$C_{19,19',23,23'}$, 129.61:$C_{15,15'}$, 130.00:$C_{9,9'}$, 130.97:$C_{4,4'}$, , 132.40:$C_{20,20',22,22'}$, ,135.62:$C_{14,14',16,16'}$, ,138.65:$C_{12,12'}$,138.75:$C_{18,18'}$, 139.96:$C_{21,21'}$,158.69:$C_{6,6'}$, 158.78:$C_{11,11'}$, 159.71:$C_{2,2'}$, 170.54:$C_{10,10'}$.

*(5,5'-(((Ethane-1,2-diylbis(oxy)) bis(3,1-phenylene)) bis(methaneylylidene)) bis(2-(4-bromophenyl) -3-(4-ethoxyphenyl)-3,5-dihydro-4H-imidazol-4-one) (**5c**).* $C_{50}H_{40}Br_2N_4O_6$: yield: 85.8%, m.p. (257-259) °C, ^{1}H-NMR (δ, ppm). 1.47 (t, 6H, $C_{25,25'}$, -CH_3), 4.07 (q, 4H, $C_{24,24'}$, -OCH_2), 4.5 (s, 4H, $C_{1,1'}$, -OCH2), 6.95 (s, 2H, $C_{3,3'}$), 7.02 (s, 2H, $C_{7,7'}$), 7.1 (d, 2H, $C_{5,5'}$), 7.3 (t, 2H, $C_{4,4'}$), 7.43 (d, 4H, $C_{19,19',23,23'}$), 7.75 (d, 4H, $C_{20,20',22,22'}$), 7.77 (d, 4H, $C_{14,14',16,16'}$), 8.14 (d, 4H, $C_{13,13',17,17'}$), 10.01(s, 2H, $C_{8,8'}$), ^{13}C-NMR (δ, ppm): 14.78:$C_{25,25'}$ 63.80:$C_{24,24'}$, 66.54:$C_{1,1'}$,15.36:$C_{7,7'}$, 117.60:$C_{3,3'}$, 119.3:$C_{8,8'}$ 124.01:$C_{5,5'}$, 126.54:$C_{13,13',17,17'}$, 128.71:$C_{19,19',23,23'}$, 129.22:$C_{15,15'}$, 129.45:$C_{9,9'}$, 129.78:$C_{4,4'}$, , 130.28:$C_{20,20',22,22'}$, ,130.61:$C_{14,14',16,16'}$, ,131.65:$C_{12,12'}$,135.64:$C_{18,18'}$, 138.64:$C_{21,21'}$,158.77:$C_{6,6'}$, 159.00:$C_{11,11'}$, 159.76:$C_{2,2'}$, 170.73:$C_{10,10'}$.

*(5,5'-(((Ethane-1,2-diylbis(oxy)) bis(3,1-phenylene)) bis(methaneylylidene)) bis(2-(4-bromophenyl)-3-(5-chloro-2,4-dimethoxyphenyl)-3,5-dihydro-4H-imidazol-4-one) (**5d**).*

$C_{50}H_{38}Br_2Cl_2N_4O_8$: yield: 88%, m.p. (265-266) °C, ^{1}H-NMR (δ, ppm): 3.86 (s, 6H, $C_{24,24',25,25'}$, -OCH_3), 4.48 (s, 4H, $C_{1,1'}$, -OCH_2), 6.51 (s, 2H, $C_{3,3'}$), 7.08 (s, 2H, $C_{7,7'}$), 7.23 (d, 2H, $C_{5,5'}$), 7.26 (t, 2H, $C_{4,4'}$), 7.77 (d, 4H, $C_{22,22'}$), 7.79 (d, 4H, $C_{14,14',16,16'}$), 8.11(d, 4H, $C_{13,13',17,17'}$), 8.39 (s, 2H $C_{19,19'}$), 9.99 (s, 2H, $C_{8,8'}$). ^{13}C-NMR (δ, ppm): 55.96:$C_{24,24'}$, 56.04:$C_{25,25'}$, 66.56:$C_{1,1'}$, 97.23:$C_{,22,22'}$, 112.86:$C_{7,7',18,18',20,20'}$, 117.47:$C_{3,3'}$, 117.80:$C_{8,8'}$ 122.16:$C_{5,5'}$, 125.97:$C_{19,19'}$,128.72:$C_{15,15'}$, 128.98:$C_{13,13',17,17'}$, 130.20:$C_{4,4'}$, 131.66:$C_{9,9'}$, ,135.60:$C_{14,14',16,16'}$, 138.56:$C_{12,12'}$, 156.56:$C_{6,6'}$, 159.22:$C_{21,21'}$, 160.33:$C_{11,11'}$, 170.84:$C_{23,23'}$, 170.85:$C_{2,2'}$, 192.06:$C_{10,10'}$.

*(5,5'-(((Ethane-1,2-diylbis(oxy)) bis(3,1-phenylene)) bis(methaneylylidene)) bis(2-(4-bromophenyl)-3-(4-((4-chlorobenzyl) oxy) phenyl)-3,5-dihydro-4H-imidazol-4-one) (**5e**).* $C_{60}H_{42}Br_2Cl_2N_4O_6$: yield: 93%, m.p. (258-260) °C, ^{1}H-NMR (δ, ppm): 4.47 (s, 4H, $C_{1,1'}$, -OCH2), 5.07 (s, 4H, $C_{24,24'}$, -OCH_2), 6.95 (s, 2H, $C_{3,3'}$), 7.01 (d,4H, $C_{20,20',22,22'}$), 7.10 (s, 2H, $C_{7,7'}$), 7.13 (d, 2H, $C_{5,5'}$), 7.26 (d, 4H, $C_{19,19',23,23'}$), 7.33 (d, 4H, $C_{26,26',30,30'}$), 7.36 (d, 4H, $C_{27,27',29,29'}$), 7.39 (t, 2H, $C_{4,4'}$), 7.44 (d, 4H, $C_{14,14',16,16'}$), 7.47 (d, 4H, $C_{13,13',17,17'}$), 8.47(s, 2H, $C_{8,8'}$).^{13}C-NMR (δ, ppm): 66.54:$C_{1,1'}$, 69.55:$C_{24,24'}$, 112.48:$C_{7,7'}$, 115.40:$C_{3,3'}$, 117.61:$C_{20,20',22,22'}$, 118.72:$C_{8,8'}$, 122.59:$C_{5,5'}$, 128.71:$C_{13,13',17,17'}$, 128.67:$C_{19,19',23,23'}$, 129.40:$C_{15,15'}$, 129.49:$C_{9,9',18,18'}$, 129.82:$C_{4,4'}$, 131.42:$C_{14,14',16,16'}$, 133.80:$C_{12,12'}$, 134.06:$C_{27,27',29,29'}$, 135.45:$C_{26,26',30,30'}$, 138.57:$C_{25,25',28,28'}$, 157.24:$C_{21,21'}$,158.78:$C_{6,6'}$, 159.04:$C_{11,11'}$, 159.63:$C_{2,2'}$, 170.67:$C_{10,10'}$.

CONCLUSIONS

In conclusion, we have demonstrated the effective synthesis and characterization of eleven compounds: bis-oxazolone **1**, a series of bis-benzamide **5**, and a series of bis-imidazolone **5**. We have assessed their antibacterial properties, revealing that the newly synthesized bis-oxazolone, bis-benzamide, and bis-imidazolone compounds showcase promising antimicrobial potential, as evidenced by antimicrobial assays. According to this study, we have noticed that our newly synthesized benzamide derivatives mostly showed significantly antibacterial activity against Gram-positives than Gram-negatives.

Molecular docking analyzed the interaction between optimized compounds and DNA gyrase, crucial for bacterial growth, revealing higher binding affinities for (**3, 4a**, and **5e**) compared to penicillin G and ampicillin (-7.7 and -7.9 kcal/mol, respectively). These findings suggest their potential in inhibiting essential bacterial targets like DNAG and PBP1a, for antibacterial drug development, as depicted in Tables (1-4) and Figures (4-6). All investigated substances have displayed moderate to high antimicrobial activity against at least two species (*E. coli* and *S. aureus*), positioning them as excellent candidates for further biological screening tests, particularly for external wound applications.

In the DPPH antioxidant activity evaluation, bis-compounds showed excellent results, comparable to ascorbic acid. Their superior radical scavenging capabilities, due to enhanced functionality and conjugation, highlight their potential as effective antioxidant agents. These findings encourage further research to explore their therapeutic applications and develop potent antioxidant compounds for medical use. These findings highlight the multifaceted pharmacological potential of these compounds and warrant further exploration for their clinical applications in combating microbial infections and oxidative stress-related diseases.

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