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Synthesis, Antibacterial, and Molecular Docking Study of Novel 2-Chloro-8-Methoxy-3-Aryl-[1,3] Benzoxazine Derivatives using Vilsmeier Reagent

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ABSTRACT

Reducing of ethyl 4-((2-hydroxy-3-methoxybenzylidene)amino)benzoate (1) afford ethyl 4-((2-hydroxy-3-methoxybenzyl) amino)benzoate (2). Reaction of this compound with Vilsmeier reagent affords novel 2-chloro-[1,3] benzoxazine ring (3). The corresponding acid hydrazide of compound 3 was synthesized from reaction of compound (3) with hydrazine hydrate. Newly series of hydrazones (5a–i) were synthesized from reaction of acid hydrazide with various aryl aldehydes. Antibacterial activity of the hydrazones was secerned utilizing gram-negative and gram-positive bacteria. Compound (5b) and (5c) exhibited significant antibacterial ability against both gram-negative and gram-positive bacteria, while the compounds (5a) showed mild antibacterial activity. Compounds (5d–i) did not display notable activity. The molecular docking of synthesised compounds were tested inside the pocket of bacterial gyrase enzyme target site by using MOE 2015 software, which acts as Adenosine triphosphate (ATP)-binding domain bacterial gyrase enzyme pocket and novobiocin was used as reference.

Keywords: Antibacterial, Benzoxazine, Hydrazone, Molecular docking, Vilsmeier.

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INTRODUCTION

Vilsmeier reagent is considered one of the most important reagents in organic chemistry. By utilizing this reagent, more than fifty functional groups and more than fifty different ring systems can be formed.¹ Many heterocyclic rings and their derivatives have been synthesized utilizing Vilsmeier reagent.²⁻⁵ Furthermore, these compounds exhibited various biological activity, such as, antimicrobial agents,^{6,7} antiproliferative activity,⁸ antioxidants,⁹ and anticancer.¹⁰ On the other hand, the 1,3-benzoxazine and their derivatives as well showed interesting biological activities, such as, antischistosomal activity, anticonvulsant, and antitumor activities,¹¹ antimicrobial activity,¹² fungicidal activity,¹³ and photochromic activity.¹⁴ In addition to 1,3-benzoxazine, the hydrazone derivatives possess diverse biological activities, such as, antimicrobial,¹⁵ α -glucosidase inhibitors,¹⁶ anti-Alzheimer's,¹⁷ and anticancer.¹⁸ It is clearly notable that the existence of more than one active functional group in structure could augment their biological activity.^{19,20} In modern researches, the molecular docking studies are extremely helpful to give a prediction of the ligand-receptor complex structure utilizing computation methods. Taking into

account ligand-receptor interaction, two points: strength of the interaction (what is known as "affinity") and kind of an effect at the biochemical, electrophysiological, and behavioral level triggered by the ligand (what we refer to as "intrinsic activity"). To gain an prudence into the type of chemical forces implicated in the interaction, first one may build models of a specific pharmacophore or construct models of a receptor protein and inspect the binding forces. Interaction of a ligand with a receptor may consequence in receptor activation (agonists).²¹ The extreme common path to predict the correct binding pose and binding affinity (BA) is protein-based modeling (docking) in physicochemical interactions amid a ligand and receptor by deduced from the 3D structures of both molecules. In this work, docking method was performed to predict the antibacterial activity of the synthesized compounds.²² This work presents green method for synthesis of 2-chloro-8-methoxy-3-aryl-[1,3] benzoxazine utilizing Vilsmeier reagent and synthesized their corresponding hydrazones. These compounds were characterized from their fourier transform infrared spectroscopy (FTIR), ¹H, ¹³C NMR besides EIMS spectra. The antibacterial activities of these compounds were screened against gram-negative and gram-positive bacteria,

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as well as, molecular docking was done by using MOE 2015 software, which acts as ATP-binding domain bacterial gyrase enzyme pocket and using novobiocin as reference.

MATERIAL AND METHOD

General

The melting point of synthesized compounds was established by open capillary tube using OMEGA MPS10 apparatus and it is uncorrected. The end of the reactions was monitored by thin-layer chromatography (TLC). Plates, brand Merck spot located with UV lights at 254 nm. The FTIR spectrums were affirmed with Perkin Elmer 400 spectrometer. The NMR spectra were recorded on Bruker spectrometer 300 MHz (AL-Bayt University, Jordan) in DMSO- d_6 with tetramethylsilane as internal standard. The mass spectrum was recorded using Shimadzu GC MS-QP2010 Ultra (Al-Mustansiriyah University, College of Science, Department of Chemistry).

Synthesis of Ethyl 4-((2-Hydroxy-3-Methoxybenzylidene) Amino)Benzoate (1)

Ethyl-4-aminobenzoate (6 grams, 36.36 mmol) in 50 mL absolute ethanol was added in small portions to a stirring ethanolic solution of 3-methoxy-2-hydroxy benzaldehyde (5.52 grams, 36.36 mmol) with three drops of acetic acid. The mixture was heated under reflux for 7 hours. Upon cooling, the precipitate was filtrated and washed with cold water and crystallized from methanol to afford orange nidel crystal. MP 98 to 101°C. Yield 83%, FTIR (KBr, U_{max}/cm^{-1}); 3379 (OH), 3045 (CH_{Ar}), 2958, 2831 (CH_{alph}), 1705 (C=O), 1620 (C=N), 1595, 1502 (C=C); 1H NMR (300 Mz, $CDCl_3$) δ : 1.24 (t, J 7.0, 3H, CH_3), 3.83 (s, 3H OCH_3), 4.13 (q, J 7.01, OCH_2), 6.60 (d, J 8.4, 2H, H_9), 6.67 (t, J 6.8, 1H, H_5) 6.71(d, J 7.1, 1H H_4), 6.85 (d, J 7.7, 1H, H_6), 7.65 (d, J 8.4, 2H, H_{10}), 8.67 (s, 1H, H_7 , CH=N), 9.83 (bs, 1H, OH). ^{13}C NMR (75 MHz, DMSO- d_6) δ : 14.87 (1C, CH_3), 56.56 (1C, OCH_3), 60.09 (1C, OCH_2), 114.89 (1C, C_3), 117.71 (1C, C_4), 120.07 (1C, C_6), 123.29 (2C, C_9), 125.18 (1C, C_5), 127.69 (1C, C_{11}), 132 (2C, C_{10}), 149.08 (1C, C_2), 151.23 (1C, C_1), 152.77 (1C, C_9), 156.82 (1C, C_7), 166.27 (1C, C=O), EIMs = 299.2 for $C_{17}H_{17}NO_4$.

Synthesis of Ethyl 4-((2-Hydroxy-3-Methoxybenzyl) Amino)Benzoate (2)

This compound has been synthesized according to procedure described by Shakir *et al.*²³ The resulting product purified by recrystallization from ethanol to obtain white precipitate. MP 198 to 199°C. Yield 79 %, FTIR (KBr, U_{max}/cm^{-1}); 3406 (OH), 3307 (NH), 3057(CH_{Ar}), 2993, 2848 (CH_{alph}), 1674 (C=O), 1601, 1529 (C=C); 1H NMR (300 MHz, $CDCl_3$) δ : 1.26 (t, J 7.1, 3H, CH_3), 3.79 (s, 3H OCH_3), 4.18 (q, J 7.2, 2H, OCH_2) 4.26 (s, 2H, CH_2NH), 4.83 (bs, 1H, NH), 6.59 (d, J 8.7, 2H, H_9), 6.67 to 6.68 (m, 2H, H_4 and H_5), 6.85 (d, J 7.7, 1H, H_6), 7.65 (d, J 8.7, 1H, H_{10}) 10.06 (bs, 1H, OH). ^{13}C NMR (75 MHz, DMSO- d_6) δ : 14.59 (1C, CH_3), 42.51 (1C, CH_2NH), 56.67 (1C, OCH_3), 60.18 (1C, OCH_2), 115.77 (1C, C_3), 117.98 (1C, C_4), 121.23 (1C, C_6), 123.55 (2C, C_9), 126.11 (1C, C_5), 128.48 (1C, C_{11}), 132.83 (2C, C_{10}), 149.35 (1C, C_2), 150.21 (1C, C_1),

152.50 (1C, C_9), 155.96 (1C, C_7), 164.17 (1C, C=O), EIMs 301.1 for $C_{17}H_{19}NO_4$.

Synthesis of Ethyl 4-(2-Chloro-8-Methoxy-2H-Benzo[E][1,3]Oxazin-3(4H)-Yl)Benzoate (3)

A solution of ethyl 4-((2-hydroxy-3-methoxybenzyl)amino) benzoate (6 grams, 19.93 mmol) in DMF (1.45 grams, 19.93 mmol) was cooled to 0°C with continuous stirring. $POCl_3$ (phosphorus oxychloride) (3.04 grams, 19.93 mmol) at 0°C was added dropwise. After that, mixture stirred for one hour at ambient temperature, then heated to 90°C for 3 hours. Upon cooling, the mixture poured into 20 mL crushed ice, then neutralized to pH 7 by sodium hydrogen carbonate. The precipitate was collected after washing with cooled water. The crud product purified by column chromatograph hexane:ethyl acetate (4:1) to afford white precipitate. MP 159 to 161°C, FTIR (KBr, U_{max}/cm^{-1}); 3010 (CH_{Ar}), 2962, 2889 (CH_{alph}), 1722 (C=O), 1601, 1485 (C=C), 1H NMR (300 MHz, DMSO- d_6) δ : 1.29 (t, J 7.1, 3H, CH_3), 3.81 (s, 3H, OCH_3), 4.28 (q, J 7.0, 2H, OCH_2), 5.02 (dd, J 6.96, 1.84, 2H, CH_2_{oxazin}), 5.91 (s, 1H, CH_{oxazin}), 6.55 (d, J 7.24, 1H, H_5), 6.65 (t, J 7.4, 1H, H_6), 6.82 (d, J 7.28, 1H, H_7) 7.45 (d, J 8.7, 2H, H_{10}), 7.91(d, J 8.4, 2H, H_{11}). ^{13}C NMR (75 MHz, DMSO- d_6) δ : 14.62 (1C, CH_3), 54.81 (1C, $CH_2_{oxazine}$), 56.62 (1C, OCH_3), 60.17 (1C, OCH_2), 111.54 (2C, C_{10}), 113.32 (1C, C_7), 119.73 (1C, C_{12}), 121.41 (1C, C_5), 122.08 (1C, C_6), 123.17 (1C, C_{4a}), 124.68 (1C, C_2), 130.52 (2C, C_{11}), 145.31 (1C, C_8), 149.61 (1C, C_{8a}), 153.18 (1C, C_9), 165.93 (1C, C=O), EIMs 347.09 for $C_{18}H_{18}ClNO_4$.

Synthesis of 4-(2-Chloro-8-Methoxy-2H-Benzo[1,3] Oxazin-3(4H)-Yl) Benzohydrazide (4)

Hydrazine hydrate (80%) (0.57 grams, 11.5 mmol) was added to a solution of ethyl 4-(2-chloro-8-methoxy-2H-benzo[1,3] oxazin-3(4H)-yl)benzoate (4 grams, 11.5 mmol) in 50 mL ethanol at ambient temperature. The mixture left to stand 18 hours under vigorous stirring. The precipitated was collected by filtration and washed with cooled water to obtain white precipitated. The crud compound was recrystallized from aqueous methanol to obtain off white. MP 199 to 201°C. Yield 67%. FTIR (KBr, U_{max}/cm^{-1}); 3398, 3309 (NH_2), 3209 (NH), 3028 (CH_{Ar}), 2939, 2841 (CH_{alph}), 1648 (C=O), 1585, 1479 (C=C), 1H NMR (300 MHz, DMSO- d_6) δ : 3.83 (s, 3H, OCH_3), 4.07 (bs, 2H, NH_2), 5.00 (dd, J 7.0 1.86, 2H, $CH_2_{oxazine}$), 5.93 (s, 1H, $CH_{oxazine}$), 6.58 (d, J 7.40, 1H, H_5), 6.65 (t, J 7.46, 1H, H_6), 6.9 (d, J 7.44, 1H, H_7), 7.42 (d, J 8.6, 2H, H_{10}), 7.88 (d, J 8.6, 2H, H_{11}), 9.83 (bs, 1H, NH) ^{13}C NMR (75 MHz, DMSO- d_6) δ : 54.32 (1C, $CH_2_{oxazine}$), 56.13 (1C, OCH_3), 111.52 (2C, C_{10}), 113.14 (1C, C_7), 120.49 (1C, C_{12}), 121.25 (1C, C_5), 122.82 (1C, C_6), 123.02 (1C, C_{4a}), 124.26 (1C, C_2), 130.17 (2C, C_{11}), 145.93 (1C, C_8), 149.32 (1C, C_{8a}), 153.63 (1C, C_9), 168.21 (1C, C=O). EIMs 333.09 for $C_{16}H_{16}ClN_3O_3$.

General Synthesis of 4-(2-Chloro-8-Methoxy-2H-Benzo[1,3]Oxazin-3(4H)-Yl)-N'-(Substituted Benzylidene) Benzohydrazide (5a-i)

Aryl aldehyde (0.75 mmol) in ethanol 5 mL was added in few portions to a solution of 4-(2-chloro-8-methoxy-2H-benzo[1,3]

oxazin-3(4H)-yl)benzohydrazide (0.25-gram, 0.75 mmol) in 5 mL acetic acid. The mixture was heated under reflux for 5 hours. After cooling, the precipitate was collected and washed with warm methanol, then with cold water. The crude product is purified by crystallization from a suitable solvent.

4-(2-Chloro-8-Methoxy-2H-Benzo[1,3]Oxazin-3(4H)-Yl)-N'-(4-Chlorobenzylidene) Benzohydrazide (5a)

Recrystallized from aqueous DMF to afford white amorphous, Yield 86 %, MP 195-196 °C, FTIR (KBr, U_{\max}/cm^{-1}); 3309 (NH), 3032 (CH_{Ar}), 2939, 2838 (CH_{aliph}), 1646 (C=O), 1606 (C=N), 1574, 1483 (C=C). ^1H NMR (300 MHz, DMSO- d_6) δ ; 3.84(s, 3H, OCH₃), 5.12 (dd, J 7.2 1.9, 2H, CH₂ oxazine), 5.98 (s, 1H, CH oxazine), 6.54 (d, J 7.36, 1H, H₅), 6.66 (t, J 7.42, 1H, H₆), 6.89 (d, J 7.32, 1H, H₇), 7.44 (d, J 8.7, 2H, H₁₀), 7.52 (d, J 8.52, 2H, H₁₇), 7.82 to 7.88 (m, 4H, H₁₁, H₁₆), 8.56 (s, 1H, H₁₄, CH=N) 9.87 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO- d_6) δ ; 53.32 (1C, C₄), 56.7 (1C, OCH₃), 109.67 (2C, C₁₀), 116.03 (1C, C₇), 118.32 (1C, C₂), 119.38 (2C, C₁₁), 120.89 (1C, C₁₂), 121.14 (1C, C₆), 121.99 (1C, C₅), 123.11 (1C, C₁₅), 123.97 (1C, C_{4a}), 127.78 (2C, C₁₆, C₂₀), 128.23 (2C, C₁₇, C₁₉), 136.41 (1C, C₁₈), 144.69 (1C, C₈), 149.13 (1C, C_{8a}), 150.77 (1C, C₉), 151.44 (1C, C=N), 166.11 (1C, C=O). EIMs = 455.1 calculated for C₂₃H₁₉C₁₂N₃O₃.

4-(2-Chloro-8-Methoxy-2H-Benzo[1,3]Oxazin-3(4H)-Yl)-N'-(4-Nitrobenzylidene) Benzohydrazide (5b)

Crude product recrystallized from ethanol to obtain yellow precipitate. Yield 89%, MP 203 to 206°C. FTIR (KBr, U_{\max}/cm^{-1}), 3313 (NH), 3030 (CH_{Ar}), 2941, 2843 (CH_{aliph}), 1651 (C=O), 1612 (C=N) 1595, 1481 (C=C), 1468 (asymmetric NO₂), 1349 (symmetric NO₂); ^1H NMR (300 MHz, DMSO- d_6) δ ; 3.79 (s, 3H, OCH₃), 4.94 (dd, J 7.0 1.84, 2H, CH₂ oxazine), 5.95 (s, 1H, CH oxazine), 6.55 (d, J 7.36, 1H, H₅), 6.63 (t, J 7.26, 1H, H₆), 6.86 (d, J 7.36, 1H, H₇), 7.48 (d, J 8.6, 2H, H₁₀), 7.84 (d, 2H, H₁₁), 7.96 (d, J 8.2, 2H, H₁₆), 8.23 (d, J 8.22, 2H, H₁₇), 8.64 (s, 1H, H₁₄, CH=N) 9.83 (bs, 1H, NH), ^{13}C NMR (75 MHz, DMSO- d_6) δ ; 54.06 (1C, C₄), 57.26 (1C, OCH₃), 109.23 (2C, C₁₀), 116.58 (1C, C₇), 118.53 (2C, C₁₁), 119.74 (1C, C₂), 120.21 (1C, C₁₂), 122.44 (1C, C₆), 122.96 (1C, C₅), 123.09 (1C, C_{4a}), 124.87 (2C, C₁₇, C₁₉), 130.39 (2C, C₁₆, C₂₀), 139.55 (1C, C₁₅), 144.48 (1C, C₈), 148.94 (1C, C_{8a}), 149.39 (1C, C₉), 150.67 (1C, C₁₈), 152.49 (1C, C=N), 167.02 (1C, C=O). EIMs = 466 calculated for C₂₃H₁₉ClN₄O₅.

4-(2-Chloro-2H-Benzo[E][1,3]Oxazin-3(4H)-Yl)-N'-(3,5-Dichlorobenzylidene) Benzohydrazide (5c)

Crude product was recrystallized by acetonitrile to afford pale yellow amorphous. MP 226 to 228°C. Yield 83%, FTIR (KBr, U_{\max}/cm^{-1}), 3246 (NH), 3051 (CH_{Ar}), 2963, 2871 ($\text{CH}_{\text{aliphatic}}$), 1645 (C=O), 1627 (C=N), 1597, 1487 (C=C), ^1H NMR (300 MHz, DMSO- d_6) δ ; 3.84 (s, 3H, OCH₃), 5.12 (dd, J 7.24 1.88, 2H, CH₂ oxazine), 6.07 (s, 1H, CH oxazine), 6.53 (d, J 7.1, 1H, H₅), 6.64 (t, J 7.26, 1H, H₆), 6.83 (m, 3H, H₇, H₁₇), 7.52 (m, 3H, H₁₀, and H₁₈), 7.62 (d, J 8.22, 2H, H₁₁), 8.04 (t, 2H, J 1.72, H₁₆, and H₂₀), 8.59 (s, 1H, H₁₄, CH=N), 9.65 (bs, 1H, NH); ^{13}C NMR (75 MHz, DMSO- d_6) δ ; 54.23 (1C, C₄), 57.03 (1C,

OCH₃), 111.42 (2C, C₁₀), 113.33 (1C, C₇), 120.62 (1C, C₁₂), 121.86 (1C, C₅), 122.03 (1C, C₆), 123.17 (1C, C_{4a}), 124.74 (1C, C₂), 125.02 (2C, C₁₆, C₂₀), 126.87 (1C, C₁₅), 130.45 (2C, C₁₁), 131.24 (1C, C₁₈), 135.92 (2C, C₁₇, C₁₉), 146.22 (1C, C₈), 149.11 (1C, C_{8a}), 152.8 (1C, C=N), 153.72 (1C, C₉), 167.16 (1C, C=O). EIMs = 489.1 calculated for C₂₃H₁₈Cl₃N₃O₃.

4-(2-Chloro-8-Methoxy-2H-Benzo[1,3]Oxazin-3(4H)-Yl)-N'-(4-Hydroxybenzylidene) Benzohydrazide (5d)

Crude product was recrystallized from ethanol to afford white crystal. MP 178 to 180°C. Yield 77%, FTIR (KBr, U_{\max}/cm^{-1}); 3312 (OH), 3257 (NH), 3066 (CH_{Ar}), 2971, 2845 (CH_{aliph}), 1658 (C=O), 1625 (C=N), 1958, 1464 (C=C). ^1H NMR (300 MHz, DMSO- d_6) δ ; 3.81 (s, 3H, OCH₃), 5.1 (dd, J 7.22 1.84, 2H, CH₂ oxazine), 6 (s, 1H, CH oxazine), 6.49 (d, J 7.1, 1H, H₅), 6.6 (t, J 7.24, 1H, H₆), 6.83 (m, 3H, H₇, H₁₇), 7.52 (d, J 8.4, 2H, H₁₀), 7.62 (d, J 8.56, 2H, H₁₆), 7.83 (d, 8.42, 2H, H₁₁), 8.61 (s, 1H, H₁₄, CH=N), 9.67 (bs, 1H, NH), 10.83 (bs, 1H, OH), ^{13}C NMR (75 MHz, DMSO- d_6) δ ; 54.51 (1C, C₄), 57.11 (1C, OCH₃), 110.44 (2C, C₁₀), 113.54 (1C, C₇), 115.73 (1C, C₁₅), 117.03 (2C, C₁₆, C₂₀), 120.21 (1C, C₁₂), 121.08 (1C, C₆), 121.33 (1C, C₅), 123.54 (1C, C_{4a}), 128.98 (2C, C₁₇, C₁₉), 129.34 (2C, C₁₁), 145.72 (1C, C₈), 148.32 (1C, C_{8a}), 153.48 (1C, C₉), 154.01 (1C, C=N), 159.38 (1C, C₁₈), 166.15 (1C, C=O). EIMs = 437 calculated for C₂₃H₂₀ClN₃O₄.

4-(2-Chloro-8-Methoxy-2H-Benzo[1,3]Oxazin-3(4H)-Yl)-N'-(2-Hydroxy-3-Methoxybenzylidene) Benzohydrazide (5e)

Recrystallized from methanol to afford pale yellow. MP 173 to 176°C. Yield 75%, FTIR (KBr, U_{\max}/cm^{-1}), 3411 (OH), 3249 (NH), 3080 (CH_{Ar}), 2962, 2839 (CH_{aliph}), 1644 (C=O), 1624 (C=N), 1577, 1462 (C=C), ^1H NMR (300 MHz, DMSO- d_6) δ ; 3.81 (s, 3H, OCH₃), 3.88 (s, 3H, OCH₃), 4.96 (dd, J 7.10 1.92, 2H, CH₂ oxazine), 5.97 (s, 1H, CH oxazine), 6.51 (d, J 7.14, 1H, H₅), 6.57 (t, J 7.1, 1H, H₆), 6.83 (m, 3H, H₇, H₁₈), 7.14 (dd, J 7.9, 1.78, 1H, H₁₉), 7.39 (m, 3H, H₁₀, H₂₀), 7.83 (d, J 8.42, 2H, H₁₁), 8.45 (s, 1H, H₁₄, CH=N), 9.63 (bs, 1H, NH), 10.9 (bs, 1H, OH). ^{13}C NMR (75 MHz, DMSO- d_6) δ ; 54.89 (1C, C₄), 56.34 (1C, OCH₃), 59.52 (1C, C₂₁, OCH₃), 111.79 (2C, C₁₀), 113.21 (1C, C₇), 114.57 (1C, C₁₈), 119.41 (1C, C₁₉), 120.36 (1C, C₁₂), 121.27 (1C, C₅), 122.17 (1C, C₆), 123.42 (1C, C_{4a}), 124.65 (1C, C₂), 127.19 (1C, C₂₀), 129.63 (1C, C₁₅), 130.56 (2C, C₁₁), 146.48 (1C, C₈), 147.05 (1C, C₁₇), 149.12 (1C, C_{8a}), 152.02 (1C, C₁₆), 152.78 (1C, C=N), 153.56 (1C, C₉), 167.12 (1C, C=O). EIMs = 467 calculated for C₂₄H₂₂ClN₃O₅.

4-(2-Chloro-8-Methoxy-2H-Benzo[1,3]Oxazin-3(4H)-Yl)-N'-(3-Ethoxy-4-Hydroxybenzylidene) Benzohydrazide (5f)

The crude product recrystallized from acetonitrile to obtain pale yellow needle crystal. MP 232 to 233°C. Yield 80%, FTIR (KBr, U_{\max}/cm^{-1}); 3324 (OH), 3292 (NH), 3006 (CH_{Ar}), 2964, 2837 (CH_{aliph}), 1648 (C=O), 1610 (C=N), 1558, 1510 (C=C). ^1H NMR (300 MHz, DMSO- d_6) δ ; 1.56 (t, J 7.48, 3H, OCH₃), 3.8 (s, 3H, OCH₃), 4.12 (q, J 7.6, 2H, OCH₂), 5.02 (dd, J 7.2 1.87, 2H, CH₂ oxazine), 5.95 (s, 1H, CH oxazine), 6.5 (d, J 7.22,

1H, H₅), 6.61 (t, *J* 7.2, 1H, H₆), 6.8 to 6.89 (m, 3H, H₇, H₁₆), 7.02 (dd, *J* 7.4, 1.88, 1H, H₁₉), 7.39 to 7.44 (m, 3H, H₁₀, H₂₀), 7.78 (d, 8.6, 2H, H₁₁), 8.52 (s, 1H, H₁₄, CH=N), 9.59 (bs, 1H, NH), 9.83 (bs, 1H, OH). ¹³C NMR (75 MHz, DMSO-d₆) δ, 13.58 (1C, CH₃), 54.07 (1C, C₄), 57.37 (1C, OCH₃), 63.41 (1C, OCH₂), 108.93 (1C, C₁₆), 110.18 (2C, C₁₀), 114.41 (1C, C₁₉), 115.23 (1C, C₇), 117.66 (1C, C₂), 118.97 (2C, C₁₁), 121.05 (1C, C₁₂), 121.87 (1C, C₆), 122.31 (1C, C₅), 123.77 (1C, C_{4a}), 126.61 (1C, C₂₀), 129.87 (1C, C₁₅), 144.95 (1C, C₈), 146.17 (1C, C₁₇), 148.56 (1C, C_{8a}), 151.13 (1C, C₉), 152.23 (1C, C₁₈), 152.67 (1C, C=N), 167.36 (1C, C=O). EIMS = 481 calculated for C₂₅H₂₄ClN₃O₅.

4-(2-Chloro-8-Methoxy-2H-Benzo[1,3]Oxazin-3(4H)-Yl)-N'-(4-Hydroxy-3,5-Dimethoxybenzylidene) Benzohydrazide (5g)

Recrystallized from ethanol to afford pale yellow amorphous. MP 121 to 124°C. Yield 76%, FTIR (KBr, U_{max}/cm⁻¹), 3396 (OH), 3257 (NH), 3030 (CH_{Ar}), 2964, 2841 (CH_{aliph}), 1640 (C=O), 1606 (C=N), 1550, 1483 (C=C), ¹H NMR (300 MHz, DMSO-d₆) δ, 3.81 (s, 3H, OCH₃), 3.89 (s, 6H, 2 × OCH₃), 4.98 (dd, *J* 7.24, 1.88, 2H, CH₂ oxazine), 6.04 (s, 1H, CH oxazine), 6.54 to 6.63 (m, 4H, H₅, H₆, H₁₆, H₂₀), 6.83 (d, *J* 7.12, 1H, H₇), 7.39 (d, *J* 8.22, 2H, H₁₀), 7.79 (d, *J* 8.4, 2H, H₁₁), 8.52 (s, 1H, H₁₄, CH=N), 9.72 (bs, 1H, NH), 10.78 (bs, 1H, OH), ¹³C NMR (75 MHz, DMSO-d₆) δ, 55.08 (1C, C₄), 56.71 (2C, OCH₃), 57.23 (1C, OCH₃), 105.86 (2C, C₁₆, C₂₀), 109.78 (2C, C₁₀), 113.91 (1C, C₇), 118.13 (1C, C₂), 119.81 (2C, C₁₁), 121.05 (1C, C₁₂), 121.51 (1C, C₆), 121.75 (1C, C₅), 123.28 (1C, C_{4a}), 124.34 (1C, C₁₅), 141.43 (1C, C₁₈), 146.21 (1C, C₈), 148.57 (1C, C_{8a}), 150.66 (2C, C₁₇, C₁₉), 151.54 (1C, C₉), 152.11 (1C, C=N), 166.49 (1C, C=O). EIMS = 497 calculated for C₂₅H₂₄ClN₃O₆.

4-(2-Chloro-8-Methoxy-2H-Benzo[1,3]Oxazin-3(4H)-Yl)-N'-(3,5-Di-Tert-Butyl-2-Hydroxybenzylidene) Benzohydrazide (5h)

Crude product recrystallized from ethanol to obtain white crystal. MP 227 to 230°C. Yield 82%, FTIR (KBr, U_{max}/cm⁻¹); 3423 (OH), 3205 (NH), 3033 (CH_{Ar}), 2985, 2868 (CH_{aliph}), 1641 (C=O), 1606 (C=N), 1552, 1481 (C=C), ¹H NMR (300 MHz, DMSO-d₆) δ, 1.32 (s, 9H, tBu), 1.43 (s, 9H, tBu), 3.82 (s, 3H, OCH₃), 4.95 (dd, *J* 7.22, 1.90, 2H, CH₂ oxazine), 6.01 (s, 1H, CH oxazine), 6.51 (d, *J* 7.14, 1H, H₅), 6.57 (t, *J* 7.1, 1H, H₆), 6.83 (d, *J* 7.2, 1H, H₇), 7.27 (d, *J* 2.2, 1H, H₁₈), 7.39 (d, *J* 8.7, 2H, H₁₀), 7.76 (d, *J* 2.25, 1H, H₂₀), 7.87 (d, 8.6, 2H, H₁₁), 8.56 (s, 1H, H₁₄, CH=N), 9.48 (bs, 1H, NH), 10.87 (bs, 1H, OH). ¹³C NMR (75 MHz, DMSO-d₆) δ, 29.25 (3C, CH₃), 31.13 (3C, CH₃), 33.48 (1C, C₂₃, C(CH₃)₃), 34.55 (1C, C₂₁, C(CH₃)₃), 55.08 (1C, C₄), 57.23 (1C, OCH₃), 107.1 (1C, C₁₅), 111.34 (2C, C₁₀), 113.84 (1C, C₇), 120.11 (1C, C₁₂), 121.19 (1C, C₅), 121.73 (1C, C₆), 123.63 (1C, C_{4a}), 124.71 (1C, C₂), 124.89 (1C, C₂₀), 128.94 (1C, C₁₈), 130.88 (2C, C₁₁), 137.68 (1C, C₁₇), 141.31 (1C, C₁₉), 146.09 (1C, C₈), 149.25 (1C, C_{8a}), 152.34 (1C, C=N), 153.11 (1C, C₉), 154.98 (1C, C₁₆), 167.03 (1C, C=O). EIMS = 549 calculated for C₃₁H₃₆ClN₃O₄.

4-(2-Chloro-8-Methoxy-2H-Benzo[1,3]Oxazin-3(4H)-Yl)-N'-(3,5-Di-Tert-Butyl-4-Hydroxybenzylidene) Benzohydrazide (5i)

Recrystallized from chloroform-methanol(1-2) to afford white powder. MP 191 to 193°C. Yield 80%, FTIR (KBr, U_{max}/cm⁻¹), 3400 (OH), 3217 (NH), 3031 (CH_{Ar}), 2943, 2843 (CH_{aliph}), 1647 (C=O), 1608 (C=N), 1553, 1467 (C=C), ¹H NMR (300 MHz, DMSO-d₆) δ, 1.44 (s, 18H, 2 × tBu), 3.85 (s, 3H, OCH₃), 5.07 (dd, *J* 7.14, 1.88, 2H, CH₂ oxazine), 5.58 (s, 1H, OH), 5.98 (s, 1H, CH oxazine), 6.48 (d, *J* 7.26, 1H, H₅), 6.59 (t, *J* 7.1, 1H, H₆), 6.89 (d, *J* 7.2, 1H, H₇), 7.39 (d, *J* 8.6, 2H, H₁₀), 7.85 (d, 8.44, 2H, H₁₁), 7.89 (s, 2H, H₁₆), 8.6 (s, 1H, H₁₄, CH=N), 9.58 (bs, 1H, NH), 10.88 (bs, 1H, OH). ¹³C NMR (75 MHz, DMSO-d₆) δ, 30.35 (6C, CH₃), 34.65 (2C, C(CH₃)₃), 54.89 (1C, C₄), 56.73 (1C, OCH₃), 111.45 (2C, C₁₀), 113.91 (1C, C₇), 115.18 (1C, C₁₅), 120.44 (1C, C₁₂), 121.08 (1C, C₅), 122.11 (1C, C₆), 123.48 (1C, C_{4a}), 124.03 (1C, C₂), 124.28 (2C, C₁₆, C₂₀), 130.47 (2C, C₁₁), 136.78 (2C, C₁₇, C₁₉), 145.81 (1C, C₈), 149.05 (1C, C_{8a}), 152.13 (1C, C=N), 153.36 (1C, C₉), 157.09 (1C, C₁₈), 166.95 (1C, C=O). EIMS = 549 calculated for C₃₁H₃₆ClN₃O₄.

Screening Antibacterial Activity

The antibacterial activities of the synthesized compounds (5a–i) were screened against standard typed strains of four gram-negative bacteria, which are *Acinetobacter calcoaceticus* ATCC 23055, *Escherichia coli* ATCC 10538, *Pseudomonas aeruginosa* ATCC 15442, and *Salmonella typhimurium* ATCC 14028, as well as, four gram-positive bacteria, which are *Bacillus subtilis* ATCC 6051, *Enterococcus faecalis* ATCC 29212, *Streptococcus pyogenes* ATCC 19615, and *Staphylococcus aureus* ATCC 29213. These microorganisms were acquired from the central service laboratory-college education for pure science-Ibn Al-Haitham. The antibacterial activities were assayed in terms of minimal inhibitory concentrations (MICs) by employing the dilution assays according to the CLSI²⁴ and the same procedure described by Saoud SA *et al.*²⁵

RESULT AND DISCUSSION

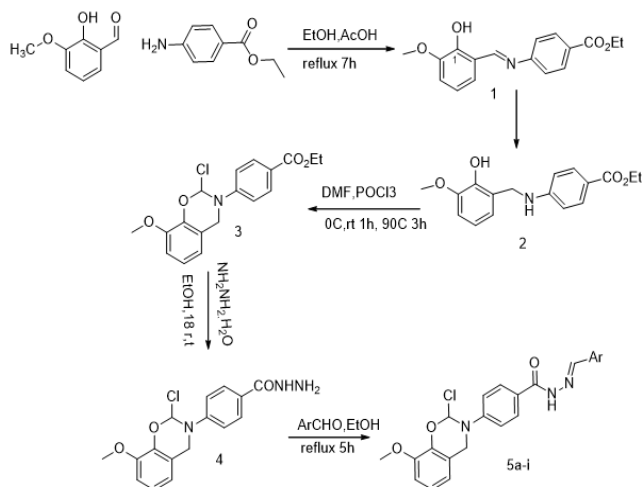
Novel 2-chloro-benzo-1,3-oxazine (3) was synthesized from the reaction of (2) with Vilsmeier reagent as depicted in Scheme 1. The acid hydrazide derivative of the corresponding 2-chloro-benzo-1,3-oxazine was synthesized from the reaction of hydrazine hydrate with compound (3) at ambient temperature. Nine new hydrazides were synthesized from reaction of compound (4) with nine aryl aldehyde, as depicted in Scheme 1. All synthesized compounds were characterized by FTIR, ¹H NMR, ¹³C NMR, and EIMS.

The FTIR of compound (1) showed peak at 3,379 cm⁻¹ for phenolic OH and peak at 1,705 cm⁻¹ attributed to carbonyl of ester. New interesting peak of imine group was located at 1,620 cm⁻¹. The ¹H NMR of this compound exhibited the all aromatic protons at their expected regions. Furthermore, the spectrum exhibited the protons of an ethoxy group of ester and interesting peak at 8.67 ppm attributed for CH=N as well, displaying to a broad peak at 9.83 ppm for phenolic OH. The

^{13}C NMR as well showed all expected peak beside the carbon of imine group were located at 156.82 ppm and the peak of $\text{C}=\text{O}$ was located at 166.27 ppm. The IR of compound (2) showed disappearing proton of imine group, as well as, the ^1H NMR spectrum confirm the success of reduction by exhibiting a new peak at 4.26 ppm attributed to CH_2NH as well, exhibiting a new broad peak at 4.83 ppm for NH. The ^{13}C NMR spectrum showed the disappearing carbon of imine group carbon and new carbon was located at 42.51 ppm for CH_2NH . The disappearance of OH and NH signals from the FTIR spectrum of compound (3) indicates that the reaction between compound (2) and Vilsmeier reagent was successful. Furthermore, the ^1H NMR of compound (3) exhibited the disappearance of the NH and OH peaks, moreover rising new peaks for 1,3-oxazine ring. The double doublet peak (due to 1, 3 splitting) with integral of two protons at 5.02 ppm clearly refers to two protons attached C_4 of 1,3-oxazine ring. The singlet peak for one proton at 5.91 ppm refers to existence of one proton for position two of 1,3-oxazine ring. The ^{13}C NMR as well confirms formation of 1,3-oxazine ring and exhibited two new peaks at 54.81 ppm attributed to C_4 and 124.68 ppm attributed to C_2 . The presence of C_2 at a lower magnetic field refers to the existence of chloride atom and this fact confirmed by sodium fusion test, as well as, EIMS confirm the existence of chloride by exhibiting the molecular ion corresponding to the mass of the suggested structure. The base peak and some fragments also confirm the existence of chloride atom. The proposed mechanism for the formation of a 2-chloro-1,3-oxazine ring was depicted in Figure 1.

The FTIR spectrum of compound (4) exhibited the successful convert the ester to corresponding acid hydrazide. The peaks of NH and NH_2 were located at 3,209, 3,398, and 3,309 cm^{-1} . The peak of carbonyl amide was located at 1,648 cm^{-1} . Furthermore, the ^1H NMR spectrum was in agreement with the FTIR spectrum. The ^1H NMR spectrum showed a new broad peak at 4.07 ppm for two protons attributed to NH_2 and the second interesting peak assigned at 9.83 ppm attributed for NH. The basic protons were located

at their expected regions. On the other hand, the ^{13}C NMR confirms conversion of the ester to their corresponding acid hydrazide. Exhibiting the carbon of amide at 168.21 ppm, while the carbonyl of ester at compounds (1–3) was located at 166.27 to 164.17 ppm, was good evidence. The EIMS spectrum confirms the structure and exhibited the molecular ion M^+ peak and the base peak (100%). The FTIR spectra of compounds (5a–i) displayed no existence of the NH_2 at range as well. It showed a new peak at range 1,606 to 1,627 cm^{-1} attributed to $\text{CH}=\text{N}$ of hydrazone. Furthermore, the FTIR exhibited the peaks of substituted, such as, the peaks of OH for compounds (5d, 5e, 5f, 5g, 5h, and 5i) at 3,312, 3,411, 3,324, 3,396, 3,423, and 3,400 cm^{-1} , respectively. The two peaks of asymmetrical and symmetrical NO_2 for compound (5b) appeared at 1,468 and 1,349 cm^{-1} , respectively. The ^1H NMR of these compounds showed the proton of imine group of the hydrazone at 8.45 to 8.64 ppm with integral of one proton. The ^1H NMR as well displayed the aromatic protons for the hydrazone and they are substituted, for instance, the OH of compound (5d) was located at 10.83 ppm. The proton of 2-hydroxy group for (5e) was located at 10.90 ppm, as well as, two methoxy groups were located at 3.81 and 3.88 ppm. The proton of 4-hydroxy group for compound (5f) was located at 9.83 ppm. The triplet peak at 1.56 ppm and quartite at 4.12 ppm assigned to five protons of 3-ethoxy group for same compound. The ^1H NMR spectrum for compound (5g) exhibited the proton of hydroxyl group at 10.78 ppm and the two methoxy group was located at 3.89 ppm with integral of six protons. The proton of 2-hydroxy group of compound (5h) was located at 10.87 ppm. The two singlets at 1.32 and 1.43 ppm were assigned for two *tert*-butyl groups with an integral equal nine for each peak. The proton of 4-hydroxy of compound (5i) was assigned at 5.58 ppm and that attributed to satirical hindrances by two *tert*-butyl group.²⁶ The eighteen protons of two symmetrical di-*tert*-butyl groups were assigned as singlet peak at 1.44 ppm. The ^{13}C NMR spectra of (5a–i) (Figure 2 and 3) showed shifting in value of $\text{C}=\text{O}$ from 168.21 to between 166.11 and 167.16 ppm, after conversion of compound (4) to their



Scheme 1: General synthetic route of 2-chloro-1,3-benzoxazine and their derivatives

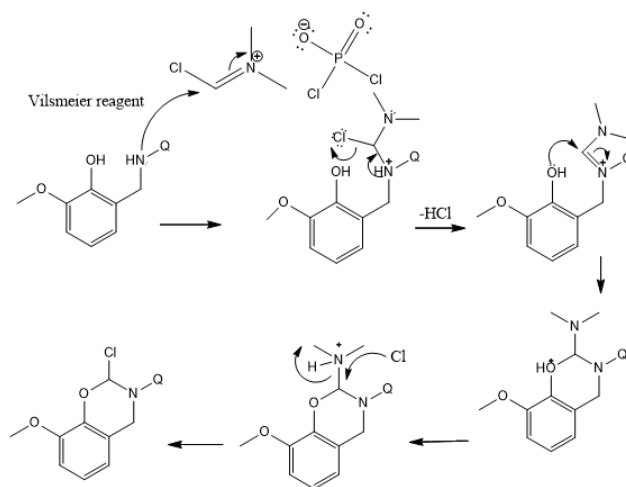


Figure 1: Proposed mechanism for synthesized 2-chloro-1,3-benzoxazine using Vilsmeier reagent

corresponding hydrazones. Furthermore, the ^{13}C spectra of these compounds displayed the new peaks attributed to new phenyl of hydrazone and their substituted group and the interesting carbons, which are attached to these groups besides peak of imine group. For instance, showed the C=N

peak at 151.44 ppm and carbon attached with 4-chloro was located at 136.41 ppm. Compound (5b) (Figure 4) showed the peak of C=N at 152.49 ppm and the peak of carbon attached with 4-nitro was located at 150.67 ppm. The peak of C=N for compound (5c) (Figure 5) was located at 152.80 besides the

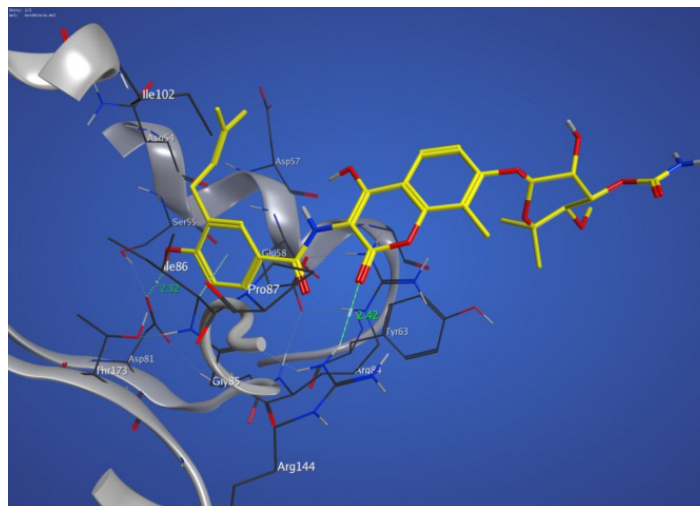
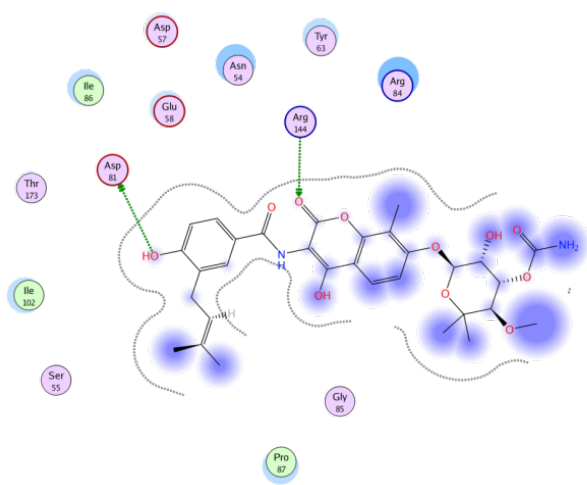


Figure 2: (a) (2D) novobiocin as potent inhibitor against ATP-binding domain bacterial gyrase enzyme pocket (b) (3D) novobiocin as potent inhibitor against ATP-binding domain bacterial gyrase enzyme pocket

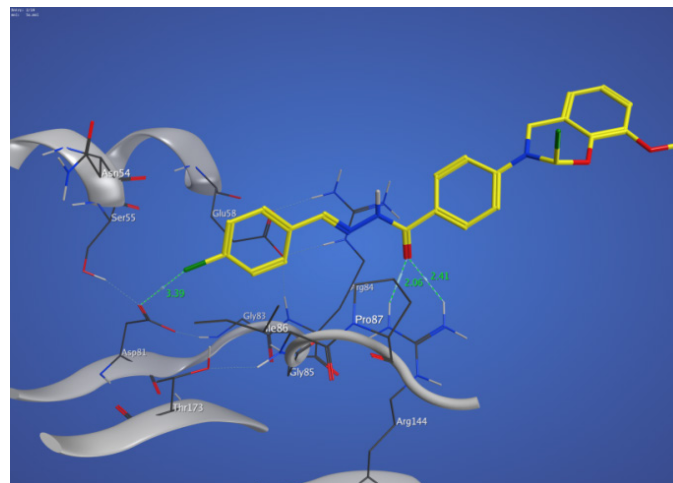
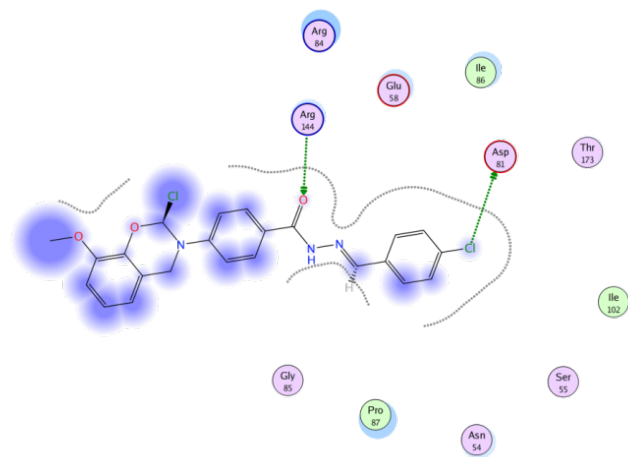


Figure 3: Compound (5a) 3D and 2D interaction

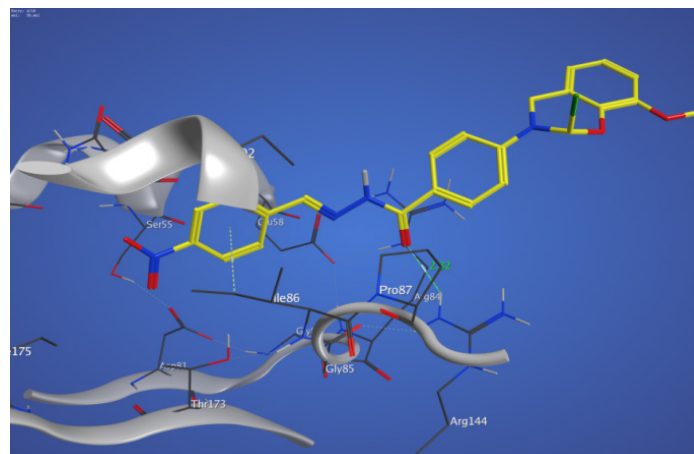
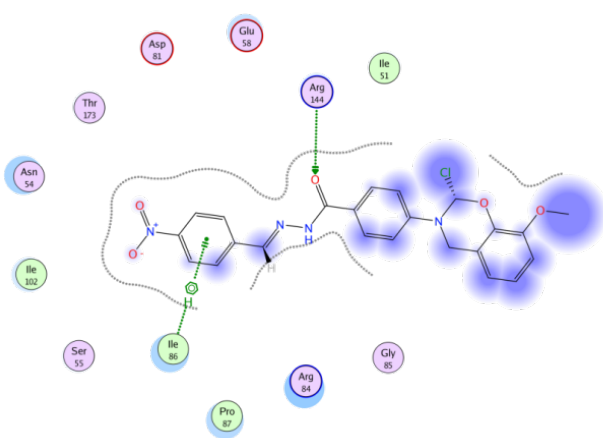


Figure 4: Compound (5b) 3D and 2D interaction

two carbons attached two groups of chloride at positions meta was located at 135.45 ppm. The peaks assigned at 154.01 and 159.38 ppm for compound (5d) (Figure 6) attributed to C=N

and C₁₈, which are attached to hydroxyl group. The spectrum of compound (5e) (Figure 7) exhibited peak of C=N at 152.78 ppm and the peak of carbon attached to the methoxy was located

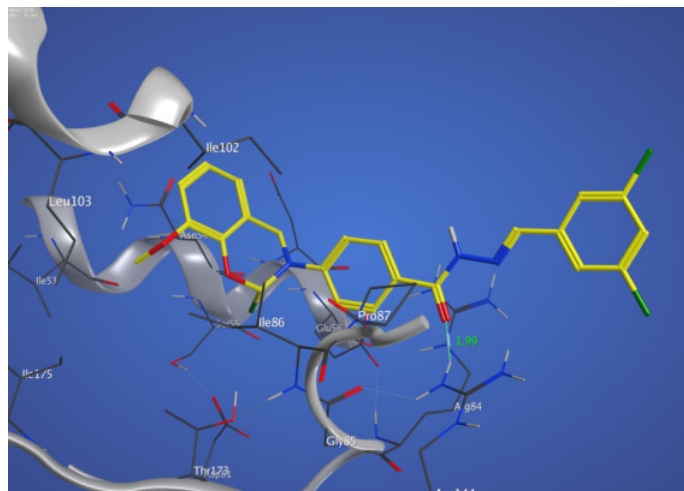
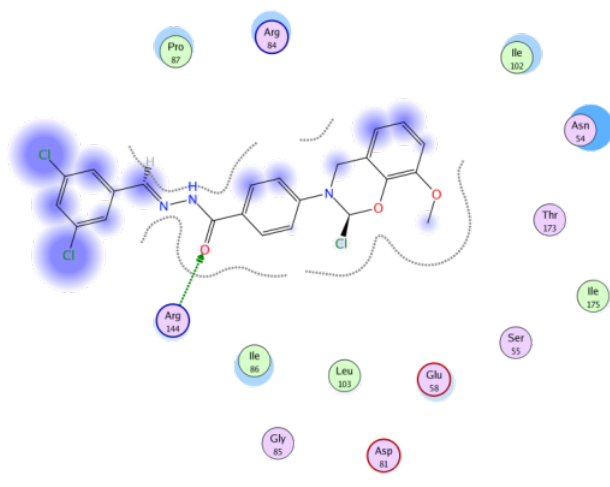


Figure 5: Compound (5c) 3D and 2D interaction

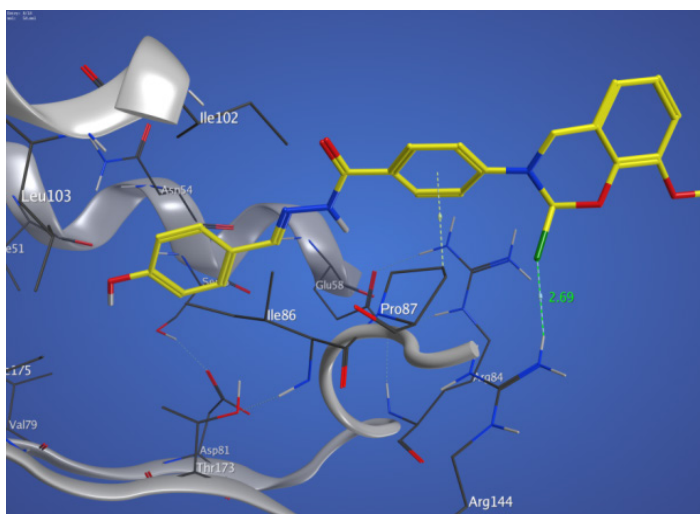
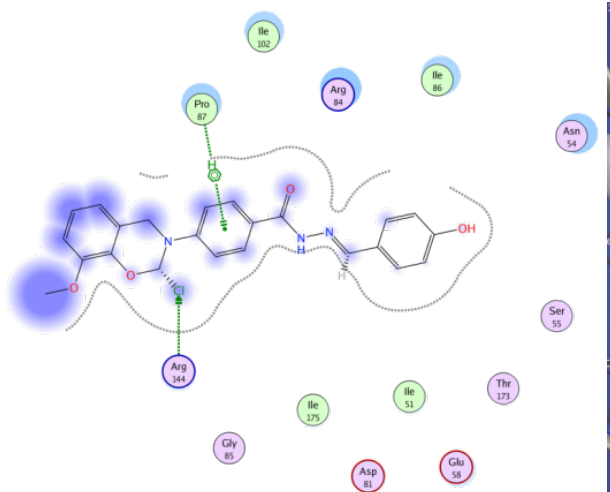


Figure 6: Compound (5d) 3D and 2D interaction

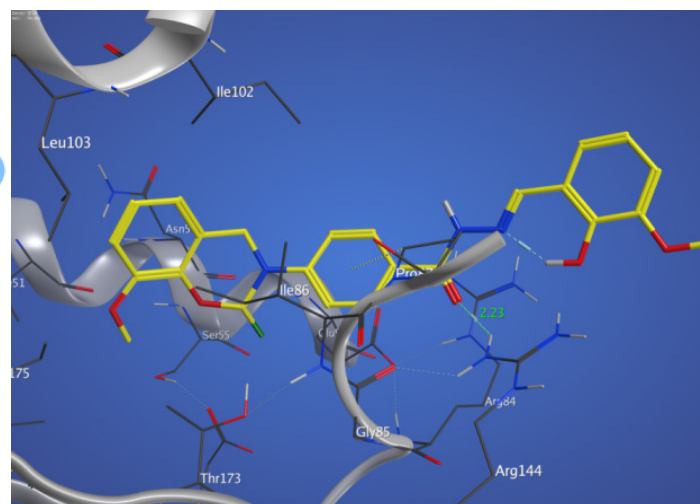
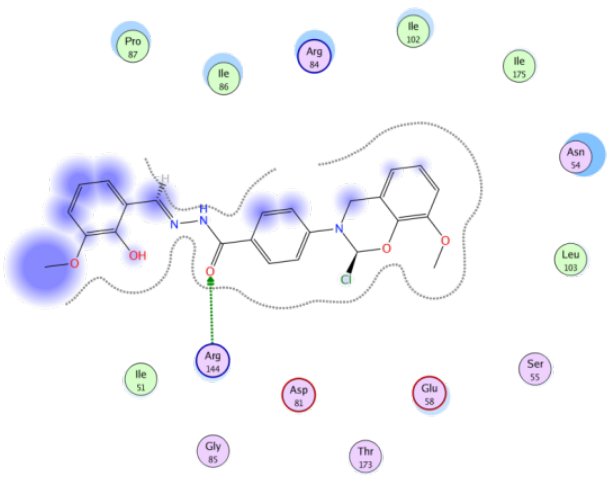


Figure 7: Compound (5e) 3D and 2D interaction

at 147.05 ppm besides carbon-attached hydroxyl group was located at 152.02 ppm. The same results were exhibited at ^{13}C NMR spectra for rest compounds. The EIMS spectra of all compounds were in agreement with FTIR, ^1H NMR, and ^{13}C NMR. The structure and some physical properties were tabulated in Table 1. The EIMS exhibited the value of molecular ion matched to the calculated value. The EIMS showed the base peak all fragmentations harmonized with the suggested structure.

Antibacterial Activity

The antibacterial activities for synthesized compounds (5a–i) were tested utilizing micro broth dilution assays. Standard strains of gram-negative and gram-positive bacteria were utilized. Commercial kanamycin and amoxicillin were utilized as references. The MIC (mg/mL) of newly synthesized compounds against the test microorganisms were determined. The results of the antibacterial capability are tabulated in Table 2. Compounds (5b) and (5c) (Figure 7)

Table 1: Structure and some physical properties of synthesized compounds (5a–i)

No.	Structure	Yield %	MP ($^{\circ}\text{C}$)	Chemical formula	EIMS
5a		86	195–196	$\text{C}_{23}\text{H}_{19}\text{Cl}_2\text{N}_3\text{O}_3$	455
5b		89	203–206	$\text{C}_{23}\text{H}_{19}\text{ClN}_4\text{O}_5$	466
5c		83	226–228	$\text{C}_{23}\text{H}_{18}\text{Cl}_3\text{N}_3\text{O}_3$	489.1
5d		77	235–237	$\text{C}_{23}\text{H}_{20}\text{ClN}_3\text{O}_4$	437
5e		75	173–176	$\text{C}_{24}\text{H}_{22}\text{ClN}_3\text{O}_5$	467
5f		80	232–233	$\text{C}_{25}\text{H}_{24}\text{ClN}_3\text{O}_5$	481
5g		76	121–124	$\text{C}_{25}\text{H}_{24}\text{ClN}_3\text{O}_6$	497
5h		82	227–230	$\text{C}_{31}\text{H}_{36}\text{ClN}_3\text{O}_4$	549
5i		80	191–193	$\text{C}_{31}\text{H}_{36}\text{ClN}_3\text{O}_4$	549

showed significant antibacterial ability against both gram-negative and gram-positive bacteria. This efficiency could be attributed to the existence of electron-withdrawing substitution, which enhanced the antibacterial activity rather than electron-donating substitution.²⁷⁻²⁹ Compound (5a) exhibited mild antibacterial activity toward gram-positive bacteria and weak antibacterial ability toward gram-negative bacteria. Compounds (5e–i) (Figure 7), which consist of phenolic hydroxide, showed mild to low antibacterial capability against gram-positive and did not show any notable antibacterial capability against gram-negative bacteria. It is obvious that existence of hydroxyl group reduces the antioxidant ability.³⁰ Furthermore, the antibacterial capability of these compounds diminution with an increase in the satirical hindrance around the phenolic hydroxyl.²⁵

Molecular Docking Study

Choosing Molecular Targets

By comparing our tested compounds with other ligands and determining the pharmacophoric feature that can bind with critical amino acid in the target site, target site selection has been done by (<https://www.rcsb.org/>) protein data bank. Our compounds tested practically against many target sites, then good results determine suitable protein for doing docking studies.

Preparation of Receptor for Virtual Screening

After choosing protein of target site some processes should be done to give insights of molecular binding modes of the tested compounds inside the pocket of bacterial gyrase enzyme target site by using MOE 2015 software, which acts as ATP-binding domain bacterial gyrase enzyme pocket. The binding sites were generated from the co-crystallized ligand, within crystal protein (PDB codes: 5 ctu). At first, water molecules were removed from the complex. Then, the crystallographic

disorders and unfilled valence atoms were corrected using protein reports and utility, and clean protein options.

Protein energy was minimized by applying CHARMM and MMFF94 force fields. The rigid binding site structure of protein was obtained by applying fixed atom constraint. The protein essential amino acid is defined and prepared for the docking process. 2D structures of tested compounds were drawn using Chem-Bio Draw Ultra 14.0 and saved in the MDL-SD file format. From MOE 2015 software, the saved file was opened, 3D structures were protonated and energy was minimized by applying 0.05 RMSD kcal/mol CHARMM force field. Then, the minimized structures were prepared for docking using prepares ligand protocol.

Molecular Docking Processes

The docking process was carried out using CDOCKER protocol. CDOCKER is a grid-based molecular docking method that employs CHARMM-based molecular dynamics (MD) scheme to dock ligands into a receptor-binding site. The receptor was held rigid, while the ligands were allowed to be flexible during the refinement. Each molecule was allowed to produce seven different interaction poses with the protein. Then docking scores (CDOCKER interaction energy) of the best-fitted poses with the active site at bacterial gyrase enzyme was recorded (Table 3). We used all these processes to predict the proposed binding mode, affinity, preferred orientation of each docking pose, and binding free energy (ΔG) of the tested compounds with bacterial gyrase enzyme. The calculated interaction energies for the tested compounds were in complete agreement with the experimental results, which showed that our compounds care potent inhibitors against bacterial gyrase enzyme.

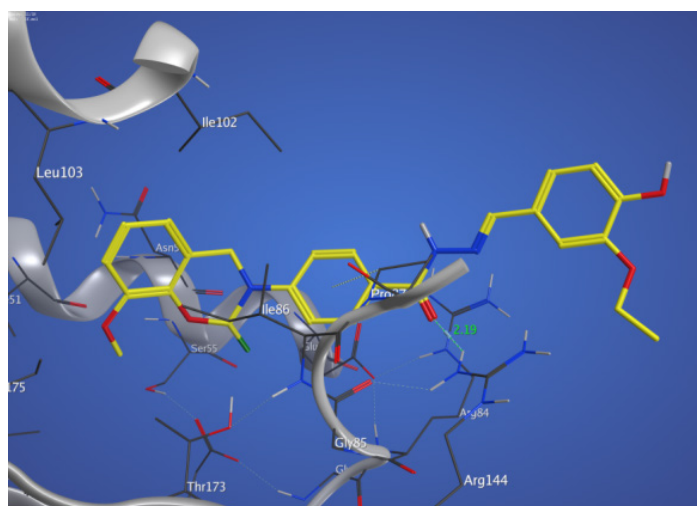
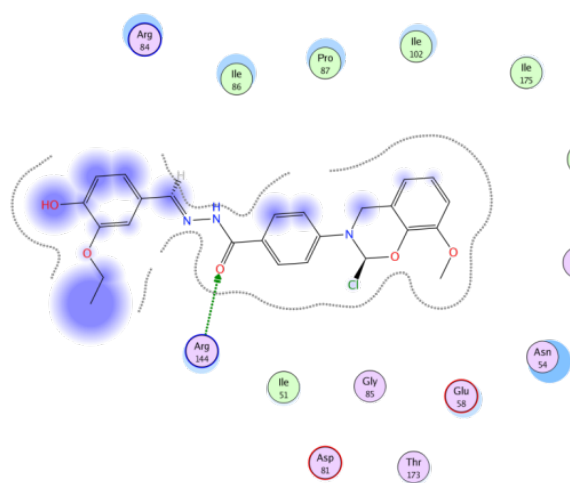
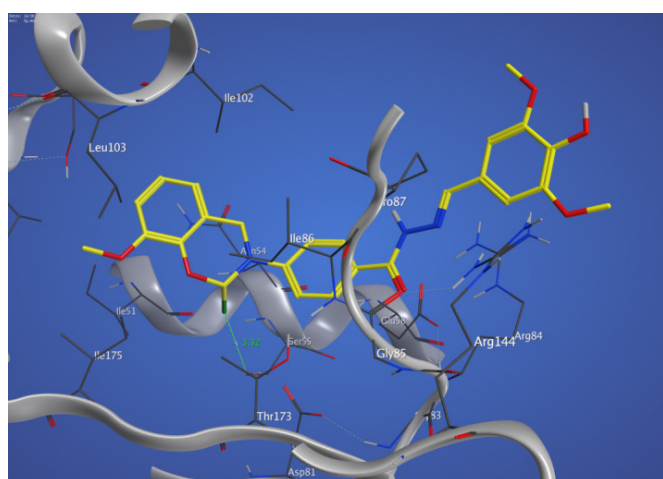
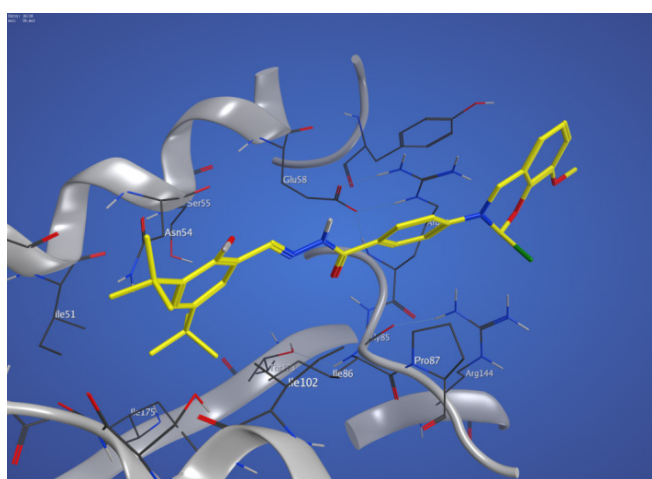
Firstly, the key binding site of bacterial gyrase enzyme has been reported by the literature, consisting of amino Arg84, Ile86, Arg144, Asp81, pro87, and Gly85. The next Figure 8

Table 2: Antibacterial activity of synthesized compounds (5a–i)

No.	Bacteria/MICs (mg/mL)							
	Gram-negative bacteria				Gram-positive bacteria			
	<i>Escherichia coli</i>	<i>Acinetobacter calcoaceticus</i>	<i>Salmonella typhimurium</i>	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Streptococcus pyogenes</i>	<i>Enterococcus faecalis</i>	<i>Staphylococcus aureus</i>
5a	> 0.5	0.5	0.25	0.2	0.5	0.4	0.35	0.35
5b	< 0.05	0.15	0.2	0.1	0.05	0.05	0.1	0.25
5c	0.15	0.25	0.25	0.01	0.2	0.25	0.3	0.2
5d	0.25	> 0.5	0.45	0.3	0.25	0.5	0.45	0.3
5e	0.4	> 0.5	0.45	0.4	0.3	0.25	0.3	> 0.5
5f	0.5	ND	0.5	0.35	0.45	> 0.5	ND	> 0.5
5g	> 0.5	> 0.5	ND	0.5	0.35	0.5	ND	ND
5h	0.5	> 0.5	ND	ND	0.4	0.45	ND	> 0.5
5i	ND	> 0.5	ND	> 0.5	0.5	0.5	0.4	ND
Amoxicillin	< 0.05	< 0.05	ND	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Kanamycin	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	> 0.5	< 0.05	< 0.05

Table 3: (DG) kcal/mol of tested compounds against bacterial gyrase enzyme target site PDB ID: 5 ctu

Compound No.	Bonds No.		Interaction with key amino acids	Score (DG) kcal/mol	RMSD value	E-place	Bonds length range/ Å
	H.B	Pi					
5a	2	-	Arg144–Asp81	-5.39	1.44	-58.33	2.02–2.41
5b	1	1	Arg144–Ile86	-5.1	2.89	-45.7	2.32
5c	1	-	Arg144	-5.72	2.58	-75.96	1.99
5d	1	1	Arg144–Pro87	-6.06	2.37	-67.61	2.69
5e	1	-	Arg144	-6.89	2.56	-83.52	2.23
5f	1	-	Arg144	-6.81	1.22	-72.87	2.19
5g	1	-	Ser55	-6.26	1.75	-89.9	3.32
5h	-	-	No interaction	-	-	-	-
5i	-	2	Arg144– Ile86	-6.44	1.7	-101.69	-
Novobiocin	2	-	Arg144–Asp81	-7.02	3.08	-84.7	2.32–2.42


Figure 8: Compound (5f) 3D and 2D interaction

Figure 9: Compound (5g) 3D interaction

Figure 10: Compound (5h) 3D interaction

show the reference compound (novobiocin) binding mode 2D and 3D simulation attachment against bacterial gyrase enzyme critical amino acids. Secondly, the next 3D and 2D Figure 9 for molecular docking simulation of our tested compounds show the binding mode, molecular surface area, and length of hydrogen bonds/ Å.

After doing docking study for our compounds against bacterial gyrase enzyme as ATP-binding domain bacterial gyrase enzyme inhibitors, it shows that most of our compounds have good binding and attachment with the target site. Compounds (5a, 5b, and 5i) have excellent result compared with novobiocin as ATP-binding domain bacterial gyrase enzyme inhibitors (Figure 10-11).

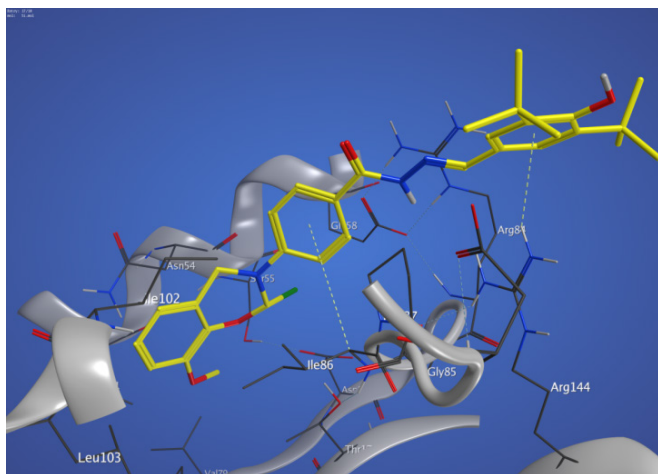


Figure 11: Compound (5i) 3D interaction

CONCLUSION

The novel compound 4-(2-chloro-8-methoxy-2H-benzo[e][1,3]oxazin-3(4H)-yl) benzoate (3) was successfully synthesized from reaction ethyl 4-((2-hydroxy-3-methoxybenzyl)amino) benzoate with Vilsmeier reagent. This compound (3) was converted to their corresponding acid hydrazide (4). New series of hydrazones (5a–i) were successfully synthesized and characterized. The antibacterial activity of these hydrazones was tested using MIC method. Compounds (5d) and (5c) showed significant antibacterial ability toward all microorganisms under study. The phenolic hydroxyl group, electro donating group, and satirical hindrance around phenolic hydroxyl constrict the antibacterial activity. The molecular structure docking shows (5a), (5b), and (5i) have excellent results compared with novobiocin as ATP-binding domain bacterial gyrase enzyme inhibitors.

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REFERENCES

- Marson, C. M., *Synthesis Using Vilsmeier Reagents*. CRC Press: 2018.
- Fadavi A, Zarei M. One-pot Vilsmeier reagent-mediated multicomponent reaction: A direct synthesis of oxazolones and Erlenmeyer azlactones from carboxylic acids. *Comptes Rendus Chimie*. 2018 Jan 1;21(1):9-13.
- Zaman AU, Ahmad I, Pervaiz M, Ahmad S, Kiran S, Khan MA, Gulzar T, Kamal T. A novel synthetic approach for the synthesis of pyrano [3, 2-c] quinolone-3-carbaldehydes by using modified Vilsmeier Haack reaction, as potent antimicrobial agents. *Journal of Molecular Structure*. 2019 Mar 15;1180:227-236.
- Zarei M. One-pot synthesis of 1, 3, 4-thiadiazoles using Vilsmeier reagent as a versatile cyclodehydration agent. *Tetrahedron*. 2017 Apr 6;73(14):1867-1872.
- Damodiran M, Selvam NP, Perumal PT. Synthesis of highly functionalized oxazines by Vilsmeier cyclization of amidoalkyl naphthols. *Tetrahedron Letters*. 2009 Sep 30;50(39):5474-5478.
- Nayak G, Shrivastava B, Singhai AK. Synthesis and Antimicrobial Activity of Azetidin-2-one Fused 2-Chloro-3-formyl Quinoline Derivatives. *Oriental Journal of Chemistry*. 2016 Aug 23;32(4):1977-1982.
- AL-Thamiar BF, Sabiha F, Saleh MM. Synthesis And Antimicrobial Activity Of Some (2-Amino-5-Thiol-1, 3, 4-Thiadiazole Derivatives. *Baghdad Science Journal*. 2007;4(1):89-94.
- Li J, Huo H, Guo R, Liu B, Li L, Dan W, Xiao X, Zhang J, Shi B. Facile and efficient access to Androsten-17-(1', 3', 4')-pyrazoles and Androst-17 β -(1', 3', 4')-pyrazoles via Vilsmeier reagents, and their antiproliferative activity evaluation in vitro. *European Journal of Medicinal Chemistry*. 2017 Apr 21;130:1-14.
- Khammas SJ, Hamood AJ. Synthesis, Cytotoxicity, Xanthine Oxidase Inhibition, Antioxidant of New Pyrazolo {3, 4 d} Pyrimidine Derivatives. *Baghdad Science Journal*. 2019;16(4 Supplement):1003-1009.
- Bingul M, Tan O, Gardner CR, Sutton SK, Arndt GM, Marshall GM, Cheung BB, Kumar N, Black DS. Synthesis, characterization and anti-cancer activity of hydrazide derivatives incorporating a quinoline moiety. *Molecules*. 2016 Jul;21(7):916.
- de Brito MR, Peláez WJ, Faillace MS, Militão GC, Almeida JR, Argüello GA, Szakonyi Z, Fülöp F, Salvadori MC, Teixeira FS, Freitas RM. Cyclohexene-fused 1, 3-oxazines with selective antibacterial and antiparasitic action and low cytotoxic effects. *Toxicology in Vitro*. 2017 Oct 1;44:273-279.
- Verma V, Singh K, Kumar D, Narasimhan B. QSAR studies of antimicrobial activity of 1, 3-disubstituted-1H-naphtho [1, 2-c] [1, 3] oxazines using topological descriptors. *Arabian Journal of Chemistry*. 2017 Feb 1;10:S747-S756.
- Tang ZL, Xia ZW, Chang SH, Wang ZX. Synthesis and fungicidal activity of novel 2-aryl-3-(1, 3, 4-thiadiazolyl)-6 (8)-methyl-1, 3-benzoxazines. *Bioorganic & medicinal chemistry letters*. 2015 Aug 15;25(16):3378-3381.
- Ozhogin IV, Dorogan IV, Lukyanov BS, Mukhanov EL, Tkachev VV, Chernyshev AV, Lukyanova MB, Aldoshin SM, Minkin VI. New spiopyrans based on 1, 3-benzoxazine-2-one: acid catalyzed synthesis and theoretical insight into the photochromic activity. *Tetrahedron Letters*. 2016 Jun 1;57(22):2382-2385.
- He LY, Qiu XY, Cheng JY, Liu SJ, Wu SM. Synthesis, characterization and crystal structures of vanadium (V) complexes derived from halido-substituted tridentate hydrazone compounds with antimicrobial activity. *Polyhedron*. 2018 Dec 1;156:105-110.
- Malik S, Khan A, Naseer MM, Khan SU, Ashraf A, Ashraf M, Rafiq M, Mahmood K, Tahir MN, Shafiq Z. Xanthenone-based hydrazones as potent α -glucosidase inhibitors: synthesis, solid state self-assembly and in silico studies. *Bioorganic chemistry*. 2019 Mar 1;84:372-383.
- Parlar S, Sayar G, Tarikogullari AH, Karadagli SS, Alptuzun V, Erciyas E, Holzgrabe U. Synthesis, bioactivity and molecular modeling studies on potential anti-Alzheimer piperidinehydrazide-hydrazones. *Bioorganic chemistry*. 2019 Jun 1;87:888-900.
- Fekri R, Salehi M, Asadi A, Kubicki M. Synthesis, characterization, anticancer and antibacterial evaluation of Schiff

- base ligands derived from hydrazone and their transition metal complexes. *Inorganica Chimica Acta*. 2019 Jan 1;484:245-54.
19. Kris-Etherton PM, Lefevre M, Beecher GR, Gross MD, Keen CL, Etherton TD. Bioactive compounds in nutrition and health-research methodologies for establishing biological function: the antioxidant and anti-inflammatory effects of flavonoids on atherosclerosis. *Annu. Rev. Nutr.* 2004 Jul 14;24:511-38.
 20. Alex AA, Beaumont K, Kalgutkar A, Walker D, Dalvie D, Prakash C, Alfin V, Gardner I, Obach RS, Smith DA, Miao Z. Metabolism, pharmacokinetics and toxicity of functional groups: impact of chemical building blocks on ADMET. *Royal Society of Chemistry*; 2010 Apr 9.
 21. El-Kerdawy MM, El-Bendary ER, Alaa AM, El-Wasseef DR, Abd El-Aziz NI. Synthesis and pharmacological evaluation of novel fused thiophene derivatives as 5-HT_{2A} receptor antagonists: molecular modeling study. *European journal of medicinal chemistry*. 2010 May 1;45(5):1805-1820.
 22. El-Helby AG, Ayyad RR, Sakr HM, Abdelrahim AS, El-Adl K, Sherbiny FS, Eissa IH, Khalifa MM. Design, synthesis, molecular modeling and biological evaluation of novel 2, 3-dihydrophthalazine-1, 4-dione derivatives as potential anticonvulsant agents. *Journal of Molecular Structure*. 2017 Feb 15;1130:333-351.
 23. Shakir RM, Ariffin A, Ng SW. Ethyl 4-[(3, 5-di-tert-butyl-2-hydroxybenzylidene) amino] benzoate. *Acta Crystallographica Section E: Structure Reports Online*. 2010 Nov 1;66(11):o2915-o2916.
 24. Wikler MA. Performance standards for antimicrobial susceptibility testing Sixteenth informational supplement. M 100-S 16. 2006.
 25. Saoud SA, Ali KF, Shakir RM. Relationship Between the structure of Newly Synthesized derivatives of 1, 3, 4-oxadiazole Containing 2-Methylphenol and their Antioxidant and Antibacterial Activities. *Oriental Journal of Chemistry*. 2017 Aug 10;33(4):1781-1798.
 26. Abraham RJ, Mobli M. An NMR, IR and theoretical investigation of ¹H Chemical Shifts and hydrogen bonding in phenols. *Magnetic Resonance in Chemistry*. 2007 Oct;45(10):865-877.
 27. Gu W, Wu R, Qi S, Gu C, Si F, Chen Z. Synthesis and antibacterial evaluation of new N-acylhydrazone derivatives from dehydroabiatic acid. *Molecules*. 2012 Apr;17(4):4634-4650.
 28. Kossakowski J, Krawiecka M, Kuran B, Stefańska J, Wolska I. Synthesis and preliminary evaluation of the antimicrobial activity of selected 3-benzofurancarboxylic acid derivatives. *Molecules*. 2010 Jul;15(7):4737-4749.
 29. Plech T, Wujec M, Siwek A, Kosikowska U, Malm A. Synthesis and antimicrobial activity of thiosemicarbazides, s-triazoles and their Mannich bases bearing 3-chlorophenyl moiety. *European Journal of Medicinal Chemistry*. 2011 Jan 1;46(1):241-248.
 30. Sánchez-Maldonado AF, Schieber A, Gänzle MG. Structure–function relationships of the antibacterial activity of phenolic acids and their metabolism by lactic acid bacteria. *Journal of applied microbiology*. 2011 Nov;111(5):1176-1184.