



Ultrasonic and solvent free Synthesis of Regioselective Diastereomeric Adducts and Heterocyclic Products as antibacterial agent

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ABSTRACT

Oxirane ring containing the carboxylic group in the α,β -position are useful intermediates in the synthesis of biologically active compounds. Epoxidation of 4-(4-acetylmino and/or bromophenyl)-4-oxo-but-2-enoic acids via ultrasound condition afforded α -oxirane carboxylic acid followed by regioselective diastereomeric adducts of camphor. The steric factor plays an important role in regioselectivity. Formation of oxirane and furan derivatives via ultrasonic condition was considered as key steps for synthesis of some important heterocyclic compounds. The structure of new synthesized compounds 2-9a,b were elucidated by elemental analysis and spectroscopic data. The antibacterial activity for the synthesized compounds was evaluated.

KEYWORDS: Epoxide, Ultrasonic irradiation, MW, Furanones, Pyridazinone, Oxazinones

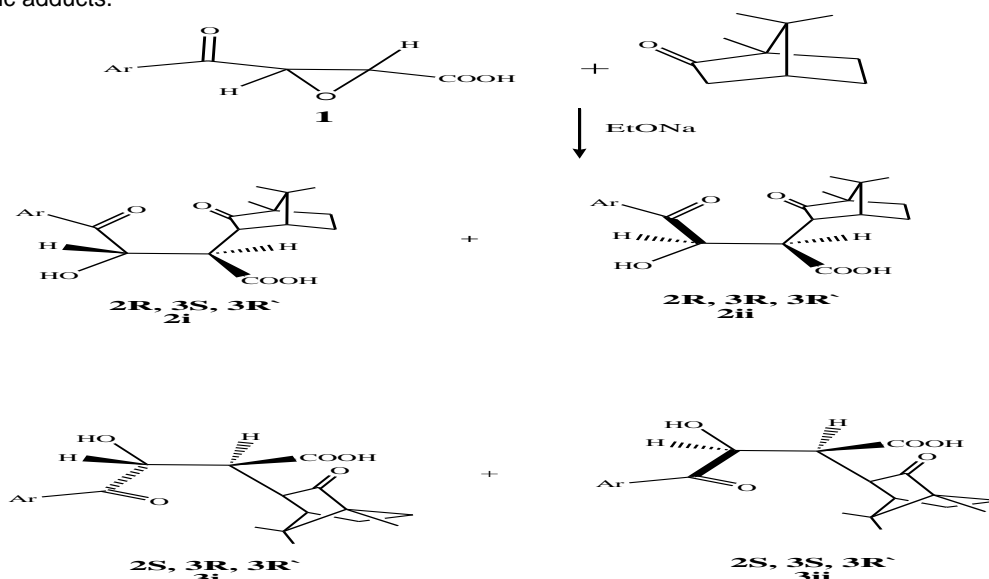
INTRODUCTION

A diversity of therapeutics in use newly that are representative both high antitumor activity and restriction of tumor metastasis such family of medicinal fungi has been derived from the species of epoxide and furanone 1-6, where the earlier medicinal species used for the treatment of fester, bellyache, oxidizing enzyme and bloody gonorrhea 7-11. Also, Phelligridin G is one of furanone derivative shows important antioxidant activity, inhibiting rat liver microsomal lipid peroxidation and reveal moderate selective cytotoxic activity against a human ovarian (A2780) and colon cancer cell line (HCT-8) 12-17. Green heterocyclic chemistry has recently attracted considerable interest from chemists, who have used this technique to achieve the green synthesis of several heterocyclic systems like ultrasonic and microwave free-solvent technique. The importance of the ultrasonic and solvent free reactions in various laboratories develop a consistent one-pot method which would be less cumbersome and lead to a significant rate enhancement with an environmentally benign method by which reagents are placed inside a vessel along with a ball-bearing that is shaken at high speeds in the absence of solvent 18-25. The ultrasonic instrument has enough force to make an amorphous mixture of the reagents which in turn facilitates a green chemical reaction with relatively new to organic synthesis 26-30.

RESULTS AND DISCUSSION

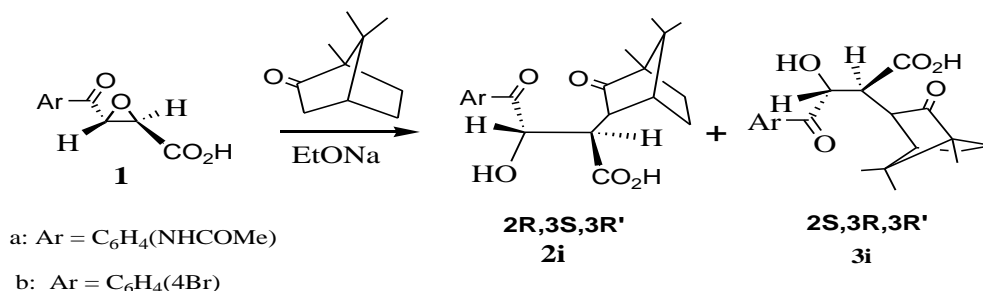
Chemistry

Recently, we reported the behavior of 4-(4-acetylaminophenyl)-4-oxo-2-butenic and 4-(4-bromophenyl)-4-oxo-2-butenic acids toward some electrophilic and nucleophilic reagents. They were allowed to react with carbon and nitrogen nucleophiles, e.g. reactive aromatic hydrocarbons, 5-methyl-2,4-dihydro-3H-pyrazol-3-one, 5-phenyl-2,4-dihydro-3H-pyrazol-3-one, barbituric acid, quinazolinone derivatives and (1R,4R) (+)-camphor in different reaction conditions under Michael, aza-Michael and Friedel-Crafts reaction conditions [31-38]. Synthesis of the Diastereomeric Michael adducts [42] have been thought us to make the reaction of the (+)-camphor with their epoxides [31]. The mechanism of epoxidation via reaction of urea and hydrogen peroxide within ultrasound basic conditions was outlined in the following (Scheme 1) where the regioselectivity of the oxirane derivatives (1) in position α toward the carboxylic group might be afforded four diastereomeric adducts.



Scheme 1: Outline the possibility of the diastereomeric adducts via reaction of the epoxide 1 with (1R,4R)(+)-camphor under Michael reaction condition

The anti aroyl and carboxylic groups for isomers **2i** and **3i** were more favored than syn isomers **2ii** and **3ii**, where the repulsion force between aroyl and carboxylic groups caused the lower stability in syn isomers **2ii** and **3ii**. In addition to the energy gaps between HOMO and LUMO values reflected the higher stability of isomers **2i** and **3i** (Figure 1). This explained that two diastereomers **2i** (2R,3S,3R') and **3i** (2S,3R,3R') can be isolated only via ultrasonic condition (Scheme 2).



Scheme 2: Outline the diastereomeric adducts via reaction of aroylacrylic acid with (1R,4R)(+)-camphor under ultrasound condition

The yield of isomer **2i** (2R,3S,3R') in case of acetamido phenyl moiety of derivative (**2a**) was 44% more than the bromo derivative (**2b**) was 32% and vice versa in isomer **3a** was 21% less than **3b** 41% due to maximum repulsion between acetamido phenyl and dimethyl groups of R(+) camphor precursor.

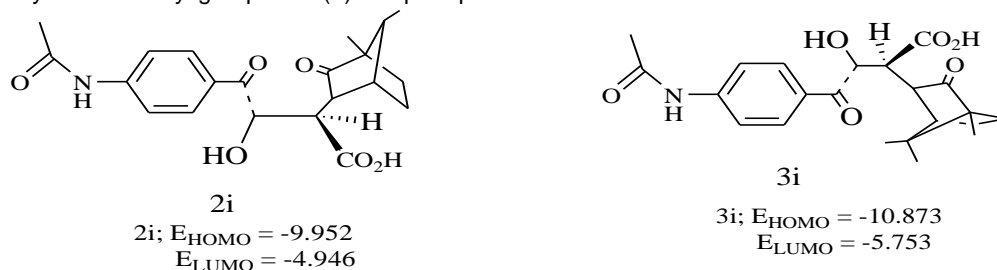
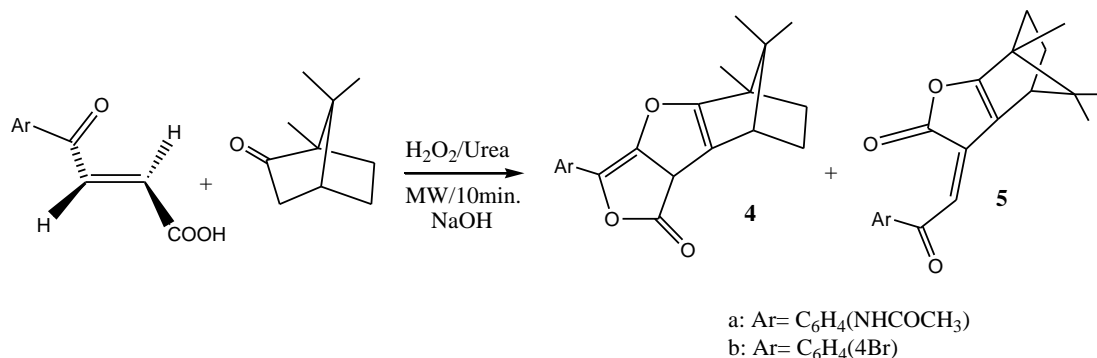


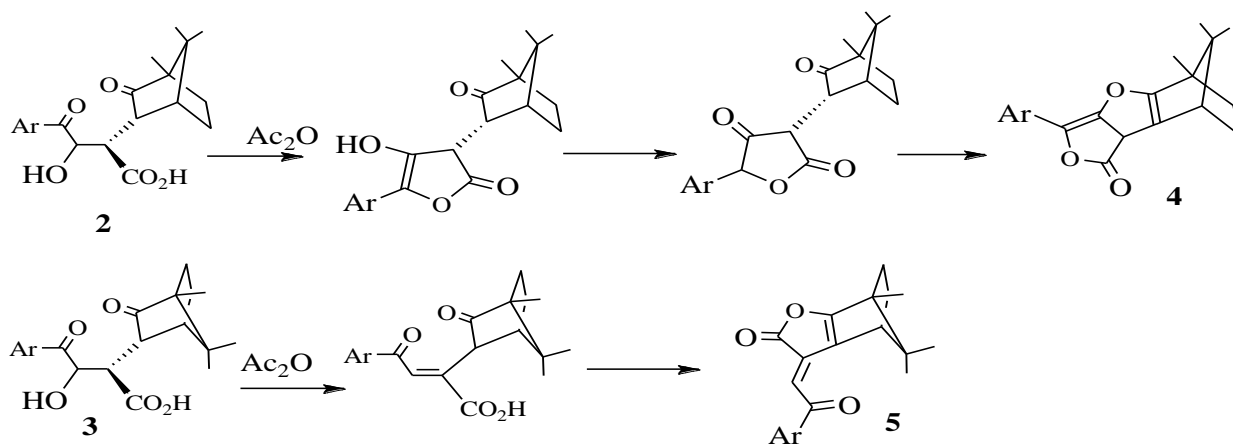
Figure 1: Outlines HOMO and LUMO values of the favored isomers that reflect their more stabilization.

On the other hand, the application of one-pot multicomponent reactions (MCRs) and microwave-assisted have been demonstrated to offer smooth reaction conditions, higher overall yield and afforded new products when they compared to classical synthesis methodologies. In our research, in one pot reaction of 4-aryl-4-oxo-2-butenoic acids, camphor, urea, hydrogen peroxide at pH 12, in MW irradiation (W 250 and T 1500C) was affording furanone **4**, and arylidene **5** (Scheme 3).



Scheme 3: Outline the diastereomeric adducts via reaction of the aroyl acrylic acid with (1R,4R)(+)-camphor under microwave condition

The mechanism of one pot syntheses of furanone derivatives **4a,b**, and **5a,b** were known to be through the formation of the epoxide product via the reaction α,β -unsaturated ketones and urea hydrogen peroxide reagent and it reacts with active methylene containing ketones using catalytic amount of strong bases like NaOH. This reaction is followed by condensation, cyclization, and dehydration to afford the corresponding furanone derivatives **4a,b**, and **5a,b**.

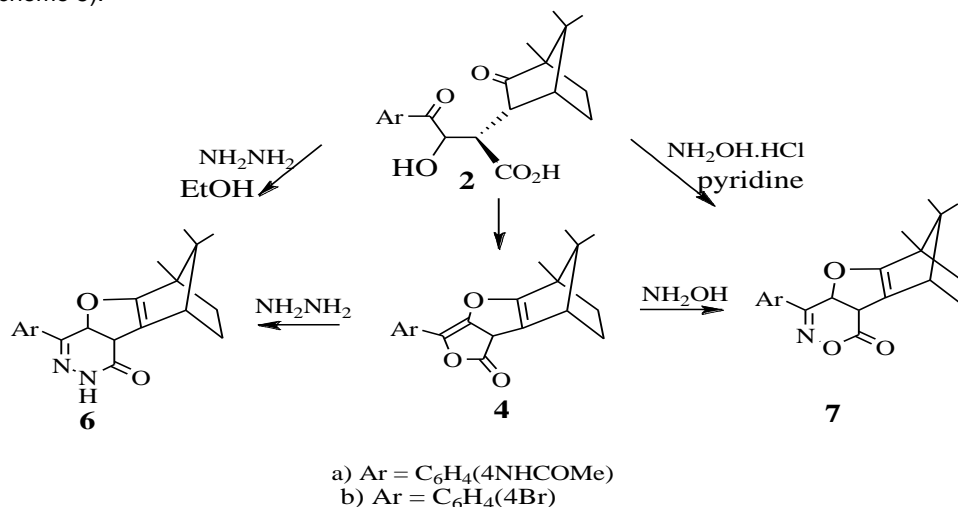


a: Ar= C₆H₄(NHCOCH₃)
b: Ar= C₆H₄(Br)

Scheme 4: Outline the synthetic route of the furanone 4a,b and 5a,b

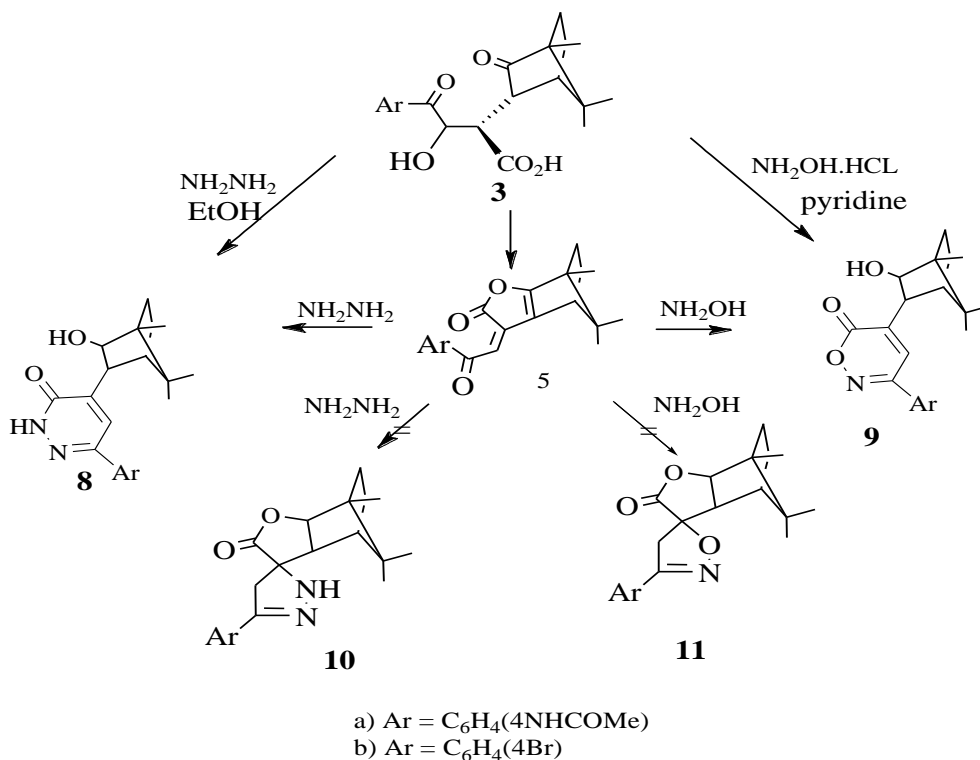
When the (2R,3S,3R') acids 2a,b and/or (2S,3R,3R') acids 3a,b were allowed to react with acetic anhydride, in water bath 2h afforded the furanone derivatives 4a,b and 5a,b respectively (Scheme 4). The steric crowding due to the bridged methyl group was outweighed the reactivity of carbonyl of camphor moiety. Therefore, the isomers 2a,b can be preferred cyclization with the carboxylic group affording furanone 4a,b and the absence of steric crowding of bridged methyl group in camphor moiety of the acids 3a,b became a driving force to afford regioselective isomers 5a,b (Scheme 4).

Moreover, when the (2R,3S,3R')-(+) acids 2a,b were allowed to react with hydrazine hydrate in boiling ethanol and/or hydroxylamine in boiling pyridine, either can be reacted directly or via their furanone derivatives 4a,b, they afforded 5,7a-dihydrofuro[2,3-d]pyridazin-4(3aH)-one derivatives 6a,b and 5,7a-dihydrofuro[2,3-d]1,2-oxazin-4(3aH)-one derivatives 7a,b respectively (Scheme 5).



Scheme 5: Synthetic route for compounds 6a,b & 7a,b

When the (2S,3R,3R')-(-) acids 3a,b were allowed to react with hydrazine hydrate in boiling ethanol and hydroxylamine in boiling pyridine, afforded the pyridazinone 8a,b and 2,1-oxazinone 9a,b derivatives respectively (Scheme 6). The authors expected formation of the spiro products 10a,b and 11a,b respectively but, the steric crowding due to the bridged methyl group was outweighed and so, the reaction of isomers 3a,b with hydrazine hydrate and/or hydroxylamine can be preferred the 1,2 addition followed by the cyclization with lactonic group.



Scheme 6: Synthetic route for compounds 8a,b & 9a,b

Antibacterial Activity Evaluation

Filter Paper Disc-Diffusion Method

The newly synthesized heterocyclic compounds listed in Table 1 were tested for their antibacterial activity against Gram positive bacteria [*Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 10987)], Gram negative bacteria [*Serratia marcescens* (ATCC 274) and *Proteus mirabilis* (SM514)] at a concentration of 100 µg/mL in dimethyl sulfoxide (DMSO). Nutrient agar and potato dextrose agars were used to culture the bacteria and fungi, respectively. The plates were inoculated by the bacteria or fungi and incubated for 24 h at 37°C for bacteria and for 72 h at 28°C for fungi and then the inhibition zones of microbial growth surrounding the filter paper disc (5 mm) were measured in millimeters. Ampicillin and Nystatin, at a concentration 100 µg/mL, were used as standard against bacteria respectively. The most active compounds were 2, 3, and 5 (a,b) which were strongly inhibitory to all or some of the tested bacteria. The authors can be explained the highly antibacterial activity for the compounds 2,3 and 5 (a,b) by quantum chemical parameter that small LUMO-HOMO energy gap implies that the molecule has high chemical reactivity and low kinetic stability as bioactive material [32] and they would be inhibited the enzyme of bacteria due to the presence of the activated double bond. The rest of compounds showed moderate activities against the tested bacteria under investigation. (Figure 2).

Table 1: Antibacterial activity of the synthesized compounds

Comp. No.	Inhibition zone (mm)			
	Staph. aureus	B. cereus	S. marcescens	Prot. mirabilis
2a	17 ± 0.12	16 ± 0.21	15 ± 0.21	16 ± 0.17
2b	18 ± 0.18	17 ± 0.15	16 ± 0.17	16 ± 0.15
3a	17 ± 0.21	16 ± 0.24	16 ± 0.18	16 ± 0.13
3b	16 ± 0.11	16 ± 0.12	15 ± 0.07	15 ± 0.21
4a	11 ± 0.16	10 ± 0.03	10 ± 0.01	10 ± 0.11
4b	11 ± 0.17	11 ± 0.02	10 ± 0.14	09 ± 0.13
5a	17 ± 0.15	18 ± 0.20	17 ± 0.11	18 ± 0.04
5b	17 ± 0.23	19 ± 0.08	18 ± 0.13	18 ± 0.22
6a	08 ± 0.03	08 ± 0.12	08 ± 0.09	08 ± 0.15
6b	09 ± 0.13	08 ± 0.20	07 ± 0.02	06 ± 0.05
7a	06 ± 0.02	08 ± 0.01	07 ± 0.02	06 ± 0.01
7b	05 ± 0.12	04 ± 0.06	05 ± 0.12	04 ± 0.10
8a	13 ± 0.15	12 ± 0.13	11 ± 0.11	10 ± 0.15
8b	03 ± 0.05	04 ± 0.21	05 ± 0.06	05 ± 0.10

9a	07 ± 0.12	08 ± 0.11	09 ± 0.08	07 ± 0.02
9b	05 ± 0.11	07 ± 0.12	08 ± 0.08	05 ± 0.20
Ampicillin	18 ± 0.11	19 ± 0.10	20 ± 0.10	19 ± 0.12
Chloramphenicol	19 ± 0.12	20 ± 0.16	20 ± 0.11	20 ± 0.11

Sensitivity of microorganisms to the tested compounds is identified in the following manner: Highly sensitive = Inhibition zone 15-20mm; Moderately sensitive = Inhibition zone 10-15mm; Slightly sensitive = Inhibition zone 5-10mm; Not sensitive = Inhibition zone 0-5mm; Each results represents the average of triplicate readings

The results are shown in Table 2, screen the Minimum Inhibitory Concentration (MIC) and calculated values of ADME, HOMO, and κ^2 that are used to generate the QSAR model. When HOMO is large in negative, it indicates high ADME, therefore it will contribute as good antibacterial. The high degree of bonding linearity (κ^2 index) with groups that increase molecular weight (high value of ADME Weight) represents a positive contribution to the antibacterial activity [32]. The effect of optically active adducts 2a, 3a and furanone derivatives 5a were stronger than furanone 4a, pyridazinone 6a, 8a and oxazinone 7a, 9a derivatives that outlined the strong antibacterial activity of the synthesized compounds.

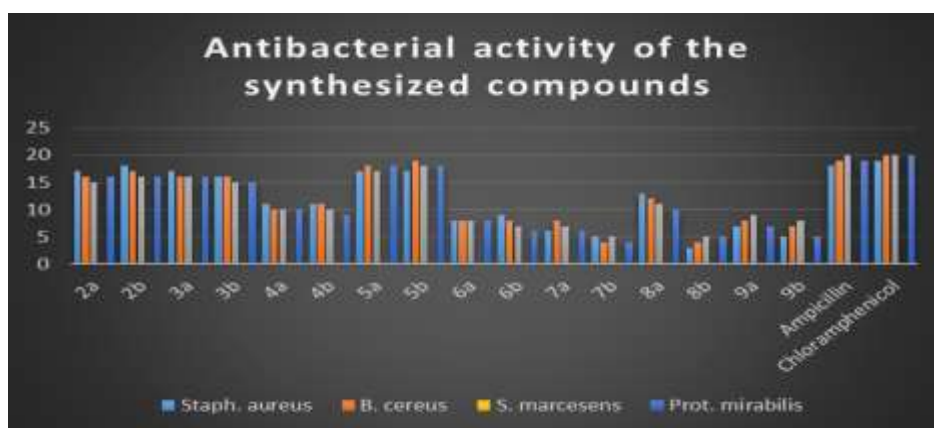
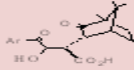
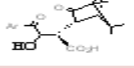
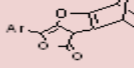
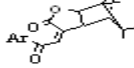
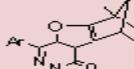





Figure 3: Outline statistics of antibacterial activity of the synthesized compounds

Table 2. Minimum Inhibitory Concentration; ^aCalculated values used to generate QSAR models.

Comp. N	Chemical Structure	MIC (ug/mL)	ADME ^a Weight	HOMO ^a	κ^2 Index ^a
2a		700	334.2	- 9.952	7.832
3a		600	413.5	- 10.873	8.657
4a		800	253.4	- 6.229	6.236
5a		500	456.2	- 11.140	9.856
6a		800	283.2	- 7.322	6.534
7a		800	275.3	- 6.718	6.103
8a		800	301.5	- 7.752	7.110
9a		800	267.5	- 6.859	6.532

ADME:

Absorption Distribution Metabolism Exertion

The obtained results are compiled in Table 2 indicate the most of the compounds have moderate to strong antimicrobial activity. Compounds 2a, 3a and 5a showed antimicrobial activity higher than the standard compounds, which means that they could be considered as promising antimicrobial agents. The minimum inhibition concentrations for the most potent compounds are recorded in Table 3 using the two fold dilution method. These potent compounds showed higher activity against Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

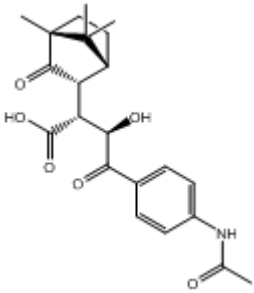

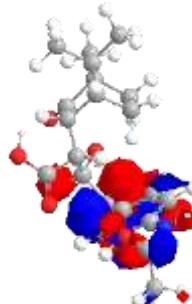
Table 3. MICs for the Most Potent Compounds ($\mu\text{g/mL}$)

Compound number	Gram-positive bacteria		Gram-negative bacteria	
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>
2a	12.5	12.5	50	50
3a	12.5	12.5	50	50
5a	12.5	12.5	50	50

Frontier molecular orbital's

The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) are the most important orbital's in the molecule. These orbitals are named as the frontier molecular orbital's. The HOMO orbital acts as an electron donor and the LUMO orbital acts as the electron acceptor. The LUMO-HOMO energy gap plays an important role in determining the way the molecule interacts with other species and in characterising the kinetic stability and chemical reactivity of the molecule. When the LUMO-HOMO energy gap is large, this indicates that the molecule is generally associated with a low chemical reactivity and high kinetic stability. On the contrary, the small LUMO-HOMO energy gap implies that the molecule has high chemical reactivity and low kinetic stability as bioactive material [32]. As shown in Tables 4, the result of theoretical calculations using (DFT/STO-3G) method indicates that in the case of the compound 2a, 3a and 5a, the energy of highest occupied molecular orbital (EHOMO) is $[(-11.140)-(-9.952)]$ eV, and the energy of the lowest unoccupied molecular orbital (ELUMO) is $[(-6.606)-(-4.946)]$ eV. Then, the frontier orbital gaps in the compounds 2a, 3a and 5a are $[4.534-5.120]$ eV that the former compounds has high chemical reactivity and low kinetic stability. For the compound 4a, 6a, 7a, 8a and 9a, the energy of HOMO orbital (EHOMO) is $[(-7.752)-(-6.220)]$ eV, and the energy of the LUMO orbital (ELUMO) is $[(-0.296)-(-1.961)]$ eV. Thus, the frontier orbital gaps in these compounds are about $[7.026-8.938]$ eV. As a result, it can be said that the compounds 2a, 3a and 5a are chemically more reactive and kinetically less stable and the compounds 4a, 6a, 7a, 8a and 9a are chemically less reactive and kinetically more stable.

Table 4: Theoretical Calculation of LUMO-HOMO

Cpd	Chemical Structure	HOMO	LUMO	ΔE
2a				$E_{\text{LUMO}} - E_{\text{HOMO}}$ $-4.946 - (-9.95)$ $= 5.006$

3a				$E_{LUMO} - E_{HOMO} =$ -5.753 - (-10.873) = 5.120
4a				$E_{LUMO} - E_{HOMO} =$ 1.051- (-6.220) = 7.271
5a				$E_{LUMO} - E_{HOMO} =$ -6.606 -(-11.140) 4.534
6a				$E_{LUMO} - E_{HOMO} =$ -0.296- (-7.322) = 7.026

7a				$E_{LUMO} - E_{HOMO} =$ 1.696- (-6.718) = 8.414
8a				$E_{LUMO} - E_{HOMO} =$ 1.186- (-7.752) = 8.938
9a				$E_{LUMO} - E_{HOMO} =$ 1.961 -(-6.859) 8.820

Experimental

All melting points are corrected and determined on a stuart electric melting point apparatus (Microanalytical centre, ainshams university, Cairo, Egypt). Elemental analyses were carried out by Elementar Viro El-Microanalysis at the Microanalytical Center, National Research Center, Egypt. IR spectra (KBr) were recorded on infrared spectrometer FT-IR 400D (New York, NY, USA) using OMNIC program and are reported frequency of absorption in terms of cm^{-1} and $^1\text{H-NMR}$ spectra recorded on a Bruker spectrophotometer (Rheinstetten, Germany) at 400 MHz using TMS as internal standard and with residual signals of the deuterated solvent $\delta = 7.26$ ppm for CDCl_3 and $\delta 2.51$ ppm for DMSO-d_6 . $^{13}\text{C-NMR}$ spectra were recorded on the same spectrometer (Rheinstetten, Germany) at 100MHz and referenced to solvent signals $\delta = 77$ ppm for CDCl_3 and $\delta 39.50$ ppm for DMSO-d_6 . DEPT 135 NMR spectroscopy were used where appropriate to aid the assignment of signals in the $^1\text{H-}$ and $^{13}\text{C-NMR}$ spectra. The mass spectra were recorded on Shimadzu GCMS-QP-1000 EX mass spectrometer (Kyoto, Japan) used the electron ionization technique at 70 e.v.

Preparation of urea- H_2O_2 (UHP). In 100 mL pyrex flask 12g urea and 34 mL hydrogen peroxide (30%) were added together. The reaction mixture was stirred at 60 oC for 10min., after cooling it was transferred to crystallizing dish for slow evaporation.



(9S)-(+)-4-(4-Bromophenyl)-6,10,10-trimethyl-4a,6,7,8,9-hexahydro-6,9-methanobenzofuro[2,3-d]pyridazin-1(2H)-one (6b): A mixture of 4-(4-bromophenyl)-2-oxoethylidene-2,3,4,5-tetrahydro-4,7-methanobenzofuran-2(3H)-one (4b) (0.01 mol) and hydrazine hydrate (0.5 mL; 0.01 mol) in ethanol (30 mL) and was heated under reflux for 5h. The reaction mixture was allowed to cool and the separated solid was filtered, dried and were recrystallized from ethanol. Yield, 75% (2.85 g), mp 216-218°C (dioxane), $[\alpha]_D^{20} = +102^\circ$. IR (KBr), ν , cm^{-1} : 3362 (NH), 1718, 1676 (CO). 1H NMR (400MHz, DMSO- d_6) δ 1.04 (s, 3H, CH₃a), 1.11 (s, 3H, CH₃b), 1.16 (s, 3H, CH₃c), 2.18-2.21 (m, 4H, 2CH₂), 3.17 (m, 1H, methine bridgehead), 4.11 (d, $J = 7.2$ Hz, 1H, CH-CO), 5.12 (d, $J = 7.2$ Hz, CH(O)-C=N), 6.62-7.31 (m, 4H, Ar-H). Anal. Calc. for C₂₂H₂₅N₃O₃: C, 69.47; H, 6.84; N, 11.69. found C, 69.45; H, 6.83; N, 11.68. MS: m/z 379 (M⁺, 100).

N-(4-((9S)-(+)-4a,6,7,8,9,9b-Hexahydro-6,10,10-trimethyl-1-oxo-1H-6,9-methanobenzofuro[2,3-d][1,2]oxazin-4-yl)phenyl)acetamide (7a): White crystals, yield, 65% (2.45 g), mp 212-214°C (dioxane), $[\alpha]_D^{20} = +102^\circ$. IR (KBr), ν , cm^{-1} : 3362 (NH), 1718, 1676 (CO). 1H NMR (400MHz, DMSO- d_6) 1.04 (s, 3H, CH₃a), 1.11 (s, 3H, CH₃b), 1.16 (s, 3H, CH₃c), 2.18-2.21 (m, 4H, 2CH₂), 3.17 (m, 1H, methine bridgehead), 4.11 (d, $J = 7.2$ Hz, 1H, CH-CO), 5.12 (d, $J = 7.2$ Hz, CH(O)-C=N), 6.62-7.31 (m, 4H, Ar-H). Anal. Calc. for C₂₂H₂₅N₃O₃: C, 69.47; H, 6.84; N, 11.69. found C, 69.45; H, 6.83; N, 11.68. MS: m/z 379 (M⁺, 100).

(9S)-(+)-4-(4-Bromophenyl)-6,10,10-trimethyl-4a,6,7,8,9,9b-hexahydro-6,9-methanobenzofuro[2,3-d][1,2]oxazin-1(2H)-one (6b): White crystals, yield, 75% (2.73 g), mp 216-218°C (dioxane), $[\alpha]_D^{20} = +102^\circ$. IR (KBr), ν , cm^{-1} : 3362 (NH), 1718, 1676 (CO). 1H NMR (400MHz, DMSO- d_6): δ 1.06 (s, 3H, CH₃a), 1.2 (s, 3H, CH₃b), 1.26 (s, 3H, CH₃c), 1.80-1.84 (m, 4H, 2CH₂), 2.93 (dd, $J = 1.1$ Hz, 1H, CH-CO), 3.93 (dd, $J = 1.1$ Hz, 1H, CH-CO), 4.93 (t, $J = 6.1$ Hz, 1H, methine), 6.67-7.71 (m, 4H, Ar-H aromatic protons), and ^{13}C NMR (DMSO- d_6) δ 38.4, 39.6, 46.6, 48.8, 50.2, 56.8, 62.9, 64.9, 67.2, 104.2, 136.2. Anal. Calc. for C₂₂H₂₅N₃O₃: C, 69.47; H, 6.84; N, 11.69. found C, 69.46; H, 6.83; N, 11.68. MS: m/z 379 (M⁺, 100).

(9S)-(+)-4-(4-Bromophenyl)-6,10,10-trimethyl-4a,6,7,8,9,9b-hexahydro-6,9-methanobenzofuro[2,3-d][1,2]oxazin-1(2H)-one (6b): White crystals, yield, 75% (2.73 g), mp 216-218°C (dioxane), $[\alpha]_D^{20} = +102^\circ$. IR (KBr), ν , cm^{-1} : 3362 (NH), 1718, 1690 (CO). 1H NMR (DMSO- d_6) 1.1 (s, 3H, CH₃a), 1.16 (s, 3H, CH₃b), 1.26 (s, 3H, CH₃c), 1.59-1.71 (m, 4H, 2CH₂), 1.98 (t, $J = 6.1$ Hz, 1H, methine), 3.27 (d, $J = 8.1$ Hz, 1H, CH-CO), 4.37 (d, $J = 8.1$ Hz, CH(O)-C=N), 7.71-7.79 (m, 4H, Ar-H), and ^{13}C NMR (DMSO- d_6) δ 18.3(2), 19.7, 21.9, 26.8, 37.3, 38.4, 44.6, 45.8, 48.2, 56.8, 129.7(2), 130.4, 131.8, 136.2, 144.3, 154.4, 168.2, 178.8. Anal. Calc. for C₂₀H₂₀N₃O₃Br: C, 59.85; H, 4.98. found C, 59.86; H, 4.96. MS: m/z 403 (M⁺, 60), 401 (M⁺, 100), 250 (66).

General Procedure for synthesis of compounds 8. A mixture of 3 (0.01 mol) and hydrazine hydrate (0.5 mL; 0.01 mol) in ethanol (30 mL) and was heated under reflux for 5h. The reaction mixture was allowed to cool and the separated solid was filtered, dried and were recrystallized from ethanol. Yield, 80% (3 g), mp 232-234°C (dioxane), $[\alpha]_D^{20} = -54.15$ (c 1.5, CHCl₃). IR (KBr), ν , cm^{-1} : 3362 (NH), 1718, 1676 (CO). 1H NMR (400MHz, DMSO- d_6): δ 1.2 (s, 3H, CH₃a), 1.26 (s, 3H, CH₃b), 1.59-1.71 (m, 4H, 2CH₂), 1.98 (t, $J = 6.1$ Hz, 1H, methine), 3.27 (d, $J = 8.1$ Hz, 1H, CH-CO), 4.37 (d, $J = 8.1$ Hz, CH(O)-C=N), 7.71-7.79 (m, 4H, Ar-H), and ^{13}C NMR (DMSO- d_6) δ 18.3(2), 19.7, 21.9, 26.8, 37.3, 38.4, 44.6, 45.8, 48.2, 56.8, 129.7(2), 130.4, 131.8, 136.2, 144.3, 154.4, 168.2, 178.8. Anal. Calc. for C₂₀H₂₀N₃O₃Br: C, 59.85; H, 4.98. found C, 59.86; H, 4.96. MS: m/z 403 (M⁺, 60), 401 (M⁺, 100), 250 (66).

General Procedure for synthesis of compounds 9. A mixture of 3 (0.01 mol) and hydrazine hydrate (0.5 mL; 0.01 mol) in ethanol (30 mL) and was heated under reflux for 5h. The reaction mixture was allowed to cool and the separated solid was filtered, dried and were recrystallized from ethanol. Yield, 75% (2.85 g), mp 216-218°C (dioxane), $[\alpha]_D^{20} = +102^\circ$. IR (KBr), ν , cm^{-1} : 3362 (NH), 1718, 1676 (CO). 1H NMR (400MHz, DMSO- d_6): δ 1.04 (s, 3H, CH₃a), 1.11 (s, 3H, CH₃b), 1.16 (s, 3H, CH₃c), 2.18-2.31 (m, 4H, 2CH₂), 3.17 (m, 1H, methine bridgehead), 4.06 (d, $J = 7.8$ Hz, 1H, CH-CO), 5.12 (d, $J = 7.8$ Hz, CH(O)-C=N), 6.78-7.56 (m, 4H, Ar-H), 9.14 (bs, 2H, 2NH of acetamido and pyridazine moieties) and ^{13}C NMR (DMSO- d_6) δ 19.8(2), 20.3, 23.7, 28.9, 36.8, 37.3, 38.4, 39.6, 44.6, 45.8, 48.2, 56.8, 125.7, 128.2(2), 130.4, 131.8, 136.2, 145.2, 155.3, 178.8. Anal. Calc. for C₂₂H₂₅N₃O₃: C, 69.65; H, 6.59. found C, 69.65; H, 6.58. MS: m/z 379 (M⁺, 100).



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6-(4-Bromophenyl)-4-((1S,3R,4S)-(-)-3-hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)pyridazin-3(2H)-one (8b):

White crystals, yield, 82% (3.12 g), mp 184–186°C(ethanol), $[\alpha]_D^{20} = -58^\circ$. IR (KBr), ν , cm^{-1} : 3303 (NH), 1708 (CO). $^1\text{H NMR}$ (400MHz, $\text{DMSO-}d_6$) 1.12 (s, 3H, CH_3a), 1.23(s, 3H, CH_3b), 1.30 (s, 3H, CH_3), 1.52–1.78(m, 5H, $\text{CH-CH}_2\text{-CH}_2$), 3.00(m, 1H, attached camph), 3.24(d, $J = 5.7\text{Hz}$, 1H, CHOH), 5.72(bs, 1H, OH), 7.48–7.56 (m, 5H, Ar-H), 11.05(s, 1H, NH pyridazinone moiety) and $^{13}\text{C NMR}$ ($\text{DMSO-}d_6$) δ 19.7(2), 21.9, 23.6, 26.8, 37.3, 38.4, 45.8, 48.2, 56.8, 74.7, 121.4, 129.7, 130.4(2), 131.8, 136.2, 142.4, 153.2, 165.8. Anal. Calc. for $\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}_2\text{Br}$: C, 59.70; H, 5.72. found C, 59.75; H, 5.72. MS m/z 402(M^+ , 22), 251(100), 175(77), 156(47).

General Procedure for synthesis of compounds 9. A mixture of 3 (0.01 mol) and hydroxyl amine (1.03 g; 0.015 mol) in pyridine (20 mL) and then refluxed for 3h. The reaction mixture was poured onto ice/HCl and the separated solid was filtered, dried and were recrystallized from ethanol.

N-(4-(5-((1S,3R,4S)-(-)-3-Hydroxy-4,7,7-trimethylbicyclo[2.2.1]heptan-2-yl)-6-oxo-6H-1,2-oxazin-3-yl)phenyl)acetamide(9a):

White crystals, yield, 70% (2.67 g), mp 234–236°C(ethanol), $[\alpha]_D^{20} = -67^\circ$. IR (KBr), ν , cm^{-1} : 3420 (OH), 1710, 1676 (CO). $^1\text{H NMR}$ (400MHz, $\text{DMSO-}d_6$) 1.62 (s, 3H, CH_3a), 1.67(s, 3H, CH_3b), 1.80 (s, 3H, CH_3), 2.12–2.18(m, 5H, $\text{CH-CH}_2\text{-CH}_2$), 2.45(s, 3H, CH_3CO), 3.00(m, 1H, attached camph), 3.84(m, 1H, CHOH), 5.12(bs, 1H, OH), 6.68–7.56 (m, 5H, Ar-H), 8.34 (s, 1H, NH of acetamido moiety), and $^{13}\text{C-NMR}$ ($\text{DMSO-}d_6$) δ 20.3(2), 23.7, 26.8, 37.3, 38.4, 39.6, 44.6, 45.8, 48.2, 56.8, 75.8, 125.2, 129.7(2), 130.4, 131.8, 136.2, 147.3, 156.3, 166.1(2). Anal. Calc. for $\text{C}_{22}\text{H}_{26}\text{N}_2\text{O}_4$: C, 69.11; H, 6.81. found C, 69.17; H, 6.81. MS m/z 382(M^+ , 100).



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