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Design, Synthesis, Molecular docking ADMET and anti-bacterial activities of some new benzamides and their corresponding quinazolinone derivatives

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Abstract

In this study, novel benzamide derivatives were synthesized from the ring opening of benzoxazin-4-one in conduction with primary aromatic amines in relatively short reaction durations (1-5) minutes. In just a few minutes, sulphuric acid was utilized to cyclodehydration of benzamide derivatives into quinazolinones intramolecularly. FT-IR, 1H-NMR, 13C-NMR, and DEPT - 135- 13C-NMR were used to characterize the structure of newly synthesized compounds, and single crystal X-Ray was used to confirm the structure of compound 3a. The synthesized compounds were shown to have potent antibacterial activity against microorganisms, and a molecular docking analysis revealed a favorable binding contact with the target bacterial DNA gyrase (PDB ID: 1KZN) of S. aureus. Furthermore, in silico ADMET calculations were performed for all synthesized compounds that showed promise when compared to conventional ciprofloxacin.

keywords: benzamides, quinazolinone, antibacterial, Docking study, In silico ADMET, X-ray crystallography.

Introduction

For nearly a century, 4H-3,1-benzoxazinones have been known, 1,3-oxazin-6-one is the heterocyclic six membered ring synthesized from Schotten Baumann reaction of substituted benzoyl chloride with 2-amino benzoic acid[1]. They were frequently employed as suitable skeletons for the planning of biologically dynamic combinations since they are found in nature. They were also used in natural union to create both natural and manufactured chemicals. As a result, they are classified as compound synthons with various physiological meanings and therapeutic applications[2].

The heterocyclic molecules containing nitrogen atoms, particularly Quinazoline and quinazolinone, are arranged biologically active chemicals within numerous groups of heterocyclic compounds[3]. Quinazoline derivatives stand out as important molecules with a wide range of intriguing pharmacological activities and one-of-a-kind physicochemical properties **Figure 1**[4].

Due to a wide range of pharmacological practices, the core of quinazolinone has gotten a lot of attention. such as anti-oxidant[5–7], antitumor [8–13]antiviral [14–18] ,Anti HCC and HBV [19],Insecticidal activity[20], and anti-lieshmancidal activity[21] Nowadays, the chemistry of quinazolinone has as of late became a new way because of a certain similarity to folic acid [22].

Many literature reviews in recent decades revealed that numerous researchers throughout the world have been synthesizing quinazolinone for a long period of time (4-18) hrs. [1,2,23–29].

In face of all of this information, we report here the synthesis of series of benzamide derivatives by ring opening of benzo[d]-1,3-oxazinone and corresponding their quinazolin-*3H*-4-one derivatives. For the first time we used sulphuric acid as a convent, and available compound, which showed dramatic result within short time (5.0 min<) during synthesis of quinazolin-*3H*-4-one derivatives from benzamide by the cyclodehydration. As a preliminary investigation for future studies in the process of our drug discovery, the newly synthesized compounds exhibited good antibacterial activity.

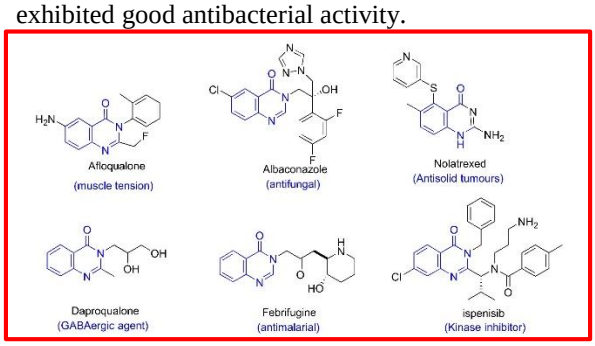

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Figure (1): Some Examples of biological active 4-(3H)-quinazolinone

Experimental

Chemistry

The chemicals that used in analytical grade from different brand (Fluka, Scharlau, Riedel-de Haen). Bruker spectrometer (400 MHz) were used to record a spectra of Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) with $CDCl_3$ and DMSO as solvents. Chemical shifts are showed in ppm. IR affinity-1 (Shimadzu) spectrometers with KBr pellets were used to record Infrared spectra. Shimadzu UV-1800 Series single beam UV/VIS recording spectrophotometer used to record the UV. Electro-thermal melting point devise 9100 were used to record the melting point of compounds (uncorrected).

Preparation of 2-(3-nitrophenyl)-4H-benzo[d][1,3]oxazin-4-one(2)

Method A

This compound 2-(3-nitrophenyl)-4H-benzo[d][1,3]oxazin-4-one was prepared according to reported method[30] . A solution of compound (**1)** (0.05 mol) was refluxed with acetic anhydride for4.0 hrs. The solution left to cool, filtered, washed several times with cold water and ethanol. The product was recrystallized from ethanol to give 2-(3-nitrophenyl)-4H-benzo[d][1,3]oxazin-4-one as a pale yellow ppt. **(2)** .

Method B

Anthranilic acid (0.01 mol) was added to 40mL of dry pyridine (*i.e.* 100g of molecular sieve 0.4nm was activated by heating between 100-150 ºC for 1h then 200mL of pyridine was added then filtered) and cooled it 0-5 ºC , then 3-nitrobenzyl chloride was added dropwise during 30 minutes, after that the stirring continued for 3h at room temperature , the pale yellow precipitate was formed washed several times with cold water and ethanol washed, then the product was recrystallized from ethanol to give 2-(3-nitrophenyl)-4H-benzo[d][1,3]oxazin-4-one as a pale yellow ppt. **(2)** [31].

$C_{14}H_8N_2O_4$ yield: (90%), MP 123ºC) **FT-IR (cm-^{1}):** 1758(carbonyl cyclic lactone) 1602 1587 (C=C), 1577.

^{1}H-NMR (δ, ppm): 7.58 (t, 1H, C10 J=10.32Hz), 7.72 (t, 1H, C9, J=10.32 Hz), 7.72 (d, 1H, C11 J=10.32 Hz), 7.88 (t, 1H, C4, J=7.4 Hz),8.24 (d, 1H, C5, J=7.8 Hz), 8.41 (d, 1H, C12, J=10.32 Hz), 8.61 (d, 1H, C3 J=7.8 Hz), 9.10 (s, 1H, C1).

13C-NMR (δ, ppm):117.15:C_{13}, 123.31:C_1, 126.88:C_{11}, 127.59:C_3, 128.84:C_9, 129.19:C_{12}, 130.02:C_4, 132.18:C_6,133.68:C_5,136.95:C_{10},146.31:C_8,148.7:C_2 , 154.85:C_7,158.7:C_{14}.

Synthesis of benzamides (3a-d)

N-(substituted phenyl) 2-(3-nitrobenzamido)benzamide

According to modified procedure[32], compound 2 (0.01 mol) with (0.01 mol) of aromatic amine were mixed in 20 mL glacial acetic acid for a 1-3 min , the solid was immediately formed during the heating, the completion of reaction and the purity of product was confirmed by TLC plate (hexan: sodium acetate 1:1) the product filtered out and recrystallized from ethanol to give the target compounds (see **Table 1**).

3a. N-(3-chloro-4-methylphenyl)-2-(3-nitrobenzamido) benzamide

^{1}H-NMR (δ, ppm): 2.44(s, 3H, Ar-CH3), 7.21 (t,1H, C11, J=7.8 Hz), 7.31(d, 2H, C20, J=7.7 Hz), 7.49(d,1H, C19, J=9 Hz), 7.61(t,1H, C10, J=7.8 Hz), 7.71(d,1H, C12, J=7.8 Hz), 7.79(t,1H, C4, J=7.8 Hz), 8.22(s,1H, C1), 8.39(d,1H, C5, J=7.3 Hz), 8.48(d,1H, C3 J=8.1 Hz),8.80(d,1H, C9, J=8.1 Hz),8.98(s,1H,NH2), 12.14 (s,1H,HN1).

13C-NMR (δ, ppm): 19.65: Ar-**C**H$_3$,119.29:C_9,120.66:C_{20},121.64:C_{16}, 122.19:C_{13}, 123.2: C_1,123.76:C_{11},126.46:C_3, 126.72:C_{12}, 130.12:C_4, 131.34:C_{19},132.68:C_{18},133.34:C_{10}, 133.54:C_5, 135:C_{17}, 135.83:C_{15}, 136.63:C_{10},139.8:C_8,148.78:C_2, 163.31:C_7,167.48:C_{14} (C=O).

3b. N-(p-tolyl)-2-(3-nitrobenzamido) benzamide

^{1}H-NMR (δ, ppm): 2.43(s, 3H, Ar-C**H$_3$**), 7.14 (t,1H, C_{11}), 7.32(d, 2H, $C_{17,17'}$), 7.45(d,1H, C_{12}), 7.56(t,1H, C_{10}), 7.63(d,1H, $C_{16,16'}$), 7.70(d,1H, C_5), 7.79(t,1H, C_4), 8.35(s,1H, C_1), 8.47(d,1H, C_3),8.72(d,1H, C_9),8.97(s,1H,NH2), 12.19 (s,1H,HN1).

^{13}C-NMR (δ, ppm): 21.05: Ar-**C**H$_3$, 121.07:$C_{16,16'}$, 121.09:C_9, 122.01:C_{13}, 123.2: C_1, ,123.65:C_{11}, 126.43:C_3, 126.96:C_{12}, 129.87:$C_{17,17'}$, 130.09:C_4, 132.7:C_{10},133.00:C_5, 134.65:C_{15}, 135.32:C_{18}, 136.62:C_6,139.75:C_8 ,148.75:C_2 ,163.37:C_7,167.45:C_{14} (C=O).

3c. N-(4-methoxyphenyl)-2-(3-nitrobenzamido) benzamide

^{1}H-NMR (δ, ppm): 3.9 (s, 3H, O-CH3),7.07(d, 2H, C17, 17', J=8.7 Hz),7.22(t, 1H, C11 J=7.3 Hz),7.62(t, 1H, :C10, J=7.4 Hz), 7.62(d, 2H, :C16,16' J=8.7 Hz),7.74(t, 1H, :C4, J=8.1 Hz), 7.78(d, 1H, C12, J=8.1 Hz),8.15(s, 1H, :C1),8.36(d, 1H, :C5, J=7.3 Hz),8.46(d, 1H, C3, J=8.1 Hz),8.82(d, 1H, C9 J=7.9 Hz),8.98(s, 1H, NH2),12.29(s, 1H, NH1).

13C-NMR (δ, ppm): 55.61: O-**C**H$_3$,114.54:$C_{17,17'}$,122:C_9,122.04:$C_{16,16'}$, 122.98:C_{13}, 122.98: C_1,123.18:C_{11},123.68:C_{15},126.38:C_{12},126.79:C_3, 130.06:C_4, 132.74:C_{10}, 133.12:C_5, 136.69:C_6, 139.58:C_8, 148.75:C_2, 157.41: C_{18}, 163.33:C_7, 167.41:C_{14} (C=O).

3d. N-(4-ethoxyphenyl)-2-(3-nitrobenzamido) benzamide

^{1}H-NMR (δ, ppm): 1.5 (t, 3H, OCH2CH3, J=6.7 Hz),4.12(q, 2H, OCH2CH3, J=6.7 Hz),7.01(d, 2H, C17,17', J=8.4 Hz), 7.22 (t,1H, C11 J=7.4 Hz), 7.6(t,1H, C10, J=8.4 Hz), 7.6(d,1H, C16,16' J=8.4 Hz),7.75(t,1H, C4 J=8.4Hz), 7.77(d,1H, C12 J=8.2Hz),8.16(s,1H, C1),8.35(d,1H, C5, J=7.35Hz), 8.46(d,1H, C3 J=7.35 Hz),8.8(d,1H, C9, J=8.3 Hz),8.97(s,1H,NH2), 12.28 (s,1H,HN1)

13C-NMR (δ, ppm): 14.87: $OCH_2\mathbf{C}H_3$ 63.85: O-$\underline{\mathbf{C}}H_2CH_3$, 115.12:$C_{17,17'}$,120.91 :$C_9$, 122.05:$C_{16,16'}$, 123.01:$C_{13}$, 123.18: C_1,123.69:C_{11}, 126.34: C_3,126.65:C_{12}, 129.5:C_{15},130.03:C_4, 132.74:C_{10}, 133.25:C_5,136.74:C_6, 139.77:C_8,148.73: C_2,156.83:C_{18},163.26:C_7,167.4:C_{14} (C=O).

^{13}C-DEPT-NMR (δ, ppm): 14.87: $OCH_2\mathbf{C}H_3$ - 63.85: O-$\underline{\mathbf{C}}H_2CH_3$,115.08:$C_{17,17'}$,120.95 :$C_9$, 122.00:$C_{16,16'}$, 122.93: C_1,123.17:C_{11}, 126.35:C_3, 126.7:C_{12}, 130.03:C_4,1 32.71:C_{10}, 133.11:C_5.

3a crystallographic data

X-AREA and X-RED32 [33]; program(s) used to solve structure: SHELXT2018 [34]; program(s) used to refine structure: SHELXL2018 [35]; molecular graphics: ORTEP-3 for Windows[36]; software used to prepare material for publication.

3a crystal data

$C_{21}H_{16}ClN_3O_4$; M =409.82; Orthorhombic, space group Pbca; a = 13.0327 (9) Å, b= 13.7645 (7) Å, c= 21.7510 (12) Å, V = 3901.9 (4) Å3; Z = 8; D_x = 1.395 Mg m^{-3}; F (000) =1696; colorless prism, size 0.63 × 0.56 × 0.52 mm; 4191 independent measured reflections, refinement based on F_2 to give R [$F_2 > 2\sigma(F_2)$] = 0.048; wR(F_2) = 0.140 for 27 302 observed reelections, and 264 parameters[37].

Synthesis of quinazolin-3H-4-one 4(a-d) from benzamides 3(a-d)

3-(substituted phenyl)-2-(3-nitrophenyl) quinazolin-4(3H)-one

benzamide derivatives (0.01 mol) were dissolved in acetic acid, 0.5 mL of concentrated sulphuric acid added immediately, the color of the solution changed to darken as the solubility of the compound increased. The completion of reaction and the purity of product were confirmed by TLC plate (hexane: sodium acetate 2:1), the solution concentrated and the water added. The formed solid product neutralized with 15 mL of 15% of Na_2CO_3, then crude product washed several times with cold ethanol and recrystallized from absolute ethanol to give the quinazolinones4 (a-d) **Table 2**.

4a. 3-(3-chloro-4-methylphenyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one

^{1}H-NMR (δ, ppm): 2.38(s, 3H, Ar-CH3), 7.05(d, 1H, C19 J=6.65 Hz), 7.28 (s, 1H, C16 J=8.8Hz), 7.28 (d, 1H, C20), 7.51(t, 1H,C11, J=7.5 Hz), 7.64(t, 1H, :C4, J=7.5 Hz), 7.74(d, 1H, C9 J=6.65 Hz),7.89(t, 1H, :C10 J=8.1Hz), 7.91(d, 1H, C12 J=8.1 Hz), 8.23(d, 1H, :C5 J=7.1Hz),8.38(s, 1H, :C1), 8.42(d, 1H, C3 J=7.5Hz).

13C-NMR (δ, ppm): 19.99: Ar-$\underline{\mathbf{C}}H_3$, 121.09:$C_{13}$, 124.52: C_1,124.56:C_5,127.44:C_3, 127.55:C_{20}, 128.08:C_{12}, 128.31:C_9, 129.46:C_{11}, 129.68:C_{16}, 131.65:C_{19}, 134.9:C_4,135.26:C_6, 135.38:C_{10}, 135.72:C_{18}, 136.84:C_{17}, 137.66:C_{15}, 147.16:C_2, 147.96:C_8, 152.47:C_7, 160.03:C_{14} (C=O).

4b. 2-(3-nitrophenyl)-3-(p-tolyl)quinazolin-4(3H)-one

^{1}H-NMR (δ, ppm): 2.37(s, 3H, Ar-CH3), 7.13(d, 2H, C16,16' J=8.1Hz), 7.22 (d, 2H, C17, 17', J=8.1Hz), 7.47(t, 1H,C11 J=8.1Hz), 7.64(t, 1H, :C4 J=7.3Hz), 7.74(d, 1H, C9 J=7.3Hz),7.89(t, 1H, :C10 ,J=7.5Hz), 7.93(d, 1H, C12 ,J=7.7Hz), 8.21(d, 1H, :C5 ,J=8.1 Hz),8.36(s, 1H, :C1), 8.45(d, 1H, C3 ,J=8.1 Hz).

^{13}C-NMR (δ, ppm): 21.34: Ar-$\underline{\mathbf{C}}H_3$, 121.3:$C_{13}$, 124.27: C_1,124.61:C_5,127.57:C_3,128.01:C_{12}, 128.11: C_9, 128.89: $C_{16,16'}$, 129.25:C_{11}, 130.31:$C_{17,17'}$, 134.53:C_4,134.99:C_6, 135.18:C_{10}, 137.26:C_{15}, 139.36:C_{18}, 147.31:C_2, 147.87:C_8, 152.99:C_7, 162.23:C_{14} (C=O).

4c. 3-(4-methoxyphenyl)-2-(3-nitrophenyl)quinazolin-4(3H)-one

^{1}H-NMR (δ, ppm): 3.83 (s, 3H, O-CH3),6.92(d, 2H, C17, 17' ,J =8.5 Hz), 7.16(d, 2H, :C16,16' ,J= 8.5Hz), 7.48 (t, 1H,C11 ,J=8.15Hz), 7.64(t, 1H, :C4,J=7.00 Hz), 7.73(d, 1H, C9,J=7.8 Hz),7.88(t, 1H, :C10,J=8.7 Hz), 7.92(d, 1H, C12,J=7.8 Hz), 8.21(d, 1H, :C 5,J=8.1 Hz),8.37(s, 1H, :C1), 8.43(d, 1H, C3,J=8.1 Hz).

13C-NMR (δ, ppm): 55.67: O-$\underline{\mathbf{C}}H_3$, 114.89: $C_{17,17'}$,121.3: C_{13}, 124.24: C_1, 124.61:C_5, 127.55:C_3,128.03:C_{12},128.09:C_9, 129.3:C_{11}, 129.73:C_6, 130.21:$C_{16,16'}$,134.96:C_4, 135.15:C_{10}, 137.35:C_{15},147.32:C_2, 147.92:C_8, 153.12:C_7, 159.83:C_{18}, 162.36:C_{14} (C=O).

^{13}C-DEPT-NMR (δ, ppm): 55.67: O-$\underline{\mathbf{C}}H_3$, 114.9:$C_{17,17'}$, 124.25: C_1,124.62:C_5, 127.56:C_3, 128.04:C_{12}, 128.10:C_9, 129.31:C_{11}, 130.22:$C_{16,16'}$,134.9:C_4, 135.16:C_{10}.

4d. 3-(4-ethoxyphenyl)-2-(3-nitrophenyl) quinazolin-4(3H)-one

^{1}H-NMR (δ, ppm): 1.44 (t, 3H, OCH2CH3,J=6.5 Hz),4.03(q, 2H, OCH2CH3,J=6.5 Hz), 6.9 (d, 2H, C17, 17,J=8.4 Hz '), 7.13(d, 2H, :C16,16',J=8.4 Hz),7.47(t, 1H,C11), 7.63(t, 1H, :C4), 7.73(d, 1H, C9),7.87(t, 1H, :C10), 7.91(d, 1H, C12,), 8.20(d, 1H, :C5, ,J=7.6 Hz),8.36(s, 1H, :C1), 8.43(d, 1H, C3, ,J=7.6 Hz).

13C-NMR (δ, ppm): 14.84: $OCH_2\underline{C}H_3$ 63.95: O-$\underline{C}H_2CH_3$, 115.35:$C_{17,17'}$,121.3:C_{13}, 124.22: C_1,124.60:C_5,127.55:C_3,128.02:C_{12}, 128.08:C_9, 129.3:C_{11}, 129.52:C_6, 130.17:$C_{16,16'}$,134.97:C_4, 135.14:C_{10}, 137.36:C_{15},147.32:C_2, 147.92:C_8, 153.12:C_7, 159.22:C_{18}, 162.37:C_{14} (C=O).

Biological Evolution

Antibacterial activity

To determine the minimum inhibitory concentration (MIC) the microtitre assay was used, the tested bacterial *Pseudomonas aeruginosa* with *ATCC* **(29213) as** Gram-negative and *Staphylococcus aureus* with *ATCC* **(25923)** as Gram-positive were performed in this investigation. The bacterial inoculation (1.5×10^8 CFU/mL) with a standard Mc-farland (0.5) was used. the stock solution of synthesized compound (2048 µg/mL in DMSO was prepared. The serial dilution of synthesized compound with concentrations (1024,512,128,64,32,16, and 8 µg/mL) were prepared and 100 µL added to the 96 well plate (see figure 2) except positive control (i.e. contain only microorganisms and broth). 100µL of Moller Hinton broth as a growth medium of microorganism was added to the 96 well plate flat shape. And added 35 µL of bacterial adjusted with (0.5) Mc-farland added to all wells except negative control, the plates were covered and shake well by using Eliza , then incubated for 24h at 37ºC, after incubation ,the results recorded by Eliza and MIC was determined as minimum concentration of drug inhibit the growth of microorganisms the results showed in **Table 3**, The inhibition was measured by the absorption at 630 nm using a microtitre assay (ELISA) reader the results triplicated and averaged[38–40].

The percent of inhibition was determined with the same as above but, the inhibition was measured by the absorption at 480 nm using a microtitre assay (ELISA) reader the results triplicated and averaged (see **Figure 8 and 9**).

Molecular Docking

The Molecular Operating Environment (Moe-Dock 2015.10) software was used to perform molecular docking techniques on all compounds 2, 3(a-d) and 4(a-d). The structure of the compounds were painted by using ChemDraw professional (2019) and saved in (mol.) files. [41] Furthermore, the structures of these compounds were subjected to energy reduction using the MOE program Amber10: EHT force field in order to prepare them for docking tests. DNA Gyrase Subunit B , 3D crystallographic structure The Protein Data Bank (PDB ID: 1KZN) was used to obtain this information. It was utilized as a model for the target the pocket of coumarin-based inhibitor was used to produce the active site of 1KZN by site finder of MOE program, and then the MOE Dock was used to dock ligands within it. The best score between the ligands and active site interactions was also calculated using MOE. The five ligands conformers with the highest and greatest score were kept by default, indicating the best ligand active site interactions. The docking procedure was validated by re-docking the ligand into the active site, which revealed identical binding interactions to a co-crystallized ligand with a root mean square deviation (RMSD) of less than 2.[42].

In silico ADME study

The Swiss Institute of Bioinformatics' (SIB) free online web application Swiss ADME was used to calculate physicochemical descriptors and forecast ADME parameters, pharmacokinetic properties, drug-like nature, and medicinal chemistry friendliness of the most powerful freshly synthesized compounds [43–46]. The structures of the compounds were translated to SMILES notations and then submitted for calculation to an internet server http://www.swissadme.ch/index.php . Absorption (% ABS) was calculated as follows:…

% ABS = **109 -(0.345∗(TPSA)** [47]

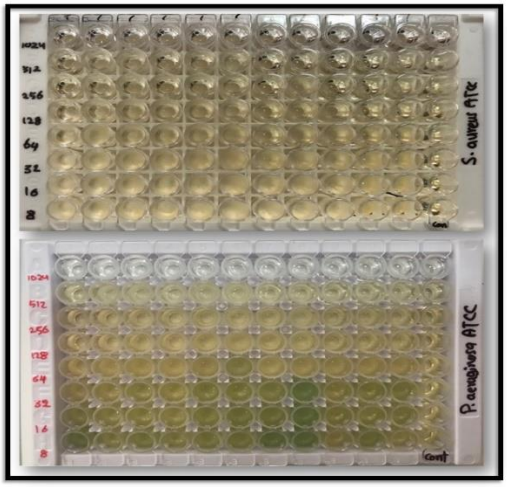


Figure (2): microtitre plate of both P. aeruginosa ATCC (29213) and S. aureus ATCC (25923)

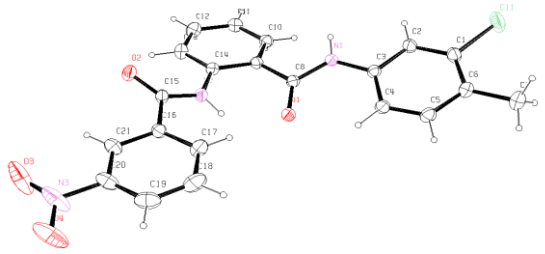


Figure 3: ORTEP diagram strained with 15% ellipsoid probability the crystal structure of 3a benzamide was performed at 296 K.

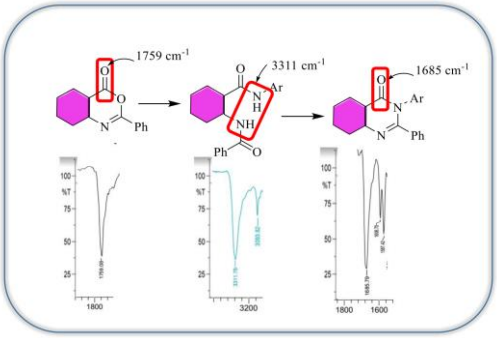


Figure 4: FT-IR spectra shifting signals of compound (2) to (4).

Results and discussion

Chemistry

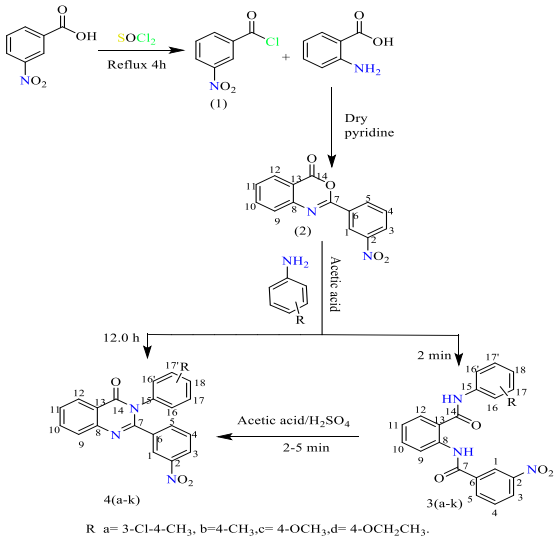
The development of a new compound from simple one and their modification to improve a new functional property is crucial for their bioactivity and its uses as biomedical and drugs.

Herein , we synthesized a new quinazolinone derivatives as shown in **Scheme 1**, benzoxazinone was synthesized from anthranilic acid with 3-nitro benzoyl chloride via Schotten Bauman benzoylation, followed by cyclodehydration to give benzoxazin-4-one , this reactive benzoxazinone treated with glacial acetic acid in presence of various aromatic amines comprises ring opening benzamides in very short reaction time, the ring closing benzamides were done by using sulphuric acid as catalyst which give target molecules quinazolinones ,adding sulphuric acid improved the intramolecular cyclodehydration in seconds, faster than any methods done before.

The chemical structure of All synthesized compounds were characterized by using spectroscopic data analysis (FT-IR, ^{1}H NMR, ^{13}C-NMR and DEPT 135- ^{13}C-NMR) **Figures 12-18** and their physical properties.

Also the structure of compound 3a unambiguously confirmed by single crystal X-Ray with CCDC No. (2095652) as shown in **Figure 3**.

The FT-IR spectra were used to observe shifting the interest signals of compound (2) to (4) as in **Figure 4.**



Scheme 1. Stepwise chemical reaction for synthesis of 2, (3a–d) and (4a–d. Note; The numbers (1,2,3,…,20) indicated on the structures are used just to interpreting the 1H NMR and 13C NMR spectra.

The UV –visible absorption spectra of benzamides derivatives 3(a-d) figure 5 and quinazolinone derivatives 4(a-d) figure 6 were recorded in DMSO at 25ºC quartz cuvette (C = 50 ppm), in the mainly all compounds show two absorption bands at 345-350 nm and 367-375 corresponds to π-π* and n-π* respectively , the π-π* relates to C=C transition of aromatic ring and n-π* to the nonbonding electrons of carbonyl groups transitions[23]. The blue shift will occur during the ring closing of benzamide to quinazolinone derivatives.

Biological evaluation

Antibacterial activity

The synthesized compounds 3a,3b,3c,3d ,4a,4b, 4c and 4d were screened for their anti-bacterial activities against P. aeruginosa ATCC (29213) as a Gram negative and S. aureus ATCC (25923) as a Gram positive by micro titer assay , the minimum inhibition concentration of the synthesized compounds were determined in µg/mL, the results of this screen as showed in **Table (3)** ,tested compounds had good activity against both microorganisms and also the compounds bearing the electro donating group had a better activity than unsubstituted group , the 3a and 4a showed better activity than other similar to the ampicillin as standard drug, and also the para substituted group demonstrated lower activity in comparison ortho and meta counter parts , due to the conformation and configuration.

The percent inhibition of growth of both microorganisms also determined most of synthesized

compounds the concentration (512-1024 µg/mL) enough to kill near 100% of bacteria typically 4c against both organisms (**Figure 7&8**).

Molecular Docking

Bacterial DNA gyrase is required for bacterial growth. Researchers have recently looked into a variety of synthetic inhibitors that target DNA gyrase as antibacterial medicines[48]. As a result, we performed a molecular docking analysis on the synthesized compounds to determine their DNA gyrase binding interactions and compared them to the clinical pharmacological inhibitor (ciprofloxacin).

The goal of this study was to better understand the ligand-receptor interactions of 3(a-d) and 4(a-d) against the target enzyme bacterial DNA gyrase. The highest binding energy of the synthesized compounds 3 (a-d) and 4(a-d) ranged from -6.5 to -7.9 kcal/mol (**Table 4**), with compound 3j (-7.9 kcal/mol) achieving the greatest result. **(Table 4)** shows the binding affinity, H-bond, and residual interaction between the produced molecule and ciprofloxacin (**Table 4**), (1) The majority of compounds are listed in (**Table 3**), with predicted binding energies lower than ciprofloxacin; (2) hydrogen bonding interactions were found for the ring opening benzamides between the of Asp73, Gly 77, Ser 121, Asp71 Asn 46,Val120, Val71, and Arg136 of DNA Gyrase Subunit B in the majority of cases (PDB ID: 1KZN) ((3) The quinazolinone derivatives were hydrogen bound to DNA Gyrase's Asn 46 and Val120.

As shown in **Figure 9**, the pot of ligand interaction of 3a revealed one H-bond acceptor with Ser121 and three pi-H interactions with Pro79, and Ile 90 of the active site of DNA Gyrase.

. These results are consistent with the goal of our design and observed in vitro antibacterial results, because both parts of the designed compounds participated in bond interactions with active sites of bacterial resistance. Compounds 3a in the binding site of DNA Gyrase Subunit B (PDB ID: 1KZN) are illustrated in three-dimensional (3D) diagrams illustrated in **Figure 10**.

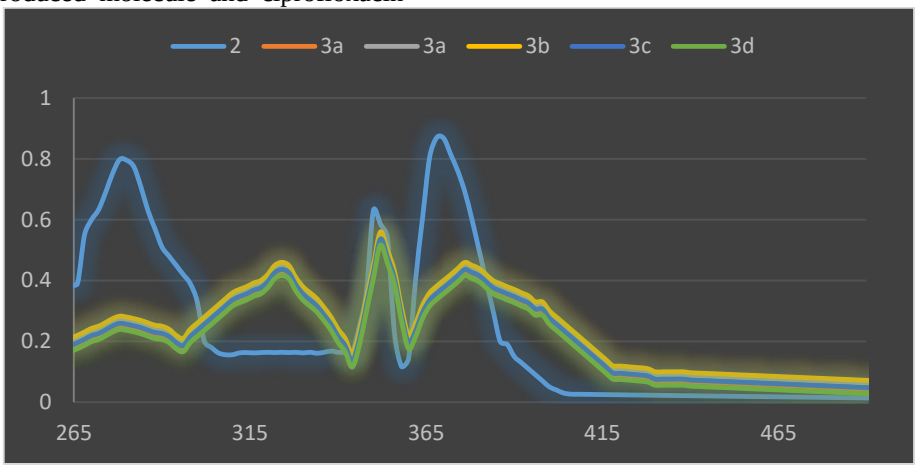


Figure 5: absorption spectra of benzamide derivatives3(a-k) and benzoxazinone (2)

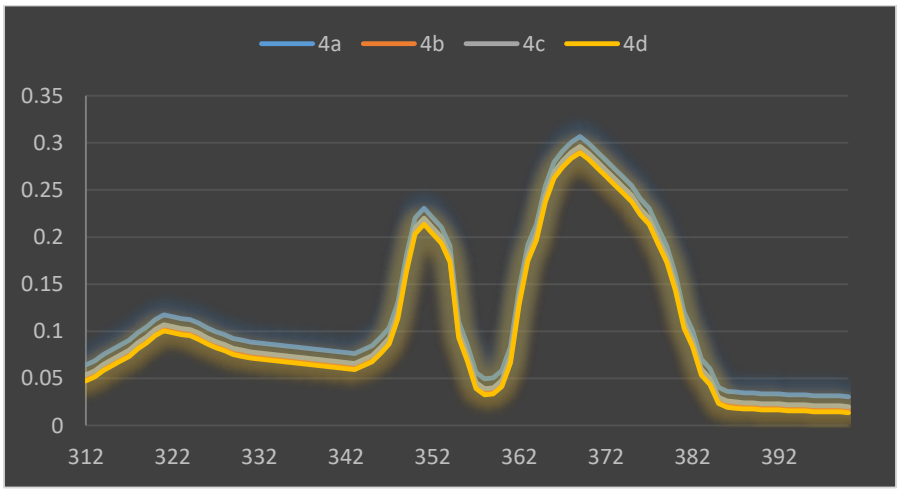


Figure 6: Absorption spectra of quinazolinone derivatives 4(a-k)

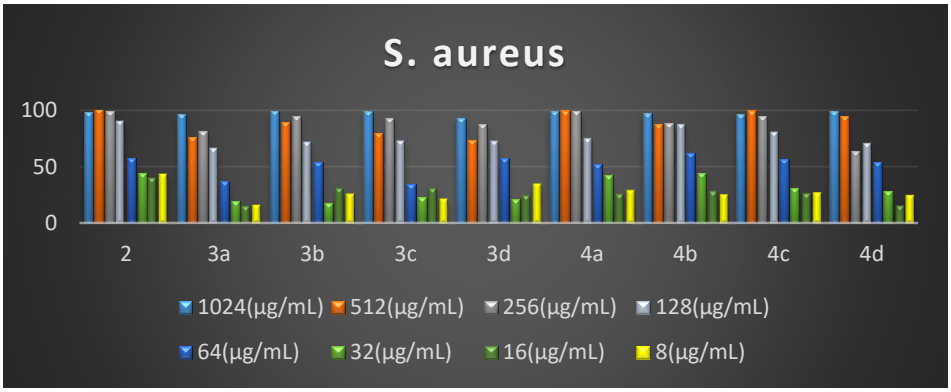


Figure 7: in vitro antibacterial activity of synthesized compounds against S. aureus (% of inhibition)

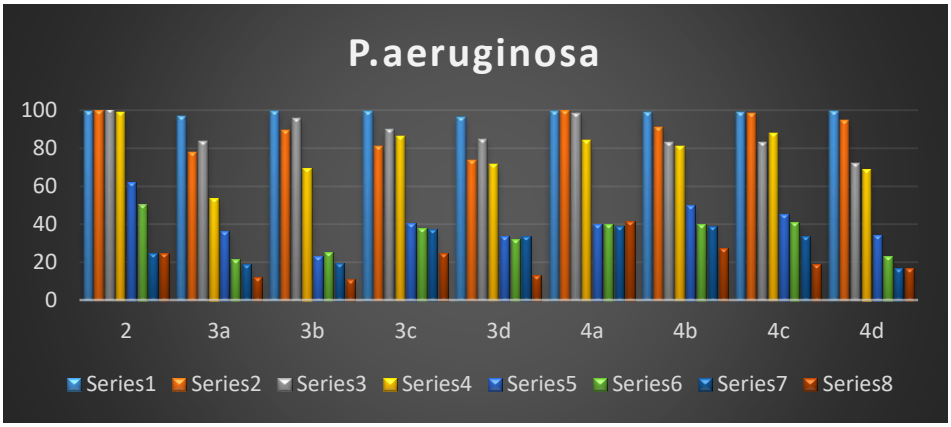


Figure 8: in vitro antibacterial activity of synthesized compounds against P.aeruginosa (% of inhibition)

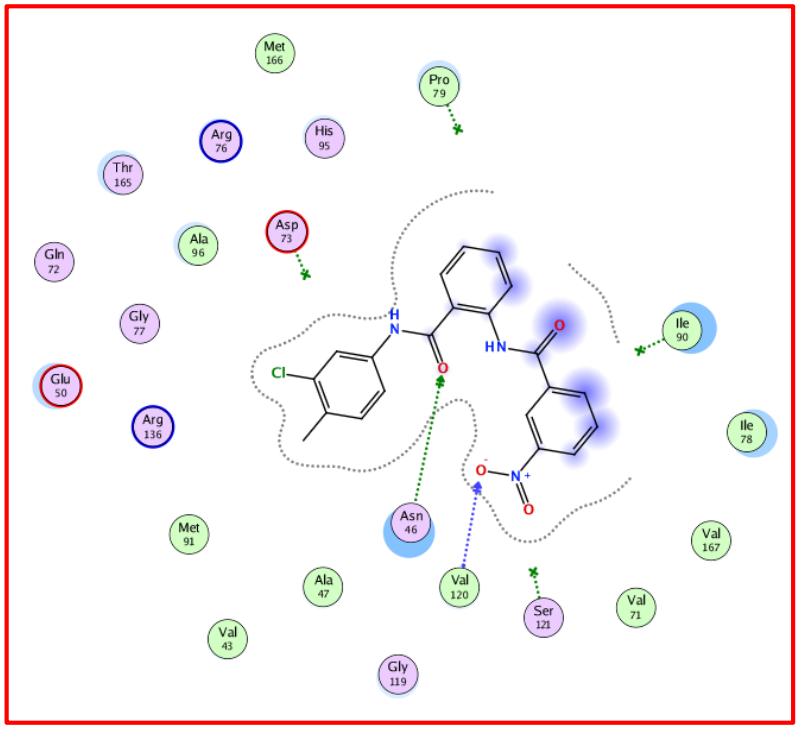


Figure 9: showed ligand interaction of 3a

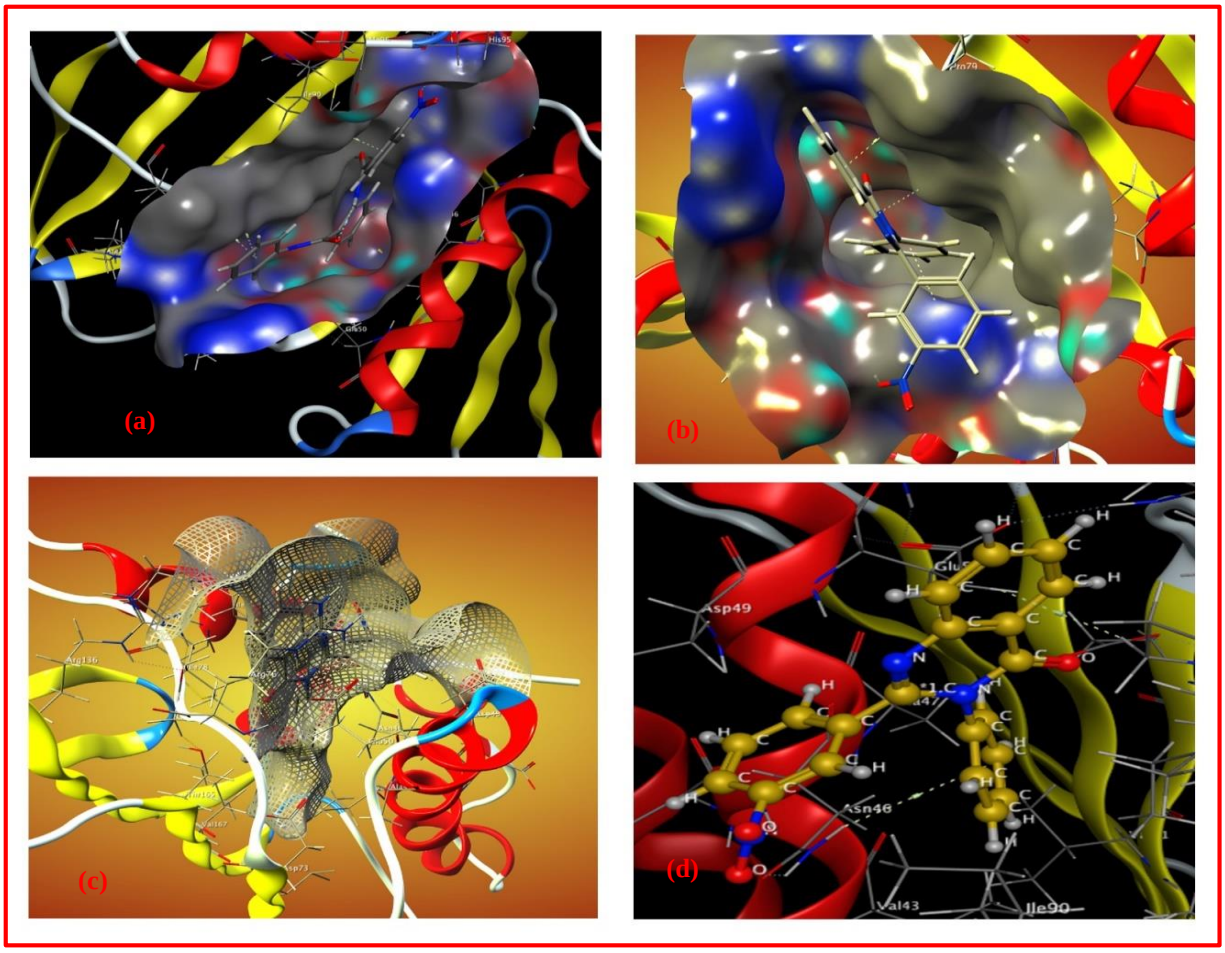


Figure 10: three dimensional structure of 3a and 4a : a,b surface cavity of docked into active site of (1KZN) c-active site of site of (1KZN), and d-ligand active site interaction

In silico ADME study

Drug-like compounds' ADME (absorption, distribution, metabolism, and excretion) properties are a crucial stage in drug development.

Bioactive compounds' oral bioavailability is a critical aspect in their development as medicinal treatments. Lipinski and Veber proposed a set of measures for evaluating the prospective oral bioavailability and drug-likeness characteristics of compounds that are orally active in humans. If the violation greater than one, it could indicate an issue with the drug's bioavailability.

Table 1: some physical properties and FT-IR Data of newly synthesized benzamide derivatives (3a-k)

Pro.	R	Chemical formula	Time (min)	M.P. ºC	Yield (%)	IR	
						NH	C=O
3a	3-Cl,4-CH_3	$C_{21}H_{16}ClN_3O_4$	2	231-232	82	3292	1662
3b	4-CH_3	$C_{21}H_{17}N_3O_4$	1	192-193	89.3	3311	1685
3c	4-OCH_3	$C_{21}H_{17}N_3O_5$	1	200-202	98	3292	1681
3d	4-O CH_2CH_3	$C_{22}H_{19}N_3O_5$	1	216-218	91	3311	1685

Table 2 : some physical properties and FT-IR Data of newly synthesized quinazolinone derivatives (4a-k)

Pro.	R	Chemical formula	Time (min)	M.P. ºC	Yield (%)	IR	
						C=O	C=N
4f	3-Cl,4-CH_3	$C_{21}H_{14}ClN_3O_3$	2	202-204	79	1685	1608

4g	4-CH_3	$C_{21}H_{15}N_3O_3$	3	180-182	84	1685	1606
4i	4-OCH_3	$C_{21}H_{15}N_3O_4$	1	171-172	98	1685	1608
4j	4-O CH_2CH_3	$C_{22}H_{17}N_3O_4$	1	181-182	95	1685	1608

Table 3: MIC of some newly synthesized compounds

	R	MIC (µg/mL)	
		P. aeruginosa ATCC (29213)	S. aureus ATCC (25923)
2	-	128	128
3a	**3-Cl,4- CH_3**	128	128
3b	**4-CH_3**	256	256
3c	**4-OCH_3**	256	128
3d	**4-OCH_2CH_3**	128	256
4a	**3-Cl,4- CH_3**	128	128
4b	**4-CH_3**	512	128
4c	**4-OCH_3**	512	512
4d	**4-OCH_2CH_3**	128	256
	Ampicillin	128	128

Table 4 :**The Docking study output of the synthesized compounds in which docked against S. Aureus DNA gyrase B (PDB ID: 1KZN).**

Entry	ΔGbinding (kcal/mol)	Hydrogen bond contacts	Arene- Arene contacts	Arene-H contacts
2	**-6.6620**	**Asp73 , Gly 77**	**--**	**Asn 44**
3a	**-7.3847**	**Ser 121**	**--**	**Pro79, Ile 90**
3b	**-7.2510**	**Ser 121**	**--**	**Pro79, Ile 90**
3c	**-7.4518**	**Arg136**	**--**	**Pro79, Ile 78**
3d	**-7.9676**	**Asp73**	**--**	**Ile 90**
4a	**-7.1766**	**Val120**	**--**	**Ile 78**
4b	**-6.5517**	**Asn 46**	**--**	**Asn 46, Pro79**
4c	**-6.8841**	**Asn 46**	**--**	**Asn 46, Pro79**
4d	**-7.3148**	**Asn 46**	**--**	**Asn 46, Ile 78 ,Pro79**
Ciprofloxacin	**-6.7022**	**--**	**--**	**Asn 46**

The ADME study of all synthesized compounds were summarized in **Table 5**. according to the results obtained showed that all synthesized compounds are fully agreement to Lipinski's rule of five. Where the M.wt. > 500, the number of rotatable bond lower than 10, exhibiting sufficient molecular flexibility, with good permeability and oral bioavailability expected as a result

The topological surface area of all synthesized compounds were <140 $Å^2$, the percentage of oral absorption %ABS ranged from (66-81%), that either may have a higher passive oral absorption rate than azithromycin (39.73 percent). It's important to note that the control chemical Tanespimycin fails to meet three criteria: MW, HBA, and TPSA, which could explain its bad oral bioavailability. Topological surface area against WLog p[49] ,from the graph all synthesized compounds placed in region of human intestinal absorption not with no blood brain barrier permeability, the boiled egg also showed non substrate P-glycoprotein PGP-), therefore they are immune to the efflux mechanism used by this transporter, which is used as a drug-resistance mechanism by many tumor cell lines[50]. Because all of the synthesized compounds met the criteria for an orally active medicine, they can be further developed as oral drug candidates. The results of the in silico ADME prediction analysis suggest that the compounds acquired match the computational assessment, indicating the pharmacologically active framework that was evaluated for moving forward with further possible hits. Because of their adverse absorption, distribution, metabolism, elimination, and cytotoxic (ADMET) characteristics, many putative therapeutic medicines fail to be a viable clinical option. [51] As a result, a significant number of in-silico ADME models have been constructed for early pharmacokinetic property prediction.

Table 5: In silico study of all newly synthesized compounds

	TPSA(Å²)[a]	NRB[b]	%ABS[c]	MW[d]	Logs[e]	Logp[f]	HBA[g]	HBD[h]	N violations	drug LIKENESS					Bioavailability Score
										Lipinski	Ghose	Veber	Egan	Muegge	
2	88.92	2	78.32	268.22	-3.7	2.31	5	0	0	Yes	Yes	Yes	Yes	Yes	0.55
3a	104.02	7	73.11	409.82	-5.05	3.55	4	2	0	Yes	Yes	Yes	Yes	Yes	0.55
3b	104.02	7	73.11	375.38	-4.92	3.11	4	2	0	Yes	Yes	Yes	Yes	Yes	0.55
3c	113.25	8	69.93	391.38	5.23	2.91	5	2	0	Yes	Yes	Yes	Yes	Yes	0.55
3d	113.25	9	69.93	405.40	-4.92	3.01	5	2	0	Yes	Yes	Yes	Yes	Yes	0.55
4a	80.71	3	81.16	391.81	-5.56	3.82	4	0	0	Yes	Yes	Yes	Yes	Yes	0.55
4b	80.71	3	81.16	357.36	-5.16	3.40	4	0	0	Yes	Yes	Yes	Yes	Yes	0.55
4c	89.94	4	77.97	373.36	-4.92	2.98	5	0	0	Yes	Yes	Yes	Yes	Yes	0.55
4d	89.94	5	77.97	387.39	-5.16	3.31	5	0	0	Yes	Yes	Yes	Yes	Yes	0.55
std	180.08	7	39.73	748.98	-6.55	5	14	5	2	-	-	-	-	-	-

Std =azithromycin a= topological polar surface area **Å² <140**, b= number of rotatable bond ≤ 10, c= percentage of oral absorption, d= molecular weight≤ 500, f= lipophilicity octanol/water coefficient ≤6, f= aqueous solubility (from -6.5 to 0.5),g= number of hydrogen bond accepter ≤ 10, h= number of hydrogen bond donor ≤ 5.

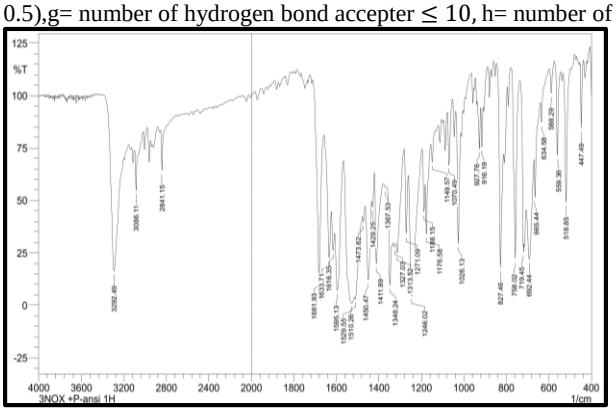


Figure 11: FT-IR spectrum of compound 3c

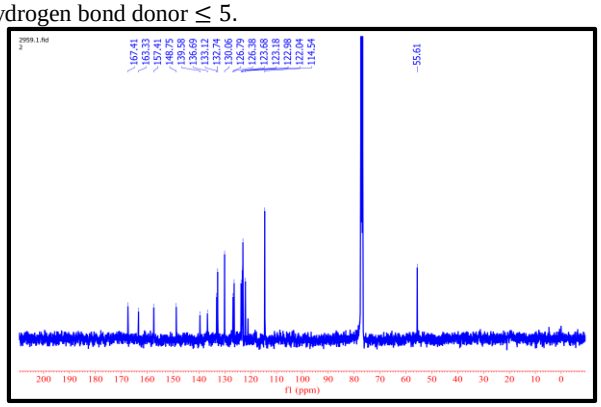


Figure 13: C^{13}-NMR spectrum of compound 3c

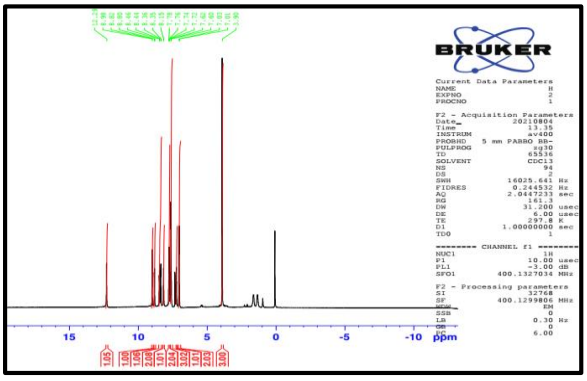


Figure 12: ^{1}H-NMR spectrum of compound 3c

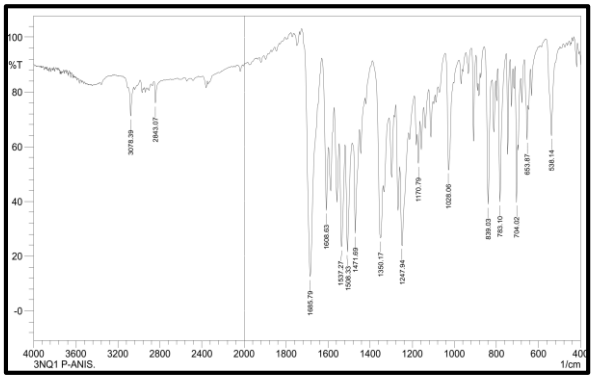


Figure 14: FT-IR spectrum of compound 4c

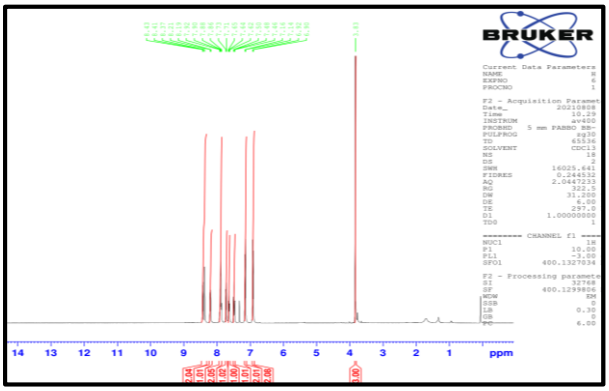


Figure 15: ^{1}H-NMR spectrum of compound 4c

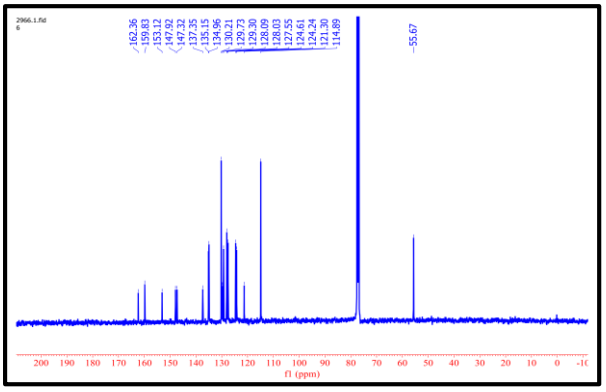


Figure 16: ^{13}C-NMR spectrum of compound 4c

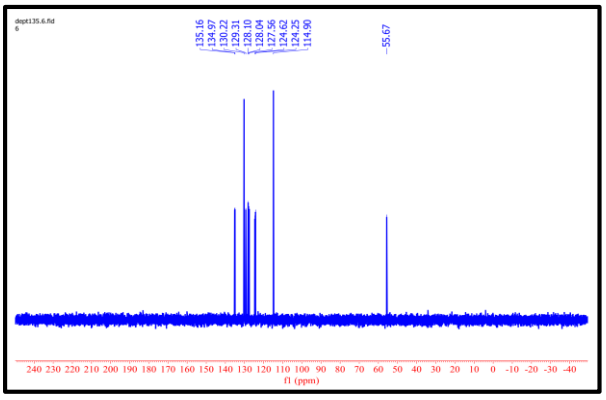


Figure 17: DEPT 135-C^{13}-NMR spectrum of compound 4c

Conclusion

From the results obtained in this study, we concluded that electron with drawing groups have a lower affinity for making benzamides, the ring closing benzamides into quinazolinones were done by using a sulphuric acid catalyst that promotes cyclodehydration, the sulphuric acid has provided some advantages such as a short reaction time and easy workup. , The antibacterial activity of certain freshly synthesized compounds were evaluated in vitro, and the results show that the tested molecules have good activity against both microorganisms and that compounds with the electro donating group had better activity than unsubstituted group. A molecular docking study was conducted to verify the interactions with the target, and to better understand the binding model, and good binding interactions were obtained. All of the synthesized compounds had the essential physicochemical and pharmacokinetic profiles to be developed as potential drug candidates. This study reveals us how our technique enables the discovery of novel promising and privileged structures based on these overall results. Finally, the findings suggest that these novel compounds could be used as a fresh lead for further research and development.

Availability of data and materials

The data that support this study are available in the article and accompanying online supplementary material.

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Conflict of interest

Authors have no conflict of interest.

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