Synthesis, Characterization, ADME Study and Anti-proliferative evaluation against MCF-7 breast cancer cell line of new analog of a 4-aminophenyl quinazolinone derivative

Zainab A. Jabbar* , Monther F. Mahdi*, Basma M. Abd Razik *

Department of Pharmaceutical Chemistry, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq

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Corresponding Author email:
Dr.monther.f71@gmail.com
Orcid: https://orcid.org/0000-0002-2069-4121

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Abstract:

New series of 4-aminophenyl quinazolinone attached to an aromatic aldehyde moiety has been designed. Compound (ZA) was synthesized by a reaction of benzene-1,4-diamine with 2-aminobenzoic acid.

The reaction between (ZA) intermediate and different substituted aromatic aldehydes (R1-R6) is considered one of the most common chemical reactions for the synthesis of imine compounds (Schiff bases) to produce compound (ZA1-ZA6). FTIR, ^{1}H-NMR, and ^{13}C-NMR have been used to confirm the chemical structures of various substances. MTT assay was used to assess in *vitro* anti-proliferative action for estrogen receptor alpha. The anti-proliferative study discovered a dose-dependent effect on cell proliferation in breast cancer (MCF-7) with inhibitory concentration In comparison to the reference medication tamoxifen (IC_{50} of 133.4µg\mL), IC_{50} of the compounds (ZA1, ZA2, ZA3) was 0.07964, 57.43 & 0.002717 µg\mL, respectively at 72 hours on same cell line mentioned above, that also signifies that compound ZA1 has a significantly greater effect on this cell line type.

Key words: Human estrogen receptor-alpfa , MCF-7,quinazolinone.

التوليف و التوصيف و تقيم مكافحة التكاثر ضد خط خلايا سرطان الثدي (MCF-7) من نظير جديد لمشتق ٤-امينوفينيل كينازولينو

زينب عباس جبار*، منذر فيصل مهدي *، بسمة منجد عبد الرزاق*

فرع الكيمياء الصيدلانية، كلية الصيلة ،الجامعة المستنصرية، بغداد ، العراق.

الخلاصة:

تم تصميم سلسلة جديدة من ٤-امينوفينيل كينازولينون مرتبطة بشق الالديهايد الاروماتي زتم تصنيع المركب (ZA) عن طريق تفاعل البنزين-١-٤-ديامين مع حمض ٢-١-امينوبنزويك. يعتبر التفاعل بين الالديهايدات الاروماتية و المركب الوسطي (ZA) احد التفاعلات الكيميائية الاكثر شيوعا لتخليق مركب الايمين (قواعد شيف بيس) لانتاج المركبات (ZA1-ZA6). تم استخدام (FTIR, ^{1}H-CNMR, ^{13}C-NMR). لتاكيد الهياكل الكيميائية للمواد المختلفة.التقيم في المختبر كنشاط مضاد للتكاثر لمستقبلات هرمون الاستروجين الفا باستخدام مقايسة(MTT).كشفت دراسة مكافحة التكاثر عن تاثير يعتمد على الجرعة على الخلايا السرطانية لسرطان الثدي (MCF-7) مع تركيزمثبط بالمقارنة مع الدواء المرجعي تاموكسيفين (IC_{50} من ٤.١٣٣ µg\mL) ، كشفت مقايسة السمية الخلوية أن IC_{50} للمركبات (ZA1، ZA2، ZA3) كان ٠,٠٧٩٦٤ ، ٥٧,٤٣ و٠,٠٠٢٧١٧ µg\mL ، على التوالي في٧٢ ساعة على نفس خط الخلية المذكور اعلاه مما يشير الى تاثير اعلى بكثير لمركب (ZA1) على نوع خط الخلية هذه.

الكلمات المفتاحية: استروجين ريسبتر الفا,MCF-7, كوينازولينون

INTRODUCTION

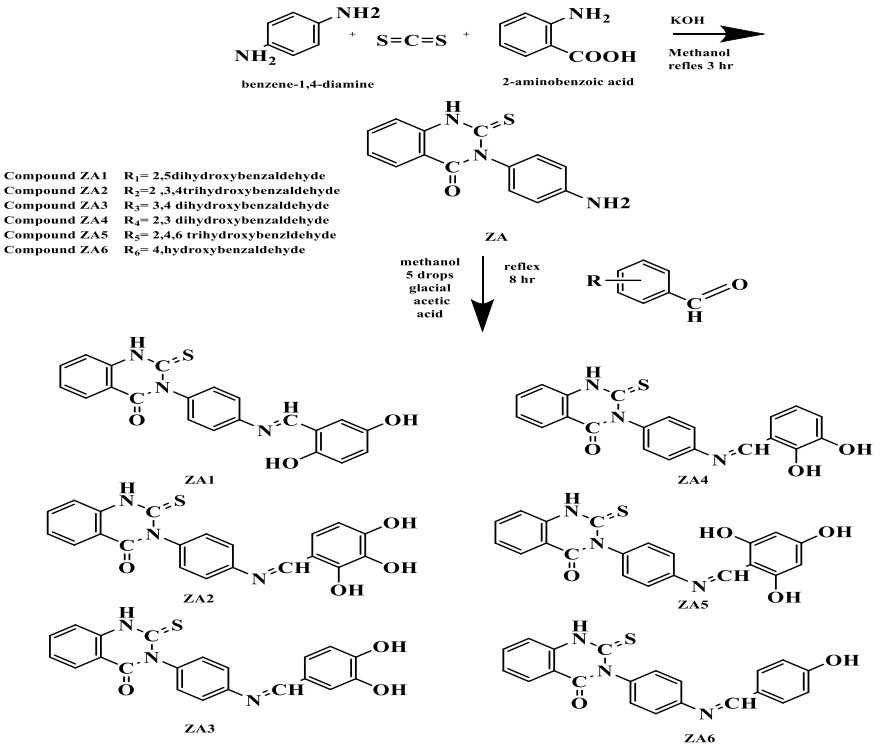
One of the most important causes of mortality in women is breast cancer. The critical biomarkers connected to the cause of breast cancer (BC) are, estrogen receptor, epidermal growth factor-2 and progesterone receptor [1, 2] .BC is a complex disease that results from numerous sequential and physiologic alterations within cells. Food additives, non-ionizing electromagnetic exposure, and anxiety are all outlined as cancer risk factors, with the least well-known being low fruit and vegetable intake and overweight[3,4]. Estrogen replacement medication has been widely used as an excellent drug of choice as a replacement therapy for menopausal women for relieve unwanted symptom like (hot flash, osteoporosis and urogenital atrophy)[5]. Estrogen-based hormonal replacement therapy has been shown to be highly connected to enhance the growth of reproductive cancer cells in specific organs[6]. Estrogen action is controlled by important endogenous regulators. Estrogen receptor known as nuclear-hormone-receptors has two isoform exist (estrogen receptor-alpha, beta) [7]. One of the most important form of breast cancer is related to estrogen receptor alpha(ER-α) known as estrogen receptor-α positive breast cancer. Therefore nowadays successfully ER-α targeting therapy is playing a critical role in controlling breast cancer [8]. Tamoxifen is the most popular ER-α target for treatment of BC estrogen receptor alpha positive. Tamoxifen operates in a highly competitive with estrogen for binding to estrogen receptors alpha (ER) in the mammary glands, restricting Gene transcription and proliferation while inducing apoptosis in breast cancer cells. Its agonist impact in the uterus, on the other hand, may cause endometrial changes such as polyps, cancer, hyperplasia, and uterine sarcoma [9,10] . Fulvestrant, a selective estrogen receptor down regulator, has an anti-estrogen effect but appears to have a low bioavailability [11] . Problems related to hormonal managements of ER-α positive breast cancer nowadays being most important concern ,therefore new analogues candidate is important for prevention unwanted symptoms and drug related resistance[12]. By studying the ER scaffold and the SAR of quinazolinone derivatives has been shown to has promising anticancer activity , design, characterize and synthesis for new analogues of 4-aminophenyl quinazolinone targeting ER-α and screening for their anti-proliferate evaluation is done. The discovery of febrifugine, a quinazolinone alkaloid found in the Chinese plant Aseru (*Dichroa febrifuga Lour)*, marked the beginning of the evolution of quinazolines[13]. At the moment, scientists are focusing their efforts on creating new anticancer medicines and heterocyclic chemicals that have been widely researched for this purpose[14]. In order to create a stable compound, molecular docking is a form of data analysis modeling that estimates a ligand's preferred orientation in reference to a receptor (Protein). The favoured orientation may be employed to determine the degree of the interaction or binding ability with both ligand and protein using scoring methods[15]. Because it can predict the binding mechanism and energy of receptor-ligand complexes, molecular docking is now a common approach in computer-aided drug design (CADD)[16]. The docking browser helps determine the binding affinity and specificity of the designed compounds to protein (ER-α) by analyzing the chemical bonding between the protein's active binding sites and the chemical compounds [17]. Many pharmaceutical compounds also contain the chemicals quinazoline and quinazolinone, which are used to make a variety of biomaterials for synthetic chemistry. All the chemical compounds that make up Schiff's base group All have

crucial pharmacophores. It can be used as a suitable lead structure in the development of agrochemicals and pharmaceuticals such fungicides, bactericides, antivirals, antioxidants, antiproliferative, and anti-microbial medicines. In addition, due to the wide range of pharmacological effects that the quinazolinone nucleus and its derivatives exhibit, they have been extensively studied. As medicines, several of them have effects that are antitubercular, anti-inflammatory, anticonvulsant, antidepressant, anti-ulcer, anti-tuberculosis, anticonvulsant, and analgesic [18,19] .

MATERIALS AND METHODS

Ingredients used in biosynthesis as well as the solvents needed for product assessment, purification, and recrystallization, were derived from common sources (HyperChem-China, Sigma Aldrich-Germany, BDH-England, Himedia-India, CDH-India). At the Department of pharmaceutical Chemistry department /College of pharmacy /University of AL-Mustansiriyah/Iraq, the melting points of the produced compounds were calculated in an uncontrolled manner using the (Digital Stuart scientific SMP30). At the BPC analysis center in Iraq, a Shimadzu FT-IR spectrophotometer was used to examine the infrared spectra of the as well as produced substances using a disc of KBr in the range 4000-400 cm as a Bruker device. The ^{1}H-NMR and ^{13}C-NMR spectra were measured at Thran University of in Iran using a 500MHz Bruker DMX-500 NMR spectrophotometer and DMSO-d6 solution.and TMS (tetramethylsilane) as an internal reference.

CHEMICAL SYNTHESIS



Scheme: (1) the synthesis of the final products and intermediates.

Synthesis of 3-(4-aminophenyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one (ZA) :

Mixture of Anthranilic acids (1.371 g, 10 mmol) and potassium hydroxide (0.6733 g, 12 mmol) were refluxed in 10 mL of

methanol, then drop wise addition of benzene-1,4-diamine (1.2977gm,12 mmol) dissolved in carbon disulfide(1.812 ml, 30 mmol).The final mixture was heated for three hours at reflux, and the resulting solid was then filtered, washed with methanol, and dried. The resulting product then dissolved in a 10% potassium hydroxide solution (strong base able to remove a multitude of contaminants) , filtered, and then (2–3 mL) of concentrated hydrochloric acid was added drop wise to the filtrate, the resultant white precipitate was filtered, distal water-washed, and dried as compound (ZA) [20].

General procedure for the synthesis of quinazolinone Schiff base derivatives (ZA1-ZA6):

Mixture of compound (ZA) (0.001mol, 0.269gm) and (0.001mol) of one of the hydroxyl-aldehydes (R_1= 2,5dihydroxybenzaldehyde, R_2=2,3dihydroxybenzaldehyde - R_6) were dissolved in 10 ml of methanol one by one, then about (2-5) drops of glacial acetic acid were further added to the aldehyde solution with stirring for 15 minutes. The solution of compound (ZA) was gradually added to the aldehyde-containing acid solution while stirring. At the end of this time, the cooling of the mixture with crushed ice yielded the colored powder, which was separated by filtration and then recrystallized using absolute% ethanol to obtain the final compounds (ZA1-ZA6) [21].

Compound (ZA) 3-(4-aminophenyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one. Light yellow powder, yield =84 %, m.p. = 248-250°C, FTIR v (cm^{-1}): 3423.23, 3340.71 (N-H stretching of primary amide), 3224.98 (N-H stretching of dihydro- quinazoline), 3035.96 (C-H for the aromatic ring), 1687.36 (C=O stretching of carbonyl group),1512.19, 1515 (C=C stretching of Ar-ring) and 1483.40 (-N-C=S stretching of thioamide). ^{1}H -NMR(DMSO-d_6,500MHz)(ppm): 5.40 (2H, s, NH_2), and 10.23 (1H,s for NH). ^{13}C-NMR (DMSO-d6, ppm, 125 MHz):160.42 (C=O), 177.25 (C=S), calculated M.W (269.06 g/mol).

Compound (ZA1) (E)-3-(4-((2,5-dihydroxybenzylidene)amino)phenyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one. Orange powder, yield =72 %, m.p. = 170-172°C, FTIR v (cm^{-1}): 3217.27 (OH stretching of phenol), 3196.22(C-H stretching of secondary amine), 3028.24 (C-H for the Ar-ring), 1662 (C=O stretching of carbonyl group),1620 (C=N stretching of imine), 1530-1560 (C=C stretching of Ar-ring) 1484.01 (-N-C=S streatching of thioamide) and 1408.04(C=S stretching).^{1}H-NMR(DMSO-d_6,500MHz)(ppm): 8.94(1H, s,C-Hof CH=N), 10.80 (1H, s, NH), 9.92-10.2(1H, s, OH). ^{13}C-NMR (DMSO-d6, ppm, 125 MHz):162.62 (C=O), 179 (C=S) and 160.42 (C=N), calculated M.W (391.45 g/mol).

Compound(ZA2)(E)-2-thioxo-3-(4-((2,3,4-trihydroxybenzylidene)amino)phenyl)-2,3-dihydroquinazolin-4(1H)-one. Yellow Orange powder, yield =90 %, m.p. = 211-213°C, FTIR v (cm^{-1}): 3236.55 (OH stretching of phenol), 3154.34(C-H stretching of secondary amine), 3032.10 (C-H for the Ar- ring), 1662 (C=O stretching of carbonyl group),1622 (C=N streatching of imine), 1531-1535 (C=C stretching of Ar-ring), 1484.01 (-N-C=S stretching of thioamide) and 1408.04 (C=S stretching). ^{1}H -NMR(DMSO-d_6,500MHz): 10.19 (1H, s, CH of CH=N), 13.09 (1H, s, NH), 9.78-9.79(1H, s, OH). ^{13}C-NMR (DMSO-d6, ppm, 125 MHz): 160.41 (C=O), 179.93 (C=S) and 153 (C=N), calculated M.W (407.44 g/mol).

Compound (ZA3) (E)-3-(4-((3,4-dihydroxybenzylidene)amino)phenyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one. Orange powder, yield =87 %, m.p. = 216-218°C. FTIR v (cm^{-1}): 3224.98 (OH stretching of phenol), 3163.24(C-H

stretching of secondary amine), 3051.39 (C-H for the Ar- ring), 1654 (C=O stretching of carbonyl group),1620 (C=N streatching of imine), 1508.33-1510 (C=C stretching of Ar-ring), 1472.40 (-N-C=S stretching of thioamide) and 1408.04 (C=S stretching). ^{1}H-NMR(DMSO-d_6,500MHz)(ppm):8.45 (1H, s, for C-H of CH=N) 10 (1H, s, NH) and 9.72(1H, s, OH), ^{13}C-NMR (DMSO-d6, ppm, 125 MHz): 162.42 (C=O), 179.91 (C=S) and 159.96 (C=N), calculated M.W (391.45 g/mol).

Compound (ZA4) (E)-3-(4-((2,3-dihydroxybenzylidene)amino)phenyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one. Bright florescent Orange powder, yield =78 %, m.p. = 2019-221°C. FTIR v (cm^{-1}): 3217.27 (OH stretching of phenol), 3171.10(C-H stretching of secondary amine), 3041.90 (C-H for theAr-ring), 1694 (C=O stretching of carbonyl group),1620.21 (C=N stretching of imine), 1516.05-1520 (C=C streatching of Ar-ring), 1455.33 (-N-C=S streatching of thioamide) and 1400.32 (C=S streatching).^{1}H-NMR(DMSO-d_6,500MHz)(ppm): 8.96 (1H,s, C-H of CH=N), 10.10 (1H,s, NH), 9.72-13.8(1H,s, OH) . ^{13}C-NMR (DMSO-d6, ppm, 125 MHz): 163.31 (C=O), 179.89(C=S) and 160.41 (C=N), calculated M.W (391.45 g/mol).

Compound (ZA5) (E)-2-thioxo-3-(4-((2,4,6-trihydroxybenzylidene)amino)phenyl)-2,3-dihydroquinazolin-4(1H)-one. Light brown powder, yield =94 %, m.p. = 224-225°C. FTIR v (cm^{-1}): 3221.12 (OH stretching of phenol), 3171.10(C-H stretching of secondary amine), 3028.24 (C-H for Ar- ring), 1662 (C=O stretching of carbonyl group),1620.21 (C=N stretching of imine), 1508-1513 (C=C stretching of Ar- ring), 1458.40 (-N-C=S stretching of thioamide) and 1408.32 (C=S stretching).^{1}H -NMR(DMSO-d_6,500MHz)(ppm): 8.65 (1H, s, C-H of CH=N), 10.11 (1H, s, NH), 9.93(1H, s, OH) and 7.8(1H, s, OH). ^{13}C-NMR (DMSO-d6, ppm, 125 MHz): 160 (C=O), 179.92(C=S) and 160.41 (C=N), calculated M.W (407.44 g/mol).

Compound (ZA6) (E)-3-(4-((4-hydroxybenzylidene)amino)phenyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one. Light brown powder, yield =70 %, m.p. = 230-231°C. FTIR v (cm^{-1}): 3020.53 (OH stretching of phenol), 3180.59(C-H stretching of secondary amine), 3020 (C-H for the Ar-ring), 1694 (C=O stretching of carbonyl group),1621 (C=N stretching of imine), 1520-1527 (C=C stretching of Ar- ring), 1450 (-N-C=S stretching of thioamide) and 1401 (C=S stretching). ^{1}H -NMR(DMSO-d_6,500MHz)(ppm): 8.68 (1H, s, C-H of CH=N), 10.22 (1H,s, NH) and 8.68(1H,s, OH). ^{13}C-NMR (DMSO-d6, ppm, 125 MHz): 163.31 (C=O), 179.89(C=S) and 160.41 (C=N), calculated M.W (375.45 g/mol).

CYTOTOXICITY ASSAY METHOD

An in vitro method was used to test how the generated compounds affected the MCF-7 cancer cell line. The Biotechnology Research Center of Al-Nahrain University provided the human breast cancer cell MCF-7 culture. They were preserved in the tissue culture research facility's cell bank at Al-Nahrain University College of Biotechnology.

Cell Line Maintenance

Confluent monolayer stage was followed by the following procedure: The cell sheet was evacuated of the growth medium, and then washed in PBS. Two to three mL of the trypsin/versine solution was injected into the cell. The monolayer was entirely covered by turning the jar over and gently rocking it. The vessel was incubated at 37 degrees Celsius for one to two minutes. Before the cells began to detach from the vessel. After adding fresh (15–20 mL) RPMI media, pipetting was employed to distribute the cells from the wedding

surface into the growth medium. If required, cells were rearranged into plates, flasks, or other culture vessels at the required concentration and incubated at 37 °C with 5% CO_2.cellular count was achieved by using the hemocytometer to count the cells and the following formula: Total Cell Count/ml: cell count multiplied by the sample volume (or dilution factor) multiplied by 10^4 [22].

MTT assay

Using an MTT kit package, the toxic effects of various doses (400, 200, 100, 50, and 25 µg/mL) of unpurified and purified compounds (ZA1-ZA6) were assessed:

A-Kit Components 10 bottles of the 1 ml MTT solution, then50 ml of the solubilization solution in two bottles.

B-Assay [22]

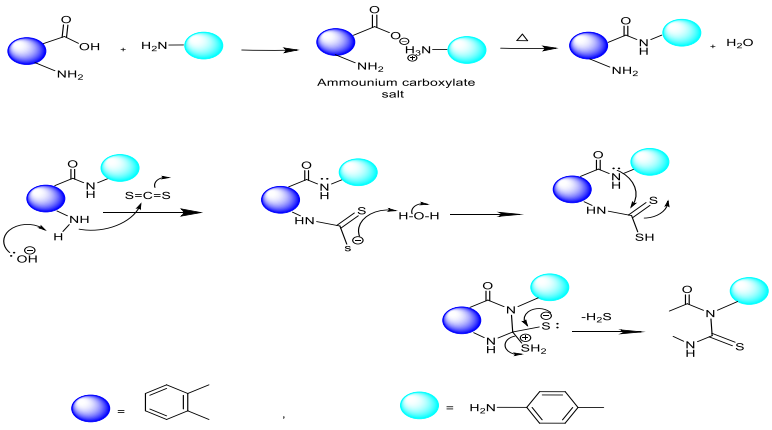
1- In 96 flat well micro-titer plates with a final volume of 200 L complete culture media for each well, tumor cells ($1x10^4$-$1x10^6$ cells/mL) were cultured. Para film that had been disinfected and gently shacked encircled the micro plate.
2- For 24 hours, plates were incubated at 37 °C with 5% CO_2.
3- The media was withdrawn, and the required concentrations of the compounds (ZA1-ZA6) (400, 200, 100, 50, and 25 g/mL) were put into the wells in two-fold serial dilutions.
4- For each concentration and the controls, triplicates were employed (cells treated with serum-free medium). Plates were incubated for the chosen exposure time at 37 °C and 5% CO_2 for 24 hours.
5- To each well, 10 mL of the MTT solution was added. Plates were then incubated for a further 4 hours at 37 °C and 5% CO_2.
6- The media were carefully removed after incubation, and 100 ML of solubilization solution was added to each well for 5 minutes.
7- By measuring at a 575 nm wavelength with an ELISA reader. To determine the concentration of chemicals needed to result in a 50% loss in viable cells for each cell line, the optical density data were statistically analyzed.

RESULT AND DISCUSSION

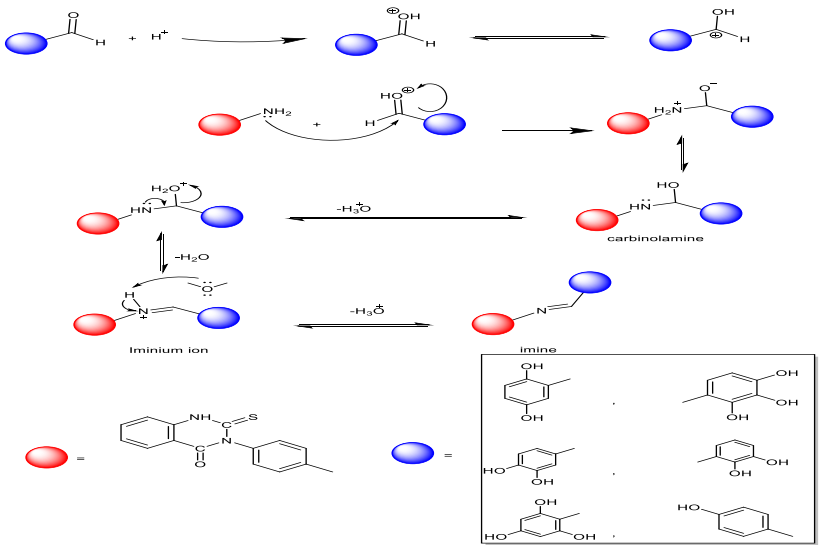
Synthetic Route Strategy

The synthesis of the target compounds (ZA1-ZA6) through their intermediates (ZA) was achieved successfully. In the current work, we predict the synthesis of new 4-aminophenyl quinazolinone derivatives. One of the most frequent chemical processes for producing imine compounds is the reaction of the (ZA) intermediate with various substituted aromatic aldehydes (R1- R6) (Schiff bases or imine). The compound (ZA) was synthesized by reacting benzene-1,4-diamine with 2-aminobenzoic acid, resulting in salt, and then removing water and synthesizing amide under heating; the second step reaction occurs between primary amine from step one and activation by base lead amine attack the carbon atom of carbon disulfide compound. Then, amide's spontaneous intermolecular nucleophilic attack on C=S results in cyclisation and the formation of the dihydroquinazoline ring.. The H_2S is then lost, yielding 3-(4-aminophenyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one(ZA),the mechanisim of reaction for synthesis of compound (ZA) shown in scheme (2). Schiff bases are formed in a reversible manner that is dependent on acids (glacial acetic acid, which catalyzes the process that began with primary amine addition and was triggered by the nucleophilic attack of the aromatic aldehyde's carbonyl (C=O) group, which produced carbinolamine by the proton's migration from nitrogen to oxygen). By oxygen protonating carbinolamine with acidic catalysis, the hydroxyl group is made a good group to depart, and when hydrogen hydroxide is released, an iminium ion is created. Finally, the final

product was created by recovering the proton loss from nitrogen and regeneration of an acid catalyst as described in scheme (3).



Scheme (2): Mechanism of synthesis (ZA) compound [23,24]



Scheme (3): Schiff base Mechanism of reaction [25]

The FT-IR spectrum of compound **(ZA)** in figure (1-1) showed appearance of the broad band of NH stretching of primary amine at 3423.23-3340.71 cm^{-1} .The ^{1}H -NMR spectra of compound **ZA** showed the appearance of (s, NH_2) at 5.40 (δ, ppm), as shown in figure (1-8). ^{13}C-NMR spectra show no band for C=N as shown in figure (1-15). The FT-IR spectrum of compound **(ZA1)** showed the appearance of 1620.21 cm^{-1} for (C=N stretching of imine) as shown in figure (1-2) .The ^{1}H-NMR spectra of compound **(ZA1)** showed the appearance of (s, CH of CH=N) at 8.94 (δ, ppm) as shown in figure (1-9). ^{13}C-NMR: the appearance of(C=N) 160.42(δ, ppm), as shown in figure (1-16). The FT-IR spectrum of compound **(ZA2)** in figure(1-3) showed the appearance 1620 cm^{-1} (C=N stretching of imine) .The ^{1}H -NMR spectra of compound **(ZA2)**showed the appearance of (s, CH of CH=N) at 8.94 (δ, ppm) as in figure (1-10).^{13}C-NMR: unlike compound (**ZA**) the appearance of (C=N) 153.76(δ, ppm), as shown in figure (1-17). The FT-IR spectrum of compound **(ZA3)** showed the appearance of 1620 cm^{-1} (C=N stretching of imine) as in figure (1-4).

The[1]H-NMR spectra of compound **(ZA3)** showed the appearance of (s, CH of CH=N) at 8.45 (δ, ppm) as in figure (1-11). [13]C-NMR: unlike compound (**ZA**) the appearance of(C=N) 159.96(δ, ppm) as in figure (1-18). The FT-IR spectrum of compound **(ZA4)** showed the appearance of 1620 cm^{-1} (C=N stretching of imine) showing in figure (1-5) .The [1]H -NMR spectra of compound **(ZA4)** showed the appearance of (s, CH of CH=N) at 8.96 (δ, ppm) as in figure (1-12).[13]C-NMR: unlike compound (**ZA**) the appearance of(C=N) at 160.41(δ, ppm) as seen in figure (1-19). The FT-IR spectrum of compound **(ZA5)** in figure (1-6) showed the appearance of band of 1620.21 cm^{-1} (C=N stretching of imine). In figure (1-13) the [1]H -NMR spectra of compound **(ZA5)** showed the appearance of (s, CH of CH=N) at 8.65 (δ, ppm). [13]C-NMR: unlike compound (**ZA**) the appearance of(C=N) at 160.41(δ, ppm) as in figure (1-20). The FT-IR spectrum of compound **(ZA6)** showed the appearance of band of 1620.21 cm^{-1} C=N stretching of imine as in figure (1-7). The [1]H -NMR spectra of compound **(ZA6)** in figure (1-14) showed the appearance of (s, CH of CH=N) at 8.68 (δ, ppm). [13]C-NMR: unlike compound (**ZA**) the appearance of(C=N) at 160.25(δ, ppm) as in figure (1-21).

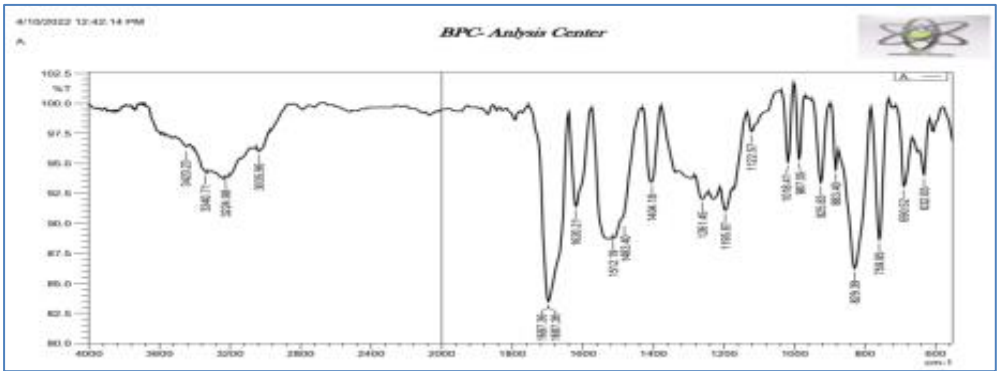


Figure (1-1): FT-IR Spectrum of compound (ZA)

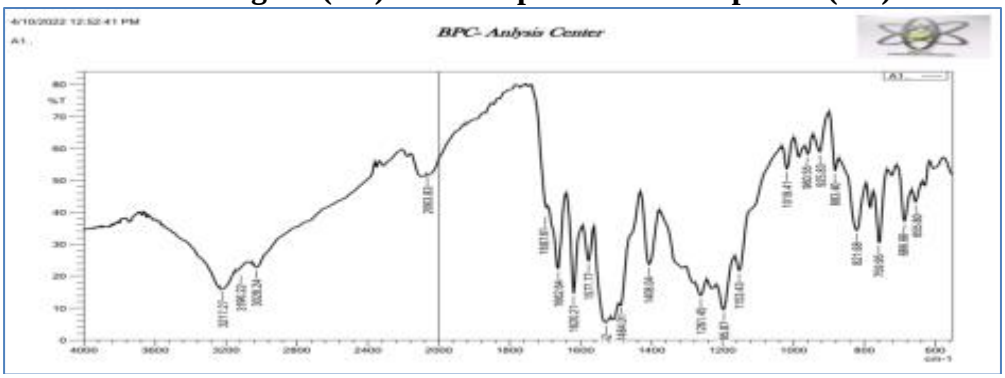


Figure (1-2): FT-IR Spectrum of compound (ZA1)

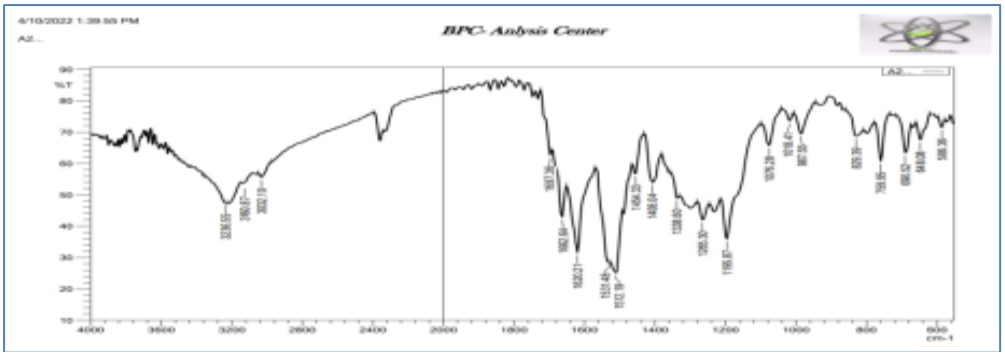


Figure (1-3): FT-IR Spectrum of compound (ZA2)

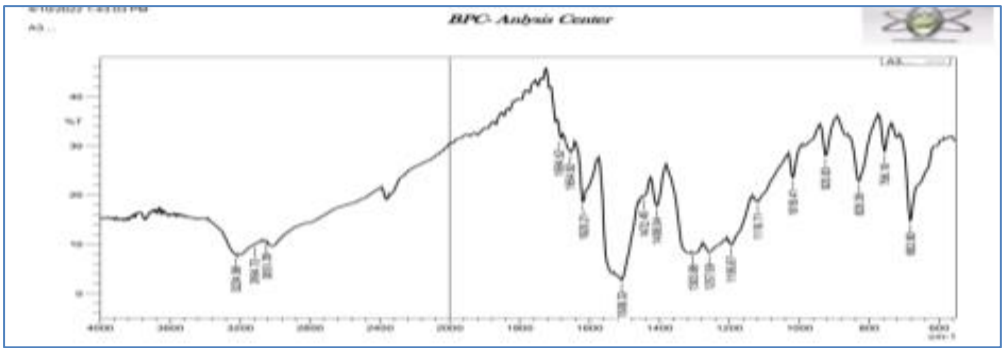


Figure (1-4): FT-IR Spectrum of compound (ZA3)

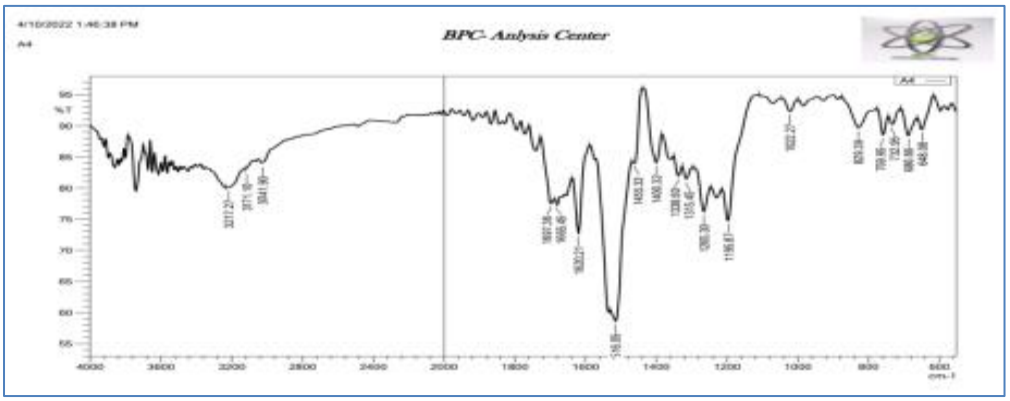


Figure (1-5): FT-IR Spectrum of compound (ZA4)

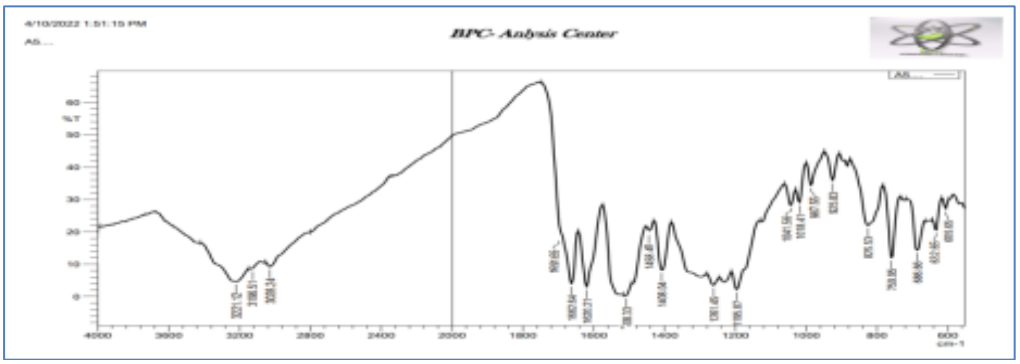


Figure (1-6): FT-IR Spectrum of compound (ZA5)

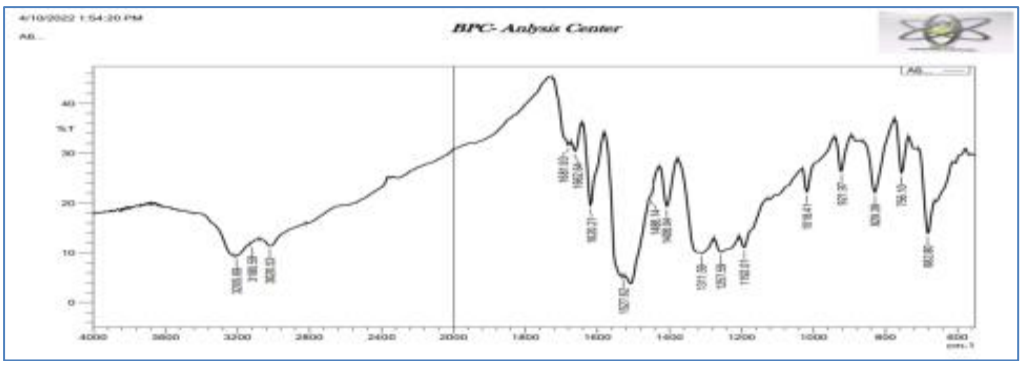


Figure (1-7): FT-IR Spectrum of compound (ZA6)

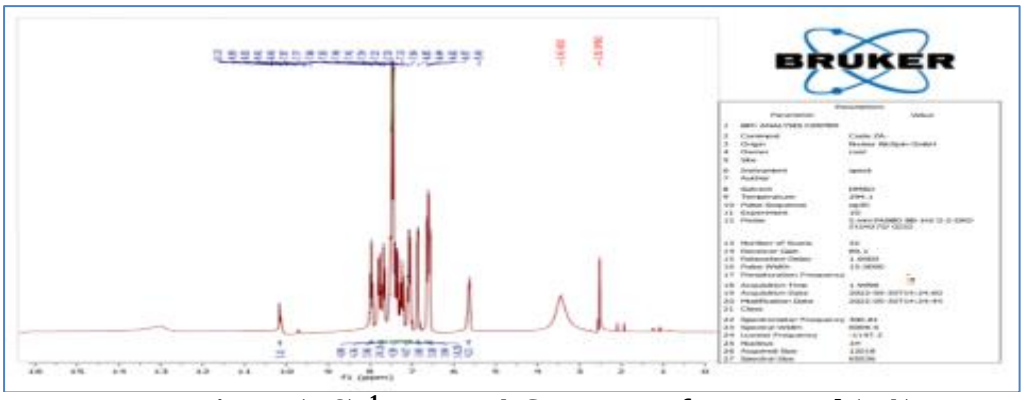


Figure (1-8):^{1}H-NMRA Spectrum of compound (ZA)

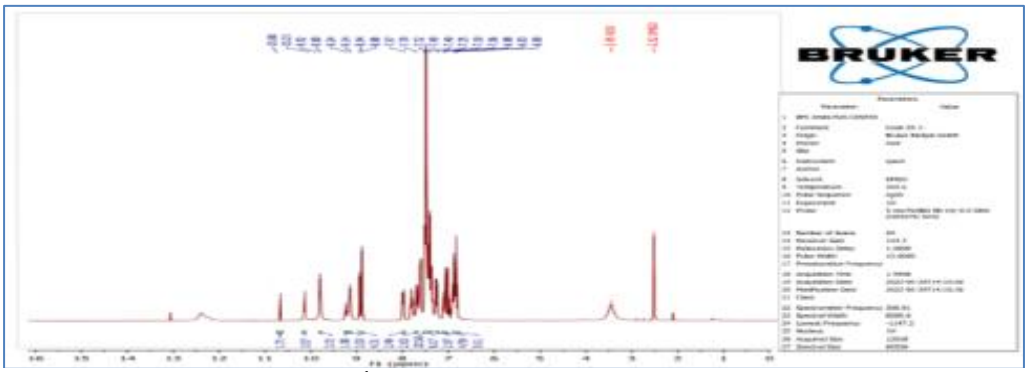


Figure (1-9):^{1}H-NMRA Spectrum of compound (ZA1)

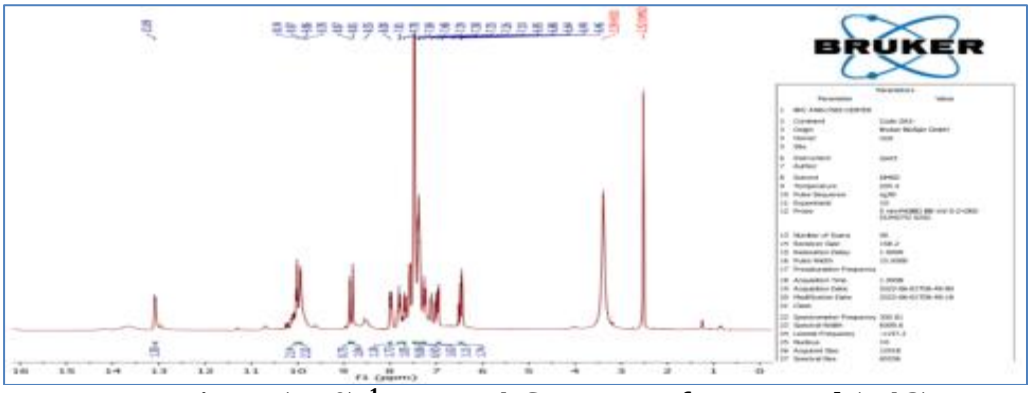


Figure (1-10):^{1}H-NMRA Spectrum of compound (ZA2)

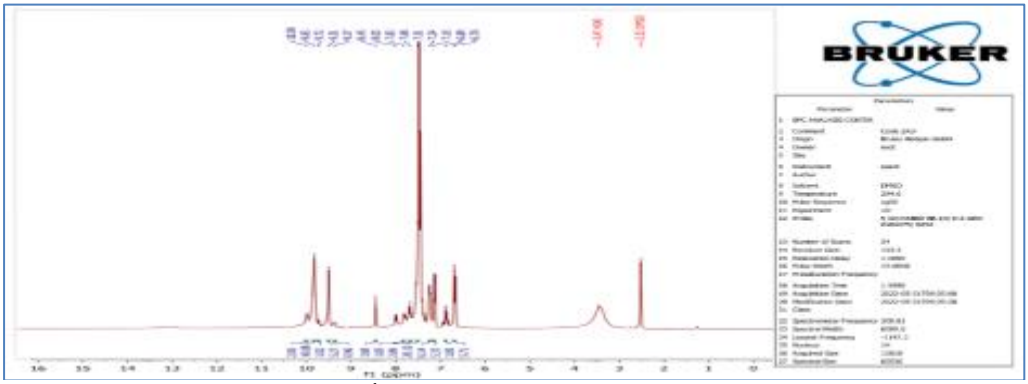


Figure (1-11):^{1}H-NMRA Spectrum of compound (ZA3)

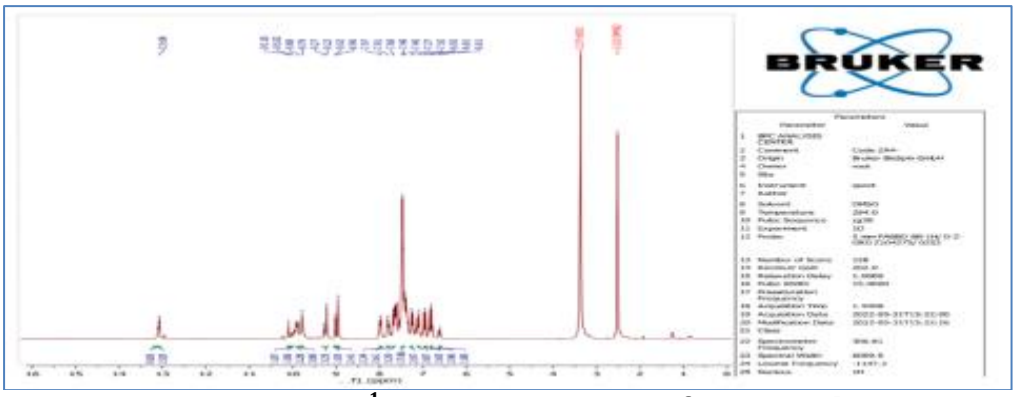


Figure (1-12):^{1}H-NMRA Spectrum of compound (ZA4)

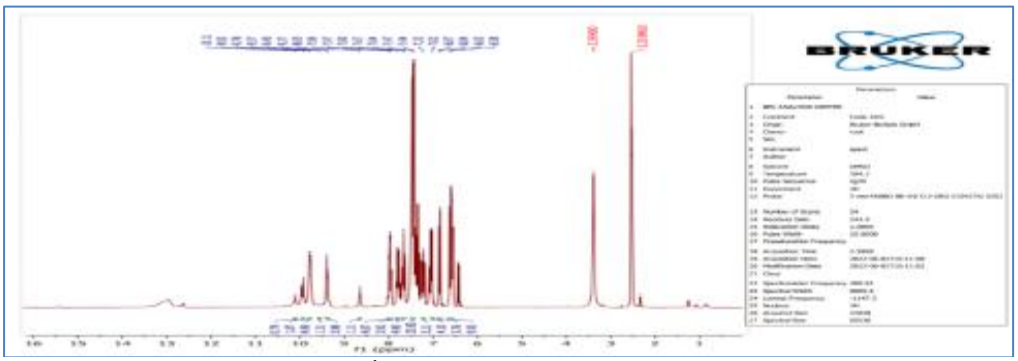


Figure (1-13):^{1}H-NMRA Spectrum of compound (ZA5)

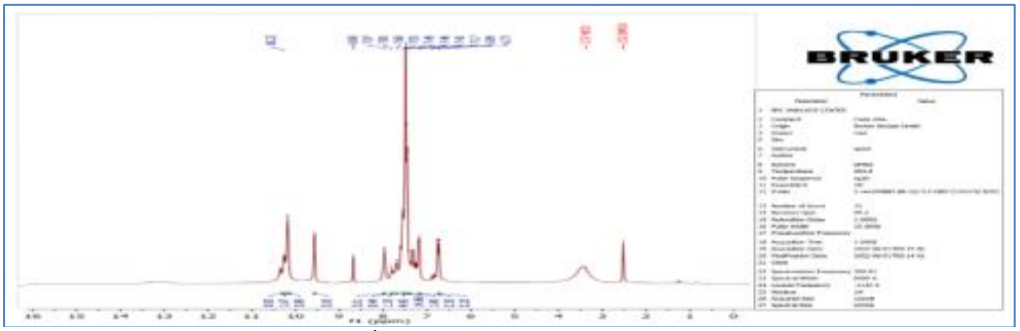


Figure (1-14):^{1}H-NMRA Spectrum of compound (ZA6)

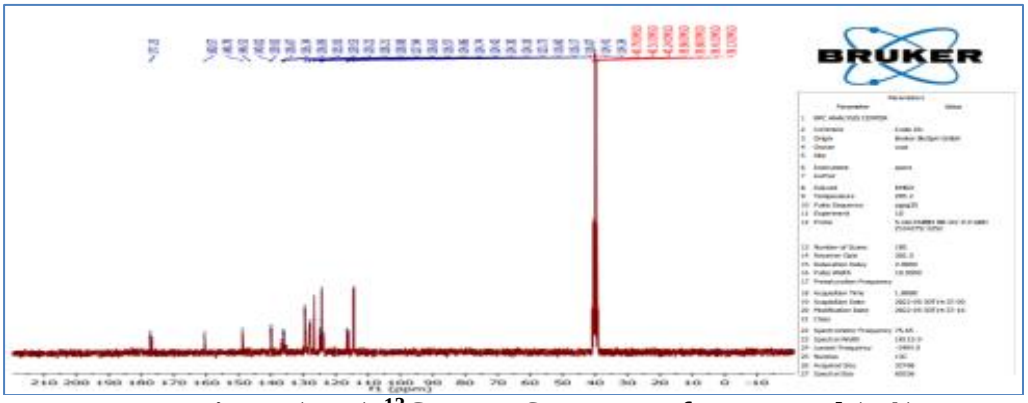


Figure (1-15):^{13}C-NMR Spectrum of compound (ZA)

Figure (1-16):^{13}C-NMR Spectrum of compound (ZA1)

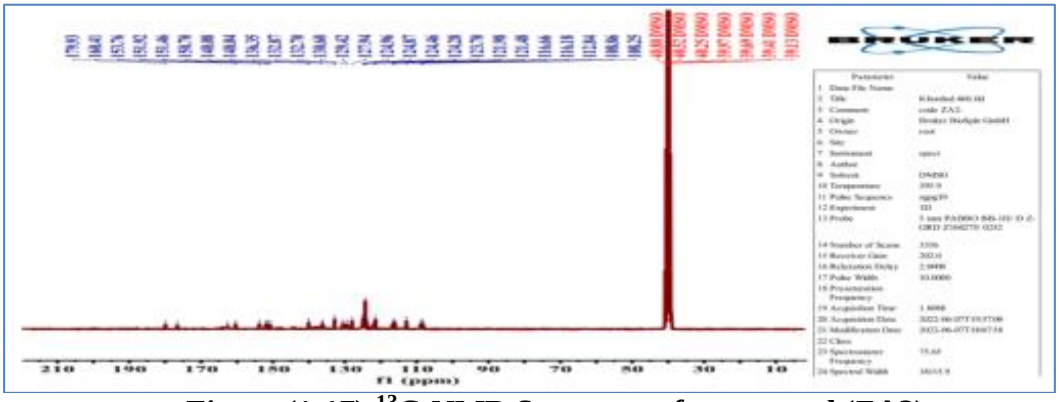


Figure (1-17):^{13}C-NMR Spectrum of compound (ZA2)

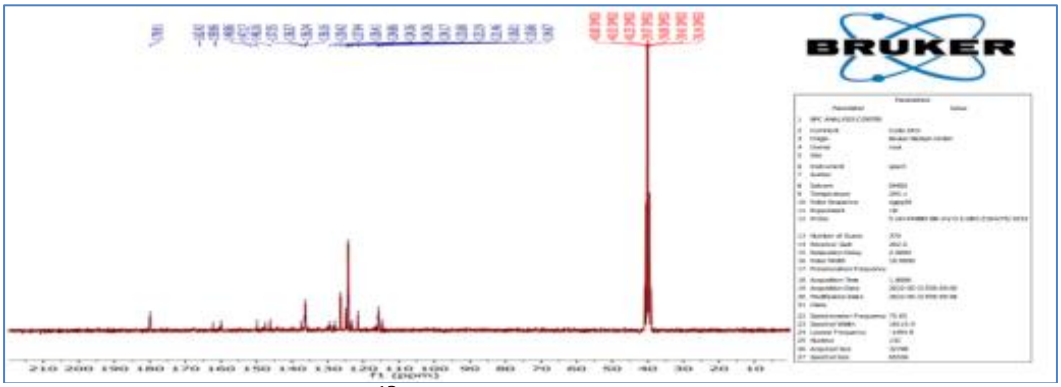


Figure (1-18):^{13}C-NMR Spectrum of compound (ZA3)

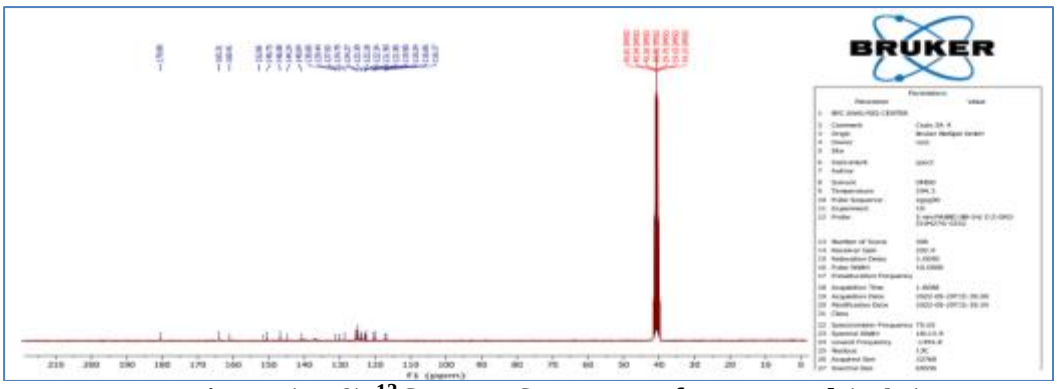


Figure (1-19):^{13}C-NMR Spectrum of compound (ZA4)

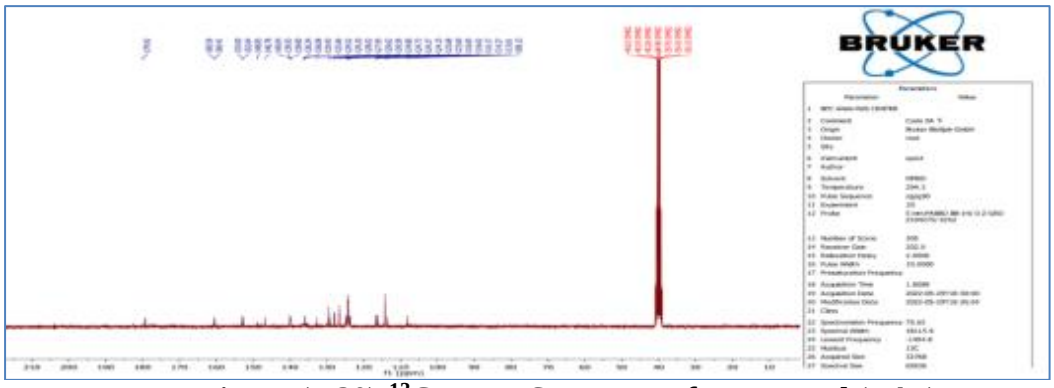


Figure (1-20):^{13}C-NMR Spectrum of compound (ZA5)

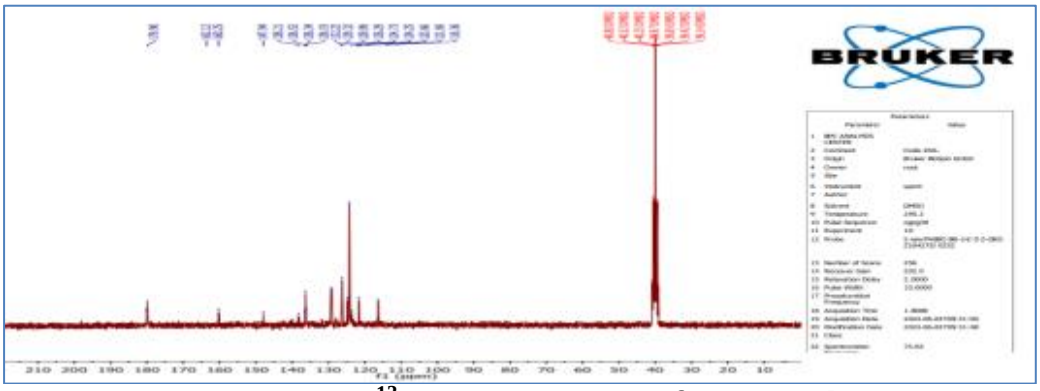
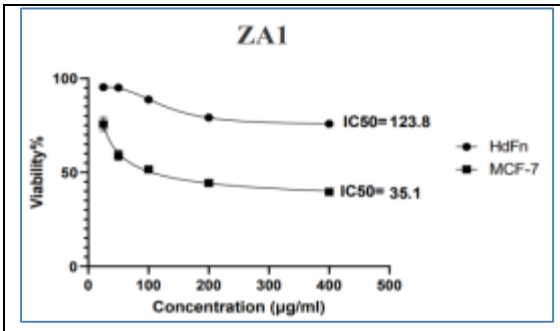


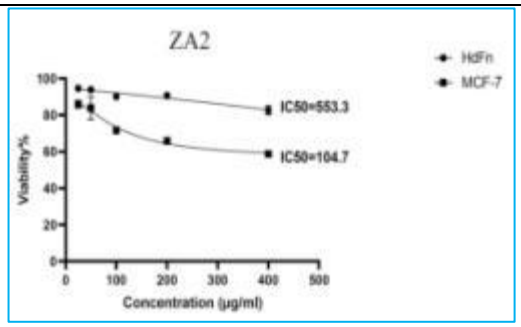
Figure (1-21):^{13}C-NMR Spectrum of compound (ZA6)

-Cell-prolifration-assay

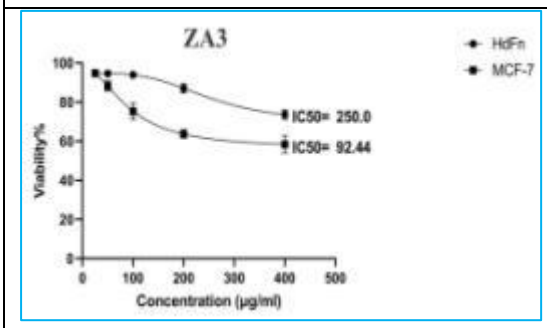
The IC_{50} values of synthesized compounds (ZA1-6) were evaluated using the MTT test, which was carried out in 96-well flat plates with a range of concentrations (400–25 µg\mL) of compounds. The IC_{50} values of examined compounds were compared to that of anticancer medication Tamoxifen as a reference. Prism Pad 8.1 was used to produce dose-response curves for compounds in MCF-7 cells using nonlinear regression as shown in figure (-). It was observed that compounds (ZA1, ZA2, ZA3) had outstanding activity and showed promise against cancer on this particular type of line to treat breast cancer with IC_{50} (13.513, 79.846, 43) µg\mL at 24 hours as in figure (1-22), (5.46, 77.41, 39.22) µg/mL at 48hours as showing in figure (1-23) and (0.07964, 57.43, 0.002717) µg/mL at 72 hours respectively as in figure (1-24), based on the results of the anti-proliferation assessment on MCF-7 cell line. Tamoxifen serves as the standard reference and has an IC_{50} of (152,143,133 µg\mL) at 24,48.72 hours respectively .



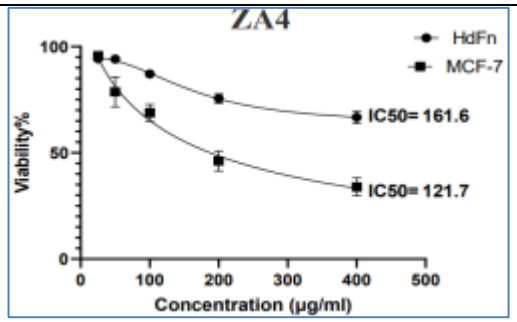
Dose response curve(ZA1) at 24hr.



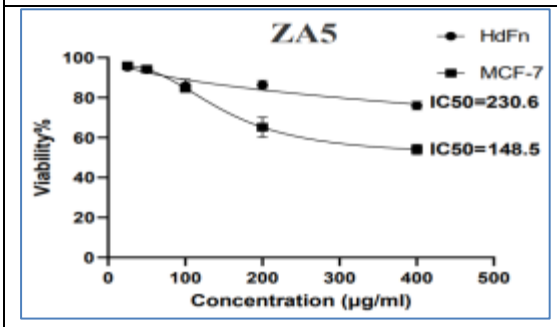
Dose response curve(ZA2) at 24hr



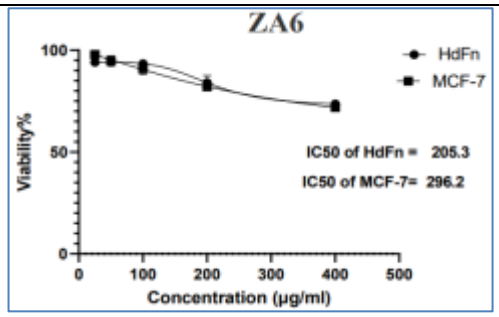
Dose response curve(ZA3) at 24hr.



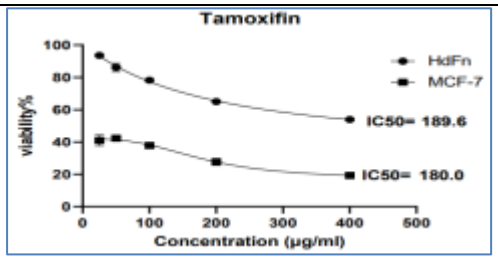
Dose response curve(ZA4) at 24hr.



Dose response curve(ZA5) at 24hr

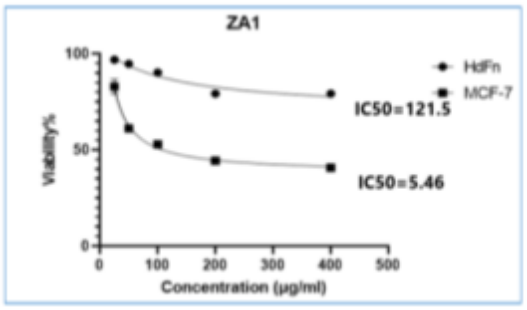


Dose response curve(ZA6) at 24hr

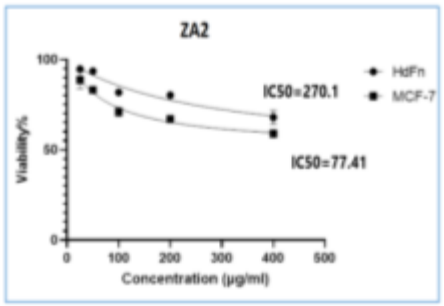


Dose response curve(tamoxifen) at 24hr

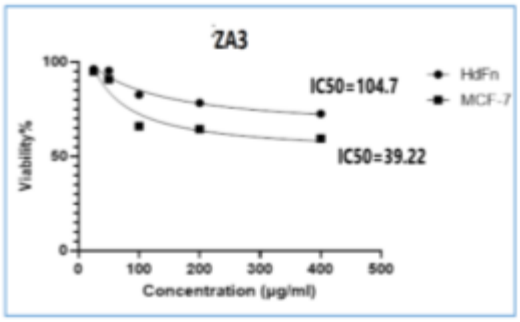
Figure (1-22): dose response curves of compounds (ZA1-ZA6) and tamoxifen At 24 hours.



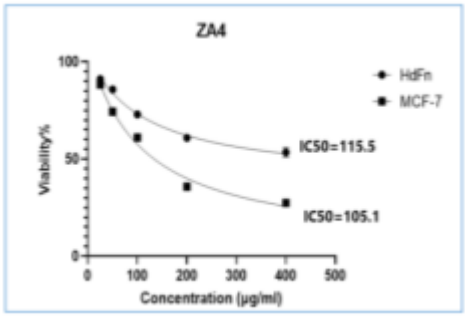
Dose response curve(ZA1) at 48hr



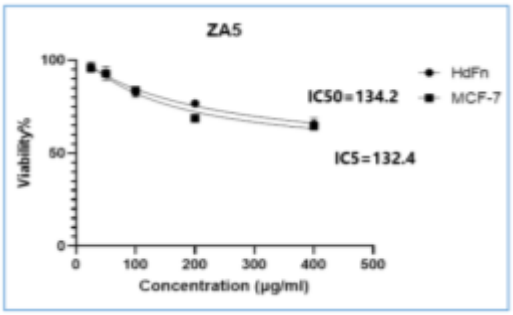
Dose response curve(ZA2) at 48hr



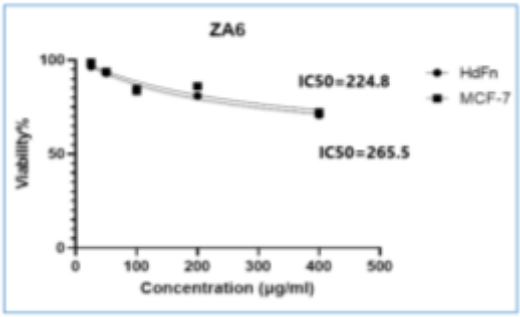
Dose response curve(ZA3) at 48hr



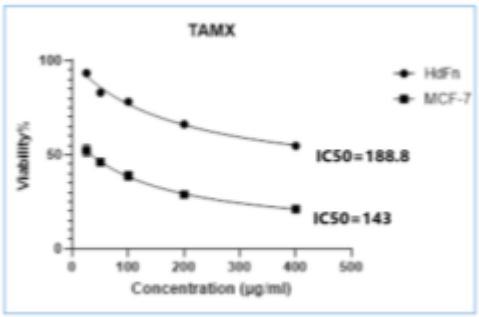
Dose response curve(ZA4) at 48hr



Dose response curve(ZA5) at 48hr

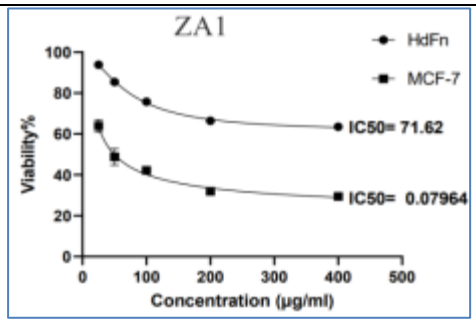


Dose response curve(ZA6) at 48hr

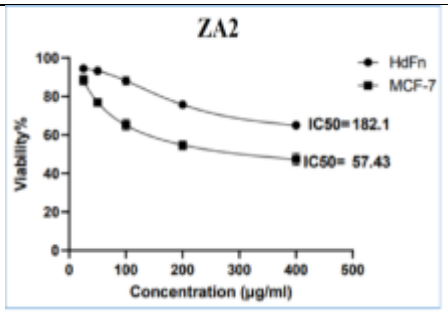


Dose response curve(tamoxifen) at 48hr

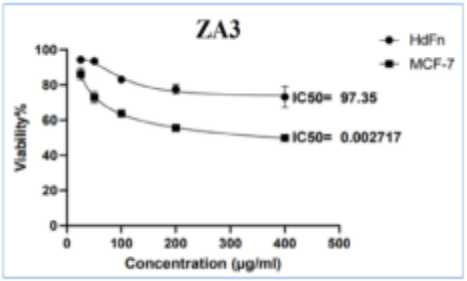
Figure (1-23): dose response curves of compounds (ZA1-ZA6) and tamoxifen At 48 hours.



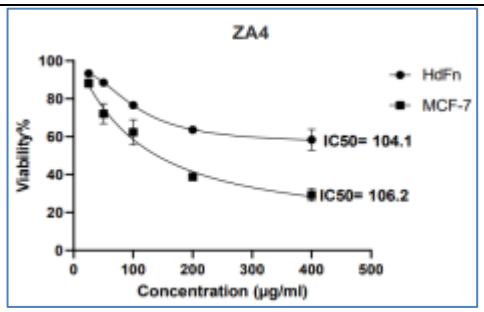
Dose response curve(ZA1) at 72hr



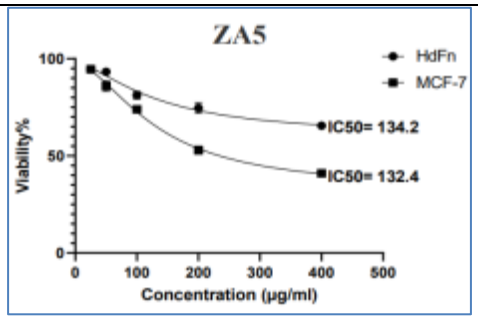
Dose response curve(ZA2) at 72hr



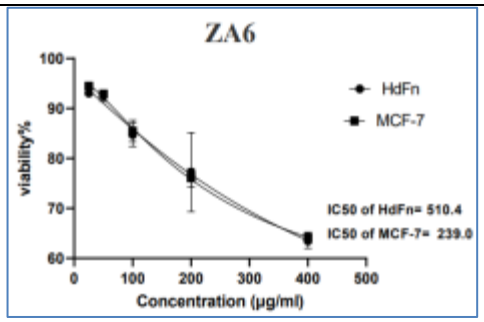
Dose response curve(ZA3) at 72hr



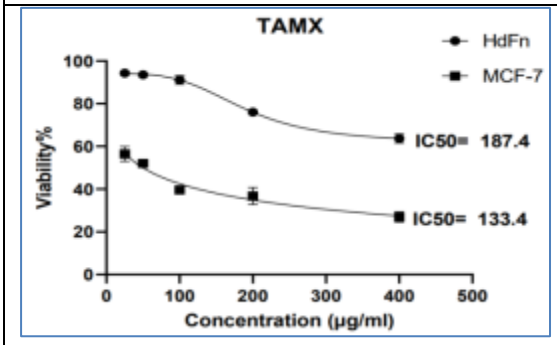
Dose response curve(ZA4) at 72hr



Dose response curve(ZA5) at 72hr



Dose response curve(ZA6) at 72hr



Dose response curve(tamoxifen) at 72hr

Figure (1-24): dose response curves of compounds (ZA1-ZA6) and tamoxifen At 72 hours.

CONCLUTION

Quinazolinone derivatives connected to the SHICFF base were successfully synthesized using FT-IR, 1H NMR, and 13C-NMR spectra. Particularly the compounds ZA1, ZA2, and ZA3, which were tested against the breast cancer MCF-7, the anti-proliferation assessment of the synthesized compounds showed excellent promising anti-cancer activity, and the outstanding final compound activity of compound ZA1 on cell line and demonstrated amazing results when compared to the standard treatment tamoxifen in the 24, 48, and 72 hours.

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