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Original Research Article

SYNTHESIS AND ASSESSMENT THE IN-VITRO ANTIOXIDANT ACTIVITY OF 2,3-DISUBTITUTED QUINAZOLIN-4(3H)-ONE DERIVATIVES

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Abstract

Quinazolinones have various biological activities such as anticancer, antibacterial, antidiabetic, anticonvulsant, antihistaminic, antiinflammatory, antifungal, anthelmintics and antiviral activities. In this research some of compounds 2,3-disubtituted quinazolin-4(3*H*)-one derivatives had been synthesized under microwave irradiation. The compounds were obtained from reaction some of benzoxazine derivatives with hydrazine hydrate using microwave irradiation and the result of reaction we obtained 82-96%. The products had been tested by IR, ^{1}H-NMR, ^{13}C-NMR and Mass Spectroscopy analysis. The using microwave irradiation was more effective and efficient to produce compounds 2,3-disubtituted quinazolin-4(3*H*)-one derivative because the reaction runs perfectly without any minor products such as compounds *N*-(2-(hydrazinecarbonyl)phenyl)benzamide derivatives.

Keywords: 2,3-disubtituted quinazolin-4(3*H*)-one derivatives; *N*-(2-(hydrazinecarbonyl) phenyl) benzamide derivatives, microwave irradiation.

INTRODUCTION

A great interest has been given to naturally occurring antioxidants, which may play important roles in inhibiting both free radicals and oxidative chain-reactions within tissues and membranes. Therefore, screening plant materials on the basis of their antioxidant potency seems to be of central importance in order to identify extracts or fractions possessing the ability either in scavenging both free radicals and chain reactions initiation or in binding with catalysts of the oxidative reactions, such as some metal. From the viewpoint of their high antioxidant potency, the consumption at high scale of many plants have been recommended, therefore, the evaluation of antioxidant activities of extracts and fractions is considered as an important step prior to the isolation of antioxidant phytochemicals they contain[1].

GLOBOCAN, International Agency for Research on Cancer (IARC) was reported 14.1 million has been indicated as a new cancer case and 8.2 million have died againts cancer. Breast cancer was indicated as higher new case than another cancer disease[4] There are many chemotherapeutic strategies for the anticancer treatment have been proposed and tested in some cases. The main procedures of cancer treatment are surgery, irradiation, and chemotherapy. Although the chemotherapeutic management has been conducted as major advances for patients, the continuous researching for new anticancer agent remains important[2,8,9]

In the course of identifying chemical agents are very importans for designing novel agents. One of agents were knowns as lead compound of anticancer drug is quinazolinone[8,9]. Quinazolinone is a fused bicyclic heterocyclic framework was known as benzo-1,3-diazanaphthalene. Numerous compounds of quinazolines have been reported have biological and pharmacological activity such as antimicrobial, antitumor, antimitotic, anticancer and others[6]

In previous of the research, the novel quinazolinones was synthesized by Nolvi and Patel in 2013. They have been using refluks as conventional method for this reaction. This reaction was produced 2 isomers includes ring opened and ring closed of quinazolinone[8] The ring closed as target of synthesis and to obtained by fusing benzoxazinone at high temperature resulting in the synthesis. The method of heating, reactants are slowly activated by conventional external heat source. Heat is driven into the substance, passing first through the walls of the vessel in order to reach the solvent and the reactants. This is a slow and inefficient method for transferring energy into the reacting system. In this research is using microwave-assisted organic chemistry. Microwave have been used to speed up chemical reactions in the laboratories to investigate the mechanism of microwave dielectric heating and to identify the advantages of the technique for chemical synthesis[5,9,10] By using microwave irradiation at hight temperature as methodology of 2,3-disubtituted quinazolin-4(3*H*)-one derivatives are simple, hight purity, improved yields, simplified and improved synthetic procedure and higher energy efficiency.

MATERIALS AND METHODS

All chemicals and solvents were purchased from Sigma Aldrich and Merck. Reactions were monitored with TLC using pre-coated aluminum sheets with GF254 silica gel. Eluen for TLC using n-hexane: ethyl acetate (1:1) and the spots were visualized in UV chamber. Melting points of the synthesized compounds were measured with an Electrothermal melting point apparatus. Infrared spectra were obtained using a Perkin Elmer Spectrum One Spectrophotometer using KBr disks. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on JEOL JNM-ECS 400 (1H-NMR: 400 MHz, 13C-NMR: 100 MHz) instrument from Institute of Tropical Disease Airlangga University, Indonesia. We used DMSO-d6 as solvent for ^{1}H- NMR and ^{13}C-NMR analysis. MS spectra were measured by a JEOL JMS 600 spectrometer by using the ESI methods.

General synthesis of 2-phenyl-benzo [1,3] oxazine-4-one derivatives (3a-c)

A mixture of 2-phenyl-benzo [1,3] oxazine-4-one derivatives (2 mmol) and hydrazine hydrate (2 mmol) was dissolved in 2 mL of DMSO. The mixture was heated in microwave irradiation at 600 W for 1 minute. The mixture was cooled and aquadest (20 mL) was added to the mixture. The separated solid was collected by filtration, washed with cooled ethanol, dried, and crystallized by ethanol 96 %.

General synthesis of 3-amino-2-phenyl quinazolin-4(3*H*)-one derivatives (4d-f)

A mixture of 2-phenyl-benzo [1,3] oxazine-4-one derivatives (2 mmol) and hydrazine hydrate (2 mmol) was dissolved in 2 mL of DMSO. The mixture was heated in microwave irradiation at 600 W for 7 minutes. The mixture was cooled and aquadest (20 mL) was added to the mixture. The separated solid was collected by filtration, washed with cooled ethanol, dried, and crystallized by ethanol 96 %.

Determination of in-vitro scavenging activities[1]:

DPPH radical scavenging activity:

The free radical scavenging activity of the extract and fractions was evaluated using the stable DPPH free radical. One mL of 0.1 mM DPPH solution in methanol was added to 1.0 mL of standard and derivatives solution at different

concentrations. The mixture was incubated for 20 min and the absorbance recorded at 517 nm. Ascorbic acid was used as positive control.

DPPH radical scavenging activity was calculated using the formula:

Percent scavenging = ((Ao – At)/Ao) × 100; where Ao =Absorbance of control (without extract) and At = Absorbance of sample. All determinations were carried out in triplicate.

ABTS radical scavenging activity:

ABTS free radical was generated by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate. The mixture was allowed to stand for 15 h in dark at room temperature. ABTS solution was diluted with methanol to obtain the absorbance of 0.7 ± 0.2 units at 750 nm. The standard/derivatives solutions were prepared at different concentrations in methanol and 20 µL of test solutions were added to 180 µL of ABTS free radical solution. The absorbance was measured after 20 minutes incubation at 750 nm. Ascorbic acid was used as positive control. **The ABTS free radical scavenging activity was calculated using the formula:**

Percent scavenging = ((Ao – At)/Ao)× 100; where Ao = Absorbance of control (without extract) and At = Absorbance of sample. All the tests were performed in triplicate.

Total antioxidant capacity:

The total antioxidant capacities of the derivatives were determined using phosphomolybdenum method. Briefly, 0.1 mL of standard/derivatives solution was mixed with 0.3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min. The mixture was cooled down to room temperature and absorbance recorded at 695 nm. The blank solution contained all the reagents except the test sample. Ascorbic acid was used to plot the standard curve. The results were expressed as ascorbic acid equivalents. All the tests were performed in triplicate.

o-Phenanthroline assay/Iron chelating activity:

The 1, l0-Phenanthroline-iron (III) reagent was prepared by mixing 0.198 g of l, l0-phenanthroline monohydrate, 2 mL of 1 M hydrochloric acid and 0.16 g of ferric ammonium sulphate in 100 mL water. Briefly, 0.2 mL standard/extracts were mixed with 0.2 mL 1, l0- phenanthroline-iron (III) reagent, 0.6 mL methanol and 4 mL water. The solutions were incubated at 50°C for 30 min and absorbance read at 510 nm. Ascorbic acid was used as positive control. A higher absorbance indicated higher iron chelating activity

Percentage scavenging was calculated by using the following formula:

Percent scavenging = ((At – Ao)/At) × 100; where Ao = Absorbance of control (without extract) and At = Absorbance of sample. All the tests were performed in triplicate.

RESULTS AND DISCUSSION

Reaction hydrazine hydrate with some of 2-phenyl-benzo [1,3] oxazine-4-one derivatives such as 2-phenyl-4H-benzo[d][1,3]oxazin-4-one; 2-(3,4-dichlorophenyl)-4H-benz[1,3]oxazin-4-one; 2-(2,4-dichlorophenyl)-4H-benzo[1,3]oxazin-4-one was dissolved in 2 mL of DMSO and it was heated in microwave irradiation at 600 W for 1 minute. We evaluated reaction by TLC using n-hexane: ethyl acetate (1:1) as mobile phase, we got single spot (Rf = 0.26) under UV chamber. The mixture was cooled and aquadest (20 mL) was added to the mixture. The separated solid was collected by filtration, washed with cooled ethanol, dried, and crystallized by ethanol 96 %. We obtained compounds N-(2-(hydrazinecarbonyl)phenyl) benzamide derivatives. The mechanism of the reaction shown in figure 1.

The reaction mechanism between **(1)** and hydrazine hydrate was dissolved in

DMSO, we obtained a product **(3a-c)** or **(4d-f)** depending on the duration of the reaction time and the strength of the given wave irradiation. The lack of microwave irradiated power and relatively short reaction time leads to compounds **(3a-c)**. Their mechanism shown in figure 1. The nucleophile attack of hydrazine hydrate on C carbonyl lactone ring causes opening of lactone ring and forms amide groups to obtain N-(2-(hydrazinecarbonyl)phenyl)benzamide derivatives

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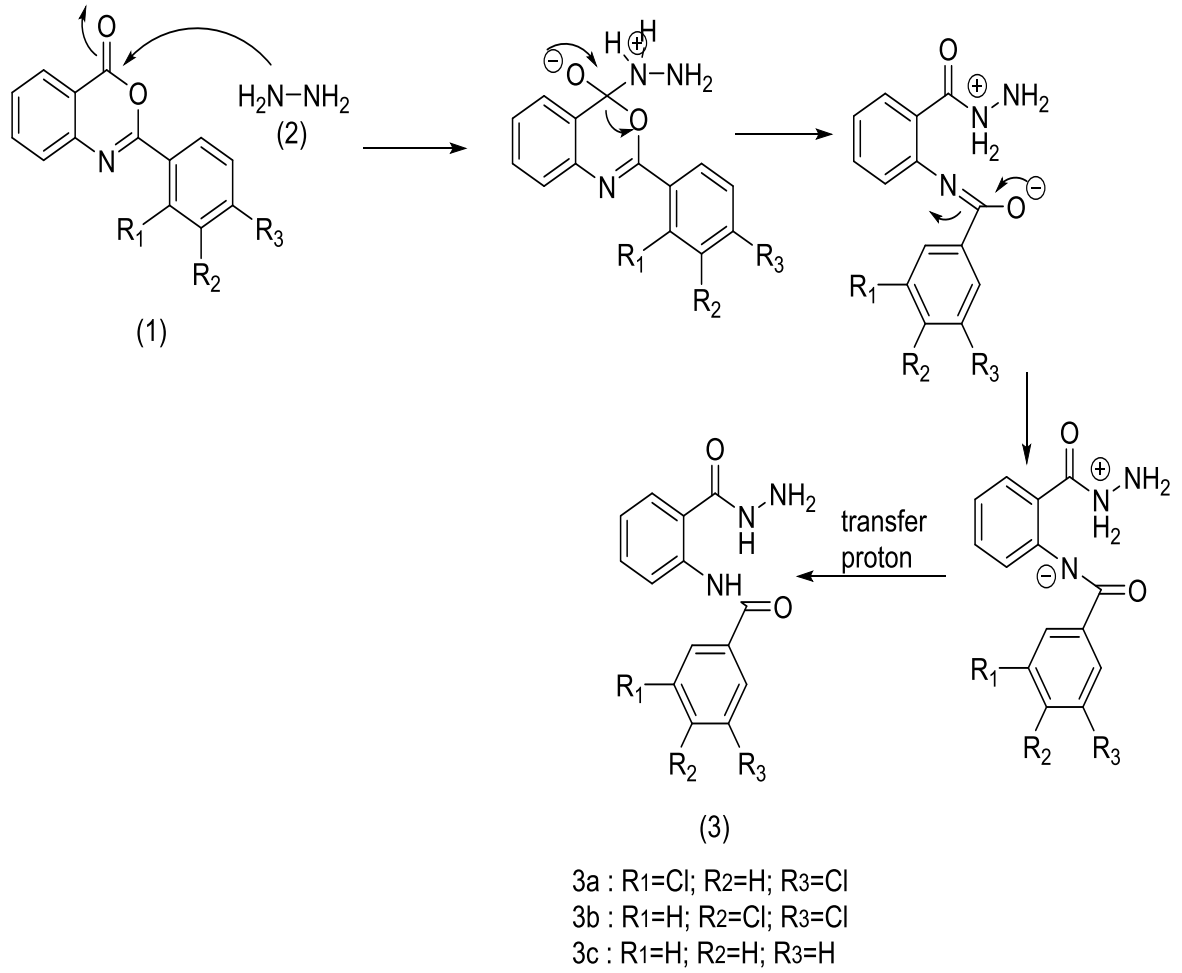

Figure 1 Hydrazine hydrate attack on carbonyl on benzoxazine ring

Detailed physicochemical and spectral data of the obtained compounds N-(2-(hydrazinecarbonyl)phenyl)benzamide derivatives are as follows.

Compound 2,4-dichloro-*N*-(2-(hydrazinecarbonyl)phenyl)benzamide (3a)

Obtained in white powders; yield 96%; mp : 119-120 ^{o}C. FT-IR (KBr) cm^{-1}: 3263 (N-H); 3052 and 675 (=C-H aromatic); 1650 (C=O amide); 1628 (C-N); 1596 and 1465 (C=C aromatic); 1311 (C-N) and 756 (C-Cl). ^{1}H-NMR (DMSO-d6, δ, ppm) :δ11.88 (1H, s); 10.07 (1H, s); δ 8.46 (1H,d, J=8.4 Hz); δ 8.75 (1H, d; J=8.4 Hz); δ 7.71-7.67 (2H, m);δ 7.56-7.50 (2H, m); δ 7.17 (1H, t, J=7.6);); δ 4.54 (2H, s). ^{13}C-NMR (DMSO-d6, δ, ppm) : δ 169.4; δ 162.6; δ 150.8; δ 139.3; δ 135.7; δ 135.4; δ 132.6; δ 132.4; δ 131.8; δ 129.7; δ 128.2; δ 127.5; δ 123.9; δ 121.1. ESI/MS m/z values (Rel. abundance): $[M+Na]^{+}$= 346 (100%); $[M^{+2}+Na]^{+}$=348 (65%); $[M^{+4}+Na]^{+}$= 350 (10%).

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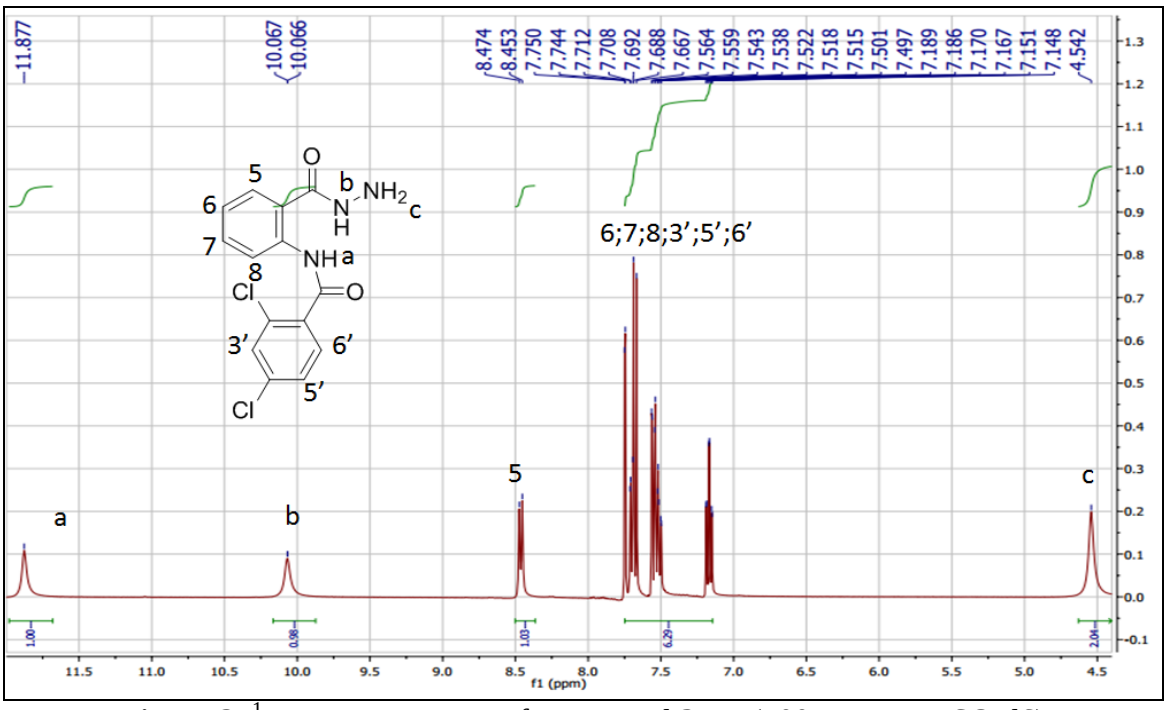

Figure 2 ^{1}H-NMR spectrum of compound 3a in (400 MHz, DMSO-d6)

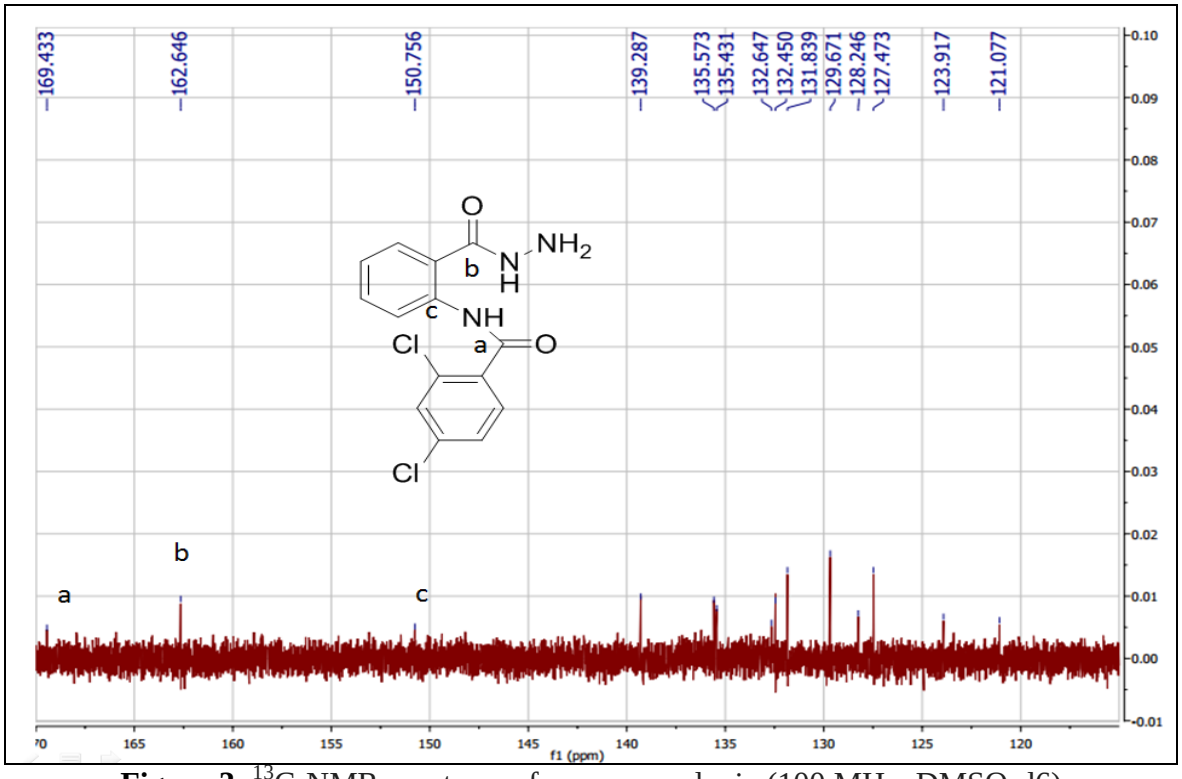

Figure 3: ^{13}C-NMR spectrum of a compound a in (100 MHz, DMSO-d6)

Compound 3,4-dichloro-*N*-(2-(hydraz inecarbonyl)phenyl)benzamide (3b)

Obtained in white powders; yield 90%; mp : 116-118 ^{o}C. FT-IR (KBr) cm^{-1}: 3384 (N-H); 3061 and 675 (=C-H aromatic); 1628 (C-N); 1666 (C=O amide); 1592 and 1450 (C=C aromatic); 1342 (C-N) and 748 (C-Cl). ^{1}H-NMR (DMSO-d6, δ, ppm) :

δ11.88 (1H, s);δ 10.07 (1H, s); δ 8.52 (1H, dd, J=8.4 Hz; 1.2 Hz); δ 8.07 (1H, t); δ 7.85 (2H, d, J=1.2 Hz,); δ 7.75 (1H, dd, J=7.6; J=1.2 Hz,); δ 7.54-7.50 (1H, m); δ 7.16 (1H, dt, J=7.6; J=1.2 Hz); δ 4.56 (2H, s). ^{13}C-NMR (DMSO-d6, δ, ppm): δ 169.4; δ 162.6; δ 150.8; δ 139.3; δ 135.7; δ 135.4; δ 132.6; δ 132.4; δ 131.8; δ 129.7; δ 128.2; δ 127.5; δ 123.9; δ 121.1. ESI/MS m/z values (Rel. abundance): $[M+Na]^+$= 346 (100%); $[M^{+2}+Na]$+=348 (65%); $[M^{+4}+Na]$= 350 (10%).

Compound *N*-(2-(hydrazinecarbonyl)-phenyl)benzamide (3c)

Obtained in white powders; yield 82%; mp : 115-117 ^{o}C. FT-IR (KBr) cm^{-1}: 3445 (N-H); 3061 and 672 (=C-H aromatic); 1660 (C=O amide); 1628 (C-N); 1592 and 1450 (C=C aromatic) and 1340 (C-N). ^{1}H-NMR (DMSO-d6, δ, ppm) : δ12.40 (1H, s); δ 10.31 (1H, s); δ 8.56 (1H, d, J=8.2 Hz); δ 8.19 (1H, s); δ 7.92 (2H, d, J=7.2 Hz,); δ 7.70 (1H, d, J=8 Hz); δ 7.58 (1H, d, J=7.2 Hz); δ 7.53(2H, t, J=7.6 Hz); δ 7.51-7.48 (1H, m); δ 4.57 (2H, s). ^{13}C-NMR (DMSO-d6, δ, ppm): δ 168.0; δ 165.0; δ 139.5; δ 134.9; δ 132.3; δ 129.5 (2C); δ 128.2; δ 127.4 (2C); δ 123.6; δ 120.8; δ 119.8. ESI/MS m/z values (Rel. abundance): $[M^{+}+Na]^{+}$= 278 (100%); $[M^{+2}+Na]^{+}$=280 (65%); $[M^{+4}+Na]^{+}$= 392 (10%).Reaction on hydrazine hydrate with some of 2-phenyl-benzo [1,3] oxazine-4-one derivatives was dissolved in 2 mL of DMSO and it was heated in microwave irradiation at 600 W for 7 minutes. We evaluated reaction by TLC using n-hexane: ethyl acetate (1:1) as mobile phase, we got single spot (Rf = 0.69) under UV chamber. The mixture was cooled and aquadest (20 mL) was added to the mixture. The separated solid was collected by filtration, washed with cooled ethanol, dried, and crystallized by ethanol 96%. We obtained compound 3-amino-2-phenyl quinazolin-4(3*H*)-one derivatives. The mechanism of the reaction if it was heated in microwave irradiation at 600 W for 7 minutes shown in figure 4.

Excessive microwave irradiation and relatively long reaction time resulted in intramolecular attack -NH at C = O amide followed by H_2O release. The nucleophile -NH attack causes the cyclicization of the quinazolinone ring and the result of compounds 3-amino-2-phenyl quinazolin-4(3*H*)-one derivatives. Their mechanism shown in figure 4.

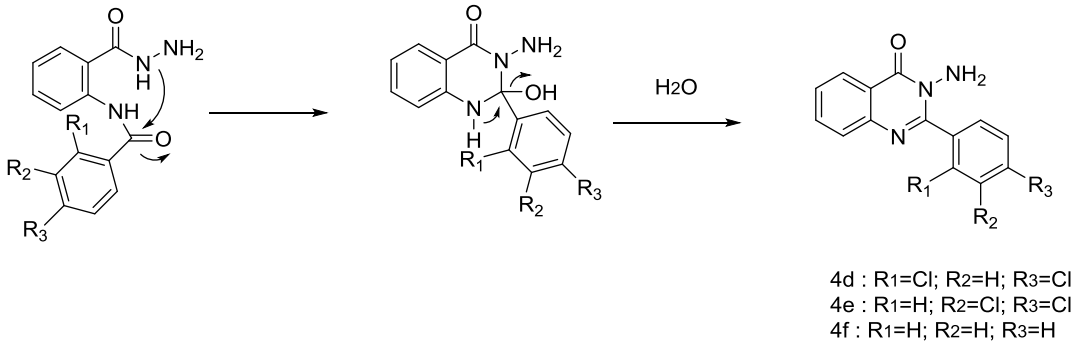

Figure 4 The nucleophile -NH attack causes the cyclicization of the quinazolinone ring

Detailed physicochemical and spectral data of the obtained compounds N-(2-(hydrazinecarbonyl)phenyl)benzamid -e derivatives are as follows.

Compound 3-amino-2-(2,4-dichloro-phenyl)quinazolin-4(3H)-one (4d)

Obtained in white powders; yield 92%; mp : 174-175 ^{o}C FT-IR (KBr) cm^{-1}: 3128 (N-H primer); 3025 dan 691 (=C-H aromatic); 1685 (C=O lactam); 1628 (C=N); 1622 and 1473 (C=C aromatic); 1324 (C-N); 1034 (C-O-C) and 775 (C-Cl). ^{1}H-NMR (DMSO, δ, ppm) : δ 8.19-8.17 (1H, dd, J=8.0 Hz; 0.4 Hz); δ 7.86-7.81 (1H, m); δ 7.73 (1H, dd, J=2.0 Hz; 0.4 Hz); δ 7.86 (1H, dd, J=8.0 Hz; 1.2 Hz); δ 7.59-7.51 (3H, m); δ 5.52 (2H, s). ^{13}C-NMR (DMSO, δ, ppm) : δ 161.5; δ 155.4; δ 147.1; δ 135.1; δ 134.9; δ 134.4; δ 133.2; δ 131.9; δ 128.9; δ 128.0; δ 127.9; δ 127.7; δ 126.6; δ 121.2. MS m/z values (Rel.abundance):[M^{+}+Na]$^{+}$=305(100%):[M^{+2} +Na]$^{+}$==307(65%); [M^{+4}+Na]$^{+}$= 309 (10%).

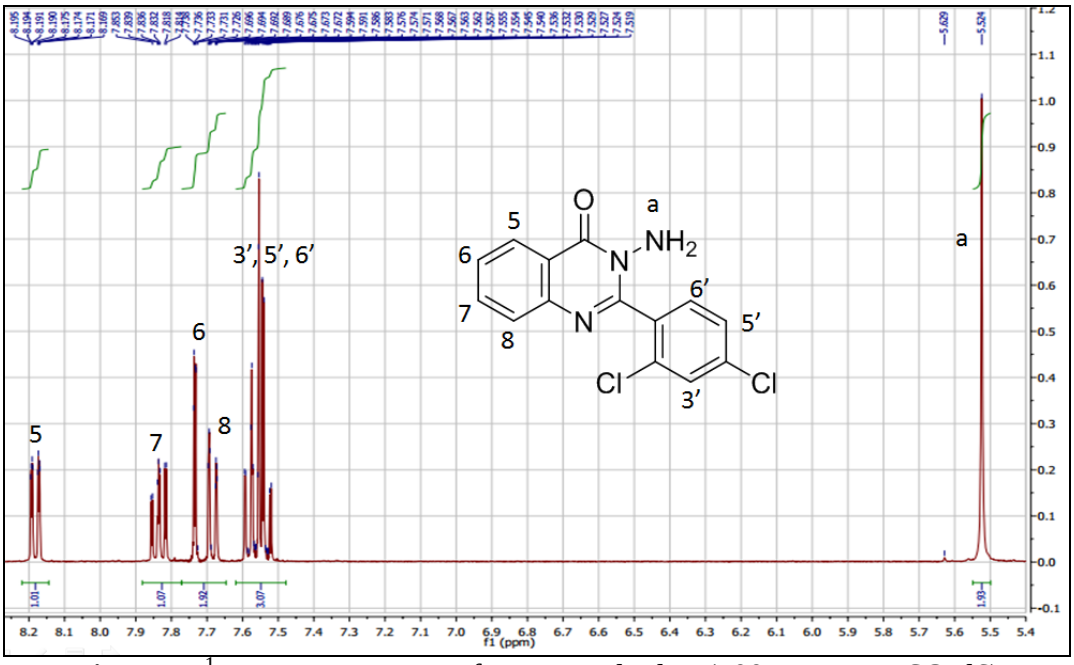

Figure 5 ^{1}H-NMR spectrum of compound 4d in (400 MHz, DMSO-d6)

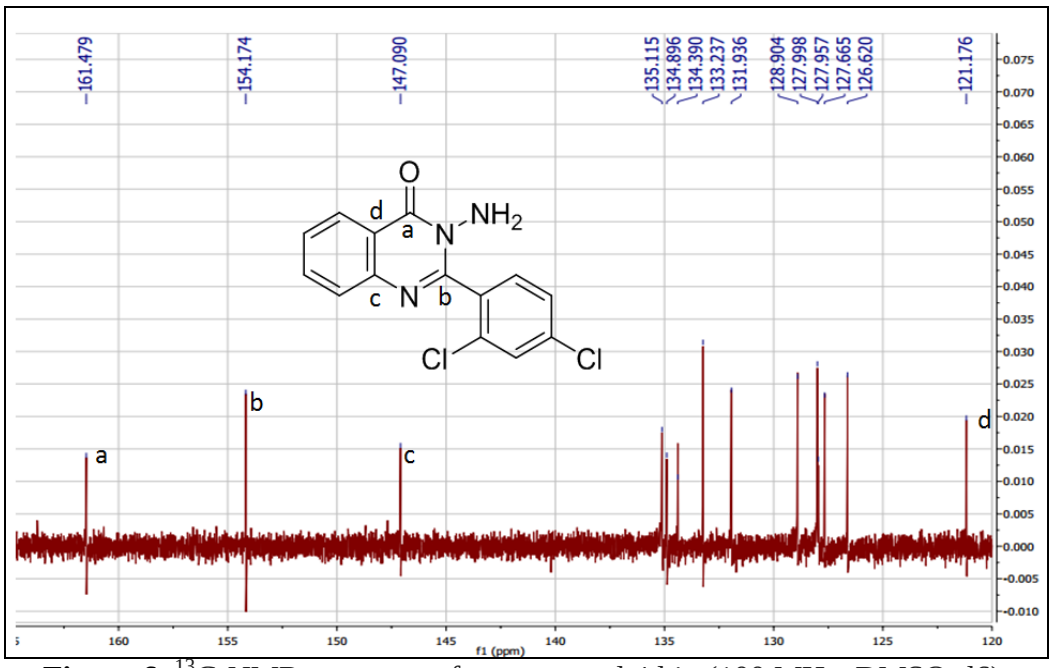

Figure 6 ^{13}C-NMR spectrum of a compound 4d in (100 MHz, DMSO-d6).

Compound3-amino-2-(3,4-dichloro-phenyl)quinazolin-4(3*H*)-one (4e)

Obtained in white powders; yield 85%; mp : 180-182 ^{o}C; FT-IR (KBr) cm^{-1}: 3025 and 691 (=C-H aromatis); 1685 (C=O lactam); 1628 (C=N); 1622 and 1473 (C=C aromatis); 1324 (C-N); 3128 (N-H primer) and 775 (C-Cl). ^{1}H-NMR (DMSO, δ, ppm) : *δ* 8.52 (1H, dd, J=8.4 Hz; 1.2 Hz); *δ* 8.07 (1H, t); *δ* 7.86 (2H, d, J=1.2 Hz); *δ* 7.76 (1H, dd, J=7.6; J=1.2 Hz); *δ* 7.55-7.50 (1H, m, Atom H); *δ* 7.17 (1H, dt, J=7.6; J=1.2 Hz); *δ* 4.56 (1H, s). ^{13}C-NMR (DMSO, δ, ppm) : *δ* 159.0; *δ* 155.0; *δ* 146.4; *δ* 137.5; *δ* 135.9; *δ* 132.5; *δ* 132.0; *δ* 131.3; *δ* 129.7; *δ* 129.6; *δ* 128.7; *δ* 128.3; *δ* 127.63; *δ* 117.6. MS m/z values (Rel. abundance) : $[M^{+}+Na]^{+}$= 305 (100%): $[M^{+2}+Na]^{+}$= 307 (65%); $[M^{+4}+Na]$ = 309 (10%).

Compound amino-2-(2,4-dichloro-phenyl)quinazolin-4(3*H*)-one (4f)

Obtained in white powders; yield 91%; mp : 170-172 ^{o}C; FT-IR (KBr) cm^{-1}: 3128 (N-H primer); 3025 dan 691 (=C-H aromatis); 1688 (C=O laktam); 1628 (C=N); 1622 and 1473 (C=C aromatis); 1324 (C-N). ^{1}H-NMR (DMSO, δ, ppm) : δ 8.56 (1H, d, J=8.2 Hz); δ 8.19 (1H, s); δ 7.92 (2H, d, J=7.2 Hz,); δ 7.70 (1H, d, J=8.0 Hz); δ 7.58 (1H, d, J=7.2 Hz); δ 7.53 (2H, t, J=7.6 Hz); δ 7.51-7.48 (1H, m); δ 4.57 (2H, s). ^{13}C-NMR (DMSO, δ, ppm): δ 165.0; δ 155.1; δ 139.5; δ 134.9; δ 132.3; δ 129.5 (2C); δ 128.2; δ 127.4 (2C); δ 123.6; δ 120.8; δ 119.8. MS m/z values (Rel. abundance): $[M^{++}+Na]^{+}$= 237 (100%): $[M^{+2+}+Na]^{+}$= 239 (65%); $[M^{+4+}+Na]^{+}$=241 (10%).

DPPH radical scavenging activity:

Scavenging of DPPH free radicals by different concentration of derivatives were evaluated. The derivatives showed

concentration dependent radical scavenging activity. IC_{50} concentration of 3a, 3b, 3c, 4d, 4e and 4f were found to be 44.08, 17.73, 13.58, 8.94,12.13 and 16.86 µg/mL respectively **(Table 2).**

Table 1: Effect of different derivatives on DPPH free radical scavenging activity

Concentration (µg/mL)	Percentage scavenging					
	3a	3b	3c	4d	4e	4f
6.25	39.24±0.00	35.71±0.00	44.62±0.00	45.45±0.00	44.19±0.00	43.31±0.00
12.5	45.86±0.00	46.27±0.00	50.00±0.00	55.28±0.00	50.34±0.00	51.02±0.00
25	48.20±0.00	49.30±0.00	54.14±0.00	56.10±0.00	56.89±0.00	53.25±0.00
50	51.35±0.00	65.05±0.00	59.32±0.00	64.18±0.00	62.30±0.00	54.43±0.00
100	53.25±0.00	75.51±0.01	62.30±0.00	69.62±0.00	66.82±0.00	56.36±0.00
200	56.10±0.00	96.51±0.29	72.93±0.00	70.49±0.00	75.51±0.00	60.00±0.00

All the values are in mean±SEM

ABTS radical scavenging activity:
Distinctive derivatives demonstrated concentration dependent ABTS radical scavenging activity. IC_{50} concentration of 3a, 3b, 3c, 4d, 4e and 4f were found to be 7.208, 7.912, 5.505, 3.227, 6.198 and 4.584 µg/mL respectively **(Table 3).**

Table 2: Effect of different derivatives on ABTS radical scavenging activity

Concentration (µg/ml)	Percentage scavenging					
	3a	3b	3c	4d	4e	4f
6.25	43.61±0.03	13.45±0.03	62.58±0.03	61.92±0.00	74.83±0.01	65.45±0.07
12.5	68.04±0.01	19.51±0.00	48.40±0.03	68.91±0.01	75.70±0.03	48.67±0.04
25	91.15±0.01	28.03±0.03	71.90±0.01	91.08±0.01	90.75±0.00	73.77±0.05
50	91.08±0.00	37.35±0.03	91.74±0.01	91.15±0.00	91.41±0.00	90.88±0.00
100	91.81±0.01	60.92±0.00	91.41±0.00	89.88±0.00	90.28±0.00	90.88±0.00
200	87.68±0.00	64.38±0.01	90.95±0.00	88.55±0.00	89.48±0.00	89.48±0.00

All the values are in mean±SEM

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Phenanthroline assay:

Ferric ion reduction potential of the various derivatives was investigated. The derivatives exhibited concentration dependent response. The IC_{50} of 3a, 3b, 3c, 4d, 4e and 4f were found to be 10.95, 11.73, 14.14, 8.913, 6.366 and 10.63 respectively **(Table 4).**

Table 3: Effect of different derivatives on ferric ion reduction potential

Concentration (µg/ml)	Percentage scavenging					
	3a	3b	3c	4d	4e	4f
12.5	42.86±0.00	47.22±0.00	47.95±0.00	50.65±0.00	49.33±0.00	49.67±0.00
25	53.37±0.00	49.67±0.00	49.67±0.00	53.66±0.01	51.59±0.00	50.97±0.00
50	58.70±0.00	54.22±0.00	51.28±0.00	55.56±0.00	57.54±0.00	51.59±0.00
100	60.82±0.00	64.32±0.00	53.37±0.00	58.92±0.00	63.64±0.00	53.94±0.00
200	63.29±0.00	79.29±0.01	62.38±0.00	62.38±0.00	70.31±0.00	60.21±0.00
400	70.43±0.00	88.55±0.01	76.25±0.01	67.52±0.00	77.25±0.01	64.32±0.00

All the values are in mean±SEM

The reducing power of 2,3-disubtituted quinazolin-4(3H)-one derivatives displayed a concentration-dependent antioxidant activity there was increase in the absorbance with increase in concentration. It was observed that derivatives scavenged the radical in concentration dependent manner. The percent scavenging of DPPH radical, ABTS radicals by derivatives and ascorbic acid were found to be and respectively (**Table 2** and **3**). Ferric ions do not get chelated by o-Phenanthroline. However, ferrous ions readily get chelated by o-Phenanthroline. The absorbance of this complex is derivatives assured at 510 nm. The absorbance is directly proportional to the antioxidant activity of the test compound. The IC_{50} values of derivatives and ascorbic acid shown in **Table 5** for the reduction of ferric ions in the o-Phenanthroline assay, free radical scavenging activity by DPPH radical and ABTS radical assay. Where it was observed derivatives possess strong antioxidant activity compared to ascorbic acid in ABTS radical assay.

Table 4: IC_{50} values of different derivatives in various *in vitro* antioxidant assays

Extracts/Fractions	IC_{50} values (µg/ml)		
	DPPH	ABTS	o-Phenanthroline
3a,	44.08	7.208	10.95
3b,	17.73	7.912	11.73
3c,	13.58	5.505	14.14
4d,	8.94	3.227	8.913
4e	12.13	6.198	6.366
4f	16.86	4.584	10.63
Asc.A	8.445	13.39	1.445

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. For the measurements of the reductive ability, it has been found that the Fe3+-Fe2+ transformation occurred in the presence of derivatives samples which was postulated previously by Oyaizu Tanaka et al. have observed a direct correlation between antioxidant activities and reducing power

of certain substance/chemicals. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom.30 Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. In this assay, depending on the reducing power of antioxidant compounds, the yellow color of the test solution changes into various shades of green and blue. Therefore, by measuring the formation of Perl's Prussian blue at 700 nm, we can monitor the Fe2+ concentration. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[1].

CONCLUSION

The usingmicrowave irradiation was more effective and efficient to produce compound 2,3-disubtituted quinazolin-4(3*H*)-one derivatives and very good yield 82-96%. It demonstrates potential cell reinforcement and free radical searching movement. These in-vitro assays show that plant extracts/fractions are significant wellsprings of common cancer prevention agents, which may be helpful as preventive specialists against oxidative stress. To clarify the prime wellspring of cell reinforcement properties further examinations ought to be done with isolate active principles.

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