INTRODUCTION

Quinazolinone ring system is known to have a broad spectrum of antibacterial activity against positive Gram and negative Gram bacteria [1]. The antibacterial activity is due to its similarity to the structure of bacterial dihydrofolate reductase (bDHFR) inhibitor [2-4]. Several compounds are known to inhibit the bacterial dihydrofolate reductase enzyme in vitro [76]. One is 6-amino-3-benzyl-4H-quinazolinone. This compound has shown good activity against Staphylococcus aureus and inhibited bacterial dihydrofolate reductase at IC50 40 µM. The amino group at position 6 plays an important role of the activity. Compound analogues without the amino substituent at position 6 lacks the antibacterial activity [5]. Another compound is 3-(5-bromothiazol-2-yl)-2-(E)-styrlyquinazolin-4-one. This compound’s activity can be compared with the positive control which was used. The styrly group at position 2 and substituents at position 3 play an important role in the activity [6]. This study aimed to combine the characteristics of the two compounds to make a new quinazolinone derivate which has antibacterial activity. Several methods are possible for synthesizing quinazolinones, but microwave and ultrasonic irradiation has attracted considerable attention for rapid synthesis of organic compounds [7].

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 6-AMINO-2-[(E)-2-(4-HYDROXY-3-METHOXYPHENYL)ETHENYL]-3,4-DIHYDROQUINAZOLIN-4-ONE AND ITS INTERMEDIATE COMPOUNDS

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EXPERIMENTAL

All the solvents, reagents and chemicals were analytical or synthesis grade and used without further purification. Melting points were determined in capillary tube using melting point apparatus (Stuart Scientific) and are uncorrected. ¹H NMR, ¹³C NMR, COSY, HMQC and HMBC. Screening for antibacterial activity was performed against Staphylococcus aureus ATCC 25923, Salmonella typhimurium ATCC 14028 and Escherichia coli ATCC 25922. The results indicated that the titled compound had been successfully synthesized and purified. The structure of desired compound was confirmed based on structural elucidation analysis. The result of testing the titled compound for the antibacterial activity was negative.

Keywords: Antibacterial activity, Synthesis, 6-Amino-2-[(E)-(4-hydroxy-3-methoxyphenyl)ethenyl]quinazolinone, Quinazolinone.
3,4-dihydroquinazolin-4-one (1) under microwave irradiation [8]. Second step was synthesis of 2-methyl-6-nitro-3,4-dihydroquinazolin-4-one (2) [9]. Third step was synthesis of 2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-6-nitro-3,4-dihydroquinazolin-4-one (3) under microwave irradiation [6,10]. Forth step was synthesis of 6-amino-2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-6-nitro-3,4-dihydroquinazolin-4-one (4) under ultrasonic irradiation [11,12].

**Synthesis of 2-methyl-3,4-dihydroquinazolin-4-one (1):** Compound 1 was synthesized under microwave irradiation according to reported method [8].

**Synthesis of 2-methyl-6-nitro-3,4-dihydroquinazolin-4-one (2):** Compound 2 was synthesized by nitration of compound 1 according to method for nitro derivatives of quinazolinone-4-one [9]. To the solution of compound 1 (8 g, 50 mmol) in 133.26 mL concentrated sulphuric acid (98%) was added dropwise of fuming nitric acid (11.18 mL, 250 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 4 h and then poured into crushed ice (50 g). The precipitate was filtered and washed with cold purified water and dried in vacuum drying oven at 70 °C for 1 h. The solid was recrystallized from ethanol.

**Synthesis of 2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-6-nitro-3,4-dihydroquinazolin-4-one (3):** Compound 3 was synthesized using modified method of Knoevenagel reaction between compound 2 and aromatic aldehyde [10]. Solution of compound 2 (824.6 mg, 4 mmol), anhydrous sodium acetate (820.5 mg) and vanillin (6.08 g, 40 mmol) in 20 mL glacial acetic acid was irradiated under microwave for 10 times of 10 min with 5 min interval each at power level 30 % (240 Watt). The precipitate was formed and filtered off, washed with cold purified water and dried in vacuum drying oven at 70 °C for 1 h. The solid was recrystallized from glacial acetic acid.

**Synthesis of 6-amino-2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-6-nitro-3,4-dihydroquinazolin-4-one (4):** Compound 4 was synthesized by reduction of compound 3 using modified method of aryl nitro reduction as reported earlier [11,12]. To a suspension of compound 3 (101.72 mg, 0.3 mmol) in 30 mL the mixture of hydrochloric acid-ethanol (1:1) was added reduced iron powder (251.28 mg, 4.5 mmol). 0.3 mmol) in 30 mL the mixture of hydrochloric acid-ethanol (1:1) was added reduced iron powder (251.28 mg, 4.5 mmol). The suspension was exposed to ultrasonic irradiation for 2 h (1:1) was added reduced iron powder (251.28 mg, 4.5 mmol). The suspension was exposed to ultrasonic irradiation for 2 h (1:1) was added reduced iron powder (251.28 mg, 4.5 mmol). The suspension was exposed to ultrasonic irradiation for 2 h.

**Antibacterial activity:** Antibacterial activity test was performed by disc diffusion method with nutrient agar medium [13]. Antibacterial activity was performed against *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC 25922. Inoculum was prepared by suspending bacteria (in the form of freeze-dried) into nutrient broth medium and diluted to obtain a concentration of 1 × 10^6 bacteria/mL. 5 mg of synthesized compound and positive control (trimethoprim) were carefully weighed and dissolved in 5 mL of DMSO and then diluted with 45 mL sterile purified water in order to obtain a concentration of 100 mg/mL. The solution was sterilized by filtration and created a serial dilution with mixture of DMSO-H2O (1:1) to obtain the following concentrations 50 mg/mL; 25 mg/mL; 12.5 mg/mL; 6.25 mg/mL. Compound 3 was made in the same way but with the solvent DMSO. Negative control was made in form of DMSO. 20 µL of each dilution test solution and control solution were dripped on the 6 mm paper disc. The paper discs were placed in a petri dish containing nutrient agar and inoculated bacteria and then incubated for 18-24 h at 37 °C.

**RESULTS AND DISCUSSION**

The titled compound was synthesized stepwise as shown by Scheme-I. In first step, anthranilic acid was reacted with acetic anhydride (Ac2O) under microwave irradiation for 20 min at power level 50 % (400 Watt) to obtain an intermediate compound 2-methylbenzoazoxin-4-one. Ammonium acetate was added to the mixture (30 %) and the microwave irradiation was continued for 20 min at power level (240 Watt) to provide compound 1 [8]. This compound was treated with fuming nitric acid in concentrated sulphuric acid at room temperature for 4 h in order to obtain compound 2 [9].

Then compound 2 was treated with vanillin in glacial acetic acid in presence of sodium acetate as catalyst and irradiated with microwave to obtain compound 3. The irradiation was performed for 10 times for 10 min at intervals of 5 min each. Physical data and analytical data of the synthesized compounds is given in Table-1. The UV-visible and IR spectra data of the synthesized compounds are given in Table-2. The NMR spectra data of the synthesized compounds are given in Table-3.

2-[(E)-2-(4-Hydroxy-3-methoxyphenyl)ethenyl]-6-nitro-3,4-dihydroquinazolin-4-one (3): Compound 3 appeared in the form of red-orange powder with protonated molecular mass (M+H) ESI 320.27. spectrum UV-visible in aceto-nitrile showed peak absorption at λ (nm) 240.0 (maks); 329.5; 399.5. The IR spectrum (KBr) of the compound 3 showed absorption bands at 1609 cm⁻¹ due to the presence of C=O quinazolinone, the NH quinazolinone could not be identified because of the overlap with phenolic hydroxy; 3176 cm⁻¹ (OH phenolic); 2964 cm⁻¹ (C-H alkene); 1609 cm⁻¹ (C=O amide); 1599 cm⁻¹ (C=C aromatic); 1562 cm⁻¹ and 1516 cm⁻¹ (NO₂ aromatic). In ¹H NMR spectrum (DMSO-d₆), a proton of NH quinazolinone appeared as one broad singlet at δ 12.63 ppm; and the tree protons of quinazolinone ring were observed as doublet at δ 8.76 ppm (1H; J = 2.6; H-5); double doublets at δ 8.49 ppm (1H; J = 9.1; J = 2.6; H-7); and doublet at δ 7.76 ppm (1H; J = 9.1; H-8). Protons of olifin group were observed as doublet at δ 6.85 ppm (1H; J = 15.5; H-11) and doublet at δ 7.98 ppm (1H; J = 16.3; H-12). Protons of vanillin group were observed as doublet at δ 7.26 ppm (1H; J = 1.9; H-14); doublets at δ 6.86 ppm (1H; J = 8.5; H-17); and δ 7.12 ppm (1H J = 8.5; J = 1.9; H-18). Proton of OH vanillin appeared as one broad singlet at δ 9.73 ppm, while three protons of methoxy group were appeared as singlet at δ 3.85 ppm. The ¹³C NMR spectra (DMSO-d₆) carbon quinazolinone ring appeared at δ 155.4 ppm (C-2); δ 161.1 ppm (C=O; C-4); δ 121.1 ppm (CH;
δ 153.6 ppm (C-NO2; C-6); δ 128.4 ppm (CH; C-7); δ 128.3 ppm (CH; C-8); δ 143.9 ppm (C; C-9); δ 120.7 ppm (C; C-10); carbon olefin appeared at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring apperaed at δ 116.8 ppm (CH; C-11) and δ 141.5 ppm (CH; C-12); carbon vanillin ring ap...
6-Amino-2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-6-nitro-3,4-dihydroquinazolin-4-one (4): Compound 4 appeared in the form of red-brown powder with protonated molecular mass (M+H)+ ESI m/z 310.10. Spectrum UV-visible in acetonitrile showed peak absorption at λ (nm) 248.0; 359.5 (C=C aromatic). In the 1H NMR spectrum (DMSO-D6), absorption bands at 3348 cm−1 indicated NH quinazolinone; and 2066 (C-H alkene); 1691 (C=O amide); 1639 (C=N imine); 1597 (C=C aromatic). In the 1H NMR spectrum (DMSO-d6), two protons of primary amine were not appeared because of the iron impurities, which was having paramagnetic properties, reduce the NMR signals [12]. This effect was also happen to tertiary amine proton at quinazolinone ring. The tree protons of quinazolinone ring were observed as singlet at δ 7.28 ppm (1H; H-5); singlet at δ 7.20 ppm (1H; H-7 overlap H-14); doublet at δ 7.50 ppm (1H; J = 8.5; H-8); Protons of olefine group were observed as doublet at δ 6.81 ppm (1H; J = 16.2; H-11) and doublet at δ 7.98 ppm (1H; J = 16.2; H-12); Protons of vanillin group were observed as singlet at δ 7.20 ppm (1H; H-14 overlap H-7); doublet at δ 6.87 ppm (1H; J = 8.4; H-17); doublet at δ 7.08 ppm (1H; J = 8.4; H-17); Proton of OH vanillin appeared as one broad singlet at δ 9.77 ppm, while three protons of methoxy group were appeared as singlet at δ 3.84 ppm. The 13C NMR spectra (DMSO-d6) carbon quinazolinone ring appeared at δ 150.0 ppm (C=C-2); δ 159.8 ppm (C=O; C-4); δ 109.3 ppm (CH; C-5); δ 145.2 ppm (C-NH2; C-6); δ 124.0 ppm (CH; C-7); δ 123.5 ppm (CH; C-8); δ 142.3 ppm (C; C-9); δ 120.7 ppm (C; C-10); carbon olefin appeared at δ 111.7 ppm (CH; C-11) and δ 147.9 ppm (C; C-12); carbon vanillin ring appeared at δ 125.6 ppm (C; C-13); δ 110.6 ppm (CH; C-14); δ 147.9 ppm (C-OCH3; C-15); δ 149.8 ppm (C-CH=CH; C-16); δ 115.8 ppm (CH; C-17); δ 123.0 ppm (CH; C-18); δ 55.6 ppm (OCH3; C-19). The 13C NMR spectra of
compounds 3 and 4 as shown in Table-3 confirmed the proposed structures.

None of the synthesized compounds showed any antibacterial activity. The result of the susceptibility assay of the synthesized compounds is given in Table-4. The lack of antibacterial activity by the tested compound might be caused by several factors. One is the solubility of the tested compound: solubility in water was important, because the compounds should be spread in the medium. The process of distribution of compounds in the medium was important for the success of the disc diffusion method [13]. The disc diffusion method was chosen instead of the turbidimetry method, because the tested compounds, especially compounds 2 and 3 will undergo salting out in an aqueous medium.

The other factor is the ability of tested compounds to penetrate the bacterial cell walls. The tested compounds was supposed to target the bacterial dihydrofolate reductase enzyme found in the cytoplasm of bacteria. Therefore, the tested compound had to be able to penetrate the bacterial cell wall. The penetration ability of the tested compound was influenced by the physical and chemical properties of these compounds [2]. The results indicated that the tested compounds were not be able to penetrate the bacterial cell wall.

Conclusion

It can be concluded that the new quinazolinone derivate compounds viz., 6-amino-2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-3,4-dihydroquinazolin-4-one and 2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-6-nitro-3,4-dihydroquinazolin-4-one were successfully synthesized. The structure of these compounds was confirmed using UV-visible spectroscopy, FT-IR, NMR spectroscopy and LC-MS. Compounds 6-amino-2-[(E)-2-(4-hydroxy-3-methoxyphenyl)ethenyl]-3,4-dihydroquinazolin-4-one and its intermediate compounds did not show antibacterial activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028.

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