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Synthesis and *in-vitro* evaluation of 3-[(benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one derivatives as antioxidant agents

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Abstract

Objective: Synthesis and *in-vitro* evaluation of 3-[(benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one derivatives as antioxidant agents.

Methods: Novel 3-[(benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one derivatives were synthesized and evaluated of their scavenging activity which was used for the determination of antioxidant action against free radicals like DPPH (1,1–diphenyl-2-picrylhydrazyl). The structures of the synthesized compounds were assigned on the basis of spectroscopic analysis UV (ultraviolet), FT- IR (fourier transform infrared spectroscopy) and NMR (nuclear magnetic resonance spectroscopy) spectral data.

Results: Compounds 3-[(4-hydroxy-benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one (PT-2) and 3-[(3, 4 dimethoxy-benzylidine) -amino]-2-phenyl-3H-quinazoline-4-one (PT-3) showed good scavenging activity. Better activity of PT-2 compound may be due to the presence of hydroxyl group at the para position.

Conclusion: In the present study, four compounds have been synthesized. The synthesized compounds showed a significant antioxidant activity.

Keywords: Quinazoline, Scavenging activity, Antioxidants, Spectroscopic analysis

Introduction

Reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) superoxide anion (O_2^-) and hydrogen peroxide are often generated as the oxidation product of biological reactions or from exogenous factor. These reactive species exert oxidative damaging effects by bombarding with living cells including DNA [1]. The antioxidant defence system in most cells is composed of two components, the antioxidant enzymes component which includes enzymes such as superoxide dismutase, catalase and glutathione peroxidase and the low molecular weight antioxidants component that includes vitamins A, E, ascorbate, glutathione and thioredoxin. These substances are the body's natural defense against endogenous generated ROS and other free radicals, as well as ROS generated by external environmental factors. Oxidative stress occurs when the production of ROS exceeds the body's natural antioxidant defense mechanisms, causing damage to bio-molecules such as lipids, proteins and DNA. Oxidative DNA damage has been implied as a cause of cancer, aging and neurodegenerative diseases such as alzheimer's and parkinson's, cardiovascular diseases [2]. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and well being [3]. Antioxidant capacity is derived both exogenously (from food, beverage and sunlight) and endogenously (from enzymatic and non-enzymatic pathways). A number of oxidant food factors service antioxidant metallo enzymes. The capacity operates extra- or intracellular. Uric acid is the major antioxidant in primate blood. Uric acid synthesis is increased by dietary fructose from fruit, sugary foods and drinks. This indirect antioxidant effect of fruit is separate from that attributable to its flavonoids [4]. A number of synthetic compounds such as quinazolinones have been extremely exploited for antioxidants activity.

Chemically quinazoline is 1, 3 - diazanaphthalen; 5, 6-benzopyrimidine or benzo [a] pyrimidine or phenmiazine, and the 4-oxo derivative of quinazoline is known as 4 (3H) - quinazolinone [5]. It is a fused heterocyclic compound having diverse range of pharmacological profile like anticancer [6], anti-HIV [7], antihypertensive [8], anti-inflammatory [9], anticonvulsant [10], antimicrobial [11, 12], antimutagenic [13] and antimalarial [14] etc.

In an extensive literature survey a research article was found which consists a series of compounds containing quinazolin-4(3H)-one ring system associated with various primary aromatic amines moiety and to evaluate their antioxidant potency [15].

The stability of the quinazolinone nucleus has inspired medicinal chemists to introduce many bioactive moieties to this nucleus to synthesize new potential medicinal agents [16].

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In the present study, four compounds has been synthesized, at first step synthesis of 2-phenyl-3, 1-benzoxazin-4(3H) by reacting anthranilic acid and benzoyl chloride. When 2-phenyl-3, 1-benzoxazine 4-(3H)-one was left to react with hydrazine hydrate in n-butanol under reflux, the corresponding 3-amino-2-phenyl -4(3H)-quinazolinone was formed in good yield. For a solution of 3-amino-2 phenyl-4(3H)-quinazolinone in methanol the required aromatic aldehyde (0.005 mol) was added and then the reaction mixture was stirred up for about 3-4 h and refluxed for overnight. Consequently four compounds were synthesized.

Materials and Methods

The starting materials were commercially available and purchased from across organics. Melting points were measured on a Veego melting point apparatus. Thin layer chromatography (TLC, silica gel-G) was used to monitor reactions and check product homogeneity. The structure of synthesized compounds was determined by spectral analysis. The λmax of synthesized compound was determined by using UV-VIS Systronics Double Beem Spectrophotometer: 2203 Smart Spectrophotometer. IR spectra were recorded in Bruker spectrometer using ABS technique. ^{1}H NMR spectra were recorded on a Brucker's WM 400 FT MHz NMR instrument using $CDCl_3$ as solvent and TMS as internal reference (chemical shifts in δ ppm). Splitting patterns are described as singlet (s) and multiplet (m).

Chemistry

Step 1: Synthesis of 2-phenyl- 4H-3, 1-benzoxazin-4-one

Synthesis of quinazolinone derivatives was done in three step procedure; in first step anthranilic acid (5.74 g, 0.04 mol) was dissolved in 60 ml of dry pyridine. To this solution benzoyl chloride (4.5 ml, 0.04 mol) was added drop wise maintaining the temperature 0-6 °C. After the addition of benzoyl chloride the reaction mixture was stirred at room temperature for 3 h. Resultant reaction mixture was treated with 10% sodium bicarbonate. Reaction mixture was filtered and washed repeatedly with water to remove inorganic materials. The crude product obtained was recrystallized from ethanol. Yield: 56.12%, mp: 118 °C.

Step 2: Synthesis of 3-amino-2-phenylquinazolin-4-(3H) ones

To a stirred solution of 2-phenyl- 4H-3, 1- benzoxazin-4-one (2.24 g, 0.01 mol) in dry pyridine (20 mL), 80% hydrazine hydrate (0.15 mol) was added. The reaction mixture was stirred and refluxed for 30 min. After cooling, the crude product was obtained by filtration and recrystallized from 80% ethanol to afford 3-amino-2- phenylquinazolin-4-(3H) ones as a white solid. Yield: 62.05%, mp: 176-179 °C.

Step 3: Synthesis of 3-[(benzylidine)-amino]-2-phenyl-3Hquinazoline- 4-one derivatives

To a solution of 3-amino-2 phenyl-4(3H)-quinazolinone (1.18 g, 0.005 mol) in methanol (20 ml) required aromatic aldehyde (0.005 mol) was added and the reaction mixture was then stirred for about 3-4 h. The product obtained was filtered, washed with hot methanol and recrystallized with ethanol. The three step reaction of synthesis is given in scheme 1.

Synthesis of 3-[(4-chloro benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one (PT-1)

Yield 61.14%; mp (°C): 220-221; λ_{max} 284 nm; IR (ABS): 3067 cm^{-1} (=C-H), 1654 cm^{-1} (C=O), 1601cm^{-1} (C=0), 1529 cm^{-1} (C=C), 1173 cm^{-1} (C-C), 755 cm^{-1} (C-Cl), 695 cm^{-1} (C-H), ^{1}H NMR ($CDCl_3$): δ values 7.235-8.012 (m, 13H, Ar-H), 1.632(s, 1H, -CH).

Synthesis of 3-[(4-hydroxy-benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one (PT-2)

Yield 56.43%; mp (°C): 208-209; λ_{max} 306 nm; IR (ABS): 3055 cm^{-1} (=C-H), 2927 cm^{-1} (C-H), 1647 cm^{-1} (C=O), 1600 cm^{-1} (C=N), 1518 cm^{-1} (C=C), 1282 cm^{-1} (C-C), 759 cm^{-1} (C-H), ^{1}H NMR ($CDCl_3$): δ values 7.078-7.965 (m, 13H, Ar-H),7.9-8.3 (m, 1H, Ar-OH), 1.553(s, 1H, -CH).

Synthesis 3-[(3, 4 dimethoxy-benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one (PT-3)

Yield 53.08%; mp (°C): 155-156; λ_{max} 332nm; IR (ABS): 3059 cm^{-1} (=C-H), 1648 cm^{-1} (C=O), 1597cm^{-1} (C=N), 1514 cm^{-1} (C=C), 1266 cm^{-1} (C-O-C), 664 cm^{-1} (C-H), ^{1}H NMR ($CDCl_3$): δ values 3.438-4.580 (s, 3H, O-CH_3), 7.234-7.995 (m, 13H, Ar-H).

Synthesis of 3-[(4-nitro-benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one (PT-4)

Yield 51.78%; mp (°C): 167-168; λ_{max} 272 nm; IR (ABS): 3060 cm^{-1} (=C-H), 1681 cm^{-1} (C=O), 1642 cm^{-1} (C=N), 1528 cm^{-1} (NO_2), 1161 cm^{-1} (C-C), 703 cm^{-1} (C-H), ^{1}H NMR ($CDCl_3$): δ values 7.075-7.990 (m, 13H, Ar-H), 1.637(s, 1H, -CH).

Antioxidant activities

The DPPH radical scavenging assay is a common method to evaluate the antiradical activity of numerous substances. This assay was based on the procedure described by Brand-Williams et al. (1995) [17] with some modifications. The hydrogen atoms or electrons donation ability of synthesized compounds was measured from the bleaching of purple colour methanol or ethanol solution of DPPH. At first, a standard solution of ascorbic acid, sample solution and reagent solution of DPPH methanolic solution (1x10-4M) was prepared. The subsequent procedure is following. Different concentration of synthesized compounds were prepared (200-1000 µg/ml) in methanol and 3ml of each solution was mixed with 1 ml of a 0.1 mM methanolic DPPH solution. The decrease in absorbance was measured at 515 nm after 30 min of incubation period at room temperature using a UV visible spectrophotometer. The scavenging activity of sample was expressed as the inhibition of DPPH radical and calculated according to the following formula with as the control:

Scavenging Activity (%) = $[(A_{control} - A_{sample}) / A_{control}]*100$

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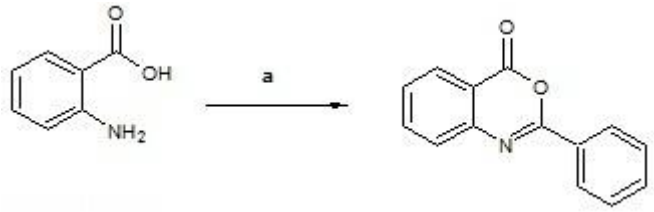
Where $A_{control}$ (containing DPPH solution adequately diluted with methanol) is the absorbance of control solution and A_{sample} is the absorbance with different dilutions of drug sample. Ascorbic acid was used as reference standard.

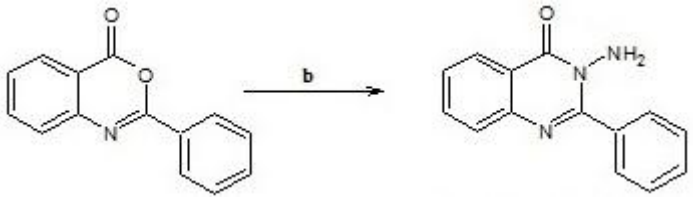
The DPPH scavenging activities of synthesized compounds at different concentrations are shown in figure 2.

Step-1: Conversion of anthranillic acid into 2-phenyl- 4H-3, 1-benzoxazin-4-one



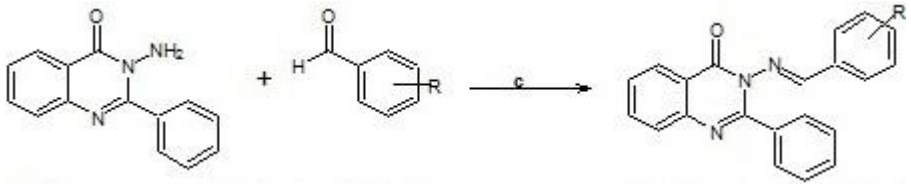
Anthranilic acid 2-phenyl-4H-3, 1-benzoxazin-4-one

Step-2: Conversion of 2-phenyl- 4H-3, 1-benzoxazin-4-one into 3-amino-2-phenylquinazolin-4-(3H) one



2-substituted 4H-3, 1-benzoxazin-4-one 3-amino-2-phenylquinazolin-4-(3H)one

Step-3: Conversion of 3-amino-2-phenyl-4(3H)-quinazolinone into 3-benzylidene-amino-2-phenyl-4(3H)-quinazolinone



3-amino-2-phenylquinazolin-4-(3H)one Aromatic aldehyde 3-benzyllidene-amino-2-phenyl-4-(3H)-quinazolinone

Scheme 1: Synthesis of compounds**.** Reagents and conditions**: a-** Dry pyridine, C_6H_5COCl, 1h (0-6 °C), 3 h stirring (RT); **b-** Hydrazine hydrate, n-butanol, reflux; **c**-methanol, 3-4 h stirring (RT)

Results and discussions

This research work was focused on the synthesis of the quinazolinone with different aldehydes derivative with improved biological activity. In this synthetic process 1st step was to synthesize the benzoxazinone compounds and 2nd step was the reaction between hydrazine hydrate and benzoxazinone which form quinazolinone and 3rd step is the synthesis of targeted compounds. By using this method four derivatives PT-1, PT-2, PT-3 and PT-4 are synthesized and list of synthesized compounds with their substitutions are given in table 1. After synthesis these were subjected to identification tests by using various methods like melting point study, thin layer chromatography, solubility study and characterization by using UV, IR and NMR spectroscopy.

All the data of compounds confirms the synthesis of the above mentioned derivatives like melting point of all the quinazolinone derivatives ranges between 155-221 °C, the thin layer chromatography by using silica gel-G as adsorbent and ethyl acetate: chloroform (1:1) as a solvent system, the result of solubility study shows that all the synthesized compounds gives maximum solubility in acetone, alcohol, benzene etc, which confirm that the synthesized compounds are semipolar in nature.

The UV spectral data represents the λmax (nm) values of the compounds. IR spectroscopy helps to identify the chemical structure of the compounds, like in the given data all the compounds show the peak values of the representing group which is present in the compounds so here all these quinazolinone derivatives show C=O stretch, C-H stretch, C-C stretch (aromatic), C-N stretch, C-H bend, C-Cl stretch, C-O-C stretch, =C-H stretch peaks that confirms the structure of the quinazolinone derivatives. NMR spectroscopy was done by using $CDCl_3$ as the solvent and tetramethylsilane (TMS) as the internal standard and the chemical shift values (ppm) gave the much more confirmation about the desired structures of the compound. After structural determination, the *in-vitro* testing of the synthesized compounds was done by using DPPH free radical scavenging activity.

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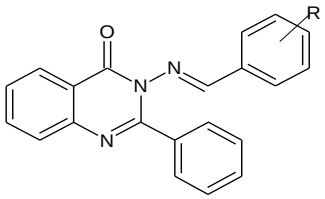

Figure 1: 3-[(benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one derivatives

Table 1: Synthesized compounds with their substitutions

S. No.	Compounds	R
1	PT-1	4-Cl
2	PT-2	4-OH
3	PT-3	3,4 $(OCH_3)_2$
4	PT-4	4-NO_2

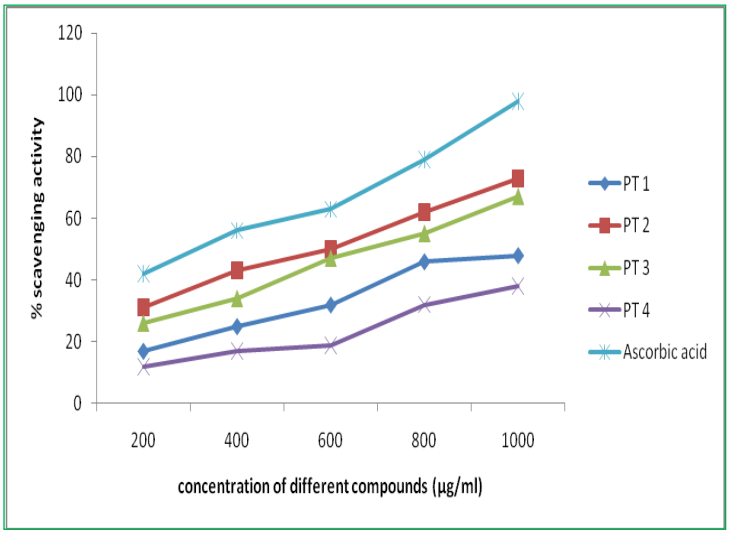

Figure 2: Graph representing % scavenging activity of synthesized compounds at different concentrations

Results are given in mean of triplicate. Error bars was omitted for the simple presentation of graph.

Conclusion

The quinazolinone derivatives were synthesized and characterized for their structure elucidation. Various chemical and spectral data supported the structure of the compounds thought of. Compounds 3-[(4-hydroxy-benzylidine)-amino]-2-phenyl-3H-quinazoline-4-one (PT-2) and 3-[(3, 4 dimethoxy-benzylidine) - amino]-2-phenyl-3H-quinazoline-4-one (PT-3) showed good scavenging activity. Better activity of PT-2 compound may be due to the presence of hydroxyl group at the para positionThe synthesized compounds showed a significant antioxidant activity.

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