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Synthesis, Characterization and Biological Evaluation of N-3 Substituted Quinazolin-4(3*H*)-ones

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A series of 3-substituted-2-phenylquinazolin-4(3*H*)-one derivatives (**4a-g**) were designed and synthesized to examine the effect of different steric and electronic substituents on their chemical and biological properties. The target compounds were obtained in good yields of 64-78% by reacting 2-phenyl-4*H*-benzo[*d*][1,3]oxazin-4-one with substituted aromatic and heteroaromatic primary amines in glacial acetic acid. Structural characterization was carried out using FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The FT-IR data established the characteristic carbonyl stretching vibration of the quinazolinone moiety within the 1682-1670 cm^{-1} range, consistent with the resonance effects of the heterocyclic ring. The synthesized derivatives exhibited moderate to good concentration-dependent antioxidant activity in the DPPH assay, while their anti-inflammatory potential was assessed through the protein denaturation assay.

Keywords: Substituted quinazolin-4(3*H*)-ones, Antioxidant, Anti-inflammatory, Thin layer chromatography.

INTRODUCTION

The quinazolin-4(3*H*)-one scaffold represents an important heterocyclic framework in medicinal chemistry and is present in numerous compounds with diverse pharmacological properties, particularly antimicrobial, antiproliferative, antioxidant and anti-inflammatory activities [1-4]. The structural versatility of this scaffold permits the incorporation of a broad range of substituents, allowing its physico-chemical and biological properties to be modulated through rational structural modification. Several conventional synthetic strategies have been reported for the preparation of quinazolin-4(3*H*)-one derivatives, with benzoxazinone intermediates serving as useful precursors through nucleophilic ring opening followed by cyclization [5].

Substituent-dependent changes in steric environment and electronic distribution can substantially influence molecular conformation, intermolecular interactions and biological activity [6-9]. Despite the extensive investigation of quinazolin-4(3*H*)-one derivatives, systematic studies that correlate localized steric and electronic modifications within a well-defined structural framework remain comparatively limited. In several reported studies, aromatic or heteroaromatic groups have been introduced at different positions of the quinazolinone nucleus, while the influence of their substitution pattern has not been examined through a consistent comparative design [10]. Such an approach is important for establishing meaningful structure-property relationships and for understanding the contribution of substituent position, steric demand and electronic characteristics to biological performance.

In the present study, a defined structural matrix was established at the N-3 position of the 2-phenylquinazolinone framework [11-14]. The design specifically incorporates positional variations of halogen substituents to compare the effects of *ortho*- and *para*-substitution, represented by 2-Cl *versus* 4-Cl and 2-F *versus* 4-F derivatives [15]. The structural series was extended to include a larger, planar and lipophilic naphthalen-1-yl group together with nitrogen containing hetero-aryl substituents, namely pyridin-2-yl, methylpyridinyl and picolinonitrile moieties, possessing distinct electronic and dipolar characteristics [16]. This systematic arrangement provides an opportunity to examine the influence of substituent size, spatial orientation and electronic character on the conformational features of the N-3 substituent and their relationship with radical-scavenging activity [17]. The synthesized derivatives (**4a-g**) were also evaluated for their antioxidant and anti-inflammatory properties using DPPH radical scavenging and protein denaturation assays, respectively.

EXPERIMENTAL

The melting points of the synthesized compounds were determined using the open-capillary tube method. The purity of the compounds was assessed by thin-layer chromatography (TLC) on silica gel G plates. The synthesized compounds were characterized by FT-IR spectroscopy using a Shimadzu instrument, ^{1}H and ^{13}C NMR spectroscopy using a Bruker instrument and mass spectrometry. These analytical techniques were used to establish the structural features and molecular composition of the synthesized compounds.

Synthesis of 2-phenyl 1,3-benzoxazine-4-one: Anthranilic acid (0.1 mol) was dissolved in dry pyridine (50 mL) and benzoyl chloride (0.2 mol) was added dropwise to the solution with continuous stirring while maintaining a low temperature. The reaction mixture was allowed to cool after complete addi-tion of benzoyl chloride and was then treated with 10% aqueous Na_2CO_3 solution (15 mL). After the evolution of gas, the reaction mixture was filtered and the solid residue was washed repeatedly with water to remove inorganic impuri-ties (**Scheme-I**). The crude product was purified by recrysta-llization from ethanol.

Synthesis of 3-substituted-2-phenyl quinazoiln-4(3*H*)-one derivatives: Equimolar amounts (0.01 mol each) of 2-phenyl-1,3-benzoxazin-4-one and the appropriate substituted aromatic or heteroaromatic primary amine were refluxed in ethanol (10 mL) for 6 h. After completion of the reaction, the mixture was allowed to cool to room temperature and then poured onto crushed ice (**Scheme-I**) [11]. The precipitated solid was collected by filtration and recrystallized from absolute ethanol.

3-(4-Methyl-pyridin-2-yl)-2-phenyl-3*H*-quinazolin-4-one (4a): Yield: 58%; R_f: 0.5; FT-IR (KBr, ν_{max}, cm^{-1}): 1745 (-C=O), 1514 (-C=N), 1435 (-C=C Ar), 690 (-Ar-H), 501 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.29-8.44 (m, 13H, Ar-H); Mass (*m/z*): 314 $[M–H]^+$.

3-(2-Cyno-pyridin-2-yl)-2-phenyl-3*H*-quinazolin-4-one (4b): Yield: 55%; R_f: 0.7; FT-IR (KBr, ν_{max}, cm^{-1}): 1762 (-C=O), 1604 (-C=N), 1467 (-C=C Ar), 763, 678 (-Ar-H); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.21-8.75 (m, 13H, Ar-H); Mass (*m/z*): 324 $[M]^+$.

3-(Naphthalen-1-yl)-2-phenylquinazolin-4(3*H*)-one (4c): Yield: 59%; m.p.: 167 ºC; R_f: 0.2; FT-IR (KBr, ν_{max}, cm^{-1}): 1687 (C=O), 1610 (C=N), 1512 (C=C Ar), 759 (Ar-H), 682 (C-F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.21-8.36 (m, 13H, Ar-H); Mass (*m/z*): 324 $[M]^+$.

3-(4-Chlorophenyl)-2-phenyl-3-hydroquinazolin-4-one (4d): Yield: 65%; m.p.: 168 ºC; R_f: 0.2; FT-IR (KBr, ν_{max}, cm^{-1}): 1680 (C=O), 1510 (C=N), 1417 (C=C Ar), 690 (Ar-H), 518 (C-Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.24-8.73 (m, 13H, Ar-H); Mass (*m/z*): 333 $[M–H]^+$.

3-(4-Flurophenyl)-2-phenylquinazolin-4(3*H*)-one (4e): Yield: 62%; m.p.: 170 ºC; R_f: 0.4; FT-IR (KBr, ν_{max}, cm^{-1}): 1681 (C=O), 1517 (C=N), 1438 (C=C Ar), 694 (Ar-H), 509 (C-N or C–F); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.50-8.60 (m, 13H, Ar-H); Mass (*m/z*): 317 $[M+H]^+$.

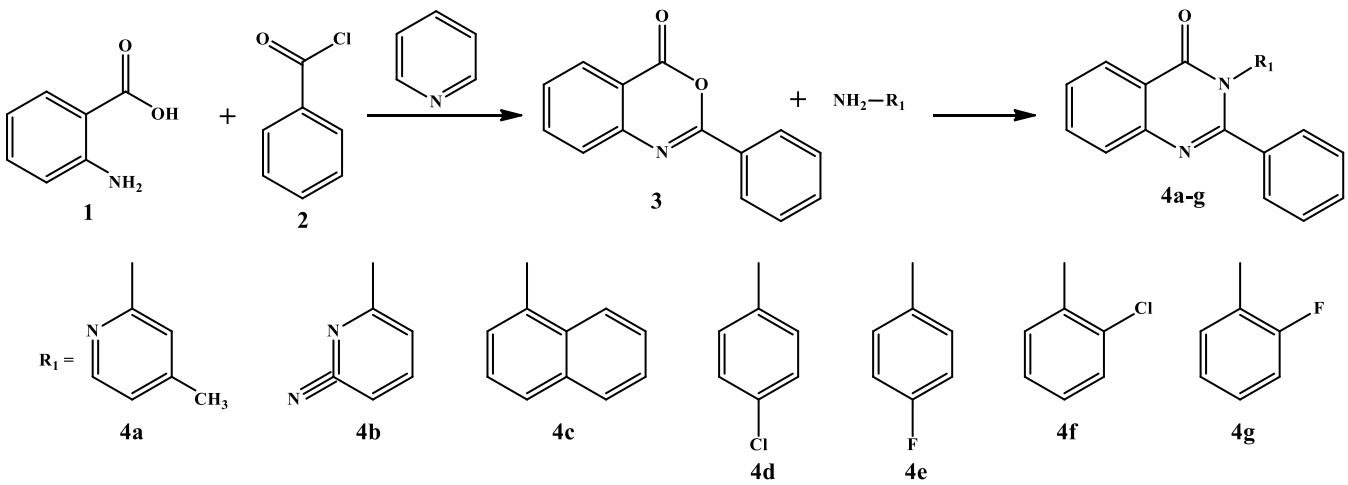
3-(2-Chlorophenyl)-2-phenylquinazolin-4(3*H*)-one (4f): Yield: 57%; m.p.: 149 ºC; R_f: 0.2; FT-IR (KBr, ν_{max}, cm^{-1}): 1653 (C=N), 1394 (C=C), 876 (Ar–H); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 6.23-8.07 (m, 15 H, Ar-H); Mass (*m/z*): 333 $[M–H]^+$.

3-(2-Flurophenyl)-2-phenylquinazolin-4(3*H*)-one (4g): Yield: 63%; m.p.: 171 ºC; R_f: 0.2; FT-IR (KBr, ν_{max}, cm^{-1}): 2929 (C–H), 1666 (C=N), 1577 (C=C), 759 (Ar–H); ^{1}H NMR 400 MHz, $CDCl_3$, δ ppm): 7.32-8.68 (m, 11H, Ar-H); Mass (*m/z*): 317 $[M+H]^+$.

Antioxidant activity: A DPPH solution (6×10^{-5} M) was prepared in methanol. Test solutions at different concentrations were prepared and 500 µL of each solution was transferred into separate Eppendorf tubes. Each concentration was tested in triplicate. DPPH solution (500 µL) was added to each test solution and the mixtures were mixed thoroughly and incubated at room temperature for 30 min. The absorbance was measured at 520 nm. Ascorbic acid was used as the positive control, while purified water served as the negative control. The Graph Pad Prism 9 software was employed for this purpose [18,19].

The percentage inhibition values were calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Blank DPPH solution absorbance} - \text{Actual absorbance}}{\text{Blank DPPH solution absorbance}} \times 100$$



Scheme-I: Synthesis of 2-phenyl-3-substituted-quinazolin-4(3*H*)-one derivatives

***In vitro* anti-inflammatory activity-inhibition of albumin denaturation:** The reaction mixture was prepared by mixing 0.5 mL of the test compound solution with 0.45 mL of 5% bovine serum albumin (BSA) solution. The pH of the mixture was adjusted to 6.3 using 0.1 N HCl and maintained at 37 ºC for 20 min. The mixture was then heated at 57 ºC for 30 min and allowed to cool to room temperature. Aliquots of the resulting solution were transferred to a 96-well microplate and the absorbance was measured at 660 nm. Diclofenac sodium (1000 µg/mL) was used as the standard, while the control contained 0.05 mL of distilled water [20]. The percentage inhibition of protein denaturation was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{test sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where $\text{Abs}_{\text{control}}$ = absorbance of the control, $\text{Abs}_{\text{test sample}}$ = absorbance of the test sample.

RESULTS AND DISCUSSION

The designed substitution pattern at the N-3 position of the 2-phenylquinazolin-4(3*H*)-one scaffold afforded quinazolinone derivatives (**4a-g**) with isolated yields ranging from 50 to 65%. The *ortho*-halogenated derivatives **4f** and **4g** gave comparatively higher yields of 65 and 62%, respectively. The higher yield of these derivatives may be associated with the substitution pattern around the N-3-linked aromatic ring, which can influence the reaction environment and the crystallization behaviour of the final products. TLC was performed on silica gel G plates (0.25 mm) using *n*-hexane (1:9) as the mobile phase, with visualization under UV light. The synthesized compounds gave distinct spots with R_f values in the range of 0.20-0.70, supporting the formation of individual products with satisfactory chromatographic purity.

The structures of the synthesized quinazolinone derivatives were established by FT-IR and ^{1}H NMR spectroscopy. The FT-IR spectra provided useful information on the characteristic functional groups of the quinazolinone nucleus. The amide carbonyl (C=O) stretching vibration appeared within the 1682-1670 cm^{-1} range for the synthesized derivatives. This lower carbonyl frequency is consistent with the resonance interaction of the carbonyl group with the adjacent nitrogen atoms of the quinazolinone ring, which reduces the effective C=O bond order. The C=N stretching vibration was observed in the 1528-1512 cm^{-1} region, while the aromatic C=C vibration occurred near 1435 cm^{-1}. The ^{1}H NMR spectra were consistent with the proposed structures, with the aromatic protons appearing as multiplet signals in the range of δ 7.287-8.444 ppm and accounting for the expected 13H aromatic proton environment. The combined spectroscopic data support the formation of the cyclized quinazolinone framework.

Antioxidant activity: The antioxidant properties of the synthesized quinazolinone derivatives were evaluated by the DPPH radical-scavenging assay. The compounds displayed concentration-dependent radical-scavenging activity, although the magnitude of the response varied considerably with the nature and position of the N-3 substituent (Table-1). The difference between the substituted derivatives points to a positional contribution to radical-scavenging behaviour rather than an effect arising solely from the presence of a particular substituent. The heteroaryl-substituted derivatives exhibited substantial DPPH activity as well. The IC_{50} values of compounds **4b**, **4c**, **4d**, **4e**, **4f** and **4g** were lower than that of the reference compound, vitamin C (70.7170 mg/mL), indicating stronger antioxidant activity under the present experimental conditions. In contrast, compound **4a** exhibited a higher IC_{50} value (91.8067 mg/mL), indicating comparatively lower antioxidant activity. The observed activity agrees with previous reports on the free-radical-scavenging properties of quinazolinone and related heterocyclic systems [21-23]. The differences in antioxidant activity across the present series may be associated with variations in steric accessibility, electronic distribution and the spatial orientation of the N-3 substituent, which may affect its interaction with the DPPH radical.

TABLE-1
In vitro ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY DATA OF SYNTHESIZED COMPOUNDS

Compd.	IC_{50} value (mg/mL)	
	Antioxidant activity	Anti-inflammatory activity
4a	91.8067	24.1937
4b	55.5954	45.2367
4c	30.5152	83.7917
4d	24.0052	21.5197
4e	20.3526	24.9155
4f	22.5114	44.7666
4g	47.2830	50.8559
Vitamin C	70.7170	–
Diclofenac sodium	–	70.7017

Anti-inflammatory activity: The anti-inflammatory activity of the synthesized compounds was evaluated using the albumin protein-denaturation assay. Compounds **4d**, **4e**, and **4a** showed the lowest IC_{50} values of 21.5197, 24.9155 and 24.1937 mg/mL, respectively, which indicate higher activity than diclofenac sodium (70.7107 mg/mL). Compounds **4f**, **4b** and **4g** showed intermediate activity, with IC_{50} values of 44.7666, 45.2367 and 50.8559 mg/mL, respectively, whereas **4c** exhibited the highest IC_{50} (83.7917 mg/mL) and the lowest activity. Moreover, compound **4d** showed the most potent anti-inflammatory activity, while compound **4e** showed the most potent antioxidant activity (IC_{50} = 20.3526 mg/mL). The variation in activity may be associated with structural differences in the quinazolinone derivatives [24-28].

Conclusion

A newly developed 3-substituted-2-phenylquinazolin-4(3*H*)-one derivatives (**4a-g**) was successfully synthesized and characterized, establishing a defined structural matrix at the N-3 position of the 2-phenylquinazolinone framework. Spectroscopic analysis resolved a significant discrepancy in the reported literature concerning the resonance-stabilized cyclic amide stretching band, which was assigned within the range of 1682-1670 cm^{-1}. The *in vitro* biological evaluation established a clear dependence of antioxidant and anti-inflammatory activities on the nature of the N-3 substituent and the concentration employed. Among the synthesized deriva-

tives, compound **4e** exhibited the most potent antioxidant activity, with an IC_{50} value of 20.3526 mg/mL, followed by compounds **4f** and **4d** with IC_{50} values of 22.5114 and 24.0052 mg/mL, respectively. In the anti-inflammatory assay, compound **4d** exhibited the highest activity, with an IC_{50} value of 21.5197 mg/mL, followed by compounds **4a** and **4e** with IC_{50} values of 24.1937 and 24.9155 mg/mL, respectively. Several synthesized derivatives showed lower IC_{50} values than the respective reference compounds, Vitamin C and diclofenac sodium, under the same assay conditions. The variation in biological responses across the series provides a preliminary structure-activity relation-ship for N-3 substitution within the 2-phenylquinazolinone framework.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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