

Microwave-Assisted One-Pot Synthesis and Characterization and Biological Evaluation of N-Mannich Base of Quinazolin-4(3H)-one Derivatives as Potential Anti-Glioblastoma and Anti-Inflammatory Agents

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Abstract: Introduction: The quinazolin-4(3H)-one core is a crucial structural motif in medicinal chemistry, widely recognized for its exceptional chemical stability and a broad spectrum of pharmacological properties. Incorporating bioactive moieties via chemical modification, such as Mannich base synthesis, represents a powerful strategy for developing novel therapeutic agents. **Methods:** In this study, a series of six novel quinazolin-4(3H)-one-based N-Mannich base derivatives (3A–3F) were designed and successfully synthesized through a green, microwave-assisted one-pot method by reacting quinazolin-4(3H)-one, formaldehyde, and various primary aromatic amines. The chemical structures of all synthesized compounds were validated using Fourier-transform infrared (FT-IR), nuclear magnetic resonance (¹H-NMR), and high-resolution mass spectrometry (HRMS) analyses. The derivatives were evaluated in vitro for their anti-glioblastoma potential against U-87 Malignant Glioma cells using the MTT colorimetric assay, and for their anti-inflammatory activity using the egg albumin protein denaturation method with diclofenac sodium as a standard. **Results:** The microwave-assisted synthesis delivered high yields ranging from 75% to 95%. In biological evaluations, compound 3E—selected via prior computational docking investigations demonstrated moderate concentration-dependent anti-glioblastoma activity with an IC₅₀ value of 41.52 µg/mL. Furthermore, all six derivatives exhibited concentration-dependent anti-inflammatory efficacy by protecting protein from heat-induced denaturation. At the highest tested concentration (400 µg/mL), compound 3F exhibited the highest anti-inflammatory protection (87.58%), followed by 3E (83.00%) and 3D (80.71%), performing closely to the standard drug diclofenac (93.13%). Notably, at lower concentrations (100 µg/mL and 200 µg/mL), compounds 3E and 3F showed inhibition comparable to or higher than diclofenac at 100 µg/mL, and 3E matched diclofenac at 200 µg/mL. **Conclusion:** The findings indicate that the nature of the aromatic amine substituents attached to the quinazolin-4(3H)-one core heavily modulates biological potency. These derivatives, particularly 3E and 3F, represent promising lead candidates for advanced multi-target drug development in oncology and inflammatory disorders.

Keywords: Quinazolin-4(3H)-one, Mannich base, Microwave synthesis, Anti-glioblastoma, Anti-inflammatory, U-87 MG cells.

INTRODUCTION

Quinazolinone chemistry goes back to 1869, when anthranilic acid was treated with cyanogen in ethanol to yield a 2-ethoxy-4(3H)-quinazolinone product [1].

The basic structure of quinazolinone consists of a quinazoline ring and a ketone group. This bicyclic compound contains a pyrimidine ring fused with a benzene ring at the 4 and 8 positions[2].

The quinazoline ring comprises six carbon atoms and two nitrogen atoms located at the 1 and 4 positions [3]. As an oxidized form of quinazoline, quinazolinone retains the nitrogen atoms and is categorized as a nitrogen-containing heterocyclic compound[4]. The quinazolinone ring is notably stable toward oxidation, reduction and hydrolysis, and its breakdown by simple chemical oxidation has not been reported [5]. The stability of the quinazolinone nucleus has inspired medicinal chemists to introduce many bioactive moieties to this nucleus to synthesize new potential medicinal agents. The quinazolinone skeleton is a frequently encountered heterocycle in medicinal chemistry literature with applications including antibacterial, analgesic, anti-inflammatory, antifungal, antimalarial, antihypertensive, CNS depressant, anticonvulsant, antihistaminic, antiparkinsonism, antiviral and anticancer activities[5]. Chlorination proceeds because the methyl group is lost, but when the methyl group is present in the 2-position, the tautomeric effect is extended by generating an exomethylene carbon. These extended tautomeric effects are significant because they enhance the reactivity of the substituted 4(3H)-quinazolinones[6,7]. The methaqualone was synthesized and it is the most well-known 4(3H)-quinazolinone based drug and has sedative and muscle relaxant effects[8]. Structure-activity relationship (SAR) studies have significance of substituents at the 2, 6, and 8 positions of the quinazoline ring in determining pharmacological activity[9].

SAR of quinazoline-3(4H)-one

Position 2 Modifications

- **Anti-cancer activity:** Presence of alkyl or heteroaryl group at position 2 significantly enhances microtubule and receptor binding affinity[10].
- **Antimicrobial activity:** Having a methyl, amine, or thiol group at position 2 is necessary for strong antimicrobial action[11].
- **EGFR Selectivity:** Linking a 5-methylfuran-3-yl ring or complex heterocyclic rings to position 2 using a short thio-linker makes the drug highly active against cancer (EGFR) and matches clinical drugs like erlotinib[12][13].
- **Pyrazoline Tail Effects:** Adding a pyrazoline nucleus at position 2 gives different results based on its tail: a p-

chlorophenyl group increases anti-cancer activity, but a p-fluoro group reduces it [13].

Position 3 Modifications

- **Anti-cancer vs. Anticonvulsant:** Adding a bulky side chain (like a phenyl group) at position 3 gives good anti-cancer activity, while adding a butyl group or 5-membered heterocycles at position 3 optimizes its anticonvulsant effects [6].
- **Antimicrobial activity:** Combining a substituted aromatic ring at position 3 with specific groups at position 2 is essential for fighting microbes[11].

Position 4 Modifications

- **Antimicrobial performance:** Putting an amine or a substituted amine group at the 4th position optimizes antimicrobial activity[11].

Positions 6 and 7 Modifications

- **Pharmacokinetics:** Substitutions here are not necessary for the main action, but they control central nervous system (CNS) penetration, bioavailability, and in vivo toxicity [6].
- **Ring Reactivity:** Adding electron-withdrawing halogens increases ring reactivity, while adding methoxy groups decreases it [6].
- **Anti-inflammatory activity:** Adding halogens (chlorine or fluorine) at position R1 (C6/C7) boosts anti-inflammatory action, with a fluorine atom at C7 giving the strongest binding to the NF- κ B receptor[14].
- **Binding Strength:** Adding hydrophobic groups (halogens or phenyl groups) at position 7 improves drug-receptor binding via strong π - π stacking in active pockets like the ATP-binding site of EGFR [10].

Position 8 Modifications

- **Specific targets:** This position is highly specific. Adding bromine or an alkyl group here helps target tuberculosis (anti-TB) or high blood pressure (antihypertensive) activities [6].

Combined Combinations & Substituent Effects

- **Solubility & Bioavailability:** While putting alkyl/heteroaryl groups at position 2 improves binding, adding polar groups (like amino, hydroxyl, or amide) at

position 6 or 8 improves water solubility and delivery in the body [10].

- **Halogen Synergism:** Adding halogen atoms (especially iodine or bromine) at both positions 6 and 8 significantly improves antibacterial action[11].
- **Aliphatic Chain Length (R3 position):** Longer carbon chains increase hydrophobic interaction and gene inhibition. The best activity is seen with a 6-carbon chain (n-hexyl); extending it to 8 carbons creates steric hindrance and hurts activity [14].
- **Aromatic Ring Patterns (R3 position):** An electron-withdrawing group (-CF₃) improves iNOS gene inhibition, a meta-methyl group yields excellent COX-2 inhibition, but a para-methoxy group completely destroys receptor binding [14].
- **Electronic effects on the Benzene Ring:** Adding electron-donating groups (hydroxyl or methoxyl) creates powerful radical-scavenging antioxidants. Conversely, adding electron-withdrawing groups (chlorine or nitro) delivers excellent anti-inflammatory activity [15].

In this study, a series of novel quinazolin-4(3H)-one-based Mannich base derivatives were strategically designed and successfully synthesized utilizing quinazolin-4(3H)-one, structural amines, and aldehydes under microwave irradiation conditions. The chemical structures of the newly synthesized target compounds were systematically elucidated and validated using FT-IR, NMR, and Mass spectral analyses. The preliminary in vitro biological activity of the synthesized N-Mannich bases of quinazolin-4(3H)-one was evaluated for anti-glioblastoma and anti-inflammatory potential. The anti-glioblastoma activity of the most promising docked candidate, compound 3E, was assessed in U-87 MG human glioma cells using the MTT colorimetric assay, with the objective of determining whether 3E produced a concentration-dependent reduction in cell viability relative to untreated control cells. Cells were exposed to 3E at 10-100 µg/mL for 24 h, and viability was calculated from the absorbance of solubilized formazan at 570 nm. The IC₅₀, obtained by nonlinear regression (sigmoidal dose-response, variable slope; GraphPad Prism 6), was 41.52 µg/mL, indicating moderate cytotoxic activity. The anti-inflammatory activity of all six derivatives (3A-3F) was

assessed using the egg albumin protein denaturation assay, with diclofenac sodium as the reference drug, to determine whether the compounds protect protein from heat-induced denaturation, a process closely linked to inflammation, in a concentration-dependent manner. The compounds and diclofenac were tested at 100, 200 and 400 µg/mL in a 5 mL reaction mixture (egg albumin, PBS pH 6.4), incubated at 37 ± 2 °C for 15 min and then heated at 70 °C for 5 min. Absorbance was read at 660 nm, and percentage inhibition was calculated relative to the untreated control. All compounds inhibited denaturation in a concentration-dependent manner. At 400 µg/mL, 3F showed the highest inhibition (87.58%), followed by 3E (83.00%) and 3D (80.71%), against 93.13% for diclofenac, while 3A was the least active (58.17%). At 100 µg/mL, 3E and 3F (53.92% and 56.20%) exceeded diclofenac (50.98%), while 3C (45.09%) was slightly lower, and at 200 µg/mL 3E (76.47%) was almost identical to diclofenac (76.14%). These results suggest that the substituents on the quinazolin-4(3H)-one nucleus influence anti-inflammatory potential.

2. Materials and Methods

2.1 Materials

All the chemicals, solvents and reagents used in the synthesis of compounds were highly pure and of analytical grade. Quinazolin-4(3H)-one, Formaldehyde solution, P- amino benzoic acid, P- nitro aniline, Aniline, Sulphanilic acid, Benzidine, Sulphanilamide, ethanol were purchased from Loba Chemie pvt ltd with high purity and used without any further purification. Ethanol was purchased from Jones chemicals pvt. Ltd. The synthesized mannich reaction completion was analyzed using coated TLC plates. Deep Vision 230 V Melting point apparatus was used to record the melting point. Bruker Alpha 2 (Opus software) FTIR Spectrophotometer with a scan range of 4000-400 cm was used to record IR Spectrum, Bruker Avance III HDNanobay 400 MHz FT-NMR Spectrophotometer was used to measure NMR Signals and Waters - Xevo G2-XS-QToF Mass Spectrometer was used to record the mass spectra of the compounds.

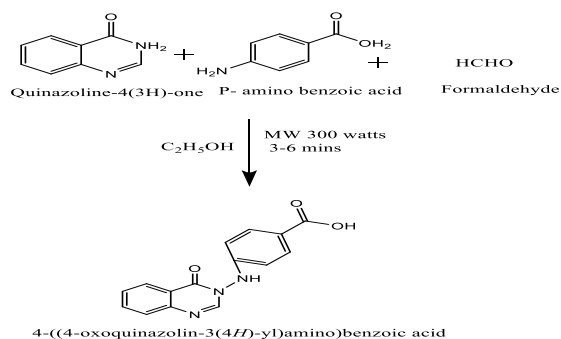
2.2 General procedure for the synthesis of N-mannich base of Quinazolin-4(3H)-one derivatives

The N-mannich base derivatives were synthesized by employing the existing literature with minor modifications [16]. An

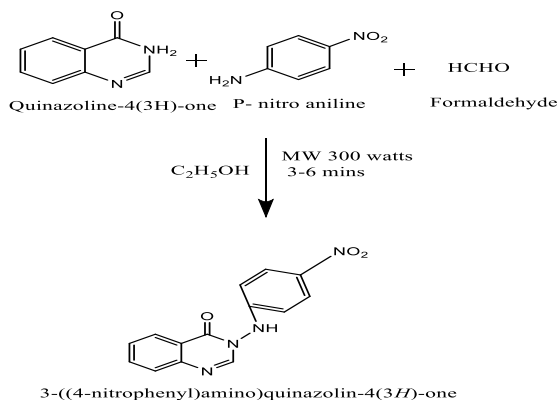
equimolar amount of Quinazoline-4(3H)-one, Formaldehyde solution, and various primary aromatic amines such as P-amino benzoic acid, P-nitro aniline, Aniline, Sulphanilic acid, Benzidine, Sulphanilamide, in ethanol were heated in microwave at the power of 300 watts for 3-6 mins. The completion of the reaction was monitored by TLC using precoated TLC plates. After the completion of the reaction the reaction mixture was cooled and kept overnight in refrigeration the product was collected by evaporating the solvent, and dried products were recrystallized using ethanol.

Scheme of Reaction

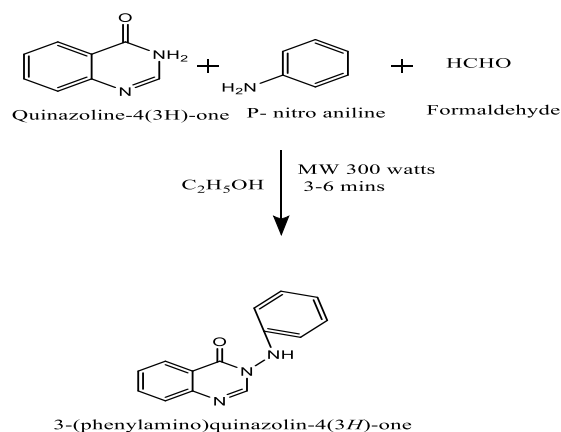
Compound 3A: Synthesis of [4-[(4-oxoquinazolin-3(4H)-yl)methyl]amino]benzoic acid]



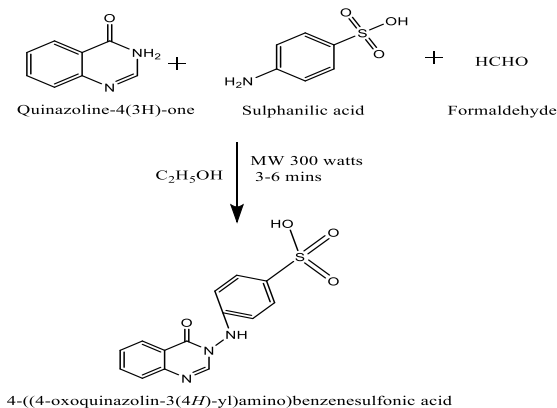
Compound 3B: Synthesis of [3-[(4-nitrophenyl)amino]methyl]quinazolin-4(3H)-one]



Compound 3C: Synthesis of [3-[(phenylamino)methyl]quinazolin-4(3H)-one]



Compound 3D: Synthesis of [4-[(4-oxoquinazolin-3(4H)methyl]amino] benzene sulfonic acid]



Compound 3E: Synthesis of [3-[(4'-amino-[1,1'-biphenyl]-4-yl)amino]methyl] quinazolin-4(3H)-one]

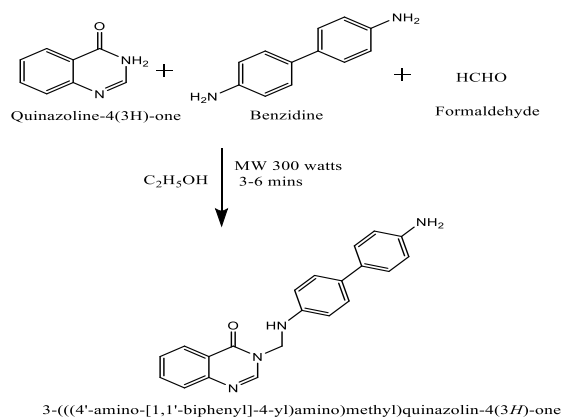




Figure 1. Photographic representation of synthesis of N-mannich base of quinazoline-4(3H)-one derivatives

2.3 Characterization

The synthesized N-mannich base of Quinazoline-4(3H)-one derivatives were characterized by melting-point determination, UV-visible spectroscopy, FT-IR spectroscopy, ^1H NMR, and mass spectrometry.

2.4.1 Anti-glioblastoma activity by using invitro cytotoxicity assay on u-87 cell line (mtt assay) [17,18]

Name of the study: Invitro Cytotoxicity activity of Synthetic compound 3E

Cell culture: U87 Cells line

Cytotoxicity testing is a key early step in judging the safety and therapeutic promise of new compounds. The MTT assay measures cell viability through metabolic activity. In viable cells, mitochondrial dehydrogenase enzymes convert the yellow, water-soluble tetrazolium dye MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into insoluble purple formazan, a step that dead or damaged cells cannot perform. The formazan was dissolved in DMSO and its absorbance, which correlates with the number of viable cells, was recorded [17,18].

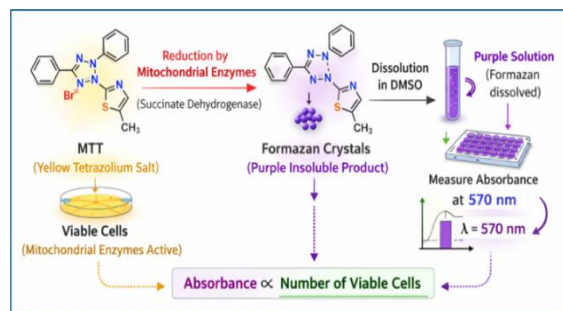


Figure 2. Reaction mechanism of MTT Assay

Materials Required

Chemicals and Reagents

- MTT reagent (5 mg/ml stock solution, (MP, India)
- Dimethyl sulfoxide (DMSO), (MP, India)
- Phosphate Buffered Saline (PBS), (Himedia, India)
- Cell culture medium (DMEM or appropriate medium), (MP, India)
- Trypsin-EDTA solution (Himedia, India)
- Test compound or treatment agent

Instruments

- CO₂ incubator ((EPPENDROF, Germany)
- 96-well cell culture plates (ABDOS, India)
- Microplate reader (ALTA ADX-150, India)
- Micropipettes and sterile tips (ABDOS India)
- Centrifuge (EPPENDROF, Germany)
- Inverted microscope (Olympus CKX53, Japan)

Procedure

• Cell seeding

Cells were seeded in 96-well plates at an appropriate density (typically 5×10^3 to 1×10^4 cells per well) and incubated overnight at 37°C in a humidified atmosphere containing 5% CO₂ to allow cell attachment.

• Treatment of cells

After incubation, cells were treated with different concentrations of the test compound and incubated for the required time period (usually 24–48 hours).

• Addition of MTT reagent

Following treatment, 10–20 µl of MTT solution (5 mg/ml) was added to each well containing culture medium.

• Incubation

Cells were incubated for 3–4 hours at 37°C to allow metabolically active cells to convert MTT into purple formazan crystals.

• Solubilization of formazan

After incubation, the culture medium containing MTT was carefully removed.

100–200 µl of DMSO was added to each well to dissolve the formazan crystals.

• Measurement of absorbance

The absorbance was measured using a microplate reader at 570 nm.

Control wells containing untreated cells were used as the reference for calculating cell viability.

$$\text{Cell Viability Inhibition (\%)} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

2.4.2 In vitro anti-inflammatory activity study by using Egg albumin denaturation assay

Inhibition of protein denaturation

Each 5 mL reaction mixture was prepared with 0.2 mL of egg albumin, 2.8 mL of phosphate-buffered saline (pH 6.4) and 2 mL of test sample at 100, 200 or 400 µg/mL; the control contained the same volume of double-distilled water in place of the sample. The mixtures were incubated in a BOD incubator at 37 ± 2 oC for 15 min and then heated at 70 oC for 5 min. Once cooled, absorbance was read at 660 nm against a vehicle blank. Diclofenac sodium (100, 200 and 400 µg/mL) served as the reference standard and was processed in the same way [19]. Percentage inhibition was calculated as:

$$\% \text{ of inhibition} = (\text{Abs of control} - \text{Abs of test}) / \text{Abs of control} \times 100$$

3. Results

3.1 Physical Characterization of Synthesized N-Mannich Base of quinazoline-4(3H)-one Derivatives

Table 1. Physical characterization data of synthesized derivatives

S.No	Compound ID	Molecular Formula	Molecular Weight (g/mol)	Colour	% Yield (W/W)	Melting point Range °C
1	1	3A	C16H13N3O3	295.29	Off white	93.84 % 186-189°C
2		3B	C15H12N4O3	296.28	Bright yellow	75.37 % 173-179°C
3		3C	C15H13N3O	251.29	white	78.63 % 162-168°C
4		3D	C15H13N3O4 _S	331.35	Off white	83.22 % 165-170°C
5		3E	C21H18N4O	342.39	Yellowish brown	95.42 % 205-211°C
6		3F	C15H14N4O3 _S	330.36	white	81.34 % 156-161°C

3.2 TLC Profile

TLC was carried out by using suitable various solvent system. N-Mannich bases of quinazoline-4(3H)-one derivatives were synthesized ,recrystallized and purified. The purity was confirmed by TLC .

Table 2. TLC Data and Rf Values of Synthesized quinazoline-4(3H)-one Derivatives

COMPOUND ID	MOBILE PHASE	Rf value
3A	Benzene: chloroform (5:5)	0.68
3B	Benzene: chloroform (5:5)	0.69
3C	Benzene: chloroform (5:5)	0.72
3D	Benzene: chloroform (5:5)	0.76
3E	Benzene: chloroform (5:5)	0.66
3F	Benzene: chloroform (5:5)	0.67

3.3 Solubility Studies

Table 3. solubilities study data of synthesized compounds (3A-3F)

S. No	Compound ID	SOLVENTS					
		Water	Ethyl acetate	Acetone	Ethanol	Chloroform	DM F
1	3A	++	++	+++	++	+++	+++
2	3B	++	++	+++	++	+++	+++
3	3C	++	++	+++	++	+++	+++
4	3D	++	++	+++	++	+++	+++
5	3E	++	++	+++	++	+++	+++
6	3F	++	++	+++	++	+++	+++

The solubility studies were performed for the synthesized compounds by using solvents of varying polarities. The results are summarized in Table 3.

Key words: +++ - Freely soluble ++ - Sparingly soluble

3.4 Characterization

Compound 3A: 4-((4-oxoquinazolin-3(4H)-yl)methyl)amino)benzoic acid: UV λmax (nm): 285. FT-IR (KBr, v, cm-1): 3452–3371 (O–H str of carboxylic acid overlapping with N–H str), 3170–3122 (secondary N–H str), 3045–3005 (aromatic C–H str), 2905–2806 (aliphatic C–H str), 1706 (C=O str of carboxylic acid), 1678 (C=O str of quinazolin-4-one), 1605 (C=N str), 1564–1450 (aromatic C=C str), 1379–1246 (C–N/C–O str). 1H-NMR (400 MHz, CDCl3, δ, ppm): 8.4 (s, 1H, C2-H of quinazolinone), 7.2–8.2 (m, 8H, Ar-H), 5.3 (s, 2H, -N-CH2-N-). HRMS (ESI-ToF) m/z: Found: 297.23 [M+H]+.

Compound 3B: 3 - (((4-nitrophenyl) amino) methyl) quinazolin – 4 (3H) - one: UV λmax (nm): 331. FT-IR (KBr, v, cm-1): 3362 (secondary N–H str), 3256–3140 (N–H/aromatic C–H str), 3072–3042 (aromatic C–H str), 2972–2818 (aliphatic C–H str), 1676 (C=O str of quinazolin-4-one), 1603 (C=N str), 1533 (asymmetric N–O str of –NO2 overlapping with aromatic C=C), 1389 (symmetric N–O str of –NO2), 1329–1252 (C–N str), 1184–1063 (C–N str). 1H-NMR (400 MHz, CDCl3, δ, ppm): 8.5 (s, 1H, C2-H of quinazolinone), 7.0–8.3 (m, 8H, Ar-H), 5.4 (s, 2H, -N-CH2-N-

), 5.1 (s, 1H, -NH-). HRMS (ESI-ToF) m/z: Found: 296.25 [M+H]⁺.

Compound 3C: 3-((phenylamino)methyl)quinazolin-4(3H)-one: UV λ_{max} (nm): 348. FT-IR (KBr, ν , cm⁻¹): 3276 (secondary N-H str), 3178–3115 (aromatic C-H str), 3057–3040 (aromatic C-H str), 2955–2845 (aliphatic C-H str), 1668 (C=O str of quinazolin-4-one), 1605 (C=N str), 1564–1458 (aromatic C=C str), 1369–1321 (C-N str), 1259–1022 (C-N/C-O str). 1H-NMR (400 MHz, CDCl₃, δ , ppm): 8.3 (s, 1H, C2-H of quinazolinone), 6.6–8.1 (m, 9H, Ar-H), 5.2 (s, 2H, -N-CH₂-N-), 4.5 (s, 1H, -NH-). HRMS (ESI-ToF) m/z: Found: 259.02 [M+H]⁺.

Compound 3D: 4 - (((4-oxoquinazolin - 3 (4H) -yl) methyl) amino) benzenesulfonic acid : UV λ_{max} (nm): 315. FT-IR (KBr, ν , cm⁻¹): 3418–3377 (O-H str of -SO₃H overlapping with N-H str), 3204–3132 (N-H str), 3092–3042 (aromatic C-H str), 2979–2868 (aliphatic C-H str), 1663 (C=O str of quinazolin-4-one), 1610 (C=N str), 1531–1468 (aromatic C=C str), 1406–1304 (asymmetric S=O str of -SO₃H), 1253–1171 (symmetric S=O str of -SO₃H), 1122–1034 (C-N str). 1H-NMR (400 MHz, CDCl₃, δ , ppm): 8.4 (s, 1H, C2-H of quinazolinone), 7.5 (s, 1H, -SO₃H), 6.9–8.2 (m, 8H, Ar-H), 5.3 (s, 2H, -N-CH₂-N-). HRMS (ESI-ToF) m/z: Found: 333.25 [M+H]⁺.

Compound 3E: 3-(((4' - amino - [1,1' - biphenyl] - 4 -yl) amino) methyl) quinazolin - 4 (3H) - one : UV λ_{max} (nm): 345. FT-IR (KBr, ν , cm⁻¹): 3371, 3348, 3305 (NH₂ str), 3238–3161 (N-H str), 3049, 3032 (aromatic C-H str), 2974–2837 (aliphatic C-H str), 1674 (C=O str of quinazolin-4-one), 1610 (C=N str), 1504, 1473 (aromatic C=C str), 1355–1242 (C-N str). 1H-NMR (400 MHz, CDCl₃, δ , ppm): 8.3 (s, 1H, C2-H of quinazolinone), 7.1–8.1 (m, 12H, Ar-H), 5.2 (s, 2H, -N-CH₂-N-), 4.6 (s, 1H, -NH-), 3.8 (s, 2H, -NH₂). HRMS (ESI-ToF) m/z: Found: 344.26 [M+H]⁺.

Compound 3F: 4 - (((4 - oxoquinazolin - 3 (4H) -yl) methyl) amino) benzene sulfonamide : UV λ_{max} (nm): 369. FT-IR (KBr, ν , cm⁻¹): 3373–3317 (N-H str of sulfonamide and secondary amine), 3205–3134 (N-H str), 3072 (aromatic C-H str), 2979–2845 (aliphatic C-H str), 1663 (C=O str of quinazolin-4-one), 1609 (C=N str), 1597–1499 (aromatic C=C

str), 1371–1323 (asymmetric S=O str of sulfonamide), 1249–1198 and 1151 (symmetric S=O str of sulfonamide), 1088–1011 (C-N str). 1H-NMR (400 MHz, CDCl₃, δ , ppm): 8.4 (s, 1H, C2-H of quinazolinone), 7.1–8.2 (m, 8H, Ar-H), 7.3 (s, 2H, -SO₂NH₂). HRMS (ESI-ToF) m/z: Found: 331.42 [M+H]⁺.

The Six N-Mannich bases of Quinazolin-4(3H)-one derivatives were synthesized through green microwave assisted one pot synthesis and all the synthesized compounds (3A-3F) were confirmed by using a capillary Melting Point Apparatus, IR, NMR, and Mass Spectroscopy.

Anti-Glioblastoma Activity by using Invitro Cytotoxicity Assay on U-87 Cell Line (MTT Assay)

Based on the preliminary in silico investigations, including target prediction and molecular docking profiles, the synthesized compound 3E exhibited the highest binding affinity and favorable interaction scores against glioblastoma-associated targets. Guided by these computational insights, the cytotoxic potential of the synthesized compounds 3E was evaluated against human cancer cell line U-87 Malignant Glioma for Anti- Glioblastoma activity. Cell viability was determined based on the absorbance at 570 nm of dissolved formazan crystals produced by metabolically active cells of the U-87 MG cell line. Higher absorbance values indicate higher cell viability, whereas reduced absorbance reflects increased cytotoxicity. Treatment with increasing concentrations of synthesized compound 3E (10–100 $\mu\text{g/mL}$) for 24 h, resulted in concentration-dependent changes in the viability of the U-87 MG cells compared with the untreated control group.

The percentage of cell viability was calculated and plotted to determine the cytotoxic effect of the treatment.

CELL VIABILITY INHIBITION(%) =

$$\frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100$$

Statistical Evaluation

IC₅₀ Value

- The half maximal inhibitory concentration (IC₅₀) represents the concentration of a compound required to inhibit 50% of cell viability under the experimental conditions. It is a widely used quantitative parameter to determine the cytotoxic potency of a compound in biological systems.

- IC₅₀ values were determined by constructing a dose–response curve that correlates the concentration of the tested compound with the percentage of cell viability obtained from the MTT assay.
- The cytotoxic effect of the compound increases as the concentration increases, leading to a reduction in viable cells. For the present study, U87 cells were treated with different concentrations of the sample (10–100 µg/mL) and cell viability was determined using the MTT assay. The absorbance values measured at 570 nm were converted to percentage cell viability relative to untreated control cells.
- The IC₅₀ value was calculated using nonlinear regression analysis based on a sigmoid dose–response (variable slope) model using GraphPad Prism version 6 (GraphPad Software, San Diego, CA, USA). The calculated IC₅₀ value for sample against U87 cells, indicating moderate cytotoxic activity.

MTT Assay Absorption Value

Table 4. In vitro cytotoxic activity of compound 3E against U-87 MG cells.

CELLS LINE : U87					SAMPLE NAME : 3E						
Blank	0	10	20	30	40	50	60	70	80	90	100
0.001	0.857	0.772	0.667	0.592	0.481	0.399	0.263	0.183	0.091	0.016	0.001
0.005	0.855	0.774	0.669	0.596	0.483	0.395	0.266	0.187	0.095	0.014	0.004
0.003	0.859	0.776	0.665	0.594	0.485	0.397	0.269	0.185	0.093	0.012	0.003
Ave	0.857	0.774	0.667	0.594	0.483	0.397	0.266	0.185	0.093	0.014	0.003
Percentage	0	10	22	31	44	54	69	78	89	98	100
Viability	100	90	78	69	56	46	31	22	11	2	0

IC₅₀ = 41.52 µg/mL

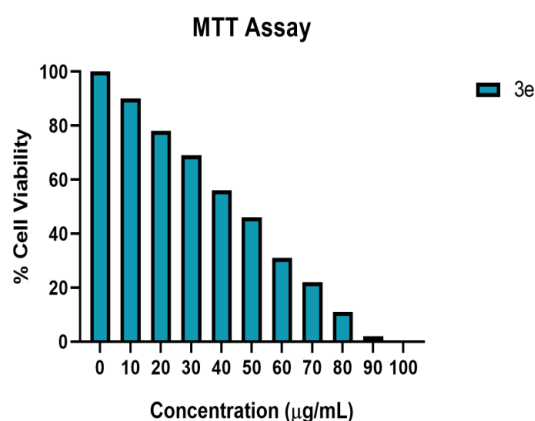


Figure 3. Cell viability percentage at different concentrations of synthesized compound 3E

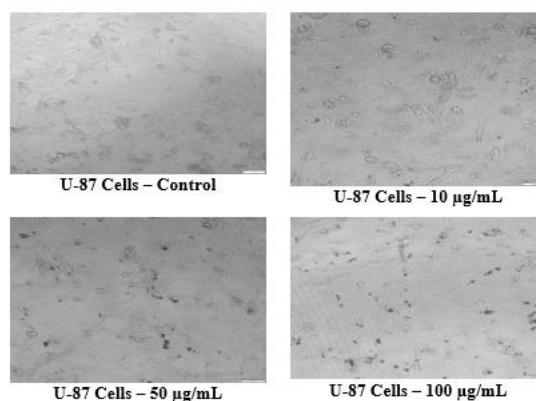


Figure 4. Microscopic image of U-87 GM cells under dose-dependent cytotoxicity assay

In-Vitro Anti-Inflammatory Activity using Egg Serum Albumin Protein Denaturation Assay

All the synthesized N-mannich bases of quinazoline-4(3H)-one derivatives, compounds (3A-3F) were evaluated for in-vitro anti-inflammatory activity. The six synthesized compounds of concentration 100 µg/ml, 200 µg/ml, 400 µg/ml were studied for inhibition and the values were compared against the standard drug Diclofenac and the results were shown in Table 5.

$$\% \text{ of inhibition} = (\text{Abs of control} - \text{Abs of test}) / \text{Abs of control} \times 100$$

Table 5. In-vitro anti-inflammatory activity using Egg albumin

S. No	Compound ID	Concentration (µg/ml)	Absorbance	% of protein inhibition
1	Control	-	0.306	-
2	Compound 3A	100	0.206	32.67%
		200	0.175	42.81%
		400	0.128	58.17%
3	Compound 3B	100	0.214	30.06%
		200	0.179	41.50%
		400	0.068	77.78%
4	Compound 3C	100	0.168	45.09%
		200	0.113	63.07%
		400	0.091	70.26%
5	Compound 3D	100	0.198	35.29%
		200	0.087	71.56%
		400	0.059	80.71%
6	Compound 3E	100	0.141	53.92%
		200	0.072	76.47%
		400	0.052	83.00%
7	Compound 3F	100	0.134	56.20%
		200	0.091	70.26%
		400	0.038	87.58%
8	Standard (Diclofenac)	100	0.150	50.98%
		200	0.073	76.14%
		400	0.021	93.13%

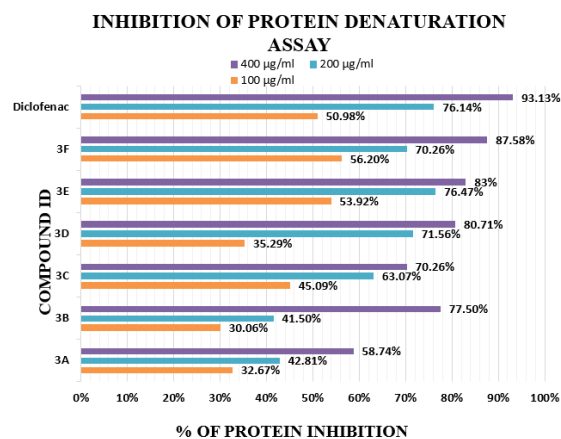


Figure 5. In vitro anti-inflammatory profile of synthesized derivatives (3A–3F) via inhibition of protein denaturation assay.

The in-vitro anti-inflammatory activity of N-Mannich base of quinazoline-4(3H)-one derivatives (3A-3F) was assessed in this study using egg albumin protein denaturation assay, a commonly used technique to evaluate a compound's efficiency to inhibit protein denaturation, a crucial step linked to inflammation. The inhibition of protein denaturation was observed in all tested quinazoline-4(3H)-one derivatives in a concentration-dependent manner. As shown in Fig. 5, Compound 3F showed the highest inhibition of any of the derivatives (87.58% at 400 µg/ml), followed by Compound 3E (83.00%) and Compound 3D (80.71%), which are similar to the standard drug diclofenac (93.13% at 400 µg/ml). Compound 3A exhibited the least amount of activity (58.17% at 400 µg/ml). The results clearly demonstrate that the specific substitutions on the core structure exhibited varying degrees of anti-inflammatory activity, indicating that both the type and chemical nature of substituents on the quinazoline-4(3H)-one nucleus significantly influence anti-inflammatory effects.

4. Conclusion

Six new N-Mannich bases of quinazolin-4(3H)-one (3A-3F) were synthesized by a quick, green microwave method in good yields (75-95%), and their structures were confirmed by FT-IR, NMR and mass spectra. Compound 3E reduced the viability of U-87 glioblastoma cells in a concentration-dependent manner ($IC_{50} = 41.52 \mu\text{g/mL}$). All six compounds also showed anti-inflammatory activity in the egg albumin denaturation assay, with 3F, 3E and 3D the most active and close to diclofenac.

These preliminary results suggest that quinazolin-4(3H)-one Mannich bases, especially 3E, are promising leads for further study, including tests on normal cells and mechanism studies.

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