

Synthesis, *In Vitro* Evaluation of Some Novel Quinazolin-4(3*H*)-one Derivatives as Anti-Tumor Agents

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Abstract: In an effort to develop anticancer agents, a series of Mannich bases were prepared by Mannich reaction. When one biologically active molecule is linked to another, the resultant molecule generally has increased potency. Hence two pharmacophores, *i.e.* quinazoline ring and amine moiety are fused to obtain highly potent, more specific and less toxic agent. In the present study, synthesis of novel quinazolin-4(3*H*)-one derivatives by condensation of appropriate quinazolines with various *p*-substituted primary amines in presence of glacial acetic acid and ethanol at room temperature (Mannich reaction). Synthesized compounds were characterized; both analytical and spectral data (UV, IR, GC-MS, ¹H NMR) of all derivatives were in full agreement with proposed structures. All the derivatives were tested for their anti-tumor activity by two *in vitro* studies like Brine shrimp Lethality assay and Trypan blue exclusion assay.

Keywords: Quinazolinones, Mannich reaction, Brine shrimp lethality assay, Trypan blue exclusion assay

Introduction

The search for new anticancer agent is one of the most challenging tasks to the medicinal chemist. Heterocyclic compounds are biologically important class of compounds. This prompted us to synthesize hybrid analogues of two pharmacophores *viz.*, quinazolinone and Mannich bases. In search for new bioactive potent molecule, it was thought worth while to incorporate some additional heterocyclic moieties in the quinazoline nucleus and study their biological and pharmacological activity, the extensive review of literature revealed the compounds have anticancer activity¹⁻⁴. The earliest uses in the pharmaceutical area for quinazoline are anti viral⁵, anti parkinsonism^{6,7}, anti microbial⁸⁻¹¹, anti inflammatory¹², bronchodilator¹³, anti hypertensive¹⁴. Encouraged by the above observations from the literature, it was planned to suitably incorporate the Mannich bases in to quinazolinone and to synthesize a better drug with less toxicity to the host, it is observed that chemical modification not only alters physicochemical properties but also pharmacological properties.

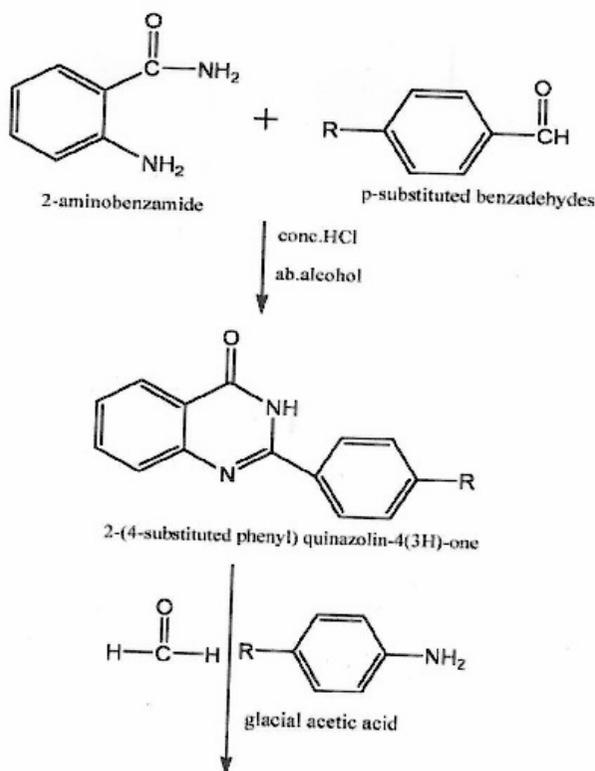
Experimental

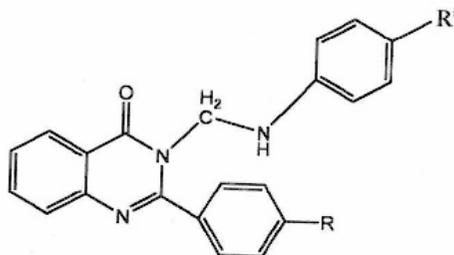
All the chemicals used in the synthesis were of Laboratory grade. The melting points were taken in open capillaries in liquid paraffin and are uncorrected. To monitor the progress of reactions and to establish the identity and purity of reactants and products, thin layer chromatography was performed on aluminium slides coated with silica gel 60, using appropriate solvent systems and the spots were visualized under ultra-violet light. IR spectra were recorded in KBr on a Shimadzu FTIR 8310 spectrometer. PMR spectrum on AMX 400 (400 MHz) instrument (chemical shift in δ ppm) using TMS as internal standard. Mass spectra were recorded in Shimadzu GC-MSQP5050. The structures of the synthesized compounds were confirmed by spectral analysis.

General procedure for the synthesis of Scheme-1 products

Step-1: Synthesis of 2-(4-substituted phenyl) quinazolin-4(3H)-one (QZ 1-4)

To the solution of 2-aminobenzamide (0.01 moles) and respective *p*-substituted benzaldehyde (0.01 moles) in 10 mL of ethanol, catalytic amount of acetic acid was added. The solution was refluxed and the progress of the reaction was monitored using TLC 15% ethyl acetate in chloroform (Solvent system: Ethyl acetate-Chloroform: 15:85). After completion of reaction, the reaction mixture was cooled to room temperature and the product obtained was filtered, washed with water and crystallized from absolute ethanol and chloroform (50:50). The physical data of the compounds synthesized were in confirmation with the literature value. The physical data of the compounds synthesized are given in Table 1.





Scheme 1

Table 1. Physicochemical data of synthesized compounds (QZM 1-12)

QZ series	Code	R	R ¹	M. F.	M.W.	R _f
1	QZ1M1	-Cl	-H	C ₂₁ ON ₃ ClH ₁₆	362	0.67
2	QZ1M2	-Cl	-CH ₃	C ₂₂ ON ₃ ClH ₁₈	376	0.63
3	QZ1M3	-Cl	-OCH ₃	C ₂₂ O ₂ N ₃ ClH ₁₈	392	0.65
4	QZ2M1	-F	-H	C ₂₁ ON ₃ FH ₁₆	345	0.58
5	QZ2M2	-F	-CH ₃	C ₂₂ ON ₃ FH ₁₈	359	0.6
6	QZ2M3	-F	-OCH ₃	C ₂₂ O ₂ N ₃ FH ₁₈	375	0.61
7	QZ3M1	-CH ₃	-H	C ₂₂ ON ₃ H ₁₉	341	0.61
8	QZ3M4	-CH ₃	-p-Cl	C ₂₂ ON ₃ ClH ₁₈	376	0.65
9	QZ3M5	-CH ₃	-p-Br	C ₂₂ ON ₃ BrH ₁₈	420	0.63
10	QZ4M1	-OCH ₃	-H	C ₂₂ O ₂ N ₃ H ₁₉	357	0.63
11	QZ4M4	-OCH ₃	-p-Cl	C ₂₂ O ₂ N ₃ ClH ₁₈	392	0.65
12	QZ4M5	-OCH ₃	-p-Br	C ₂₂ O ₂ N ₃ BrH ₁₈	436	0.63

Step-2: Synthesis of 2-(4-substituted phenyl) 3-[(4-substituted phenyl amino) methyl] quinazolin-4(3H)-one (QZM 1-12)- (Mannich reaction)¹⁵⁻¹⁷

To the solution of QZ 1-4 (0.01 moles) and respective *p*-substituted primary amines (0.01 moles) in 5 mL of glacial acetic acid, formaldehyde solution 30% (0.05 moles) was added. The solution was refluxed and the progress of the reaction is monitored using TLC Solvent system: Ethyl acetate-chloroform: 15:85. After completion of reaction, the reaction mixture was cooled and immediately poured to crushed ice. The product obtained was filtered, washed with methanol and crystallized from absolute ethanol and chloroform (50:50).

2-(4-Chloro phenyl)-3-[(phenyl amino) methyl] quinazolin-4(3H)-one (QZ1M1)

Yield 95%; m.p. 240-243 °C; IR (KBr, cm⁻¹): 3429.5 (N-H), 1670.2(C=O), 761.8 (C-Cl); H¹ NMR (CD₃OD, δ ppm): 4.97(s,2H, -CH₂), 7.1 (m,12H, Ar-H); Mass spectra(*m/z*): 363 (M+1), 119 (base peak).

*2-(4-Chloro phenyl)-3-[(*p*-tolyl amino) methyl] quinazolin-4(3H)-one (QZ1M2)*

Yield 91%; m.p. 232-233 °C; IR (KBr, cm⁻¹): 3429.2 (NH stretch), 1676.2(C=O), 2852.5 (C-CH₃), 761.8 (C-Cl); H¹NMR (CD₃OD, δ ppm): 4.97(s,2H, -CH₂), 7.1-8 (m,12H,Ar-H), 2.5 (s, 3H, Ar-CH₃); Mass spectra (*m/z*): 377 (M+1), 119 (base peak).

*2-(4-Chloro phenyl)-3-[(*p*-tolyl amino) methyl] quinazolin-4(3H)-one (QZ1M3)*

Yield 92%; m.p. 250-253 °C; IR (KBr,cm⁻¹): 3430(N-H), 1720(C=O), 1148 (C-OCH₃), 865 (C-Cl); H¹ NMR (CD₃OD, δ ppm) :3.97(s,3H,Ar OCH₃), 7.8 (m,12H, Ar-H); Mass spectra (*m/z*): 393 (M+1).

2-(4-Fluoro phenyl)-3-[(phenyl amino) methyl] quinazolin-4(3H)-one (QZ2M1)

Yield 96%; m.p. 219-223 °C; IR (KBr, cm⁻¹): 3458.1 (N-H), 1670.2(C=O), 1147.8 (C-F); H¹ NMR (CD₃OD, δ ppm): 4.97(s,2H, -CH₂), 7.4-8.3 (m,13H, Ar-H); Mass spectra (m/z): 345 (M+1).

2-(4-Fluoro phenyl)-3-[(p-tolyl amino) methyl] quinazolin-4(3H)-one (QZ2M2)

Yield 90%; m.p. 250-253 °C; IR (KBr, cm⁻¹): 3425.1 (N-H), 1675(C=O), 2920 (C-CH₃), 1145.8 (C-F); H¹ NMR (CD₃OD, δ ppm): 2.7 (s, 3H, Ar-CH₃), 4.9(s,2H, -CH₂), 7 -8.3 (m,12H, Ar-H); Mass spectra (m/z): 360 (M+1).

2-(4-Fluoro phenyl)-3-[(p-methoxy phenyl amino) methyl] quinazolin-4(3H)-one (QZ2M3)

Yield 91%; m.p. 225-228 °C; IR (KBr, cm⁻¹): 1725(C=O), 3425.1 (N-H), 1150 (C-OCH₃), 1140 (C-F); H¹ NMR (CD₃OD, δ ppm): 3.7 (s, 3H, Ar-OCH₃), 4.9(s,2H, -CH₂), 7.7 (m,12H, Ar-H); Mass spectra (m/z): 376 (M+1).

3-[(Phenyl amino) methyl]2-p-tolyl quinazolin-4(3H)-one (QZ3M1)

Yield 85%; m.p. 230-233 °C; IR (KBr, cm⁻¹): 2918 and 2854 (C-CH₃), 3458.1 (N-H), 1670.2(C=O); H¹ NMR (CD₃OD, δ ppm) : 4.97(s,2H, -CH₂), 7.4-8.3 (m,13H, Ar-H); Mass spectra (m/z): 341 (M+1).

3-[(4-Chlorophenyl amino) methyl] 2-p-tolyl quinazolin-4(3H)-one (QZ3M4)

Yield 89%; m.p. 145-148 °C; IR(KBr, cm⁻¹): 2854(C-CH₃), 3458.1(N-H), 1670.2(C=O), 865(C-Cl); H¹ NMR (CD₃OD, δ ppm) : 4.97(s,2H, -CH₂), 7.4-8.3 (m,13H, Ar-H); Mass spectra (m/z): 376 (M+1).

3-[(4-Bromo phenyl amino) methyl] 2-p-tolyl quinazolin-4(3H)-one (QZ3M5)

Yield 79%; m.p. 130-132°C; IR (KBr, cm⁻¹): 2918 (C-CH₃), 3458.1 (N-H), 753 (C-Br), 1670.2(C=O); H¹ NMR (CD₃OD, δ ppm) : 4.97(s,2H, -CH₂), 7.4-8.3 (m,13H, Ar-H); Mass spectra (m/z): 420 (M+1).

2-(4-Methoxy phenyl)-3-[(phenyl amino) methyl] quinazolin-4(3H)-one (QZ4M1)

Yield 89%; m.p. 257-259 °C; IR (KBr, cm⁻¹): 3429.2 (N-H), 1681.2(C=O), 2360 (C-CH₃); H¹ NMR (CD₃OD, δ ppm): 4.97(s,2H, -CH₂), 7.4-8.3 (m,13H, Ar-H); Mass spectra (m/z): 357 (M+1).

3-(4-Chloro phenyl amino methyl) -2-(p-methoxy phenyl) quinazolin-4(3H)-one (QZ4M4)

Yield 90%; m.p. 220-223 °C; IR (KBr, cm⁻¹): 1681.2(C=O), 3429.2 (N-H), 847 (C-Cl), 2360 (C-CH₃); H¹ NMR (CD₃OD, δ ppm) : 4.97(s,2H, -CH₂), 7.4-8.3 (m,13H, Ar-H); Mass spectra (m/z): 392 (M+1).

3-(4-Bromo phenyl amino) methyl) -2-(p-methoxy phenyl) quinazolin-4(3H)-one (QZ4M5)

Yield 92%; m.p. 240-243 °C; IR (KBr, cm⁻¹): 3429.2 (N-H), 1681.2(C=O), 784 (C-Br) , 2360 (C-CH₃); H¹ NMR (CD₃OD, δ ppm) : 4.97(s,2H, -CH₂), 7.4-8.3 (m,13H, Ar-H); Mass spectra (m/z): 436 (M+1).

Results and Discussion

Anti-tumor activity

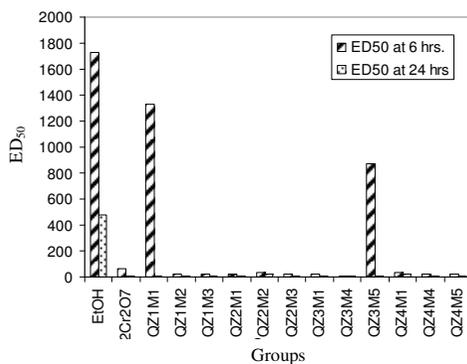
Anti-tumor activity was evaluated by two methods viz., Brine shrimp lethality bioassay and Trypan blue exclusion method.

Brine shrimp lethality bioassay

This method was developed by Mayer *et al*¹⁸. Drug samples were prepared by dissolving 10 mg of derivative in 10 mL of ethanol. Naupli were drawn in a pipette along with water and ten shrimps were transferred to each sample vial after they were counted in the stem of pipette against lighted background. The artificial sea water was added to each vial to make up to 5 mL, a drop of dry yeast suspension (3 mg in 5 mL seawater) was added to each vial as food for shrimps³. The vials were maintained under illumination. After 24 hours, survivors were counted by using 3X magnifying glass and the percent deaths, ED₅₀ values are calculated by using +ve control K₂Cr₂O₇ and -ve control ethyl alcohol and the results are shown in Table 2 and Figure 1. Composition of the artificial sea water is given in Table 3.

Table 2. ED₅₀ of synthesized compounds

Code	ED ₅₀	
	Acute toxicity (6 h)	Chronic toxicity (24 h)
QZ1M1	1328.35	6.08
QZ1M2	21.02	5.83
QZ1M3	17.98	8.14
QZ2M1	21.06	5.79
QZ2M2	32.97	21.03
QZ2M3	18.22	4.12
QZ3M1	21.48	6.63
QZ3M4	6.83	5.22
QZ3M5	874.17	6.65
QZ4M1	33.27	21.48
QZ4M4	21.02	5.79
QZ4M5	21.26	6.23
K ₂ Cr ₂ O ₇	62.76	6.23
Ethyl alcohol	1729.37	475.86

**Figure 1.** ED₅₀ of synthesized compounds*Trypan blue exclusion method*

EAC (Ehrlich Ascites Carcinoma) cells were collected by aspiration from animal peritoneum. A suspension of 1x10⁶ cells was prepared in phosphate buffer saline (PBS its composition was shown in Table 4) and the viability of the cell was checked by counting with a haemocytometer. DMSO was taken as solvent as solubilizing agent and its concentration was not allowed to go beyond 0.1% (v/v) of total volume. Various concentrations of the derivative *viz.*, 10 µg, 25 µg and 50 µg was taken in a clean test tube added 100 µL of the EAC cells

final volume was adjusted with buffer up to 1 mL respectively. All the test tubes were incubated at 37 °C for 3 h along with control and solvent control. After incubation, 100 µL of 4% trypan blue was added to each test tube. The alive cells (with out stain) and dead cells (stained) were calculated and checked for the percentage cytotoxicity¹⁹. The % cytotoxicity and IC₅₀ of synthesized compounds at different concentrations are given in Table 5. IC₅₀ of synthesized compounds against EAC Cells was graphically represented in Figure 2.

$$\% \text{ Cytotoxicity} = (T_{\text{dead}} - C_{\text{dead}}) / T_{\text{total}} \times 100$$

Where, T_{dead} = number of dead cells in the drug treated tube,
 C_{dead} = number of dead cells in the control tube,
 T_{total} = number of dead and alive cells in the drug treated tube

Table 3. Composition of the artificial sea water

Composition of artificial sea water (Modified from morel's Aquil formula)	
Components	g/L
Sodium chloride LR	24.0
Calcium chloride LR	1.5
Potassium bromide LR	0.1
Potassium chloride LR	0.7
Sodium sulphate LR	4.0
Sodium bicarbonate LR	0.2
Magnesium Chloride LR	11.0
Total salts	41.5

The pH should be adjusted to pH 7-8 with sodium bicarbonate

Table 4. Composition of the phosphate buffer saline (PBS)

Ingredients	g/1000 mL
Sodium chloride	8 g
Potassium chloride	200 mg
Disodium hydrogen phosphate	1.15 g
Calcium chloride	100 mg
Magnesium sulphate hepta hydrate	120 mg.

Table 5. % Cytotoxicity and IC₅₀ of synthesized compounds at different concentrations

Code	% Cytotoxicity			IC ₅₀
	10 µg/mL	25 µg/mL	50 µg/mL	
QZ1M1	16.58	17.88	72.91	37.87
QZ1M2	34.09	40.89	65.78	32.12
QZ1M3	19.45	75.53	62.87	25.49
QZ2M1	37.28	57.14	33.59	12.62
QZ2M2	47.43	57.86	68.79	13.01
QZ2M3	25.64	40.24	79.39	19.84
QZ3M1	24.45	74.19	72.60	21.75
QZ3M4	16.67	65.67	96.87	23.28
QZ3M5	15.52	75.08	90.34	22.41
QZ4M1	16.93	60.77	88.09	25.25
QZ4M4	11.13	64.68	88.15	25.78
QZ4M5	17.62	60.18	91.74	24.70
Control (DMSO)	10.20	12.03	13.52	500.83
Standard drug (Cisplatin)	16.28	16.2	23.53	191.16

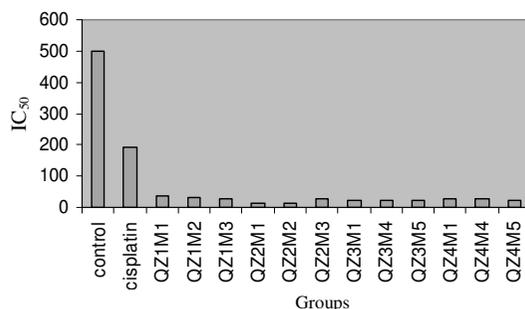


Figure 2. IC₅₀ of synthesized compounds against EAC cells

Conclusion

The synthesized derivatives were characterized and identified on the basis of physical and spectral data. The derivatives were tested for *in vitro* cytotoxic potential using brine shrimp lethality bioassay and trypan blue exclusion method. As the Brine shrimp method is preliminary investigation for the assessment of cytotoxicity, all the synthesized derivatives are screened for this bioassay. ED₅₀ values for the drugs and controls at 50, 100 and 200 ppm concentrations were calculated. ED₅₀ of +ve control (K₂Cr₂O₇) was found to be 62.76 & 6.23 in acute and chronic studies respectively. While ED₅₀ of -ve control (ethyl alcohol) was found to be 1729.37 & 475.86 in acute and chronic studies respectively. All the ED₅₀ values of the synthesized drugs were found to be significantly lower than that of -ve control ethyl alcohol and higher than the +ve control K₂Cr₂O₇. QZ1M2, QZ2M2, QZ2M3, QZ3M4, QZ3M5, QZ4M4, QZ4M1 and QZ4M5 derivatives were found to have highest cytotoxicity in Brine shrimp lethality Bioassay. Since all the compounds are active, investigation proceeded for trypan blue exclusion method using EAC cells.

All the synthesized compounds were screened for their cytotoxicity on EAC cells at 10, 25 and 50 µg/mL. The trypan blue exclusion technique indicates that the IC₅₀ of the standard drug cisplatin is 191.16 and that of control was 500.83. All the compounds displayed cell necrosis above 10 µg/mL. It reveals that cell necrosis is the main reason for the cell death. In some cases morphological changes in cells were found. It is suspected to be cell apoptosis, which might be the reason for cell death. All the synthesized drugs showed the activity. But promising anti-tumor activity was shown by compounds QZ1M2, QZ2M3, QZ3M4 and QZ4M which might be attributed to the presence of electron withdrawing and donating substitution at para or 4th position of the phenyl ring which is at position 2 of quinazolinone ring and in Mannich bases. Thus, the synthesized quinazolinone Mannich compounds containing halogens were more lipophilic than others as per the value. As the compounds are lipophilic in nature they can display good permeability through the phospholipids membranes and so a better activity can be expected.

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