SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL TRIAZOLYL QUINAZOLIN-4-ONE DERIVATIVES AS ANTICANCER AGENTS

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ABSTRACT
A novel series of triazolylquinazolin-4-one derivatives have been synthesized and characterized by TLC, melting point, FT-IR, 1H NMR and mass spectroscopy data. The synthesized series of title compounds were subjected for docking studies using Schrodinger Glide software, evaluated for their potential to inhibit enzyme EGFR-tyrosine kinase followed by in-vitro anticancer activity by SRB assay method on HeLa, MCF-7, A-549 cell lines. The series of compounds shows anticancer activity probably by inhibiting the enzyme EGFR-tyrosine kinase.

KEYWORDS: Anticancer activity, EGFR, Molecular Docking, SRB assay, Synthesis, Tyrosine Kinase, triazolylQuinazolin-4-ones.

1. INTRODUCTION
Cancer is a disease category in which unregulated cells in the body form, spreading between organ and other body bodies, according to the World Health Organization (WHO). In India as well as internationally, cancer is the leading cause of death. Cancer diagnosis and care remain a significant health concern in low- and middle-income countries. In several cell phases including metabolism, cell proliferation, apoptosis, and survival, tyrosine kinases are essential. Cancer is commonly observed in all ages and gender[1]. Tyrosine kinase's overexpression triggers the development of the tumour [2]. The best approach in designing modern cancer therapies is blocking tyrosine kinases. The main targets for cancer inhibition are EGFR, VEGFR, HER2, PDGFR, mTOR, HGF, FGFR [3]. Quinazolin-4-ones have a range of pharmacological potentials, including antimicrobial, antifungal, anticonvulsant, antifungal, anti-oxidant, alpha glucosidase inhibitor[4-7]. Nitrogen, which comprises five chemicals, is known as anti-microbial, antifungal, antitumor, antiureasis and anti-bacterial [8-11]. In our current study, we have synthesised 9 replacements, TLC, Melting point, FT-IR, 1H NMR and Mass spectral tests for triazolylquinazoline-4-ones. Molecular docking of synthesised compounds with EGFR protein was performed to control molecular interactions. EGFR tyrosin kinase was tested by enzymes, and the title compounds were inhibited. Synthesized compounds anti-cancer operation was also carried out by SRB research procedure on three separate cancer cell lines.
2. MATERIALS AND METHODS

Materials
Merck, S.D Fine Chemicals, and Loba Chemicals were the manufacturers of both the chemicals and solvents used for synthesis. A silica gel G 60 3 (Merck, Germany) was used to monitor the progression of reacción and purity of compounds. TLC was used. Melting points have been developed and uncorrected using Gallenkamp electric melting point instruments. The 1H NMR spectrum was recorded on the BRUKER AVANVEI 400 spectrophotometer (Germany) with DMSO-d6 in the form of solvent and chemical shipments expressed as τ in ppm against TMS as internal standard. The 1H NMR spectra were recorded using Shimadzu FT-IR spectrophotometer.

Methods
Synthesis of TriazolylQuinazolin-4-ones (QT01-QT09)

STEP-1: Synthesis of Benzoazizinone [12]
The benzoic acid of 2-amino (0.1 mol) was dissolved by shaking into a minimum of dry pyridine volume (30 mL). This solution was applied with a steady stirring slowly by adding benzoyl chloride (0.2 mol) in dry pyridine (30 ml). The resultant solution was vigorously agitated with the mechanical agitator after full addition. The resulting mixture has been retained and treated with 10 percent sodium bicarbonate at room temperature for 60 minutes. Sodium bicarbonate solutions were added before the evolution of carbon dioxide had stopped their effervescence. The solid was stripped and washed again and again with cold water to eliminate the scent of pyridine and unresponsive benzoyl chloride. The final benzoazizinone was dried using the diluted ethanol vacuum, which produced a pure analysis sample of 2-phenyl-benzo[d][1, 3]-oxazine-4-one as white crystalline mass.

STEP-2: Synthesis of 3-substituted-5-amino-1, 2, 4-triazole) [13]
A portion (0.1 mol) of Aminoguanidinehydrocarbonate was mixed with (0.1 mol) of substituted carboxylic acid and (0.1 mol) of a 33.5% HCl solution. The resulting mixture was heated to boiling under agitation and water was gradually evaporated until the temperature of the reaction mixture reached a value of 180°C. Then the reaction mixture was kept at 180–185°C for 3 h, cooled to approximately 100°C, and a solution (0.1 mol) of NaOH in 20 ml of water was added. The resulting mixture was boiled under agitation for 10 min, neutralized with a 20% HCl solution to pH 8–9, and cooled to 3–5°C. The precipitate formed was filtered off, recrystallized from water, and dried at 130°C.

STEP-3: Synthesis of Substituted triazolylquinazolinones[12]
Benzoazizinone and 3-substituted-5-amino-1, 2, 4-triazole mixture was fused for 30 minutes in an oil bathtub at 250oC. The mixture was cooled and the mixture was supplemented with methanol. Using ethanol, the separated solid was eventually rinsed with methanol, dried and replenished.
Scheme 1. Synthetic route for synthesis of Triazoly1-Quinazolin-4-ones

2-Phenyl-3-(1H-1,2,4-triazol-5-yl)-quinazolin-4(3H)-one (QT01)
Molecular formula: C_{16}H_{13}N_{5}O; % Yield: 73.15; MW: 289.29; M. P.: >300°C; TLC mobile phase: Toluene: Methanol(5:5) Rf value: 0.64; FT-IR (KBr, cm⁻¹): 2854, 1699, 1606, 1332; ¹H NMR (DMSO-đ₆): δ ppm: 7.25-7.42 (m, 4H, Ar-H); 7.55-7.66 (m, 5H, Ar-H); 8.21 (s, 1H, CH); 14.30 (s, 1H, NH); MS: m/z 288.12 (M-1)

2-Phenyl-3-(3-phenyl-1H-1,2,4-triazol-5-yl)-quinazolin-4(3H)-one (QT02)
Molecular formula: C_{21}H_{15}N_{5}O; % Yield: 60.20; MW: 365.39; M. P.: >300°C; TLC mobile phase: Toluene: Methanol(5:5) Rf value: 0.68; FT-IR (KBr, cm⁻¹): 3047, 1699, 1606, 1246; ¹H NMR (DMSO-đ₆): δ ppm: 7.30-7.36 (m, 5H, Ar-H); 7.48-7.52 (m, 4H, Ar-H); 7.58-7.67 (m, 5H, Ar-H); 14.77 (s, 1H, NH); MS: m/z 364.12(M-1)

2-Phenyl-3-(3-pyridin-2-yl)-1H-1,2,4-triazol-5-yl)-quinazolin-4(3H)-one (QT03)
Molecular formula: C_{21}H_{14}N_{6}O; % Yield: 85.90; MW: 366.38; M. P.: >300°C; TLC mobile phase: Toluene: Methanol(5:5) Rf value: 0.69; FT-IR (KBr, cm⁻¹): 3061, 1695, 1664, 1338; ¹H NMR (DMSO-đ₆): δ ppm: 7.31-7.37 (m, 5H, Ar-H); 7.51-7.68 (m, 4H, Ar-H); 7.96-7.98 (m, 4H, Ar-H); 13.94 (s, 1H, NH); MS: m/z 365.20(M-1)
2-Phenyl-3-(3-(pyridin-3-yl)-1H-1,2,4-triazol-5-yl)-quinazolin-4(3H)-one (QT04)
Molecular formula: C_{21}H_{14}N_{8}O; % Yield: 55.10; MW: 366.38; M. P.: >300°C; TLC mobile phase: Toluene: Methanol(5:5) Rf value: 0.69; FT-IR (KBr, cm⁻¹): 2922, 1737, 1691, 1602, 1562, 1471; ¹H NMR (DMSO-d₆): δ ppm: 7.31-7.43 (m, 5H, Ar-H); 7.60-7.74 (m, 4H, Ar-H); 8.15-8.29 (m, 4H, Ar-H); 14.32(s, 1H, NH); MS: m/z 364.12 (M-1)

2-Phenyl-3-(3-(pyridin-4-yl)-1H-1,2,4-triazol-5-yl)-quinazolin-4(3H)-one (QT05)
Molecular formula: C_{21}H_{14}N_{8}O; % Yield: 85.86; MW: 366.38; M. P.: >300°C; TLC mobile phase: Toluene: Methanol(5:5) Rf value: 0.69; FT-IR (KBr, cm⁻¹): 3072, 1691, 1600, 1288; ¹H NMR (DMSO-d₆): δ ppm: 7.21-7.35 (m, 5H, Ar-H); 7.65-7.68(m, 4H, Ar-H); 8.22-8.23 (m, 4H, Ar-H); 14.05 (s, 1H, NH); MS: m/z 365.17 (M-1)

3-(3-(2-chlorophenyl)-1H-1,2,4-triazol-5-yl)-2-phenylquinazolin-4(3H)-one (QT06)
Molecular formula: C_{22}H_{14}ClN_{3}O; % Yield: 77.60; MW: 399.83; M. P.: >300°C; TLC mobile phase: Toluene: Methanol(5:5) Rf value: 0.76; FT-IR (KBr, cm⁻¹): 3037, 1699, 1664, 1300, 700; ¹H NMR (DMSO-d₆): δ ppm: 7.32-7.40(m, 5H, Ar-H); 7.50-7.58 (m, 4H, Ar-H); 7.63-7.68(m, 4H, Ar-H)14.65(s, 1H, NH); MS: m/z 399.12 (M-1)

3-(3-(chlorophenyl)-1H-1,2,4-triazol-5-yl)-2-phenylquinazolin-4(3H)-one (QT07)
Molecular formula: C_{22}H_{14}ClN_{3}O; % Yield: 86.04; MW: 399.83; M. P.: >300°C; TLC mobile phase: Toluene: Methanol(5:5) Rf value: 0.72; FT-IR (KBr, cm⁻¹):2920, 1737, 1701, 1294, 769; ¹H NMR (DMSO-d₆): δ ppm: 7.30-7.36 (m, 5H, Ar-H); 7.55-7.59(m, 4H, Ar-H); 7.93-7.97(m, 4H, Ar-H); 13.90 (s, 1H, NH); MS: m/z 398.12 (M-1)

3-(3-(4-chlorophenyl)-1H-1,2,4-triazol-5-yl)-2-phenylquinazolin-4(3H)-one (QT08)
Molecular formula: C_{22}H_{14}ClN_{3}O; % Yield: 63.53; MW: 399.83; M. P.: >300°C; TLC mobile phase: Toluene: Methanol(8:2) Rf value: 0.69; FT-IR (KBr, cm⁻¹): 3095, 1674, 1602, 1334, 765; ¹H NMR (DMSO-d₆): δ ppm: 7.30-7.36 (m, 5H, Ar-H); 7.60-7.67(m, 4H, Ar-H); 7.91-7.97(m, 4H, Ar-H); 14.91(S, 1H, NH); MS: m/z 398.11 (M-1)

3-(2-chloro-4-fluorophenyl)-1H-1,2,4-triazol-5-yl)-2-phenylquinazolin-4(3H)-one (QT09)
Molecular formula: C_{23}H_{13}ClFN_{4}O; % Yield: 87.82; MW: 417.82; M. P.: >300°C; TLC mobile phase: Toluene: Methanol(6:4) Rf value: 0.67; FT-IR (KBr, cm⁻¹): 3066, 1699, 1606, 1330, 1141, 698; ¹H NMR (DMSO-d₆): δ ppm: 7.32-7.42(m, 5H, Ar-H); 7.65-7.72(m, 3H, Ar-H); 7.95-7.98 (m, 4H, Ar-H); 14.69 (s, 1H, NH); MS: m/z 417.14 (M-1)

Molecular Docking
Molecular docking has been carried out to detect receptor and ligand binding. This analysis was conducted on the Schrodinger module of Maestro-Glide (Schrodinger Inc., USA) [14-20]. The EGFR-tyrosin protein crystalline structure was obtained from a database of proteins (PDB Code: 1M17) (PDB). A Protein Preparation Wizard in Maestro 8.0 was used to prepare the protein. EGFR-tyrosine kinase receivers were successfully used and tested for the interactions between the docking and the docking score of all the title compounds.

EGFR-tyrosine kinase Inhibitory activity
The compounds QT01-QT09 were subjected to in vitro cell free EGFR enzyme inhibition determination [21-23] by using Z-LYTE Kinase Assay Kit according to manufacturer’s instructions by ELISA assay method.

Anticancer Activity
SRB research [24-25] assessed the anti-cancer activity. The cells were cultivated in a medium
containing RPMI 1640 foetal serum bovine and L-glutamine. 96 well microtiter plates inoculated in 90 μL with 5000 cells per well. At 37°C, 5 percent of CO2, 95 percent of air and 100 percent of humidity for 24 hours before synthesising drugs were incubated at the same time as inoculation of the cells. In order to prepare inventory of 10-2 concentrations, experimental drugs were solubilized in the correct solvent. Four series 10-fold dilutions with the complete medium were made at the time of experiment. The correct micro-titter wells previously containing 90 μl medium were applied to 10 μl quotls of the different drug dilutions resulting in required final medicinal concentrations.

Following the addition of compound plates, the addition of cold TCA was incubated for 48 hours. The cells were slowly fixed in loc by 50 μl of cold 30% TCA at a final concentration of 10% TCA and incubated 1 hour at 4°C. The cells were fixed in situ. The top layer has been discarded and the panels have been rinsed and tap water has been dried several times. A solution of sulforhodamine B (SRB) at a concentration of 50 μl at 0.4% (w/v) was applied to each well at 1% acetic acid. Plates again incubated at the room temperature for 20 minutes. With 1% acetic acid and air dry, the unbound colouring has been eliminated. Subsequently, bound stain was eluted with 10 mM trizma base and absorption read at 540 nm wavelength with reference wavelength of 690 nm on an Elisa reader.

The growth of percentage of wells connected to control wells was measured on a platform-by-plate basis. The growth of percentage was indicated as the ratio of average test uptake well to average control well absorption*100. The percent growth of each medication concentration level has been measured using the six absorptive measurements [time zero (Tz), control growth (C), and test growth in the drug's presence at the four concentration levels (Ti)]. For each test article, the dose response parameters have been determined. Calculated from, 50% inhibition of growth (GI50),

\[ \frac{(T_i - T_z)}{(C - T_z)} \times 100 = 50 \]  ---Formula 1

Which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from,

\[ T_i = T_z. \]  ---Formula 2

The LC50 (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from,

\[ \frac{(T_i - T_z)}{T_z} \times 100 = -50. \]  ---Formula 3

Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the values for that parameter were expressed as greater or less than the maximum or minimum concentration tested.

3. RESULTS AND DISCUSSION

Table 1: The physical properties of title compounds

<table>
<thead>
<tr>
<th>COMP CODE</th>
<th>MOL FORMULA</th>
<th>MOL WEIGHT gm/mole</th>
<th>MELTING POINT°C</th>
<th>% YEILD</th>
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<tbody>
<tr>
<td>QT-01</td>
<td>C_{16}H_{11}N_{2}O</td>
<td>289.29</td>
<td>&gt;300</td>
<td>73.15</td>
</tr>
<tr>
<td>Compound Code</td>
<td>Structure</td>
<td>Docking score</td>
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<td>---------------</td>
<td>-----------</td>
<td>---------------</td>
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<td></td>
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<td></td>
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<td>-6.285</td>
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Table 2: Docking study of compounds against the EGFR Tyrosine Kinase (PDB Code: 1M17)
<table>
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<th>QT 06</th>
<th>![Chemical Structure]</th>
<th>-6.235</th>
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<td>QT 07</td>
<td>![Chemical Structure]</td>
<td>-7.465</td>
</tr>
<tr>
<td>QT 08</td>
<td>![Chemical Structure]</td>
<td>-6.457</td>
</tr>
<tr>
<td>QT 09</td>
<td>![Chemical Structure]</td>
<td>-5.784</td>
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</table>
Figure 1: Molecular docking of synthesized compounds with EGFR-tyrosine kinase and along with possible hydrogen and hydrophobic bonding interactions. The SP Docking Protocol of the Maestro glide module was used for molecular quinazoline docking studies (Figure 1). EGFR Tyrosine Kinase crystal structure was extracted from the protein database (PDB ID:1M17). The important affinity towards EGFR Tyrosine Kinase has been demonstrated among the quinazoline derivatives QT07, QT06, QT04, QT03 (Table 2).
EGFR Inhibition Activity:
An additional EGFR kinase trial to research the postulated mechanism of action of the present synthesized compounds was performed for their EGFR over-expression. QT01-QT09 compounds have all been subjected by the manufacturer to inhibition determination in vitro cell free EGFR enzyme by the Z-LYTE Kinase Assay Kit as per the ELISA assay method directions. Table 3 indicates the percentage of inhibition of title compounds of EGFR-tyrosine kinase. It shows that compound QT07 showed high EGFR inhibition (% Inhibition=75.54%), compared to normal (percent Inhibition=96%). The findings of enzyme tests suggest that the title compound sequence shows strong activity of EGFR-inhibitors and may have good anti-cancer activity in various cancer cell lines.

Table 3: The % inhibition of EGFR-tyrosine kinase by title compounds.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Compound Code</th>
<th>% Inhibition</th>
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<tbody>
<tr>
<td>1</td>
<td>QT01</td>
<td>73.1</td>
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<tr>
<td>2</td>
<td>QT02</td>
<td>48.56</td>
</tr>
<tr>
<td>3</td>
<td>QT03</td>
<td>29.64</td>
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<td>QT09</td>
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<tr>
<td>10</td>
<td>Standard</td>
<td>96</td>
</tr>
</tbody>
</table>

Anticancer Activity (SRB Assay)

Table 4: The % growth inhibition of synthesized compounds on different cell lines a) Hela b) MCF-7 c) A-549

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample Code</th>
<th>Hela</th>
<th>MCF-7</th>
<th>A-549</th>
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<tr>
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<td>3</td>
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<td>NE</td>
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<td>4</td>
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<td>NE</td>
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<td>NE</td>
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<td>6</td>
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<td>NE</td>
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<td>7</td>
<td>QT07</td>
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<td>1.54</td>
<td>0.154</td>
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</tr>
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<td>9</td>
<td>QT09</td>
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<tr>
<td>10</td>
<td>ADR</td>
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<td>9.588³</td>
<td>NE</td>
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</table>
Figure 2: Growth Curve: Anticancer activity on Human Cervical Cancer Cell Line HeLa

Figure 3: Growth Curve: Anticancer activity on Human Breast Cancer Cell Line MCF-7

Figure 4: Growth Curve: Anticancer activity on Human Lung Cancer Cell Line A-549
Figure 5: Morphological changes after treatment. Morphological changes of cells were observed under a transmission electron micrograph.

The tested series of title compounds showed varied level of % growth inhibition with different anticancer cell lines (Table 4) such as Hela, MCF-7 and A-549 respectively as shown in Figure 2, Figure 3, Figure 4. Anticancer activity of title compounds was compared with the standard compound Adriamycin as shown in Figure 5. The compound QT07 showed good anticancer activity ($LC_{50}$= HeLa-0.044; MCF-7-1.54, A-549-0.154).

4. CONCLUSION

The present study is based on the standard method used to compose ninetriazolyquinazoline-4-one derivatives. TLC, melting point, FT-IR and 1H NMR and mass spectroscopy confirmed the structures of synthesised derivatives. The EGFR-tyrosine kinase was used for molecular docking of the title compounds with the aid of Schrodinger Glide Tools. The percent inhibition of EGFR was determined by an enzyme assay which reveals good inhibition of EGFR with title compounds. The compound QT07 revealed good EGFR inhibition activity (percent inhibition =75.54 percent). Title compounds were finally tested for the effects of anti-cancer activity against the different cancers. Compound QT07 revealed good EGFR inhibition activity (percent inhibition=75%) (A-549). QT07 compounds have strong activity against all three lines of the cells ($LC_{50}$-0.044, 1.54, 0.154). The QT03, QT04 and QT08 compounds also demonstrate mild activity against all three cell lines. A minimum of activity against all cell lines is also demonstrated in compounds QT01, QT02, QT05, QT06 and
The title compounds series possibly demonstrate anti-cancer activity by inhibiting the EGFR-tyrosine kinase enzyme. The 3-(2-(chloro-4-fluorophenyl)-1H-1,2,4-triazol-5-yl)-2-phenylquinazolin-4(3H)-on could be an advantage for the production of anticancer agent by EGFR-tyrosine kinase inhibition. This sequence has excellent directions for anticancer products by inhibiting EGFR-tyrosine kinase in the future.

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CONFLICT OF INTEREST
The authors have declared that no competing interest exists.

6. REFERENCES


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