# Synthesis leading to novel 2,4,6-trisubstituted quinazoline derivatives, their antibacterial and cytotoxic activity against THP-1, HL-60 and A375 cell lines[†]

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A series of novel 2,4,6-trisubstituted quinazoline derivatives **6** have been synthesized from anthranilic acids **1** in five steps *via* benzoxazinones **2**, N-benzoyl benzamides **3**, quinazol-4-ones **4**, 4-chloroquinazolines **5**. Products **6** have been screened for antibacterial and cytotoxic activity, promising compounds have been identified.

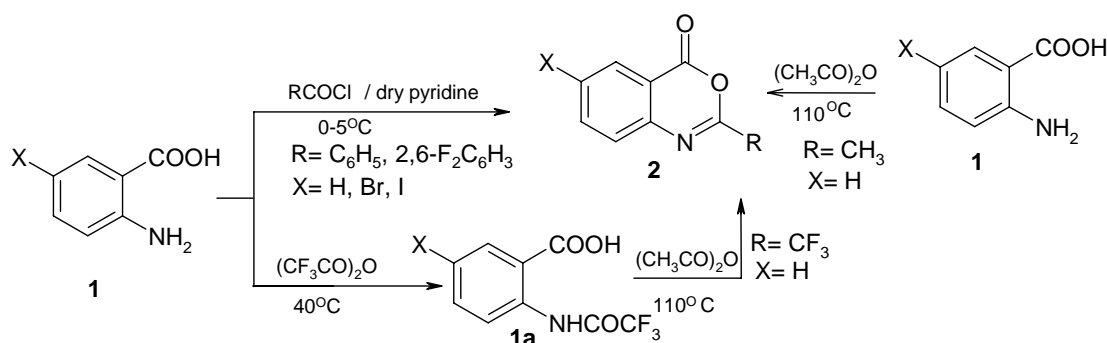**Keywords**: Benzoylation, acylation, cyclisation, quinazolones, quinazolines

The development of multifunctional therapeutic tools, *i.e.*, single compound able to interact with multiple altered pathogenetic pathways, for the multifactorial mechanistic nature of cancer cells, is of current interest. Following this rationale, we designed 4-long chain amino quinazolines, able to irreversibly block epidermal growth factor receptor (EGFR), and to induce apoptosis in tumor cell lines. Recent evidence also suggests that, combination therapy of cancer with receptor tyrosine kinase (RTK) inhibitors, are usually cytotoxic and conventional chemotherapeutic agents provide an improved treatment option. Several successful attempts have been recorded in literature demon-strating promising outcomes[1-3]. Quinazoline deri-vatives have a therapeutic benefit as an antiinvasive agents with potential activity in early and advanced solid tumors, metastatic bone disease and leukemias[4,5]. Some of the known quinazoline deri-vatives are reported to exhibit remarkable anticancer activity[6-11]. More specifically trimetrexate (TMQ) and piritrexim (PTX) considered as new generation potent lipophilic DHFR inhibitors[12-14]. Further 4-anilinoquinazolines are considered to be potent and highly selective inhibitors of tyrosine kinase

activity[15-17]. Aliphatic branched chain on position-4 of quinazoline nucleus has been found to show modest potency for cyclic dependent kinases[18]. Antibiotic resistance in the community is a growing public health concern due to the continual emergence of bacterial strains that demonstrate multi drug resistance[19]. Quinazoline derivatives also known to show antibacterial activity[20-26]. In view of the above, the design and synthesis of newer antibacterials is an area of immense significance and continues to attract the attention of increasing number of medicinal chemists. Earlier reports on synthesis of quinazoline derivatives is mainly starting from anthranilic acid[27-32], benzonitrile[33,34] and so on. In continuation of our efforts[35-40], we report here the synthesis of novel 2,4,6-trisubstituted quinazoline derivatives, their antibacterial and anticancer activities. Compounds with promising activity have been identified.
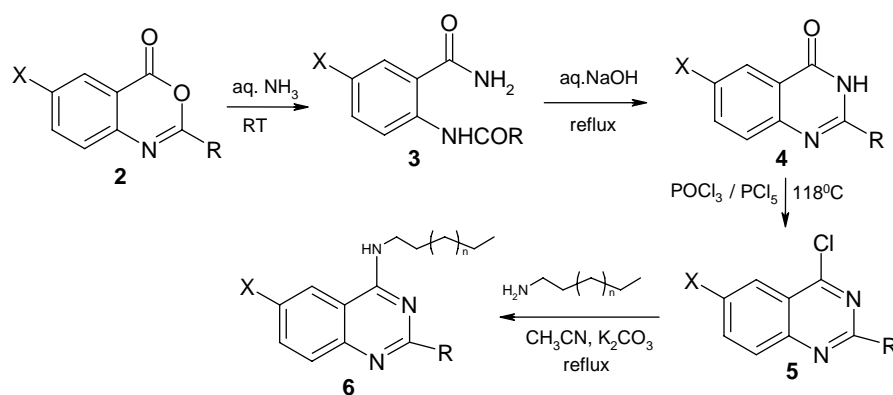
## Results and Discussion

Quinazoline derivatives with appropriate substi-tuent mainly amine or substituted amine on 4th position and either halogens or electron rich substituents on 6th position known to promote activity against bacteria or cancer cell lines. In order to find a potent molecule, several 2,4,6-trisubstituted quina

**Scheme I**



**Scheme II**

**Table I** — Physical data of 2, 6-disubstituted
1, 3-benzoxazin-4-ones, **2a-f**

| Entry | Compd | X | R | m.p. °C | Yield (%) |
|-------|-------|---|---|---------|-----------|
| 1 | **2a** | H | $C_6H_5$ | 122 (123-24) \ (Ref. 32) | 82 |
| 2 | **2b** | H | 2,6-$C_6H_3F_2$ | 192 | 81 |
| 3 | **2c** | H | $CH_3$ | 80 (80-82) (Ref. 33) | 73 |
| 4 | **2d** | H | $CF_3$ | 49 | 80 |
| 5 | **2e** | I | $C_6H_5$ | 199 (Ref. 28) | 82 |
| 6 | **2f** | Br | $C_6H_5$ | 182 (Ref. 28) | 80 |

zoline derivatives **6** have been prepared starting from anthranilic acid **1** in five steps and studied their activity. Initially compound **1** was benzoylated using benzoyl chloride/2,6-difluoro benzoyl chloride followed by dehydration in dry pyridine at 0-5°C for 4 hr and obtained respective benzoxazinones **2**. Alternately compound **1** was independently reacted with acetic anhydride at 110°C or trifluoroacetic anhydride at 40°C temperature to have $CH_3$ or $CF_3$ respectively in position 2 of compound **2**. In case of reaction of acetic anhydride, compound **2** is formed

directly, whereas in reaction of trifluoro acetic anhydride, an intermediate N-acyl derivative **1a** is formed. It is further cyclised to compound **2** in presence of acetic anhydride. The details of reactions have been drawn below in **Scheme I** and products are tabulated in **Table I**.

2,6-Disubstituted benzoxazinones **2** are further treated with aqueous ammonia at room temperature, resulted amide derivatives **3**, followed by refluxing in aqueous sodium hydroxide, formed quinazole-4-ones **4**. However in case of 2-trifluoromethyl benzoxazin-ones on treatment with aq.ammonia, direct product **4** is formed without isolating intermediate amide derivative **3**. It is attributed to change in mode of nucleophilic attack on carbon attached to $CF_3$ group instead of carbonyl, as a result product **4** is formed in single step. It is in consistence with our earlier report[39] on reaction of different nucleophiles with pyridofluorooxazines. The quinazole-4-ones **4** were chlorinated using $POCl_3/PCl_5$ under reflux conditions and obtained respective 4-chloro derivatives **5** and are tagged with long chain amines such as decyl-amine/haxadecylamine to have 4-amino derivatives **6**. The sequence of reactions is drawn in **Scheme II** and products are tabulated in **Tables II**, **III** and **IV**.

**Table II** — Synthesis of compounds **4** and **5**

| Entry | Compd | X | R | m.p. °C | Yield (%) |
|---|---|---|---|---|---|
| 1 | **4a** | H | $C_6H_5$ | 226 | 84 |
| 2 | **4b** | H | $2,6\text{-}C_6H_3F_2$ | 234 | 85 |
| 3 | **4c** | H | $CH_3$ | 235(237-39) (Ref. 33) | 76 |
| 4 | **4d** | H | $CF_3$ | 231 | 81 |
| 5 | **4e** | I | $C_6H_5$ | 241 | 81 |
| 6 | **4f** | Br | $C_6H_5$ | 230 | 80 |
| 7 | **5a** | H | $C_6H_5$ | 124 | 80 |
| 8 | **5b** | H | $2,6\text{-}C_6H_3F_2$ | 116 | 82 |
| 9 | **5c** | H | $CH_3$ | 098 | 78 |
| 10 | **5d** | H | $CF_3$ | 064 | 81 |
| 11 | **5e** | I | $C_6H_5$ | 135 | 78 |
| 12 | **5f** | Br | $C_6H_5$ | 129 | 75 |

**Table III** — Synthesis of 2, 4, 6 trisubstituted quinazoline derivatives **6**.

| Entry | Compd | X | R | n | m.p. °C | Yield (%) |
|---|---|---|---|---|---|---|
| 1 | **6a** | H | $C_6H_5$ | 06 | 094.9 | 91 |
| 2 | **6b** | H | $C_6H_5$ | 12 | 094.0 | 85 |
| 3 | **6c** | H | $2,6\text{-}C_6H_3F_2$ | 06 | 097.5 | 93 |
| 4 | **6d** | H | $2,6\text{-}C_6H_3F_2$ | 12 | 098.0 | 85 |
| 5 | **6e** | H | $CH_3$ | 06 | 103.8 | 81 |
| 6 | **6f** | H | $CH_3$ | 12 | 094.3 | 88 |
| 7 | **6g** | H | $CF_3$ | 06 | 057.3 | 87 |
| 8 | **6h** | H | $CF_3$ | 12 | 063.0 | 82 |
| 9 | **6i** | I | $C_6H_5$ | 06 | 080.3 | 95 |
| 10 | **6j** | I | $C_6H_5$ | 12 | 084.0 | 80 |
| 11 | **6k** | Br | $C_6H_5$ | 06 | 097.6 | 93 |
| 12 | **6l** | Br | $C_6H_5$ | 12 | 080.8 | 83 |

## Pharmacology

Quinazoline derivatives being considered as potent antibacterial and anticancer agents, several new quinazoline derivatives **6a-l** were synthesized and screened for antibacterial and anticancer activities. The response of all the compounds to antibacterial activity is moderate to good, however some of the compounds showed promising anticancer activity. The details of activity results are outlined below.

## Antibacterial activity

Antibacterial activity of all the test compounds was determined by agar well diffusion method[41], as recommended by the National Committee for Clinical Laboratory Standards against Gram positive micro organisms such as *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermis* (MTCC 435), *Escherichia coli* (MTCC 443) and Gram negative micro organisms such as *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (MTCC 109) at 100 μg/mL concentration, using dimethyl sulfoxide (DMSO) as solvent. The bacteria were sub-cultured on Mueller Hinton agar medium. Standard anti-bacterial streptomycin was also screened under similar conditions at a concentration of 50 μg/mL for comparison. Solvent control was also maintained under similar conditions. All the compounds **6a-l** except **6e** and **6f** showed good activity against Gram-positive bacteria such as *Staphylococcus epidermis* and Gram-negative bacteria such as *Pseudomonas aeruginosa*. Almost all the compounds showed moderate activity against other bacterial strains. The activity data generated is tabulated in **Table V**.

## Anticancer activity (cytotoxic activity)

The anticancer activity of compounds **6a-l** was screened against on THP-1 (Human acute mono-cytic leukemia)**,** HL-60 (Human promyelocytic leukemia) and A375 (Human malignant mela-noma) cells by known method[42] in order to find a promising anti-cancer agent. Three experiments with test compounds were performed in triplicate for each assay and the percent inhibition of cell viability was determined and compared with the data available for standard Amphotericin in literature against THP-1. However, data against HL-60 and A375 for standard amphotericin is not available.The data were subjected to linear regression analysis and the regression lines were plotted for the best fit straight line. The $IC_{50}$ (inhibition of cell viability) concentrations were calculated based on the regression equation. Based on the $IC_{50}$ values, it is concluded that the compound **6e** showed highest activity against THP-1 and HL-60 among all the compounds. Compounds **6a, 6c** and **6h** showed moderate activity. The activity is attributed to the presence of various substituents on position 4, 6 and independent of position 2. In all the active compounds, the presence of decyl amine group in position 4 promotes activity and iodo group in position 6 is detrimental to activity. The details are tabulated in **Table VI**.

## Experimental Section

Melting points were recorded on Casia-Siamia (VMP-AM) melting point apparatus and are

**Table IV** — Spectral analysis of compounds **4b, 4d, 5b, 5d** and **6a-l**

| Compd | H$^1$ NMR | MS($m/z$)(M$^+$+1) |
|---|---|---|
| **4a** | (200MHz, DMSO-$d_6$): δ 7.60 (4H, m, Ar-H), 7.85 (1H, m, Ar-H), 7.95 (1H, m, Ar-H), 8.25 (3H, m, ArH), 12.75(1H, s, NH). | 223 |
| **4b** | (200MHz, DMSO-$d_6$): δ 7.10 (t, $J$=11.53 Hz, 2H, Ar-H), 7.75 (quintet, $J$=7.69 Hz, 2H,Ar-H), 7.79 (quintet, $J$=7.69 Hz, 2H, Ar-H), 8.25 (d, $J$=15.38 Hz, 1H, Ar-H) | 259 |
| **4d** | (200MHz, DMSO-$d_6$): δ 7.69 (t, $J$=9.67 Hz, 1H, Ar-H), 7.89 (m, 2H, Ar-H), 8.25 (t, $J$=9.67 Hz, 1H, Ar-H) | 215 |
| **4e** | δ 7.62 (2H, d, $J$=10Hz, Ar-H), 7.80 ( 1H, s, Ar-H), 7.93 (3H, m, Ar-H), 8.22( 2H, m, Ar-H), 12.73(1H, s,NH). | 349 |
| **5a** | δ 7.50(2H, m, Ar-H), 7.70 (2H, m, Ar-H), 8.0(1H, m,Ar-H), 8.1 (1H,d, $J$=20Hz, Ar-H), 8.3 (1H,d, $J$=20Hz, Ar-H), 8.60(2H, m, Ar-H) | 240 (M$^+$) |
| **5b** | (300MHz, CDCl$_3$): δ 7.10 (quintet, $J$=9 Hz, 2H, Ar-H), 7.41 (m, 1H, Ar-H), 7.80 (t, $J$=18 Hz, 1H, Ar-H), 8.0 (t, $J$=18 Hz, 1H, Ar-H), 8.19 (d, $J$=24 Hz, 1H, Ar-H), 8.38 (d, $J$=24 Hz, 1H, Ar-H) | 277 |
| **5c** | δ 2.21 (s, 3H, CH$_3$), 7.52 (t, $J$=9.63 Hz, 1H, Ar-H), 7.72 (m, 2H, Ar-H), 7.93 (d, $J$=16.34 Hz, 1H, Ar-H) | 179 |
| **5d** | (200MHz, CDCl$_3$): δ 7.90 (m, 1H, Ar-H), 8.10 (t, $J$=10.71 Hz, 1H, Ar-H), 8.26 (d, $J$=21.42 Hz, 1H, Ar-H), 8.40 (d, $J$=21.42 Hz, 1H, Ar-H) | 233 |
| **5e** | δ 7.53 (2H, m, Ar-H), 7.74 (2H, m, Ar-H), 8.1(1H, s,Ar-H), 8.3 (1H,d, $J$=20Hz, Ar-H), 8.6 (1H,d, $J$=20Hz, Ar-H), 8.67 (1H, m, Ar-H) | 367 |
| **5f** | δ 7.51(2H, m, Ar-H), 7.75 (1H, d, $J$=10Hz,Ar-H), 8.6(1H,s,Ar-H), 8.5(1H,d, $J$=20Hz, Ar-H), 8.8 (1H,d, $J$=20Hz, Ar-H), 8.74 (2H, m, Ar-H) | 320 |
| **6a** | (300MHz, CDCl$_3$): δ 0.90 (t, $J$=15.51 Hz, 3H, CH$_3$), 1.35 (s, 10H, (CH$_2$)$_5$), 1.50 (s, 4H, (CH$_2$)$_2$), 1.80 (quintet, $J$=10.34 Hz, 2H,CH$_2$), 3.78 (q, $J$=10.34 Hz, 2H, CH$_2$), 5.60 (br., 1H, NH), 7.43 (m, 4H, Ar-H), 7.67 (m, 2H, Ar-H), 7.90 (d, $J$=20.33 Hz, 1H, Ar-H), 8.55 (d, $J$=20.33 Hz, 2H, Ar-H) | 362 |
| **6b** | (300MHz, CDCl$_3$): δ 0.88 (t, $J$=15 Hz, 3H, CH$_3$), 1.25 (s, 22H, (CH$_2$)$_{11}$), 1.45 (s, (4H, CH$_2$)$_2$), 1.79 (quintet, $J$=10 Hz, 2H, CH$_2$), 3.80 (q, $J$=10Hz, 2H, CH$_2$), 5.60 (br., s, 1H, NH), 7.43 (m, 4H, Ar-H), 7.67 (m, 2H, Ar-H), 7.90 (d, $J$=20 Hz, 1H, Ar-H), 8.55 (d, $J$=20 Hz, 2H, Ar-H) | 446 |
| **6c** | (200MHz, CDCl$_3$): δ 0.90 (t, $J$=9.37 Hz, 3H, CH$_3$), 1.25 (s, 10H, (CH$_2$)$_5$), 1.38 (s, 4H, (CH$_2$)$_2$), 1.70 (quintet, $J$=7.81 Hz, 2H, CH$_2$), 3.65 (q, $J$=8.34 Hz, 2H, CH$_2$), 5.80 (t, $J$=9.37 Hz, 1H, NH), 6.95 (m, 2H, Ar-H), 7.40 (m, 2H, Ar-H), 7.70 (m, 2H, Ar-H), 7.90 (d, $J$=12.50 Hz, 1H, Ar-H) | 398 |
| **6d** | (300MHz, CDCl$_3$): δ 0.90 (t, $J$=11.12 Hz, 3H, CH$_3$), 1.30 (s, 22H, (CH$_2$)$_{11}$), 1.50 (s, 4H, (CH$_2$)$_2$), 1.70 (quintet, $J$=8.34 Hz, 2H, CH$_2$), 3.62 (q, $J$=11.12 Hz, 2H, CH$_2$), 5.72 (br., s, 1H, NH), 6.93 (m, 2H, Ar-H), 7.30 (m, 1H, Ar-H), 7.41 (t, $J$=11.12 Hz, 1H, Ar-H), 7.70 (m, 2H, Ar-H), 7.90 (d, $J$=16.67 Hz, 1H, Ar-H) | 482 |
| **6e** | (300MHz, CDCl$_3$): δ 0.90 (t, $J$=14.50 Hz, 3H, CH$_3$), 1.37 (s, 10H, (CH$_2$)$_5$), 1.41 (s, 4H, (CH$_2$)$_2$), 1.70 (quintet, $J$=9.67 Hz, 2H, CH$_2$), 2.20 (s, 3H, CH$_3$ ), 3.70 (q, $J$=9.67 Hz, 2H, CH$_2$), 5.90 (s, 1H, NH), 7.50 (t, $J$=9.67 Hz, 1H, Ar-H), 7.75 (m, 2H, Ar-H), 7.98 (d, $J$=16.01 Hz, 1H, Ar-H) | 300 |
| **6f** | (300MHz, CDCl$_3$): δ 0.90 (t, $J$=14.40 Hz, 3H, CH$_3$), 1.31 (s, 22H, (CH$_2$)$_{11}$), 1.40 (s, 4H, (CH$_2$)$_2$), 1.74 (quintet, $J$=9.64 Hz, 2H, CH$_2$), 2.21 (s, 3H, CH$_3$ ), 3.72 (q, $J$=9.64 Hz, 2H, CH$_2$), 5.90 (br., s, 1H, NH), 7.52 (t, $J$=9.64 Hz, 1H, Ar-H), 7.75 (m, 2H, Ar-H), 7.95 (d, $J$=16.34 Hz, 1H, Ar-H) | 384 |
| **6g** | (300MHz, CDCl$_3$): δ 0.90 (t, $J$=14.51 Hz, 3H, CH$_3$), 1.30 (s, 10H, (CH$_2$)$_5$), 1.40 (s, 4H, (CH$_2$)$_2$), 1.70 (quintet, $J$=9.67 Hz, 2H, CH$_2$), 3.70 (q, $J$=9.67 Hz, 2H, CH$_2$), 6.15 (br., s, 1H, NH), 7.50 (t, $J$=9.67 Hz, 1H, Ar-H), 7.75 (t, $J$=9.67 Hz, 2H, Ar-H), 7.90 (d, $J$=19.35 Hz, 1H, Ar-H) | 354 |
| **6h** | (300MHz, CDCl$_3$): δ 0.90 (t, $J$=10.71 Hz, 3H, CH$_3$), 1.30 (s, 22H, (CH$_2$)$_{11}$), 1.40 (s, 4H, (CH$_2$)$_2$), 1.75 (quintet, $J$=8.03 Hz, 2H, CH$_2$), 3.70 (q, $J$=8.92 Hz, 2H, CH$_2$), 5.91 (br., s, 1H,NH), 7.55 (t, $J$=10.71 Hz, 1H, Ar-H), 7.75 (m, 2H, Ar-H), 7.95 (d, $J$=16.07 Hz, 1H, Ar-H) | 438 |
| **6i** | (300MHz, CDCl$_3$): δ 0.90 (t, $J$=18 Hz, 3H, CH$_3$), 1.33 (s, 10H, (CH$_2$)$_5$), 1.42 (s, 4H, (CH$_2$)$_2$), 1.80 (quintet, $J$=9.0 Hz, 2H, CH$_2$), 3.78 (q, $J$=12 Hz, 2H, CH$_2$), 5.52 (br., s, 1H, NH), 7.42 (d, $J$=18 Hz, 3H, Ar-H), 7.62 (t, $J$=9.0 Hz, 2H, Ar-H), 7.90 (m, 1H, Ar-H), 8.55 (d, $J$=18 Hz, 2H, Ar-H) | 488 |
| **6j** | (200MHz, CDCl$_3$): δ 0.90 (t, $J$=10.71 Hz, 3H, CH$_3$), 1.32 (s, 22H, (CH$_2$)$_{11}$), 1.45 (s, 4H, (CH$_2$)$_2$),1.80 (quintet, $J$=8.92 Hz, 2H, CH$_2$), 3.75 (q, $J$=9.52 Hz, 2H, CH$_2$), 5.95 (br., s, 1H, NH), 7.50 (m, 4H, Ar-H), 7.70 (m, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 8.55 (t, $J$=7.14 Hz, 2H,Ar-H) | 572 |
| **6k** | (400MHz, CDCl$_3$): δ 0.90 (t, $J$=13.34 Hz, 3H, CH$_3$), 1.32 (s, 10H, (CH$_2$)$_5$), 1.45 (s, 4H, (CH$_2$)$_2$), 1.79 (quintet, $J$=10 Hz, 2H, CH$_2$), 3.75 (q, $J$=8.89 Hz, 2H, CH$_2$), 5.51 (t, $J$=13.34 Hz, 1H, NH), 7.49 (d, $J$=13.34 Hz, 4H, Ar-H), 7.64 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 8.59 (m, 2H, Ar-H) | 440 |
| **6l** | (400MHz, CDCl$_3$): δ 0.88 (t, $J$=12 Hz, 3H, CH$_3$), 1.30 (s, 22H, (CH$_2$)$_{11}$), 1.45 (m, 4H, (CH$_2$)$_2$), 1.75(quintet, $J$=8.0 Hz, 2H, CH$_2$), 3.71 (q, $J$=8.0 Hz, 2H, CH$_2$), 5.50 (t, $J$=8.0 Hz, 1H, NH), 7.40 (m, 4H, Ar-H), 7.69 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 8.59 (m, 2H, Ar-H) | 524 |

**Table V** — The antibacterial activity of test compounds **6a-l**.
Zone of inhibition (Diameter in mm)

| Entry | Compd | Gram positive strains | | | Gram negative strains | | |
|---|---|---|---|---|---|---|---|
| | | *B. subtilis* | *S. aureus* | *S. epidermis* | *E. coli* | *P. aeruginosa* | *K. pneumoniae* |
| 1 | **6a** | 09 | 08 | 18 | 10 | 18 | 10 |
| 2 | **6b** | 15 | 10 | 14 | 08 | 14 | 10 |
| 3 | **6c** | 10 | 10 | 14 | 10 | 14 | 10 |
| 4 | **6d** | 14 | 13 | 16 | 10 | 16 | 12 |
| 5 | **6e** | 09 | 10 | 10 | 09 | 13 | 12 |
| 6 | **6f** | 10 | 11 | 08 | 10 | 11 | 16 |
| 7 | **6g** | 12 | 10 | 20 | 10 | 15 | 10 |
| 8 | **6h** | 10 | 10 | 14 | 10 | 20 | 12 |
| 9 | **6i** | 09 | 08 | 18 | 12 | 16 | 10 |
| 10 | **6j** | 12 | 10 | 21 | 12 | 18 | 10 |
| 11 | **6k** | 08 | 08 | 16 | 12 | 16 | 12 |
| 12 | **6l** | 80 | 08 | 16 | 10 | 15 | 14 |
| 13 | **Streptomycin (Std).** | 24 | 21 | 23 | 29 | 24 | 23 |

**Std.**: Streptomycin-positive control (conc. 50 µg/mL); DMSO-negative control showed no activity; Inhibitory zone diameters in mm (conc. 100 µg/mL)

**Table VI** — Cytotoxic activity of compounds on cancer cell growth *in vitro*

| Compd | THP-1 IC$_{50}$ (µg/mL) | HL-60 IC$_{50}$ (µg/mL) | A375 IC$_{50}$ (µg/mL) |
|---|---|---|---|
| **6e** | 00.66±0.09 | 00.70±0.11 | 07.71±0.10 |
| **6h** | 16.51±1.21 | 07.25±0.63 | NS |
| **6c** | 28.32±3.98 | 10.57±1.14 | 13.11±0.46 |
| **6a** | 8.44±0.77 | 17.78±1.42 | 09.53±0.31 |
| **6g** | 34.32±1.71 | 68.58±3.21 | 28.14±1.0 |
| **6i** | 50.17±1.57 | 69.89±4.0 | NS |
| **6b, 6d, 6f, 6j, 6k, 6l** | NS | NS | NS |
| **Camptothecin (Std).** | 3.34 | NA | NA |

NS-Not significant. NA- Not available
Control experiments were performed with carrier solvents alone and found to be insignificant difference with "normal" (without solvent).

uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectro photometer using KBr optics. $^1$H NMR spectra were recorded on Gemini Varian 200 MHz, Bruker AV 300 MHz and Unity 400 MHz spectrometer in DMSO-$d_6$ or CDCl$_3$ using TMS as an internal standard. Electron impact (EI) and chemical ionization mass spectra were recorded on a VG 7070 H instrument at 70 eV. All reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F$_{254}$ (mesh), Spots were visualized under UV light. Merck silica gel (100-200 mesh) was used for chromatography. CHN analyses were recorded on a Vario EL analyzer.

**Preparation of quinazolin-4 (3*H*)-one derivative, 4**

The 4*H*-(1,3)-benzooxazin-4-one **2** (1.93 mmole), was treated with aqueous ammonia at RT while stirring for 4 hr. Excess ammonia was removed by evaporation and quenched with water. The solid obtained was filtered and crude product phenylamido benzamide derivatives **3** thus formed was refluxed in presence of aq. NaOH for 2 hr. The reaction mixture was neutralized with acid and separated solid product was filtered, washed with water and dried. The crude product was purified passing through a column packed with silica gel (mesh 60/120) and solvents hexane : ethyl acetate (90:10) as eluents.

**2-Phenyl quinazolin-4(3*H*)-one 4a**: Yield: 0.359 g, Anal. Calcd for C$_{14}$H$_{10}$N$_2$O: C, 75.66; H, 4.54; N, 12.60. Found: C, 75.94; H, 4.80; N, 12.88%.

**2-(2, 6-Difluorophenyl) quinazolin-4 (3*H*)-one 4b**: Yield: 0.42g, Anal. Calcd for C$_{14}$H$_8$F$_2$N$_2$O: C, 65.12; H, 3.12; N, 10.85. Found: C, 65.0; H, 3.01; N, 10.55%.

**6-Iodo, 2-Phenyl quinazolin-4(3*H*)-one 4e**: Yield: 0.544g, Anal. Calcd for C$_{14}$H$_9$N$_2$OI: C, 48.30; H, 2.61; N, 8.05. Found: C, 48.52; H, 2.84; N, 8.26%.

**6-Bromo, 2-Phenyl quinazolin-4(3*H*)-one 4f**: Yield: 0.359g, Anal. Calcd for C$_{14}$H$_9$N$_2$OBr: C, 75.66;

H, 4.54; N, 12.60. Found: C, 75.94; H, 4.80; N, 12.88%.

## Preparation of 2-(trifluoromethyl) quinazoline-4 (3*H*)-one, 4d

2-(Trifluoromethyl)-4*H*-(1,3)-benzooxazin-4-one **2** (2.31 mmole) was treated with aqueous ammonia at RT while stirring for 4 hr. Excess ammonia was removed and residue is treated with water. The solid product is filtered, washed with water and dried. The crude product is purified by column chromatography using silica gel (60:120 mesh) with 92:8 (hexane:ethyl acetate) solvent system. Yield: 0.40 g. Anal. Calcd for $C_9H_5F_3N_2O$: C, 50.48; H, 2.35; N, 13.08. Found: C, 50.32; H, 2.20; N, 12.85%.

## 4-Chloro-2-substituted quinazoline, 5
## General procedure

2-Substituted quinazoline-4 (3*H*)-one **4** (0.01 mole), phosphorous pentachloride (0.05 mole) and phosphorous oxychloride (12 mL) were heated under reflux for 4 hr at 115-18ºC. Excess of phosphorous oxychloride was removed by distillation under reduced pressure. The residue was extracted three times with ethyl acetate. The combined organic extracts were made acid-free by washing with sodium bicarbonate solution (5%). The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product thus obtained was purified by column chromatography (60/120) using hexane:ethyl acetate (98:2) mixture as eluents.

**4-Chloro-2-phenyl quinazoline-4 (3*H*)-one, 5a**: Yield: 1.92g, Anal. Calcd. for $C_{14}H_9N_2Cl$: C, 69.86; H, 3.77; N, 11.64. Found: C, 69.62; H, 3.51; N, 11.43%.

**4-Chloro-2-(2,6-difluorophenyl) quinazoline, 5b**: Yield: 2.26g. Anal. Calcd. for $C_{14}H_7ClF_2N_2$: C, 60.78; H, 2.55; N, 10.13. Found: C, 60.87; H, 2.72; N, 10.33%.

**4-Chloro-2-methyl quinazoline, 5c**: Yield: 1.38g, Anal. Calcd for $C_9H_7N_2Cl$: C, 60.52; H, 3.95; N, 15.68. Found: C, 60.74; H, 4.20; N, 15.92%.

**4-Chloro-2-(trifluoromethyl) quinazoline, 5d**: Yield: 1.87g. Anal. Calcd. for $C_9H_4ClF_3N_2$: C, 46.48; H, 1.73; N, 12.04. Found: C, 46.30; H, 1.52; N, 11.93%.

**6-Iodo 4-chloro-2-phenyl quinazoline-4 (3*H*)-one, 5e**: Yield: 2.85 g, Anal. Calcd. for $C_{14}H_8N_2ClI$: C, 45.87; H, 2.20; N, 7.64. Found: C, 45.68; H, 2.02; N, 7.41%.

**6-Bromo 4-chloro-2-phenyl quinazoline-4 (3*H*)-one, 5f**: Yield: 2.39 g, Anal. Calcd for $C_{14}H_8N_2ClBr$: C, 52.62; H, 2.52; N, 8.77 Found: C, 52.85; H, 2.77; N, 8.94%.

## 2, 4, 6-Trisubstituted quinazoline derivatives, 6a-l

## General procedure

4-Chloro-2,6-disubstituted quinazoline (1 mole) was dissolved in acetonitrile (6 mL) and was added equimolar quantity of 1-decylamine/1-hexadecyl-amine (1.2 mole), followed by potassium carbonate (1 mole). The reaction-mixture was then heated under reflux for 5 hr at 80ºC. After cooling to RT, solvent was removed under reduced pressure and the resultant residue was extracted with ethyl acetate 2-3 times (15 mL each time). Combined organic extracts were washed with distilled water until washings are neutral to *p*H. The organic layer was dried over anhydrous sodium sulphate and concentrated over rotavapor. The dried crude product thus obtained was purified by column chromatography. The compounds were eluted using hexane:ethylacetate in different ratios. Compounds **6a**, **6b** with 92:8, **6c** and **6d** with 85:15, **6e-h** with 95:5 and **6i-l** with 98:2.

**N-Decyl-N-(2-phenyl-4-quinazolinyl) amine, 6a**. Yield: 0.65 g. Anal. Calcd. for $C_{24}H_{31}N_3$: C, 79.73; H, 8.64; N, 11.62. Found: C, 79.55; H, 8.49; N, 11.44%.

**N-Hexadecyl-N-(2-phenyl-4-quinazolinyl)-amine, 6b**. Yield: 0.76 g. Anal. Calcd. for $C_{30}H_{43}N_3$: C, 80.85; H, 9.72; N, 9.43. Found: C, 80.68; H, 9.50; N, 9.28%.

**N-Decyl-N-[2-(2, 6-difluorophenyl)-4-quinazolinyl] amine, 6c**. Yield: 0.735 g. Anal. Calcd. for $C_{24}H_{29}F_2N_3$: C, 72.52; H, 7.35; N, 10.57. Found: C, 72.69; H, 7.47; N, 10.65%.

**N-Hexadecyl-N-[2-(2,6-difluorophenyl)-4-quinazolinyl] amine, 6d**. Yield: 0.81 g. Anal. Calcd. for $C_{30}H_{41}F_2N_3$: C, 74.81; H, 8.58; N, 8.72. Found: C, 74.53; H, 8.28; N, 8.50%.

**N-Decyl-N-(2-methyl-4-quinazolinyl) amine, 6e**. Yield: 0.48 g. Anal. Calcd..for $C_{19}H_{29}N_3$: C, 76.21; H, 9.76; N, 14.03. Found: C, 76.01; H, 9.58; N, 13.89%.

**N-Hexadecyl-N-(2-methyl-4-quinazolinyl) amine, 6f**. Yield: 0.67 g. Anal. Calcd for $C_{25}H_{41}N_3$: C, 78.27; H, 10.77; N, 10.95. Found: C, 78.01; H, 10.58; N, 10.79%.

**N-Decyl-N-[2-(trifluoromethyl)-4-quinazolinyl] amine, 6g**. Yield: 0.61 g. Anal. Calcd. for $C_{19}H_{26}F_3N_3$: C, 64.57; H, 7.41; N, 11.89. Found: C, 64.70; H, 7.58; N, 11.99%.

*N*-Hexadecyl-*N*-[2-(trifluoromethyl)-4-quinazo-linyl] amine, 6h. Yield: 0.71 g. Anal. Calcd for $C_{25}H_{38}F_3N_3$: C, 68.62; H, 8.75; N, 9.60. Found: C, 68.45; H, 8.60; N, 9.47%.

*N*-Decyl-*N*-(6-iodo-2-phenyl-4-quinazolinyl) am-ine, 6i. Yield: 0.92 g. Anal. Calcd for $C_{24}H_{30}IN_3$: C, 59.14; H, 6.20; N, 8.62. Found: C, 59.26; H, 6.33; N, 8.75%.

*N*-Hexadecyl-*N*-(6-iodo-2-phenyl-4-quinazolinyl) amine, 6j. Yield: 0.91 g. Anal. Calcd for $C_{30}H_{42}IN_3$: C, 63.04; H, 7.41; N, 7.35. Found: C, 63.20; H, 7.56; N, 7.51%.

N-(6-Bromo-2-phenyl-4-quinazolinyl)-*N*-decyla-mine, 6k. Yield: 0.81 g. Anal. Calcd for $C_{24}H_{30}BrN_3$: C, 65.45; H, 6.87; N, 9.54. Found: C, 65.58; H, 6.95; N, 9.66%.

N-(6-Bromo-2-phenyl-4-quinazolinyl)-*N*-hexade-cyl, 6l. Yield: 0.87 g. Anal. Calcd for $C_{30}H_{42}BrN_3$: C, 68.69; H, 8.07; N, 8.01. Found: C, 68.57; H, 7.95; N, 7.93%.

### Antibacterial activity (*in vitro*)

The antibacterial activity was determined by the well diffusion method according to NCCLS[41]. Three to five identical colonies from each agar plate were lifted with a sterile wire loop and transferred into a tube containing 5 mL of Nutrient Agar. The turbidity of each bacterial suspension was adjusted to reach an optical comparison to that of a 0.5 McFarland standard; resulting in a suspension containing approximately 1 to $2 \times 10^{-8}$ CFU/mL. Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking 2 more times, rotating the plate approximately 60° each time to ensure even distribution of the inoculum. As a final step, the rim of the agar was also swabbed. After allowing the inoculum to dry at RT, 6 mm diameter wells were bored in the agar. Each extract was checked for anti bacterial activity by introducing 50 µL of a 100 mg/mL concentration into duplicate wells. The plates were allowed to stand at RT for 1 hr for extract to diffuse into the agar and then they were incubated at 37°C for 18 hr. Subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone diameter (IZD) measured to the nearest millimeter.

### Cytotoxic activity (*in vitro*)

In all experiments, A375, THP-1 and HL-60 cells were seeded at a final density of $2 \times 10^4$ cells/well in 96 well microtiter plates and were treated with test compounds. Ten dilutions were used for each compound. After 48 hr of incubation, 10 µL of MTT (3-4,5-dimethyl 2-thiazolyl 2,5-diphenyl-2*H*-tetra-zolium bromide) solution in phosphate buffer saline, PBS (5 mg/mL) was added to each well and the wells were then exposed to 37°C for 4 hr. This colorimetric assay is based on the ability of live and metabolically unimpaired tumor-cell targets to reduce MTT to a blue formazan product[42] then 100 µL of DMSO was added to each well. After a vigorous shaking, the absorbance was measured on a microculture plate reader (Spectro-max Plus; Molecular Devices, Sunny-vale, CA, USA) at 570 nm.

### Conclusion

A series of novel 2,4,6-trisubstituted quinazoline derivatives have been synthesized through a facile strategy and screened for antibacterial and anticancer (cytotoxic) activities. Compounds which showed promising antibacterial and anticancer activities have been identified.

### Acknowledgements

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