HL **ISSN: 2231 – 3087(print) / 2230 – 9632 (Online)**
http://heteroletters.org **Vol. 3: (1), 2013, 9-16**

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL N-(SUBSTITUTED BENZYL)-N-PROPYL-2-(TRICHLOROMETHYL) QUINAZOLIN-4-AMINE DERIVATIVES AS CYTOTOXIC AGENTS.

Maneesh Kumar Srivastav, N. Srinivasulu, S. M. Shantakumar*

Department of Pharmaceutical Chemistry, V. L. College of Pharmacy, Raichur-584103, Karnataka, India.

Abstract:
A series of novel 2-trichloromethyl quinazoline derivatives bearing tertiary amine group at the 4th position were synthesized and evaluated for their *in-vitro* cytotoxicity against human lung carcinoma cell line A549 and Human colorectal adernocarcinoma cell line HT-29 by MTT assay method. All the molecules were designed on the basis of Hydrogen bond acceptor, trichloro methyl group and presence of aromatic ring. The targeted compounds (**8a-o**) were synthesized from 4-chloro-2-(trichloromethyl) quinazolines and N-(substituted benzyl) propan-1-amines in alcoholic medium under reflux for 15-18 h. Compounds **8f** and **8l** exhibited highest cytotoxicity against human lung carcinoma A549 cell line (IC_{50} 7.5 and 6.8 μG) and human colon carcinoma HT-29 cell line (IC_{50} 8.2 and 8.0 μG) among the derivatives.

Keywords: Cytotoxicity, 2-trichlomethyl quinazoline, MTT assay, A549 cell lines, HT-29 cell lines.

Introduction:

Cancer is the worldwide health problem and the most frightening disease of human. It represents the second leading cause of human mortality after cardiovascular disease[i,ii]. The identification of novel structures that can act as more effective and reliable anticancer agents is still a major challenge to medicinal chemistry researcher[iii]. It is well known that quinazoline derivatives are potent inhibitors of epidermal growth factor receptor (EGFR)[iv-xii]. The epidermal growth factor receptor (EGFR) is cellular trans-membrane tyrosine kinases that is over expressed in a significant number of human tumors (eg., breast, ovarian, colon, and prostate), their expression level often correlate with vascularity, and is associated with poor prognosis in patients[xiii-xvi]. A number of small molecules EGFR kinase inhibitors have been evaluated in cancer clinical trials[iv-xvi], anilinoquinazoline- containing compounds Gefitinib (Iresa™)[iv-xvi], Erlotinib (Tarceva™)[vii], Lapatinib (Tykerb™) and Vandetanib (Zactima™) were recently approved for the treatment of HER2-positive metastatic breast cancer[xiv-xvi]. Many more compounds are still under evaluation in clinical trials for the treatment of cancer. In view of the previous rationale, we designed a new series of novel N-(substituted benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine derivatives **(8a-o)** and have been synthesized, screened for their cytotoxic activity. Since small substitution changes of structure might affect the activity of target molecules and drug properties,

and hence various quinazolines were synthesized by substituting at C-4 position of the quinazoline ring with tertiary amines containing aromatic ring and alkyl side chains, where as trichloromethyl group was introduced at C-2 position.

Experimental:

All the reagents and chemicals were procured from Sigma-Aldrich Company and used without further purification. Melting points were determined by using an open capillary tube method and are uncorrected. IR spectra (V_{max} in cm^{-1}) were recorded on a Shimadzu FT-IR 8300 Spectrophotometer using KBr pellets technique. ^{1}H-NMR Spectra were recorded using Bruker WM-400 spectrophotometer using $CDCl_3$ as the solvent and Chemical Shifts were expressed in δ values (ppm) relative to tetramethylsilane (TMS) as internal standard. The purity of new synthesized compounds was checked by TLC using silica gel G60 (Merck, Germany) plates in hexane-ethyl acetate (9:1) co-solvent system and spots were visualized under UV light.

N-(substituted benzyl) propan-1-amines (3a-o): Appropriate benzaldehyde (3.0 mmol) was dissolved in anhydrous methanol (20 mL) and cooled to 0 ^oC. To this cold mixture n-propylamine (0.35 gm, 6.0 mmol) was added drop wise and stirring continued at 0 ^oC for 30 min, then at ambient temperature for 3 h. The reaction mixture was again cooled to 0 ^oC and $NaBH_4$ was added (0.17 gm, 4.5 mmol) in three portions at 10 min intervals. Then it was stirred at room temperature for another 1 h and the solvent was evaporated to 1/3 of its original volume under vacuum by maintaining the bath temperature not more than 40 ^oC. Cold water (5 mL) and $NaHCO_3$ (0.25 gm, 3.0 mmol) were added, stirred for 10 min and extracted with dichloromethane (3x50 mL). Combined organic phase was dried over Na_2SO_4 and evaporated the solvent under vacuum to get colored liquid.

2-acetamidobenzoic acid (5): Anthranilic acid (1.37 gm, 10.0 mmol) was taken in acetic anhydride (4.08 mL, 40.0 mmol) and refluxed under anhydrous conditions for 4 h. Excess of acetic anhydride was distilled off under reduced pressure and obtained solid was kept in petroleum ether for 1 h, filtered and washed thoroughly with cold water. Dried and recrystallized to give compound **5** in yield 85%, m.p: 181-183 ^oC (Aq. EtOH); IR (KBr, cm^{-1}): 2945 (Aromatic CH stretching), 1475 (C=N); ^{1}H NMR ($CDCl_3$) δ 2.3 (s, 3H of CH_3), 7.3-8.9 (m, 4H of Ar-H), 10.8 (s, 1H of NH), 12.8 (s, 1H of OH); MS (m/z): M^{+1}= 180.

2-methylquinazolin-4(3*H*)-one (6): 2-Acetamidobenzoic acid **5** (1.79 gm, 10.0 mmol) and formamide (4.5 gm, 100.0 mmol) were heated in an oil bath at 155- 160 ^oC for 5 h. The yellow residue obtained after cooling was pulverized and washed with 5% sodium bicarbonate solution, then with cold water, dried and recrystallized to give compound **6** in yield 81 %, m.p: 232-234 ^oC(Aq. EtOH); IR (KBr, cm^{-1}): 2940 (Aromatic CH stretching), 1465 (C=N); ^{1}H-NMR ($CDCl_3$) δ 2.4 (s, 3H of CH_3), 7.6-8.3 (m, 4H of Ar-H), 10.9 (s, 1H of NH); MS (m/z): M^{+1}= 161.

4-chloro-2-(trichloromethyl) quinazoline (7): 2-Methyl quinazolin-4(3*H*)-one **6** (1.6 gm, 10.0 mmol), phosphorous pentachloride (10.41 gm, 50 mmol) and phosphorous oxychloride (20 mL) were heated under reflux for 6 h at 115-118 °C. Excess of phosphorous oxychloride was removed by distillation under reduced pressure. The residue was extracted three times with ethyl acetate. The combined organic phase was washed with sodium bicarbonate solution (5.0%), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude product thus obtained was purified by column chromatography (60/120) using n-hexane-ethyl acetate (9:1) mixture as eluent to give compound **7** in yield 47 %, m.p: 118-119 ^oC(Aq. EtOH); IR (KBr, cm^{-1}): 2965 (Aromatic CH stretching), 1475 (C=N); ^{1}H-NMR ($CDCl_3$) δ 7.5-8.3 (m, 4H of Ar-H); MS (m/z): M^{+1}= 283.

N-(substituted benzyl)–N–propyl–2-(trichloromethyl) quinazolin-4-amines (8a-o): 4-chloro-2-(trichloromethyl) quinazoline (2.81gm, 10 mmol) (**7**), and N (substituted benzyl) propan-1-amines (20 mmol) (**3a-o**) was dissolve in 30 mL of dried alcohol and refluxed for 15-18h. After completion of reaction (TLC monitored) the solution was poured in ice cold water and treated with 5% of dil HCl. The obtained solid was filtered, washed with cold water, dried and recrystallized to get solid compounds in yields 58-79%. (Table 1)

N-(benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8a): Reddish color solid, yield 79%, m.p. 88-90 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2957 (Aromatic CH stretching), 1476 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.9-1.0 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.8-1.9 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.7-3.8 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 4.3 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.3-8.3 (m, 9H of Ar-H); MS (m/z): M^{+1}= 395.

N-(2-nitro benzyl)-N-propyl-2-(trichloromethyl) quinazoline-4-amine (8b): Brown color solid, yield 73 %, m.p. 58-60 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2924 (Aromatic CH stretching), 1474 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.8-0.9 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.6-1.7 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.4-3.5 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.3 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.3-8.3 (m, 8H of Ar-H); MS (m/z): M^{+1}= 441.

N-(3-nitro benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8c): Brown solid, yield 75 %, m.p. 52-54 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2926 (Aromatic CH stretching), 1477 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.7-0.8 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.7-1.8 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.5-3.6 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.2 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.2-8.3 (m, 8H of Ar-H); MS (m/z): M^{+1}= 441.

N-(2-chloro benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8d): Reddish color solid, yield 63 %, m.p. 78-80 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2937 (Aromatic CH stretching), 1479 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.9-1.0 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.7-1.8 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.6-3.7 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.3 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.34-8.4 (m, 8H of Ar-H); MS (m/z): M^{+1}= 430.

N-(3-chloro benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8e): Reddish color solid, yield 65 %, m.p. 96-98 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2933 (Aromatic CH stretching), 1475 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.9-1.0 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.8-1.9 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.5-3.6 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.4 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.4-8.5 (m, 8H of Ar-H); MS (m/z): M^{+1}= 430.

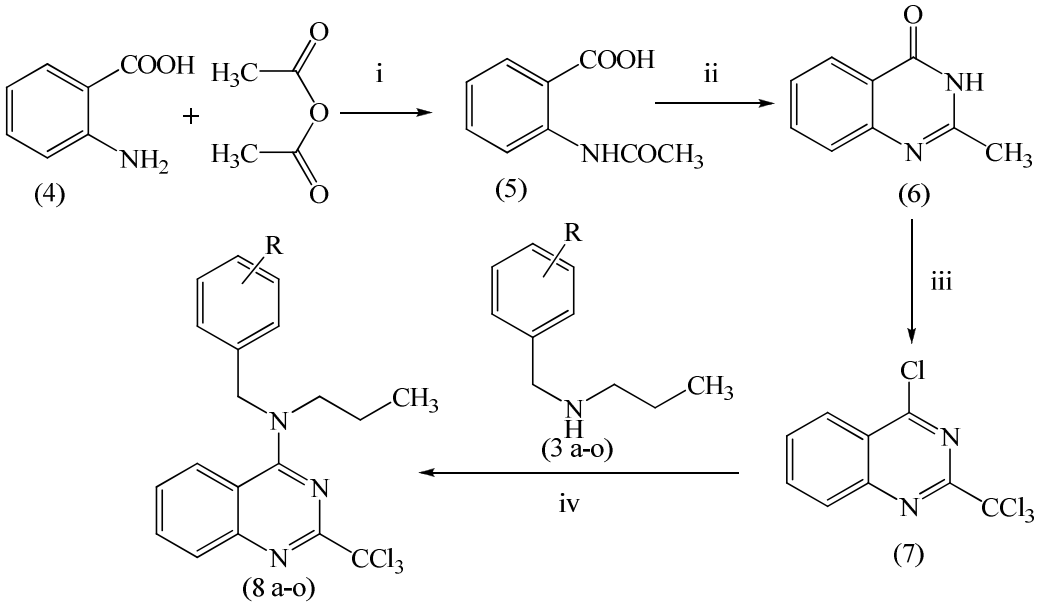
N-(4-chloro benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8f): Reddish color solid, yield 71 %, m.p. 56-58 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2943 (Aromatic CH stretching), 1485 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.8-0.9 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.7-1.8 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.4-3.6 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.2 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.2-8.0 (m, 8H of Ar-H); MS (m/z): M^{+1}= 430.

N-(2-fluoro benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8g): Brown solid, yield 65 %, m.p. 82-84 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2934 (Aromatic CH stretching), 1454 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.8-0.9 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.5-1.7 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.3-3.5 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.3 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.1-8.3 (m, 8H of Ar-H); MS (m/z): M^{+1}= 414.

N-(4-fluoro benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8h): Brown solid, yield: 69 %, m.p. 76-78 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2944 (Aromatic CH stretching), 1444 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.8-0.9 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.4-1.6 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.2-3.3 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.1 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.2-8.3 (m, 8H of Ar-H); MS (m/z): M^{+1}= 414.

N-(2-hydroxy benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8i): Reddish yellow solid, yield 58 %, m.p. 92-94 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2934 (Aromatic CH stretching), 1454 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.7-0.8 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.4-1.6 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.3-3.5 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.4 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.3-8.3 (m, 8H of Ar-H),12.1 (s, 1H of OH); MS (m/z): M^{+1}= 412.

Scheme-1

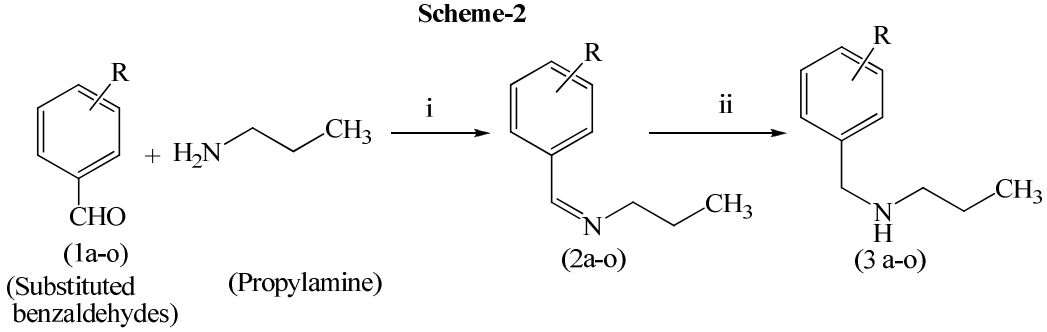


Reaction conditions and reagents: (i) reflux, 4 h, 85%; (ii) formamide, reflux, 5 h, 81%; (iii) $POCl_3$, PCl_5, reflux, 6 h, 47%; (iv) dry ethanol, reflux, 15-18 h, 58-79%.

8: R= a:H; b:2-NO_2; c:3-NO_3; d:2-Cl; e:3-Cl; f:4-Cl; g:2-F; h:4-F; i:2-OH; j:3-OH; k:4-OH; l:4-CH_3; m:4-OCH_3; n:4-C_2H_5; o:4-OC_2H_5.

Scheme1. Synthesis of novel N-(substituted benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine derivatives (8a-o).

Scheme-2



Reaction conditions and reagents: (i) methanol, stirring, 3 h; (ii) $NaBH_4$, stirring 2 h.

3: R= a:H; b:2-NO_2; c:3-NO_3; d:2-Cl; e:3-Cl; f:4-Cl; g:2-F; h:4-F; i:2-OH; j:3-OH; k:4-OH; l:4-CH_3; m:4-OCH_3; n:4-C_2H_5; o:4-OC_2H_5.

Scheme 2. The synthesis of N-(substituted benzyl) propan-1-amines.

N-(3-hydroxy benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8j): Reddish yellow solid, yield 66 %, m.p. 170-172 ^{o}C(Aq. EtOH); IR (KBr, cm^{-1}): 2939 (Aromatic CH stretching), 1465 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.8-0.9 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.4-1.6 (m, 2H of

CH_2 of CH_2-**CH_2**-CH_3), 3.4-3.5 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.4 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.2-8.3 (m, 8H of Ar-H),12.0 (s, 1H of OH); MS (m/z): M^{+1}= 412.

N-(4-hydroxy benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8k): Reddish yellow solid, yield 68 %, m.p. 74-76 ^oC(Aq. EtOH); IR (KBr, cm^{-1}): 2934 (Aromatic CH stretching), 1454 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.8-0.9 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.4-1.6 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.3-3.4 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 5.3 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.1-8.0 (m, 8H of Ar-H),12.2 (s, 1H of OH); MS (m/z): M^{+1}= 412.

N-(4-methyl benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8l): Reddish yellow solid, yield 72 %, m.p. 98-100 ^oC(Aq. EtOH); IR (KBr, cm^{-1}): 2915 (Aromatic CH stretching), 1468 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.9-1.0 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.5-1.6 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 2.3 (s, 3H of Ar-CH_3), 3.5-3.6 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 4.9 (s, 2H of CH_2 of N-**CH_2**-Ar), 7.1-8.1 (m, 8H of Ar-H); MS (m/z): M^{+1}= 410.

N-(4-methoxy benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8m): Reddish yellow solid, yield 75 %, m.p. 82-84 ^oC(Aq. EtOH); IR (KBr, cm^{-1}): 2917 (Aromatic CH stretching), 1447 (C=N); ^{1}H-NMR ($CDCl_3$) δ 0.8-0.9 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.6-1.7 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.5-3.6 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 3.9 (s, 3H of OCH_3) 5.4 (s, 2H of CH_2 of N-**CH_2**-Ar), 6.9-8.1 (m, 8H of Ar- H); MS (m/z): M^{+1}= 426.

N-(4-ethylbenzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8n): Reddish yellow solid, yield 77 %, m.p. 64-66 ^oC(Aq. EtOH); IR (KBr, cm^{-1}): 2927 (Aromatic CH stretching), 1469 (C=N); ^{1}H-NMR (DMSO.d_6) δ 0.8-0.9 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.3 (s, 3H of Ar-CH_3), 1.6-1.8 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.6-3.7 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 4.2-4.4 (q, 2H of Ar-**CH_2**-CH_3), 4.9 (s, 2H of CH_2 of N-**CH_2**-Ar), 6.8-7.9 (m, 8H of Ar-H); MS: (m/z): M^{+1}= 424.

N-(4-ethoxy benzyl)-N-propyl-2-(trichloromethyl) quinazolin-4-amine (8o): Reddish yellow solid, yield 79 %, m.p. 54-56 ^oC(Aq. EtOH); IR (KBr, cm^{-1}): 2929 (Aromatic CH stretching), 1459 (C=N); ^{1}H-NMR (DMSO.d_6) δ 0.8-0.9 (t, 3H of CH_3 of –CH_2-CH_2-**CH_3**), 1.4 (s, 3H of Ar-OCH_2-**CH_3**), 1.7-1.8 (m, 2H of CH_2 of CH_2-**CH_2**-CH_3), 3.5-3.7 (t, 2H of CH_2 of N-**CH_2**-CH_2-CH_3), 4.2-4.4 (q, 2H of Ar-**OCH_2**-CH_3), 4.7 (s, 2H of CH_2 of N-**CH_2**-Ar), 6.9-8.0 (m, 8H of Ar-H); MS: (m/z): M^{+1}= 440.

***In vitro* Cytotoxicity Assay**

The cytotoxicity of the newly synthesized compounds was evaluated by MTT assay according to the Mossman's method[xvii] against Human lung carcinoma A549, colon carcinoma HT-29 cell lines. The MTT assay is based on the reduction of the soluble 3-(4,5-methyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) into a blue-purple formazan products, mainly by mitochondrial reductase activity inside living cells. The cells used in cytotoxicity assay were cultured in MEM media supplemented with 10 % fetal bovine serum. Cells suspended in the medium (10^4 cells/well in 100 µl) were placed in 96-well culture plates and incubated at 37 ^oC in a 5 % CO_2 incubator. After 12 h, the test sample (10, 20 and 30 µG) was added to the cells (10^4 cells/well in 100 µl) in 96-well plate and cultured at 37 ^oC for 3 days. The cultured cell were mixed with 100 µL of MTT solution and incubated for 4 h at 37 ^oC. The supernatant was carefully removed from each well and 100 µL of DMSO were added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by an ELISA microplate reader using a test wavelength of 490 nm. The results were expressed as the IC_{50}, which inducing a 50% inhibition of cell growth of treated cells when compared to the growth of control cells. The drug concentration that causes 50% cell growth inhibition after 72 h of continuous exposure

to the test compounds (IC_{50}) was determined by plotting the graph of concentration of the drug against the percent cytotoxicity.

Table-1. Physical Characteristic data of the synthesized compounds (**8a-o**).

S.No	Compound code	R	Mol. Formula	Mol. weight	Yield %	M.P °C
1	**8a**	H	$C_{19}H_{18}Cl_3N_3$	395	79	88-90
2	**8b**	2-NO_2	$C_{19}H_{17}Cl_3N_4O_2$	440	73	58-60
3	**8c**	3-NO_2	$C_{19}H_{17}Cl_3N_4O_2$	440	75	52-54
4	**8d**	2-Cl	$C_{19}H_{17}Cl_4N_3$	429	63	78-80
5	**8e**	3-Cl	$C_{19}H_{17}Cl_4N_3$	429	65	96-98
6	**8f**	4-Cl	$C_{19}H_{17}Cl_4N_3$	429	71	56-58
7	**8g**	2-F	$C_{19}H_{17}Cl_3FN_3$	413	65	82-84
8	**8h**	4-F	$C_{19}H_{17}Cl_3FN_3$	413	69	76-78
9	**8i**	2-OH	$C_{19}H_{18}Cl_3N_3O$	411	58	92-94
10	**8j**	3-OH	$C_{19}H_{18}Cl_3N_3O$	411	66	170-172
11	**8k**	4-OH	$C_{19}H_{18}Cl_3N_3O$	411	68	74-76
12	**8l**	4-CH_3	$C_{20}H_{20}Cl_3N_3$	409	72	98-100
13	**8m**	4-OCH_3	$C_{20}H_{20}Cl_3N_3O$	425	73	82-84
14	**8n**	4-C_2H_5	$C_{21}H_{22}Cl_3N_3$	423	77	64-66
15	**8o**	4-OC_2H_5	$C_{21}H_{22}Cl_3N_3O$	439	79	54-56

Results and discussion:

The synthetic strategies adopted for the preparation of intermediate and targeted compounds are depicted in scheme 1 and scheme 2. The intermediate N-(substituted benzyl) propan-1-amines (**3a-o**) were synthesized by treating n-propylamine with various substituted benzaldehydes in methanol, followed by sodium borohydrate reduction. The 4-chloro-2-(trichloromethyl) quinazoline (**7)** was prepared by following Kato[xviii] protocol permitting a trichlorination of the activated methyl group in α-position of the sp^2 nitrogen atom in pyridine and quinoline series. This method uses phosphorous pentachloride as a powerful ionic chlorinating agent, and phosphorous oxychloride as a solvent, the reaction mixture being heated to reflux for not less than 5 h and not more than 7 h, with longer times the yields of the reaction decreases because of the high reactive nature of the refluxed reaction mixture that damages the product previously formed. Optimal PCl_5 quantity required for the chlorination of methyl group has to be always superior to the number of hydrogen atom that's have to be substituted by chlorine ones, knowing that each PCl_5 molecule is able to liberate a single chlorine atom for the chlorination reaction. Consequently, in order to trichlorinate our substrates, the use of 5 equiv of PCl_5 usually provided the good optimal yields. The titled compounds were synthesized smoothly by treating the ethanolic solution of compound **7** with respective N (substituted benzyl) proane-1-amines **(3a-o)**. The yields of the synthesized compounds were in the range of 58-79%, the higher yields were observed with the compounds containing electron donating substituent's, which may be increasing the reactivity of secondary amino group.

In vitro MTT Cytotoxic activity

Fifteen analogs namely **8a, 8b, 8c, 8d, 8e, 8f, 8g, 8h, 8i, 8j, 8k, 8l, 8m, 8n and 8o** were evaluated for their *in vitro* cytotoxic activity by the standard MTT (3-(4, 5-methyl-2-thiazolyl)-2, 5-diphenyl-2*H*-tetrazolium bromide) assay method against a panel of two human tumor cell lines

namely; Human lung carcinoma A549, colon carcinoma HT-29. The results of *in vitro* cytotoxic activity were presented in table 2 as IC_{50} (µG) value, which is the inhibitory concentration of the compounds, which cause inhibition of 50% the cells in 24h. The obtained data revealed that, the two tested human tumor cell lines exhibited variable degree of sensitivity profiles towards seven of the tested compounds namely; **8a, 8b, 8f, 8g, 8h, 8l** and **8m**. Among these, the A549 cell lines showed pronounced sensitivity against compounds **8f** and **8l** with IC_{50} values of 7.5 and 6.8 µG respectively. Moreover a remarkable cytotoxic potential was displayed by compounds **8b** and **8g** against the same cell line (12.8 and 11.3 µG). Meanwhile, compound **8a, 8h** and **8m** revealed an obvious cytotoxicity profile against A549 cell lines with IC_{50} values 17.4, 17.7 and 16.5 µG, respectively. However compounds **8c, 8d, 8e, 8i, 8j, 8k, 8n, 8o** showed insignificant activity against the same cell lines. The *in vitro* cytotoxic activity data against HT-29 cell line reveals that the compounds **8b, 8f**, and **8l** exhibited outstanding potential as evidenced from their IC_{50} values (8.9, 8.2 and 8.0 µG). Where as the compounds **8a, 8g** and **8h** displayed pronounced sensitivity with IC_{50} values 10.4, 10.5 and 12.5 µG respectively. However, compound **8m** exhibited moderate activity with IC_{50} 25.5 µG. while the compounds **8c, 8d, 8e, 8i, 8j, 8k, 8n** and **8o** showed insignificant activity. In particular, compounds **8f** and **8l** proved to be the most active members in this study with a broad spectrum of activity against the tested cell lines, with special effectiveness against the human lung carcinoma A549 cell line (IC_{50} values 7.5 and 6.8 µG) and human colon carcinoma HT-29 cell line (IC_{50} values 8.2 and 8.0 µG, respectively) (Table 2). A close examination of the structure of the active compounds showed that the 4-chlorophenyl and 4-methylphenyl counterpart at postion-4 of the 2-trilchlorometyl quinazoline skeleton is the most favorable position when compared with the other analogues. The differences in the IC_{50} values may be attributed to factors such as substitution on the phenyl ring attached at 4^{th} position of quinazoline nucleus, genetic and biochemical background of the cell lines.

Table 2. Cytotoxic activity data (IC_{50} values) of synthesized compounds 8a-o against human cancer cell lines.

Compd No.	Human lung carcinoma A549 (µG)	Human colon carcinoma HT-29 (µG)
8a	17.4	10.4
8b	12.8	08.9
8c	>100	>100
8d	>100	>100
8e	>100	>100
8f	07.5	08.2
8g	11.3	10.5
8h	17.7	12.5
8i	>100	>100
8j	>100	>100
8k	>100	>100
8l	06.8	08.0
8m	16.5	25.5
8n	>100	>100
8o	>100	>100

IC_{50}: Inhibition concentration of the compound which causes lysis of 50% of cells in 24h (µG).

Conclusion:

The present paper describes the synthesis of N-(substituted benzyl)-N-propyl-2-(trichloromethyl) quinazoline-4-amine derivatives as possible anticancer agents. The investigation of anticancer activity data revealed that the compounds **8f** and **8l** displayed excellent activity. These lead molecules **8f** and **8l** could be further utilized for designing newer anticancer agents.

Acknowledgment:

The authors are thankful to the management of V. L. College of Pharmacy, Raichur for providing the necessary facilities to carry out the research work. The authors are highly indebted to the SAIF Punjab University, Chandigarh and Oxygen Health Care Research Pvt. Ltd. Ahmedabad for providing NMR, LCMS and IR Spectra. The authors express sincere thanks to Dr. Kishore G. Bhat, Maratha Mandal Dental College Belgaum for their help in *in-vitro* cytotoxicity studies.

References:

i. J. B. Gibbs, Science. 287, 1969 (2000).
ii. C. Unger, Drug future. 22, 1337 (1997).
iii. P. Heffeter, M.A. Jakupec, W. Orner, S. Wild, N.G. Keyserlingk, L. Elbing, H. Zorbas, A.Korynevska, S. Knasmuller, H. Sutterluty, M. Micksche, B.K. Keppler, W. Berger, Biochem. Pharmacol. 71, 426 (2006).
iv. F. Barlesi, C. Tchouhadjian, C. Doddoli, P. Villani, L. Greillier, J. Kleisbauer, P.Thomas, P. Astoul, Fundam. Clin. Pharmacol. 19, 385(2005).
v. C.L. Arteaga, D.H. Johnson, Curr. Opin. Oncol. 13, 491 (2001).
vi. A.J. Baker, K.H. Gibson, W. Grundy, A.A. Godfrey, J.J. Barlow, M.P. Healy, J.R. Woodburn, S.E. Ashton, B.J. Curry, L. Scarlett, L. Henthorn, L. Richards, Bioorg. Med. Chem. Lett. 11, 1911 (2001).
vii. K.N. Ganjoo, H. Wakelee, Biologics. Targets Therapy. 1, 335 (2007).
viii. L. Kopper, Pathol. Oncol. Res. 14, 1 (2008).
ix. S. Dhillon, A.J. Wagstaff, Drugs. 67, 2101(2007).
x. H.A. Burris, H. I. Hurwitz, E.C. Dees, A. Dowlati, K.L. Blackwell, B. Oneil, P.K. Marcom, M.J. Ellis, B. Overmoyer, S.F. Jones, J.L. Harris, D.A. Smith, K.M. Koch, A. Stead, S. Mangum, N.L. Spector, J. Clin. Oncol. 23, 5305 (2005).
xi. E.R. Wood, A.T. Truesdale, O.B. Mcdonald, D. Yuan, A. Hassell, S.H. Dickerson, B. Ellis, C. Pennisi, E. Horne, K. Lackey, K.J. Alligood, D.W. Rusnak, T.M. Gilmer, L. Shewchuk, Cancer Res. 64, 6652 (2004).
xii. L.F. Hennequin, E.S.E. Stokes, A.P. Thomas, C. Johnstone, P.A. Ple, D.J. Ogilvie, M. Dukes, S.R. Wedge, J. Kendrew, J.O. Curwen, J. Med. Chem. 45, 1300 (2002).
xiii. J. Fricker, Lancet. Oncol. 7, 621 (2006).
xiv. S. Garofalo, R. Rosa, R. Bianco, G. Tortora, Expert. Opin. Ther. Patents. 18, 889 (2008).
xv. M. Srinivasan, S.G. Trivadi, Clin. Biochem. 37, 618 (2004).
xvi. G.S. Cockerill, K.E. Lackey, Curr. Top. Med. Chem. 2, 1001 (2002).
xvii. T. Mosmann, J. Immunol. Meth. 65, 55 (1983).
xviii. T. Kato, N. Katagiri, A. Wagai, Tetrahedron. 34, 3445 (1978).

Received on December 17, 2012.