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Design, Synthesis and Pharmacological Evaluation of 2-Phenyl Quinazolin-4-one Derivatives as Anticolorectal Cancer and Anti-Inflammatory Agent

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Quinazoline derivatives are heterocyclic compounds that acts as important structural lead for the discovery of effective therapeutic agents. The anti-inflammatory activity along with cytotoxicity helps to reduce the inflammation and pain associated with carcinoma. Derivatives of quinazoline-4-one were preliminary screened and *in silico* molecular modelling studies using Autodock were performed. In the docking study, ligands were docked against anticancer and anti-inflammatory targets. *In silico* analysis revealed that the compounds with aromatic substitution at 3rd and halogen substitution at 6th or 7th positions possess lesser side effects and have more potent antitumor activity. Based upon these results 12 compounds were selected, synthesized, characterized and screened for their *in vitro*, anti-inflammatory, antioxidant and anticancer activities. From the *in vitro* studies, compounds **QA4**, **QA7** and **QB1** showed good anticancer, anti-inflammatory and anti-oxidant activity when compared to the standard.

Keywords: Quinazolin-4-one, Colorectal cancer, Drug designing, Molecular docking, Anticancer activity.

INTRODUCTION

The practice of cancer medicine has faced drastic transformations over the past decade with the development of a large number of curative and palliative medicines. However, none of the available therapy has proven to alleviate cancer and prevent its remission. Also with the advancement of the modern lifestyle, newer forms of cancer and related mutations are cropping up. This provides a need for development of a novel therapeutic agent for cancer therapy.

Quinazoline is one of the widest spread scaffolds among natural and synthetic bioactive compounds. With the chemical formula $C_8H_6N_2$, this heterocyclic compound is made up of two fused rings a benzene ring and a pyrimidine ring which are both six-membered aromatic ring. Quinazolines are insoluble in most of the organic solvents and water but soluble in aqueous alkali and are always high melting crystalline solids. Keto quinazolines otherwise simply termed as quinazolinones is one of the most important therapeutically active derivative of quinazoline system. Quinazolin-4-(3*H*)-one has been considered as an important research compound as it carries several therapeutic uses such as anticancer [1], anti-inflammatory [2,3], antibacterial [4], analgesia [2], antivirus [5], anti-cytotoxin [6], antioxidation [7] and antidiabetes [8] effects. Quinazolinones are also the fundamental part of about 150 natural alkaloids occurring in some families of plants, animals and microorganisms [9]. In addition, the nucleus constructs an intrinsic part in many drugs currently used in numerous clinical medicine [10]. The relevance of quinazoline derivatives in biology has been thoroughly examined they earned increasing attentions since they were proven to be promising anticancer agents.

Colorectal cancer starts in the colon or rectum, which can be either inherited or due to alterations in the genomes. First stage in colorectal cancer is the formation of polyps on the inner lining of colon or rectum and develops into cancer slowly. The development of cancer may take several years. The major cause of morbidity and mortality all over the world is colorectal cancer. Of all cancer cases it accounts for over 9 %. Structure activity relationship studies showed that substitutions at 2 and 3 positions of quinazolin-4-one ring are related to cytotoxic activity of these derivatives. In the present investigation we reported some 2-phenyl 3-(substituted) quinazolin-4-(3*H*)-one derivatives and screened them for anticancer properties and

anti-inflammatory properties. Antioxidant activity of the active derivatives was also examined.

EXPERIMENTAL

Design of the derivatives: Software's used for ligand designing are ChemSketch, Corina, Mol inspiration, Molsoft, Swiss PDB Viewer, Cygwin and AutoDock. From the literature review, quinazoline-4-one was selected for the further study. By giving different substitutions at 3rd, 6th and 7th positions of 2-phenyl quinazolin-4-one ligands were designed. From the online software CORINA 3D structure were generated and the molecular structure were drawn by using ChemSketch software.

Lipinski's rule of five by using Molinspiration online software was calculated by giving the structures of the molecules as the input. The druglikeness of the molecule in terms of hydrogen bond acceptors, donors, molecular weight and physiological log P would be provided as the output. AutoDock was used for the docking studies, which is a free molecular docking package. Docking score of each drug molecule against all the targets were analyzed.

Synthesis and characterization

General information for synthesis and characterization: All the chemicals used were commercially procured from Spectrum Reagents and Chemicals Pvt. Ltd. Cochin, Nice Chemicals Pvt. Ltd. Cochin, Fisher Scientific Mumbai, Sigma-Aldrich Bangalore, Loba Chemie Pvt. Ltd. Mumbai and Alfa-Aesar USA and were of analytical grade. Using thermo electric melting point apparatus, melting point apparatus, roy capillary, melting points were determined and were uncorrected. For TLC Silica gelG plates (3 cm × 8 cm) were used and using UV and iodine chamber spots were located. The derivatives were purified by performing column chromatography in silica gel (100-200 mesh). The IR spectra were determined on FT-IR Shimadzu spectrometer and the values were expressed in cm^{-1}. ^1H NMR and ^{13}C NMR were recorded in DMSO solvents using TMS as internal reference with Bruker Fourier-NMR spectrometer in 400 MHz. On ABSCIEX triple quadrupole MS mass spectra were recorded on with Waters ACQUITY ultra performance LC.

Synthesis of anthranilic acid: Weighed accurately 30 g of sodium hydroxide and transferred into a 350 mL conical flask containing 120 mL distilled water. This was allowed to cool to 0 °C or below in a bath of ice and salt. 8.4 mL of bromine was added in portions to the above mixture with continuous shaking till all the bromine is reacted to provide with the sodium hypobromite solution. Upon the addition of bromine, the temperature of the reaction will rise and again cool the solution to 0 °C or below. Meanwhile a solution was prepared by dissolving 22 g of sodium hydroxide in 80 mL water. To the cold solution of sodium hypobromite, added 24 g of powdered pthalimide in one portion to form a smooth paste with continuous stirring. The flask was removed from the ice bath and shaken vigorously for approximately 5 min to get a clear yellow solution. The NaOH solution prepared earlier was added in one portion and the solution was heated to 80 °C for 2 min. The solution was cooled in ice and neutralized by slow addition of concentrated hydrochloric acid. Anthranilic acid was completely precipitated by the gradual addition of 25 mL of glacial acetic acid. The crude product was recrystallized from hot water with the addition of little amount of charcoal. The recrystallized product was collected and dried at 100 °C [11]. m.f.: $C_7H_7N_2O$, m.w.: 137, colour and appearance: light yellow colour crystals, m.p.: 144 °C, yield: 80 %, R_f value: 0.6 (hexane:ethyl acetate (8:2)), UV λ_{max} (methanol) 310 nm, IR (KBr, ν_{max}, cm^{-1}): 3608 (NH_2) 3030 (OH), 2916 (C-H, Ar-H), 1764 (C=O).

Synthesis of 2-phenyl-4(3*H*)-3,1-benzoxazinone: 0.05 mol of benzoyl chloride was added to a solution of equimolar anthranilic acid in 25 mL of pyridine drop-wise with the temperature maintained between 0-5 °C for 1 h. The stirring was continued for another 2 h at room temperature to get a solid product. Upon neutralization of the above reaction mixture with sodium bicarbonate solution yields pale yellow solid crude product (compound **1**). This separated product can be filtered, washed with water and then recrystallized from ethanol [12]. m.f.: $C_{21}H_{16}N_2O$, m.w.: 223, Colour and Appearance: cream colour crystals, m.p.: 120 °C, yield: 78 %, R_f value: 0.70 [toluene:chloroform (8:2)], UV λ_{max} (methanol) 313 nm, IR (KBr, ν_{max}, cm^{-1}): 3030 (C-H, Ar-H), 1764 (C=O), 1614 (C=N), 1254 (C-O).

Synthesis of QA1-QA9: An equimolar mixture of 2-phenyl-4(3*H*)-3,1-benzoxazinone and substituted anilines were taken in a round bottom flask containing glacial acetic acid and refluxed for 6 h. TLC was performed in a timely manner to determine the progress and completion of the reaction. Upon completion of the reaction, the reaction mixture was poured onto crushed ice to for solid mass. This crude product obtained was filtered, washed with water and recrystallized from warm ethanol [11].

3-(2-Methylphenyl)-2-phenylquinazolin-4(3*H*)-one (QA1): m.f.: $C_{21}H_{16}N_2O$; m.w.: 312.46; colour and appearance: cream colour crystals; m.p.: 110 °C; yield: 80 %, R_f value: 0.82 (acetonitrile:ethyl acetate (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3030 (Ar-H str), 1764 (C=O), 1614 (C=N), 1180 (C-N), 1254 (C-O), 2850 (C-H str), C-C (1269), 2732 (C-CH_3); ^1H NMR (DMSO, 400 MHz) δ 8.28 (s, 2H), 7.39 (d, *J* = 6 Hz, 1H), 7.10 (d, *J* = 6.2 Hz, 1H), 6.86 (s, 2H), 6.62 (d, *J* = 7.4 Hz, 1H); ^{13}C NMR (DMSO, 100 MHz) δ 160.33, 157.28, 149.09, 148.79, 130.30, 129.94, 129.87, 129.57, 126.56, 121.80, 120.43, 112.94, 112.51, 102.24, 55.99, 45.60; MS *m/z* (%): 312 (100), 313 (M^{+1}, 20), 314 (M^{+2}, 4).

3-(4-Chlorophenyl)-2-phenylquinazoline-4(3*H*)-one (QA2): m.f.: $C_{20}H_{13}N_2OCl$; m.w.: 332; colour and appearance: off white crystals; m.p.: 190 °C; yield: 68.56 %; R_f value: 0.75 (*n*-hexane:ethyl acetate [1:1]); IR (KBr, ν_{max}, cm^{-1}): 3026 (Ar-H str), 822 (C-C), 1179 (C-N), 1607 (C=N), 1665 (C=O), 686 (C-Cl); ^1H NMR (DMSO, 400 MHz) δ 9.62 (s, 2H), 8.15 (d, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 6.2 Hz, 1H), 6.80 (s, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 160.33, 157.27, 148.83, 148.80, 133.10, 130.57, 129.86, 127.08, 126.67, 126.16, 120.43, 112.94, 112.51, 102.23, 55.99, 45.58; MS *m/z* (%): 332 (100), 334 (M^{+4},34).

3-(2-Ethyl-6-methylphenyl)-2-phenylquinazolin-4(3*H*)-one (QA3): m.f.: $C_{23}H_{20}N_2O$; m.w.: 340; colour and appearance: pinkish white crystals; m.p.: 130 °C; yield: 74.83 %; R_f value:

0.98 (acetonitrile:ethyl acetate (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3016 (Ar-H str), 819 (C-C), 1180 (C-N), 1603 (C=N), 1720 (C=O), 2732 (C-CH_3); ^{1}H NMR (DMSO, 400 MHz) δ 7.42 (s, 3H), 7.36 (s, 2H), 7.29 (t, *J* = 6.24 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 6.81 (s, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 160.28, 159.04, 149.09, 148.89, 133.07, 130.94, 129.96, 129.66, 126.56, 121.84, 120.33, 114.61, 111.43, 100.98, 55.57, 55.37, 45.53; MS *m/z* (%) 340 (100), 341 (M^{+1}, 28), 342 (M^{+2}, 4).

7-Chloro-3-(4-methylphenyl)-2-phenylquinazolin-4(3*H*)-one (QA4): m.f.: $C_{21}H_{15}N_2OCl$; m.w.: 346.8; colour and appearance: light brown crystals; m.p.: 150 °C; yield: 65.35 %; R_f value: 0.71 (*n*-hexane:ethyl acetate (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3024 (Ar-H str), 819 (C-C), 1180 (C-N), 1603 (C=N), 1662 (C=O), 646 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ 8.18 (s, 2H), 7.29 (d, *J* = 6 Hz, 1H), 7.08 (d, *J* = 6.2 Hz, 1H), 6.92 (s,2H), 6.72 (d, *J* = 7.4 Hz, 1H); ^{13}C NMR (DMSO, 100 MHz) δ 160.29, 159.04, 148.88, 148.63, 133.15, 130.62, 129.93, 127.10, 126.76, 126,16, 120.32, 114.61, 111.42, 100.98, 55.57, 55.37, 45.50; MS *m/z* (%) 346 (100), 347 (M^{+1}, 22), 348 (M^{+2}, 34).

7-Chloro-3-(4-chlorophenyl)-2-phenylquinazolin-4(3*H*)-one (QA5): m.f.: $C_{20}H_{12}N_2OCl_2$; m.w.: 366.22; colour and appearance: off white crystals; m.p.: 210 °C; yield: 45.26 %; R_f value: 0.74 (ethyl acetate:chloroform (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3020 (Ar-H str), 822 (C-C), 1179 (C-N), 1607 (C=N), 1665 (C=O), 606 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ 9.62 (s, 2H), 8.15 (d, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 6.2 Hz, 1H), 6.80 (s, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 163.43, 161.01, 160.34, 157.23, 149.27, 149.20, 148.81, 130.61, 130.52, 129.83, 123.44, 123.42, 120.45, 114.09, 113.88, 113.55, 113.34, 113.05, 112.45, 102.02, 55.99, 45.66; MS *m/z* (%) 366 (100), 368 (M+2, 64).

7-Chloro-3-(2-ethyl-6-methylphenyl)-2-phenylquinazolin-4(3*H*)-one (QA6): m.f.: $C_{23}H_{19}N_2OCl$; m.w.: 374.86; colour and appearance: pinkish white crystals; m.p.: 185 °C, yield: 23.28 %; R_f value: 0.72 (*n*-hexane:ethanol(1:1)); IR (KBr, ν_{max}, cm^{-1}): 3010 (Ar-H str), 819 (C-C), 1180 (C-N), 1603 (C=N), 1662 (C=O), 648(C- Cl); ^{1}H NMR (DMSO, 400 MHz) δ 7.44 (s, 3H), 7.38 (s, 2H), 7.24 (t, *J* = 6.24 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 6.81 (s, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 163.44, 161.01, 160.29, 159.02, 149.06, 149.00, 148.88, 130.68, 130.59, 129.91, 123.46, 120.35, 114.70, 114.12, 113.91, 113.65, 113.43, 111.36, 100.96, 55.60, 55.37, 45.67; MS *m/z* (%) 374 (100) 376 (M^{+2}, 36).

6-Chloro-3-(4-methylphenyl)-2-phenylquinazolin-4(3*H*)-one (QA7): m.f.: $C_{21}H_{15}N_2OCl$; m.w.: 346.8; colour and appearance: off white crystals; m.p.: 155 °C; yield: 20.49 %; R_f value: 0.64 (ethyl acetate:chloroform (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3012 (Ar-H str), 819 (C-C), 1180 (C-N), 1603 (C=N), 1662 (C=O), 2732 (C-CH_3); ^{1}H NMR (DMSO, 400 MHz) δ 8.18 (s, 2H), 7.60 (d, *J* = 6 Hz, 1H), 7.12 (d, *J* = 6.4 Hz, 1H), 6.94 (s, 2H), 6.66 (d, *J* = 7.2 Hz, 1H); ^{13}C NMR (DMSO, 100 MHz) δ 160.49, 150.31, 149.12, 148.92, 133.07, 130.51, 129.37, 127.04, 126.56, 126.13, 120.59, 109.61, 109. 45, 98.48, 55.72, 45.94, 45.43; MS *m/z* (%) 346 (100), 348 (M^{+2}, 34).

6-Chloro-2-(4-chlorophenyl)-3-phenylquinazolin-4(3*H*)-one (QA8): m.f.: $C_{20}H_{12}N_2OCl_2$; m.w.: 366.22; colour and appearance: off white crystals; m.p.: 190 °C; yield: 53.05 %; R_f value: 0.71 (ethyl acetate: chloroform (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3020 (Ar-H str), 822 (C-C), 1179 (C-N), 1607 (C=N), 1665 (C=O), 606 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ 9.64 (s, 2H), 8.18 (d, *J* = 7.6 Hz, 1H), 7.16 (d, *J* = 6.2 Hz, 1H),6.70 (s, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 160.33, 157.28, 149.09, 148.79, 130.30, 129.94, 129.87, 129.57, 126.56, 121.80, 120.43, 112.94, 112.51, 102.24, 55.99, 45.60; MS *m/z* (%) 366 (100), 368 (M+2, 64).

6-Chloro-2-(2-ethyl-6-methylphenyl)-3-phenylquinazolin-4(3*H*)-one (QA9): m.f.: $C_{23}H_{19}N_2OCl$; m.w.: 374.86; colour and appearance: light violet crystals; m.p.: 228 °C; yield: 28.07 %; R_f value: 0.64 (*n*-hexane:ethanol (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3010 (Ar-H str), 819 (C-C), 1180 (C-N), 1603 (C=N), 1662 (C=O), 2732 (C-CH_3); ^{1}H NMR (DMSO, 400 MHz) δ 7.42 (s, 3H), 7.36 (s, 2H), 7.29 (t, *J* = 6.24 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 6.81 (s, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 160.28, 159.04, 149.09, 148.89, 133.07, 130.94, 129.96, 129.66, 126.56, 121.84, 120.33, 114.61, 111.43, 100.98, 55.57, 55.37, 45.53; MS *m/z* (%) 374 (8) 369 (2), 354 (15), 340 (8), 325 (2), 262 (16), 160 (100), 120 (20).

Synthesis of 3-amino-2-phenyl-4(3*H*)-quinazolinone: To a solution of 0.05 mol of compound **1** in 20 mL pyridine, added 0.15 mol of 80 % hydrazine hydrate solution. The mixture was stirred well and refluxed at 117 °C for about 2 h. after refluxed is stopped, the reaction mixture was cooled and filtered to get the crude product. The pure compound (compound **2**) can be obtained by recrystallization from warm ethanol [12]. m.f.: $C_{14}H_{11}N_3O$, m.w.: 237.25, colour and appearance: lemon yellow crystals, m.p.: 172 °C, yield: 83 %, R_f value: 0.72 (toluene:chloroform (8:2)), UV λ_{max} (methanol) 270 nm, IR (KBr, ν_{max}, cm^{-1}): 3316 (NH_2), 1659 (C=O), 1602 (C=N).

Synthesis of QB1-QB3: 0.47 g of compound **2**, 0.002 mol of substituted aryl aldehydes and 2-3 drops of glacial acetic acid was taken in a round bottom flask and refluxed for 3-4 h. After the completion of the reaction, which is monitored by performing TLC, the solvent was removed and the final product was recrystallized from ethanol [13].

3-{(*Z*)-[2-(3-Chlorophenyl)ethylidene]amino}-2-phenylquinazolin-4(3*H*)-one (QB1): m.f.: $C_{21}H_{14}N_3OCl$; m.w.: 359.8; colour and appearance: white crystals; m.p.: 214 °C; yield: 13.89 %; R_f value: 0.72 (dimethyl formamide:ethyl acetate (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3028(Ar-H str), 1654 (C=O), 1604 (C=N), 1180 (C-N), 697 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ 8.96 (s, 2H), 7.83 (d, *J* = 7.4 Hz, 1H), 6.50 (d, *J* = 2.4, 2H), 6.21 (d, *J* = 2.4 Hz, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 163.43, 161.01, 160.34, 157.23, 149.27, 149.20, 148.81, 130.61, 130.52, 129.83, 123.44, 123.42, 120.45, 114.09, 113.88, 113.55, 113.34, 113.05, 112.45, 102.02, 55.99, 45.66; MS *m/z*, 359 (M+, 100), 360 (M+1, 22), 361 (M+2, 34).

3-[(*E*)-(3-Bromobenzylidene)amino]-7-chloro-2-phenylquinazolin-4(3*H*)-one (QB2): m.f.: $C_{21}H_{13}N_3OBrCl$; m.w.: 438.7; colour and appearance: cream crystals; m.p.: 210 °C; yield: 24.28 %; R_f value: 0.71 (acetonitrile:ethyl acetate (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3030 (Ar-H str), 1654 (C=O), 1604 (C=N), 1174 (C-N), 697 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ 8.86 (s, 2H), 7.73 (d, *J* = 9.4 Hz, 1H), 6.60 (d, *J* =4.4, 2H), 6.51 (d, *J* = 6.4 Hz, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 160.33, 157.27, 148.83, 148.80, 133.10, 130.57, 129.86,

127.08, 126.67, 126.16, 120.43, 112.94, 112.51, 102.23, 55.99, 45.58; MS *m/z*, 439 (M+, 100), 441 (M+2, 26), 437 (74).

3-[(*E*)-(3-Bromobenzylidene)amino]-6-chloro-2-phenylquinazolin-4(3*H*)-one (QB3): m.f.: $C_{21}H_{13}N_3OBrCl$; m.w.: 438.7; colour and appearance: cream crystals; m.p.: 210 °C; yield: 24.28 %; R_f value: 0.71 (acetonitrile:ethyl acetate (1:1)); IR (KBr, ν_{max}, cm^{-1}): 3024 (Ar-H str), 1684 (C=O), 1624 (C=N), 1180 (C-N), 697 (C-Cl); ^{1}H NMR (DMSO, 400 MHz) δ 8.76 (s, 2H), 7.43 (d, *J* = 7.2 Hz, 1H), 6.40 (d, *J* = 2.8, 2H), 6.11 (d, *J* = 2.6 Hz, 2H); ^{13}C NMR (DMSO, 100 MHz) δ 160.33, 157.28, 149.09, 148.79, 130.30, 129.94, 129.87, 129.57, 126.56, 121.80, 120.43, 112.94, 112.51, 102.24, 55.99, 45.60; MS *m/z*: 439 (M+, 100), 441 (M+2, 26), 437 (74).

Antioxidant activity

Nitric acid free radical scavenging method: Nitric oxide was generated from sodium nitroprusside and measured by the Griess reagent. 0.5 mL of the sample was mixed with 0.5 mL of phosphate buffer (pH 7.4) and 2 mL sodium nitroprusside solution. The mixture was incubated at 25 °C for 2 h. 0.5 mL of the reaction mixture was pipetted out and mixed with 1 mL pf sulphanilic acid and allowed to stand for 5 min for complete diazotization. Then 1 mL of 0.1 % N-naphthylethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 min to form pink coloured chromophore. Absorbance was then measured at 530 nm against the corresponding blank solution.

Hydrogen peroxide free radical scavenging method: 1 mL of standard ascorbic acid and test solution was added to 0.6 mL hydrogen peroxide solution. After 10 min the absorbance of the solution was measured at 230 nm using UV/visible spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of both and test compound were determined.

***In vitro* anti-inflammatory activity:** 2 mL of each sample was mixed with 2 mL of 2 mM of BSA (1.329 g in 10 mL of phosphate buffer) and incubated at 27 ± 1 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 60 ± 1 °C in electronic water bath for 10 min. After cooling, the turbidity was measured at 660 nm. The percentage inhibitions of all test samples were determined.

***In vitro* cytotoxicity-MTT assay:** Cell lines used were HT-29 cell line was obtained from National Centre for Cell Sciences, Pune, India. Stock cells was cultured in culture medium composed of DMEM supplemented with 10 % inactivated foetal bovine serum, streptomycin (100 µg/mL), penicillin (100 IU/mL) and amphotericin B (5 µg/mL) in a moist atmosphere of 5 % CO_2 at 37 °C until consistent. The cells were disassociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and 96 microtitre plates were used to carry out the experiment [14-18].

Procedure: The cell culture was trypsinized and by using DMEM containing 10 % FBS the cell count was adjusted to 1.0×10^5 cells/mL. 0.1 mL of the diluted cell suspension were added to each well of 96 well microtitre plate. A partial monolayer was developed after 24 h, the supernatant flicked off, washed the monolayer once with medium and 100 µL each of different test concentrations of the corresponding test drugs were added on the partial monolayer in microtitre plates. Then it was incubated for 3 days at 37 °C in 5 % CO_2 atmosphere and microscopic examinations were carried out every 24 h. The drug solutions in the wells were discarded after 72 h and 50 µL of MTT in PBS was added to each well. Then the plates were shaken gently and incubated at 37 °C for 3 h in 5 % CO_2 atmosphere. The supernatant was removed and then propanol of volume 100 µL was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured at a wavelength of 540 nm using a micro plate reader [15,16]. The percentage growth inhibition was calculated and from the dose-response curves concentration of test drug required to inhibit cell growth by 50 % (CTC_{50}) values for each cell line are generated [17].

RESULTS AND DISCUSSION

Design of novel quinazoline-4-ones: 2-Phenyl-quinazolin-4-one derivatives were docked against the three known targets, thymidylate synthase (1JU6) and focal adhesion kinase receptor (1MP8) were anticancer targets and cyclooxygenase 2 receptor (1CX2) was the anti-inflammatory protein target. The ligands were validated by docking operation using AutoDock. Reference standards were used to compare docking score of the ligands. The bound analogues were examined for their hydrogen bonding and binding energies, which was shown in the Table-1.

TABLE-1
AUTODOCK DOCKING SCORES

S. No.	Compd. code	Docking score (kcal/mol)		
		1JU6	1MP8	1CX2
1	**QA1**	-7.41	-6.41	-5.86
2	**QA2**	-7.10	-7.23	-6.41
3	**QA3**	-6.23	-7.44	-6.82
4	**QA4**	-6.16	-6.45	-6.61
5	**QA5**	-5.99	-7.06	-5.49
6	**QA6**	-5.98	-6.26	-6.37
7	**QA7**	-7.45	-5.89	-5.16
8	**QA8**	-7.05	-6.34	-6.83
9	**QA9**	-6.35	-6.99	-6.81
10	**QB1**	-6.10	-7.21	-6.84
11	**QB2**	-6.92	-7.24	-6.42
12	**QB3**	-6.99	-6.48	-5.86
13	Raltitrexed	-4.83	-4.31	-3.21
14	Ibuprofen	–	–	-4.12

AutoDock results showed that most of the derivatives have hydrogen bonding between the ligand-target interactions (Fig. 1). These hydrogen bonding interactions helped to increase the binding energy of ligand-protein interactions.

Lipinski rule of 5 and their extension parameters like number of rotatable bonds and TPSA were used to evaluate the drug likeness of the derived analogues. The drug-likeness assessments of the compounds were shown in Table-2.

These results revealed that the value of all the derivatives were within the optimal range of molecular weight less 500 Daltons and possessed number of hydrogen bond donors (HBD) and hydrogen bond acceptors (HBA) of all the analogues below 5 and 10, respectively. All the number of rotatable bonds and

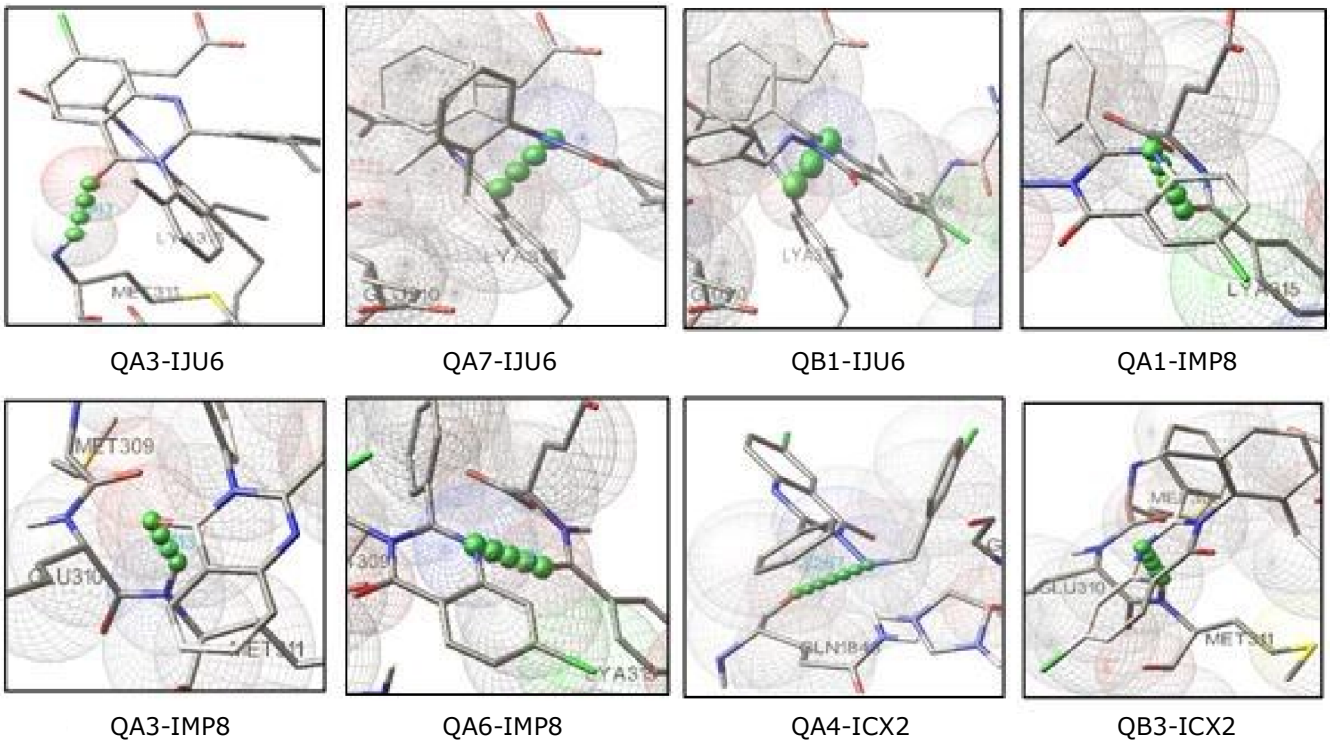
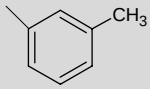
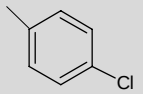
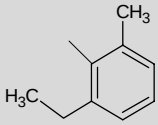
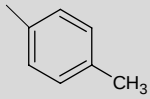
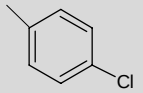
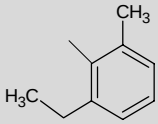
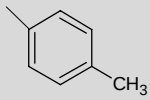
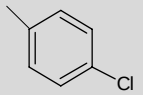
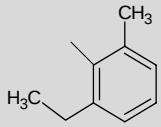
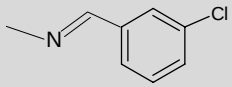
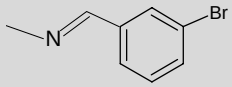
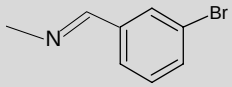


Fig. 1. Ligand-target complexes with H_2 bonding interactions by AutoDock 4.0 program

TABLE-2
DRUG LIKENESS ASSESSMENTS OF THE 2-PHENYL-QUINAZOLIN-4-ONE DERIVATIVES

Compd. code	R_1	R_2	R_3	Number of HBA	Number of HBD	C log P	Number of rotatable bonds	TPSA (Å^2)
QA1	H	H		2	0	4.37	2	69.277
QA2	H	H		2	0	4.80	2	74.277
QA3	H	H		2	0	3.15	2	62.283
QA4	Cl	H		2	0	4.20	2	69.283
QA5	Cl	H		2	0	3.52	2	76.277
QA6	Cl	H		2	0	3.86	3	62.287
QA7	H	Cl		2	0	4.20	2	69.283
QA8	H	Cl		2	0	3.52	2	76.277

QA9	H	Cl		2	0	3.86	3	62.287
QB1	H	H		3	0	4.91	3	68.682
QB2	Cl	H		3	0	4.76	3	70.471
QB3	H	Cl		3	0	4.76	3	70.471

values of partition coefficient were under the limit of 10 and 5, respectively. The polarity of the molecules is explained by the physico-chemical property called topological polar surface area, none of the analogues exhibited TPSA greater than 140 Å^2. This parameter has been found to associate with the gastro-intestinal absorption, Caco-2 permeability and blood-brain barrier penetration. All these data revealed that having no more violations makes it likely to be an orally active drug.

Substituted or unsubstituted anthranilic acid reacts with benzoyl chloride followed by dehydration to form the benzoxazinone intermediate. The subsequent addition of an amine or an aldehyde to the above mixture provides the fused quinazolinones. Reaction for the synthesis was shown in Fig. 2.

IR spectrum showed characteristic bands at 3150-3050 and 1600-1400 cm^{-1} indicating the presence of aromatic –CH stretching and C=C stretching respectively. The characteristic

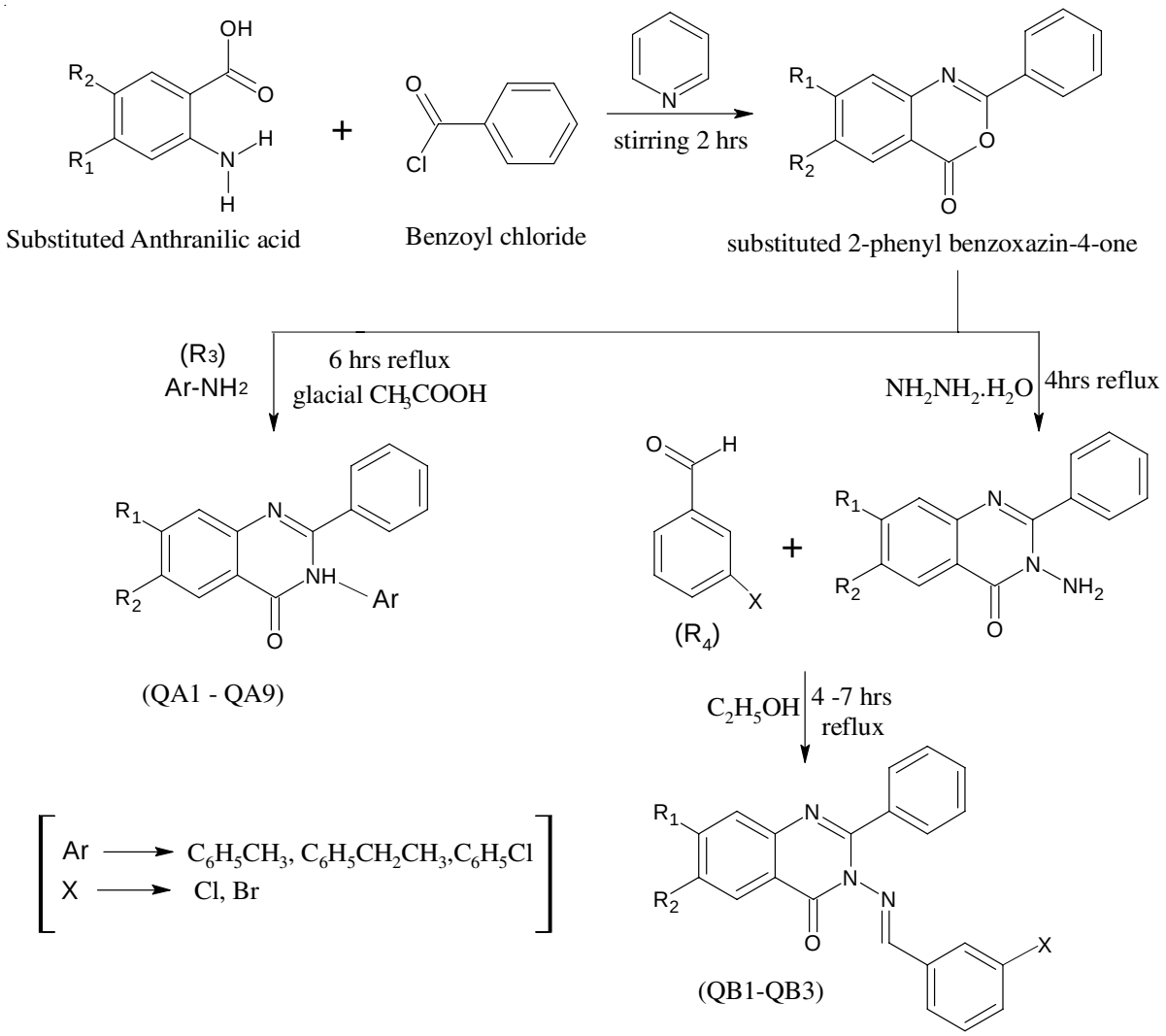


Fig. 2. Preparation of 2-phenyl-3-substituted quinazoline-4-one

bands for alkyl –CH were seen at 3000-2800 cm^{-1}. The characteristic bands for halogen groups like chlorine and bromine were found at 800-680 cm^{-1} and 616-620 cm^{-1}, respectively. For C=O of amide, the characteristic peaks were observed at 1670-1630 cm^{-1}. The characteristic peak for C-N and C=N were found at 1280-1180 and 1640-1600 cm^{-1}, respectively. This clearly suggests the formation of expected compounds.

^{1}H and ^{13}C were carried out using DMSO as solvent. The characteristic signals for the protons nearer to electron withdrawing groups were found at higher chemical shift range and signlas for the proton nearer to electron donating groups were found at lower chemical shift range. ^{1}H NMR showed peaks for the protons of alkyl group at 2-4 δ ppm. For aryl protons peaks were found at 6-10 δ ppm. First and second hydrogens singlet or doublet peaks depended upon the substitutions on the adjacent carbon atom. For 4th, 5th and 6th hydrogens a single triplet peak and for 3rd and 5th hydrogens a single doublet peaks were seen. The δ value were found at 6.5-8.1 ppm for above hydrogens. ^{13}C NMR signals assigned to confirm the number of carbon atoms present in the prepared compound. Aromatic carbon aatoms on the ring were seen in the range of 120-150 δ ppm. Aliphatic carbon atoms were found at 2-4 δ ppm. This ^{1}H and ^{13}C NMR spectral data led to confirm the structure of the targeted compounds.

Formation of the derivatives were also confirmed by mass spectrometric analysis. Mass spectroscopy of these compounds were recorded in the positive ion mode. Base peak and molecular ion peak were same. Halogens were present in majority of the compound and gave characteristic M+2 peaks.

***In vitro* analysis:** The *in vitro* anti-inflammatory activity of 2-phenyl quinazolin-one derivatives was evaluated by albumin denaturation method and is compared with that of the standard ibuprofen. Results were summarized in the Table-3.

All the compounds showed significant level of anti-inflammatory activity, but the level of percentage inhibition was less than the reference standard. The results states that the molecules **QA7**, **QA4** and **QA9** having a very low IC_{50} value when compared to the other molecules.

In vitro antioxidant activity of 2-phenyl quinazoln-4-one derivatives was assessed by hydrogen peroxide and nitric oxide free radical scavenging activity. Ascorbic acid and butylated hydroxytoluene (BHT) were used as reference standards. Percentage inhibitions of all the derivatives found out by hydrogen peroxide method and nitric oxide radical scavenging method were shown in the Tables 4 and 5, respectively.

TABLE-3
INHIBITION (%) SHOWED BY SYNTHESIZED COMPOUNDS (ALBUMIN DENATURATION METHOD)

Compound code	Percentage inhibition ± SEM			IC_{50} µg/mL
	10 µg/mL	20 µg/mL	30 µg/mL	
QA1	33.20±0.28	48.40±0.22	59.73±0.26	20.8
QA2	36.19±0.69	52.00±0.52	66.94±0.39	18.4
QA3	32.67±0.13	56.66±0.38	67.79±0.33	16.8
QA4	29.41±0.08	54.13±0.04	67.77±0.39	14.2
QA5	31.64±0.38	54.31±0.61	63.81±0.48	18.0
QA6	31.66±0.39	52.30±0.67	58.24±0.62	16.6
QA7	35.41±0.48	69.99±0.77	83.19±0.69	13.8
QA8	37.62±0.17	47.76±0.20	64.66±0.32	20.4
QA9	41.44±0.22	56.24±0.66	64.58±0.08	14.4
QB1	41.18±0.68	65.12±0.34	70.34±0.16	16.2
QB2	31.56±0.21	45.76±0.25	68.46±0.32	22.6
QB3	40.70±0.01	48.53±0.01	56.12±0.03	20.2
Ibuprofen	80.35±0.07	80.80±0.04	84.22±0.06	11.8

Nitric oxide scavenging activity displayed by **QA4**, **QA3** and **QA1** were higher when compared to the other compounds. From the results of hydrogen peroxide scavenging activity, it was observed that maximum scavenging activity was exhibited by **QA4**, **QA7** and **QA1** when compared to standard ascorbic acid in hydrogen peroxide scavenging method.

In vitro anticancer screening was carried out by MTT assay in colorectal cancer (HT-29) cell lines. Twelve of the synthesized compounds were submitted to cytotoxicity study. Medium of cancer cell lines without samples were served as control. From the dose-response curves concentration of test drug needed to inhibit cell growth by 50 % (CTC_{50}) was identified. Dose dependent curve was plotted between % Cell inhibition and concentration and CTC_{50} was determined from the curve by using Graph Pad Prism software. The results were shown in Table-6.

In vitro activity screening was done for all the compounds at different concentrations of 100, 50, 25, 12.5 and 6.25 µg/mL. All the compounds possessed moderate to good anticancer

TABLE-4
PERCENTAGE INHIBITION SHOWED BY SYNTHESIZED DERIVATIVES (N_2O SCAVENGING METHOD)

Compound code	Percentage inhibition ± SEM					IC_{50} µg/mL
	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	
QA1	39.45 ± 0.78	45.60 ± 0.18	53.77 ± 0.86	58.52 ± 0.83	63.18 ± 0.79	25.4
QA2	25.45 ± 0.07	39.38 ± 0.10	48.61 ± 0.11	61.57 ± 0.11	70.65 ± o.11	30.4
QA3	39.57 ± 0.95	47.72 ± 0.58	53.94 ± 0.20	62.95 ± 0.81	72.92 ± 0.20	24.8
QA4	26.82 ± 0.10	44.13 ± 0.09	55.50 ± 0.11	65.42 ± 0.26	72.69 ± 0.09	23.8
QA5	30.30 ± 0.20	46.18 ± 0.14	59.41 ± 0.13	67.42 ± 0.06	76.67 ± 0.09	26.2
QA6	39.29 ± 0.59	44.36 ± 0.67	52.18 ± 0.47	62.17 ± 0.32	74.09 ± 0.27	29.4
QA7	32.91 ± 0.85	40.25 ± 0.97	49.08 ± 0.06	54.32 ± 0.41	60.39 ± 0.38	30.2
QA8	36.16 ± 0.11	43.63 ± 0.47	51.00 ± 0.43	55.95 ± 0.68	63.61 ± 0.09	29.2
QA9	28.51 ± 0.14	45.41 ± 0.14	54.28 ± 0.18	68.63 ± 0.14	74.68 ± 0.10	25.6
QB1	29.42 ± 0.82	38.76 ± 0.20	50.37 ± 0.48	55.76 ± 0.95	65.05 ± 0.36	29.2
QB2	33.24 ± 0.58	40.23 ± 0.69	53.32 ± 0.44	56.35 ± 0.25	66.85 ± 0.06	29.2
QB3	29.60 ± 0.18	42.06 ± 0.04	53.47 ± 0.12	66.61 ± 0.11	75.37 ± 0.18	27.3
BHT	31.75 ± 0.14	38.55 ± 0.08	49.65 ± 0.04	58.40 ± 0.48	66.28 ± 0.06	30.4
Ascorbic acid	34.77 ± 0.05	50.53 ± 0.05	68.47 ± 0.06	77.82 ± 0.04	89.55 ± 0.17	20.6

TABLE-5
PERCENTAGE INHIBITION SHOWED BY SYNTHESIZED DERIVATIVES (H_2O_2 SCAVENGING METHOD)

Compound code	Percentage inhibition ± SEM					IC_{50} (µg/mL)
	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	
QA1	25.46 ± 0.06	42.75 ± 0.06	56.35 ± 0.05	65.35 ± 0.09	71.83 ± 0.08	25.4
QA2	35.72 ± 0.94	41.20 ± 0.64	49.77 ± 0.45	56.08 ± 0.43	64.92 ± 0.86	31.6
QA3	23.64 ± 0.06	37.26 ± 0.07	49.14 ± 0.05	60.83 ± 0.05	70.84 ± 0.04	30.4
QA4	27.26 ± 0.05	46.46 ± 0.08	55.45 ± 0.08	63.69 ± 0.06	74.83 ± 0.08	24.2
QA5	32.04 ± 0.43	42.19 ± 0.81	47.50 ± 0.64	59.16 ± 0.99	69.03 ± 0.46	30.8
QA6	31.70 ± 0.19	38.21 ± 0.46	48.41 ± 0.77	53.95 ± 0.11	62.31 ± 0.68	30.2
QA7	29.88 ± 0.79	42.65 ± 0.60	52.93 ± 0.47	63.48 ± 0.66	68.41 ± 0.39	24.6
QA8	35.44 ± 0.77	40.42 ± 0.52	52.29 ± 0.55	60.02 ± 0.91	70.29 ± 0.49	26.4
QA9	29.35 ± 0.19	44.55 ± 0.04	56.37 ± 0.17	68.35 ± 0.14	76.46 ± 0.18	24.6
QB1	34.55 ± 0.14	38.77 ± 0.12	53.04 ± 0.48	58.45 ± 0.61	66.71 ± 0.34	26.0
QB2	26.44 ± 0.07	41.75 ± 0.22	53.61 ± 0.21	66.45 ± 0.05	74.55 ± 0.04	29.4
QB3	28.21 ± 0.04	43.65 ± 0.04	54.57 ± 0.08	67.33 ± 0.05	75.47 ± 0.08	28.0
BHT	29.85 ± 0.22	37.86 ± 0.05	48.44 ± 0.07	59.35 ± 0.08	68.14 ± 0.08	31.4
Ascorbic acid	26.89 ± 0.88	41.25 ± 0.06	56.76 ± 0.23	68.83 ± 0.03	86.72 ± 0.06	24.8

TABLE-6
PERCENTAGE CYTOTOXICITY OF PREPARED COMPOUNDS AGAINST COLORECTAL CANCER (HT-29) CELL LINES

Compound code	Test concentration (µg/mL)	Cytotoxicity (%)	CTC_{50} (µg/mL)	Compound code	Test concentration (µg/mL)	Cytotoxicity (%)	CTC_{50} (µg/mL)
	100	70.73			100	89.37	
	50	62.87			50	70.94	
QA1	25	48.97	25.8	**QA7**	25	67.33	14.8
	12.5	33.64			12.5	44.50	
	6.25	21.70			6.25	33.16	
	100	76.50			100	71.40	
	50	61.89			50	53.78	
QA2	25	41.68	33.6	**QA8**	25	32.86	46.4
	12.5	28.66			12.5	27.97	
	6.25	11.71			6.25	15.21	
	100	74.81			100	77.51	
	50	64.31			50	62.90	
QA3	25	45.73	28.2	**QA9**	25	42.69	33.4
	12.5	20.38			12.5	29.67	
	6.25	10.38			6.25	12.72	
	100	81.14			100	80.59	
	50	71.37			50	70.21	
QA4	25	66.85	11.2	**QB1**	25	59.22	17.6
	12.5	51.87			12.5	41.96	
	6.25	35.71			6.25	34.65	
	100	68.50			100	83.80	
	50	56.19			50	76.14	
QA5	25	37.68	41.6	**QB2**	25	58.53	19.8
	12.5	28.27			12.5	32.77	
	6.25	10.31			6.25	28.64	
	100	71.85			100	84.97	
	50	67.24			50	66.83	
QA6	25	44.96	29.4	**QB3**	25	53.17	19.8
	12.5	20.31			12.5	43.64	
	6.25	16.18			6.25	21.76	

activity. Compounds **QA4**, **QA7** and **QB1** were the most active compounds against HT-29 cell lines with IC_{50} of 11.2, 14.8 and 17.6 /mL, respectively. So these compounds were found to be more potent than the remaining compounds.

Conclusion

Few quinazoline derivatives were successfully synthesized in this work. Analytical and spectral analysis were performed for the same. The structural elucidation was done with the help of spectral data. These synthesized molecules were screened for anti-inflammatory, antioxidant and anticancer activity. It was concluded that all the compounds showed potent anticancer activity against HT-29 cell line with IC_{50} less than 50 µg/mL. These compounds possessed significant level of anti-infla-mmatory and antioxidant activity. Compounds **QA4**, **QA7** and **QB1** showed good anticancer and anti-inflammatory activity compared to other compounds. Cytotoxic effects of these compounds in cancer cells indicated that they were good candidates for further pharmacological studies to discover effective chemotherapeutic agents for the treatment of human colorectal carcinoma.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. V. Chandregowda, A.K. Kush and G. Chandrasekara Reddy, *Eur. J. Med. Chem.*, **44**, 3046 (2009); https://doi.org/10.1016/j.ejmech.2008.07.023.
2. V. Alagarsamy, V. Raja Solomon and K. Dhanabal, *Bioorg. Med. Chem.*, **15**, 235 (2007); https://doi.org/10.1016/j.bmc.2006.09.065.
3. A. Baba, N. Kawamura, H. Makino, Y. Ohta, S. Taketomi and T. Sohda, *J. Med. Chem.*, **39**, 5176 (1996); https://doi.org/10.1021/jm9509408.
4. A. Jose, A.B. Chittethu, S. Sankaran, S.T. Suja and K. Prem Ekambaram, *J. Pharm. Res.*, **6**, 933 (2013); https://doi.org/10.1016/j.jopr.2013.07.033.
5. H. Liu, R. Huang, D. Qiu, Z. Yang and X. Liu, *Prog. Nat. Sci.*, **8**, 359 (1998).
6. P.M. Chandrika, T. Yakaiah, B. Narsaiah, V. Sridhar, G. Venugopal, J.V. Rao, K.P. Kumar, U.S.N. Murthy and A.R.R. Rao, *Indian J. Chem.*, **48B**, 840 (2009).
7. G. Saravanan, V. Alagarsamy and C.R. Prakash, *Int. J. Pharm. Pharm. Sci.*, **2**, 83 (2010).
8. M.S. Malamas and J. Millen, *J. Med. Chem.*, **34**, 1492 (1991); https://doi.org/10.1021/jm00108a038.
9. S.B. Mhaske and N.P. Argade, *Tetrahedron*, **62**, 9787 (2006); https://doi.org/10.1016/j.tet.2006.07.098.
10. P.C. Sharma, G. Kaur, R. Pahwa, A. Sharma and H. Rajak, *Curr. Med. Chem.*, **18**, 4786 (2011); https://doi.org/10.2174/092986711797535326.
11. K. Manasa, R.V. Sidhaye, G. Radhika and C.N. Nalini, *Curr. Pharm. Res.*, **1**, 101 (2011).
12. N.A. Salih, *J. Al-Nahrain University*, **11**, 16 (2008); https://doi.org/10.22401/JNUS.11.2.03.
13. A.K. Nanda, S. Ganguli and R. Chakraborty, *Molecules*, **12**, 2413 (2007); https://doi.org/10.3390/12102413.
14. N. Thomas and S.M. Zachriah, *Int. J. Pharm. Sci. Rev. Res.*, **22**, 50 (2013).
15. F. Denizot and R.J. Lang, *Immunomethods*, **89**, 271 (1986).
16. H.A. Ali, N.H.A. Orooba and W.A. Khulood, *J. Assn. Arab. Univ. Basic Appl. Sci.*, **14**, 87 (2013).
17. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney and M.R. Boyd, *J. Natl. Cancer Inst.*, **82**, 1107 (1990); https://doi.org/10.1093/jnci/82.13.1107.
18. A.B. Chittethu, S. Sathianarayanan, A. Nair, E. Varghese, R.V. Gopal, and K.S. Sreelakshmi, *Int. J. Pharmacol. Biol. Sci.*, **5**, 75 (2011).