Research Article

Novel Quinolone Substituted Quinazolin-4(3H)-Ones as Anti-Inflammatory, Anticancer Agents: Synthesis and Biological Screening

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ABSTRACT

Background: Quinolones and, quinazolinones are important pharmacodynamic heterocyclic nuclei which when incorporated into different heterocyclic templates, have been reported to possess potent anti-inflammatory and anticancer properties. The activity of these compounds were associated with inhibition of nuclear factor-kappaB (NF-κB) which is one of the important targets studied for designing of anti-inflammatory and antitumor drug. Further, the combination of two pharmacophores on the same molecule is a well-established approach for designing of potent molecules and may further enhance their activity. **Objectives:** Aim of the study is to synthesis a series of quinolone substituted quinazolinones and evaluation of their anti-inflammatory and anticancer activity. **Methods:** Quinolone substituted quinazolin-4(3H)-ones were synthesized in two steps, first step involves synthesis of 7-amino-4-methyl-quinolin-2(1H)-one. Second step involves aminolysis of 7-amino-4-methyl-quinoline-2(1H)-one with substituted benzoxazines afforded quinolone substituted quinazolinones. Structures of synthesized compounds were characterized by spectral techniques. The synthesized compounds were evaluated for anti-inflammatory activity by carrageenan-induced rat paw oedema test and anticancer activity was assessed by evaluating the cytotoxic effect of synthesized compounds on BT-549 and HeLa, human cancer cell lines by MTT assay. **Results:** The synthesized compounds; 6, 7-dimethoxy-2-methyl-3-(4-methyl-2-oxo-1, 2-dihydroquinolin-7-yl)quinazolin-4(3H)-one (6d) was the most cytotoxic compound against HeLa and BT-549 human cancer cell lines. While the compounds 6,8-dibromo-2-methyl-3-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)quinazolin-4(3H)-one (6f) and 2-methyl-3-(4-methyl-2-oxo-1,2-dihydroquinolin-7-yl)-7-nitroquinazolin-4(3H)-one (6g) exhibited the highest anti-inflammatory activity in carrageenan-induced paw oedema model. **Conclusion:** Molecular docking studies revealed that combination of two pharmacophores was crucial for binding of quinolone substituted quinazolin-4(3H)-ones on NF-κB and good correlation was observed between docking scores and biological activity of synthesized compounds.

Key words: Anti-inflammatory, Anticancer, Docking, Quinolone, Quinazolinone, NF-κB.

INTRODUCTION

In spite of substantial advancement attained in anticancer therapy, high incidence of side effects and acquired drug resistance has become the major challenge in the treatment of cancer. Nevertheless most cancer treatments, involve chemotherapy, radiotherapy, and surgery, with chemotherapy remains the most significant pharmacological approach for anticancer therapy. Owing to limitations of existing chemotherapeutic agents like side effects and acquired resistance discovery of new chemotherapeutic agents with therapeutic profile is a need of the hour. Research over the years has established a strong

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link between inflammation and cancer.[1-2] Disease states like cancer and inflammation is strongly associated with activation of transcription factors belonging to the nuclear kappa B (NF-κB) family. Accordingly, molecules' targeting NF-κB dependent biological functions are considered of great interest and therefore are attractive therapeutic target for cancer and inflammation. Even though there is considerable progress in almost all the aspects of cancer research, the current therapeutic options have many disadvantages, including low efficacy and high degree of toxicity. Many cancer chemotherapeutic agents are themselves carcinogenic.[3] Therefore, there is a need to rationally design novel, targeted anti-cancer therapeutics, which are selective, less toxic and ultimately more effective than existing treatments.

Quinazolin-4(3H)-ones is a versatile fused heterocyclic scaffold frequently used for designing bioactive agents. Among the class of compounds containing quinazoline nucleus most important compounds are those which have hydroxyl group in the 2 or 4 positions in the quinazoline ring, adjacent to a heterocyclic nitrogen atom. This hydroxyl group on 2nd or 4th position is capable of exhibiting keto-enol tautomerism. Thus depending on the position of this tautomeric keto group these compounds may be classified in two types: 2-(1H) quinazolinones and 4-(3H) quinazolinones and therefore 4-hydroxyquinazoline, tautomeric with 4-keto-3, 4- dihydroquinazoline, is commonly named 4(3H)-quinazolone. This scaffold is found in more than 200 naturally occurring alkaloids and are not only synthetically important but also reported to possess a wide range of biological activity like anticancer,[4-6] anti-inflammatory,[7-8] antibacterial,[9] antifungal,[10] anticonvulsants[11-13] and antihypertensive.[14] More recent literature reports also shows that the anticancer properties of quinazolin-4(3H)-ones are associated with inhibition of NF-κB dependent gene transcription and thus initiating apoptosis.[15] On the other hand Quinolin-2(1H)-one skeleton which is present in many alkaloids and organic compounds of biological importance. Quinolin-2(1H)-one are isosteric with coumarins and isomeric to quinolin-4(1H)-ones. Various quinoline-2(1H)-ones derivatives were found to be associated with various biological activities such as antitumor,[16] antibacterial and anti-inflammatory activities[17] and many substituted quinolin-2-one derivatives have recently created great interest in chemotherapy as antitumor drugs.[16] Ruiz *et al*. reported cytotoxic mechanism of quinolone derivatives as alkylating agents.[18] The combination of two pharmacophores on the same molecule popularly called as hybrid pharmacophore approach is a well-known approach for the designing of more active and effective drugs with more predictable pharmacokinetic and pharmacodynamics properties. As quinazolinones and quinolones moieties are experimentally proven anticancer and anti-inflammatory pharmacophores, we thought of combining these pharmacophore and additional substitutions on this scaffolds would may further enhance their activity as anticancer and anti-inflammatory agents. Hence we thought of synthesizing a series of novel quinolone substituted quinazolinones and screen them for their anti-inflammatory and anticancer activity.

MATERIALS AND METHODS

Chemistry

All chemicals and solvents used were of analytical or reagent grades and were used without further purification. All the chemicals were purchased from Aldrich, Sigma-aldrich, Spectrochem, Himedia and SD Fine chemicals limited, India. The purity of all compounds was established by single spot on the pre-coated silica gel plates (TLC silica gel F_{254}, Merck, Germany). The TLC solvent systems used were chloroform: methanol (8:2), chloroform: acetone (3:7), hexane: ethyl acetate (7:3). Iodine vapour was used as developing reagent. Infrared spectra were recorded on a Shimadzu FTIR-8310 (Shimadzu, Japan) using potassium bromide discs. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrophotometer (Bruker, USA) in. Chemical shifts are reported in parts per million (δ) units relative to an internal standard of tetramethylsilane. Coupling constants are given in Hz and the relative peaks areas were in agreement with all assignments. Mass spectra were recorded on a Waters Q-TOF Premier spectrometer (Waters, Milford, MA, USA). Elemental analyses were performed on Leco CHNS-932 (Leco, St. Joseph, MI, USA). Melting points were determined on a capillary melting point apparatus (Shital Scientific Industries, India) and are uncorrected.

Synthesis of 7-amino-4-methyl-quinoline-2(1H)-one (3)

10.8 g (0.1 mol) of m-phenylenediamine (1) and 12.64 ml (0.1 mol) of ethylacetoacetate (2) were taken in a 250 ml round bottom flask. The reaction mixture was refluxed for 48 h. At the end of reaction period, 200 ml of water was added to the flask and the contents were heated to the boiling temperature of water. The mixture was then filtered; the filtrate was chilled in refrigerator till the precipitation appeared. The precipitate was collected, dried in air, recrystallized from methanol and

was characterized. (M. P. 270-272 °C, TLC Solvent system-Acetone: Chloroform- 7: 3).[19]

Synthesis of 2-methyl-4H-1, 3-benzooxazin-4-ones (5a-5g)

A mixture of antranilic acid or substituted anthranilic acid (0.01 M), acetic anhydride (0.02 M) and acetic acid (0.01 M) were taken in a 250 ml round bottom flask. The reaction mixture was refluxed for 4 h. At the end of reaction period, acetic acid together with acetic anhydride was distilled off under reduced pressure and the solid separated was immediately used for next step. (TLC Solvent system-Hexane: Ethyl acetate- 7: 3).[20]

Synthesis of Quinolone substituted quinazolin-4(3H)-ones (6a-6g)

General method: To a solution of 2-methyl-4H-1,3-benzooxazin-4-one (0.005 M) in 15 mL of glacial acetic acid, added 7-amino-4-methyl-quinoline-2(1H)-one (0.005 M) in portions with constant stirring for 10 minutes. The reaction mixture was refluxed for 9 h. The hot solution was added to a beaker containing 100 g of crushed ice. The solid separated was filtered, dried and recrystallized from methanol to give pure product. (TLC Solvent system- Chloroform: Methanol - 8: 2).[20]

2-methyl-3-(4-methyl-2-oxo-1, 2-dihydroquinolin-7-yl)quinazolin-4(3H)-one (6a)

Crystallized from methanol to give white crystals, yield 62%; mp 171-172 (°C); IR (KBR, γ, cm^{-1}): 3435.22 (N-H str.), 1660.75 (C=O str. of quinolone ring), 1598.99 (C=N str.), 1560.41 (C=C str.),1402.25 (C-N str.); ^{1}H NMR (DMSO-*d6*, δppm, 400 MHz): 11.843 (s, 1H, -NH-), 7.18-8.15 (m, 7H, Ar-H), 6.48 (s, 1H, =CH- of quinolone ring), 2.51(s, 3H, -CH_3 of quinazolinone) 2.23 (s, 3H, -CH_3); TOF MS ES$^+$ (*m/z*): 318 $[M+1]^+$, 340 $[M+Na]^+$; Analysis for $C_{19}H_{15}N_3O_2$: Calcd % C, 71.91; H, 4.76; N, 13.24. Found %: C, 71.88; H, 4.74; N, 12.94.

8-chloro-2-methyl-3-(4-methyl-2-oxo-1, 2-dihydroquinolin-7-yl) quinazolin-4(3H)-one (6b)

Crystallized from methanol to give white crystals, yield 58%; mp 180-182 (°C); IR (KBR, γ, cm^{-1}): 3415.93 (N-H str.), 1672.28 (C=O str. of quinolone ring), 1618.28 (C=N str.), 1575.84 (C=C str.), 1400.32 (C-N str.); ^{1}H NMR (DMSO-*d6*, δppm, 400 MHz): 11.518 (s, 1H, -NH-), 7.33-7.995 (m, 6H, Ar-H), 6.25 (s, 1H, =CH- of quinolone ring), 2.41 (s, 3H, -CH_3 of quinazolinone), 2.13 (s, 3H, -CH_3); TOF MS ES$^+$ (*m/z*): 352 $[M+1]^+$, 374 $[M+Na]^+$; Analysis for $C_{19}H_{14}ClN_3O_2$: Calcd % C, 64.87; H, 4.01; N, 11.94. Found %: C, 64.81; H, 4.04; N, 11.89.

7-chloro-2-methyl-3-(4-methyl-2-oxo-1, 2-dihydroquinolin-7-yl) quinazolin-4(3H)-one (6c)

Crystallized from methanol to give white crystals, yield 62%; mp 175-178 (°C); IR (KBR, γ, cm^{-1}): 3413.71 (N-H str.), 1675.95 (C=O str. of quinolone ring), 1617.32 (C=N str.), 1575.14 (C=C str.), 1402.11 (C-N str.); ^{1}H NMR (DMSO-*d6*, δppm, 400 MHz): 11.517 (s, 1H, -NH-), 7.34-7.998 (m, 6H, Ar-H), 6.31 (s, 1H, =CH- of quinolone ring), 2.48 (s, 3H, -CH_3 of quinazolinone), 2.19 (s, 3H, -CH_3); TOF MS ES$^+$ (*m/z*): 352 $[M+1]^+$, 369 $[M+H_2O]^+$. Analysis for $C_{19}H_{14}ClN_3O_2$: Calcd % C, 64.87; H, 4.01; N, 11.94. Found %: C, 64.78; H, 4.00; N, 11.87.

6, 7-dimethoxy-2-methyl-3-(4-methyl-2-oxo-1, 2-dihydroquinolin-7-yl) quinazolin-4(3H)-one (6d)

Crystallized from methanol to give white crystals, yield 78%; mp 186-187 (°C); IR (KBR, γ, cm^{-1}): 3392.72 (N-H str.), 1676.14 (C=O str. of quinolone ring), 1600.92 (C=N str.), 1563.28 (C=C str.), 1402.25 (C-N str.); ^{1}H NMR (DMSO-*d6*, δppm, 400 MHz): 11.538 (s, 1H, -NH-), 7.31-7.891 (m, 6H, Ar-H), 6.21 (s, 1H, =CH- of quinolone ring), 3.91(s, 6H, -$(OCH_3)_2$) 2.47 (s, 3H, -CH_3 of quinazolinone), 2.19 (s, 3H, -CH_3); TOF MS ES$^+$ (*m/z*): 378 $[M+1]^+$, 401 $[M+Na]^+$;
Analysis for $C_{21}H_{19}N_3O_4$: Calcd % C, 66.83; H, 5.07; N, 11.13. Found %: C, 66.78; H, 5.01; N, 11.09.

2, 6-dimethyl-3-(4-methyl-2-oxo-1, 2-dihydroquinolin-7-yl) quinazolin-4(3H)-one (6e)

Crystallized from methanol to give white crystals, yield 60%; mp 195-197 (°C); IR (KBR, γ, cm^{-1}): 3425.58 (N-H str.), 1683.86 (C=O str. of quinolone ring), 1595.83 (C=N str.), 1523.76 (C=C str.), 1408.04 (C-N str.); ^{1}H NMR (DMSO-*d6*, δppm, 400 MHz): 11.513 (s, 1H, -NH-), 7.32-7.899 (m, 6H, Ar-H), 6.23 (s, 1H, =CH- of quinolone ring), 2.45 (s, 3H, -CH_3 of quinazolinone), 2.31(s, 3H, -CH_3 of quinazolinone at 6th position) 2.17 (s, 3H, -CH_3). TOF MS ES$^+$ (*m/z*): 332 $[M+1]^+$; Analysis for $C_{20}H_{17}N_3O_2$: Calcd % C, 72.49; H, 5.17; N, 12.68. Found %: C, 72.38; H, 5.11; N, 12.59.

6, 8-dibromo-2-methyl-3-(4-methyl-2-oxo-1, 2-dihydroquinolin-7-yl) quinazolin-4(3H)-one(6f)

Crystallized from methanol to give white crystals, yield 68%; mp 195-196 (°C); IR (KBR, γ, cm^{-1}): 3415.35 (N-H str.), 1678.52 (C=O str. of quinolone ring), 1619.17 (C=N str.), 1535.11 (C=C str.), 1401.42 (C-N str.); ^{1}H NMR (DMSO-*d6*, δppm, 400 MHz): 11.521 (s, 1H, -NH-), 7.35-8.173 (m, 5H, Ar-H), 6.35 (s, 1H, =CH- of quinolone ring), 2.48 (s, 3H, -CH_3 of quinazolinone), 2.17 (s, 3H, -CH_3); TOF MS ES$^+$ (*m/z*): 477

$[M+2]^+$; Analysis for $C_{19}H_{13}Br_2N_3O_2$: Calcd % C, 48.03; H, 2.76; N, 8.84. Found %: C, 47.97; H, 2.55; N, 8.63

2-methyl-3-(4-methyl-2-oxo-1, 2-dihydroquinolin-7-yl)-7-nitroquinazolin-4(3H)-one (6g)

Crystallized from methanol to give white crystals, yield 63%; mp 225-227 (°C); IR (KBR, γ, cm^{-1}): 3415.51 (N-H str.), 1677.75 (C=O str. of quinolone ring), 1619.51 (C=N str.), 1567.34 (C=C str.), 1401.69 (C-N str.); ^{1}H NMR (DMSO-*d6*, δppm, 400 MHz): 11.533 (s, 1H, -NH-), 7.37-8.191 (m, 6H, Ar-H), 6.37 (s, 1H, =CH- of quinolone ring), 2.48 (s, 3H, $-CH_3$ of quinazolinone), 2.19 (s, 3H, $-CH_3$); TOF MS ES^+ (*m/z*): 363 $[M+1]^+$; Analysis for $C_{19}H_{14}N_4O_4$: Calcd % C, 62.98; H, 3.89; N, 15.46. Found %: C, 62.89; H, 3.76; N, 15.15.

Biological Evaluation

Animals

Animal care and handling were done as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals, (CPCSEA), Govt. of India. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC), Kasturba Medical College, Manipal-5761004, India (Registration No. IAEC/KMC/04/2013-2014). Albino rats of Charles foster species were used for anti-inflammatory studies. The animals were acclimatized to the experimental room having temperature 27 ±3°C, controlled humidity conditions, and 14:10 h light and dark cycle. The four animals were housed in each polypropylene cage containing paddy husk as bedding. The mice were fed on autoclaved standard mice food pellets (Hindustan Lever Ltd., New Delhi) and water ad libitum.

Toxicological study (OECD 425 Guidelines)

Acute toxicity study was done and safe dose was calculated as per OECD test guideline 425.[21]

Anti-inflammatory activity

Carrageenin-induced paw oedema test was performed on albino rats of Charles foster species. Groups of six rats of both sex (body weight 150–200 g), pregnant females excluded, were given a 100 mg/kg oral dose of a test compounds. 1 h later, 0.2 mL of 1% carrageenan suspension in 0.9% NaCl solution was injected subcutaneously, into the sub plantar tissue of the right hind paw of each mouse and the paw volume was measured with a plethysmometer (UGO Basile 7140, model-7141, Biological research apparatus, Italy) and then measured again 3 h later. Indomethacin (10 mg/kg) was used as reference drug. The mean increase of paw volume was compared with that of control group (six rats received only carrageenan, but not test compounds) and percent inhibition values were calculated by the formula: % anti-inflammatory activity = $1–D_t/D_c \times 100$. Where D_t represents the percentage difference in increased paw volume after the administration of test drugs to the rats and D_c represents the percentage difference of increased volume in the control group.[22]

Anticancer study

Cell lines

Human breast carcinoma cell line (BT-549), Human cervical adenocarcinoma cell line (HeLa) were procured from National Centre for Cell Science, Pune, India. The BT-549 and HeLa were cultured in the RPMI-1640 medium with 2mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and supplemented with 10% FBS, 10000 U/ml penicillin and 10mg/ml streptomycin. Both cell lines were incubated at 37°C, in 5% CO_2 humidified atmosphere.

In vitro cytotoxic study (MTT assay)

In vitro cytotoxicity of synthesized compounds was evaluated by MTT assay.[23] In brief, exponentially growing BT-549 and HeLa cells were plated in 96-well plates (10^4 cells/well, in 100 µl of media) and incubated for 24 h to obtain 60-80% confluency. The test compounds were solubilized in 50 µl DMSO and were further diluted with media so as to get DMSO concentration less than 0.25%.The cells were then exposed to different concentration of test compounds (500, 250, 125, 62.5, 31.25, 15.62, 7.81 µg/ml) in the volume of 100 µl /well. The control wells received only cells containing media and 0.25% DMSO. Cisplatin was used as positive control. Media was removed after 48 h, and 100 µl MTT reagent (1 mg/ml) was added to cell cultures, and then kept for incubation at 37°C for 4 h. The viable cells developed formazan complex; was solubilized by addition of 100 µl DMSO. The plates were placed on micro-vibrator for 5 min. The absorbance was recorded on BIOTEK EL X800- MS microtiter plate reader at 540 nm and percentage cytotoxicity was calculated as (control-test/control) ×100.

Statistical analysis

The statistical analyses were performed by one way analysis of variance, followed by Tukey's post hoc test using GraphPad Prism 5.02. The results are expressed as the mean± S.D. Statistical significance was set at $p<0.05$ level.

Molecular docking into DNA binding site of NF-κB

Library of thirty one quinolone substituted quinazolin-4(3H)-one derivatives was prepared by using chemdraw ultra 11.0. These derivatives were differing in their substitution at 5th, 6th, 7th and 8th position of quinolone substituted quinazolin-4(3H)-one moiety. The 3D crystal structures of the NF-κB were obtained from Protein Data Bank (PDB codes: 1NFK and 1LE9).[24-25] The 3D structures of NF-κB, p50-p50 homodimer (from 1NFK), p50 monomer chain A (p50a from 1NFK), p50 monomer chain B (p50b from 1NFK; p50b from 1LE9) were used for virtual screening. The co-crystallized DNA and water molecules were removed and AutoDock 4.2 was used for molecular docking studies. Docking parameters were set to default values on the basis of Lamarckian genetic algorithm principle.[26] The seven compounds with highest estimated free energy of binding were selected for wet lab synthesis. Synthesized compounds were biologically evaluated for anti-inflammatory and anticancer study and attempt was made to establish structure-activity relations of compounds to explore new lead compounds.

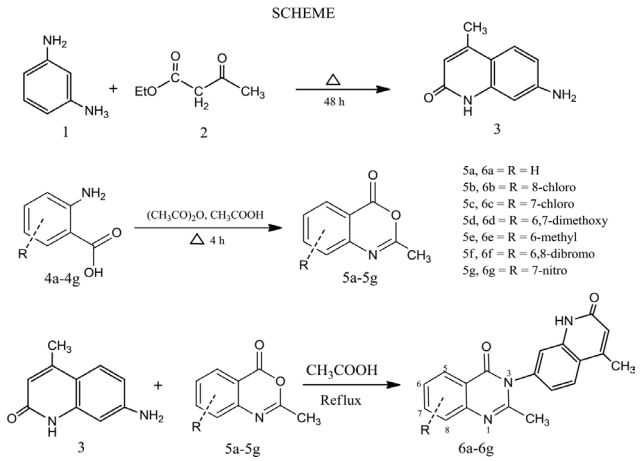
RESULTS AND DISCUSSIONS

Chemistry

Synthesis of quinolone substituted quinazolin-4(3H)-one involves 3 steps. In first step, 7-amino-4-methyl-quinoline-2(1*H*)-one (3) was synthesized by cyclocondensation of m-phenylenediamine (1) and ethylacetoacetate (2) (Scheme). Step-2 involves synthesis of 2-methyl-4*H*-1, 3-benzooxazin-4-ones (5a-5g) derivatives by cyclocondensation of various substituted antranilic acids (4a-4g) with acetic anhydride (Scheme). While in step-3 aminolysis 2-methyl-4*H*-1,3-benzooxazin-4-ones (5a-5g) derivatives with 7-amino-4-methyl-quinoline-2(1*H*)-one (3) by refluxing in glacial acetic acid yielded the title compounds quinolone substituted quinazolin-4(3H)-one (6a-6g). The reactions proceed by initial nucleophilic attack of amino group of 7-amino-4-methyl-quinoline-2(1*H*)-one on the carbonyl carbon of 2-methyl-4*H*-1,3-benzooxazin-4-one followed by ring opening by the breakage of C-O bond followed by intramolecular cyclization with elimination of water. Progresses of the reactions were monitored by TLC using chloroform: methanol (8:2) as mobile phase. The synthesized compounds were purified by column chromatography using silica gel 100-200 mesh and later by recrystallization in acetone to afford white/ yellowish-white products. The yields of synthesized compounds ranged from 58-78%.

Scheme

Synthetic scheme of quinolone substituted quinazolin-4(3H)-ones (6a-6g)



Structures of synthesized title compounds were confirmed by IR, NMR and Mass techniques. The FT-IR (KBr) spectra displayed characteristic absorption bands corresponding to N-H, C=O, C=C, C=N and C-N, stretching vibrations. IR spectra of all quinolone substituted thiazolidin-4-ones showed N-H stretching in the range of 3392.79-3435.22 cm^{-1}. The vibrational frequency at 1660.00–1683.86 cm^{-1} confirmed the presence of carbonyl group in synthesized compounds. Other characteristic peaks C=C, C=N, and C-N appeared at 1523.76–1581.63, 1595.83–1618.28, and 1400.32–1408.04 cm^{-1} respectively. NMR spectra of all the compounds showed NH proton in the range of 11.413–11.843 ppm as a broad signal. The methine proton (=CH-) of quinolone appeared as a singlet between 6.25-6.48 ppm. The methyl ($-CH_3$) protons of quinolone and quinazolin-4(3H)-one appeared as a singlet between 2.13-2.23 and 2.41-2.51 ppm respectively. TOF MS ES$^+$ spectra showed characteristic M^+/ $[M+1]^+$ peaks corresponding to molecular weight of synthesized compounds.

Biological evaluation

Anti-inflammatory activity

The anti-inflammatory activity of newly synthesized seven compounds was evaluated by carrageenan-induced paw oedema model in rats using indomethacin as a reference drug. Results are expressed as mean ± S.D. (Table 1). Differences between control and treatment groups were tested using one way analysis of variance followed by tukey's test. The test compounds administered 1 h prior to carrageenan injection at a dose of 100 mg/kg body wt. caused significant inhibition of paw oedema volume. Compound 6f, possessing

Table 1: Effect of test compounds on carrageenan induced paw oedema in rats.

Compound	Dose (mg/kg po)	%Oedema inhibition ± SD[b]
6a	100	13.39±1.28[a]
6b	100	21.45±1.39[a]
6c	100	25.71±1.17[a]
6d	100	21.34±2.11[a]
6e	100	11.61±1.93
6f	100	29.77±1.15[a]
6g	100	31.39±2.25[a]
Indomethacin	10	39.57±2.37[a]

[a] One way ANOVA followed by Tukey's test , a = p<0.05 Vs Control group
[b] Average of six determinations.

Table 2: *In vitro* cytotoxicity of compounds towards BT-549 and HeLa cells.

Compound	IC_{50} µg/ml± SD[a]	
	BT-549	HeLa
6a	29.54±2.11	33.87±3.17
6b	53.11±3.15	51.73±3.75
6c	54.85±4.23	48.72±3.91
6d	18.11±1.24	20.31±1.82
6e	51.33±3.57	65.95±4.89
6f	33.75±2.45	31.35±2.65
6g	23.62±2.31	25.51±2.18
Cisplatin	3.62±0.75	5.51±0.71

[a] Average of three determinations.

6,8-dibromo substitution on parent quinazolin-4(3H)-one moiety confers maximum anti-inflammatory activity followed by 6g bearing 7-nitro substituted quinazolin-4(3H)-one. In general compounds with electron withdrawing groups such as bromine/nitro substituted quinazolinones showed better anti-inflammatory activity. Pattern of percentage oedema inhibition among test compounds bearing halogen substituted quinazolinone ring was seen as: 6, 8-dibromo >7-nitro>7-chloro>8-chloro substitution. The unsubstituted quinazolinone ring possessing pharmacophore was least active among the synthesized compounds.

In vitro cytotoxic studies (MTT assay)

In the present work, newly synthesized seven compounds were screened for their *in vitro* growth inhibitory activities against two human cultured cell lines namely, human breast carcinoma cell line (BT-549) and Human cervical adenocarcinoma cell line (HeLa) at concentrations ranging from 7.81-500 µg/ml by MTT assay method. Results are shown as concentration (µg/ml ±S.D) which exhibited 50% cell death (IC_{50}) after 48 h of incubation (Table 2). Among the synthesized compounds compound 6d with 6, 7-dimethoxy substitution on parent quinazolin-4(3H)-one moiety confers maximum cytotoxicity followed by 6g bearing 7-nitro substituted quinazolin-4(3H)-one exhibited maximum cytotoxicity with an IC_{50} value of 20.31±1.82 and 25.51±2.18 µg/ml respectively on HeLa cells. While on BT-549 cells the same compounds 6d and 6g exhibited maximum cytotoxicity with an IC_{50} value of 18.11±1.24 and 23.62±2.31 µg/ml respectively. Thus the methoxy substituted quinazolinone exhibited maximum cytotoxic potential and 6, 8 dibromo substituted and 7-nitro substituted quinazolin-4(3H)-ones exhibited moderate growth inhibitory activity against HeLa and BT-549 cells.

Table 3: Estimated free energy of binding of synthesized compounds on the target nf-κb as dimer (p50-p50).

Compound	Estimated free energy of binding (kcal/mol)
6a	-9.71
6b	-7.11
6c	-8.14
6d	-10.91
6e	-7.85
6f	-9.97
6g	-10.22

Molecular docking study

The 3D structures of NF-κB-DNA complex was taken from Protein Data Bank (PDB code: 1NFK) and co-crystallized DNA was removed. The p50 dimer and p50 monomers (chains A and B) were used for the virtual screening and structures were prepared by using Chem Draw Ultra 11.0. All the water molecules were removed and hydrogens were added to protein. Library of thirty one molecules was docked into the binding site of receptor using Autodock 4.2.[27] Virtual screening results are expressed in terms of estimated free energy of binding (docking score) in Table 3. Estimated free energy of binding for all thirty one molecules ranged from -10.91 to -4.31 kcal/mol. Compound 6d showed estimated free energy of binding, -10.91 kcal/mol. Docked structure of 6d with p50 dimer (Figure 1) reveals that carbonyl (>C=O) group of quinolone along with methoxy group of quinazolinone moiety were involved in hydrogen bonding with Lys 145 and Leu 207 residues respectively. The >C=O group of quinolone formed hydrogen bond with -NH_2 group of Lys 145 (O…H-N: 1.966 Å), while 6-methoxy group of quinazolinone moiety interacted

with –NH_2 group of Leu 207 (O…H-N: 1.702 Å) by hydrogen bonding. The methyl group of quinazolinone moiety of 6d was accommodated in a hydrophobic pocket surrounded by Met 205, Lys 203, and Thr 202, similarly the methyl group of quinolone moiety was accommodated in a hydrophobic pocket surrounded by Val 147 and hydrophobic Phe 148 (Figure 2). Visual inspection of docked structure of 6d with receptor shows carbonyl oxygen of quinolone ring and methoxy substitution on quinazolinone contributed for the hydrogen bonding interaction. Thus the binding conformation of compound 6d shows that combination of two pharmacophores quinolone and quinazolinone might have played a crucial role in pharmacological profile of quinolone substituted quinazolin-4(3H)-ones.

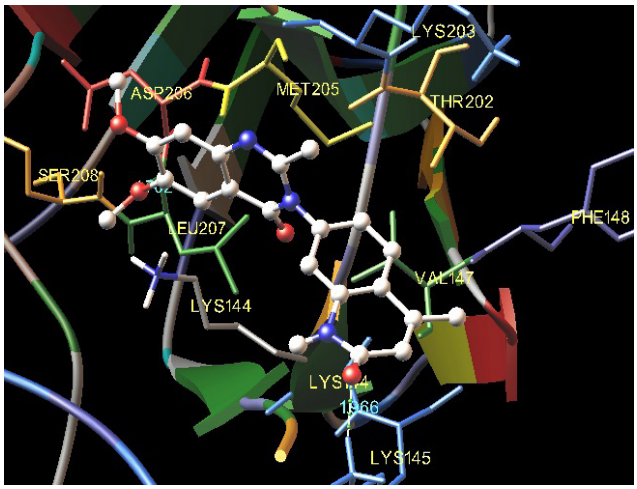


Figure 1: Stereoview of the complex formed by NF-κB homodimer and the docked compound 6d. The amino acids Lys 145, and Leu 207 were involved in hydrogen bonding interaction with the compound 6d.

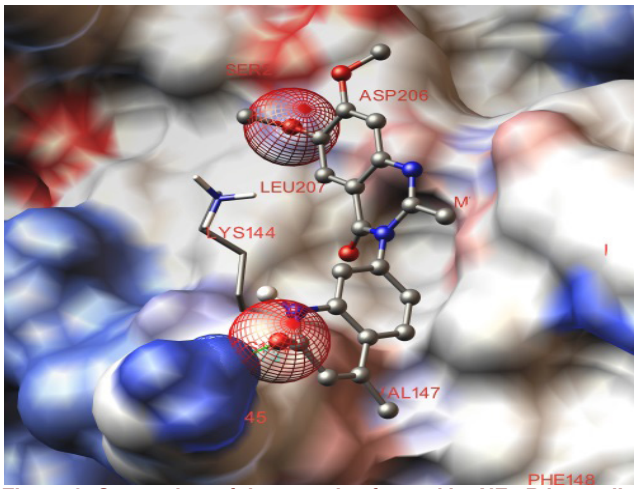


Figure 2: Stereoview of the complex formed by NF-κB homodimer and the docked compound 6d. The methyl group of quinolone and quinazolinone moiety of 6d was accommodated in a hydrophobic pockets surrounded by Met 205 and Val 147 respectively.

CONCLUSION

Virtual screening of quinolone substituted quinazolin-4(3H)-ones library resulted into identification of seven compounds. These seven compounds were selected based on their docking scores indicating their ability to bind with NF-κB protein. Further; results of docking studies confirm our hypothesis that conjugation of two pharmacophores might improve pharmacological profile of resulted/final pharmacophore. As carbonyl oxygen of quinolone ring and methoxy substitution on quinazolinone contributed for the hydrogen bonding interaction with various amino acids of receptor which resulted into better binding of compounds with NF-κB. Selected compounds were further synthesized in lab and biologically evaluated for anti-inflammatory and anticancer activity. Cyclocondensation of m-phenylenediamine with ethylacetoacetate yielded 7-amino-4-methyl-quinoline-2(1*H*)-one. In Step-2 synthesis of 2-methyl-4*H*-1, 3-benzooxazin-4-ones derivatives were carried out by cyclocondensation of various substituted antranilic acids with acetic anhydride. While in step-3 aminolysis 2-methyl-4*H*-1,3-benzooxazin-4-ones derivatives with 7-amino-4-methyl-quinoline-2(1*H*)-one by refluxing in glacial acetic acid yielded the title compounds quinolone substituted quinazolin-4(3H)-one Synthesis of test compounds was confirmed by IR, NMR and Mass spectra. *In vivo* anti-inflammatory activity of test drugs in carrageenan-induced paw oedema model identified compounds 6f and 6g as potent anti-inflammatory agents. Very good agreement existed between docking score and anti-inflammatory potency of screened drugs. *In vitro* anticancer activity of synthesized compounds was evaluated against BT-549 and HeLa human cancer cell lines. A fair correlation was observed between docking scores and anticancer potency of synthesized compounds. Among the synthesized compounds 6d and 6g exhibited maximum cytotoxic effect against BT-549 and HeLa human cancer cell lines. Hence; 6d and 6g are distinctly potent cytotoxic and anti-inflammatory agent as exhibited by *in vitro* and *in vivo* anti-inflammatory study these compounds may be developed as lead molecules for anticancer and anti-inflammatory activities respectively.

ABBREVIATIONS

FTIR: Fourier transform infrared spectroscopy; **NMR:** Nuclear magnetic resonance spectroscopy; **Q-TOF:** Quadrupole time of flight mass spectrometry; **TLC:** Thin layer chromatography; **MTT:** 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium bromide **OECD:** Organization for economic co-operation and development; **DMSO:** Dimethyl sulphoxide.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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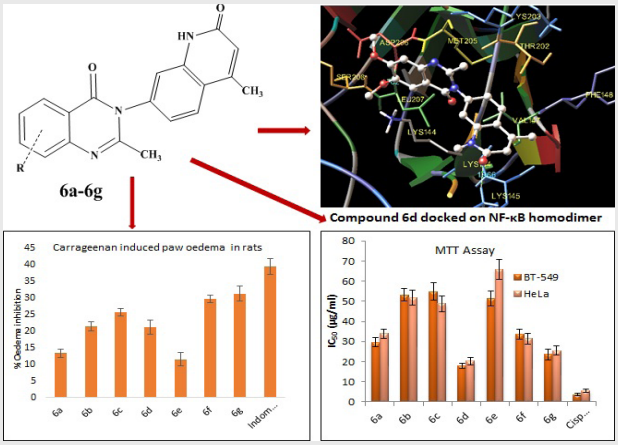
PICTORIAL ABSTRACT



SUMMARY

- Molecular docking studies of a library of quinolone substituted quinazolin-4(3H)-ones enabled to rank compounds according to their binding affinity towards NF-κB protein.
- Top ranked novel seven compounds with highest docking scores were synthesized and characterized by spectral techniques.
- The synthesized compounds were evaluated for anti-inflammatory activity by carrageenan-induced rat paw oedema test.
- The anticancer activity was assessed by evaluating the cytotoxic effect of synthesized compounds on BT-549 and HeLa, human cancer cell lines.
- • Virtual screening studies reveal that combination of two pharmacophores was crucial for binding of quinolone substituted quinazolin-4(3H)-ones on NF-κB.

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