Research Article

SYNTHESIS, EVALUATION AND PHARMACOLOGICAL SCREENING OF 2, 3-BENZOQUINOLINE DERIVATIVES FOR THEIR POSSIBLE ANTI-INFLAMMATORY AND ANTI-CANCER ACTIVITY

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ABSTRACT

The 2, 3-Benzoquinoline derivatives have been synthesized by condensation of dimedone, aldehydes and aniline through conventional methods. Their chemical structures were identified by spectral analysis like FT-IR, ¹H-NMR, and Mass spectrum. All the synthesized compounds of 2, 3-Benzoquinoline derivatives were screened for their possible anti-inflammatory and anticancer activity. In the present investigation test compounds R1, R2, and R3 were evaluated for in-vitro anti-inflammatory activity and in-vivo anticancer activity to provide the possible link between Inflammation and Inflammation Induced cancer. The results have clearly demonstrated that the compounds R1, R2, and R3 at different concentrations have good anti-protein denaturation activity; however it exhibited prominent anti-protein denaturation activity at its lowest concentration. The IC₅₀ values of R1, R2, and R3 of the synthetic test compounds were found to be 115µg/ml, 99µg/ml, and 96µg/ml against MCF-7 cell. The study also demonstrated that the test compound R3 had greater inhibitory effects on the proliferation of the breast cancer cell line by inducing apoptosis as revealed by the expression of the apoptotic genes.

Keywords: 2, 3-Benzoquinoline, Spectral analysis, Anti-inflammatory activity, anticancer activity.

INTRODUCTION

Inflammation is a physiological process in response to tissue damage resulting from microbial pathogen infection, chemical irritation or wounding. At the initial stage of inflammation, as primary response the neutrophils migrate to the inflammatory sites under the regulation of rapidly responding macrophages and mast cells pre-stained in tissues. As the inflammation progresses, it leads to activation and migration of various cells like leukocytes, lymphocytes, and other inflammatory cells to the inflamed site by signaling pathways involving complex growth factors, cytokines and chemokines. All cells recruited to the inflammatory site contribute to tissue breakdown and are beneficial by strengthening and maintaining the defense against infection.¹ Acridine is an organic compound and a nitrogen heterocycles with the formula C₁₃H₉N. Acridines are the substituted derivatives of the parent ring. The synthesis of acridine and analogues has attracted considerable attention from organic and medicinal chemists for many years, as a number of natural sources have been reported to have this heterocyclic nucleus. Structure activity relationship of acridine was established by an Australian chemist Adrien Albert.² The finding of his study indicated that cationic ionization and planar molecular surface area = 370Å is necessary for antibacterial activity. Acridine and related derivatives bind to DNA and RNA due to their abilities to intercalate. There are few reports in the literature on the three-component Hantzsch-type condensation of aromatic aldehydes, anilines and dimedone via the traditional organic solvents under microwave irradiation and in ionic leading to acridines.³ However, the contemporary antibacterial therapy suspended the acridine based therapy. But in recent times, exponential increase in drug resistant bacterial infections has diverted the attention toward Acridine once again. In the literature it is evident that Acridine derivatives possesses wide array of

activities such as anti-inflammatory and anticancer, anthelmintic, fungicidal and antitumor activities.⁴ The range of pharmacological activities had been attributed to acridine derivatives. The anti-inflammatory compounds are being considered regularly for development of anticancer agents. Hence, present investigation was planned to determine possible role of acridine to control inflammation and cancer like life threatening conditions.

Substituted 3, 4-Dihydro-3, 3-Dimethyl-9-(Substituted phenyl) acridine-1(2H, 9H, 10H)-One nucleus

Substituted 3, 4-dihydro-3, 3-dimethyl-9-(substituted phenyl)-acridine-1(2H, 9H, 10H)-one nucleus commonly called as 1-oxo-tetrahydroacridine. Substituted 3, 4-dihydro-3, 3-dimethyl-9-(substituted phenyl)-acridine-1(2H, 9H, 10H)-one nucleus has marked pharmacological application due to its anticancer, antifungal, antibacterial and anti-inflammatory activities.⁵ In continuation of efforts to identify new candidates that may be of value in designing new potent, selective and less toxic antimicrobial agent, and the synthesis of some new heterocyclic incorporated the acridine moiety are reported.⁶ On the basis of

these observations we have the impetus to synthesize some novel acridine derivatives and subsequently evaluate their in vitro antimycobacterial activity. This important group of substances has found practical applications in number of fields.⁷

Structure activity relationship of substituted 3, 4-dihydro-3, 3-dimethyl-9- (substituted phenyl) acridine-1(2H, 9H, 10H)-one

The Structure Activity Relationship (SAR) of 3, 3-dihydro-3, 3-dimethyl-9-phenyl acridine-1(2H, 9H, 10H)-one is essential for activities.⁸ It has carbonyl group at 9th positions and nitrogen at 10th position it is oxidized product of Acridine. The substituted at N10-position with substituted tertiary amino propyl and tertiary amino butyl side chain also shows cytotoxic activity.⁹ The synthesized some new substituted tetrahydroacridin-1-ones and evaluate them for in vitro anti-microbial activities.¹⁰ Good inhibitory effect against the microorganisms such as *Escherichia coli*, *Klebsiella aerogenes*, *salmonella typhimurium*, *Bacillus subtilis* (Synthesized a novel series of 10-benzyl-9(10H)-acridine and 1-benzyl-4-piperidones and screened them for their in vitro

antitumor activities. The all synthesized compounds showed greater inhibitory effects.¹¹

The development of cancer in various organs is often associated with chronic inflammation, suggesting a strong relationship between inflammation and carcinogenesis via. transcription factors and inflammatory mediators such as TNF, IL-1, IL-6, IL-10, NF- κ B, STAT3, chemokines and COX-2. The nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin and ibuprofen are potent inhibitors of cyclooxygenase (COX). They block the synthesis of PGG and hence PGS, TX and PGI. Hence anti-inflammatory drugs can be used as anticancer therapy. Anti-inflammatory activity for Acridine (2, 3-Benzoquinoline)¹² gives an opportunity for further evaluation of anticancer activity. So, we have planned anti-inflammatory and anticancer activity of test compounds R1, R2 and R3, in vitro and in vivo model.

Scheme of Synthesis

Scheme for synthesis of substituted 3, 3-dimethyl-9-(substituted phenyl)-3, 4, 9, 10-tetrahydroacridine-1(2H)-one derivatives.

Table 1: Synthesis of substituted 3, 4-dihydro-3,3-dimethyl-9-(substituted phenyl)acridin-1(2H, 9H, 10H)-one derivatives.

Compound	R	R'	Structure
R1	3-CH ₃ O 4-CH ₃ O	2-Cl 4-Cl	5,7-dichloro-3,4-dihydro-9-(3,4-dimethoxyphenyl)-3,3-dimethylacridin-1(2H,9H,10H)-one
R2	3-CH ₃ O 4-CH ₃ O	3-NO ₂	3,4-dihydro-9-(3,4-dimethoxyphenyl)-3,3-dimethyl-6-nitroacridin-1(2H,9H,10H)-one
R3	3-CH ₃ O 4-CH ₃ O	2-NO ₂ 4-Cl	7-chloro-3,4-dihydro-9-(3,4-dimethoxyphenyl)-3,3-dimethyl-5-nitroacridin-1(2H,9H,10H)-one

MATERIAL AND METHODS

Methods for Synthesis

General procedure for synthesis of substituted 3, 3-dimethyl-9-(substituted- phenyl)-3, 4, 9, 10-tetrahydroacridine-(2H)-one derivatives.

The mixture of 5, 5-dimethylcyclohexane-1, 3-dione (1mmol), substituted aldehydes, (1 mmol) and substituted aniline (1mmol)

were added in 20 ml methanol and stirred for 90 min. The resulting mixture was stirred for 90 min. After completion of reaction (monitored by TLC), reaction mixture was poured in ice and neutralized with dilute HCl. The product was separated by using whatmann filter paper. Crude product was further purified by crystallization from hot ethanol. The product was confirmed by melting point, IR, ¹H NMR and Mass spectrometer. The mobile phase used for thin layer chromatography was pet ether; ethyl acetate; methanol (1:1:0.25 v/v).¹³

Physicochemical Data of Synthesized Compound

Table 2: Physicochemical data of synthesized substituted 3, 3-dimethyl-9-(substituted phenyl) 3, 4, 10-tetrahydroacridin-1(2H)-one derivatives.

Comp. code	Nature	Colour	Molecular formula	M. W.	R _f value (cm)	M. P. (°C)	Yield (%)
R1	Solid	White	C ₂₃ H ₂₁ Cl ₂ NO ₃	430.32	3.0	145	50
R2	Solid	Cream	C ₂₃ H ₂₄ N ₂ O ₅	408.44	1.2	250	68
R3	Solid	Orange	C ₂₃ H ₂₃ ClN ₂ O ₅	442.89	1.3	246	54

All the derivatives were found to be soluble in DMSO. The mobile phase used for thin layer chromatography was pet Ether: Ethyl acetate: Methanol (1:1:0.25 v/v).

Identification and Characterization of Synthesized Compounds

Compound-R1: **5, 7-dichloro-3,4-dihydro-9-(3,4-dimethylphenyl)-3,3-dimethylacridin-1(2H, 9H, 10H)-one**; IR (KBr); ν cm^{-1} 3095 (N-H Stretching), 2949 (CH_3 Stretching), 1734 (C=O Stretching), 1527 ($-\text{NO}_2$ Stretching), 1471 (C=C Stretching) 725(C-Cl Stretching); The ^1H NMR (DMSO) δ ppm; 10.153 (s, 1H, N-H), 6.526 (m, 5H, Ar-H) 5.522 (s, 1H, CH), 1.036 (d, 4H, CH_2), 2.084 (s, 6H, CH_3), 3.210 (s, 6H, OCH_3); Mass Spectrum shows the formation of molecular ion peak at $m/z = 433.5$

Compound-R2: **3,4-dihydro-9-(3,4-dimethylphenyl)-3,3-dimethyl-6-nitroacridin-1(2H, 9H,10H)-one**; IR (KBr); ν cm^{-1} 3111 (N-H Stretching), 3024 (Ar-Stretching), 2956 ($-\text{CH}_3$ Stretching); 1761 (C=O Stretching), 1699($-\text{NO}_2$ Stretching) 1473

(C=C Stretching); The ^1H NMR (DMSO) δ ppm; 11.816 (s, 1H, N-H), 7.77 (s, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 7.07 (s, 1H, Ar-H), 6.79 (s, 1H, Ar-H), 6.56 (s, 1H, Ar-H), 6.45 (s, 1H, Ar-H), 5.3 (s, 1H, CH), 1.07(q, 4H, CH_2 , $J=18.24$), 2.2(t, 9H, CH_3), 3.5(d, 6H, OCH_3); Mass Spectrum shows the formation of molecular ion peak at $m/z = 408.4$.

Compound-R3: **7-chloro-3,4-dihydro-9-(3,4-dimethylphenyl)-3,3-dimethyl-5 nitroacridin-1(2H, 9H, 10H)-one**; IR (KBr); ν cm^{-1} 3080 (N-H Stretching), 2943 ($-\text{CH}_3$ Stretching), 1716 (C=O Stretching), 1541 ($-\text{NO}_2$ Stretching), 1506 (C=C Stretching), 704 (C-Cl Stretching); The ^1H NMR (DMSO) δ ppm; 12.05(s, 1H, N-H), 8.11 (d, 2H, Ar-H, $J=0$), 7.28 (s, 1H, Ar-H), 6.7 (s, 1H, Ar-H), 6.65 (s, 1H, Ar-H), 5.5(s, 1H, CH), 3.37 (s, 1H, OCH_3), 2.38 (q, 4H, CH_2 , $J=9.2$), 1.12(q, 6H, CH_3), Mass Spectrum shows the formation of molecular ion peak at $m/z = 441.9$.

SPECTRUM OF SYNTHESIZED COMPOUND

Fourier Transforms Infra-Red Spectrum IR Spectrum of Compound-R1

The formation of compound R1 is confirmed from the spectroscopic and physicochemical data observed as the vibrational modes in IR spectrum (cm^{-1}) showed N-H stretching at 3096, CH_3 stretching 2949, C=O stretching 1749, NO_2 stretching 1527, C=C stretching 1421, C-Cl stretching 725.

Fig. 1: IR Spectrum of Compound-R1

IR Spectrum of Compound-R2

The formation of compound R2 is confirmed from the spectroscopic and physicochemical data observed as the vibrational modes in IR spectrum (cm^{-1}) showed N-H stretching at 3111, Ar-stretching 3024, CH_3 stretching 2956, C=O stretching 1761, NO_2 stretching 1699, C=C stretching 1473.

Fig. 2: IR Spectrum of Compound-R2

IR Spectrum of Compound-R3

The formation of compound R3 is confirmed from the spectroscopic and physicochemical data observed as the vibrational modes in IR spectrum (cm^{-1}) showed N-H stretching 3080, CH_3 stretching 2943, C=O stretching 1716, NO_2 stretching 1541, C=C stretching, C-Cl stretching.

Fig. No.03: IR Spectrum of Compound-R3

¹H NMR Spectrum of Compound

¹H NMR Spectrum of Compound-R1

The ^1H NMR (DMSO) δ ppm; 10.153 (S, 1H, N-H), 6.526 (M, 5H, Ar-H) 5.522 (S, 1H, CH), 1.036 (d, 4H, CH_2), 2.084 (S, 6H, CH_3), 3.210 (S, 6H, OCH_3).

Fig. 4: ^1H NMR Spectrum of Compound-R1

¹H NMR Spectrum of Compound-R2

The ^1H NMR (DMSO) δ ppm; 11.816 (S, 1H, N-H), 7.77 (S, 1H, Ar-H), 7.27 (S, 1H, Ar-H), 7.07 (S, 1H, Ar-H), 6.79 (S, 1H, Ar-H), 6.56 (S, 1H, Ar-H), 6.45 (S, 1H, Ar-H), 5.3 (S, 1H, CH), 1.07(q, 4H, CH_2 , $J=18.24$), 2.2(T, 9H, CH_3), 3.5(d, 6H, OCH_3).

Fig. 5: ^1H NMR Spectrum of Compound-R2

¹H NMR Spectrum of Compound-R3

The ^1H NMR (DMSO) δ ppm; 12.05(S, 1H, N-H), 8.11 (d, 2H, Ar-H, J=0), 7.28 (S, 1H, Ar-H), 6.7 (S, 1H, Ar-H), 6.65 (S, 1H, Ar-H), 5.5(S, 1H, CH), 3.37 (S, 1H, OCH₃), 2.38 (q, 4H, CH₂, J=9.2), 1.12(q, 6H, CH₃).

Fig. 6: ¹H NMR Spectrum of Compound-R3

Mass Spectrum of Compounds

Mass Spectrum of Compound-R1

Mass Spectrum shows the formation of molecular ion peak at $m/z = 433.5$. Which is equal to calculated molecular weight and hence compound-3 is confirmed. The yield of compound was found to be 50%.

PHARMACOLOGICAL SCREENING METHODS

In-Vitro Anti-inflammatory Activity

Egg albumin protein denaturation

Egg albumin (0.2 ml), phosphate buffer saline of pH 6.4 (2.8 ml), and varying concentration (200, 400 and 800 µg/ml) of test sample (2 ml) were used to prepare the reaction mixture (5ml). The mixtures were incubated at 37°C for 15 min. and then heated at 70°C for 5 min. After cooling, the absorbance was measured at 660 nm by using vehicle as blank. Diclofenac at concentration 200, 400 and 800 µg/ml was used as reference drug.¹⁴ The percent inhibition of protein denaturation was calculated by following equation:

$$\% \text{ inhibition} = 100 \times [A_t / A_c - 1]$$

Where, A_t = absorbance of test sample, A_c = absorbance of control.

Anticancer Activity

In-Vitro Anticancer Activity

MTT solution preparation: 5 mg in 1 ml of Phosphate Buffer Saline (PBS – Ph7.4).

Cytotoxicity Assay

In vitro growth inhibition effect of test compound was assessed by colorimetric or spectrophotometric determination of conversion of MTT into “Formazan blue” by living cells.

Procedure

50µl of 1×10^5 cells/ml cell suspension was seeded into each well in a 96 well microtiter plate and final volume was made up to 150 µl by adding DMEM media. Dilutions of the test compounds were prepared in DMEM media. 100µl of the test compounds of different concentrations [1000, 500, 250, 125, 62.5, 31.25 µg/ml] was added to the wells and incubated for 24 hrs, in presence of 5 % CO₂, at 37°C into CO₂ incubator. After 24 hrs, 20µl of 5 mg/ml MTT reagent was added to the wells. The plate was kept for 4 hrs incubation in dark place at room temperature. (The plate was covered with aluminum foil, since MTT reagent is

photosensitive.) The supernatant was carefully removed without disturbing the precipitated Formazan crystals and 200 µl of DMSO was added to dissolve the crystals formed. The optical density (OD) was measured at wavelength of 492 nm. The study was performed in triplicates and represents the mean of three readings.^{15, 16}

Formula:

$$\text{Cell viability (\%)} = \frac{\text{Abs. of treated cells}}{\text{Abs. of control cells}} \times 100$$

Evaluation parameters

For in-vitro anti-inflammatory activity by egg albumin protein denaturation, the percent inhibition of protein denaturation was calculated.

For in-vitro anticancer activity by MTT assay, the percent of cell viability or percent of inhibitory activity on MCF-7 breast cancer cell with different concentrations were calculated.

Statistical test

All the data were statistically analyzed using INSTAT Graphpad. Values were expressed as mean ± SEM. (n=6), ANOVA followed by Dunnett test. *p<0.05, **p<0.01, ***p<0.001 when compared with control.

RESULTS

In-Vitro Anti-inflammatory Activity

Egg Albumin Protein Denaturation

The anti-inflammatory activity of test compounds (R1, R2, and R3) are shown in the Table No. 03,04 & 05 whereas, compound R3 showed more effective inhibition of protein denaturation 49.59% at 300 µg/ml. R1 and R3 showed 47.15% and 39.83% inhibition at concentration of 300 µg/ml respectively. All the test compounds revealed significant anti-inflammatory effect against the egg albumin denaturation model as, while Diclofenac demonstrated 72.9% inhibition of protein denaturation at 300µg/ml which was higher than all fractions.

Table 3: Effect of test compound (R1) & Standard on Protein denaturation

Design of treatment	Concentration (µg/ml)	Mean ± SEM	% Inhibition
Group-I (Control)	-	1.21± 0.054	-
Group-II (Diclofenac)	50	0.5533±0.01	51.46
	100	0.493±0.04	57.01
	150	0.426±0.33	66.4
	200	0.4±0.08	68
	250	0.36±0.32	70.73
Group-III (R1)	50	0.71±0.02	37.71
	100	0.7±0.02	38.59
	150	0.7±0.01	44
	200	0.68±0.01	45.6
	250	0.68±0.03	44.71

Table 4: Effect of test compound (R2) & Standard on Protein denaturation

Design of treatment	Concentration (µg/ml)	Mean ± SEM	% Inhibition
Group-I (Control)	-	1.21± 0.054	-
Group-II (Diclofenac)	50	0.5533±0.01	51.46
	100	0.493±0.04	57.01
	150	0.426±0.33	66.4
	200	0.4±0.08	68
	250	0.36±0.32	70.73
Group-IV (R2)	50	0.83±0.02	27.19
	100	0.82±0.03	28.07
	150	0.80±0.02	36
	200	0.78±0.02	37.6
	250	0.75±0.03	39.02

Table 5: Effect of test compound (R3) and Standard on Protein denaturation

Design of treatment	Concentration (µg/ml)	Mean ± SEM	% Inhibition
Group-I (Control)	-	1.21± 0.054	-
Group-II (Diclofenac)	50	0.5533±0.01	51.46
	100	0.493±0.04	57.01
	150	0.426±0.33	66.4
	200	0.4±0.08	68
	250	0.36±0.32	70.73
Group- V (R3)	50	0.72±0.02	36.84
	100	0.70±0.02	38.59
	150	0.67±0.03	46.6
	200	0.66±0.03	47.2
	250	0.64±0.02	47.96

In-Vitro Anticancer Activity

The anticancer activity of all the synthesized compounds (R1, R2, and R3) was evaluated against MCF-7 cancer cell line using MTT assay. In the present study MCF-7 cells showed growth inhibition in a dose dependent manner when treated with test compounds (R1, R2, and R3) at concentrations ranging from 31.25 - 1000 µg/ml. (Table 6)

The IC₅₀ values of R1, R2, and R3 of the synthetic test compounds were found to be 115 µg/ml, 99 µg/ml, and 96 µg/ml against MCF-7 cell. The IC₅₀ of these compounds were comparable with known anticancer agent Methotrexate (IC₅₀ value 45.42 µg/ml). Many anticancer drugs are effective against MCF-7 cells by causing apoptosis through the expression of caspase-3, generating reactive oxygen species (ROS) and damaging DNA. The results indicate that the anticancer activity of derivatives varied with concentration. The following Graph No.01 and Table No.06 shows the % inhibitory activity with different dilutions (100, 500, 250, 125, 62.5, 32.25 µg/ml).

Graph 1: Percentage Inhibitory activity of tests compounds (R1, R2, and R3) with different concentrations on MCF-7 cell Breast cancer.

The concentration required for a 50% inhibition of viability (IC₅₀) was determined graphically. Standard Graph was plotted by taking concentration of the drug in X axis and relative cell viability in Y axis.

The following formula is used to calculate cell viability from the absorbance of samples:

$$\text{Cell viability (\%)} = \frac{\text{Abs. of treated cells}}{\text{Abs. of control cells}} \times 100.$$

Table 6: % Cell Viability and % Inhibition Activity of compounds (R1, R2, and R3) with different concentrations on MCF-7 Cell breast cancer

Compound	Concentration [µg/ml]	% Cell Viability	% Inhibition Activity	IC ₅₀
R1	1000	18.07	81.93	115 (µg/ml)
	500	21.49	78.51	
	250	22.20	77.08	
	125	46.11	53.89	
	62.5	73.44	26.56	
	31.25	80.62	19.38	
R2	1000	17.7	82.93	99 (µg/ml)
	500	23.04	76.96	
	250	26.43	73.57	
	125	37.41	62.59	
	62.5	60.70	39.03	
	31.25	66.25	33.75	
R3	1000	17.33	82.67	96 (µg/ml)
	500	21.49	78.51	
	250	24.98	75.02	
	125	35.37	64.63	
	62.5	78.09	21.91	
	31.25	79.05	20.95	

DISCUSSION

In the present investigation test compounds R1, R2, and R3 were evaluated for in-vitro anti-inflammatory activity and in-vivo anticancer activity to provide the possible link between Inflammation and Inflammation Induced cancer (IIC).

The mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen species (ROS) from activated neutrophils and macrophages. This over production leads to tissue injury by damaging the macromolecule and lipid per oxidation of membranes. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as interleukin- I, tumor necrosis factor-α, and interferon-γ, which stimulate

recruitment of additional neutrophils and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation, the investigation is based on the quest for newer anti-inflammatory drugs from the synthetic sources with significant activity and lesser side effects as substitutes for other drugs. Heterocyclic compounds constitute the largest and most varied family of organic compounds. Many broader aspects of heterocyclic chemistry are recognized as disciplines of general significance that impose on almost all aspects of modern organic chemistry, medicinal chemistry and biochemistry. Heterocyclic compounds offer a high degree of structural diversity and have proven to be broadly and economically useful as therapeutic agents. Substituted 3, 3-dihydro-3, 3-dimethyl-9-phenyl acridine-1(2H, 9H, 10H)-one nucleus is the subject of considerable interest from both academic and industrial level. Among the various classes of the nitrogen containing heterocyclic compounds substituted 3, 3-dihydro-3, 3-dimethyl-9-phenyl acridine-1(2H, 9H, 10H)-one nucleus is important component of several pharmacological active compounds. The different test compounds were synthesized from the acridine moiety and subjected to TLC. However the test compounds R1, R2, and R3 shown prominent bands which were further purified by passing through different column. The purity of the compounds R1, R2, and R3 thus obtained was checked by analytical standardized methods such as NMR, Mass and IR. Spectral values of compounds R1, R2, and R3 was found to be same as that of acridine, hence it was assumed as acridine (2, 3 benzoquinoline) derivative.

Maintenance of structural hierarchy of proteins by cell system is necessary for proper functioning of metabolic activities. Denaturation of tissue proteins is one of the well documented causes of inflammatory and arthritic diseases.¹⁷ Production of auto-antigens in certain arthritic diseases may be due to denaturation of tissue proteins.¹⁸ The various concentrations of compounds R1, R2, and R3 ranging from 50-300µg/ml were tested for its in-vitro Egg Albumin Protein Denaturation activity. The results have clearly demonstrated that the compounds R1, R2, and R3 at different concentrations have good anti-protein denaturation activity; however it exhibited prominent anti-protein denaturation activity at its lowest concentration.

The compound R1, R2, and R3 has protected Bovine serum albumin (BSA) against heat denaturation. The compound R3 has shown highest 47.96% and 49.59% anti-protein denaturation activity on BSA (Bovin Serum Albumin) at concentrations of 250µg/ml and 300µg/ml respectively compare to control. The compound R1 has shown 44.71% and 47.15% anti-protein denaturation activity on BSA at concentrations of 250µg/ml and 300µg/ml respectively. Compound R1 has lesser activity than R3 when compare with control. The another compound R2 have shown 39.02% and 39.83% anti-protein denaturation activity on BSA (Bovin Serum Albumin) at concentrations of 250µg/ml and 300µg/ml respectively compare to compound R3, R1 and control at same concentrations. The standard Diclofenac shown greater 70.73% and 72.09% anti-protein denaturation activity on BSA at concentrations of 250µg/ml and 300µg/ml respectively compare to control. The carbonyl group at 9th position and nitrogen at 10th position with side chain may be responsible for anti-protein denaturation activity as anti-inflammatory action.¹⁹

In sub-acute inflammatory model, Inflammation has different phases; the first phase is caused by an increase in vascular permeability, the second one by infiltration of leukocytes and the third one by granuloma formation.²⁰ In present study, we have examined the Inhibitory effects of test compounds R1, R2, and

R3 (10mg/kg and 20mg/kg) on these phases of inflammation (Table No.03-05). The anticancer activity of all the synthesized compounds (R1, R2, and R3) was evaluated against MCF-7 cancer cell line using MTT assay. In the present study MCF-7 cells showed growth inhibition in a dose dependent manner when treated with test compounds (R1, R2, and R3) at concentrations ranging from 31.25 - 1000 µg/ml.

The IC₅₀ values of R1, R2, and R3 of the synthetic test compounds were found to be 115µg/ml, 99µg/ml, and 96µg/ml against MCF-7 cell. The IC₅₀ of these compounds were comparable with known anticancer agent Methotrexate (IC₅₀ value 45.42 µg/ml). The test compound R1 having significant anti-breast cancer activity at 115µg/ml concentration as compare to other test compounds and control. Many anticancer drugs are effective against MCF-7 cells by causing apoptosis through the expression of caspase-3, generating reactive oxygen species (ROS) and damaging DNA. The results indicate that the anticancer activity of derivatives varied with concentration. The Graph 01 and Table No. 06 shows the % inhibitory activity with different dilutions (100, 500, 250, 125, 62.5, 32.25 µg/ml). These results may have clinical significance in understanding test compound R1 treatment to breast cancer. The study also demonstrated that the test compound R1 had greater inhibitory effects on the proliferation of lung cancer cells than the breast cancer cell line by inducing apoptosis as revealed by the expression of the apoptotic genes.

CONCLUSION

On the basis of obtained results from anti-inflammatory and anti-cancer activity, it can be concluded that, the synthesize 2, 3-benzoquinoline possess prominent anti-inflammatory and anti-cancer activity owing to its ability to inhibit various inflammatory mediators like interleukins, prostaglandins etc. Further study needs to evaluate molecular mechanism for in-vivo anti-inflammatory and anti-cancer activity.

REFERENCES

1. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002; 420:860 – 7.
2. Eicher T, Hauptmann S. *The Chemistry of Heterocycles. Structure, Reactions, Syntheses and Applications.* George Thieme Verlag, Stuttgart, New York. 1995; 354-358.
3. Joseph Lakowicz. *Principles of Fluorescence Spectroscopy* 3rd edition. Springer 2006; Page 260.
4. Srivastava A, Nizamuddin. Synthesis and fungicidal activity of some acridine derivatives. *Indian Journal of Heterocyclic Chemistry.* 2004; 13 , 3 ; 261-264
5. Sayed A, Laila A, Sayed I, Omayma B. Microwave irradiation for accelerating the synthesis of acridine and xanthenes derivatives from dimedone. *ARKIVOC* 2006 ;178-186
6. Gniazdowski M, Szmigiero L. Nitracrine and its congeners- An overview *General Pharmacology: The Vascular System.* 1995; 26, 473.
7. Yongfeng Wang, Malcolm Stevens, William T. Thomson, Bruce P. Shutt. Antitumour imidazo tetrazines. Part 33. New syntheses of the antitumour drug temozolomide using 'masked' methyl isocyanates. *Journal of the Chemical Society, Perkin Transactions.* 1995; 2783-2787
8. Ramesh Kumar, Mandeep Kaur and Meena Kumari. Acridine: A Versatile heterocyclic nucleus, *Acta Poloniae Pharmaceutica - Drug Research*, 2012; Vol. 69 No. 1, 3-9.
9. Ramesh Kumar and Meena Kumari. Chemistry of Acridone and its analogues: A review *Journal of Chemical and Pharmaceutical Research.* 2011; 3(1):217-230

10. Jawaidd Akhtar, Ahsan Ahmed Khan, Zulphikar Ali, Rafi Haider et al., Structure-activity relationship (SAR) study and design strategies of nitrogen-containing heterocyclic moieties for their anticancer activities. *European Journal of Medicinal Chemistry* 2017; 143-189
11. Moussa Z, Sharief M, Sharief A. Synthesis and characterization of new types of halogenated and alkylated imidazolidine iminothiones and a comparative study of their antitumor, antibacterial, and antifungal activities. *European Journal of Medicinal Chemistry*. 2011; 46(6):2280-9
12. Alpana Asnani, Shashikant Sahare. Synthesis and Pharmacological Studies of Some Novel Benzoquinoline Derivatives. *Asian Journal of Pharmaceutical and Clinical Research*. 2013; Vol 6, Suppl 2, 303-308
13. Shankar Hangirgekar, Shankar Phulwale. Synthesis, Characterization and Biological evaluation of some new Acridine Derivatives. *Heterocyclic Letters*. 2018; Vol. 8. No.1.95-104
14. Sangita Chandra, Priyanka Chatterjee, Protapaditya Dey, Sanjib Bhattacharya, Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein, *Asian Pacific Journal of Tropical Biomedicine*. 2012; S178-S180
15. Senthilraja P, Kathiresan K. In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF -7 cell lines study of Marine Yeast. *Journal of Applied Pharmaceutical Science*. 2015; Vol. 5 (03), page no. 080-084
16. Pavan Kumar Kalla, Sashikanth Chitti, Seyedeh Tahereh Aghamirzaei, Radhakrishnan Senthilkumar and SelvamArjunan. Anti-Cancer Activity of Silymarin on MCF-7 and NCIH-23 Cell Lines, *Advan. Biol. Res.*, 2014; 8 (2): 57-61
17. Malcolm S. *Cancer encyclopedia of life science*, Nature, 2001
18. Porter, P. Westernizing women's risks? Breast cancer in lower-income countries. *N. Eng. J. Med.*, 2008; 358(3), 213-216.
19. Rajeswaramma G, Jayasree D .In vitro Anti-Inflammatory Activity of Anacardium Occidentale Seed Extract. *Journal of Dental and Medical Sciences (IOSR-JDMS)*. 2018; vol. 17. 18-22
20. Frederic Buxant1, Nadège Kindt, Guy Laurent, Jean-Christophe Noël And Sven Saussez, Antiproliferative Effect Of Dexamethasone In The MCF-7 Breast Cancer Cell Line, *Molecular Medicine Reports*.2015; 12(3): 4051–4054

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