International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

© International Scientific Organization

Design, Synthesis, *In-Vitro* Antioxidant, Anti-inflammatory and Antibacterial Activity of Novel Indenoquinoxaline Derivatives

Chirra Ramakrishna[1*], P.T.S.R.K Prasada Rao[2]

[1]*Department of Chemistry, Krishna University, Machilipatnam-521004*

[2]*Department of Chemistry, PB Siddhartha college of Arts and Science, Vijayawada-520010*

Abstract

Quinoxaline is an excellent lead for designing potential drug candidates and their derivative indenoquinoxaline is a generation hit to modify the potential drug candidate. The present work is to design novel indenoquinoxaline derivatives and screen for free radical scavenging activity, and anti-inflammatory and antibacterial activity to explore therapeutic potential. Since antioxidant activity is a good biological indicator for the assessment of therapeutic activity of new compounds. Indenoquinoxaline derivatives were also tested for *in-vitro* anti-oxidant activity by DPPH assay, anti-inflammatory activity done by protein denaturation assay, and antibacterial activity against *Streptococcuspneumoniae* and *Klebsiellapneumoniae* to explore pharmacological profile. Selected derivatives of synthesized compounds and lead investigated for *in-vitro* antibacterial activity against human pathogenic bacteria like *Klebsiellapneumoniae* and *Streptococcuspneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* and compared with standard streptomycin under similar conditions. Indenoquinoxaline derivatives had significant antioxidant activity and good anti-inflammatory activity. From antibacterial activity lead molecule (QN) derivatives such as QN-4FA, QN-2CA, and QN-2FA were found to be highly active. The synthesized derivatives QN-2FA, QN-4FA & QN-4CA have zones of inhibition of 23mm, 22mm & 21mm respectively against *Streptococcuspneumoniae.* The synthesized derivatives QN-2FA, QN-4FA, QN-4CA, QN-2APY & QN-NDMAP have zones of inhibition of 20mm, 28mm, 27mm, 32mm& 33mm respectively against *Klebsiella pneumoniae.* The standard streptomycin has a zone of inhibition (ZoI) of 32mm against tested *Klebsiella pneumoniae*. Compounds QN-CREAT and QN-NNA have zone of inhibition 18 and 28mm respectively against *Staphylococcus aureus.* Presence of chlorine and fluorine substituted aniline derivatives of QN is more active and potential derivatives for further investigation.

Keywords: DPPH Assay, Well-plate method, Protein Denaturation, lead, Indenoquinoxaline

Full length article **Corresponding Author*, e-mail: brk.ram10@gmail.com

1. Introduction

Indenoquinoxaline and its derivatives have been investigated for a wide variety of biological activity as a versatile lead molecule for possible bioactive compounds [1-2]. The therapeutic efficacy of indenoquinoxaline derivatives is essential at the present moment or needs of hour to explore the newer drugs against emerging diseases. Newly designed compounds were also tested for *in-vitro* antibacterial activity against highly pathogenic bacteria like *Streptococcus pneumoniae* and *Klebsiellapneumoniae*, compared with standard antibiotic streptomycin under similar conditions. Indenoquinoxaline is an excellent lead molecule for the creation of brand-new bioactive substances. Their derivatives were documented for a large number of pharmacological actions including anticancer, antiviral, antimicrobial, and other activity [3-5]. Lead molecule indenoquinoxaline synthesized by reaction between keto groups of Ninhydrin (Indane-1,2,3-trione) with O-phenylenediamine by dehydrative cyclization [6]. Newer derivatives were prepared by Schiff reaction of aryl hetero/aryl amine with keto group of indenoquinoxaline. By using spectrum analysis, newly synthesized chemical structures were identified. By using the *In-vitro* DPPH assay, synthetic substances were tested for their ability to scavenge DPPH free radicals. Compounds with potential free radical scavenging activity are indicators for wide-spectrum biological activity [7]. The goal of the current effort is to develop, synthesize, and characterize new Indeno-quinoxaline derivatives using the Schiff reaction and spectrum analysis. Ascorbic acid was used as the standard in a DPPH experiment to test the antioxidant capability of synthesized compounds, which were also tested for their *in-vitro* anti-inflammatory and antibacterial properties. Compounds with antioxidant activity along with anti-inflammatory an important factor for accelerating the wound healing activity in microbial infections and diabetic wounds, this fact's present work is to study, so synthesized

compounds investigated for antioxidant, and anti-inflammatory activity along with antibacterial activity in this investigation.

2. Materials and Methods

2.1. Synthesis of Indenoquinoxaline

Equimolar concentrations of O-phenylenediamine and Ninhydrin (Indane-1,2,3-trione) (0.01M) were added to 10mL of glacial acetic acid, refluxed in a water bath for two hours, and then allowed to cool overnight. The product is filtered and recrystallized from dimethylformamide (DMF) [8-17].The scheme of the reaction is presented in scheme 1.

2.2. Synthesis of Indenoquinoxaline derivatives

Equimolar quantities of indenoquinoxaline (0.01M) and primary amine derivatives (arylamine/heteroarylamine) (0.01M) were refluxed in 10mL of glacial acetic acid for 2 to 4hours and allowed to cool. The product is filtered and re-crystallized from ethanol. The scheme of the reaction is presented in scheme 2.

2.3. Physiochemical properties of synthesized compounds

The molecular weights of the synthesized compound are determined by the mass spectrometer. The melting points of the synthesized compounds are determined by melting point apparatus. The Rf value of the synthesized compound are determined by Thin layer chromatography method using silica gel G as the stationary phase and methanol as the mobile phase. The spots are detected by Iodine chamber method. The % yields of the synthesized compound are determined by calculating the theoretical yield and practical yield. The result is presented in Table 1.

2.4. In-vitro antioxidant activity by DPPH assay method

10mg of the compound was mixed with the 10mL of methanol; it will give the 1mg/mL concentration of the compound. From this solution, 1mL was taken and made up to volume 10mL with the methanol which gave 100µg/ mL concentration of compound. 10mg of the Ascorbic acid was mixed with the 10mL of methanol; it will give the 1mg/mL concentration. From this 1mL was taken and made up to volume 10mL with the methanol which gives 100µg/mL concentration of ascorbic acid. Using an electronic balance, 2mg of DPPH powder was measured and combined with 100mL of methanol to produce a 20µg/mL DPPH solution. It should be stored in a dark, dry, and cold area [10-12], [32-34].

2.5. Assay of free radical scavenging activity

In three separate test tube, 2mL of the 100µg/mL compounds and ascorbic acid in methanol were taken, and they were combined with 3mL of the DPPH (20µg/mL in methanol). After 30minutes of reaction time at room temperature in a dark area, the absorbance was measured at 517nm using a UV-VIS spectrophotometer with methanol as the blank. The absorbance of the control (20µg/mL DPPH in methanol) was also measured at 517nm using a UV-VIS spectrophotometer with methanol as the blank. The DPPH assay was repeated three times (n=3) to get an average % Inhibition of DPPH free radical. The following formula was used to compute the percentage of free radical DPPH inhibition:I % = {(Ao – As) / Ao} X 100, Where,Ao is the absorbance of the control and As is the absorbance of the compound /Standard. The result is presented in Table 2.

2.6. In-vitro anti-inflammatory activity by Protein denaturation method

100µL of the sample, 5.6mL of phosphate-buffered saline (PBS, pH 6.4), and 0.4mL of fresh hen's egg albumin made up the reaction mixture (10mL). The reaction mixture was prepared three times for each compound separately.The same amount of double-distilled water was utilized as a control. After being incubated at (37˚C) in an incubator for 15minutes, the mixtures were heated to 70˚C for 5minutes. After cooling, their absorbance at 660nm was assessed using double-distilled water as a blank. Diclofenac sodium was used as a reference standard and treated equally for calculating absorbance. The *in-vitro* anti-inflammatory assay by protein denaturation method was repeated three times (n=3) to get the average % Inhibition of protein denaturation. The % inhibition of protein denaturation was estimated using the formula below: The Result is present in Table 3. % inhibition = C –T/ C, Where, T = absorbance of test sample, C = absorbance of control [10, 35].

2.7. Antibacterial activity by disc diffusion and well-plate method

The study utilized the following bacterial strains: *Escherichiacoli*,*Staphylococcus aureus*, *Klebsiellapneumoniae*, and *Streptococcuspneumoniae*. In this study, the antibiotic streptomycin was used as a positive control. Onto sterile Petri dishes, nutritional agar medium is added in millilitre increments, left to set, and then disposed of. After ensuring a uniform layer of medium using a spreading rod, 100µL of broth produced from a particular bacterial strain was piped on top. This process was continued until the media had dried entirely. For the last twenty-four hours, the temperature of the Petri dishes has been maintained at 37˚C. Dimethyl sulfoxide was utilized as the negative control and streptomycin at a dosage of 1mg/mL as the positive control. We measured the dimensions of the zones of inhibition to evaluate the antibacterial effectiveness. The disc diffusion method used a sterile disc with a 6-millimeter diameter. The good diffusion method requires the use of a sterile cork borer to insert the bore. The antibacterial activity of QN-2FA, QN-4FA, QN, QN-4CA, Streptomycin and negative control was determined by disc diffusion method against *Steptococcuspneumoniae.*The antibacterial activity of QN, QN-2FA, QN-4FA, QN-4CA, QN-2APY, QN-NDMAP, negative control and positive control was determined against *K.pneumoniae*by well-plate method.The antibacterial activity of QN-MET, QN-DAPS, QN-DMPA, QN-2APY, QN-CREAT, QN-NNA, negative control & positive control against *E.coli & S.aureus* was determined by well-plate method. The *in-vitro* antibacterial activity was performed only one time (n=1), the average zone of inhibition was not found. The result is presented in Tables 4 & 5.The zone of

inhibition of QN, QN-2FA, QN-4CA, QN-2APY, QN-NDMAP,+ve control, & -ve control against *S. pneumoniae*&*K. pneumoniae*was present in fig 1.The zone of inhibition of QN-MET, QN-DAPS, QN-DMPA, QN-2APY, QN-CREAT, QN-NNA,+ve Control,-ve Control against *E.coli*&*S.auerus* was present in fig 2 [11], [36-37].

3. Results and discussion

Newer indenoquinoxaline derivatives were synthesized with a good yield of 58 to 75% and the structure was characterized by spectral analysis and formation of compounds indicated by had C=N signal in FT-IR (JASCO-FTIR-4700 model) studies and structure was confirmed by LC-MS (Agilent 6530LC/Q-TOF) their corresponding molecular weight given in Table 1. The Rf values of the synthesized compounds are present between 0.68-0.92. The melting points of the synthesized compounds are present between 218-465˚C. Molecular weights of the synthesized compounds are present between 232-465, all the synthesized compounds were investigated for free radical scavenging activity by DPPH assay to explore antioxidant potential. The synthesized compound exhibits significant antioxidant activity of 50 to 75% when compared with standard vitamin-C of 81.44% at 100mg/mL, under similar conditions, and compound QN-4FA was found to be more active in this series with 75% inhibition of DPPH-free radicals (Table 2). Free radicals responsible for a wide range of biological damages lead to many diseases. The current investigation is to synthesize the Indenoquinoxaline derivatives and study for antioxidant activity and from this study, all the newer derivatives had good *in-vitro* antioxidant activity by DPPH Assay. Indenoquinoxaline is a versatile lead molecule and its derivatives are documented for excellent Pharmacological action including antioxidant activity [19-24]. Free radical scavenging activity of synthesized compounds is essential for a wide spectrum of biological activity since free radicals induce many diseases in humans [25-26]. Quinoxaline derivatives such as indeno and Indoloquinoxaline derivatives documented for anti-HIV activity10, 20. The free radical neutralization capacity of synthetic compounds essential for biological activity and antioxidant activity is also a good indicator of compounds' therapeutic and pharmacological action. The antibacterial activity of synthesized compounds tested against highly pathogenic *Streptococcuspneumoniae* and *Klebsiellapneumoniae*, and the synthesized compounds had significant antibacterial activity when compared with standard streptomycin under similar conditions. Indenoquinoxaline derivatives were reported for significant antimicrobial activity [28-29]. When compared to 100mg of normal ascorbic acid, synthetic compounds demonstrated high antioxidant activity. When tested for *in-vitro* anti-inflammatory activity using Diclofenac sodium as standard by the protein denaturation assay, few compounds were assessed and found to have significant effectiveness. Indenoquinxaline derivatives also exhibit good antibacterial activity against *Streptococcusspneumoniae* and *Klebsiella pneumoniae* when compared with standard streptomycin under similar conditions. So synthesized compounds have antioxidant, anti-inflammatory activity, and antibacterial activity. All the designed compounds have good anti-oxidant activity and selected compounds also have significant anti-inflammatory and antioxidant activity will help to reduce inflammation and microbial infections. Free radicals scavenging activity essential for neutralizing the toxic effect of free radicals and their induced damage in the system, since free radicals induce severe damage and lead to many diseases like cancer, diabetes, and nephritis [27]. Synthesized compounds demonstrate significant antioxidant activity when compared to standard ascorbic acid, and high anti-inflammatory activity, strong antibacterial action against respiratory pathogens when compared to conventional streptomycin, Indenoquinoxaline had significant antioxidant activity at 100mg when compared with standard ascorbic acid (Vitamin-C) under similar condition. Designed selected newer derivatives also tested for *in-vitro* anti-inflammatory activity, all the tested compounds exhibit good anti-inflammatory activity at 100mg when compared with standard Diclofenac sodium under similar conditions. Selected newly synthesized compounds studied for antibacterial activity against *Streptococcuspneumoniae* and *Klebsiellapneumoniae* and tested compounds documented for significant activity against pathogens when compared with standard Streptomycin under similar conditions (Table 4). *In-vitro* anti-inflammatory activity performed in protein denaturation assay using Diclofenac as standard, synthesized compounds have good activity when compared with standard under similar conditions. Indenoquinoxaline (QN) and their selected derivatives QN-2FA, QN-4FA, QN-2CA, and QN-4CA tested for *in-vitro* antibacterial activity against human respiratory pathogens like *Klebsiellapneumoniae* and *Streptococcuspneumoniae* and among the QN derivatives QN-CREAT and QN-NNA tested for *in-vitro* antibacterial activity against the bacteria *Staphylococcus aureus* and *Escherichia coli.* Indenoquinxaline derivatives also exhibit significant antibacterial activity against *Streptococcus pneumoniae* and *Klebsiella pneumoniae* when compared with standard streptomycin under similar conditions. When compared to 100mg of normal ascorbic acid, synthetic compounds demonstrated high antioxidant activity. Free radicals are reported for major diseases for humans and neutralization of free radicals may be a good solution to rectify human disease and antioxidant activity of synthesized compounds and natural products plays a vital role in the treatment of many diseases like diabetes, cancer, and neuritis8. Diabetic and cardiovascular diseases are mainly due to the free radical-induced damages and potential free radical scavenging activity of synthetic molecules needed for the hours as prophylactic and therapeutic activity via scavenging free radicals [23-35]. Synthesized compounds screened for *in-vitro* anti-inflammatory activity by protein denaturation assay using Diclofenac sodium as standard to explore preliminary anti-inflammatory potential. From these *in-vitro* anti-inflammatory studies, all the compounds exhibit good activity with 10 to 75% inhibition of protein Denaturation.

Table 1. Physiochemical Properties of Synthesized Compounds

Compound code	Molecular weight	Melting point	Rf	% Yield
QN	232	218	0.89	70
QN-2FA	325	311	0.71	62
QN-4FA	325	311	0.76	58
QN-2CA	341.80	327	0.68	62
QN-4CA	341.80	327	0.78	65
QN-2AP	308	296	0.9	60
QN-2APY	309.33	308	0.85	65
QN-PH	322	308	0.83	72
QN-DNPH	412	398	0.89	68
QN-DAPS	351	448	0.9	60
QN-BENZI	346	384	0.65	65
QN-OPD	351	299	0.79	65
QN-PABA	350	327	0.82	62
QN-ANTH	465	324	0.825	70
QN-B1	299	465	0.85	58
QN-MET	398	365	0.92	75
QN-CREAT	327	313	0.75	60
QN-NNA	336	343	0.82	75

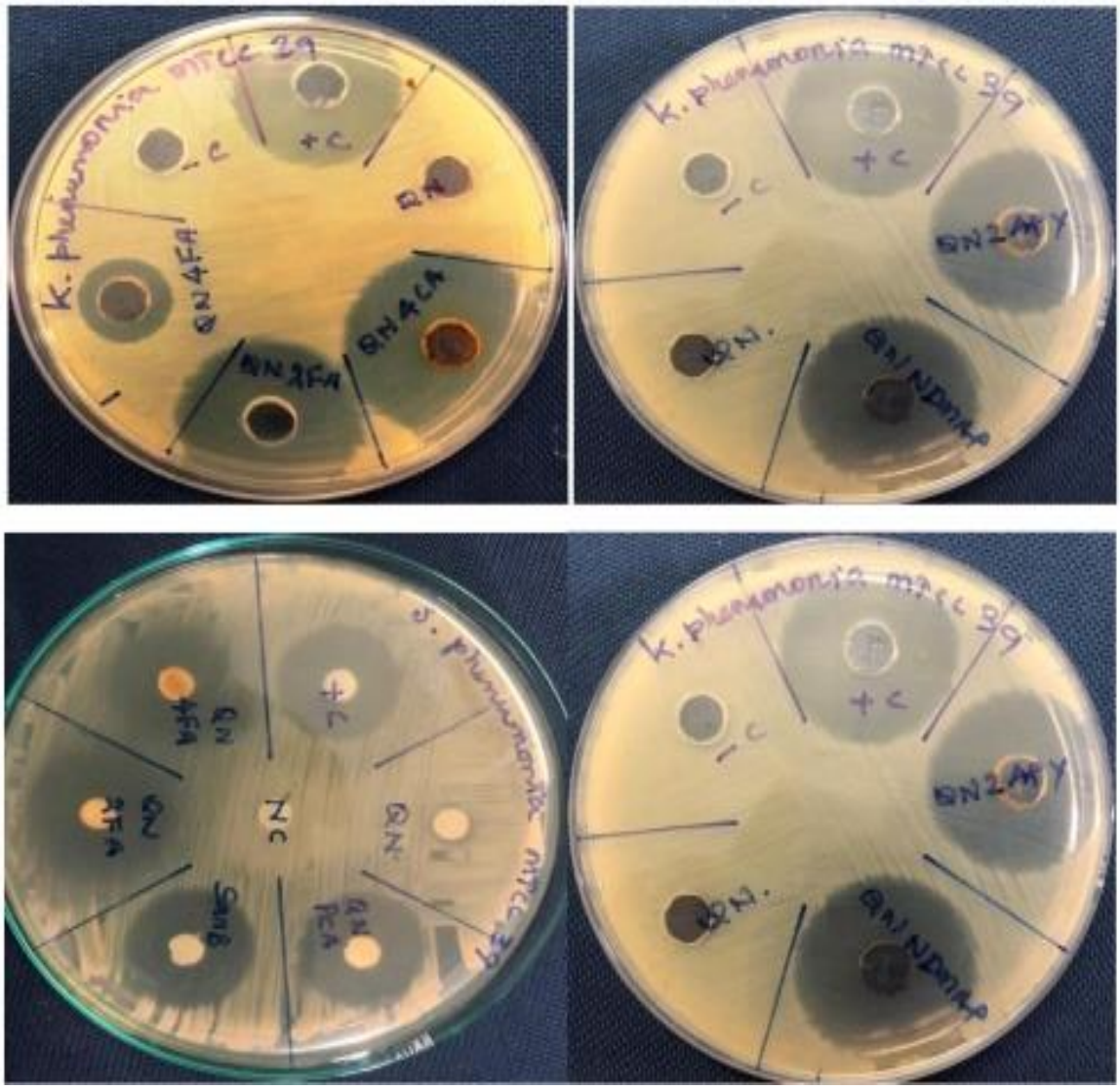


Figure 1. *In-vitro* antibacterial activity of QN, QN-2FA, QN-4CA, QN-2APY, QN-NDMAP, +ve control, & -ve control against *S. pneumoniae& K. pneumoniae*

Figure 2. *In-vitro* antibacterial activity of QN-MET, QN-DAPS, QN-DMPA, QN-2APY, QN-CREAT, QN-NNA,+ve Control,-ve Control against *E.coli&S.auerus*

Table 2. *In-vitro* Anti-oxidant activity by DPPH Assay

Compound	Molecular weight	COD	SOD	% Inhibition	Mean % Inhibition
QN	232	0.34	0.12	64.71	63.73±1.50
		0.34	0.12	61.76	
		0.34	0.12	63.73	
QN-2FA	325	0.34	0.09	73.53	75.49±6.12
		0.34	0.10	70.59	
		0.34	0.06	82.35	
QN-4FA	325	0.34	0.09	73.53	75.49±6.12
		0.34	0.10	70.59	
		0.34	0.06	82.35	
QN-2CA	341.80	0.34	0.12	64.71	63.73±1.50
		0.34	0.12	61.76	
		0.34	0.12	63.73	
QN-4CA	341.80	0.34	0.12	60	62.22±3.85
		0.34	0.10	66.67	
		0.34	0.12	60	
QN-2AP	308	0.30	0.06	80	77.15±2.71
		0.29	0.06	79.31	
		0.29	0.08	75	
QN-2APY	309.33	0.31	0.10	67.74	68.82±3.22
		0.31	0.09	70.96	
		0.31	0.11	64.52	
QN-PH	322	0.38	0.16	57.56	53.92±2.80
		0.38	0.18	52.63	
		0.38	0.19	52.78	
QN-DNPH	412	0.36	0.19	47.22	47.22±0
		0.36	0.19	47.22	
		0.36	0.19	47.22	
QN-MET	398	0.36	0.14	61.11	51.88±16.97
		0.36	0.10	72.22	
		0.36	0.22	38.89	
QN-B1	299	0.34	0.09	73.53	73.37±3.85
		0.36	0.11	69.44	
		0.35	0.08	77.14	
QN-DAPS	351	0.30	0.10	66.67	65.56±1.93
		0.30	0.11	63.33	
		0.30	0.10	66.67	

QN-OPD	351	0.36	0.15	58.33	52.78±5.56
		0.36	0.19	47.22	
		0.36	0.17	52.78	
QN-PABA	350	0.38	0.18	52.63	50.44±2.63
		0.38	0.20	47.37	
		0.38	0.19	50.0	
QN-ANTH	465	0.38	0.14	66.67	63.66±5.07
		0.38	0.16	57.89	
		0.38	0.14	66.67	
QN-BENZ	346	0.34	0.15	55.8	61.63±5.80
		0.34	0.12	67.4	
		0.34	0.13	61.7	
QN-CREAT	327	0.36	0.18	50	55.56±11.57
		0.36	0.12	66.67	
		0.36	0.20	44.44	
QN-NNA	336	0.36	0.20	44.44	42.13±1.60
		0.36	0.20	44.44	
		0.36	0.21	41.67	
QN-DMPA	346	0.36	0.20	44.44	46.29±3.21
		0.36	0.18	50.0	
		0.36	0.20	44.44	
QN-SA	437	0.31	0.08	74.19	73.12±1.86
		0.31	0.08	74.19	
		0.31	0.08	70.97	
QN-1A2SNAP	387	0.36	0.15	58.33	52+5.56
		0.36	0.19	47.22	
		0.36	0.17	52.78	
Vit C (STD)	-	0.36	0.07	80.5	81.44±1.63
		0.36	0.07	80.5	
		0.36	0.06	83.33	

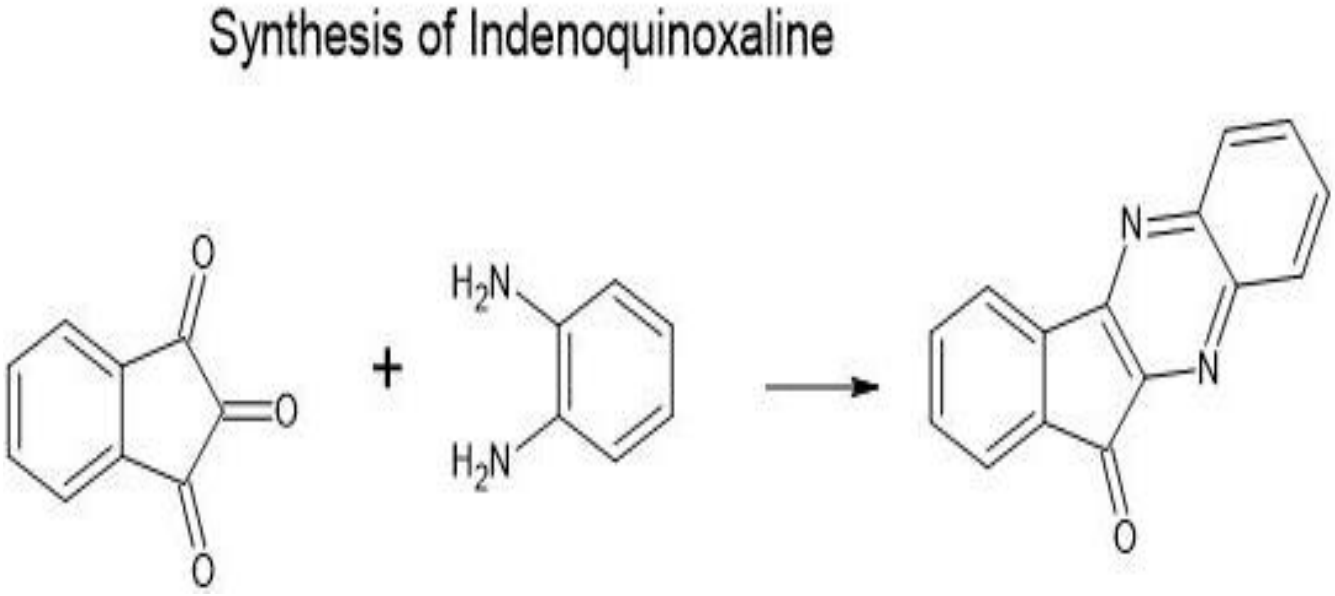


Figure 3. Synthesis of Indenoquinoxaline

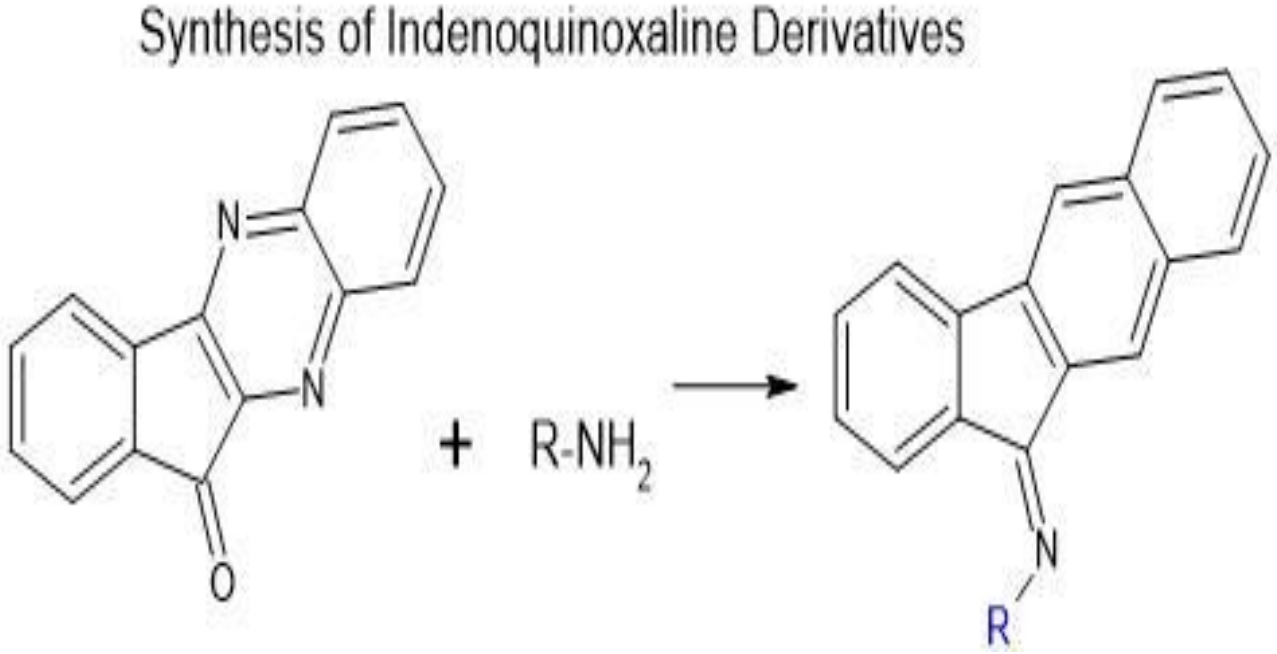
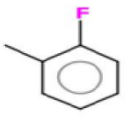
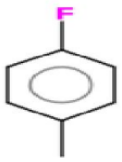
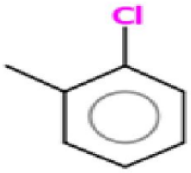
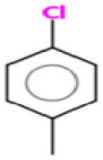
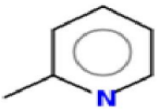
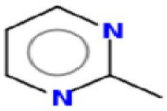
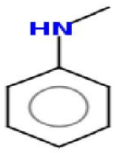
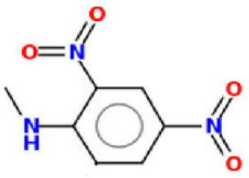
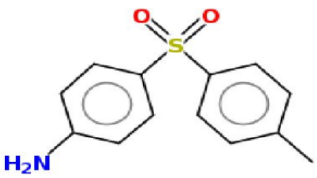


Figure 4. Synthesis of Indenoquinoxaline Derivates

Compound Code	Molecular weight	Substitution R
QN	232	=O
QN-2FA	325	
QN-4FA	325	
QN-2CA	341.80	
QN-4CA	341.80	
QN-AP	308	
QN-2APY	309.33	
QN-PH	322	
QN-DNPH	412	
QN-DAPS	462	

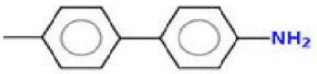
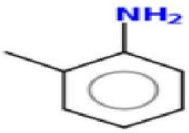
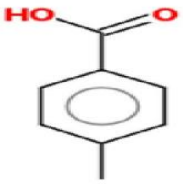
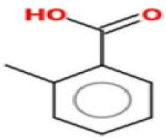
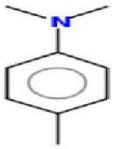
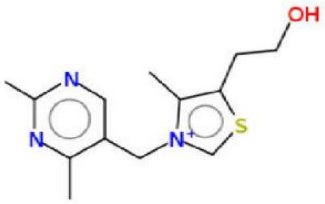
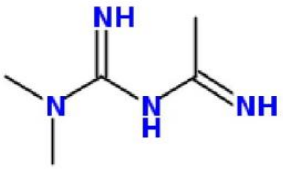
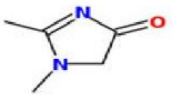
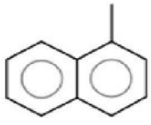
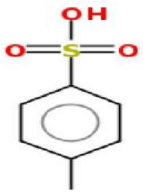
QN-BENZI	398	
QN-OPD	299	
QN-PABA	351	
QN-ANTH	351	
QN-PDMB	350	
QN-B1	465	
QN-MET	346	
QN-CREAT	327	
QN-NNA	336	
QN-SA	343	

Table 3. *In-vitro* Anti-inflammatory Activity by protein Denaturation method

Compound	Molecular weight	COD	SOD	% Inhibition	Mean % Inhibition
QN	232	0.34	0.30	11.76	11.96±0
		0.34	0.30	11.76	
		0.34	0.30	11.76	
QN-2FA	325	0.34	0.30	11.76	11.96±0
		0.34	0.30	11.76	
		0.34	0.30	11.76	
QN-4FA	325	0.34	0.28	16.65	18.63±4.64
		0.34	0.29	14.70	
		0.34	0.26	23.53	
QN-2CA	341.80	0.34	0.07	79	73.33±7.21
		0.34	0.12	64.71	
		0.34	0.09	73.53	
QN-4CA	341.80	0.34	0.30	11.76	17.33±5.24
		0.34	0.28	17.65	
		0.34	0.26	22.22	
QN-2APY	309.33	0.34	0.25	26.47	26.03±3.61
		0.34	0.24	29.41	
		0.34	0.26	22.22	
QN-NNDMPA	322	0.34	0.20	44.18	41.00±5.11
		0.34	0.19	44.11	
		0.34	0.22	35.29	
QN-PH	412	0.33	0.25	24.24	18.18±5.25
		0.33	0.28	15.15	
		0.33	0.28	15.15	
QN-DNPH	462	0.33	0.28	15.15	16.16±1.75
		0.33	0.27	18.18	
		0.33	0.28	15.15	
QN-BENZ	398	0.33	0.31	6.45	6.45±0
		0.33	0.31	6.45	
		0.33	0.31	6.45	
QN-NDMA	299	0.33	0.30	9.09	9.09±0
		0.33	0.30	9.09	
		0.33	0.30	9.09	
QN-ANTH	351	0.33	0.28	15.15	15.15±0
		0.33	0.28	15.15	
		0.33	0.28	15.15	
QN-DAPS	351	0.33	0.29	12.12	11.11±1.75
		0.33	0.30	9.09	

		0.33	0.29	12.12	
QN-PABA	350	0.33	0.30	9.09	9.09±0
		0.33	0.30	9.09	
		0.33	0.30	9.09	
QN-2AP	465	0.33	0.18	44.44	42.13±1.56
		0.33	0.18	44.44	
		0.33	0.17	41.67	
QN-BENZ	346	0.35	0.29	17.14	19.24±1.84
		0.35	0.28	20.58	
		0.35	0.28	20.00	
QN-SA	327	0.33	0.30	9.09	9.09±0
		0.33	0.30	9.09	
		0.33	0.30	9.09	
QN-1A2SNAP	336	0.33	0.30	9.09	9.09±0
		0.33	0.30	9.09	
		0.33	0.30	9.09	
QN-B1	343	0.34	0.28	16.65	20.40±4.64
		0.34	0.29	14.70	
		0.34	0.26	23.53	
QN-CREAT	437	0.34	0.24	26	26.28±0.16
		0.34	0.24	26.28	
		0.34	0.23	26	
QN-OPD	387	0.33	0.30	9.09	10.28±0
		0.33	0.30	9.09	
		0.33	0.30	9.09	
Diclofenac sodium (STD)	-	0.33	0.06	81.82	82.83±1.75
		0.33	0.06	84.85	
		0.33	0.06	81.82	

Table 4. Anti-Bacterial Activity of Indenoquinoxaline Derivatives

Compound code	Zone of inhibition (mm)	
	S. pneumoniae	***K. pneumoniae***
QN	-	-
QN-2FA	23	20
QN-4FA	22	28
QN-4CA	21	27
QN-2APY	-	32
QN-NDMAP	-	33
Streptomycin (Std.)	28	32
Negative control	-	-

Table 5. Anti-Bacterial Activity of Indenoquinoxaline Derivatives

Compound code	Sample code in Petri dish	Zone of inhibition (mm)	
		Staphylococcus aureus	***E .coli***
QN-MET	1	-	-
QN-DAPS	2	-	-
QN-DMPA	3	-	-
QN-2APY	4	-	-
QN-CRAET	1	28	-
QN-NNA	2	18	-
Streptomycin (Std.)	3	28	32
Negative control	4	-	-

4. Conclusions

Indenoquionxaline is an excellent lead molecule for designing potential bioactive agents and its derivatives are reported for wide-spectrum of activity [20-22], [29-34]. Present work to design novel indenoquinoxaline derivatives by Schiff's reaction and characterized by spectral analysis. Newly synthesized compounds were investigated for *in-vitro* antioxidant activity by DPPH assay, *in-vitro* anti-inflammatory activity by protein Denaturation method, and *in-vitro* antibacterial activity by disc diffusion and well-plate method. Newer derivatives had significant antioxidant activity and the selected compound exhibits good antibacterial and anti-inflammatory activity.

References

[1] A. Irfan, I. Sabeeh, M. Umer, A.Z. Naqvi, H. Fatima, S. Yousaf, Z. Fatima. (2017). A review on the therapeutic potential of quinoxaline derivatives. World Journal of Pharmacological Research. *6* 47-68.

[2] J.A. Pereira, A.M. Pessoa, M.N.D. Cordeiro, R. Fernandes, C. Prudêncio, J.P. Noronha, M. Vieira. (2015). Quinoxaline, its derivatives and applications: A State of the Art review. European Journal of Medicinal Chemistry. 97 664-672.

[3] M. Montana, F. Mathias, T. Terme, P. Vanelle. (2019). Antitumoral activity of quinoxaline derivatives: A systematic review. European journal of medicinal chemistry. 163 136-147.

[4] S. Tariq, K. Somakala, M. Amir. (2018). Quinoxaline: An insight into the recent pharmacological advances. European Journal of Medicinal Chemistry. 143 542-557.

[5] M.S. Khan, M.A. Munawar, M. Ashraf, U. Alam, A. Ata, A.M. Asiri, M.A. Khan. (2014). Synthesis of novel indenoquinoxaline derivatives as potent α-glucosidase inhibitors. Bioorganic & medicinal chemistry. 22 (3) 1195-1200.

[6] S. Das, A. Dutta. (2020). Ninhydrin adducts as valid synthon in organic synthesis: a review. ChemistrySelect. 5 (36) 11361-11377.

[7] V. Lobo, A. Patil, A. Phatak, N. Chandra. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews. 4 (8) 118.

[8] P. Selvam, D.R. Lakra, C. Pannecouque, E. De Clercq. (2012). Synthesis, antiviral and cytotoxicity studies of novel N-substituted indophenazine derivatives. Indian Journal of Pharmaceutical Sciences. 74 (3) 275.

[9] U. Asmat, K. Abad, K. Ismail. (2016). Diabetes mellitus and oxidative stress—A concise review. Saudi pharmaceutical journal. 24 (5) 547-553.

[10] A.R. Gangadasu, P. Selvam, R. Jat. (2021). Studies on in vitro antioxidant and anticancer potential of smilax china rhizome extracts. World Journal of Pharmacy and Pharmaceutical Sciences. 11(1) 2036-2046.

[11] S.D. Santhosam, P. Selvam, A. Danodia. (2023). In-vitro anti tumour and brine shrimp lethality assay of Abrus precatorius seeds. Tropical Journal of Pharmaceutical and Life Sciences. 10 (1) 01-10.

[12] N. Subhashini, G. Nagarajan, S. Kavimani. (2011). In vitro antioxidant and anticholinesterase activities of Garcinia combogia. Int J Pharm Pharm Sci. 3 (3) 129-132.

[13] C.H. Tseng, Y.R. Chen, C.C. Tzeng, W. Liu, C.K. Chou, C.C. Chiu, Y.L. Chen. (2016). Discovery of indeno [1, 2-b] quinoxaline derivatives as potential anticancer agents. European journal of medicinal chemistry. 108 258-273.

[14] N. Kumar, G.K. Inwati, E.M. Ahmed, C. Lal, B. Makwana, V.K. Yadav, B.H. Jeon. (2022). Modified 7-Chloro-11 H-indeno [1, 2-b] quinoxaline Heterocyclic System for Biological Activities. Catalysts. 12 (2) 213.

[15] K.S. Mani, B. Murugesapandian, W. Kaminsky, S.P. Rajendran. (2018). Enantioselective approach towards the synthesis of spiro-indeno [1, 2-b] quinoxaline pyrrolothiazoles as antioxidant and antiproliferative. Tetrahedron letters. 59 (30) 2921-2929.

[16] A. Hasaninejad, N. Golzar, A. Zare. (2013). One-Pot, Four-Component Synthesis of Novel Spiro [indeno [2, 1-b] quinoxaline-11, 4′-pyran]-2′-amines. Journal of Heterocyclic Chemistry. 50 (3) 608-614.

[17] M. Moemeni, H. Arvinnezhad, S. Samadi, M. Tajbakhsh, K. Jadidi, H.R. Khavasi. (2012). An efficient multicomponent and stereoselective synthesis of new spiro [indeno [1, 2-b] quinoxaline-11, 2′-pyrrolidine] derivatives. Journal of Heterocyclic Chemistry. 49 (1) 190-194.

[18] P. Selvam, E. De Clercq, C. Pannecouque. (2013). Design, synthesis, anti-HIV activity and cytotoxicity of novel schiff's base of indeno [1, 2-b] quinoxalin-11-one derivatives. Int J Drug Des Discov. 4 1017-1019.

[19] M.A.M. Gomaa, M.H. El-Katatny, H.A. Ali. (2020). Synthesis and characterization of N′-(11 H-indeno [1, 2-b] quinoxalin-11-ylidene) benzohydrazonamides as potential antimicrobial agents. Synthetic Communications. 50 (18) 2819-2829.

[20] B.D. Pearson, R.A. Mitsch, N.H. Cromwell. (1962). Indenoquinolines. III. Derivatives of 11H-indeno [1, 2-b] quinoxaline and related indenoquinolines. The Journal of Organic Chemistry. 27 (5) 1674-1678.

[21] S.B. Patel, B.D. Patel, C. Pannecouque, H.G. Bhatt. (2016). Design, synthesis and anti-HIV activity of novel quinoxaline derivatives. European journal of medicinal chemistry. 117 230-240.

[22] S.K. Suthar, N.S. Chundawat, G.P. Singh, J.M. Padrón, Y.K. Jhala. (2022). Quinoxaline: A comprehension of current pharmacological advancement in medicinal chemistry. European Journal of Medicinal Chemistry Reports. 5 100040.

[23] P.Y. Zhang, X. Xu, X.C. Li. (2014). Cardiovascular diseases: oxidative damage and antioxidant protection. European Review for Medical & Pharmacological Sciences. 18 (20).

[24] S.B. Nimse, D. Pal. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. RSC advances. 5 (35) 27986-28006.

[25] L.G. Liao, M.M. Song, J.F. Feng, M. Tan, F. Liu, Z.J. Qiu, B.J. Li. (2022). Green synthesis of indeno [1, 2-b] quinoxalines using β-cyclodextrin as catalyst. Molecules. 27 (2) 580.

[26] H.A. Abd El Salam, M.A. El-Bendary, M. Ibrahim, F.A. El-Samahy. (2020). Synthesis, Molecular Modeling and Biological Evaluation of Indeno [1, 2-b] quinoxaline Derivatives as Antifungal and Antibacterial Agents. Egyptian Journal of Chemistry. 63 (7) 2577-2590.

[27] J. Azizian, S. Zomorodbakhsh, M. Entezari, H. Anaraki-Ardakani. (2013). Functionalization of carboxylated multi-wall nanotubes with derivatives of N1-(11H-Indeno [1, 2-b] quinoxalin-11-ylidene) benzene-1, 4-diamine. Journal of Chemistry, 2013.

[28] C.H. Tseng, Y.L. Chen, P.J. Lu, C.N. Yang, C.C. Tzeng. (2008). Synthesis and antiproliferative evaluation of certain indeno [1, 2-c] quinoline derivatives. Bioorganic & medicinal chemistry. 16 (6) 3153-3162.

[29] N. Bouali, M.B. Hammouda, I. Ahmad, S. Ghannay, A. Thouri, A. Dbeibia, A. Kadri. (2022). Multifunctional derivatives of Spiropyrrolidine tethered Indeno-Quinoxaline heterocyclic hybrids as potent antimicrobial, antioxidant and antidiabetic agents: design, synthesis, in vitro and in silico approaches. Molecules. 27 (21) 7248.

[30] A. Rajasekaran. (2007). Synthesis, antinociceptive, antiinflammatory and antiepileptic evaluation of some novel indeno [1, 2-b] quinoxalin-11-ylidenamines.

[31] C. Muthuselvi, S.S. Pandiarajan, B. Ravikumar, S. Athimoolam, R.V. Krishnakumar. (2018). Halogen substituted indeno quinoxaline derivative crystal: a spectroscopic approach. Asian J Applied Sci. 11 29-37.

[32] S. Dudonne, X. Vitrac, P. Coutiere, M. Woillez, J.M. Mérillon. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. Journal of agricultural and food chemistry. 57 (5) 1768-1774.

[33] W. Brand-Williams, M.E. Cuvelier, C.L.W.T. Berset. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 28 (1) 25-30.

[34] S.D. Santhosam, P. Selvam, A. Danodia. (2023). Isolation and characterization of three isolates of Abrus precatorius seeds by LC-MS, 1HNMR, And 13CNMR. International Journal of Science and Research Archive. 8 (1) 404-420.

[35] P. Padmanabhan, S.N. Jangle. (2012). Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. International journal of basic and applied medical sciences. 2 (1) 109-116.

[36] S.D. Santhosam, P. Selvam, A. Danodia. Phytochemical Analysis of Abrus precatorius Seeds: A Review.

[37] A.A. Mostafa, A.A. Al-Askar, K.S. Almaary, T.M. Dawoud, E.N. Sholkamy, M.M. Bakri. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi journal of biological sciences. 25 (2) 361-366.