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Screening of antimicrobial and anti-oxidant activity of newly synthesized 1- (4- (9- bromo- 6H- indolo [2, 3-b] quinoxalin- 6- yl)- 3- oxobutanoyl)- 3 - substituted- 4, 5- dihydro- 1H- pyrazole- 4- carbaldehyde derivatives of Quinoxaline

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ABSTRACT

The Quinoxaline derivatives 1- (4- (9- bromo -6H- indolo [2,3-b] quinoxalin -6- yl)-3-oxobutanoyl) -3-substituted -4, 5- dihydro -1H- pyrazole -4- carbaldehyde (QND 1 to QND 5) were synthesized from different substituted Benzene derivatives. All the compounds were structurally elucidated with physical and analytical methods and evaluated with anti-microbial activity against a variety of bacterial strains and fungal strains and anti-oxidant activity using nitric oxide scavenging radical method and super oxide scavenging radical method. Some of these compounds have shown significant antibacterial and antifungal activities. Majority of quinoxaline derivatives when tested invitro for antioxidant activity showed recognizing activity.

Keywords: Quinoxaline derivatives, antimicrobial activity, antioxidant activity.

INTRODUCTION

Quinoxaline is heterocyclic compound containing benzo-pyrazine ring structure and also called as 1,4-diazanaphathalene is well known in pharmaceutical industry. From literature survey Quinoxaline compounds are found to be very effective against several pathogenic conditions. By virtue of its activity several scientists showed interest in designing various quinoxaline derivatives which possess several biological activities like antibacterial [1], antifungal [1], antitubercular [1], antiamoebic [2], antidepressant [3], anticancer [4], anti-inflammatory and antioxidant [5], anti HIV activity [6], antiherpes virus [7], pdgfr kinase inhibitors [8], selective SRPK-1 inhibitors [9], trypanocidal [10], influenza NS1A protein inhibitors [11], controlling ligands for zinc catalysed epoxide- CO_2- copolymerization [12], neuropharmacological [13], GABA modulators [14], antiallergic [15], NSAID and analgesic [16], antihyperglycemic [17] and so on. Keeping many points in view, it's worthwhile to design the synthesis of newer Quinoxaline derivative compounds, where in the biologically active compounds is linked to other potent moieties through various means. A walkthrough inspection of literature helped to develop innovative scheme which is so far not

seen in any article which is described in detail in this article. So it's our zeal to develop various quinoxaline derivatives from innovative procedure and finally evaluating them for antimicrobial and antioxidant activities.

MATERIALS AND METHODS

All the melting points were taken in open capillary tube and are uncorrected. The purity of the compounds was checked routinely by TLC using silica gel coated plates and spots were visualized by exposing the dry plates in iodine vapors. IR spectra ($\square_{max}$ in cm^{-1}) were recorded on FT-IR-Spercle Elmer DHF1FT-IR using KBr technique. The ^{1}HNMR and ^{13}C NMR spectra of the compounds were carried out in Bruker AMX 400 MHz NMR instrument using $CDCl_3$ or DMSO-d_6 as solvent and TMS as internal reference (chemical shifts in δ ppm). The mass spectra of the compounds were carried out in Agilent 1100 series LC-MSD.

Synthesis of 9-bromo-*6H*-indolo [2, 3-*b*] quinoxaline (1): Solution of O-phenylenediamine (0.0015mol) in rectified spirit (12ml) is added to a warm solution of 5-bromo isatin (0.0015mol) in rectified spirit (12ml). The mixture is warmed for 30 mins in a water bath/ reflux the mixture for 30mins. Addition of water drop wise results slight cloudiness persistence. Resultant solution is cooled; separated product is filtered and crystallized from alcohol. The yield, melting point range, solubility, Rf value was found to be 67%, 110-115^{0}C, ethyl acetate, 0.87 respectively.

Synthesis[18] of ethyl 4-(9-bromo-6H-indolo [2,3-b]quinoxalin-6-yl)-3-oxobutanoate (2):9-bromo-6H- indolo [2,3-b] quinoxaline (0.1 mol) in 100ml dry acetone was heated with 4- ethyl chloro acetoacetate (0.1mol) for 3 hours on water bath in presence of anhydrous potassium carbonate (7g). Reaction mixture was cooled and filtered to separate potassium carbonate. Dry acetone was removed by vacuum and product isolated from recrystallisation which is done from methanol: water. The yield, melting point range, and Rf value, was found to be 52%, 102-107^{0}C, 0.80.

Synthesis of 4-(9-bromo-6H-indolo [2,3-b]quinoxalin-6-yl)-3-oxobutanehydrazide (3): An equimolar mixture of compound **(2)** and 80% hydrazine hydrate (0.025 mol) in methanol was refluxed for 3hrs and the reaction mixture was cooled to room temperature. Filtered, washed with methanol and recrystallized from aqueous ethanol. The yield, melting point range, and Rf value was found to be 60%, 120-130^{0}C, 0.91.

Synthesis of 4-(9-bromo- 6H- indole [2.3-b] quinoxalin-6-yl)-3-oxo-N'-(1-substituted ethylidene) butanehydrazide. (4): An equimolar mixture of compound **(3)** (0.01mol) was dissolved in ethanol: water (1:1) containing few drops of glacial acetic acid to which different ketones were added. Reaction mixture was refluxed for 3hrs in ice bath. Product separated on cooling was filtered with cold methanol and recrystallized from methanol.

Synthesis of 1-(4-(9-bromo-6H-indolo [2, 3-b] quinoxalin-6-yl)-3-oxobutanoyl)-3-substituted-4, 5-dihydro-1H-pyrazole-4-carbaldehyde (5): Vilsmeier- Haack reagent is prepared by drop wise addition of $POCl_3$ (0.0012mol) to an ice cold solution of N, N- dimethyl formamide (5ml). Compound **(4)** (0.004mol) is added to Vilsmeier- Haack reagent; reaction mixture was warmed in a water bath for 2hrs and poured into ice-cold water and neutralized using an excess $NaHCO_3$. Product obtained was filtered, washed with water and recrystallized from methyl acetate. The characterization data of the resultant compounds is shown in table 1.

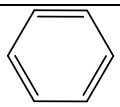
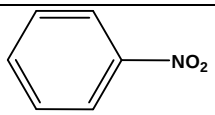
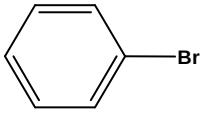
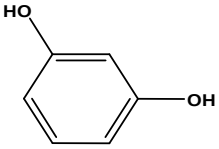
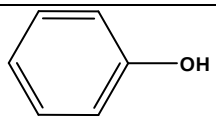
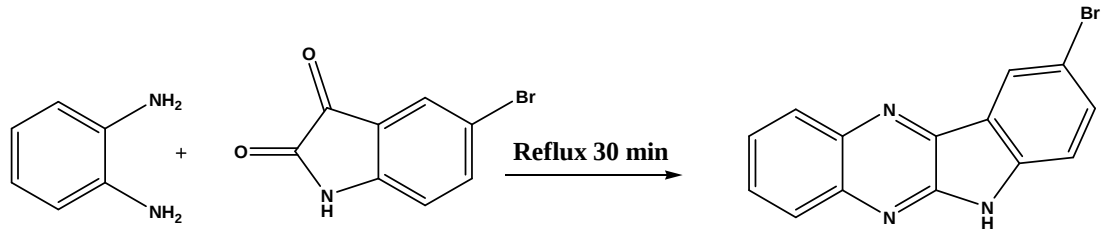
Compound Code	R
QND 01	
QND 02	
QND 03	
QND 04	
QND 05	

Table No 1: Characterization data of the synthesized derivative compounds

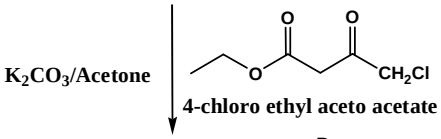
Comp. Code	Molecular Formula	Molecular Weight	Melting Point ^{O}C	% Yield (w/w)	Rf Value*
QND 01	$C_{28}H_{20}BrN_5O_3$	554.39	151	47.6	0.9
QND 02	$C_{28}H_{19}BrN_6O_5$	599.39	140	43	0.84
QND 03	$C_{28}H_{19}Br_2N_5O_3$	633.29	270	29	0.88
QND 04	$C_{28}H_{20}BrN_5O_5$	586.39	260	18.8	0.82
QND 05	$C_{28}H_{20}BrN_5O_4$	570.39	220	18.5	0.85

Recrystallisation solvent: Ethyl acetate,*Mobile phase: Ethyl acetate: Benzene (7:3)

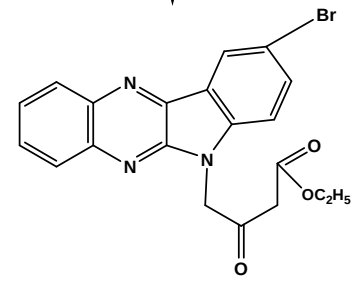


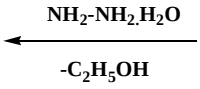
o-phenylene diamine **5-bromo isatin**

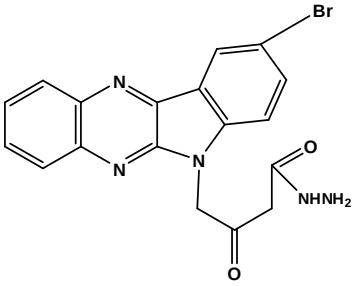
9-bromo-6*H*-indolo[2,3-*b*]quinoxaline
(1)



4-chloro ethyl aceto acetate

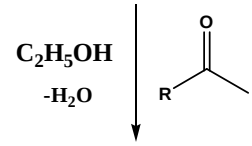


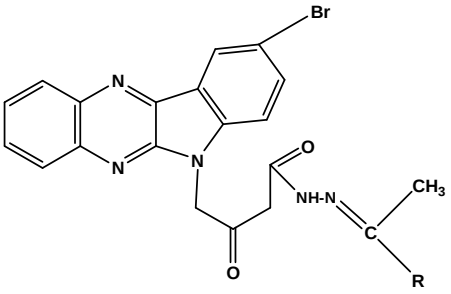


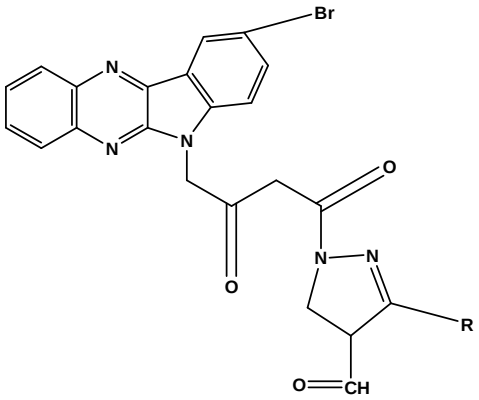


ethyl 4-(9-bromo-6*H*-indolo[2,3-*b*]quinoxalin-6-yl)-3-oxobutanoate
(2)

4-(9-bromo-6*H*-indolo[2,3-*b*]quinoxalin-6-yl)-3-oxobutanehydrazide
(3)







4-(9-bromo-6*H*-indolo[2,3-*b*] quinoxalin-6-yl)-3-oxo-*N*'-(*1-sub stituted* ethylidene)butanehydrazide
(4)

1-(4-(9-bromo-6H-indolo[2,3-b]quinoxalin -6-yl)-3-oxobutanoyl)-*3-substituted*-4,5-dihydro-1H-pyrazole-4-carbaldehyde
(5)

SCHEME 1: Synthetic route to the title compounds

Spectral data of the synthesized derivatives

QND 01: IR(υ, in KBr, cm^{-1}):577.04(str, Ar-Br), 1242.23(str, C-N), 1598.65(str, Ar, C=C), 1687.87(str, Ar, C=N), 2919.52(str, C-H), 3104.20(z), 2849.28(str, aldehyde C-H), 1775.57(str, aldehyde C=O), 1720.66 (str, Aliph, C=O).
^{1}H NMR(δ in $CDCl_3$, ppm): 1.346(m, 1H, C-H), 3.12(d, 1H, CH), 3.24(d, 2H, CH_2), 5.854(s, 2H, CH_2), 7.361(m, 1H, Ar-H) 7.414(m, 2H, Ar-H) 7.671(m, 2H, Ar-H), 7.801(m, 2H, Ar-H), 8.216(d, 2H, Ar-H), 8.71(d, 2H, Ar-H), 9.52(d, 1H, CHO)
QND 02: IR:577.04(str, Ar-Br), 1242.23(str, C-N), 1598.65(str, Ar, C=C), 1687.87(str, Ar, C=N), 2919.52(str, alip CH), 3104.20(str, Ar-H), 1339.66,1516.89(str, Asym & Sym, NO), 2849.28(str, aldehyde C-H), 1775.57(str, aldehyde C=O), 1720.66 (str, aliph, CO).
^{1}H NMR(δ in $CDCl_3$, ppm): 1.256(m, 1H, C-H), 3.0(d, 1H, CH), 3.5(d, 2H, CH_2), 5.354(s, 2H, CH_2), 7.260(m, 1H, Ar-H) 7.404(m, 2H, Ar-H) 7.674(m, 2H, Ar-H), 7.771(m, 2H, Ar-H), 8.116(d, 2H, Ar-H), 8.603(d, 2H, Ar-H), 9.52(d, 1H, CHO).
^{13}CNMR (δ in $CDCl_3$, ppm):204.70 (^{6}C), 188.22 (^{14}C), 143.50 (^{23}C), 135.72 (^{34}C), 128.99 (^{28}C), 115.54 (^{37}C), 38.94 (^{5}C).
MS: *m/z 600.3(M+1)* $^+$
QND 03: IR(υ, in **KBr, cm^{-1}):**577.04(str, Ar-Br), 1242.23(str, C-N), 1598.65(str, Ar, C=C), 1687.87(str, Ar, C=N), 2919.52(str, C-H), 3104.20(z), 2849.28(str, aldehyde C-H), 1775.57(str, aldehyde C=O), 1720.66 (str, Aliph, C=O).
^{1}H NMR (δ in $CDCl_3$, ppm):2.562(d, 1H, C-H), 3.491(d, 2H, CH), 5.242(s, 2H, CH), 7.600(d, 2H, NH), 8.068(d, 2H, Ar-H), 8.331(d, 2H, Ar-H), 9.052 (s, 1H, CHO)
QND 04: IR(υ, in **KBr, cm^{-1}):**642.24(str, Ar-Br),1204.12(str, C-O), 1272.75(str, Ar, C-N), 1515.20(str, Ar, C=C), 1653.98(str, Ar, C=N), 1701.22(C=O), 3671.52(str, Ar , OH).2759.28(str, aldehyde C-H), 1653.98(str, aldehyde C=O).
^{1}H NMR (δ in $CDCl_3$, ppm):2.562(d, 1H, C-H), 3.491(d, 2H, CH), 5.242(s, 2H, CH), 7.600(d, 2H, NH), 8.068(d, 2H, Ar-H), 8.331(d, 2H, Ar-H), 9.052 (s, 1H, CHO), 10.34 (s, 1H, –OH).
QND 05: IR(υ, inKBr, cm^{-1}) : 571.04(str, Ar-Br),1242.23(str, C-O),1339.66(str, Ar, C-N), 1516.89(str, Ar, C=C), 1598.65(str, Ar, C=N), 1720.65(C=O), 3669.82(str, Ar, OH).2711.54(str, aldehyde C-H), 1653.98(str, aldehyde C=O),3167.57(str, Ar-H), 3644.18(str, Ar, OH).
^{1}H NMR(δ in $CDCl_3$, ppm): 1.401(m, 1H, C-H), 3.212(d, 1H, CH), 3.194(d, 2H, CH_2), 5.954(s, 2H, CH_2), 7.451(m, 1H, Ar-H) 7.504(m, 2H, Ar-H) 7.701(m, 2H, Ar-H), 7.751(m, 2H, Ar-H), 8.326(d, 2H, Ar-H), 8.69(d, 2H, Ar-H), 9.48(d, 1H, CHO), 10.31 (s, 1H, –OH)

Antibacterial activity: The *invitro* antibacterial screening of all the Quinoxaline derivatives were evaluated against Gram- positive organisms *Staphylococcus aureus* (NCIM 2079), *Bacillus pimilis* (NCIM 2063) and Gram- negative organism *Escherichia coli* (MTCC 443) by cup and plate method [19]. Streptomycin is used as standard drug. Chloroform was used as control.

Antifungal activity: *Aspergillus niger* (MTCC 277) and *Candida albicans* (MTCC 227) were employed for testing antifungal activity using the cup-plate method [19]. Miconazole nitrate is used as standard and chloroform as control.

Antioxidant activity: Nitric oxide radical scavenging activity. The principle is- Nitric oxide generated as a result of decomposition of sodium nitroprusside in aqueous medium, interacts with oxygen at physiological pH to produce nitrite ions, which are measured by using Griess reaction. In this reaction, nitrite ions were subjected to diazotization followed by azo coupling reactions to yield an azo dye. Absorbance of this dye was measured at 546nm. Here the ability of the drug extract to inhibit nitric oxide generation from nitroprusside was evaluated by comparing with the control absorbance values.

REAGENTS USED	COMPOSITION
Griess Reagent	Sulphanilamide 1%, Ortho phosphricacid 2%, and Naphtyl ethylene diamine dihydrochloride 0.1% in 100ml water.
Phosphate buffer saline (PH 7.4)	Mix 2.3g of disodium hydrogen phosphate, 0.1g of potassium dihydrogen phosphate, 8g of NaCl, in 1000ml water.
Sodium nitroprusside solution	1%w/w solution (1gm/100ml water)

Absorbance was measured by Elico SL-244 double beam UV-VISIBLE SPECTROSCOPY

Preparation of working solutions for absorbance measurements -

Standard**:** ascorbic acid, standard phosphate buffer solution, 5mL of sodium nitroprusside solution, Griess' reagent was added.

Control**:** 5ml Methanol, standard phosphate buffer solution, 5mL of sodium nitroprusside solution, Griess' reagent were added.

Test**:** 5mL each of synthesized derivatives, standard phosphate buffer solution, 5mL of sodium nitroprusside solution, Griess reagent were added.

Blank**:** Methanol.

Note**:** Methanol is used to auto zero instrument and same reference shall be maintained until end**.**

Calculation:

$$\%\text{INHIBITION} = \frac{\text{ABSORBANCE(BASAL CONTROL)} - \text{ABSORBANCE(SAMPLE)}}{\text{ABSORBANCE(BASAL CONTROL)}} \text{ X } 100$$

Procedure [20]: To each solution, 1ml Sodium Nitroprusside solution was added and incubated at 37^0 C for 2.5 hrs. After incubation baseline was taken with methanol and 1ml Sodium nitroprusside solution was used as blank. GRIESS reagent and methanol was added immediately before recording of readings. Readings were recorded at 560nm.

Scavenging of superoxide radical method:

REAGENTS USED	COMPOSITION
Nitro blue tetrazolium solution	10mg nitro blue tetrazolium and 10ml DMSO
Alkaline DMSO	1ml of DMSO and 0.1ml of 5mM Noah

Preparation of working solutions:

Standard**:** To 0.3ml ascorbic acid, 0.1ml nitro blue tetrazolium solution, 1ml Alkaline DMSO were added.

Control**:** To 5ml DMSO, 0.1ml nitro blue tetrazolium solution, 1ml Alkaline DMSO was added.

Test**:** To 0.3ml each of synthesized derivatives, 0.1ml nitro blue tetrazolium solution, 1ml alkaline DMSO was added. Blank**:** DMSO.

Calculation:

$$\%\text{INHIBITION} = \frac{\text{ABSORBANCE(BASAL CONTROL)} - \text{ABSORBANCE(SAMPLE)}}{\text{ABSORBANCE(BASAL CONTROL)}} \text{ X } 100$$

Procedure: To 0.3 ml of synthesized compounds or standard in DMSO was added 0.1ml of nitro blue tetrazolium and 1ml of alkaline DMSO. The final volume was made up to of 5ml.The absorbance was measured at 560nm against basal Control.

RESULTS AND DISCUSSION

A total of 5 synthesized quinoxaline derivatives were evaluated for antibacterial activity with cup plate method at concentrations of 50 and 100 µg ml^{-1}. Streptomycin was taken as standard. Chloroform was taken as control. The results were shown in table 2.

Table 2. Zone of inhibition obtained on bacteria

S.No	Compound Code	Gram +ive				Gram -ive	
		S.aureus		*B. Pimilis*		*E.Coli*	
		50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}
1	QND 01	_	11	_	_	11	12
2	QND 02	12	17*	14	18*	17*	19*
3	QND 03	_	_	11	_	_	_
4	QND 04	_	11	_	11	_	12
5	QND 05	_	12	11	12	_	11
Control	Chloroform	_		_		_	
Standard	Streptomycin (100 µg/ml)	18		20		20	

Note: (-) not active and (*) significant zone of inhibition Bore size: 10mm

High activity was shown by QND- 02. Remaining compounds have shown moderate activity. A total of 5 synthesized quinoxaline derivatives were evaluated for antifungal activity with cup plate method at concentrations of 50 and 100 µg ml^{-1}. Miconazole nitrate was taken as standard. Control was taken as chloroform. The results were shown in table 3**.**

Table 3. Zone of inhibition on fungi

S.no	Compound Code	*Aspergillus Niger*		*Penicillum notatum*	
		50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}
1	QND 01	_	_	_	_
2	QND 02	15*	17*	17*	18*
3	QND 03	11	13*	_	_
4	QND 04	11	12	_	11
5	QND 05	_	11	11	12
Control	Chloroform	_	_	_	_
Standard	Miconazole Nitrate (50 µg/ml)	23		20	

Note: (-) not active and (*) significant zone of inhibition Bore size: 10mm

QND- 02 is effective against both *Aspergillus Niger* and *Pencillum notatum* when compared to standard miconazole nitrate. Remaining compounds were found to show minimal activity against fungi.

A total of 5 synthesized quinoxaline derivative compounds were evaluated for antioxidant activity with appropriate methods at concentrations of 25-200 µg ml^{-1}. Ascorbic acid was taken as standard. Methanol along with reagents was taken as control. The results were shown in table 4 and fig. 1 (Nitric oxide radical scavenging method) and table5 and fig. 2 **(**Super oxide radical scavenging method**).**

Table 4a. %scavenging activity by Nitric oxide radical scavenging method

Code	%Scavenging activity [mean ± SEM]				IC_{50} µ ml^{-1}
	25 µ ml^{-1}	50 µ ml^{-1}	75 µ ml^{-1}	100 µ ml^{-1}	
QND 01	29.68±0.09	34.55±0.03	38.36±0.89	46.26±0.07	125
QND 02	27.71±0.07	31.92±0.06	37.05±0.111	39.94±0.10	125
QND 03	27.31±0.14	35.07±0.65	39.55±0.19	49.68±0.19	100
QND 04	24.02±0.12	27.57±0.14	30.73±0.002	39.28±0.09	135
QND 05	24.42±0.02	30.21±0.08	36.52±0.05	45.07±0.13	128
STD*	45.21±0.19	59.23±0.15	74.34±0.23	80.23±0.01	27

Table 4b. %scavenging activity by Nitric oxide radical scavenging method

Code	%Scavenging activity [mean ± SEM]				IC_{50} µ ml^{-1}
	125 µ ml^{-1}	150 µ ml^{-1}	175 µ ml^{-1}	200 µ ml^{-1}	
QND 01	50.34±0.01	59.55±0.22	64.15±0.12	65.60±0.10	125
QND 02	49.55±0.023	57.44±0.067	64.15±0.02	69.42±0.09	125
QND 03	54.94±0.08	56.52±0.58	58.36±0.01	66.92±0.85	100
QND 04	46.00±0.23	53.76±0.11	57.71±0.15	65.34±0.98	135
QND 05	48.76±0.032	54.28±0.19	58.36±0.03	69.42±0.47	128
STD*	83.32±0.90	86.14±1.01	89.25±0.39	94.22±0.88	27

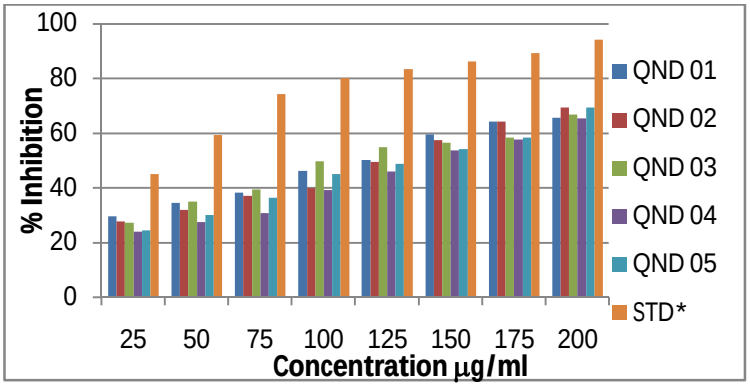


Fig 1: %scavenging activity by Nitric oxide radical scavenging method

Table 5a. %scavenging activity by Super oxide scavenging method

Code	%Scavenging activity [mean ± SEM]				IC_{50} µ ml^{-1}
	25µ ml^{-1}	50 µ ml^{-1}	75 µ ml^{-1}	100 µ ml^{-1}	
QND 01	30.68±0.29	33.54±0.13	40.36±1.79	48.36±1.70	103
QND 02	28.98±0.09	33.92±0.08	39.09±0.20	40.04±0.06	124
QND 03	30.21±1.24	39.04±1.55	43.65±1.09	51.68±1.90	85
QND 04	27.01±1.92	29.58±0.04	31.63±0.12	38.18±0.19	136
QND 05	25.22±1.22	33.11±0.29	38.42±1.15	48.12±1.03	103
STD*	36.11±0.19	44.59±0.30	58.33±1.14	63.25±1.52	56

Table 5b. %scavenging activity by Super oxide scavenging method

Code	%Scavenging activity [mean ± SEM]				IC_{50} µ ml^{-1}
	125 µ ml^{-1}	150 µ ml^{-1}	175 µ ml^{-1}	200 µ ml^{-1}	
QND 01	52.33±1.10	57.65±0.15	68.25±2.02	70.09±0.22	103
QND 02	51.65±0.20	56.44±0.09	66.15±0.02	71.42±1.80	124
QND 03	57.44±0.18	62.22±1.08	67.86±0.21	72.02±0.57	85
QND 04	45.70±0.33	55.86±0.11	60.81±0.05	67.94±0.08	136
QND 05	52.79±0.32	56.18±0.20	62.16±1.13	72.22±1.27	103
STD*	75.91±0.89	83.30±0.52	88.91±1.10	96.25±0.69	56

These compounds were found to have significant activity compared with control ($p<0.001$) and standard ($p<0.01$). Dose dependent activity was shown in both the methods.

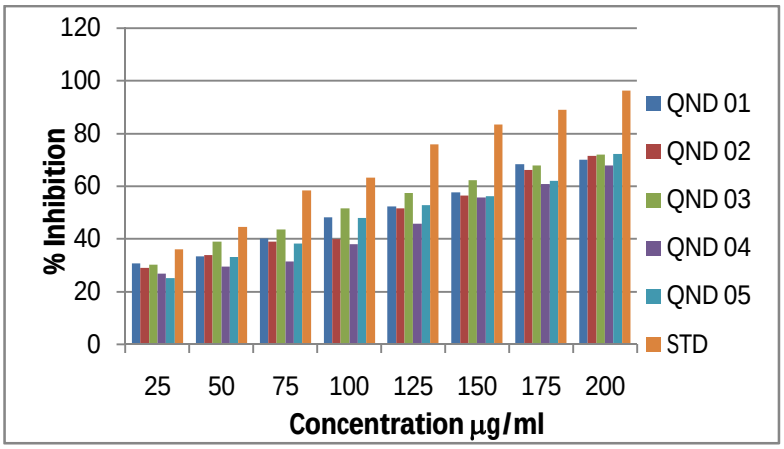


Fig 2: %scavenging activity by Super oxide scavenging method

APPLICATIONS

The prepared five quinoxaline derivatives when tested invitro for antioxidant activity showed recognizing activity.

CONCLUSIONS

1- (4- (9- bromo- 6H- indolo [2, 3-b] quinoxalin- 6- yl)- 3- oxobutanoyl)- 3- substituted-4, 5-dihydro 1H-pyrazole- 4- carbaldehyde derivatives of Quinoxaline were synthesized and characterized for their structure elucidation. All the compounds were evaluated with anti-oxidant activity using nitric oxide scavenging radical method and super oxide scavenging radical method and majority of the compounds showed recognizing activity. Antibacterial and antifungal studies of these compounds indicated that a

majority of these compounds show only moderate or less activity, however, few of them like **QND 02** has shown incredible activity.

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