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Research Article

SYNTHESIS, ANTIMICROBIAL AND WOUND HEALING ACTIVITIES OF DIPHENYL QUINOXALINE DERIVATIVES

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Abstract

O-phenylene diamine was the starting material for the synthesis of 2,3-diphenyl quinoxaline. Different derivatives were prepared by replacing hydrogen at 6th position of 2, 3-diphenyl quinoxaline by using different groups such as nitro, sulfonyl choride, sulfonic acid amide according to the literature. All compounds were synthesized in good yields and high purity. The structure of biphenyl quinoxaline and its derivatives were confirmed by M.P, TLC and spectral studies such as IR, ^{1}H NMR and Mass Spectral Studies. The synthesized compounds were evaluated against antibacterial activity employing agar diffusion method. Ofloxacin (10µg/ml) was taken as the standard for antibacterial activity and the results obtained were compared. Sulfonicacid amide derivative showed good antibacterial activity against both gram positive and gram negative organisms as compared to the standard Ofloxacin. The basic nucleus showed poor antibacterial activity as compared to standard. The wound healing activity (excision wound) of the other three derivatives was evaluated by using male Wister rats weighing about 150-200g. The drugs were administered at a dose of 200mg/kg body weight to the respective group of animals and control animals received normal saline. wound contraction was measured by polythene paper on wound ,day followed by 2, 4, 6, 8 and 10th day & subsequently on every alternate day till complete wound closure occurred. Nitro and sulfonic acid amide derivatives showed good wound healing activity compared to sulfonyl chloride derivative.

Key words: Antibacterial activity, Diphenyl quinoxaline, Ofloxacin, Wound contraction.

Introduction

Quinoxalines are an important class of nitrogen containing heterocycles with variety of biological activities. Different derivatives of quinoxalines are found to posses different activities. Quinoxalines are known to possess antimicrobial[1], antibacterial[2], bacteriostatic[3], anti-inflammatory[4], antifungal[5], CNS depressant [6], antitumor [7,] anti malarial[8], antiviral[9], antileprotic activity[10] etc. Quinoxaline ring is a part of various antibiotics, such as Echinomycin, Levomycin and Actinoleutin that are known to inhibit growth of gram positive bacteria and are active against various transplantable tumors. Meanwhile, the incidence of drug resistance in gram positive bacteria is growing rapidly and has become a significant public health threat. The aim is to synthesize various new quinoxaline analogues and to find effective antibacterial agents.The most common method for their preparation relies on the condensation of an aryl 1, 2-diamine with a 1, 2-dicarbonyl compound.For example, the condensation of 1, 2-diaminobenzene with benzil provides quinoxaline which yields ranging from 34-85% depending on the reaction conditions.Quinoxaline ring is a part of various antibiotics, such as Echinomycin, Levomycin and Actinoleutin that are known to inhibit growth of gram positive bacteria and are active against various transplantable tumors. Meanwhile, the incidence of drug resistance in gram positive bacteria is growing rapidly and has become a significant public health threat. The aim is to synthesize various new quinoxaline analogues and to find effective antibacterial agents.The most common method for their preparation relies on the condensation of an aryl 1, 2-diamine with a 1, 2-dicarbonyl compound.For example, the condensation of 1,2-diaminobenzene with benzil provides quinoxaline which yields ranging from 34-85% depending on the reaction conditions.

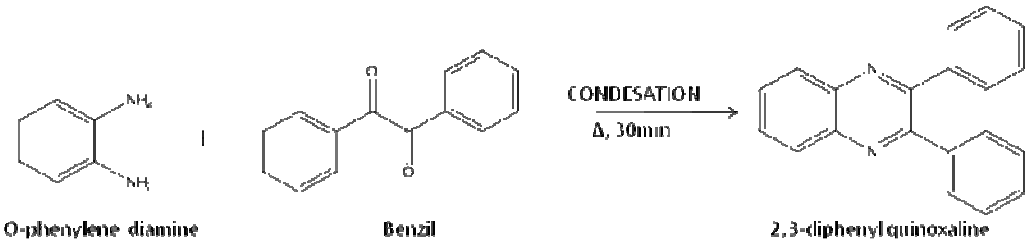
Experimental

Materials and methods:

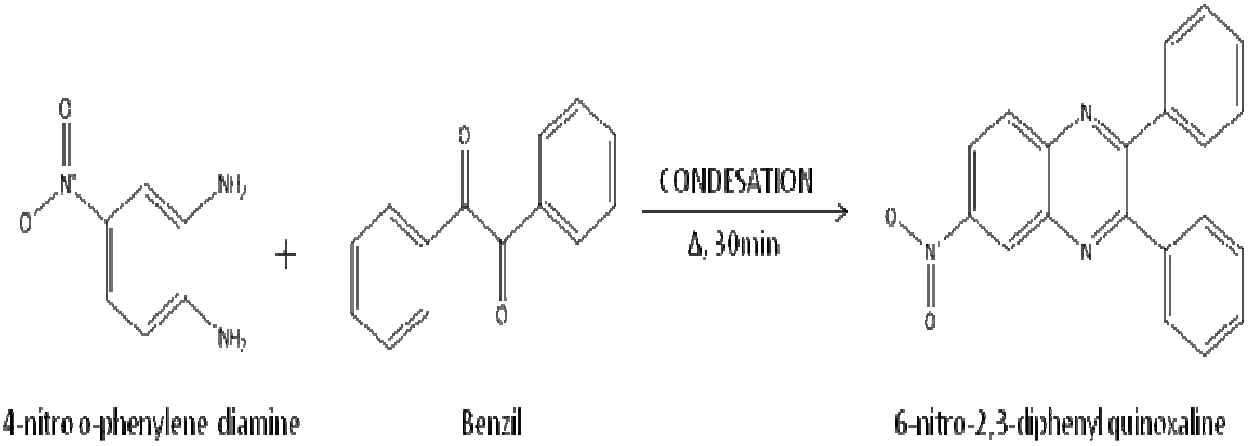
Melting points of all the compounds were determined by thiel's melting point apparatus and are uncorrected. IR spectra of the compounds were recorded on BRUKER FT-IR Spectrophotometer by using KBr discs, 1H NMR spectra were recorded on BRUKER 400.12324 MHz and Mass spectra were recorded on Aglient 1100 series. TLC using E-Merck 0.25 mm silica gel plates monitored progress of the each reaction in the present investigation.

Scheme:

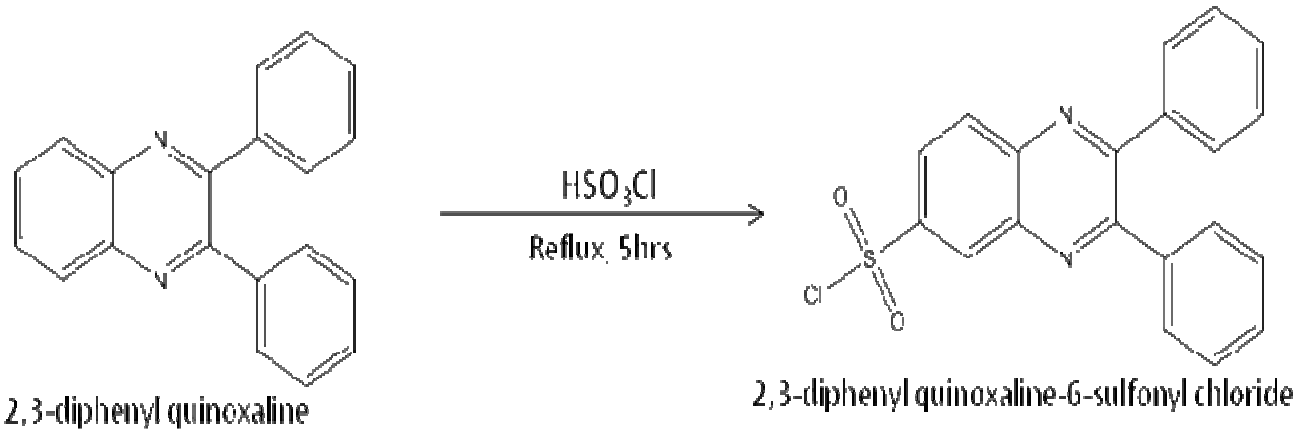
1. Synthesis of 2, 3-diphenyl quinoxaline: -



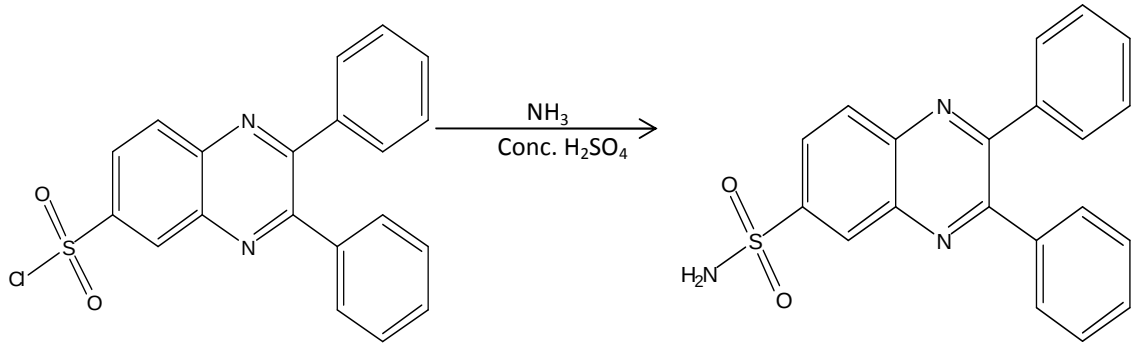
2. Synthesis of 6-nitro-2, 3-diphenyl quinoxaline: -



3. Synthesis of 2, 3-diphenyl quinoxaline-6-sulfonylchloride: -



4. Synthesis of 2, 3-diphenyl quinoxaline-6-sulfonicacid amide: -



2, 3-diphenyl quinoxaline-6-sulfonyl chloride 2, 3-diphenyl quinoxaline-6-sulfonic acid amide

Synthesis of 2, 3- diphenyl quinoxaline:-

To a warm solution of 2.1g (0.01mol) of Benzil ($C_{14}H_{10}O_2$) in 8ml of rectified spirit a solution of 1.1g (0.01mol) of o-phenylene diamine in 8ml of rectified spirit was added, warmed in water bath for 30 minutes, water was added until slight cloudiness persists and allowed to cool. Filtered and recrystallized from aqueous ethanol. The yield obtained was 1.43g (51%). The melting point was found to be 121-124ºC.

Synthesis of 6- nitro-2, 3-diphenyl quinoxaline:-

To a warm solution of 2.1g (0.01mol) of Benzil ($C_{14}H_{10}O_2$) in 8ml of rectified spirit a solution of 1.1g (0.01mol) of 4-nitro-o-phenylene diamine in 8ml of rectified spirit was added, warmed in water bath for 30 minutes, water was added until slight cloudiness persists and allowed to cool. Filtered and recrystallized from aqueous ethanol. The yield obtained was 2.56g (80%). The melting point was found to be 140-143ºC.

Synthesis of 2, 3-diphenyl quinoxaline-6-sulfonyl chloride:-

To 2, 3-diphenyl quinoxaline (0.01mol) chlorosulfonic acid (0.015mol) was added and the reaction mixture was refluxed for a period of 5hrs. The mixture was poured slowly on ice-water mixture; white solid precipitated out was filtered, washed thoroughly with cold water to make it acid free and recrystallized by using aqueous ethanol. The yield obtained was 2.7g (59%). The melting point was found to be 117-119ºC.

Synthesis of 2, 3-diphenyl quinoxaline-6-sulfonicacid amide:-

2, 3-diphenyl quinoxaline-6-sulfonyl chloride was transferred into a conical flask, to that 10ml of NH_3 and 40ml of distilled water was added. The mixture was heated on water bath until a part by suspension of the product was obtained.

To this dil.H_2SO_4 was added until blue litmus turns to red. The final product obtained was filtered, washed and recrystallized from aqueous ethanol. The yield obtained was 2.2g (81%). The melting point was found to be 112-115 ^oC.

NBPQ

IR (KBr, cm-1): 3424.88 cm^{-1} (-CH Str (aromatic)), 1447.70 cm^{-1} (-C=N- Str), 1664.26 cm^{-1} (-C=C- Str), 1590.81,1345.86 cm^{-1} (-CNO_2), **1HNMR: δ** 7.68(1H,Quinoxaline Ring), 7.989 (1H,Quinoxaline Ring), 7.97(1H,Quinoxaline Ring), 7.260(2H,Phenyl Rings), 7.497(4H,Phenyl Rings), 7.516(4H,Phenyl Rings),): **MS:** *m/z* 328.4 (M+1).

CSBPQ

IR (KBr, cm-1): 3427.30 cm^{-1} (-CH Str (aromatic)), 1471.96 cm^{-1} (-C=N- Str), 1058.55 cm^{-1} (Ar-SO_2 Str), 1344.42 cm^{-1} (SO_2 Cl Str), 1664.26 cm^{-1} (Ar-NO_2 Str), **1HNMR: δ** 8.21(1H,Quinoxaline Ring), 8.203(1H,Quinoxaline Ring), 8.194(1H,Quinoxaline Ring), 7.256(2H,Phenyl Rings), 7.323(4H,Phenyl Rings), 7.511(4H,Phenyl Rings)

BPQSA

IR (KBr, cm-1): 3054.26 cm^{-1} (-CH Str (aromatic)), 1544.74 cm^{-1} (-C=N- Str), 1064.07 cm^{-1} (Ar-SO_2 Str), 3480.40 cm^{-1} (-NH Str), 1344.42 cm^{-1} (Ar-SO_2 NH2 Str), **1HNMR: δ** 8.154(1H,Quinoxaline Ring), 8.163(1H,Quinoxaline Ring), 8.171(1H,Quinoxaline Ring), 7.332(2H,Phenyl Rings), 7.348(4H,Phenyl Rings), 7.485(4H,Phenyl Rings), 2.506(2H,Amine).

Physical data of synthesized compounds.

Code	Molecular formula	Melting point (^0C)	Yield (%)	Molecular weight	R_f value
BPQ	$C_{20}H_{14}N_2$	121-123	51	282.34	0.75
NBPQ	$C_{20}H_{13}N_30_2$	140-142	80	327.34	0.82

CSBPQ	$C_{20}H_{13}N_2SO_2Cl$	117-119	59	380.85	0.22
BPQSA	$C_{20}H_{15}N_3SO_2$	112-115	81	361.42	0.79

Results and Discussion

In recent years the heterocyclic compounds are very much used as antimicrobial agents. Quinoxalines are an important class of nitrogen containing heterocyclics with varieties of biological activities. Different derivatives of quinoxalines are found to possess different activities. Presences of phenyl groups increase the lipid solubility. Review of literature showed that the substitution at 2, 3, 6 and 7^{th} position have antimicrobial activity. The substitution was done at 6^{th} position of 2, 3-diphenyl quinoxaline by using different groups such as nitro, sulfonyl choride, sulfonicacid amide as a research work.O-phenylene diamine was the starting material for the synthesis of 2,3-diphenyl quinoxaline. The mechanism involved in the synthesis was condensation or cyclization. Different derivatives were prepared by replacing hydrogen at 6^{th} position of 2, 3-diphenyl quinoxaline by using different groups such as nitro, sulfonyl choride, sulfonicacid amide according to the literature. The above derivatives were recrystallised by using ethanol as solvent and were checked melting point, solubility and TLC. All compounds were synthesized in good yields and high purity. The products were characterized for desired structure by spectral studies such as IR, ^1H NMR and Mass spectral studies.The antimicrobial activity of the synthesized compounds was evaluated against the bacteria Proteus vulgaris, Klebsiella pneumoniae, E.coli and Bacillus subtilis employing agar diffusion method. Ofloxacin (10µg/ml) was taken as the standard for antibacterial activity and the results obtained were compared. The zone of inhibition was recorded in mm by using a scale. sulfonicacid amide derivative showed good antibacterial activity against both gram positive and gram negative organisms as compared to the standard Ofloxacin. The basic nucleus showed poor antibacterial activity as compared to standard.The wound healing activity (excision wound) of the other three derivatives was evaluated by using male wistar rats weighing about 150-200g. The wound was made for both control and tested groups on the dorsal thoracic central region 5mm away from the ears by using a round seal of 2.5 cm diameter. The wound area obtained

was about 300mm^2. The drugs were administered at a dose of 200mg/kg body weight to the respective group of animals and control animals received normal saline. The physical attribute of healing viz, wound contraction was measured by polythene paper on wound day followed by 2, 4, 6, 8 and 10th day & subsequently on every alternate day till complete wound closure occurred. Nitro and sulfonic acid amide derivatives showed good wound healing activity compared to sulfonyl chloride derivative.

Antibacterial activity

Table: Antibacterial activity of 2, 3-diphenyl quinoxaline and its derivatives.

Code No.	Dose (µg/ml)	Zone of inhibition (mm)			
		Klebsiella pneumoniae (Gram-ve)	Proteus vulgaris (Gram-ve)	E.coli (Gram-ve)	B. Subtilus (Gram+ve)
Std (0floxacin)	10	12	13	15	14
BPQ		11	12	12	11
NBPQ		12	13	14	12
CSBPQ		12	13	14	16
BPQSA		13	14	15	18

Antibacterial study of 6-substituted 2, 3-diphenyl quinoxalines (10 µg/ml) was evaluated against the bacteria, Proteus vulgaris, Klebsiella pneumoniae, E.coli and Bacillus subtilis employing agar diffusion method and the average radius of zone of inhibition was recorded. Among the derivatives screened, the following observations were made in comparison with the standard Ofloxacin (10 µg/ml). Sulfonic acid amide derivative showed more antibacterial activity as compared to the standard. Nitro and sulfonyl chloride derivatives showed equal activity against klebsiella pneumoniae, Proteus vulgaris as compared to standard. Basic nucleus showed less activity as compared to standard.

Acute toxicity study

The LD $_{50}$ values 2,3-diphenyl quinoxaline derivatives were 2000mg/kg body weight, so the dose had been selected to be 200mg/kg. At this dose no toxicity was found.

Table: Acute toxicity studies were carried out according to OECD guide lines and the LD_{50} is given below.

S.No	Code of drug	LD $_{50}$ mg/kg (Cut off value)	Test dose 1/10th of LD $_{50}$ mg/kg
1.	NBPQ	2000	200
2	CSBPQ	2000	200
3	BPQSA	2000	200

Excision wound study

Percentage closure of original wound area was calculated in mm^2 at different time intervals. The various parameters monitored include:

i) Wound contraction.
ii) Time of complete epithelialization.
iii) Scar area and shape on complete epithelialization.

i) Wound contraction studies:

Drug code	Avg. mean wound area (mm^2)					
	0th day	2ndday	4thday	6thday	8thday	10thday
control	299.63± 2.52	244.5± 1.42	234.71± 2.41	205.58± 1.12	138.46± 2.51	90.5± 0.69
NBPQ	297.83± 1.842	216.72± 1.31	153.51± 1.96	93.91± 1.86	32.2± 1.09	0.00
CSBPQ	298.91± 2.07	210.09± 2.05	126.42± 2.11	65± 2.132	29.49± 01.56	12.04± 1.34
BPQSA	302.02± 1.52	201.06± 1.12	111.9± 2.34	51.6± 2.56	15.9± 0.986	0.00

Statistical analysis of the results obtained by ANOVA followed by Dun net's "t" test showed that there was significant difference between all groups.

ii) Time of complete epithelialization:

On further follow up, mean time (days) to complete healing of control was 15.67 ± 0.422, while that of NBPQ, CSBPQ and BPQSA treated groups was 10.00 ± 0.66, 12.00 ± 0.56 & 10.00 ± 0.34 respectively indicating significant epithelialization ($p<0.01$) compared to control. Statistical analysis of the result by ANOVA followed by Dun net's "t" test showed that there was significant difference between all groups, $p<0.01$ and the nitro and sulfonic acid amide derivatives were found to be more effective than sulfonyl chloride derivative.

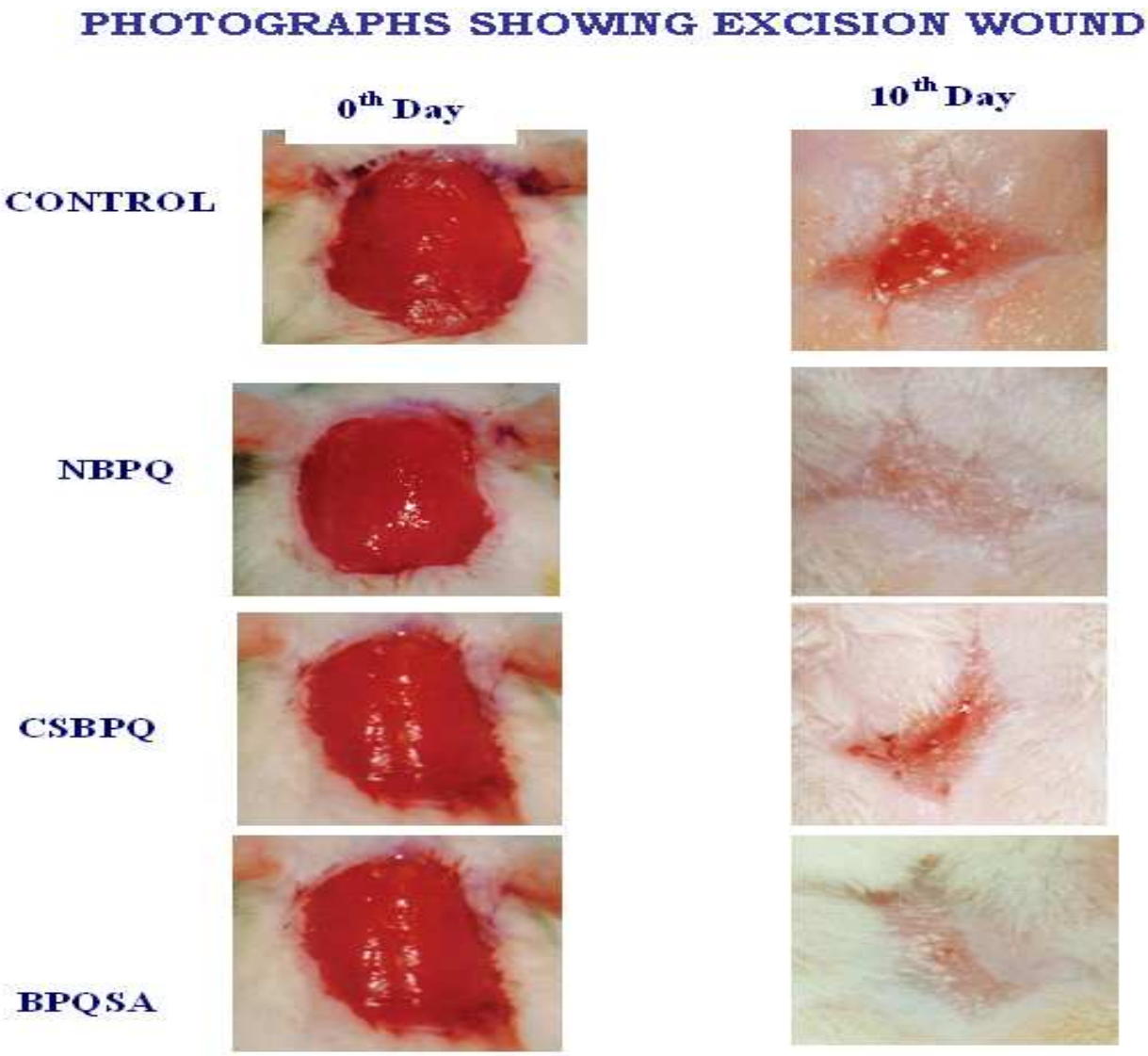


Table: Effect of 6-substituted 2, 3-diphenyl quinoxalines derivatives on excision wound model.

Group (n)	Dose (Oral)	Excision wound						
		% Wound contraction					Mean size of scar area (Mm2)	Period Of epithelialization (Days)
		2nd day	4th day	6th day	8th day	10th day		
Control	Normal saline	19.53± 1.087	22.00± 1.3082	31.33± 2.228	54.00± 2.345	68.6± 2.345	42.00± 1.072	15.67 ± 0.4223
NBPQ	200mg/kg	28.00± 1.056**	49.00± 1.329**	68.67± 2.480**	89.3± 1.233**	100± 1.02**	29.00± 1.32**	10.00 ± 0.66**
CSBPQ	200mg/kg	30.24± 1.451*	58.00± 2.124	78.44± 1.621	90.33± 0.734	96.33± 0.211	33.42± 0.921	12.00± 0.56*
BPQSA	200mg/kg	33.00± 1.023	62.67± 1.381*	82.82± 0.883*	94.72± 0.577*	100.0± 0.00**	30.12± 1.422	10.00± 0.34

Values are Mean±SEM;

Note: *p<0.05, **p<0.01

Statistical analysis by One Way ANOVA followed by Dun nets.

Conclusion

Substituting hydrogen at 6th position of 2, 3- Diphenyl quinoxaline ring by nitro, sulfonyl chloride, and sulfonic acid amide groups did the research work. Melting point, TLC, IR, 1H NMR and Mass spectral studies confirmed the derivatives formed. These derivatives showed good antibacterial and wound healing (excision wound) activities**.** Since

these derivatives are having good wound healing activity further work can be carried out on analgesic and anti-inflammatory activities**.**

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