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In Vitro Anti-inflammatory Activity of Quinoxalin Sulfonamides

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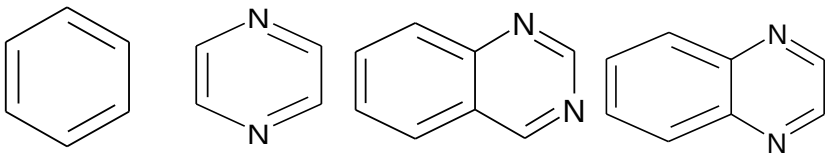
Abstract : Quinoxaline is six membered heterocyclic nitrogen containing two nitrogen atoms are based on pyrazine so also called as benzopyrazine. α dicarbonyl compounds reacts with aromatic ortho-diamine by consecutive addition-elimination mechanism to give quinoxalines. Quinoxaline have become attractive target of extensive research due to its inherent properties and therapeutic uses. Quinoxaline finds many pharmacological activities like antibacterial, antifungal, antituberculer, anti-inflammatory, antihyperglycemic, antitumor etc.
The present study includes the synthesis of sulfonamide derivatives of quinoxalines, by addition-elimination mechanism. All derivatives were characterized by TLC, IR, and MS1HNMR.Quinoxaline sulfonamide derivatives were then subjected to anti-inflammatory screening on albino rat by carageenan induced paw edema and activity was recorded by Plethysmometer (UGO Basile 7140).
Key words : Quinoxaline; Anti-inflammatory; paw edema; Anti Cancer; Anti-HIV, Anti-Oxidant.

1. Introduction:

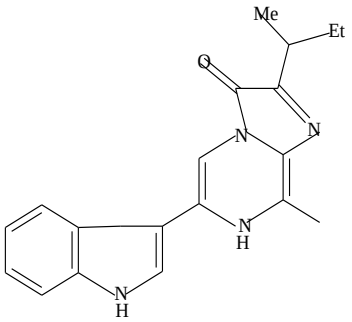
Quinoxaline is also called as benzopyrazine. It is heterocyclic compound containing benzene ring & pyrazine ring. Pyrazine are stable, colorless compound which are soluble in water. Unlike pyridine, they are expensive & not readily available & so are seldom used as starting material for synthesis of their derivative. Diazines are fused to benzene ring to form quinoxaline. The pyrazine ring system is found in the fungal metabolite aspergillic acid and in dihydro form in luciferin of several bettles including the fire fly is responsible for the chemiluminescence of this ostracod. Methoxypyrazine are very important component of aroma of many fruit's and vegetable such as Peas and Capsicum peppers and also of wines.

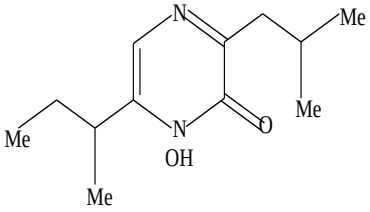


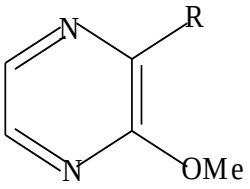
Benzene Pyrazine Quinazoline Quinoxaline

1.1 Naturally Occurring Pyrazine & Quinoxaline

Cyridina Luciferin Aspergillic acid Methoxy Pyrazine (food aroma)







1.3 Biological Activities of Quinoxaline

1.3.1 Antimalarial against Plasmodium falciparum.

Malaria is by far the world's most important tropical parasitic disease. Mortality, currently estimated at over a million people per year has risen in recent years, probably due to increasing resistance to antimalarial medicines.

E. Vicente et.al. Synthesized new active compound from lead compound 3- (4'-chloropheny) quinoxaline -2- carbonitrile 1,4- di-N-oxide, which was subjected to a structural change in order to obtain new active compounds : replacement of benzene in position 3 of the quinoxaline subunit by a heteroaromatic 5-member ring, 2-furane or 2-thiene. All the synthesized compounds were evaluated for antimalarial activity against Plasmodium falciparum. The 3-(2'-furyl) quinoxaline -2- carbonitrile 1,4-di-N-oxide derivatives appear to be a novel and promising antimalarial candidates.[2]

1.3.2 Anti-inflammatory and antioxidant

Many non-steroidal anti-inflammatory drugs have been reported to act as inhibitors of free radical production or as radical scavenger's compounds with antioxidant properties could be expected to offer protection in rheumatoid arthritis and inflammation and to lead to potentially effective drugs. Thus, Asuncion Burguete et.al. , synthesized novel ring substituted 3-phenyl -1- (1,4-di-N-oxide quinoxaline-2-yl) -2-propen-1-one derivatives and of their 4,5-dihydro-(1H)-pyrazole analogues. Synthesized compounds were evaluated for anti-inflammatory and antioxidant activity. The tested compounds inhibit the carrageenan-induced rat paw edema (4.5-56.1%) and present important scavenging activities. [3]

1.3.3 Anti- HIV

Since the human immunodeficiency 1 (HIV-1) was first confirmed as the causative agent of acquired immunodeficiency syndrome (AIDS). These are many clinical drugs, non-nucleoside reverse transcriptase inhibitors, which interact with a specific allosteric non-substrate binding site on HIV-1 reverse transcriptase, have proved to be effective anti-HIV drugs because of their high potency, low toxicities, and improved pharmacokinetics. Thus, Bailing Xu- *et al*, synthesized N^4 – (hetero) arylsulfonylquinoxalinones and their analogs and tested for anti viral activity as HIV-1 reverse transcriptase inhibitors.[4]

The anti-HIV-1 activities of all target compounds were evaluated by a cell-based HIV-1 replication pharmacological model which was set up by HIV-1 (pNL4-3) core packed with vesicular stomatitis virus glycoprotein. The level ofHIV-1 replication was presented by a reporter gene expression (i.e., luciferase activity) in infected cells.

1.3.4 Anti cancer

Sandra piras *et.al*., synthesized Methyl [4-(substituted 2-quinoxalinyloxy) phenyl] acetates and ethyl N-{[4-(substituted 2-quinoxalinyloxy) phenyl] acetyl] glutamate analogs of methotrexate and evaluated for in vitro anti cancer activity bioisosteric replacement of pteridine ring with 6 (7) – trifluoromethyl quinoxaline affords a good substrate for the classical antifolate analogs, and bioisosteric replacement of 2- NH group with an oxygen that in some cases was of relevance in anticancer activity. Quinoxalines bearing a 2 - (4 – substituted phenoxy) substituent were endowed with potent antitumor activity.[5]

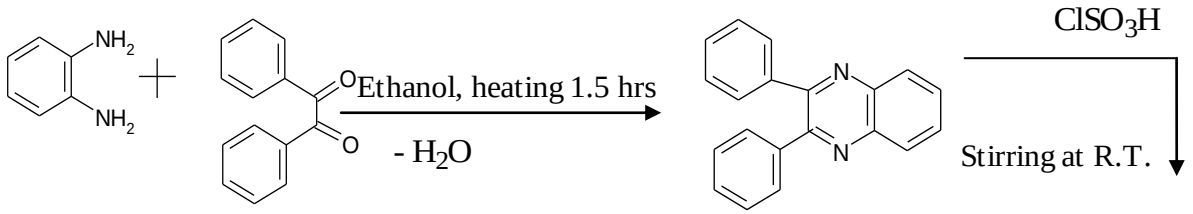
2. Material and Method

2.1 Synthesis of 2, 3-diphenylquinoxaline 7-sulfonylchloride

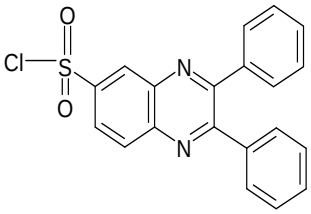
0.01 moles of 2, 3 diphenylquinoxaline (2.84g) was treated with chlorosulfonic acid under ice-cold condition in fuming cupboard with constant stirring. The stirring was continued until the reaction reaches worm temperature. The resultant mixture was poured into water to give sulfonylchoride derivative shown in fig. 1. [1]

2.1.1Scheme

Figure 1: Synthesis of 2,3-diphenylquinoxaline 7-sulfonylchloride (parent compound).



2, 3-diphenylquinoxaline



2, 3-diphenylquinoxaline 7-sulfonylchloride

2.2 General procedure for the synthesis of Quinoxaline Sulfonamides

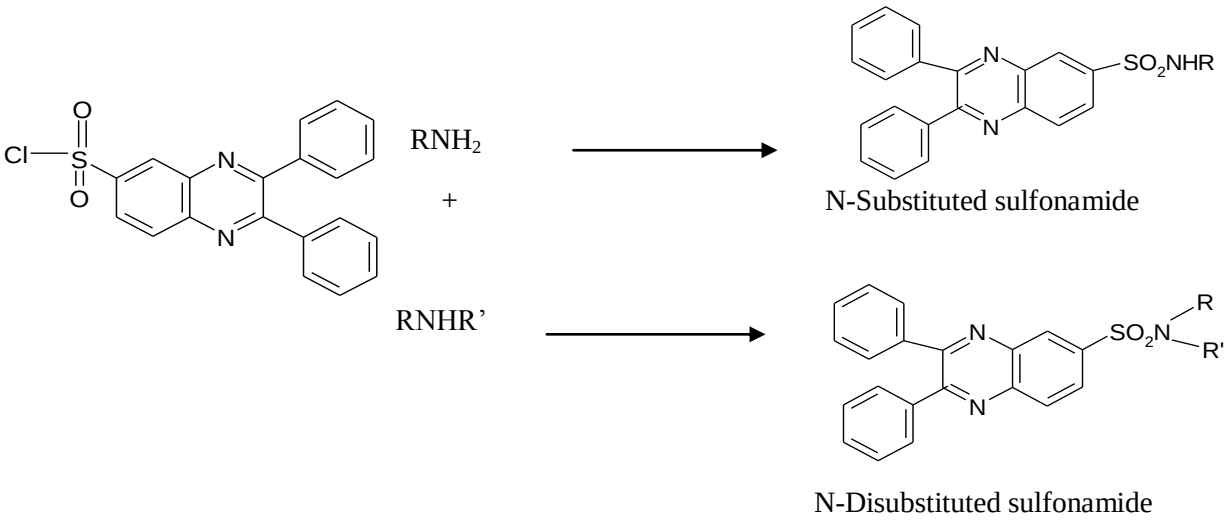
Table 1: Different derivatives prepared by above scheme

Sr. No.	Name of derivative	R	R'
L1	2,3-Diphenyl ,7-sulfonamido quinoxaline	H	-
L2	2,3-Diphenyl,7-(N phenyl)-sulfonamidoquinoxaline	C_6H_5	-
L3	2,3-Diphenyl ,7-(2,nitro,N-phenyl)-sulfonamido quinoxaline	C_6H_5-NO_2	-

Sulfonylchloride quinoxaline derivative was reacted with various primary and secondary amines as per given condition. Then reaction mixture was cooled and poured into water to get sulfonamide derivative of 2,3-diphenylquinoxaline. The crude product was recrystallized from 90% ethanol, and reaction was monitored by TLC, as per given in Fig 2 and Table 1. [1]

2.2.1 Scheme

Figure 2: Scheme for synthesis 7-sulfonamide derivatives of 2, 3-diphenylquinoxaline

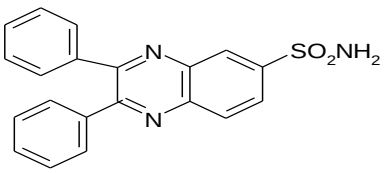


3. Result and Discussion

3.1Identification & characterization

The identification & characterization of prepared compound were carried out on the basis of physical, chemical, and spectral data given in Table 2,3,4,5 such as

1. Melting point. (MP)
2. Thin layer chromatography. (TLC)
3. Infrared Spectroscopy. (IR)
4. Mass Spectrometry.(MS)

L1: 

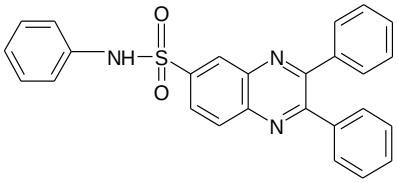
Melting point 194^0c, molecular formula $C_{12}H_{15}N_3O_2S$, Rf Value 0.39, molecular weight377

IR data:

Table 2 : Observed group frequencies in IR spectrum of 2, 3-Diphenyl, 7-(N phenyl) sulfonamido quinoxaline

Functional Group	**Frequency (cm^{-1})**
C=N	1673.98 (1690-1630)
C=C (Aromatic stretch)	1583.62 (1475-1600)
CH aromatic	3087.02 (3100-3000)
S=O	1172.56(1180-1140) 1383.77 (1370-1300)
Pri. NH_2	3423.35(3500-3300)

L2:



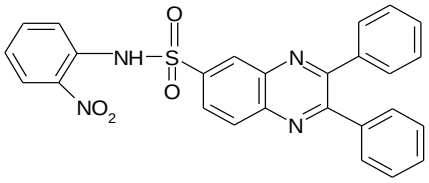
Melting point 95^0c, molecular formula $C_{26}H_{19}O_2N_3S$, Rf Value 0.42, molecular weight 466

Table 3: Observed group frequencies in IR spectrum of 2, 3-Diphenyl, 7-(N phenyl)-sulfonamido quinoxaline

Functional Group	**Frequency (cm^{-1})**
C=C (Aromatic stretch)	1496.20 (1475-1600)
S=O	1156.16(1180-1140) 1347.21 (1370-1300)
NH_2	3055.60 (3500-3300)
Mono sub. benzene	770.40 (790-770)

IR data:

L3:



Melting point 70^0c, molecular formula$C_{26}H_{19}O_4N_4S$, Rf Value 1.2, molecular weight483

Table 4: Observed group frequencies in IR spectrum of 2, 3-Diphenyl, 7-(2, nitro, N-phenyl)-sulfonamido quinoxaline

Functional Group	**Frequency (cm^{-1})**
C=C (Aromatic stretch)	1505.86 (1475-1600)
S=O	1173.30 (1180-1140) 1345.36 (1370-1300)
NH_2	3345.36 (3500-3300)
Mono sub. benzene	770.40 (790-770)
NO_2	1505.86 (1515-1560)

Table 5: mass characterization of 2, 3-Diphenyl, 7-(2,nitro,N-phenyl)-sulfonamido quinoxaline

S. no	**m/z**	**Fragment ion responsible**
1	483	M^+ (base peak)
2	344	$-NH_2C_6H_4NO_2$
3	378	-Ph CN
4	289	-A
5	405	-Ph

3.2 Anti-inflammatory Screening

This pharmacological activity was performed after obtaining approval of Institutional Animal Ethical Committee.

Synthesized compounds were subjected to testing for anti-inflammatory activity in albino rats employing the carrageenan induced rat paw edema test using Plethysmometer (UGO Basile 7140). The compounds were injected orally to the wistar albino rats. Percentage reduction in the inflammation (i.e. reduction in the hind paw edema volume of the animals) at different time interval after administration of carrageenan was recorded, and test compounds (25mg/kg body weight)were compared with that of the animals administrated with carrageenan using the reference standard Diclofenac sodium (10mg/kg body weight).

Data are expressed as Mean Paw Volume ± SEM and analyzed by One-Way ANOVA followed by Dunnett's Test to determine the significance of the difference between the control group and group treated with test compounds. All the statistical calculations were carried out using Graph Pad InStat 3 Statistical Software. The result for anti-inflammatory activity is given in Table No 6, 7,8,9,10,11.

- L1 = 2, 3-Diphenyl, 7-sulfonamido quinoxaline
- L2 = 2,3-Diphenyl,7-(N phenyl)-sulfonamidoquinoxaline
- L3 = 2,3-Diphenyl,7-(N-acetyl)-sulfonamido quinoxaline
- Standard = Diclofenac sodium

Table 6: Mean paw volume and % inhibition of compounds after 30 min.

Compounds	Mean Paw Volume (ml) ± SEM	% Inhibition of Edema
Control	1.23 ± 0.022	---
L1	0.93 ± 0.016**	61.03
L2	1.04 ± 0.033*	13.04
L3	1.08 ± 0.043**	20.06
Diclofenac sodium	0.97 ± 0.033**	6.89

Test compounds = 25 mg/kg
Reference Standard, Diclofenac Sodium = 10 mg/kg
Mean ± SEM [**p<0.01], [*p<0.05],
Statistical analysis was done by One-Way ANOVA followed by Dunnett's test.

Table 7: Mean paw volume and % inhibition of compounds after 1hr.

Compounds	Mean Paw Volume (ml) ± SEM	% Inhibition of Edema
Control	0.99 ± 0.040	……
L1	0.96 ± 0.058	4.04
L2	0.96 ± 0.052	3.82
L3	0.98 ± 0.021	1.17
Diclofenac sodium	0.85 ± 0.0085	15.15

Test compounds = 25 mg/kg
Reference Standard, Diclofenac Sodium = 10 mg/kg
Mean ± SEM [**p<0.01], [*p<0.05],
Statistical analysis was done by One-Way ANOVA followed by Dunnett's test.

Table 8: Mean paw volume and % inhibition of compounds after 2 hr.

Compounds	Mean Paw Volume (ml) ± SEM	% Inhibition of Edema
Control	1.23 ± 0.022	……..
L1	0.86 ± 0.068	10.86
L2	0.96 ± 0.062	7.84
L3	0.88 ± 0.028	5.66
Diclofenac sodium	0.87 ± 0.0089	16.06

Test compounds = 25 mg/kg
Reference Standard, Diclofenac Sodium = 10 mg/kg
Mean ± SEM [**p<0.01], [*p<0.05],
Statistical analysis was done by One-Way ANOVA followed by Dunnett's test.

Table 9 : Mean paw volume and % inhibition of compounds after 3 hr.

Compounds	Mean Paw Volume (ml) ± SEM	% Inhibition of Edema
Control	0.97± 0.042	……
L1	0.95± 0.059	12.77
L2	0.86 ± 0.058	8.66
L3	0.98 ± 0.041	9.90
Diclofenac sodium	0.85 ± 0.0095	21.5

Test compounds = 25 mg/kg
Reference Standard, Diclofenac Sodium = 10 mg/kg
Mean ± SEM [**p<0.01], [*p<0.05],
Statistical analysis was done by One-Way ANOVA followed by Dunnett's test.

Table 10: Mean paw volume and % inhibition of compounds after 6 hr.

Compounds	Mean Paw Volume (ml) ± SEM	% Inhibition of Edema
Control	0.87 ± 0.052	…..
L1	0.85 ± 0.058	19.15
L2	0.89 ± 0.057	8.16
L3	0.99 ± 0.046	17.90
Diclofenac sodium	0.89 ± 0.0098	22.95

Test compounds = 25 mg/kg
Reference Standard, Diclofenac Sodium = 10 mg/kg
Mean ± SEM [**p<0.01], [*p<0.05],
Statistical analysis was done by One-Way ANOVA followed by Dunnett's test.

Table 11: Mean paw volume and % inhibition of compounds after 24 hr.

Compounds	Mean Paw Volume (ml) ± SEM	% Inhibition of Edema
Control	0.97 ± 0.042	……
L1	0.75 ± 0.068	19.98
L2	0.88 ± 0.087	10.75
L3	0.89 ± 0.066	19.07
Diclofenac sodium	0.79 ± 0.0078	21.21

Test compounds = 25 mg/kg
Reference Standard, Diclofenac Sodium = 10 mg/kg
Mean ± SEM [**p<0.01], [*p<0.05],
Statistical analysis was done by One-Way ANOVA followed by Dunnett's test.

4. Conclusion

The plan of work of present study was synthesis of sulfonamides derivatives of quinoxaline and physicochemical and spectral characterization, *in vitro* anti-inflammatory screening.All sulfonamides derivatives of quinoxaline were synthesized on the basis of elimination-addition mechanism.

The reaction was carried out in the presence of base, (pyridine) was used, all the reaction was refluxed with pyridine on water bath. The reaction time required for each reaction was different it was depends on the amines used. After completion of reaction, reaction mixture was poured intAll the sulfonamides derivatives were confirmed by TLC, IR, MS and ^{1}HNMR. The spectral characterization revealed the formation of sulfonamide derivatives o cold water and continuously stirred until product crystallized.

Quinoxalin derivatives L1, L2, L3 were screened for in vitro anti-inflammatory activity in albino rat. All derivatives possess anti-inflammatory activity with percent inhibition ranging from2.25% to 22.95%.

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