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Synthesis Characterisation and Anti-Fungal activity of some Nitrosubstituted Quinoxalines against *Aspergillus niger*

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A B S T R A C T

Some Quinoxaline compounds containing nitro group in the nucleus have been planned to synthesise and evaluate for antifungal activity against *Aspergillus niger.* Physical treatment e.g T.L.C melting point, IR, NMR, spectroscopy and finally with alternative method of synthesis. Anti-fungal activity was performed against *Aspergillus niger* by cup-plate method (diffusion method) using griseofulvin as standard compound. *A.niger* produces most alarming infection and to protect from this infection there is a requirement to develop a new replacement drug which is effective against resistant fungi having lesser toxicity as well as economical also. In the present work efforts have been made to synthesise the proposed compounds N – 01 to N – 09 and screened for antifungal activity.

Introduction

It has been found that the development and designing of selective non nucleoside inhibitor of Vinus polymerases is and emerging area of research activity (Hospenthal *et al.*, 1998; Dagenais and Kellar, 2009; Walsh *et al.*,; Hope *et al.*,; Bradshaw *et al.*, 1998). There are various field of interest in discovering non-nucleoside inhibitors of virus polymerases increased after. The pronounced antifungal activity. In 1965 Raper and Fennel divided the genus *Aspergillus A. Ellipticus, A. Heteromorphus, A. Carbonarius, A. Japonicus* and *A. Aculeatus* as brown to black shaded spores and this group of species which are difficult to distinguish, *A ficuum, A phoenicis, A niger* and *J. Awamori* being the most prominent. *A. Niger* is able to colonise the human body and cause infection e.g lung infections, ear infections (olomyeosis) may be caused by mechanical damage of skin barrier even it was also reported that *A. Niger* is mainly responsible and consider for the most common and deadly pulmonary fungal infection world-wide through forming a dense colony of filaments embeded in a polymeric extracellular matrix in lungs. It is also reported that hundreds of *Aspergillus* inhaled by the person perday (Hospenthal *et al.*, 1998), basically involved removing *Aspergillus* spp from respiratory

tract while polymorphonuclear neutrocytes cleared germinating spores and hyphae through degranulation and the release of oxidants (Dagenais and Kellar, 2009). *Aspergillus* spp. are capable to colonise in respiratory tract, even presence of these effective clerance mechanisms in the body for the elimination of inhaled fungi from the respiratory tracts of helthy individuals. The main target site of colonising of *Aspergillus* spp is injured lung tissue and epithalia, although such colorisation aften has no clinical consequences. *Aspergillus* spp can cause a variety of clinical manifestations depend upon the imune status of the host (Walsh *et al.*,), with The discovery, antifungal drugs, which reduced the threats posed by infectious disease caused by the fungus. It is important to note that the available drugs will not be able to combat disease and alarming situation may occur in the course of resistant development by these drugs.

For a long time antimicrobial has saved the life of millions of people. Today we people are suffering for two reasons and hence this emergence, first is spreading of microbes, which makes resistant to economic and effetive first line drugs (Hope *et al.*,) and second is fungal infections are contributing most to human diseases, which emerging fungal resistant. To overcome this problem, there is requirement to develop new replacement as soon as possible, which should be effective against bacteria resistant with less toxicity as well as economical too. In recent years heterocyclic compounds analogues and derivatives especially, Quinoxalines, Tetrazines and many other nitrogeneous derivatives are known to possess a wide range of biological activities (Bradshaw *et al.,* 1998; Alang G. Kaur *et al.,* 2010; Suresh *et al.,*; Basavaraja *et al.,* 2010; Vedavathi *et al.,*; Pandurangan *et al.,*; Rajeeva *et al.,* 2009; Malik *et al.,* 2009; Patel and Shaikh,; Barot *et al.,* 2010; Dua *et al.,*; Bhusari *et al.,* 2008). In the course of last three years of study, there have been a large number of therapeutic agents have been synthesised with the help of quinoxaline nucleus (Sathe *et al.,* 2011) and consider for their systhesis for the purpose of significance in the field of medicinal treatment of organic compound due to their remarkable pharmalogical potentalities (Sathe *et al.,* 2011; Sreenivasa *et al.,* 2009; Venkatesh and Pandeya, 2009; Shashank *et al.,* 2009). The present paper concerned with the synthesis of nitrosubstituted quinoxaline derivatives, already prepared by us and presented for publication in plant archives journal and now for antifungal activity to establish, structure-biological (antifungal) acitivity relationship.

Experimental Design

Materials and Methods

All melting points are uncorrected and were obtained in capillary using paraffin bath. FT-IR spectra were recorded using KBr disc on parkin Elmer FT-IR KBr spectrophotometer and 'HNMR on Brucker advance II 400 NMR spectrometer using DMSO, CDCl3 as solvent. Purity of the compound is checked on silical gel G.glass plate using iodine vapours as a visualising agent. All aryl substances obtained in different steps were prepared by the extension of the known procedure.

In the present work synthesis and characterisation of some quinoxaline have been with all the synthesis i,e alternative method elemental analysis, melting point and mixed melting point. Finaly comparing the spectral datas to elucidate (Pandurangan *et al.,*; Rajeeva *et al.,* 2009; Malik *et al.,* 2009). The structural formula may be obtained.

Alternative method of preperation

Preparation of substituted - 1,2,3,4-tetrahydroquinoxaline by the condensation of substituted o-dibromobenzene and 1,2-diamino ethane :- (Ia)

A solution of (0.5M) of substituted of -0-dibromobenzene in dry carbontetrachloride is a round bottomed flask fitted with reflux condenser is placed Ethylene diamine hydrochloride (0.5M) solution was added drop-wise with constant stirring (Pandurangan *et al.,*). The pot was kept in ice. The

progress of reaction was made with the evolution of hydrogen bromide and further it was checked by tlc examination time to time. After completion of reaction, the reaction mixture was then poured into a mixture of ice and the ethereal extract washed with water and dried over anhydrous calcium chloride. Removal of ether and carbon disulphide by distillation left a gummy residue which crystallised on trituration with benzene and light petroleum ether. Recrystallisation from benzene gave the pure 1,2,3,4-tetrahydroquinoxaline (Rajeeva *et al.,* 2009) as yellowish brown crystals, elemental analysis and m. point is placed in table. Primary amino compounds exhibit medium to strong N-H in plane bending vibrations near 1650-1580 cm-1 which is moved during reaction to give product in which it is slightly moved to higher frequency (Patel and Shaikh).

Aromatic secondary amine absorb near 1490-1440 cm-1 and C-N stretching vibration at 1350-1310 cm-1. There is much less interaction between these modes compared to the transform. The N-H out of plane bending (wagging) vibration appears as a broad band near 800 cm-1 and aeral absorption at 3040 cm^{-1}assigned to aromatic C-H stretching frequency.

Preparation of quinoxaline by dehydrogenation of 1,2,3,4-tetrahydroquinoxaline prepared earlier (Malik *et al.,* 2009):- (IIa)

0.5 M of purified 1,2,3,4-tetrahydroquinoxaline in methanol is placed in round bottomed flask fitted with water condenser under reflux and then added 0.25g paladisedchareoal in it and allowed heating to boil for about four hours in a slow current of carbon dioxide. After completion of heating the flask was kept in a ice chest overnight. The crude product obtained was filtered off and washed with 10% sulfuric acid and then with 10% sodium bicarbonate solution, followed by water, dried with anhydrous calcium sulphate and finally recrystalised from methanol. A brown crystal of substituted quinoxaline with 76% yield. m.point and elemental analysis are placed in table

Alternative Method of Preparation of Subtituted Quinoxalines

A mixture of substituted 1,2-diaminobenzene (0.5M) and glyoxal sodiumdibisulphite (0.5M) in aqueous solution of sodium acetate and acetic acid (10 ml) was taken and small amount of cone sulphuric acid (1 ml) was added to reaction mixture kept in a small round bottomed flask and heated under reflux on a water bath15-18. The reaction was followed by t.l.c (Thin layer chromatography) which showed almost complete disappearance of the starting material after five hours of heating22-23. Acetic acid was removed by distillation. The residue on cooling deposited a pale yellow mass. This was washed on cooling deposited a pale yellow mass. This was washed with benzene and ethanol. It was then recrystallised from ethanol to furnish the pure crystals of substituted quinoxaline crystals. M. point and Mixed M. point determination of the proposed compound prepared here and earlier were found same and their elemental analysis spectral datas were also found identicals.

On the same outline all compounds (Venkatesh and Pandeya, 2009; Shashank *et al.,* 2009) were prepared and were compared physically (M-point spectral analysis and elemental analysis) and chemically (alternative method of synthesis) both.

Antifungal Screening of Synthesised Compound

The synthesised compounds are screened against selected fungal stains. *A.niger* by using diffusion method and as a standard drug. Under the aseptic condition, 48 hours old fungal culture was inoculated into the nutrient both and incubated for 48 hours at 37 ± 2^0C in an incubator. Potato-dextrose agar media (20%) mixed with inoculated culture and poured into petriplated. Five bores are made at an equal distance by using sterile steel cork borer (8 mm in diameter) after solidification. Different concentrations (50$\mu g/ml$ and 100 $\mu g/ml$) of standard drug and systhesised compounds along with control introduced in these plates and place in refrigerator at $8\text{-}10^0$C as cold incubation for two hours that allowed proper diffusion of the drug and

synthesised compounds. The petriplated were transferred to the incubator and maintained at 37 ± 2^0C for 24-36 hours after cold incubation. Zone of inhibition was observed by using vernier scale. The mean value of the zone of inhibition was measured in millimeter of two preparation of synthesised compounds (N-01to N-09) and standard drug.

Minimum inhibitory concentration (MIC) by broth dilution method

Nutrients broths (double strength) was prepared in test tubes and labled them. In first test tube (UT) inoculum is not added which is used for checking the sterility of medium and as a negative control. Other all test tubes, inoculums (three to four drops) is added to reach the final concentration of micro organisms is 106 cells/ml in all test tubes, test antimicrobal compound is added ranging from 0.5 to 5 ml except uninoculated (negative control) and control (positive) tube. The positive control tube is used to check the suitability of the medium for growth of the test microorganism and the viability of the inoculums. Adjust the final volume (10 ml) in all test tubes by using sterile water. All test tubes are properly shaken and then incubated at 37^0C for two days.

Results and Discussion

Quinoxaline[21] contains only nitrogen as hetroatom but imparts biological activity, while substitution at C_5, C_6, C_2, C_3, - positions. In the present work nitro substituted phenyl as well as only nitro group in quinoxaline nuclues derivatives were synthesised.

The novel derivatives (N – 01 to N - 09) evaluated for antifungal activity against *Aspergillus niger*. In the present work nitro and nitrophenyl groups consider as rotating basis as 5, 6,0-nitrophenyl, p-nitrophenyl and m-nitrophenyl at C_2 & C_3 positions. The reason behind considering the nitro and nitrophenyl groups as substituents is fungi rarely acquire resistance. TLC, melting point IR and 1HNMR were used for analytical characterisation as discussed in pervious pages. In the TLC, the distance travelled by compound. N - 01 to N - 09 was found to be different from that of the starting material that proved synthesised compounds were different from parent one even during TLC performance every tome single spot was obtained, hence it also reveals that synthesised compounds were free from impurity as well as reaction was completed.

Structure elucidation by IR spectra frequency range for C═N, C—NO═C was considered. In case of structure elucidation by ‘HNMR sharp characteristic signals for 8H Ar - H, 2H for heterocyclic aromatic ring was observed and considered 2,3-bis (nitrophenyl) -5-nitroquinoxaline and 2,3-bis (nitropheyl)-6-nitroquinoxalines in all the synthesised compounds as shown below in the given table – 1

Antifungal activity performed at two concentration 50µg/ml and 100 µg/ml using griseofulvin as a standard drug against *Aspergillus niger*. The result of zone of inhibition (ZOI) and (MIC) revealed that compound N-O_3 showed potent antifungal activity against A. Niger while compound. N – 05, N – 08 and N – 09 showed moderate inhibitory activity at both concentrations 50 µg/ml and 100 µg/ml as compared to standard Table – 2, Table – 3, Figure – 3 and Figure – 4. The structure acticity relationship of newly synthesised compound revealed that 2,3-bis(p-nitrophenyl)-5-nitro quinoxaline (N-03) found to more active than standard, while 2,3-bis(m-nitrophenyl)-6-nitroquinoxaline (N-05), 2-(O-nitrophenyl)-3-(nitrophenyl)-6-nitroquinoxaline (N-08) and 2-(m-nitrophenyl)-3-(p-nitrophenyl)-6-nitroquinoxaline (N-09) exhibited prominent inhibitory activity against *Aspergillus niger*.

In the present work nitrosubstituted novel-1,2-dihydro-1,2,4,5-tetrazine derivatives were synthesised and screened for antifungal acitvity against *Aspergillus niger* (N – 01 to N – 09) The paucity N – 03 exhibited more potent activity as compared to standard.

Table.1 Analytical characterisation of synthesised compounds (N – 01 to N - 09)

Compound Code	% Yield	Melting Point (OC)	TLC (Rf-Value)	IR – Spectral Study	'HNMR spectral study
N - 01	82%	166	0.52	2130 $cm^{-1}$for – N═C–C=N 3050 - 3080 cm^{-1} for aromatic C–H stretching 1580 cm^{-1} for ─N═C 1680cm^{-1} for substituted benzene ring	Double singlet at δ 4.8 three sets of aromatic protons, and had a resonance multiple at δ 8.4 to δ 7.2
N – 02	80%	112	0.54	2120 $cm^{-1}$for ─N═C–C=N─ 3040 $cm^{-1}$to 3060 cm^{-1} for C–H stretching 1585 cm^{-1} for ─N═C 1690 cm^{-1} for substituted benzene ring	Double singlet at δ 4.2 three sets of aromatic protons, and had a resonance multiple at δ 8.4 to δ 7.2
N – 03	75%	188	0.50	2125 $cm^{-1}$for ─N═C–C=N─ 3050 $cm^{-1}$to 3075 cm^{-1} for C–H 1580 cm^{-1} for ─N═C 1675 cm^{-1} for substituted benzene ring	Double singlet at δ 4.6 three sets of aromatic protons, and had a resonance multiple at δ 8.4 to δ 7.2
N – 04	72%	205	0.49	2130 cm^{-1} for – N═C–C=N 3050 - 3080 cm^{-1}	Double singlet at δ 4.34 three sets

				for aromatic C–H stretching 1580 cm^{-1} for ═N═C 1680 cm^{-1} for substituted benzene ring	of aromatic protons, and had a resonance multiple at δ 8.4 to δ 7.2
N – 05	79%	126	0.61	2120 $cm^{-1}$for ═N═C–C=N═ 3040 $cm^{-1}$to 3060 cm^{-1} for C–H stretching 1585 cm^{-1} for ═N═C 1690 cm^{-1} for substituted benzene ring	Double singlet at δ 4.20 three sets of aromatic protons, and had a resonance multiple at δ 8.4 to δ 7.2
N – 06	76%	210	0.54	2125 $cm^{-1}$for ═N═C–C=N═ 3050 $cm^{-1}$to 3075 cm^{-1} for C–H 1580 cm^{-1} for ═N═C 1675 cm^{-1} for substituted benzene ring	Double singlet at δ 4.30 three sets of aromatic protons, and had a resonance multiple at δ 8.4 to δ 7.2
N – 07	62%	244	0.55	2130 cm^{-1} for –N═C–C=N 3050 - 3080 cm^{-1} for aromatic C–H stretching 1580 cm^{-1} for ═N═C 1680 cm^{-1} for	Double singlet at δ 4.22 three sets of aromatic protons, and had a resonance multiple at δ 8.4 to δ 7.2

				substituted benzene ring	
N – 08	69%	232	0.62	2120 $cm^{-1}$for ─N═C–C=N─ 3040 $cm^{-1}$to 3060 cm^{-1} for C–H stretching 1585 cm^{-1} for ─N═C 1690 cm^{-1} for substituted benzene ring	Double singlet at δ 3.78 three sets of aromatic protons, and had a resonance multiple at δ 8.4 to δ 7.2
N – 09	74%	255	0.46	2125 $cm^{-1}$for ─N═C–C=N─ 3050 $cm^{-1}$to 3075 cm^{-1} for C–H 1580 cm^{-1} for ─N═C 1675 cm^{-1} for substituted benzene ring	Double singlet at δ 4.3 three sets of aromatic protons, and had a resonance multiple at δ 8.4 to δ 7.2

Table.2 Results of antifungal acitivity for ZOI

Compound Code	***Aspergillus* Niger**	
	Zone of Inhibition (mm)	
	50 μg/ml	100 μg/ml
	24	38
N – 01	09	15
N – 02	12	16
N – 03	32	55
N – 04	10	17
N – 05	21	34
N – 06	05	08
N – 07	11	17
N – 08	22	36
N – 09	22	32

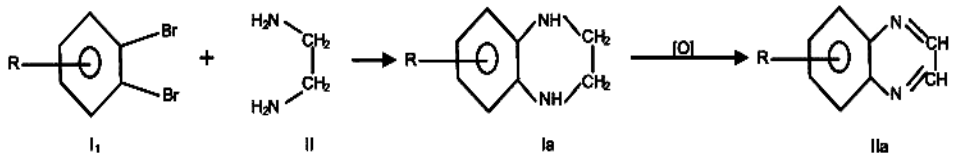
Each value is the mean of three replicates.

Table.3 Results of MIC of synthesised compound

Compound Code	Minimum Inhibitary concentration MIC μg/ml ± SD	
	Aspergillus Niger	
	50 μg/ml 24	100 μg/ml 38
	23.80 ± 0.21	35.14 ± 0.47
N – 01	07.69 ± 0.28	13.88 ± 0.63
N – 02	9.65 ± 0.14	15.22 ± 0.10
N – 03	30.57 ± 0.51	52.11 ± 0.68
N – 04	09.10 ± 0.74	14.10 ± 0.42
N – 05	19.65 ± 0.55	30.25 ± 0.33
N – 06	03.96 ± 0.32	05.02 ± 0.21
N – 07	10.53 ± 0.47	12.44 ± 0.20
N – 08	21.47 ± 0.40	34.05 ± 0.52
N – 09	20.21 ± 0.96	28.22 ± 0.78
Control	0	0

Each value is the mean of three replicates

Fig.1



Alternative method of preparation

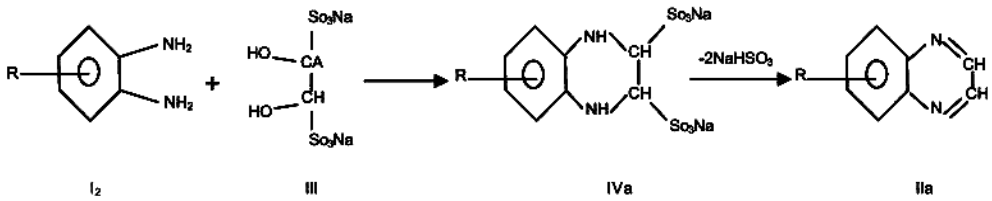
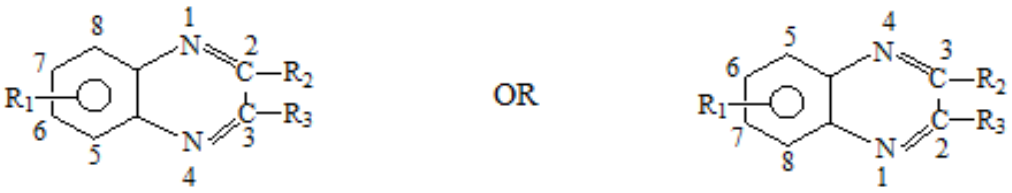
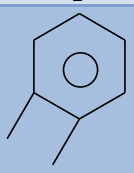
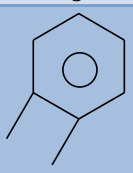
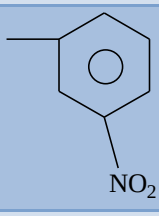
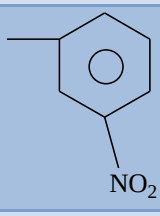
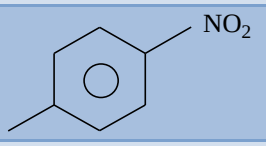
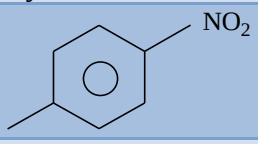
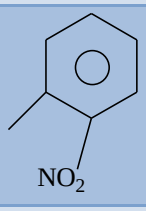
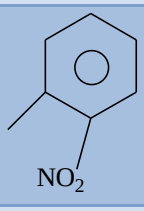
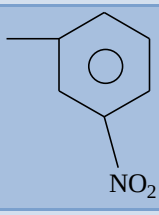
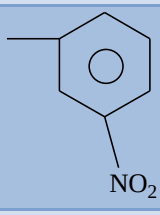
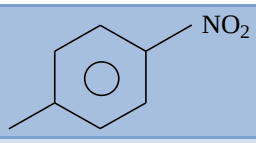
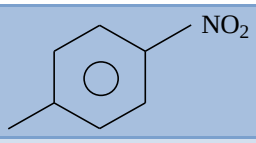
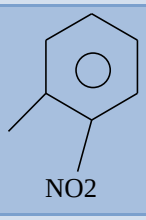
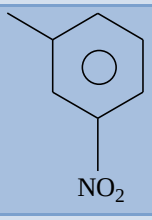
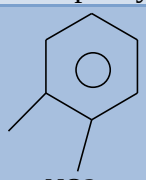
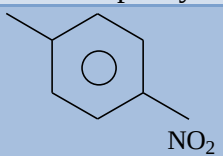
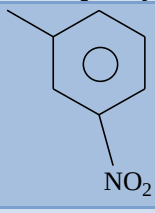
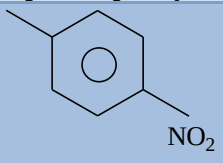


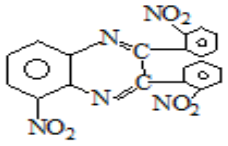
Fig.2



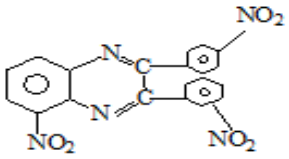
(5 = 8 & 6 = 7) (2 = 3)

Compound Code	R_1	R_2	R_3
N – 01	5-NO_2		
		o-nitrophenyl	
N – 02	5-NO_2		
		m-nitrophenyl	
N - 03	5-NO_2		
		p-nitrophenyl	
N – 04	6-NO_2		
		o-nitrophenyl	
N – 05	6-NO_2		
		m-nitrophenyl	

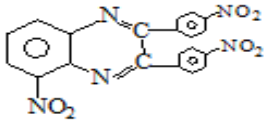
N – 06	6 – Nitro		
		p-nitrophenyl	
N – 07	6 – Nitro		
		o-nitrophenyl	m-nitrophenyl
N – 08	6 – Nitro		
		o-nitrophenyl	p-nitrophenyl
N – 09	6 – Nitro		
		m-nitrophenyl	p-nitrophenyl



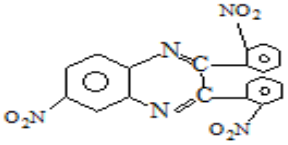
(N - 01)



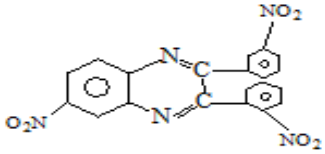
(N - 02)



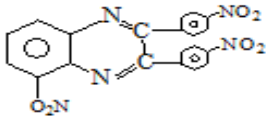
(N - 03)



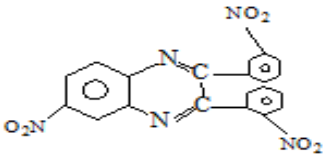
(N - 04)



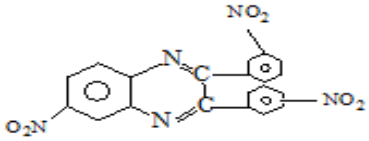
(N - 05)



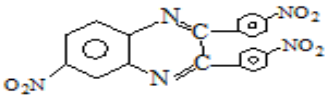
(N - 06)



(N - 07)

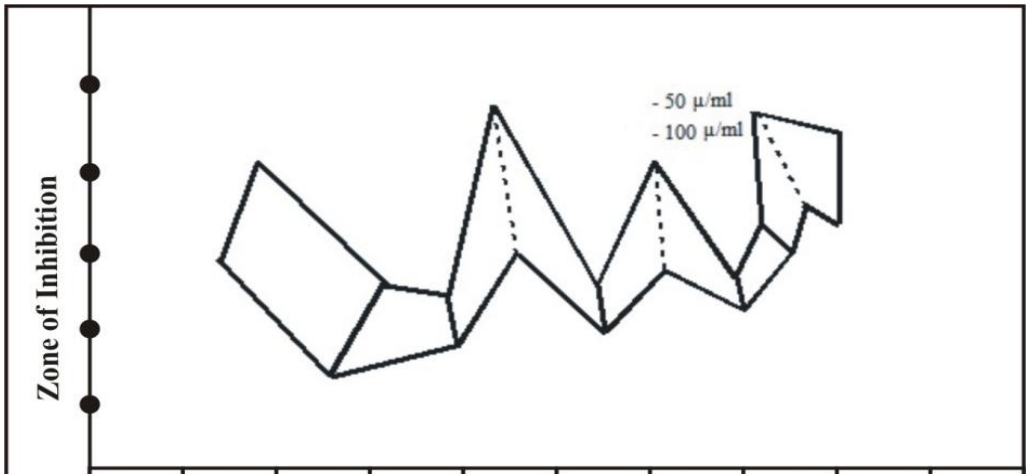


(N - 08)



(N - 09)

Fig.3

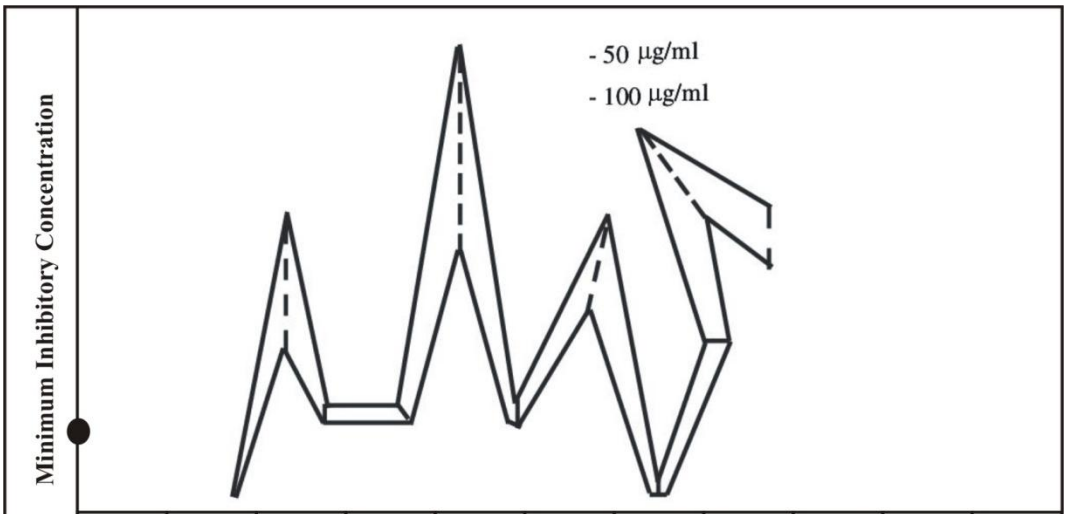


	GF	N - 01	N - 02	N - 03	N - 04	N - 05	N - 06	N - 07	N - 08	N - 09
- 50 µ/ml	24	9	12	32	10	21	5	11	22	22
- 100 µ/ml	36	15	16	55	19	34	8	17	36	32

Compound Code

Result of comparative study of novel synthesised compounds

Fig.4



	Cl	GF	N - 01	N - 02	N - 03	N - 04	N - 05	N - 06	N - 07	N - 08	N - 09
- 50 µg/ml	0	23.8	7.60	9.65	30.57	9.1	19.65	3.96	10.55	21.47	20.21
- 100 µg/ml	0	35.14	13.38	15.22	52.11	14.1	30.25	5.02	12.44	34.05	28.22

Compound Code

Result of comparative study of MIC of synthesised compounds

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