Antimicrobial activity and bio-film inhibition potential of selected heterocyclic compounds

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Abstract

Novel heterocyclic compounds indenoquinoxaline-11-one (QN)and4-[2-pyrimidinyl-2-phenylQuinazolin-4(3H)-one(Q-2APy) synthesized and investigated for anti-bacterial activity against *Staphylococcus aureus* and Multidrug resistance *Staphylocoocus aureus* (MRSA), Synthesized compounds also investigated for bio-film activity against above tested bacteria SA to explore antibacterial potency. Both QN and Q-2APY inhibits SA and MRSA bacteria. Both compounds had inhibition of bio-film produced by MRSA, but not with SA.

Key words: Indenoquinoxaline, Quinazolin-4(3H)-one, *Staphylococcus aureus*, MRSA, Bio-film

Introduction

Recently much attention has been devoted for searching of novel antibacterial agents with inhibition bio-film formation by pathogenic bacteria and present study aimed to design heterocyclic compounds with antibacterial agents along with inhibition of bio-film activity. Indenoquinoxaline and Quinazolin-4(3H)-one is a versatile lead molecule for potential bioactive agents and its derivatives were documented for board-spectrum of biological activity[1,2]. Therapeutic efficacy of indenoquinoxaline derivatives is essential at the present moments or need of hour to explore the newer drugs against emerging diseases[3-8].

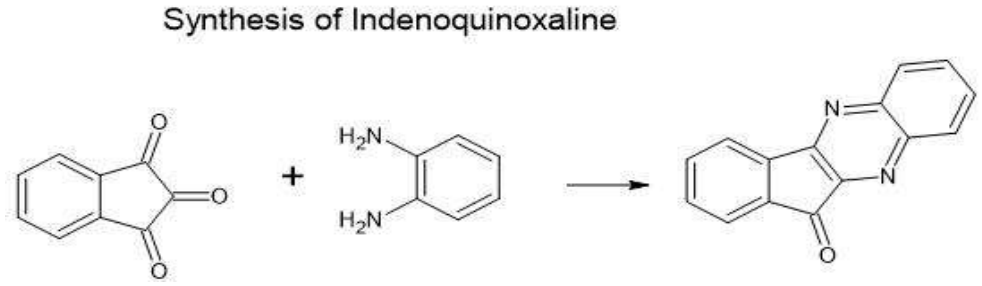
Quinazolin-4(3H)-one also documented for vide spectrum of Pharmacological actions[9-16]. Indenoquinoxaline (QN) and 4-[2-pyrimidinyl-2-phenylQuinazolin-4(3H)-one (Q-2APy), synthesized and investigated for antibacterial activity against *Staphyllococcus aureus* (SA) and Multidrug resistance *S. aureus* (MRSA). Synthesized compounds also investigated for bio-film activity to explore antibacterial potency.

Indenoquinoxaline-11-one prepared by condensation of O-phenylenediamine and ninhydrin in presence of ethanol. 4-[2-pyrimidinyl-2-phenylQuinazolin-4(3H)-one [Q-2APy] was synthesized by refluxing an equimolar mixture of 2-phenyl benzoxine and 2-aminopyrimidine using ethanol/acetic acid. In-vitro anti-bacterial activity studied by well plate method in MHA medium against SA and MRSA, assessment of Anti-biofilm assay also performed by Crystal violet staining method.

Materials and methods

Synthesis of Indenoquinoxaline

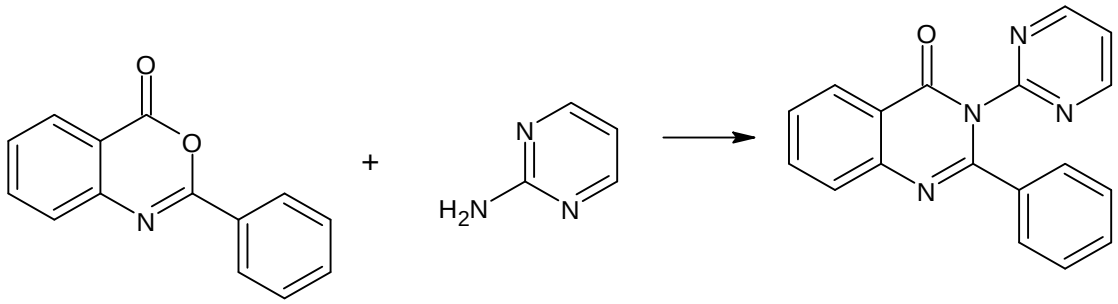
11H-Indeno [1,2-b]quinoxalin-11-one (1) was synthesized by refluxing an equimolar mixture of o-phenylenediamine (0.001M) and ninhydrin (0.001M) using ethanol/acetic acid (1:1) as a solvent.



Scheme 1 Synthesis of Indenoquinoxaline

Synthesis of4-[2-pyrimidinyl-2-phenylQuinazolin-4(3H)-one (Q-2APy)

[Q-2APy] (1) was synthesized by refluxing an equimolar mixture of 2-phenyl benzoxine (0.001M) and 2-aminopyrimidine (0.001M) using ethanol/acetic acid (1:1) as a solvent.



Scheme 2 Synthesis of4-[2-pyrimidinyl-2-phenylQuinazolin-4(3H)-one

Spectral data: FT-IR QN 1726 C=O, 1602 C=C 775 Ar-H, QN-2APy:1650C=O, 1587 C=N, 1533 C=C Ar-H, 754 Ar-H, FT-H NMR: 6.5 t, 2H,Ar-H, 6.6 s, 2H4H,Ar-H, 7.2 q,1H, Ar-H 7.6-7.7 m, 5H,Ar-H, 7.95 m,2H,Ar-H, 8.0-8.1 d,2H, Ar-H, 8.2 d,4H,Ar-H, 8.7 d,1H,Ar-H.QN NMR 7.6 t, 1H,Ar-H, 7.77-7.92 m 4H,Ar-H, 7.9 d, 1H,Ar-H, 8.1 t,1H,Ar-H, 8.2 d, 1H, Ar-H.

Assessment of Antimicrobial assay – Zone of inhibition

A 66mg of extract was dissolved in 1mL of sterile DMSO for sample QN, and 175mg of extract was dissolved in 1mLof sterile DMSO for sample Q2A.Py.Fresh broth suspensions were prepared from the test organisms and were adjusted to 0.5McFarland Standards. Lawn cultures were made onto sterile Mueller Hinton agar plates. Wells were cut with the sterile agar cutter and 50µl of each test compound at different concentrations (10mg, 5mg, and 2.5mg dissolved in sterile DMSO) was added to the respective wells. The plates were incubated at 37°C/24hrs. After incubation, the zone of inhibition/clearance around the wells was measured and recorded.

Assessment of Anti-biofilm assay – Crystal violet staining method

A 66mg of extract was dissolved in 1mL of sterile DMSO for sample QN, and 175mg of extract was dissolved in 1mL of sterile DMSO for sample Q2APy. Fresh broth suspensions were prepared from the test organisms and were adjusted to 0.5McFarland Standards. The micro-titre plate was loaded with the broth, sample, and inoculum with a total volume of 200µL (80µL broth + 100µL test sample + 20µL inoculum).

The test sample was subjected to serial dilution from 1 to 10 (10mg to 0.01mg), 11 is control, and 12 (180µL of broth + 20µL of inoculum) is sterile control (200µL of broth). Sample Q2APy was loaded and serially diluted in B (1 to 10) for MRSA, and E (1 to 10) for SA, whereas, QN was loaded and serially diluted in C (1 to 10) for MRSA, and F (1 to 10) for SA respectively. The micro-titre plate was incubated at 37°C/48hrs. After incubation, the content in the wells was removed and washed, and the 0.1% of 50µL crystal violet staining solution was added and incubated for 10min. Again washed and 100µL of 70% Ethanol was added and kept for incubation for 5min. Finally, the absorbance values were measured at 595nm using a micro-plate reader.

Results and discussion

Compounds indenoquinoxaline-11-one (QN) and Quinazolin-4(3H)-one (Q-2APY) were synthesized with good yield (Scheme 1 and 2) and structure was characterized by spectral data analysis (Figure 1-4). Synthesized compounds (QN and Q-2APy) had significant activity against *staphylococcus aureus* (SA) and MDR *staphylococcus aureus* (MRSA) [Table 1-2]. QN also had significant antibacterial activity against clinical isolate of *staphylococcus aureus*

with ZoI 20mm (Table 3). Both the QN, and Q2APy have bio-film inhibition in MRSA, whereas, no bio-film inhibition is seen in SA (Table 4).

Both QN and 2-APy had significant activity against bio-film produced by multi-drug resistance S.A (MRSA) (Table 3). This is the first report of antibacterial activity with inhibition of bio-film formation against *staphylococcus aureus* of the lead molecules. Compound indenoquinoxaline-11-one (QN) and Quinazolin-4(3H)-one (Q-2APY) in the world of Literature.

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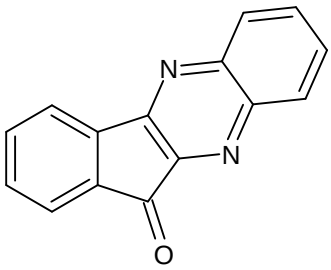


Fig A

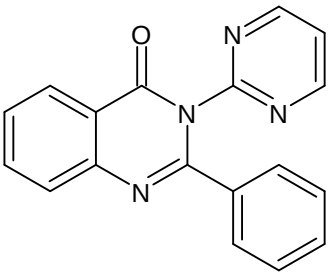


Fig. B

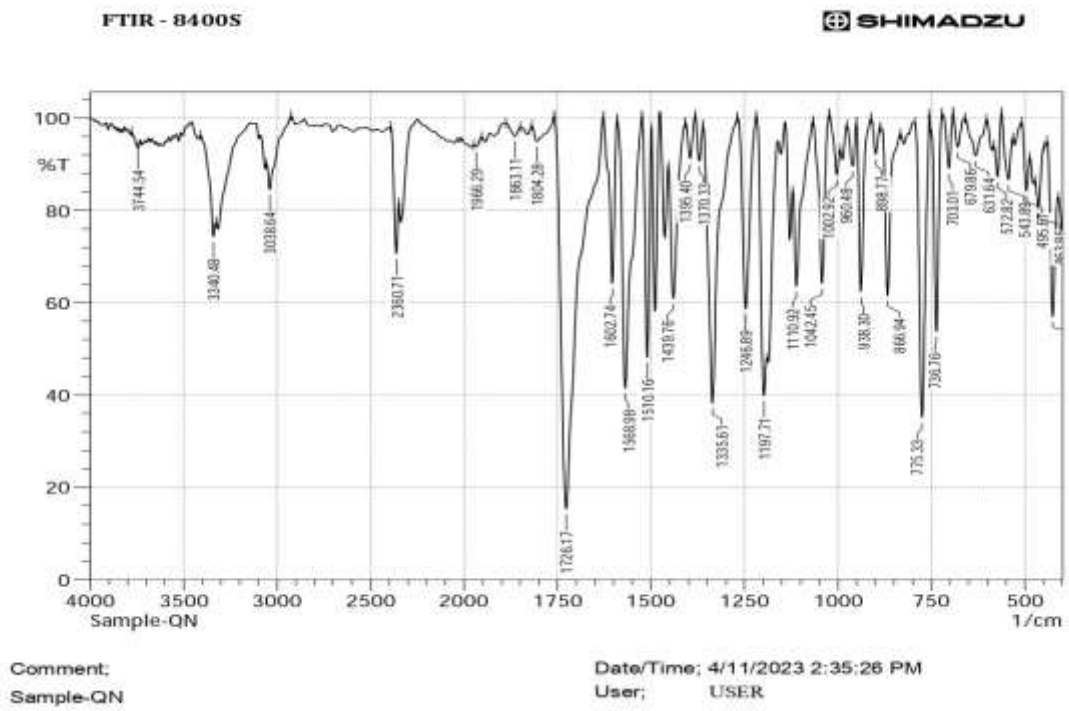


Figure 1 FTIR spectrum of QN

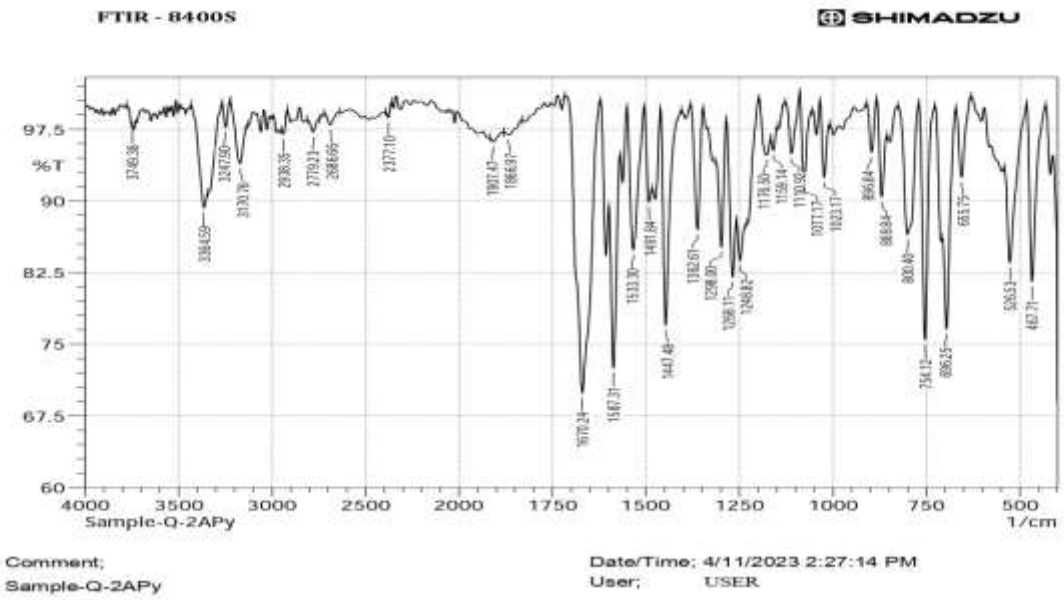
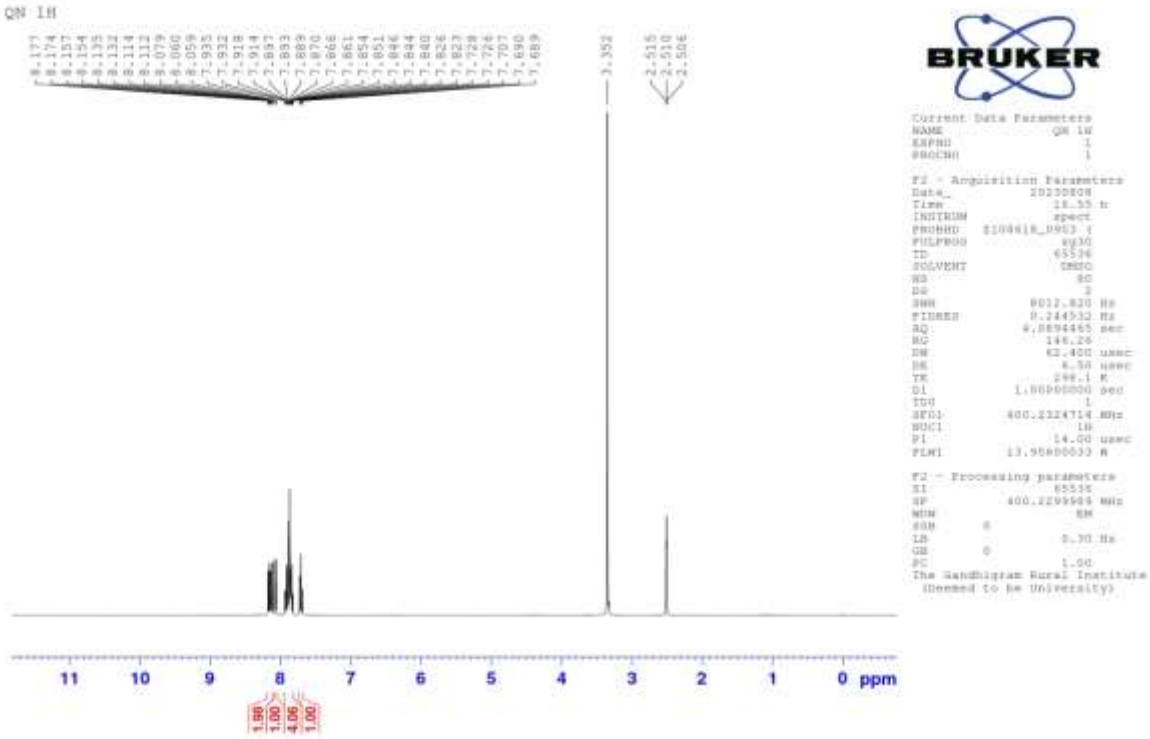


Figure 2 FTIR spectrum of Q-2APy



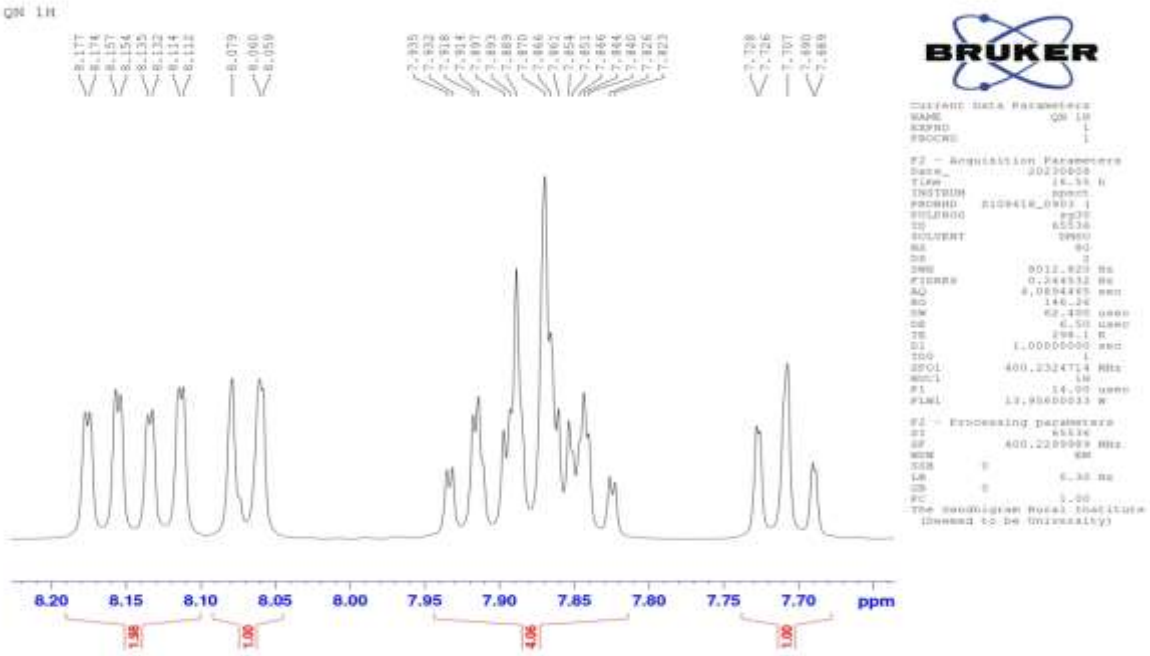
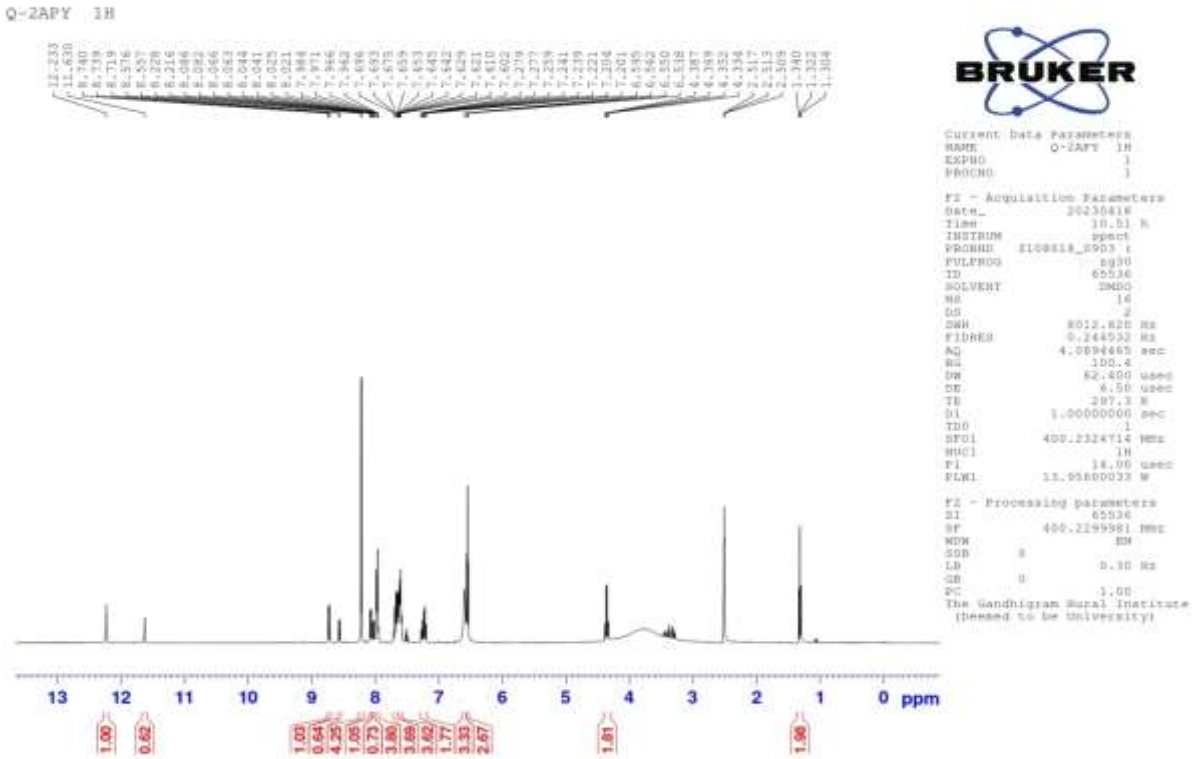


Figure 3 PMR (Proton NMR) spectrum of QN



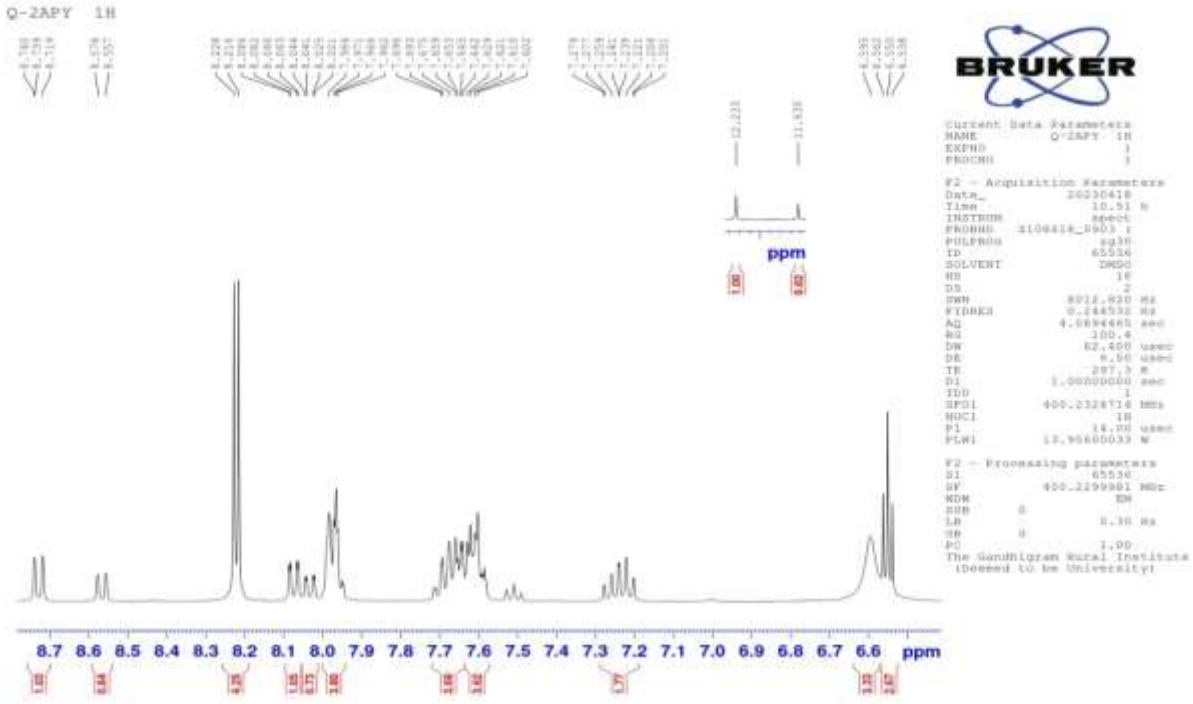


Figure 4 PMR (Proton NMR) spectrum of Q-2APy

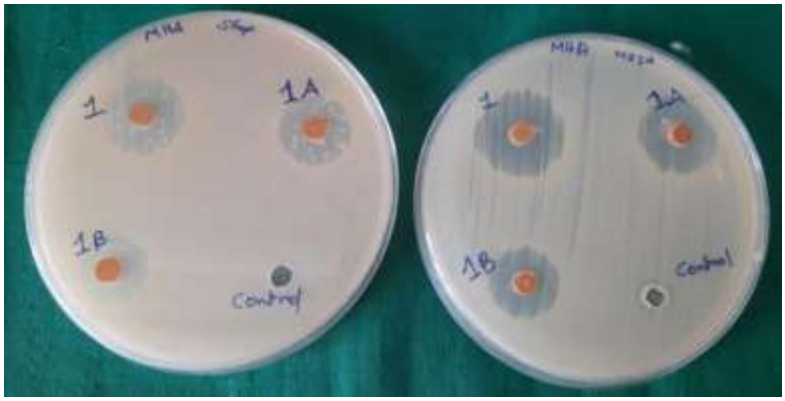


Figure 5 – Antimicrobial activity–zone of inhibition of the sample QN

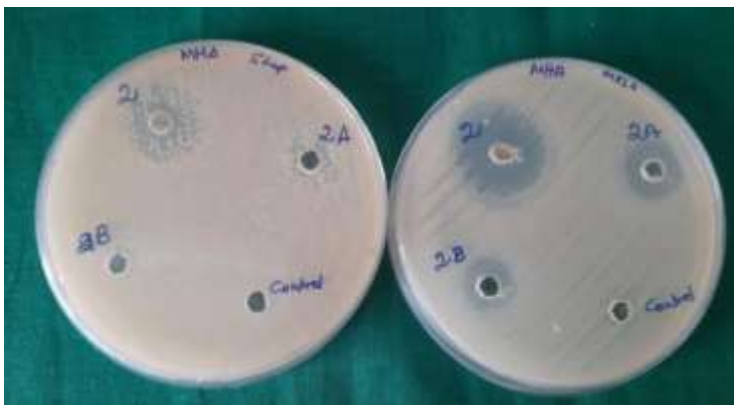


Figure 6 – Antimicrobial activity–zone of inhibition of the sample Q2APy

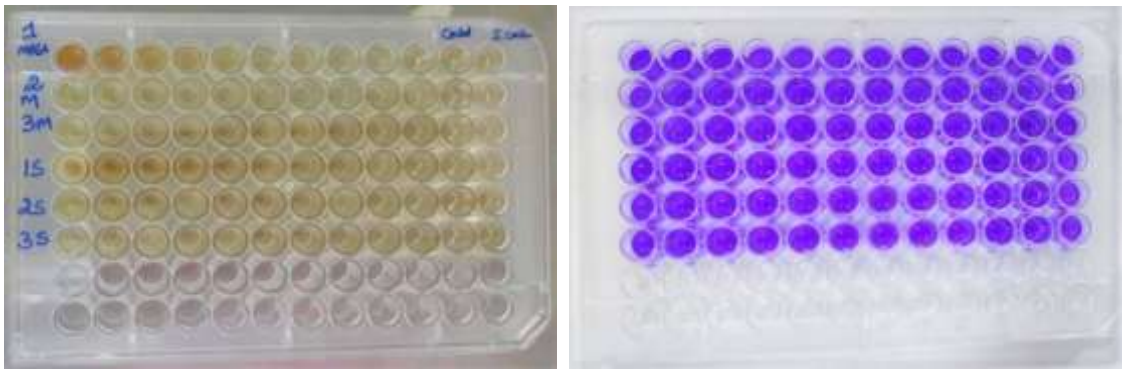


Figure 7 Micro-titer plate after crystal violet staining incubation for 48 hrs

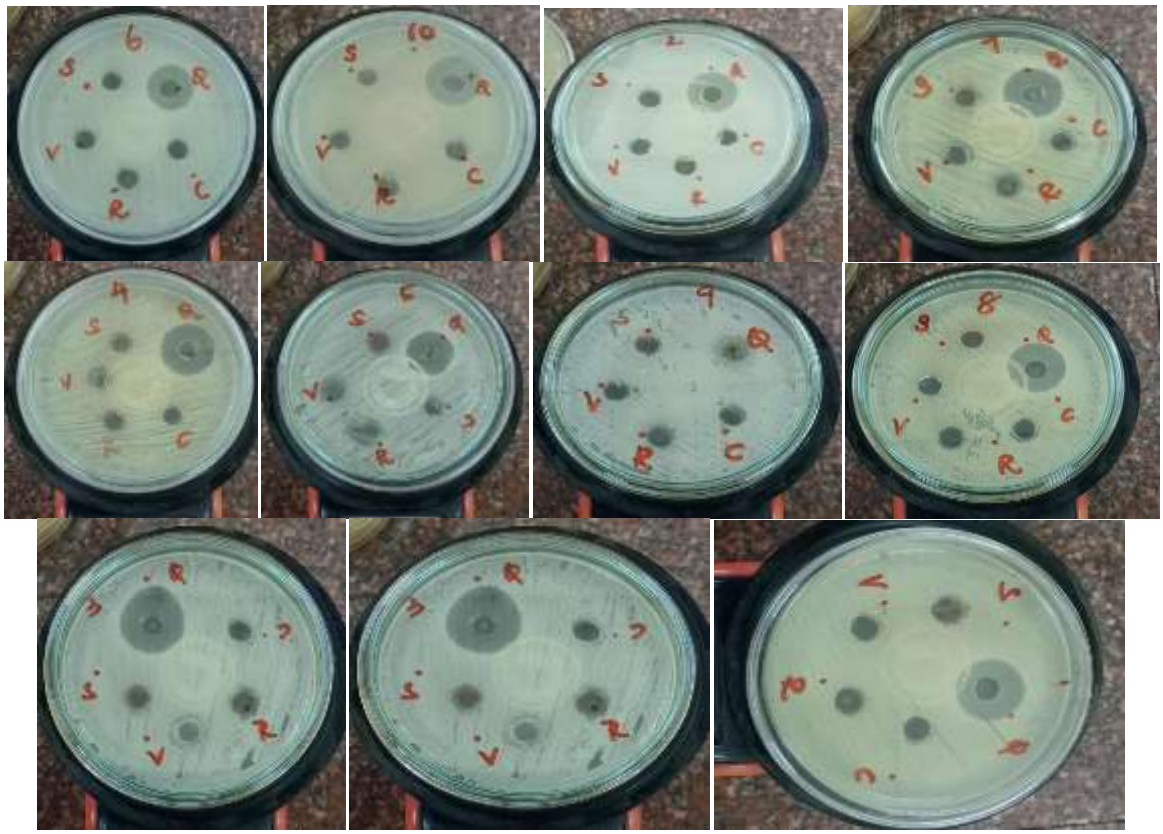


Figure 8 Antibacterial activity of QN Indenoquinoxaline *S. aureus*

Table 1 – In-vitro antibacterial activity QN

	Antimicrobial assay – Zone of inhibition (in mm)		
	Staphylococcus aureus		
Sample Name	**QN (10mg)**	**QN (5mg)**	**QN (2.5mg)**
QN1	20	18	17
QN2	21	17	16
QN3	22	19	15
Average	21	18	16
		MRSA	
Sample Name	**QN (10mg)**	**QN (5mg)**	**QN (2.5mg)**
QN1	24	21	17
QN2	23	20	16
QN3	22	22	15
Average	**23**	**21**	**16**

Table 2 – In-vitro antibacterial activity Q2APy

	Antimicrobial assay – Zone of inhibition (in mm)		
	Staphylococcus aureus		
Sample Name	**Q2APy (10mg)**	**Q2APy (5mg)**	**Q2APy (2.5mg)**
Q2APy1	24	17	13
Q2APy2	23	18	14
Q2APy3	22	16	15
Average	23	17	14
		MRSA	
Sample Name	**Q2APy (10mg)**	**Q2APy (5mg)**	**Q2APy (2.5mg)**
Q2APy1	20	13	0
Q2APy2	19	15	0
Q2APy3	18	14	0
Average	**19**	**14**	**0**

Table 3 - Assessment of Anti-biofilm assay – Crystal violet staining method

Strain used	Sample No.	Sample Name	Sample Concentration									
			10	5	2.5	1.25	0.62	0.31	0.15	0.07	0.03	0.01
			Percentage of inhibition									
MRSA	2	Q2QPy	8.71	0	0	2.09	0	1.04	0	0	0	0
MRSA	3	QN	0	4.29	0	10.42	0	3.68	0	0	0	0
SA	2	Q2QPy	0	0	0	0	0	0	0	0	0	0
SA	3	QN	0	0	0	0	0	0	0	0	0	0

Table 4 – *Invitro* antibacterial activity of QN

Antimicrobial assay – Zone of inhibition (in mm)	
	Staphylococcus aureus
SampleName	**QN (10mg)**
QN1	20
QN2	21
QN3	22
QN4	21
QN5	20
QN6	22
QN7	20
QN8	21
QN9	22
QN10	20
Average	**21**