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Spectroscopic studies and antibacterial activities of 11 H-indeno [1,2-*b*] quinoxaline-11-one

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Abstract

Morbidity and mortality induced by bacterial infection are significant health problems. To take over this challenge, new antibacterial agent discovery is a must. In this work, 11*H*-indeno[1,2-*b*]quinoxaline-11-one was chemically synthesized, and its biological activities were evaluated against *Salmonella typhimurium* (*S. typhi*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Bacillus subtilis* (*B. subtilis*). The biological activity of the compound was studied by a well diffusion method besides minimum inhibitory concentration (MIC), and resistant development evaluation. Optimization of the geometric structure was performed using DFT calculation and a comparison between calculated and experimental geometric structures and spectral data using the function BLY3P/6-311++G(d,p). MIC values against *S. typhi*, *K. pneumoniae*, and *B. subtilis* were recorded as values of 10, 20, and 20 mM respectively. Within seven days, none of these bacteria have no resistance against the tested compound at 1, 2, 3, and 4x MIC concentrations. The comparison between optimized structures geometry and spectral data (^{1}HNMR and ^{13}CNMR) with experimental data displayed acceptable agreement. Eventually, 11*H*-indeno[1,2-*b*]quinoxaline-11-one could be recommended as an effective antibacterial agent.

Keywords: 11H-indeno[1,2-b]quinoxaline-11-one; S. typhi; B. sibtilis; K. pneumoniae; resistant development; spectral analysis.

1. Introduction

Infectious diseases, food spoilage, corrosion of industrial materials, and much more are triggered by bacteria. The major global problem is bacteria that have developed and become more resistant to several antibiotics [1], due to excessive use and abuse of antibiotics [2]. To combat this, many antimicrobial agents have progressed to avoid returning to pre-antibiotic therapy.

The control of pathogenic bacteria such as *K. pneumoniae* using zirconia nanoparticles [3], and *B. subtilis* and *S. typhi* using *Senecio glaucus* L plant extract [4].

Numerous medications and chemicals that are important to biology contain heterocyclic compounds. The existence of heteroatoms or groups frequently imparts preference specificities in biological reactions. The study of heterocyclic compounds and their biological effects has long been an intriguing area of chemistry [5], and quinoxaline is one such moiety that has recently attracted attention due to its growing relevance to the study of medicinal chemistry. Quinoxalines are a group of low-molecular-weight heterocyclic scaffolds [6, 7]. This class is a key area of interest for the inquiry due to its straightforward chemical production and biological actions such as anticancer, antibacterial, antiviral, antifungal, anti-inflammatory, antidiabetic, antiparasitic, and other activities. The antimicrobials echinomycin, actinomycin, and levomycin, the active or basic substance is quinoxaline [8-10]. Many quinoxaline derivatives have been to possess antibacterial, antifungal, antiprotozoal, anticancer, and antiviral activities [11-21]. Several quinoxaline derivatives have an antibacterial activity [22] like compound 4-(2-methylphenyl)aminoacetyl-3,4-dihydroquinoxalin-2(1*H*)-one showed mild to medium activity against *B.*

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subtilis, and *S. typhi* [23], 2-methylquinoxaline [24] and 3b-chlorocholest-5-en-7-oxaazolo[4,5-b]quinoxaline [25] were in presence of metabolic activation reported to be positive in a mutagenicity test with *Salmonella typhimurium* TA98, and quinoxaline 1,4-dioxide (QdO) compounds have a robust antibacterial activity [26, 27]. Their antibacterial activities have been attributed to the inhibition of DNA synthesis, resulting in DNA breakdown in bacterial cells [28, 29]; 2-quinoxaline carboxylic acid 1,4-dioxide, *tert-butyl-3-(2-quinoxalinyl* methylene)carbazate 1,4-dioxide, l-acetyl-2-(2-quinoxalinyl methylene) hydrazine 1,4-dioxide had good activity against S. *typhi* TA98 and TA100 [30].

11*H*-Indeno[1,2-*b*]quinoxalin-2-one is vastly used for the preparation of derivatives possessing a wide range of biological activity. Schiff bases obtained from the 11*H*-indeno[1,2-*b*]quinoxalin-2-one are highly cytotoxic and possess antiviral activity [31]. They found interaction with the activity of the compound against some strains of various species of bacteria and strains of fungi such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus,* and *Candida albicans* [32].

The compound has been tested for its toxic effect on *Salmonella typhimurium (S. typhi), Klebsiella pneumoniae (K. pneumoniae),* and *Bacillus subtilis (B. subtilis)*. It was found that the compound exhibited a significant reduction in the *S. typhi, K. pneumoniae,* and *B. subtilis* viability at 20 mM. Also, the compound was investigated in a DFT-theoretical study of the structural and spectral properties. Agreeably, the optimized geometry structure was in agreement with experimental X-ray data. Moreover, calculated ^{1}H- and ^{13}C-NMR spectral data of the optimized structure exhibited a decent agreement with their experimental spectral analysis.

2. Experimental

2.1. Tested compound and Bacteria

The compound (Figure 1) was synthesized as reported [33]. The tested compound was liquefied in 1 mL absolute dimethyl sulfoxide (DMSO) at 20 mM concentration. *S. typhi, K. pneumoniae,* and *B. subtilis* used in this study were resistant to Chloramphenicol, Ampicillin, and Streptomycin and sensitive to Gentamicin and Penicillin.

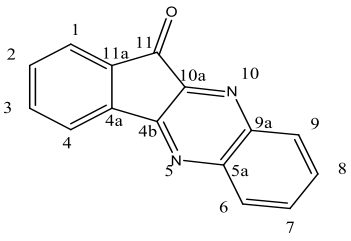


Figure 1. Chemical structure of the compound

2.2. Detecting the Antimicrobial Activity of the Tested Compound

In-vitro, the compound was screened for its antibacterial activity by agar well diffusion method [34-36]. Using the cup-plate agar diffusion method, determine the compound's antibacterial activity against *S. typhi, K. pneumoniae,* and *B. subtilis* [37]. Twenty mM concentration of the tested compound was liquefied in DMSO. Into sterilized Petri dishes, 20 ml of the LB agar was added. The agar was allowed to be set before each of these plates was individually infected with 10^7 CFU/mL of bacterial cultures and evenly dispersed throughout its whole surface. A sterile cork borer cut the 7 mm in diameter and removed the agar discs. 70 µL of the compound using a microliter pipette filled cups and allowed to diffuse at room temperature for one hour. The plates were then incubated for 24 hours in an upright posture at 37°C. The widths of the growth inhibition zones were measured after 24 hours of incubation, and the mean values were tabulated and compared to a 10% DMSO negative control. In this test, each assay was carried out three times.

2.3. Determination of MIC of the Tested Compound

The MIC of the chemical was determined using the procedures outlined in Clinical and Laboratory Standards [38]. To the previously autoclaved LB broth medium, various doses (0–20 mM) of the compound dissolved in DMSO were introduced separately. *S. typhi, K. pneumoniae*, and *B. subtilis* prepared LB cultures were used for the present test. An inoculum of 100 L seed culture of the examined organisms with roughly 10^7 colony-forming units (0.8 OD) was utilized to test the chemical. Following the preservation of respective blanks (culture and broth alone). At 37 °C overnight, every tube was incubated before having its optical density measured at 600 nm. Triplicate experiments were carried out.

2.4. Detection of Compound-Resistant Variants

Growth tests were carried out with or without a substance to assess the emergence of resistance in *S. typhi, K. pneumoniae*, and *B. subtilis* to the compound.

After centrifuging 20 mL of cells, re-suspended cells were placed in 2 mL of LB broth (Luria-Bertani broth). The inoculum density was adjusted to 10^8 CFU/mL. Different chemical concentrations (4, 3, 2, and 1 MIC) were introduced. All tubes were incubated at 37 °C, and every day for a week, the absorbance at 600 nm was measured [39, 40].

2.5. Computational studies

Gaussian 09W program software was used to operate molecular dynamic calculations [41]. The function BLY3P/6-311++G(d,p) was applied to optimize the geometry of the structures [42-44]. The solvent used to calculate ^{1}H and ^{13}C NMR chemical shifts were DMSO. The method of calculation applied was the gauge-invariant atomic orbital (GIAO) method [45]. The NMR spectral data were assigned visually by Gauss View program [46].

3. Results and Discussion

3.1. Detecting the Antimicrobial Activity of the Tested Compound

The tested compound demonstrated a broad range of antibacterial action against *S. typhi*, *K. pneumoniae*, and *B. subtilis*, while the width of the inhibition zone was measured at 27, 30, and 33 mm in (Figure 2), respectively. Consequently, the compound has considerable antimicrobial activity. Around the well-containing DMSO (control) no growth inhibition was observed. The antibacterial activity of the investigated compounds was compared to that of conventional antibiotics such as Gentamycin and Penicillin (data not shown). The antibiotics exhibited antimicrobial activities that were lower than the activity of the compound.

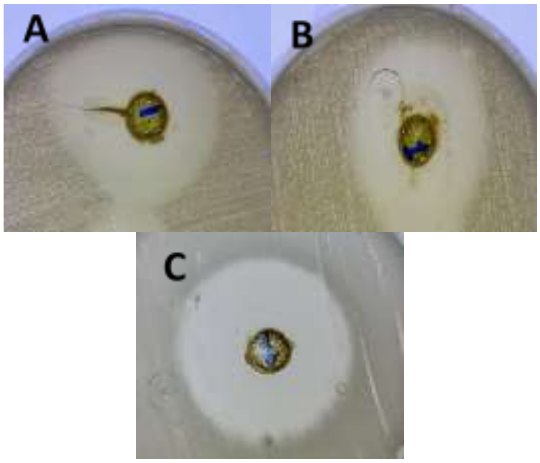


Figure 2. Susceptibility of *S. typhi* (A), *K. pneumoniae* (B), *and B. subtilis* (C) towards 11*H*-indeno[1,2-*b*]quinoxaline-11-one.

3.2. Determination of MIC of the Tested Compound

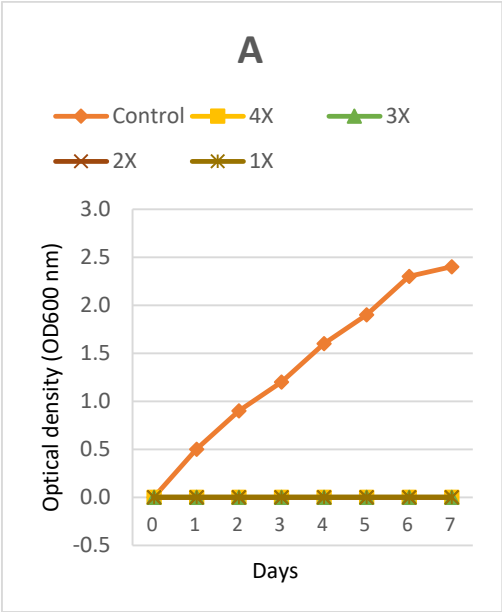
The tested substance's MIC value was recorded at a concentration low enough to prevent microbiological growth (Table 1). The drug proved efficient, with MIC values of 10, 20, and 20 mM against *S. typhi*, *K. pneumoniae*, and *B. subtilis*, respectively.

Table 1. Determination of the tested compound's MBC (mM) and MIC (mM) against bacteria.

Bacteria	MIC (mM)	MBC (mM)
S. typhi	10	10
K. pneumoniae	20	20
B. subtilis	20	20

3.3. Detection of Compound-Resistant Variants

With all doses, the substance could successfully stop *S. typhi* and *B. subtilis* development after seven days. On the other hand, *K. pneumoniae* acquired one case of resistance after five days of incubation with a one-fold concentration (1 MIC) (Figure 3).



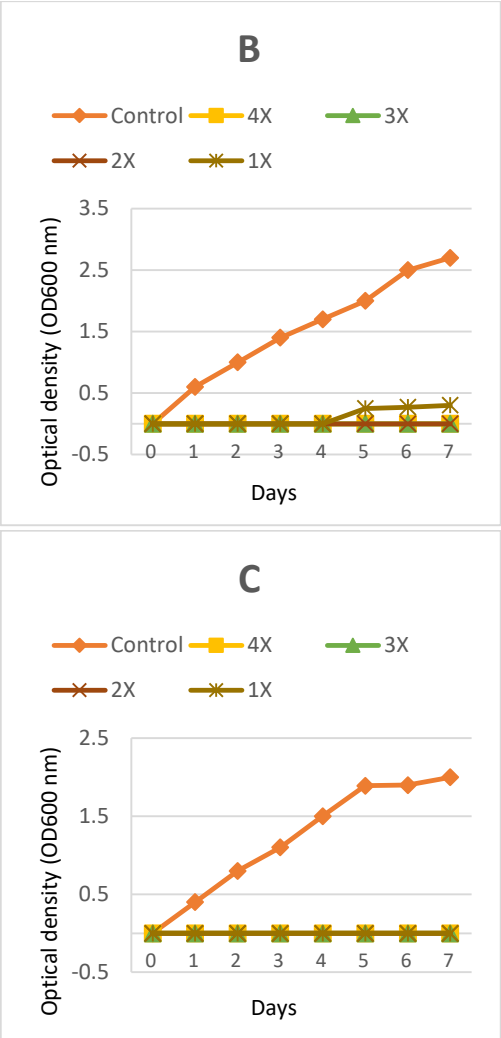


Figure 3. Development of resistance of the tested compound at (4, 3, 2, and 1× MIC) during 7 days of incubation. (A) *S. typhi*; (B) *K. pneumoniae*; (C) *B. subtilis.*

3.4. Computational study

The quantum chemical calculation study of 11*H*-indeno[1,2-*b*]quinoxalin-11-one at the DFT/B3LYP level was performed to explore the structural and spectral properties.

3.5. Geometrical parameters

The DFT optimized geometry for compound 11*H*-indeno[1,2-*b*]quinoxalin-11-one (Figure 4) was found to have bond length and bond angles very close to the corresponding x-ray reported values with deviation ~0.1Å and 0.7° [47], as shown in (Table 2). Meanwhile, the dihedral angle data showed that its structure was planar with little deviation, i.e., C4a-C11a-C11-O and O-C11-C10a-N10 were 175.12 and 3.70 while their x-ray values were 179.99 and 0.02 (Table 2).

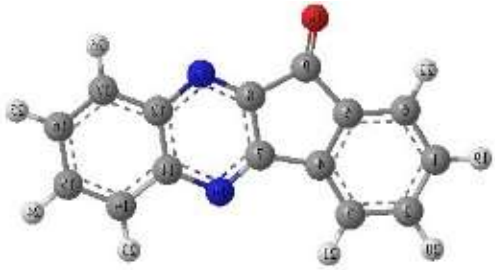


Figure 4. DFT optimized structures for the compound.

Table 2. X-ray and calculated bond length, bond angle, dihedral angle for the compound.

Bond Length	x-ray	Modelling	Bond Angels	x-ray	modelling	Dihedral Angels	x-ray	modelling
C2-C1	1.40	1.40	C1-C2-C3	121.01	120.64	C11a-C4a-C4-C3	-0.87	0.00
C3-C2	1.40	1.40	C2-C3-C4	121.36	121.23	C11a-C4a-C4-H4	-178.85	-180.00
C4-C3	1.40	1.40	C3-C4-C4a	117.56	118.08	C2-C1-C11a-C4a	-0.30	0.00
C4a-C4	1.39	1.39	C1-C11a-C4a	121.21	121.14	C3-C2-C1-C11a	-0.68	0.00
C4a-C11a	1.41	1.41	C11a-C4a-C4	121.24	120.71	C3-C2-C1-H1	179.56	-180.00
C1-C11a	1.39	1.39	C11a-C1-C2	117.61	118.22	C4a-C4-C3-C2	-0.11	0.00
C1-H1	0.97	1.08	C11a-C1-H1	125.71	120.30	C4a-C4-C3-H3	-178.11	-180.00
C2-H2	0.98	1.08	H1-C1-C2	116.69	121.48	C4-C3-C2-C1	0.90	0.00
C3-H3	0.98	1.08	H2-C2-C1	122.39	119.87	C4-C3-C2-H2	-175.46	-180.00
C4-H4	0.97	1.08	H2-C2-C3	116.49	119.50	C4-C4a-C11a-C1	1.10	0.00
C4b-C4a	1.48	1.47	H3-C3-C2	119.80	119.30	H1-C1-C11a-C11	-1.87	0.02
C4b-C10a	1.43	1.43	H3-C3-C4	118.81	119.47	H1-C1-C11a-C4a	179.43	180.00
C11-C10a	1.51	1.51	H4-C4-C3	119.94	121.27	H2-C2-C1-C11a	175.46	180.00
C11a-C11	1.49	1.50	H4-C4-C4a	122.47	120.66	H2-C2-C1-H1	-4.29	0.00
O-C11	1.22	1.21	C11-C11a-C1	128.91	128.83	H3-C3-C2-C1	178.88	180.00
N5-C4b	1.31	1.31	C11-C10a-N10	127.17	127.72	H3-C3-C2-H2	2.52	0.00
N5-C5a	1.38	1.37	C11-C11a-C4a	109.87	110.03	H4-C4-C3-C2	177.92	180.00
C9a-C5a	1.43	1.44	C11a-C11-O	128.22	127.79	H4-C4-C3-H3	-0.08	0.00
N10-C9a	1.38	1.37	C10a-C11-O	126.88	127.86	C4-C4a-C11a-C11	-177.82	179.98
N10-C10a	1.31	1.30	C11a-C4a-C4b	108.51	108.68	C1-C11a-C11-C10a	178.69	179.98
C6-C5a	1.41	1.41	C11-C10a-C4b	108.39	108.48	C1-C11a-C11-O	-3.69	0.00
C7-C6	1.38	1.38	C10a-C11-C11a	104.85	104.34	C2-C1-C11a-C11	178.40	-179.98
C8-C7	1.41	1.41	C10a-C4b-C4a	108.32	108.47	C4b-C4a-C11a-C1	-179.14	-179.99
C9-C8	1.38	1.38	C4-C4a-C4b	130.25	130.62	C10a-C4b-C4a-C4	179.19	-179.98
C9a-C9	1.42	1.41	C4a-C4b-N5	128.27	128.26	C4b-C4a-C4-C3	179.43	179.98
C6-H6	0.99	1.08	C4b-C10a-N10	124.43	123.80	C4b-C4a-C4-H4	1.46	-0.02
C7-H7	0.98	1.08	C10a-C4b-N5	123.41	123.27	C10a-C4b-C4a-C11a	-0.53	0.01
C8-H8	0.96	1.08	C10a-N10-C9a	113.99	114.85	C11a-C11-C10a-C4b	2.13	0.00
C9-H9	1.00	1.08	C4b-N5-C5a	113.99	114.81	C4a-C11a-C11-C10a	-2.50	0.00
			C5a-C9a-N10	121.84	121.52	C4a-C11a-C11-O	175.12	-179.99
			C6-C5a-N5	118.61	119.25	C4a-C4b-C10a-C11	-1.05	-0.01
			C9a-C5a-N5	122.32	121.74	C4b-C4a-C11a-C11	1.93	0.00
			C9-C9a-N10	118.60	119.03	O-C11-C10a-C4b	-175.53	179.99
			C5a-C6-C7	120.22	120.20	N5-C4b-C4a-C11a	179.22	-179.99

	C5a-C9a-C9	119.55	119.45	O-C11-C10a-N10	3.70	-0.02
	C6-C5a-C9a	119.07	119.01	N5-C4b-C10a-C11	179.18	179.99
	H6-C6-C5a	112.92	117.99	C4a-C4b-C10a-N10	179.70	-180.00
	H6-C6-C7	126.86	121.80	C11a-C11-C10a-N10	-178.64	180.00
	H7-C7-C6	124.11	119.79	C9a-N10-C10a-C11	-178.68	-179.99
	H7-C7-C8	115.19	119.44	C5a-N5-C4b-C4a	179.53	180.00
	H8-C8-C7	118.15	119.57	N5-C4b-C4a-C4	-1.05	0.03
	H8-C8-C9	121.32	120.02	C9a-N10-C10a-C4b	0.43	0.00
	H9-C9-C8	118.26	122.01	C5a-N5-C4b-C10a	-0.75	0.00
	H9-C9-C9a	121.71	117.83	N10-C9a-C5a-N5	-0.91	0.00
	C6-C7-C8	120.66	120.76	N5-C4b-C10a-N10	-0.07	-0.01
	C7-C8-C9	120.54	120.41	C4b-N5-C5a-C9a	1.21	0.00
	C8-C9-C9a	119.97	120.16	C10a-N10-C9a-C5a	0.04	0.00
				N10-C9a-C5a-C6	178.63	180.00
				H6-C6-C5a-N5	0.52	0.00
				N10-C9a-C9-C8	-178.20	-180.00
				N10-C9a-C9-H9	-1.29	0.00
				C9-C9a-C5a-N5	-179.88	180.00
				C7-C6-C5a-N5	179.43	-180.00
				C4b-N5-C5a-C6	-178.34	180.00
				C10a-N10-C9a-C9	179.02	180.00
				C5a-C9a-C9-C8	0.80	0.00
				C5a-C9a-C9-H9	177.71	180.00
				C7-C6-C5a-C9a	-0.14	0.00
				C8-C7-C6-C5a	0.16	0.00
				C8-C7-C6-H6	178.90	-180.00
				C9a-C9-C8-C7	-0.79	0.00
				C9a-C9-C8-H8	179.13	-180.00
				C9-C8-C7-C6	0.31	0.00
				C9-C8-C7-H7	178.03	180.00
				C9-C9a-C5a-C6	-0.34	0.00
				H6-C6-C5a-C9a	-179.04	180.00
				H7-C7-C6-C5a	-177.35	180.00
				H7-C7-C6-H6	1.39	0.00
				H8-C8-C7-C6	-179.61	180.00
				H8-C8-C7-H7	-1.89	0.00
				H9-C9-C8-C7	-177.81	-180.00
				H9-C9-C8-H8	2.11	0.00

3.6. NMR spectra

The comparison results between calculated ^{1}H- and ^{13}C-NMR spectral data for the compound and experimental data are shown in (Table 3). The ^{1}H-NMR DFT calculated protons displayed very good agreement with the experimental data showing 0.21-0.43 ppm deviation. For example, calculated 1-H and 6-H were present at 8.17 and 7.84 ppm whereas their experimental signals were detected at 8.51 and 8.08 ppm, respectively (Figure 5). ^{13}C-NMR DFT calculated signals showed in range 125.78–197.29 ppm exhibiting average differences 2.32-7.98 ppm. While their experimental signals observed at 122.29–189.31ppm (Figure 6).

Table 3. The DFT calculated and experimental ^{1}H-NMR and ^{13}C-NMR data of the compound.

H-atoms	Exp	DFT	C-atoms	Exp	DFT
6-H	8.17	8.51	11-C	189.31	197.29
9-H	8.13	8.51	4b-C	156.45	161.52
4-H	8.08	8.38	10a-C	149.84	152.16
7-H	7.92	8.35	5a-C	142.12	146.2
8-H	7.87	8.22	9a-C	141.82	146.03
3-H	7.87	8.21	4a-C	140.96	145.93
1-H	7.84	8.08	3-C	136.94	142.45
2-H	7.69	7.99	11a-C	136.64	140.79
			7-C	132.77	137.92
			2-C	132.44	136.99
			9-C	130.96	136.75
			8-C	130.36	134.78
			6-C	129.36	134.24
			1-C	124.22	127.92
			4-C	122.29	125.78

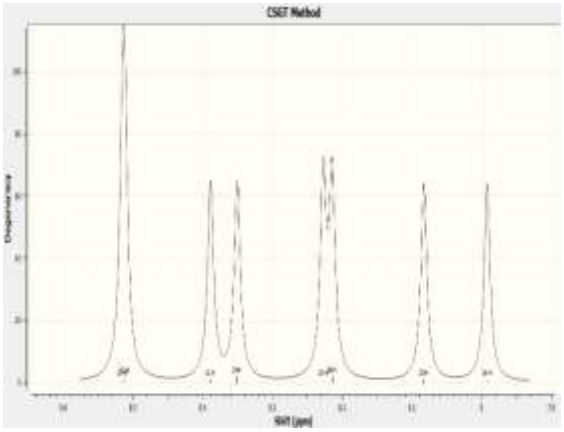


Figure 5. Calculated ^{1}HNMR spectra for the compound

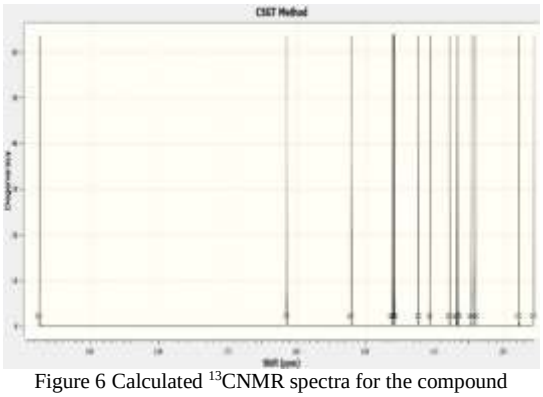


Figure 6 Calculated ^{13}CNMR spectra for the compound

4. Conclusion

This research examined the antibacterial activities of 11*H*-indeno[1,2-*b*]quinoxaline-11-one. In vitro antibacterial activities of the compound were carried out against the culture of bacteria. The compound showed remarkable antibacterial activity. The comparison between calculated optimized structures geometry and spectral data (^{1}HNMR and ^{13}CNMR) with the experimental data displayed acceptable agreement.

5. References

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