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SYNTHESIS, SPECTRAL ANALYSIS AND BIOLOGICAL STUDIES ON 6-METHYL-7, 9-DIPHENYL-1, 4, 8-TRIAZASPIRO [4.5] DECANE

N.Manivannan[1], B.Elanchezhian[1], G.Selvanathan[1], K.Pandiarajan[2]

[1]Department of Chemistry, A.V.C College (Autonomous), Mannampandal, Mayiladuthurai, Tamilnadu
[2]Department of chemistry, Annamalai University, Annamalai Nagar, Tamilnadu

E-mail of corresponding author:
chembell_2005@rediffmail.com

ABSTRACT

The title of the molecule 6-methyl-7, 9-diphenyl-1, 4, 8-triazaspiro (4.5) decane is synthesized from the condensation reaction of ethylenediamine with (t)3-methyl-r(2),c(6)-diphenyl Piperidin-4-one. The product is evidenced by IR, ^{1}H NMR and ^{13}C NMR spectra. In the ^{1}H NMR study, it is found that the ABX spin system belongs to this molecule.The second-order analysis based on the method developed by Bernstein etal is used for ABX system.The parameters of coupling constants ($^{3}J_{9a,\ 10a}$ = 11.46 Hz and $^{3}J_{9a,\ 10e}$ =2.46Hz) and origin of chemical shifts (υ_A=1.77 and υ_B=1.86ppm) are calculated using ABX system from which also found that the title molecule adopt chair conformation. These results also confirm that the substitutions of Phenyl and methyl groups are in equatorial position of the six memberd piperidine ring of the title compound. The Biological (antibacterial and antifungal) activities of title compound have also been studied.

Keywords: Ethylenediamine, triazaspiro decane, Spiro, ABX, second-order, conformation, antibacterial, antifungal.

INTRODUCTION

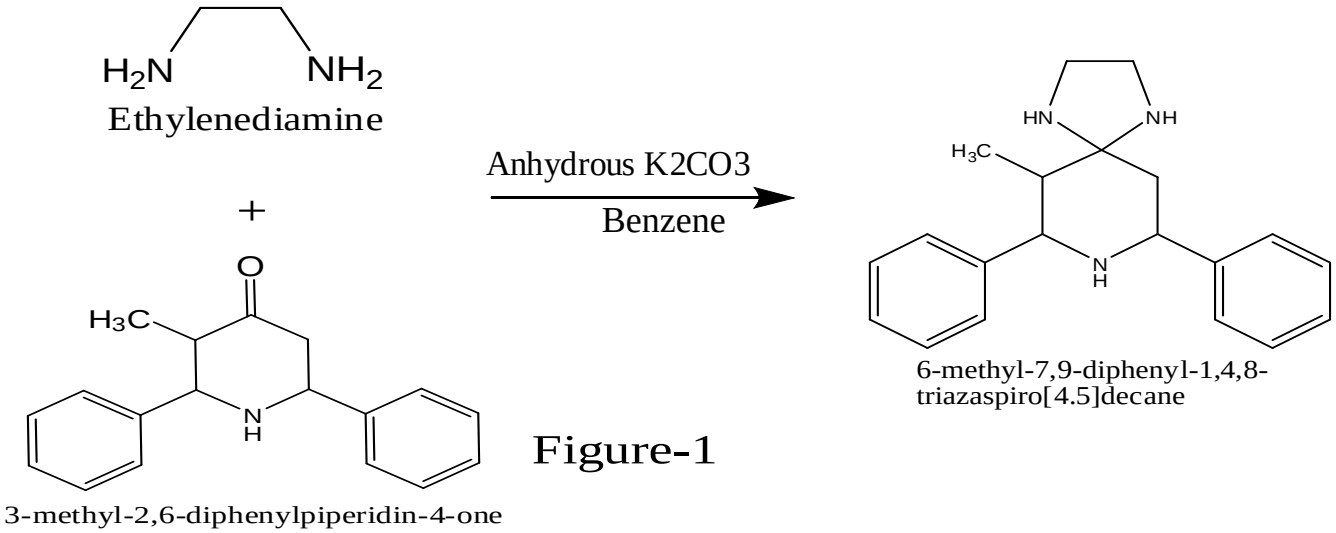
The 6-methyl-7, 9-diphenyl-1, 4, 8-triazaspiro (4.5) decane is a ABX spin systems which have attracted considerable interest from the chemistry community as they represent promising building blocks with potential applications in the field of pharmaceuticals.Artificial product synthesis also diverse key synthetic catalyst. The 6-methyl-7, 9-diphenyl-1, 4, 8-triazaspiro (4.5) decane can be consist of symmetrical substitutions of phenyl with piperidine ring as diphenyl piperidine extended with methyl group. A linear fused or bridged systems, spirocyclic core systems are less common in known drugs and natural products. Some examples of spirocyclic natural product contain ring nitrogen include cephalotaxine, halichlorine, histrionicotoxin, triazaspiro and manzamine. In the area of nonnatural compounds spirocyclic nitrogen-containing systems are fashioned into compounds displaying interesting biological activities. Such spiro scaffolds can be classified according to the ring size of the heterocyclic ring.

The interplay of the structural variation is resulted in compound ranging from the inactive to the extraordinary potent belongs to the above statement.We expect that,

fusing imidazolidine ring and piperidine ring cause new biological activity. NMR spectral studies of heterocyclic compounds have helped in understanding the influence of electronic and conformational effects on chemical shifts and coupling constants .The conformational studies of heterocyclic compounds also have been made using NMR spectra [1-2]. Several NMR spectral studies [3-5], have been reported on 2,6-diaryl piperidine derivatives. The reaction of 1,2 diamines with carbonyl compounds provide Imidazolidines. The imidazolidine is the guiding principle of bond formation occurring via reaction of a binucleophillic component with an electron-deficient bielectrophilic counter.The five membered heterocyclic belong with two heteroatom at 1 and 3 position from the reaction of a 1,4-binucleophile with a 1,1-bielectrophile [6-11]. Generally, 1,4 binucleophiles are encountered Ethylenediamine , Ethylene glycol and 1,1- Bielectrophile. Many of the common reagents are used as RCOR, RCHO, Carboxylic chlorides and phosgene. In the absence of reagent, the five membered rings are coupled by one carbon unit with piperidine ring. Because of this coupling, the piperidine ring is gained antifungal and antibacterial activities.

2. Experimental Methods

The reactant materials such as ammonium acetate, ethylmethylketone, benzaldehyde, benzene, potassium carbonate and petroleum-ether (60-80^{o} C) are purchased from Sigma Aldrich chemicals, U.S.A. which is of spectroscopic grade is used for recording the spectra as such without any further purification. The product 6-methyl-7,9-diphenyl-1,4,8-triazaspiro (4.5) decane is prepared by a Stirred solution of(AR)Ethylenediamine (15 mmol) in (AR) Benzene (45 ml), t(3)- methyl r(2) and c(6)-diphenylpiperidin-4-one (15 mmol). The reaction flask is fitted with a Dean - stark water separator.Which is charged with anhydrous K_2CO_3 and the solution is gently refluxed for 14 hrs and cooled room temperature [12-14]**.** The yellow oily solution is obtained and then (AR) petroleum ether (60-80 ^{o}c) was added. The product is separated as pale yellow solid. It is recrystallized from Benzene, petroleum - ether. After recrystallization the melting point is observed at 71-72 ^{o}C. (Yield -90%). The reaction scheme of the title molecule is given in figure 1.



3-methyl-2,6-diphenylpiperidin-4-one

6-methyl-7,9-diphenyl-1,4,8-triazaspiro[4.5]decane

Figure-1

The FT-IR spectrum of the compound is recorded in Bruker IFS 66V spectrometer in the range of 4000-100cm^{-1}. The ^{1}HNMR spectral analysis is carried out using Bruker AMX-400 NMR spectrometer operating at frequency 400 MHZ using deutriated chloroform .The ^{13}CNMR spectral analysis is also carried out using Bruker AMX- 400 NMR spectrometer operating at frequency 100 MHZ.

2.1. Biological Activities:

About 0.03g of compound 6-methyl-7,9-diphenyl-1,4,8-triazaspiro(4.5) decane is dissolved in a suitable solvent such as (AR) ethanol, (AR) petroleum ether etc Muller Hinton Agar medium (MHA) – Antibacterial. Seaboard Dextrose Agar medium (SDA) Antifungal is Carried out Agar diffusion method.

3. Results and Discussion

Ethylenediamine is condensed [15] with t(3)-methyl-2,6-diphenylpiperidin-4-one and the product is carried out by scheme-1.The product was identified as 6-methyl -7,9-diphyl-1,4,8-triazaspiro (4.5) decane by IR, ^{1}H and ^{13}C NMR spectral studies. The structure of compound–II (6-methyl-7,9-diphyl-1,4,8-triazaspiro(4.5)decane) is shown in Figure 2.

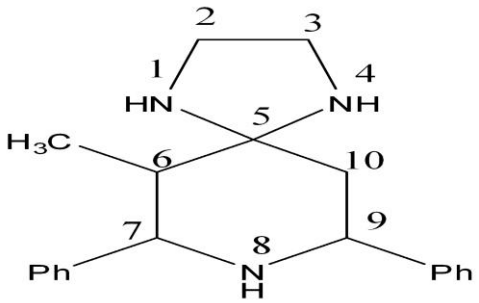


figure 2

The IR spectrum of compound (I) shown in Figure 3, a strong band at 1701 cm^{-1} is observed. But in the compound (II) that the carbonyl group weak band at 1701 cm^{-1} is observed .

IR spectrum of compound (II) is shown in Figure 4.

3273 cm^{-1} (NH, str), 3060 cm^{-1} (Aromatic CH stretching), 2967(Aliphatic CH stretching), 1437 & 1375 cm^{-1} (Anti-symmetric deformation of CH in C- CH_3), 1113cm^{-1}

(C- N stretching), 702cm^{-1} C_6 H_5 – ring deformation).

^{1}H NMR spectral data's of title compound (II)is presented in the table 1&2 and the corresponding spectrum is given in figure 5&6.H_{9a} – δ4.10 (H,dd), H_{7a} - δ 3.70 (1H.d), H_{10a} - δ 1.75 (1H,t), H_{10e} – δ 1.89(H, dd) H_{6a}- δ 2.04 (H ,m), CH_3 - δ 0.64 (3H,d),C_2 and C_3 (CH_2)- δ 3.11 - δ 2.85 (4H.m), Aromatic- δ 7.15 - δ 7.55 (10H, m), N-H- δ 1.63 (3H- broad Singlet).

^{13}C NMR spectral data's of title compound (II) is given in table-3 and the corresponding figure is presented in the figure 7. C_5 - δ 80.28, C_7 - δ 66.12, C_9- δ 59.10, C_2- δ 45.80,C_3- δ 46.0, C_6- δ 46.5, C_{10}- δ 47.85, CH3- δ 10.48, aromatic ipso δ 144.7, δ 143.7, ortho- δ 126.9, δ 128.1, meta- δ 128.38, δ 128.32, Para- δ 127.1, δ 127.4.ppm.

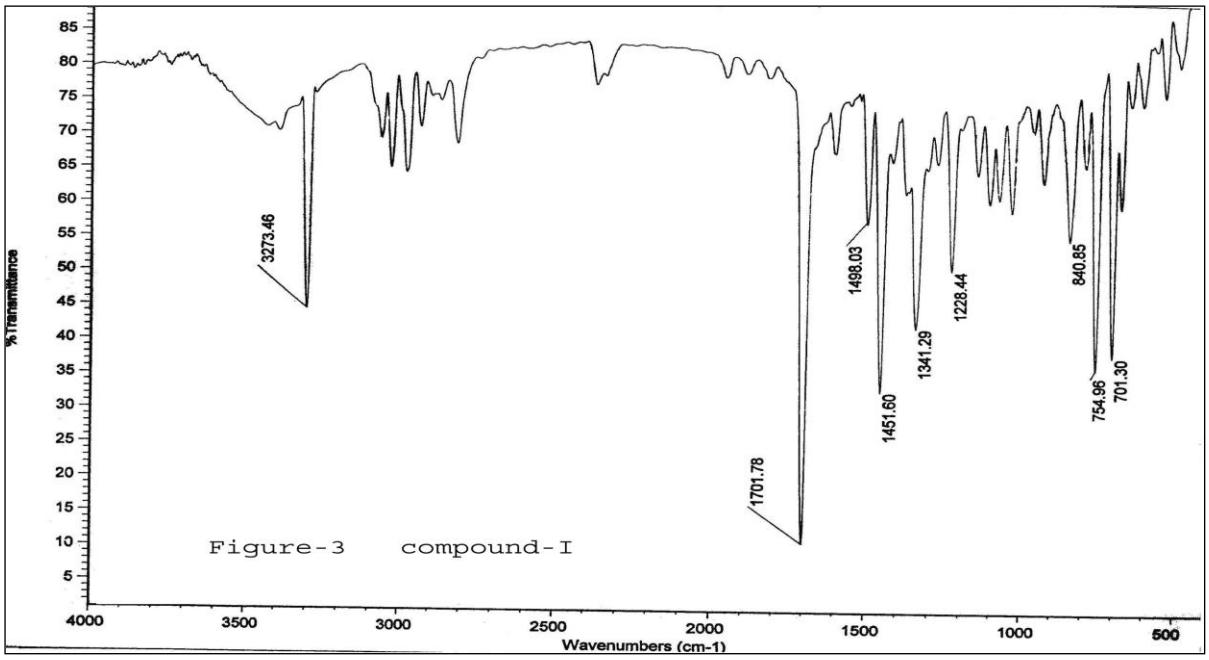


Figure-3 compound-I

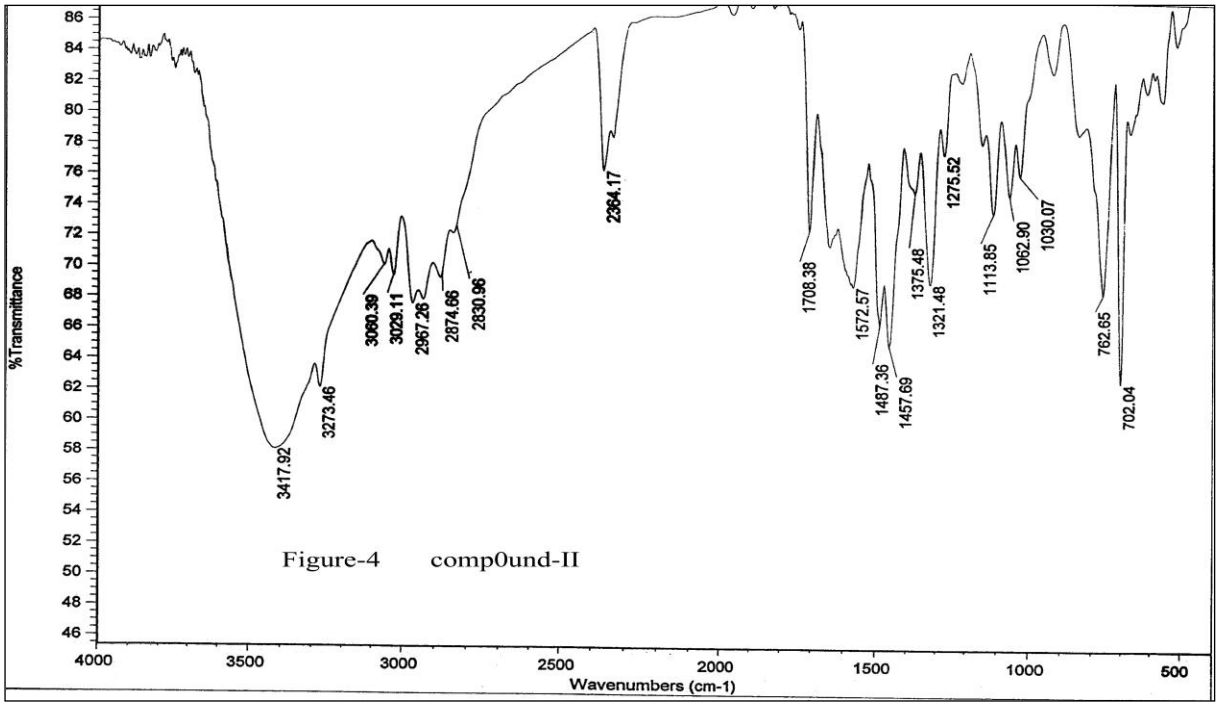


Figure-4 comp0und-II

Table-1: ^{1}H NMR spectral data's of 6-methyl-7,9-diphenyl-1,4,8-triazaspiro[4.5]decane

Protons	Chemical shifts(ppm)	Multiplicity
H_{9a}	4.10(1H)	Double-doublet
H_{7a}	3.70(1H)	Doublet
H_{10a}	1.75(1H)	Triplet
H_{10e}	1.89(1H)	Double-doublet
H_{6a}	2.04(1H)	Multiplet
H_{CH3}	0.64(3H)	Doublet
C-2 and C-3(CH_2)	3.11-2.85(4H)	Multiplet
Aromatic	7.15-7.55(10H)	Multiplet
N-H	1.63(3H)	Broad singlet

Table 2: Coupling constant of 6-methyl-7,9-diphenyl-1,4,8-triazaspiro[4.5]decane.

Protons	Chemical shift(δ)	Coupling constant Hz
$^{3}JCH_{3}$, 6a	0.64	6.8
$^{3}J_{7a},6_{a}$	3.70	10.3
$^{3}J_{9a}$, 10_{a}	1.75	11.46
$^{3}J_{9a}$, 10_{e}	1.89	2.46

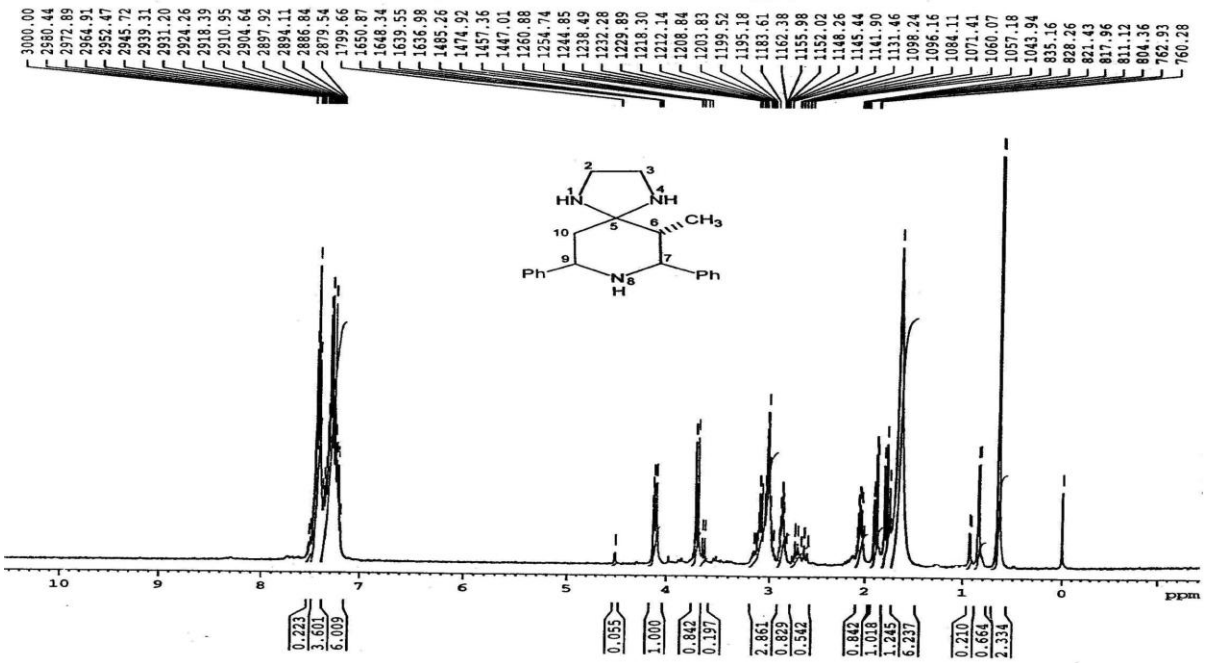


Figure-5 ^{1}H-NMR spectra of compound II

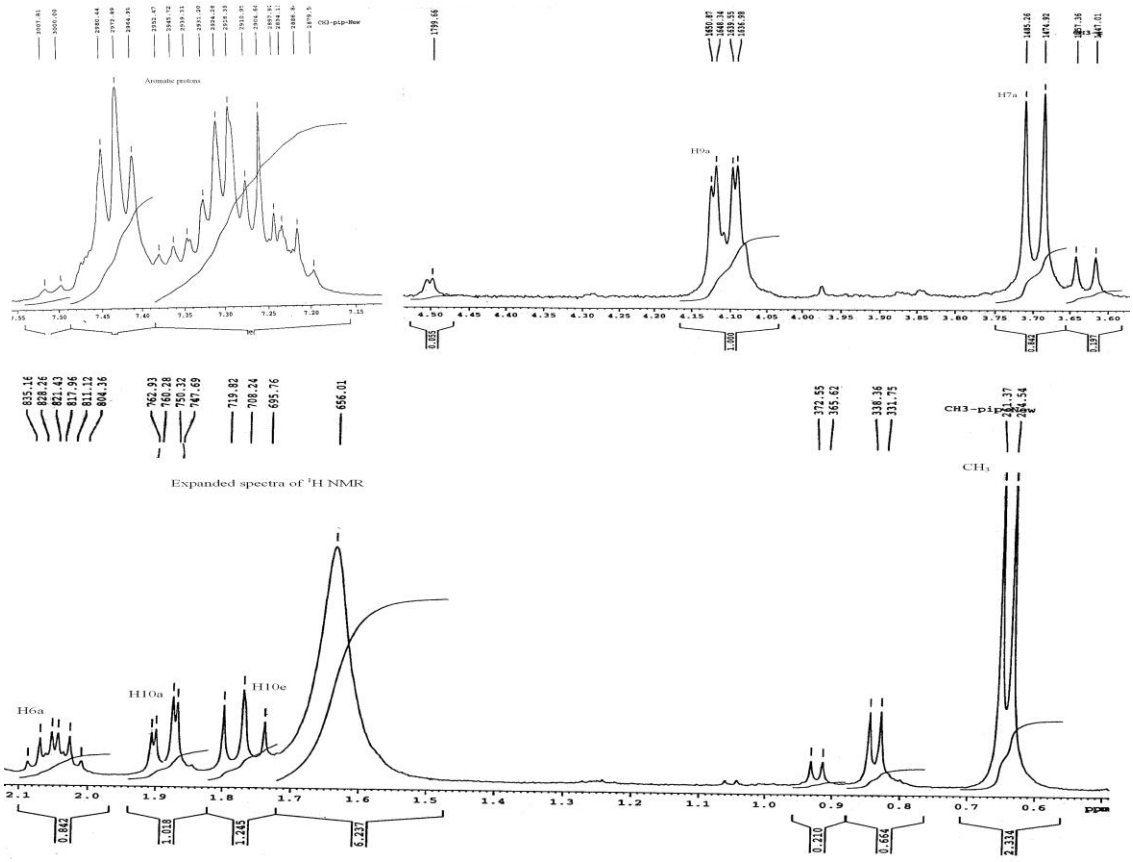


Figure-6: Expanded spectrum

Table-3: ^{13}C NMR spectral data's of 6-methyl-7,9-diphenyl-1,4,8 triazaspiro[4.5]decane

Carbon assignment	Chemical shifts(ppm)
C(5)	80.28
C(7)	66.12
C(9)	59.10
C(2),C(3),C(6) and C(10)	45.80,46.0,46.5,47.85
CH_3	10.48
Aromatic	
Ipso	144.70,143.7
Ortho	126.9,128.1
Meta	128.38,128.32
Para	127.1,127.4

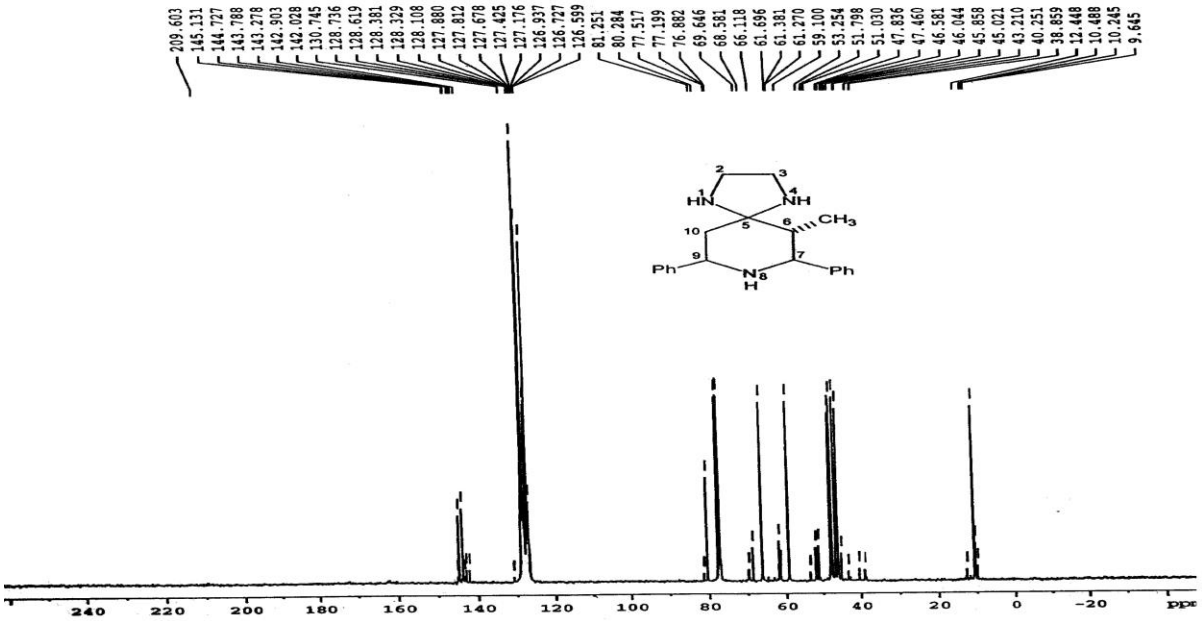


Figure 7 : 13C NMR Spectra of compound -II

AMX Calculation:

The spacing between centre of the signals are 10_a & 10_e is 40 HZ. The coupling constant between them is obtained from the signal 10_e as 12Hz. The value $\Delta\upsilon/J$ is much less than 6 Hence protons H_{9a}, 10_a and 10_e from an ABX system [16-18]. The coupling constant J_{AB} and the chemical shift of proton X can be directly calculated from the observed spectral data. The actual value of J_{AX} will be higher than that form the spectrum. The real value of J_{BX} will be less than that obtained from the spectrum. Let υ_A & υ_B are actual chemical shifts of proton A and B in Hz. Let υ'_A & υ'_B centers of the observed signal from protons A & B actual value of J_{Ax} & J_{Bx} are calculated as follows.

$$(\upsilon'_B - \upsilon'_A)^2 = (\upsilon_B - \upsilon_A)^2 + J_{AB}^2$$

$$(\upsilon_B - \upsilon_A)^2 = (755.30\text{-}707.92)^2 - 12.65^2$$

$$= 47.51^2 - 12.65^2$$

$$(\upsilon_B - \upsilon_A)^2 = 2097.18 \;;$$

$$(\upsilon_B - \upsilon_A) = 45.89$$

$$(J_{AX} - J_{BX})_{(Real)} = (J_{AX} - J_{BX})_{(observed)} \times \frac{\upsilon'_B - \upsilon'_A}{\upsilon_B - \upsilon_A}$$

$$= 11.3 - 2.65 \times \frac{47.51}{45.79}$$

$$= 8.65 \times 1.03 = 8.97 \text{ Hz}$$

$$(J_{Ax} - J_{Bx})_{(Real)} = 8.97 \text{ Hz}$$

$$J_{Ax} + J_{Bx} = 13.95 \text{ Hz}$$

$$J_{Ax} + J_{Bx} + J_{Ax} - J_{Bx} = 13.95 + 8.97 = (22.92)$$

$$2J_{Ax} = 22.92 \text{ Hz}$$

$$J_{Ax} = 11.46 \text{ Hz}$$

$$(J_{Ax} + J_{Bx}) - (J_{Ax} - J_{Bx}) = 13.95 - 8.97 = 4.98 \text{ Hz}$$

$$2J_{Bx} = 4.98 \text{ Hz}$$

$$J_{Bx} = 2.49 \text{Hz}$$

$$\upsilon'_B - \upsilon'_A = 47.51 \text{Hz}$$

$$\upsilon_B - \upsilon_A = 45.79 \text{Hz}$$

$$(\upsilon'_B - \upsilon'_A) - (\upsilon_B - \upsilon_A) = 47.51 - 45.79 \text{Hz}$$

$$\upsilon'_B - \upsilon'_A - \upsilon_B + \upsilon_A = 1.72 \text{ Hz}$$

$$(\upsilon'_B - \upsilon_B) + (\upsilon_A - \upsilon'_A) = 1.72 \text{ Hz}$$

Where $(\upsilon'_B - \upsilon_B) = \upsilon_A - \upsilon'_A$

$$2(\upsilon'_B - \upsilon_B) = 1.72 \text{ Hz}$$

$$(\upsilon'_B - \upsilon_B) = 0.86 \text{ Hz}$$

$$\upsilon_B = \upsilon'_B - 0.86 \text{ Hz}$$

$$= 755.30 - 0.86$$

$$\upsilon_B = 754.44 \text{ Hz} \div 400$$

$$\upsilon_B = 1.86 \text{ ppm}$$

$$2(\upsilon_A - \upsilon'_A) = 1.72 \text{ Hz}$$

$$\upsilon_A - \upsilon'_A = 0.86 \text{ Hz}$$

$$\upsilon_A = 0.86 + \upsilon'_A$$

$$= 0.86 + 707.92$$

$$= 708.78 \text{Hz} \div 400$$

$$\upsilon_A = 1.77 \text{ ppm.}$$

3.1. Conformation of the piperidine ring in Compound - II

The coupling constant are ^3J9_a, 10_a is 11.46 and ^3J6_a, 7_a is 10.3 Hz. The coupling constant ^3J9_a, 10_e is 2.46 HZ. These values are indicated that the piperidine ring in the title of compound adopts a chair conformation [19] as shown in Figure 8& 8(A, B&C)

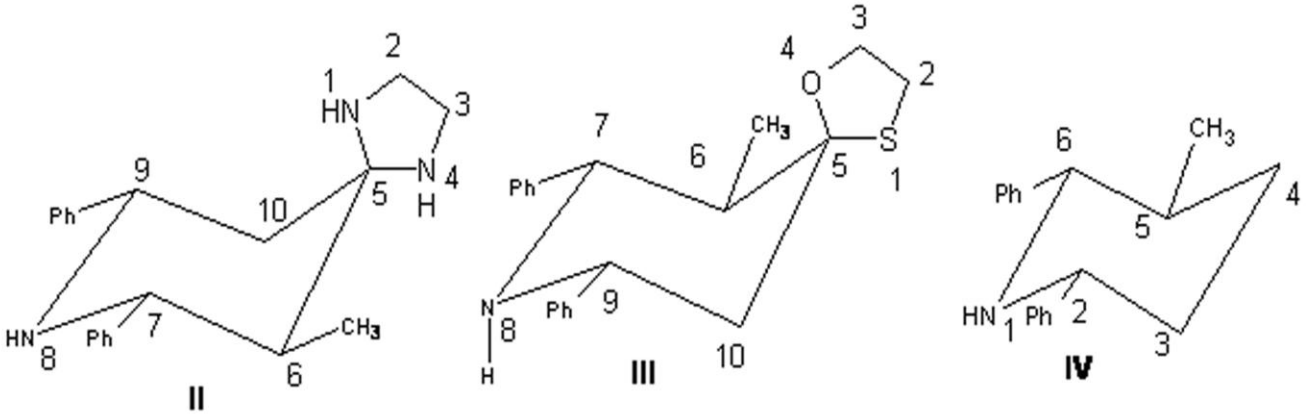


Figure 8: Chair conformation structure of title molecule(II) and compared molecule(III&IV)

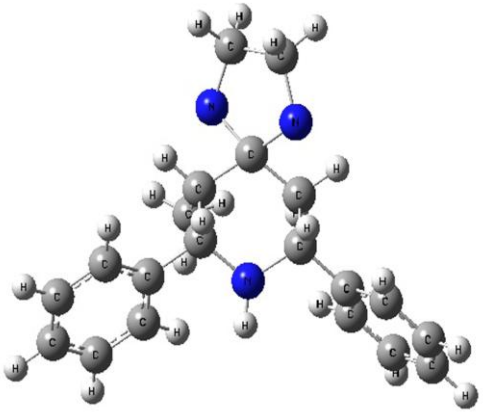


Figure 8 A

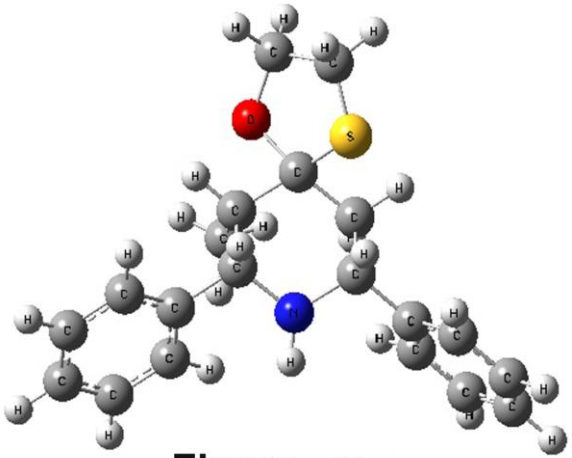


Figure .8B

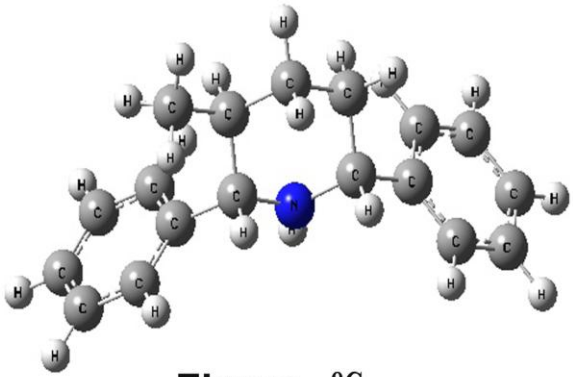


Figure .8C

It is of interest to compare the ^{1}HNMR spectral data of compound-II with those of 6-methyl-7,9-diphenyl-8-aza-4-oxa-1-thio Spiro[4.5]decane [20] (III) and 3-methyl-r(2),c(6)-diphenylpiperidine(IV) [21-23].For such comparison the ^{1}H NMR spectral data of II,III and IV are given in Table-4 and 13C NMR spectral data of II,III and IV are given in Table-5.

II- 6-methyl-7,9-diphyl-1,4,8-triazaspiro(4.5)decane.
III- 6-methyl-7,9-diphenyl-8-aza-4-oxa-1-thio Spiro[4.5]decane.
IV- 3-methyl-r(2),c(6)-diphenylpiperidine.

^{1}H NMR chemical shifts (ppm) of Compound II, III and IV

Protons H_{7a} and H_{9a} are shifted to high frequency by 0.30 and 0.46 ppm in II than in IV. This is due to proximity interaction between the axial Nitrogen at C-5 and axial Hydrogen's H_{7a} and H_{9a}. A similar shift is observed in III .The methylene protons at C-2 and C-3 appear at 2.85 -3.11 ppm as a complex pattern in II. In III protons at C-2 appear at 2.99 ppm those at C-3 appear at 4.06 ppm. This is due to a grater electro negativity of oxygen than Nitrogen.

Benzylic carbons at C-7 and C-9 are shifted to lower frequency by 4.08 and 3.60 ppm in II than in IV this is due to the proximity interaction of the axial Nitrogen at C-5, which polarizes the C-H bonds at C-7 and C-9. A partial positive charge is accumulated on protons, which shifts the protons signal to higher frequency. A partial negative charge is accumulated on carbon, which shifts the carbon signal to lower frequency.

Table-4: The comparison of ^{1}H NMR spectral data's of Compound II,III and IV

Compound No.	H_{9a}	H_{7a}	H_{6a}	H_{10} Axial	H_{10} equatorial	CH_2 C-2	CH_2 C-3	N H	CH_3
II	4.10	3.70	2.04	1.75	1.89	2.85 – 3.11		1.63	0.64
III	4.14	3.80	2.07	2.07	2.40	2.99	4.06	1.76	0.80
IV	H_{6a} 3.80	H_{2a} 3.34	-	-	-	-	-	-	-

^{13}C NMR chemical shifts (ppm) of Compound II, III and IV

Benzylic carbons at C-7 and C-9 are shifted to lower frequency by 4.08 and 3.60 ppm in II than in IV this is due to the proximity interaction of the axial Nitrogen at C-5, which polarizes the C-H bonds at C-7 and C-9. A partial positive charge is accumulated on protons, which shifts the protons signal to higher frequency. A partial negative charge is acumulated on carbon, which shifts the carbon signal to lower frequency.

Table-5: The comparison of ^{13}C NMR spectral data's of Compound II,III and IV

Compound No.	C-7	C-9	C-5	C-6	C-10	C-2	C-3	CH_3
II	66.12	59.10	80.28	45.80	46.0	46.5	47.85	10.48
III	65.67	58.64	96.92	49.22	46.88	71.39	33.89	11.70
IV	C-2 70.2	C-6 62.7	35.0	37.5	35.6	-	-	18.7

Table 6: The results of biological characteristics of bacterial-organism

S. No	Name of organisms	Gram staining	Motility test	Iodole	MR	VP	Citrate	TSI	Urease	Catalase	Oxidase
1	E.coli	GNB	+	+	+	-	-	A/A	-	+	-
2	Pseudomonas aeruginosa	GNB	+	-	-	-	+	K/K	+	-	+
3	Salmonella Spp	GNB	+	-	+	-	+	K/A	-	+	-
4	Klebsiella pneumonia	GPB	-	-	-	+	+	A/A	+	+	-
5	Bacillus cereus	GPB	-	-	-	-	-	A	-	+	-

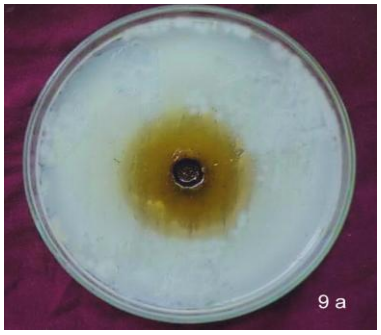
Table 7: The results of antibacterial activity

S. No	Name of organisms	Zone of Inhibition (mm)
1	E.coli	35
2	Pseudomonas aeruginnosa	40
3	Salmonella Spp	36
4	Klebsiella pneumonia	33
5	Bacillus cereus	31

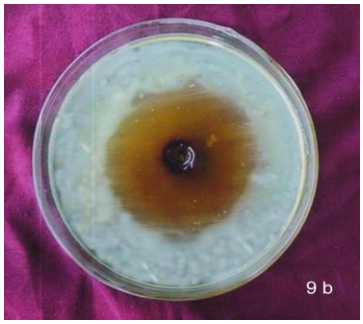
Table 8: The results of antifungal activity

S. No	Name of organisms	Zone of Inhibition (mm)
1	Alternaria spp	39
2	Fusarium Spp	36
3	Aspergillus niger	40
4	Aspergillus Flavus	37
5	Penicllium notatum	35

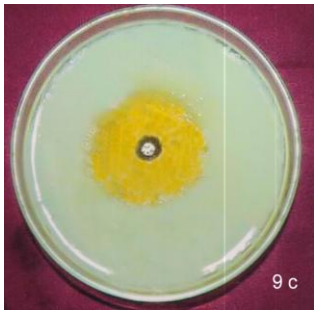
Figure 9: Antibacterial activity



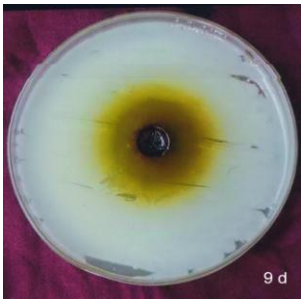
9a-Escherichia coli



9b-Pseudomonasaeruginnosa



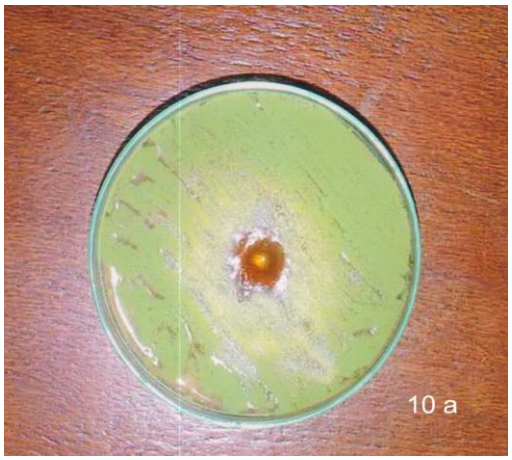
9c-Bacilluscereus



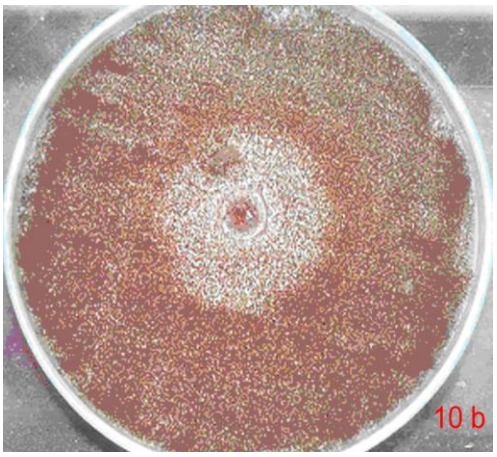
9d- salmonella spp

9e-Klebsiella pneumonia

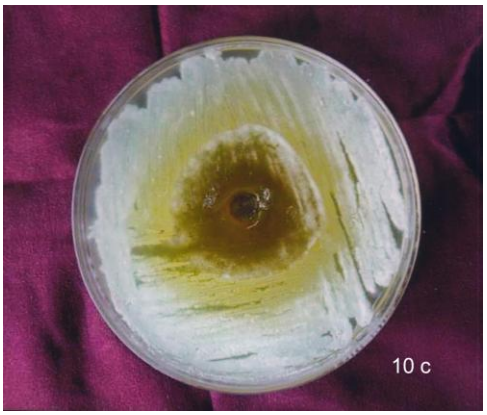
Figure 10: Antifungal activity



10a-Penicillium notatum

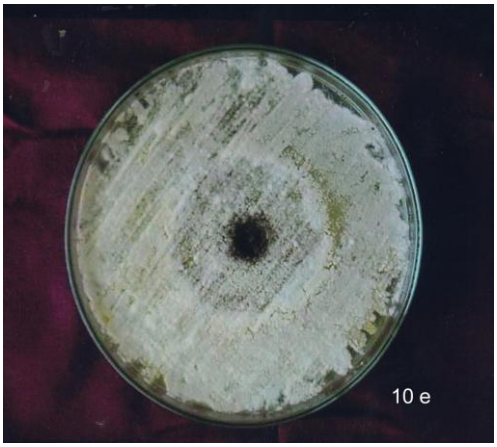


10b- Aspergillus Niger



10c-FusariumSpp

10d-Alternaria spp



10e-Aspergillus flavus

3.2. Biological activities of the compound

The compound 6-methyl-7,9-diphenyl-1,4,8-triazaspiro[4.5]decane is treated with selected bacterial [24] and fungal [25] organisms. The bacterial organisms are used in E.coli, Pseudomonas, aeruginnosa, bacillus cereus, salmonella spp and klebsiella pneumonia. The fungal organisms are used in alternaria spp, Fusarium Spp, Aspergillus Niger, Aspergillus flavus and penicillium notatum. MR. methyl red, VP-vogues proskauer,TSI – Triple sugar iron, GNB – Gram negative Bacilli,GPB – Gram positive bacilli, A/A – Acid slant bacilli, K/K- Alkaline salt alkaline bacilli ,K/A-alkaline salt Acid bacilli] .A-Acid, (+) positive, (-) – Negative.

The results of biological characteristics of bacterial-organism and antibacterial activity are presented in table 6 and 7 respectively and their photos are given in figure 9(9a,9b,9c,9d and 9d). The antifungal activity of title molecule is presented in table 8 and it's photos are given in figure 10(10a,10b,10c,10d and10e).

4. CONCLUSION

The 6-methyl-7, 9-diphenyl-1, 4, 8-triazaspiro (4.5) decane is synthesized from the condensation reaction of ethylenediamine with (t) 3-methyl- r (2), c (6) - diphenyl Piperidin-4-one. The IR, ^{1}HNMR and ^{13}C NMR spectral analyses have been carried out. The title compound is investigated for antibacterial and antifungal activities using MHA and SDA medium. The organisms like E. coli, pseudomonas aeruginnosa, salmonella spp klebsiella pneumonia and bacillus cereus for antibacterial and alternation spp, Fusarium spp, asparagus Niger, Aspergillums flavus, and penicillium notatum for antifungal. From the analyses it is found that the compound has good antibacterial and antifungal microbial activities. The vicinal coupling constants suggest that the piperidine ring of the compound has chair conformation. The methyl group at C-6 and two phenyl groups at C -7, C- 9 are equatorially oriented. Because of the wide pharmaceutical application, this compound can be used for the manufacturing the drugs.

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