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Synthesis, Spectral Studies and *in vitro* Antibacterial Evaluation of Triaza and Dioxa Aza Spiro Derivatives

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Six compounds 7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5]decane **1-6** have been synthesized by Mannich reaction and cyclo condensation. The structures and stereochemistry established by IR, NMR studies. The purities were checked by elemental analysis. The synthesized compounds **1-6** adopt chair conformation with equatorial orientation of the aryl groups. All the compounds were screened for their antibacterial activity against *Proteus mirabilis, Klebsiella oxytoca, Staphylococcus aureus* and *Salmonella paratyphi*. The compound **5** exhibited excellent *in vitro* antibacterial activity in all species.

Key Words: Triaza, Dioxa aza, Spiro, Chair conformation, Antibacterial.

INTRODUCTION

The Spiro cyclic systems containing one carbon atom common to two rings are structurally exciting[1]. The asymmetric point of the molecule due to the chiral spiro carbon is one of the important criterions of the biological activities. The occurrence of the spiro structure in various natural products also investigated in spiro compounds[2]. Spiro compounds are highly pronounced biological properties[3]. A lot of synthetic methodologies have been developed for constructing these spiro cycles; most of them prefer cyclo addition or condensation reactions[4-6].

The triaza and dioxa aza spiro compounds are adopt the chair conformation form with equatorial orientation of greater part of the substituents in six membered cyclic compounds. The conformational studies of piperidine have been evaluated by NMR studies[7,8]. All the compounds are screened for their antibacterial activity against *Proteus mirabilis, Klebsiella oxytoca, Staphylococcus aureus* and *Salmonella paratyphi*. Imidazolidines and dioxolanes are formed by the reaction of a 1,4-binucleophile with a 1,1-bielectrophile would lead to a five-membered heterocyclic with two hetero atom at 1 and 3 position[9,10]. The examples of commonly encountered 1,4-binucleophilies and 1,1-bielectrophiles given in Table-1.

EXPERIMENTAL

Acetone, ethyl methyl ketone, ammonium acetate, benzaldehyde and solvents were purchased from Sigma-Aldrich and were used as such. All the reagents and solvents were of laboratory grade.

TABLE-1
1,4-BINUCLEOPHILIES AND 1,1-BIELECTROPHILES

H_2N-$(CH_2)_2NH_2$	RCOR′
HS-$(CH_2)_2$SH	RCHO
$H_2N(CH_2)_2OH$	Carboxylic acid chlorides
$H_2N(CH_2)_2SH$	Phosgene
$HS(CH_2)_2OH$	Thiophosgene
o-Phenyl diamine	*ortho*-Esters

The melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on an AVATAR 330 FT-IR Thermo Nicolet spectrometer in KBr pellets. Elemental analysis was performed on an Elemental Vario EL III (C, H, N, O and Cl) analyzer. The purity of the compounds was checked by thin layer chromatography (TLC) with silica gel plates. ^{1}H NMR spectra were recorded on a Bruker AMX-300 NMR spectrometer operating at 300.03 MHz for ^{1}H with the following spectral parameters; acquisition time = around 3.0 s, number of scans = 100 and spectral width = 10,330 Hz. Proton decoupled ^{13}C NMR spectra were recorded on a Bruker AMX-300 NMR spectrometer operating at 75.07 MHz for ^{13}C with the following spectral parameters; acquisition time = around 0.5 s; number of scans = 1000; spectral width = 30,000 Hz. All NMR measurements were made in 5 mm NMR tubes using solutions made by dissolving about 10 mg of the material in 0.5 mL of DMSO-d_6.

Antibacterial activity: The target compounds are screened[11] against, *Proteus mirabilis, Klebsiella oxytoca, Staphylococcus aureus* and *Salmonella paratyphi*. Using the paper disc assay method, Whatman No.1 filter paper disc of 6 mm diameter was sterilized by autoclaving for 15 min at 121 ºC. The sterile discs were impregnated with different extracts (1 mg/mL). Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In all cases, the concentration was approximately 1.2108 CFU/mL. The impregnated discs were placed on the medium suitably spaced apart and the plates were incubated at 37 ºC for 24 h. Streptomycin disc was used as a positive control. The diameter (mm) of the growth inhibition halos caused by the compounds was examined.

Preparation of compounds: Piperidin-4-ones (**a-d**) is prepared by the following procedure of Noller and Baliah[12,13].

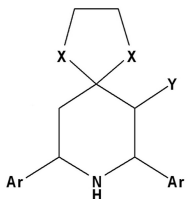
The target spiro compounds **1-6** are prepared by the procedure[14,15].

Preparation of compounds 1, 2, 4 and 6: A mixture of ethane-1,2-diol and piperidine-4-ones (**a,b,c,d**) and benzene (45 mL) on round bottom flask. The reaction flask is fixed with a Dean-stark water separator and charged with anhydrous K_2CO_3. The solution is quietly refluxed for 14 h and cooled in the room temperature and a yellow oily solution is obtained and later petroleum-ether (60-80 ºC) was added. The product is separated as pale yellow solid. This similar way follows for preparation of compounds **1**, **2**, **4** and **6**.

Preparation of compounds 3 and 5: A mixture of ethane-1,2-diamine and piperidine-4-one (**c, d**) and benzene (45 mL) placed in a round bottom flask. The reaction flask is fixed with a Dean-stark water separator and charged with anhydrous K_2CO_3. The solution is quietly refluxed for 14 h and cooled in the room temperature and a yellow oily solution is obtained and later petroleum-ether (60-80 ºC) was added. The product is separated as pale yellow solid. This method follows compounds **3** and **5**.

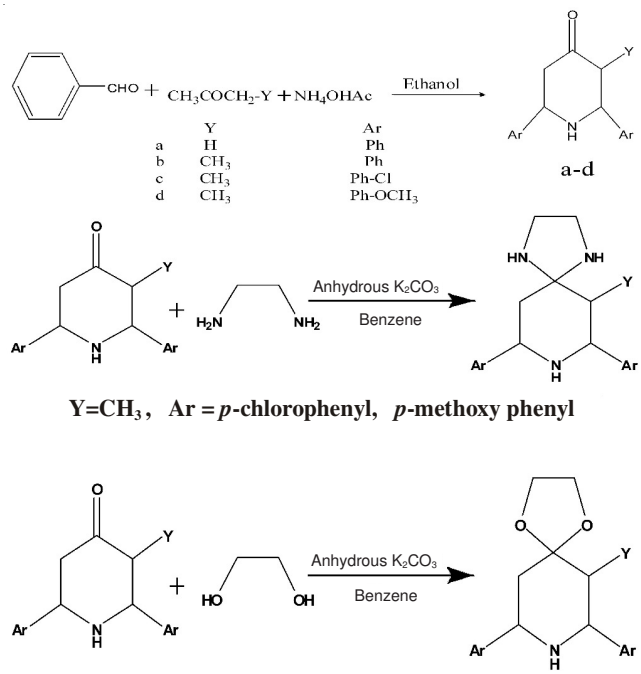
RESULTS AND DISCUSSION

Synthesis of the compounds and the numbering of atoms: Compounds 7,9-diphenyl-1,4-dioxa-8-aza spiro[4.5] decane, 6-methyl-7,9-diphenyl-1,4-dioxa-8-aza spiro[4.5] decane, 7,9-*bis*-(4-chlorophenyl)-6-methyl-1,4,8-triaza spiro [4.5]decane, 7,9-*bis*-(4-chlorophenyl)-6-methyl-1,4 -dioxa-8-aza spiro[4.5]decane,7,9-*bis*-(4-methoxyphenyl)-6-methyl-1,4,8-triazaspiro[4.5]decane and 7,9-*bis*-(4-methoxyphenyl)-6-methyl-1,4,-dioxa-8-aza spiro[4.5]decane **1-6** have been synthesized by using reactions shown in **Scheme-I**. The numbering of carbon atoms is shown in Fig. 1. The methylene protons in the cyclohexane ring are denoted as axial and equatorial protons assuming chair conformation for the cyclohexane ring.



	X	Y	Ar
1.	O	H	Phenyl
2.	O	CH_3	Phenyl
3.	NH	CH_3	*p*-Cl-Phenyl
4.	O	CH_3	*p*-Cl-Phenyl
5.	NH	CH_3	*p*-methoxy-Phenyl
6.	O	CH_3	*p*-methoxy-Phenyl

Fig. 1.



Y=CH_3, Ar = *p*-chlorophenyl, *p*-methoxy phenyl

Y = H, CH_3 Ar = Phenyl, *p*-Cl-Phenyl, *p*-methoxy-Phenyl

Scheme-I

The conformation and optimization of the target compounds structure shown that the atoms or group of atoms are arranged in molecule with reference to space. The optimization structures of favoured conformations **1-6** are given in Fig. 2.

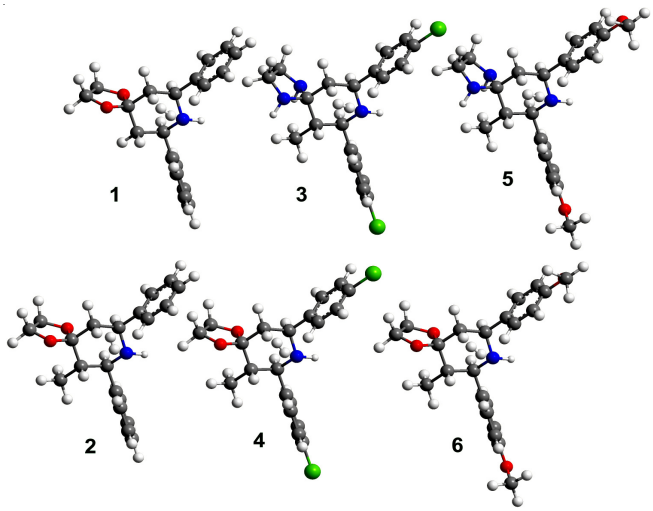


Fig. 2.

Mass-spectra and IR: Physical properties, mass spectral data and IR spectral data's are given in Table-2 and 3 respectively. In the mass spectra, for all compounds molecular ion peak corresponding to the molecular formula were observed. The carbonyl stretching frequencies of the title compounds are mislaid to compare parent compound **a-d.** The carbonyl group are involved in condensation reaction with 1,4-binucleophilic reactants.The N-H stretching frequencies for the compounds **1-6** were in the range of 3427-3300 cm^{-1}.

Assignments of ^{1}H NMR signals: ^{1}H NMR spectral data's of the products were compared with compounds **7** and **8**[16,17] are listed in Table-4. The compounds **7** and **8** chair conformation

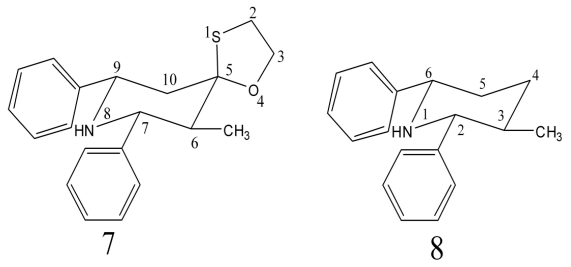
TABLE-2
PHYSICAL PROPERTIES AND MASS SPECTRAL DATA

Compounds	m.f.	m.p. (ºC)	Elemental analysis (%)				Yield (%)	Mass of parent ion
			H	C	N	Cl		
1	$C_{19}H_{21}NO_2$	96	7.17	77.26	4.74	-	70	295
2	$C_{20}H_{23}NO_2$	86	7.49	77.64	4.53	-	65	309
3	$C_{20}H_{23}N_3Cl_2$	110	6.16	63.83	11.17	18.84	60	377
4	$C_{20}H_{21}NO_2Cl_2$	94	5.60	63.50	3.70	18.74	60	379
5	$C_{22}H_{29}N_3O_2$	92	7.95	71.91	11.43	-	55	367
6	$C_{22}H_{27}NO_4$	118	7.37	71.52	3.79	-	55	369

is given in Fig. 3. The signals in the ^{1}H NMR spectra of **1-6** were assigned based on positions, multiplicities and integral values. The NMR data's of the products shown that the piperidine ring in the molecule favoured chair conformation.

TABLE-3
IR STRETCHING FREQUENCIES (cm^{-1}) OF COMPOUNDS **1-6**

Comp.	NH	Aromatic C-H	Aliphatic CH_3	C-O-C	C-N
1	3300	2956	–	513	1156, 1176
2	3309	3029	2929	513	1138
3	3424	3010	2935	–	1112, 1143
4	3301	3027	2957	1174	1143
5	3427	3022	2961	–	1142, 1112
6	3349	3015	2935	1218	1143



7= 6-methyl-,7,9-diphenyl-8-aza-4-oxa-1-thio spiro[4.5]decane 8 =3-methyl-r(2),c(6)-diphenylpiperidine

Fig. 3.

The various protons coupling constant are given in Table-5. The ^{1}H NMR results of synthesized compound **1-6** were compared with reference compound **7** and **8** shows small variations. The reference compound **7**, H-2 and H-3 protons are observed down field when compare with synthesized compounds **1-6**, due to strong electro negativity of sulphur than nitrogen and oxygen.

Analysis of coupling constant: For **1** observed one large *vicinal* coupling constant between H(6a)and H(7a) or H(9a) and H(10a) is found as 10.2 Hz and one small coupling H(6e) and H(7a) or H(9a) and H(10e) is found as 3.9 Hz. The *geminal* coupling constant H(10a) H(10e) is found as15.6 Hz. For **2-6**, H(6a) and methyl coupling constant were found as 6.6 Hz. Vicinal(axial-axial) coupling constant between H(7a) and H(6a) were found as 11.4, 10.5, 10.5, 10.2 and 7.2 Hz, another one found in H(9a) and H(10a) were 10.5, 12.3, 8.4 and 8.7 Hz respectively. The vicinal (axial-equatorial) coupling constant between H (9a) and H (10e) were found 3.9, 3.3, 3.0 and 2.7 Hz and the geminal coupling constant between H(10a) and H(10e) were found as 7.2, 12.0 and 13.5 Hz. The favoured chair conformation structures are given in Fig. 4.

Assignments of ^{13}C NMR signals: The proton- decoupled ^{13}C NMR spectral data of the products were compared with reference compounds **7** and **8** are given in Table-6. The aromatic carbons could be readily distinguished by their characteristic absorption above 100 ppm. Assignments for the benzylic

TABLE-4
PROTON CHEMICAL SHIFTS OF **1-6** AND REFERENCE COMPOUND **7, 8**

Compounds	Chemical shifts, δ(ppm)									
	H-7a	H-9a	H-6a	H-10a	H-10e	Aromatic	NH	C2-C3	CH_3	OCH_3
1	4.06		1.95			7.25-7.48	1.60	3.25-3.37	-	-
2	4.07	3.5	2.02	1.85		7.2-7.4	1.70	3.2-3.4	0.81	-
3	3.58	4.03	2.54	1.90		7.29-7.42	1.80	2.93-3.38	0.82	-
4	3.60	4.04	2.59	1.922		7.26-7.42	1.75	3.10-3.30	0.81	-
5	3.53	3.99	2.60	2.06	2.18	6.85-7.37	1.65	2.92-3.24	0.82	3.78
6	3.70	4.00	2.65	1.95	2.09	6.83-7.35	1.75	3.2-3.64	0.81	3.78
7	3.80	4.14	2.07	2.07	2.40	-	1.76	2.99-4.06	0.80	-
8	-	H_{2a}- 3.34	3.80	-	-	-				

TABLE-5
PROTON-PROTON COUPLING CONSTANTS IN **1-6**

Compounds	Coupling constants (Hz)						
	$^3J_{CH3,6a}$	$^3J_{7a,6a}$	$^3J_{9a,10a}$	$^3J_{9a,10e}$	$^2J_{10a,10e}$	$^3J_{6a,7a}$ or $^3J_{10a,9a}$*	$^3J_{6e,7a}$ or $^3J_{10e,9a}$*
1	-	-	-	-	15.6	10.2	3.9
2	6.6	11.4	10.5	3.9	7.2	-	-
3	6.6	10.5	12.3	-	-	-	-
4	6.6	10.5	-	3.3	-	-	-
5	6.6	10.2	8.4	3.0	12.0	-	-
6	6.6	7.2	8.7	2.7	13.5	-	-

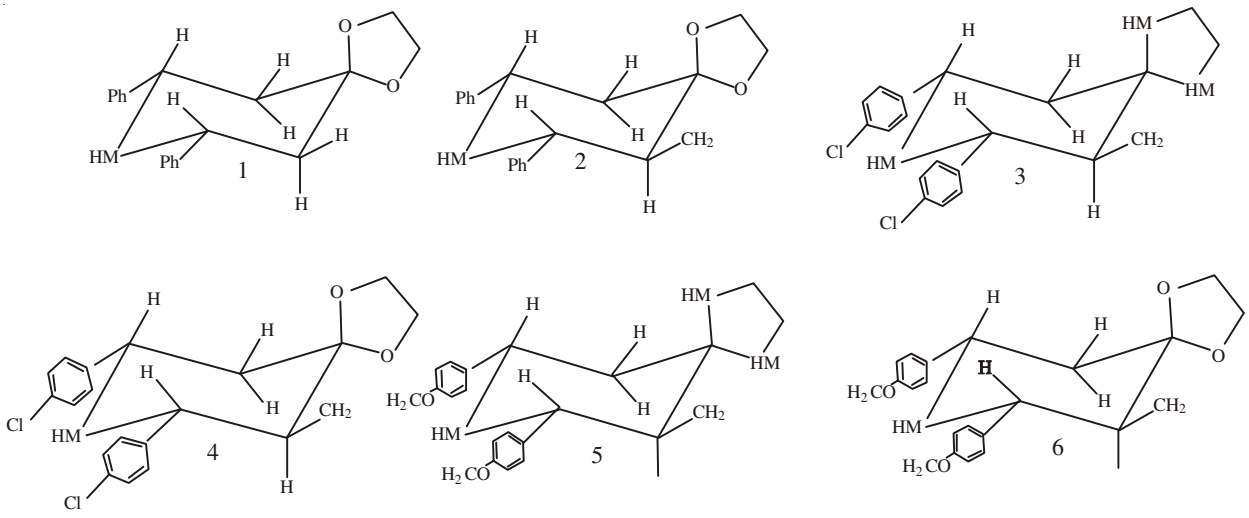


Fig. 4.

TABLE-6
^{13}C CHEMICAL SHIFTS OF COMPOUNDS **1-6** AND REFERENCE COMPOUNDS **7,8**

Comp.	C(5)	C(7)	C(9)	C(6)	C(10)	C(3)	C(2)	Aromatic carbons	Ph-ipso carbons	Ph-Cl / Ph-CH_3	CH_3	OCH_3
1	86.05	61.22		50.39		51.13	49.04	126.5-128.7	142.7	-	-	-
2	93.37	68.47	61.58	45.15	47.58	51.67	50.89	126.5-128.6	141.9, 142.7	-	10.15	-
3	82.57	67.67	60.88	51.63	50.81	48.80	46.88	127.8-129.0	133.5, 133.7	140.26, 141.23.	10.07	-
4	89.02	67.70	60.90	51.65	48.95	54.62	48.95	127.8-129.0	133.6, 133.8	140.26, 141.13.	10.08	-
5	84.89	67.89	61.00	46.85	45.07	43.85	42.09	113.8-128.7	134.2, 135.1	159.15, 159.27.	10.17	55.28
6	94.85	67.90	61.01	49.03	48.64	51.85	51.07	113.8-128.7	134.2, 135.1	159.16, 159.28.	10.18	55.29
7	96.92	65.67	58.64	49.22	46.88	33.89	71.39	-	-	-	11.70	-
8	35.0	C(2) 70.2	C(6) 62.7	C(3) 37.5	C(5) 35.6	-	-	-	-	-	18.7	

carbons have been made on the basis of known effects of alkyl substituents in six-membered ring compounds. The decoupled carbon NMR values observed for spiro ipso carbon C(5) are 86.05, 93.37, 82.57, 89.02, 84.89 and 94.85 ppm respectively. These values point out the neighboring atoms. The compounds **3** and **5** having lower chemical shift when compare with **1**, **2**, **4** and **6** value, due to nitrogen having lower electro negativity than oxygen at 1,3 position.

These values point out the neighboring atoms. The compounds **3** and **5** having lower chemical shift when compare with **1**, **2**, **4** and **6** value, due to nitrogen having lower electro negativity than oxygen at 1, 3 position. The benzylic carbons signals C(7) appears at down field to compare C(9) and C(6) appear at down field to compare C(10) due to the deshielding factor. The OCH_3 appears down field to compare CH_3 due to the more electronegative nature of methoxy. The aromatic carbons are observed around 110-130 ppm, phenyl ipso carbons observed at 142.7, 141.9, 142.7, 133.5, 133.7, 133.6, 133.8, 134.2, 135.1 and 134.2, 135.1 ppm in that order. The chloro phenyl and metonymy phenyl ipso carbons are found at 140.26, 141.23, 140.26, 141.13, 159.15, 159.27, 159.16, 159.28 ppm in that order.

^{13}C NMR results of compounds **1- 8**, C(5) observed on up field in **8** because there is no electronegative atoms in nearest position, but in **7** observed down field to compare **1-6** because the sulphur atom present in the molecule. The C(2), C(3) were observed up field, down field respectively in **7**, but **1-6** observed in between the above range due to the nitrogen and oxygen.

Antibacterial activity: For evaluating antibacterial activity streptomycin was used as the standard drug. The observed minimum inhibitory concentrations (MIC) are given in Table-7.

TABLE-7
ANTIBACTERIAL ACTIVITY OF COMPOUNDS **1-6**

Compound	Minimum inhibitory concentration (μg mL^{-1})			
	Proteous mirabilis	*Klebsiella oxytoca*	*Staphylococcus aurous*	*Salmonella paratyphoid*
1	-	6.6	-	8.1
2	-	-	9	-
3	6.3	-	6.1	6.5
4	-	-	5.5	-
5	8.2	7.8	8.7	7.9
6	7.6	8.4	7.0	-
7 [Streptomycin]	5.5	5.5	6.0	6.0

All the compounds were screened for their antibacterial activity against some significant bacterial species are *Proteus mirabilis, Klebsiella oxytoca, Staphylococcus aureus* and *Salmonella paratyphi*. Compounds **1-6** with various substituents in the aromatic ring will be useful in understanding the influence of steric and electronic effects on the biological activity. The plot diagram of compounds **1-6** are comparing with streptomycin **7**. The replacement of carbon by nitrogen and oxygen to increase the biological activity. The proportional antibacterial evaluations are given in bar diagram in Fig. 5.

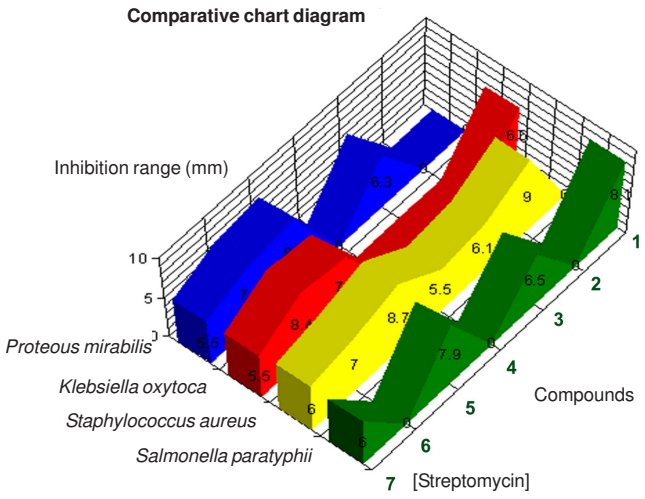


Fig. 5.

Conclusion

A series of compounds **1-6** were synthesized and screened for their antibacterial activity. The observed *vicinal* proton-proton coupling and *geminal* coupling constants suggest that in **1-6** piperidine rings adopts chair conformation with equatorial orientations of the aryl groups. The compound **5** has high antibacterial activity against the bacterial species compared with norfloxacin standard compound. The spiro products (**1-6**) are highly active against *Staphylococcus aureus*.

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