ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis and Antibacterial Evaluation of Bis-pyrrolidinyl Ketones

AA Al-Kadhimi[1*], AJ AL-Hamdany[2], SS Jasim[3]

[1]Department of Chemistry, College of Science, University of Tikrit, Iraq
[2]Department of Chemistry, College of Science, University of Mosul, Iraq
[3]Fuel & Energy Department, Technical College, Kirkuk, Iraq

ABSTRACT

A series of novel bis-pyrrolidines (9-14) were prepared by the reaction of two moles of Schiff's bases (**7** & **8**) in a 1,3-anionic cycloaddition manner with substituted (1*E*,4*E*)-1,5-diphenyl-1,4-pentadien-3-one (1-4). All new compounds were characterised using ^{1}H, ^{13}C-NMR, IR, UV and elemental analysis. The antibacterial activity of some of these compounds was also evaluated. The proposed reaction mechanism was investigated using theoretical methods such as heat of formation (H.F.) and steric energy (S.E.) calculation. MOPAC, (H.F.) and MM2 methods were also used in this study.

Keywords: 1,3-anionic cycloaddition, Bis-pyrrolidinyl Ketones, Schiff's bases, *Staphylococcus aureus*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Pyrrolidines play an important role in medicinal and pharmaceutical chemistry. These compounds show biological activities such as analgesic potency [1], antibacterial [2], dipeptidyl-4 peptidase inhibitors [3], histamine H_3-receptor ligands [4], antimicrobial [5], and antitumor [6]. Some pyrrolidines also act as potent H_3-antagonists [7].

The value of the pyrrolidines and their *N*-substituted analogues is not limited to the use of these compounds, but they can also be used as precursors for building other important compounds. These compounds undergo typical reactions of secondary or tertiary alkyl amines. Therefore, these compounds can be alkylated, acylated and nitrosated [8].
Mikhova *et al* [25] and Popandova-Yambolieva *et al* [26] prepared several of these pyrrolidinyl ketones. They [25, 26] also studied the stereochemistry and the NMR spectroscopy of these compounds in detail (Scheme **1**).

We would like to report herein the synthesis of bis-pyrrolidinyl ketones, their ^{1}H, ^{13}C-NMR as well as their antibacterial activity.

MATERIALS AND METHODS

Experimental

General: Ultra-violate spectra (UV) were obtained using Shimadzu UV-VIS spectrometer UV-160 and JASCOW32 (UV-VIS) spectrometer V-500, $CHCl_3$, Scale 200-800nm. Infrared spectra were recorded on Shimadzu FT 8400 S, Fourier Transform-Infrared Spectrometer in Tikrit University/Iraq. Proton Nuclear Magnetic Resonance (^{1}H-NMR) and Carbon Nuclear Magnetic Resonance (^{13}C-NMR) spectra were recorded on Brucker (400 MHz) using tetramethylsilane (TMS) as an internal standard, and $CDCl_3$ as a solvent in AL-Baath University/Syria and on Brucker (500 MHz) using tetramethylsilane (TMS) as an internal standard, and DMSO-d_6 as a solvent were performed in Technische University/Germany. The elemental analyses were recorded on C.H.N. Elemental analyser "FIASH AE1112 in Technische University/Germany.

Computational Work

In the present work, the CS ChemOffice (version 6.0) was used for the computational work.

Synthesis of Diaryledene acetone

*Typical procedure for the synthesis of compounds (**1-4**) [9]:*

A solution of acetone (1.4 g, 25 mmol) and benzaldehyde [(50 mmol) or substituted benzaldehyde] was added dropwise with stirring at room temperature to a stirred solution of sodium hydroxide (5 g, mmol), in [water (50 mL) and ethanol (40 mL)]. The stirring was continued for 30 min at room temperature. The resulting precipitate was filtered, washed with

ISSN: 0975-8585

cold water (3x30 mL) and dried. Recrystallization from ethanol (95%) afforded the required product. Melting point, yield and colour of these compounds are compiled in Table (**1**).

Synthesis of Schiff's bases

*Typical procedure for the synthesis of compounds (**5-8**) [10]:*

Benzylamine (800 mg, 10 mmol) and benzaldehyde (or substituted benzaldehyde) were dissolved in *n*-butanol (10 mL) and heated at (100^{o}C) for 10 min. The cold reaction mixture was filtered and dried. The product was purified by distillation at atmospheric pressure. For melting point / boiling point, yield and the colour of these compounds see (Table **2**).

Synthesis of Dipyrrolidinyl Ketone (9-15)

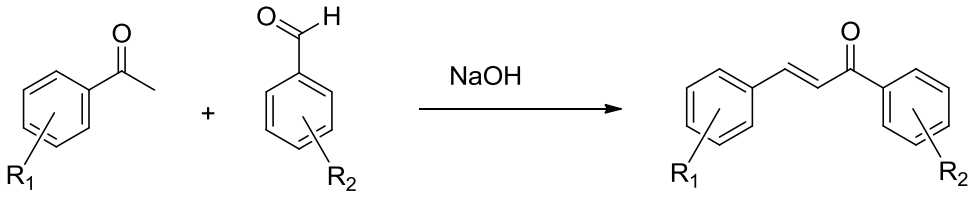
*Typical procedure for the synthesis of compounds (**9-15**) [11]:*

A mixture of Schiff's bases (**5-8**) (5 mmol), diarylidene acetone (9-15)(2.5 mmol), tetrabutylammonium bromide (TBAB) (0.12 g, 0.5 mol), sodium hydroxide (50%, 3 mL) and dimethyl sulphoxide (DMSO) (10 mL) was stirred at room temperature for 1h. The separated product was filtered, washed with water until the filtrate became clear and neutral, and then the product was dried and recrystallized from methanol / ethyl acetate. For the physical properties see (Tables **3a** and **3b**).

RESULTS AND DISCUSSION

[1] Condensation of substituted benzaldehyde with acetone

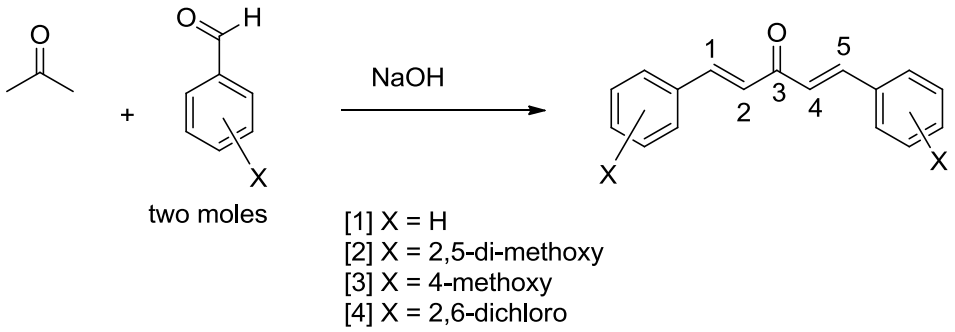
Substituted benzaldehydes were condensed with acetone using Claisen-Shmidt condensation methodology in the presence of sodium hydroxide. This reaction gave rise to the required products (**1-4**) (Scheme **2**). This was similar to the work of Mikhova *et al* [25], Khalaf *et al* [27, 28] and Popandova-Yambolieva *et al* [26] (Scheme **1**) in which they synthesised substituted (2E)-1,3-diphenyl-2-propen-1-ones.



Scheme 1. Synthesis of substituted (2E)-1,3-diphenyl-2-propen-1-one

ISSN: 0975-8585

These compounds (**1-4**) were fully characterised using ^{1}H-NMR, ^{13}C-NMR, IR, UV, and microanalysis, (Table **4**). These compounds were prepared as illustrated in (Scheme **2**) analogous to the method in (Scheme **1**).



Scheme 2. Synthesis of substituted (1E,4E)-1,5-diphenyl-1,4-pentadien-3-one.

The infra red (IR) spectra of these compounds (**1-4**) showed strong absorption bands at (1650-1681 cm^{-1}) which represent the stretching vibration of the carbonyl group (νC=O) [12]. The conjugation of carbonyl group with (C=C) bond resulted in the delocalization of the carbonyl group with the (C=C) bond which led the absorption to shift to a lower frequency [13]. The bands at (1606-1589 cm^{-1}) represent (C=C) stretching vibration (νC=C) and the bands at (1560-1492 cm^{-1}) represent aromatic ring stretching vibration (νC=C).

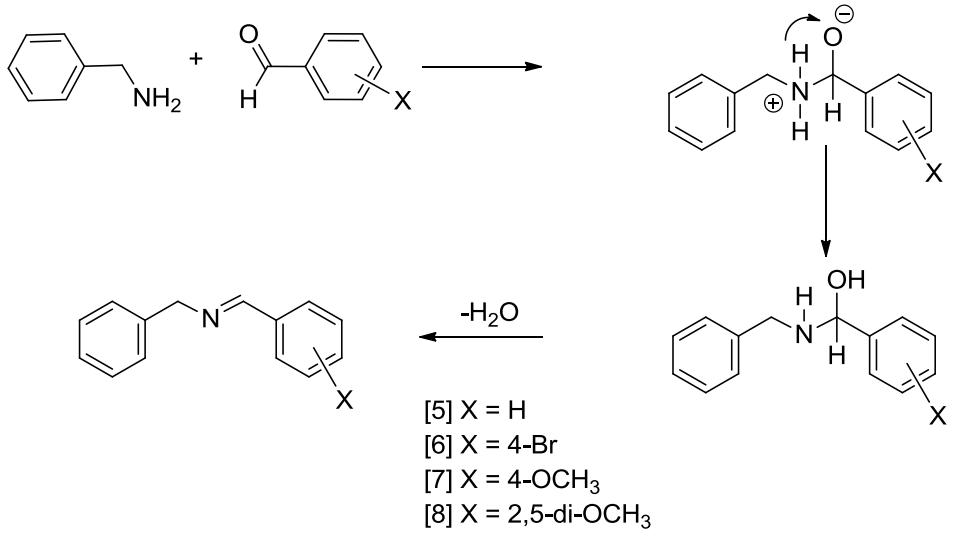
The ^{13}C NMR spectrum of compound (**2)** showed a peak at δ(55.82)ppm due to the methoxy carbon at the ortho position in the two rings; another peak at δ(56.10)ppm was attributed to the methoxy carbon at the meta position in the two rings [14]. There are four peaks at δ(112.43)ppm, δ(113.17)ppm, δ(117.19)ppm, and δ(124.46)ppm, which represent the *o, p, m* carbon atoms attached to the two olefinic carbon at C_2 and C_4. There is a peak at (138.07)ppm attributed to the ortho carbon attached to the methoxy group of the two rings. The peak at δ(153.12)ppm referred to the olefinic carbon at C_1 and C_5, while the peak at δ(153.50)ppm referred to the meta carbon attached to the methoxy group of the two rings; finally the peak at δ(189.58)ppm was due to the carbonyl carbon [15]. ^{13}C NMR data of compounds (**1, 2, 13)** are summarized in (Table **8**).

The ^{1}H NMR data of compound (**2)** are in (Table **9**). The (UV) spectra showed a (λ_{max}) in the range of (312-390)nm, which seemed in good agreement with the analogous (1*E*,4*E*)-1,5-diphenyl-1,4-pentadien-3-one [16], due to the conjugation of carbonyl group with the double bond and the spectrum showed red shift (bathochromic shift).

[2] Condensation of Benzaldehydes with Benzylamines

Benzaldehydes were condensed with benzylamine to afford substituted Schiff's bases (**5-8**) (Scheme **3**).

ISSN: 0975-8585



[5] X = H
[6] X = 4-Br
[7] X = 4-OCH_3
[8] X = 2,5-di-OCH_3

Scheme 3. Preparation of Schiff's bases

Schiff's bases were identified according to the spectroscopic data obtained (Table **5**). IR spectra showed strong absorption bands at (1650-1641 cm^{-1}) which represents the stretching vibration of the (νC=N) bond [17], while the bands at (1600-1440 cm^{-1}) related to the aromatic ring stretching vibration (νC=C). The (UV) spectra showed a (λ_{max}) in the region of (306-313 nm), which were in good agreement with analogous Schiff's bases [18].

[3] Condensation of Schiff's bases with (1E,4E)-1,5-diphenyl-1,4-pentadien-3-one

Compounds (**9-14**) were identified on the basis of spectroscopic evidence (Table **6**). The IR spectra exhibited bands in the range of (1705-1700 cm^{-1}) corresponding to the carbonyl group stretching vibration (νC=C) [19], while the weak absorption bands in the range of (3429-3326 cm^{-1}) were related to the stretching vibration of (N-H).

The (^{1}H-NMR) spectrum of compound (**13**) showed a peak at δ(2.14) ppm integrated for 2H, which attributed to the protons of the two nitrogen atoms. The peak at δ(2.85) ppm integrated for 2H corresponds to the protons at C_3 & C_3' of the two pyrrolidine rings. The other peak at δ(3.25) ppm integrated for 2H corresponds to the protons at C_4 & C_4' of the two pyrrolidine rings. The peak at δ(3.58) ppm integrated for 2H corresponds to the protons at C_2 & C_2' of the two pyrrolidine rings. The other peak at δ(3.83) ppm integrated for 2H corresponds to the protons at C_5 & C_5' of the two pyrrolidine rings. Finally, a doublet at δ(6.43) ppm and the multiplet at δ(6.82-7.49) ppm integrated for 28H corresponding to the aromatic protons.

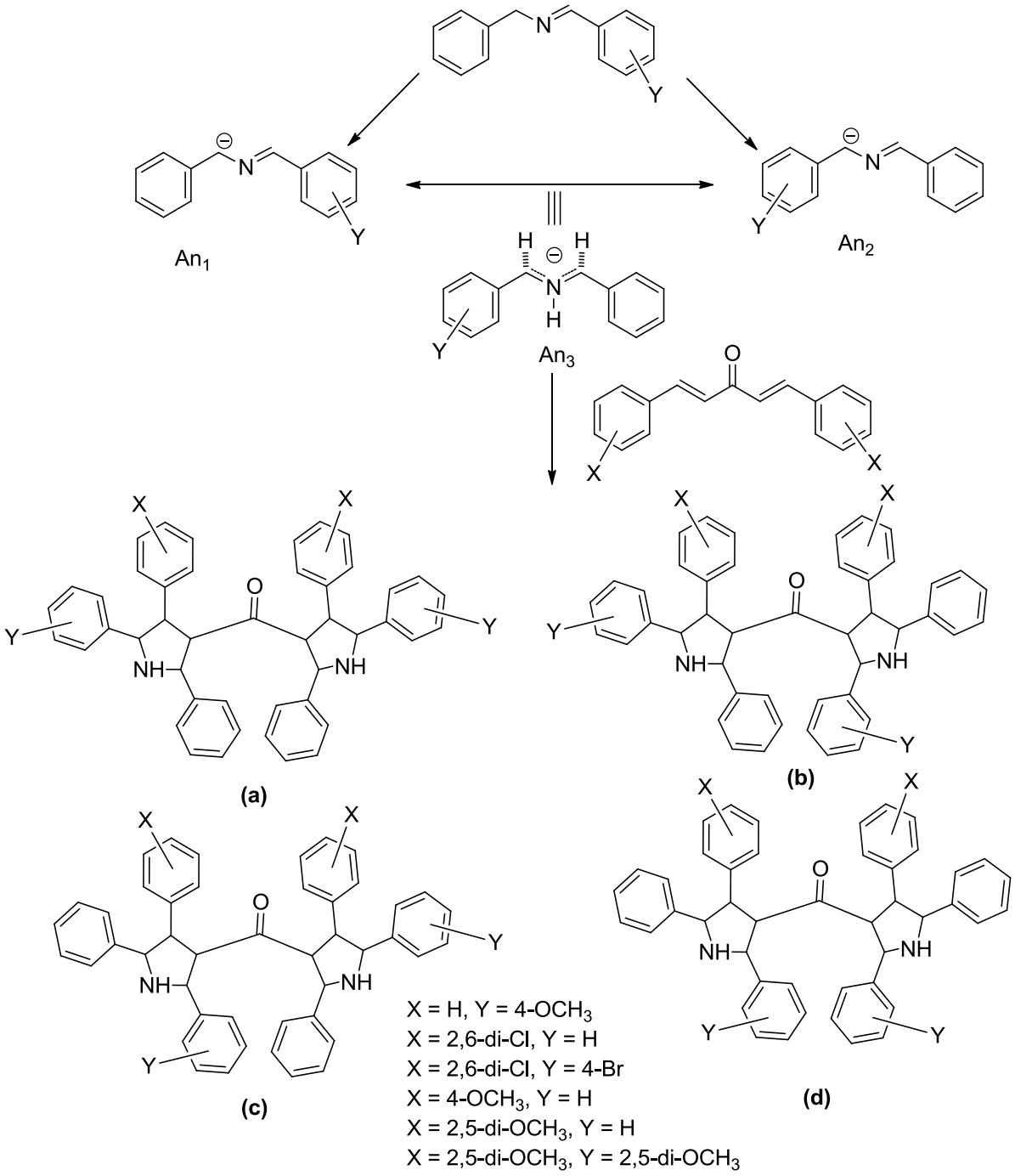
^{13}C-NMR spectrum of compound (**13**) shows a peak at δ(58.33) ppm attributed to the carbon of the two methoxy groups. There are four peaks at δ(62.08, 65.33, 65.80 and 70.52) ppm corresponding to (C_3 & C_3'), (C_2 & C_2'), (C_4 &C_4') and (C_5 & C_5') of the two pyrrolidine rings respectively. A peak at δ(116.54) ppm referred to the meta carbons of the two rings attached to the methoxy groups. The peak at δ(118.27) ppm was due to the para carbons of the phenyl rings at C_2, C_2', C_5 and C_5' of the two pyrrolidine rings. The two peaks at δ(130.40) ppm and

δ(130.70) ppm referred to the ortho carbons of the phenyl rings at C_2, $C_2^{/}$, C_5 and $C_5^{/}$ while the peak at δ(133.02) ppm corresponding to the two carbons of the two phenyl rings attached to pyrrolidine rings at C_4, $C_4^{/}$. The peak at δ(142.60) ppm due to the four carbons of the four phenyl rings attached with pyrrolidine rings at C_2, $C_2^{/}$, C_5 and $C_5^{/}$ as well as the δ(160.02) ppm attributed to the para carbons of the two phenyl rings containing the methoxy groups, and finally, the peak at δ(203.34) ppm was attributed to the carbonyl carbon [20].

The (UV/VIS) spectra (Table **6**) of the products showed a (λ_{max}) in the range of (365-420)nm indicating a red shift. The observed red shift may be attributed to the presence of additional aromatic rings [21]. The elemental analysis of compound (**13**) showed a considerable agreement with the calculated values of this compound (see Table **10**). For mp, yield, colour and nomenclature see (Tables **1**, **2**, **3a** and **3b**).

The suggested mechanism [22] for the 1,3-anionic cycloaddition reaction of two moles of Schiff's base and (1*E*,4*E*)-1,5-diphenyl-1,4-pentadien-3-one was initiated by the abstraction of the more acidic proton from Schiff's base to afford two moles of the anion An_1 or An_2 and the delocalization of the negative charge on An_1 or An_2 giving rise to the resonance hybrid An_3 (Scheme **4**), which in turn may attack the two double bonds of the α, β-unsaturated system via 1,3-anionic cycloaddition to afford one of four possible structures: a, b, c, or d. Depending on the values of (H.F) (Table **7**). It can be concluded that the final products are those having the lowest values of (H.F) as shown in (Scheme **4**).

ISSN: 0975-8585



Scheme 4. Preparation of bis pyrrolidinyl ketones

Biological Evaluation

The biological inhibitory effects of certain products such as (DAA)(2), Schiff base (**6**) and pyrrolidine (**13**) against five types of bacterial groups such as Gram-negative *E. coli, Proteus*

ISSN: 0975-8585

vulgaris, Bacillus subtilis, Klebsiella pneumonia and Gram-positive, such as *Staphylococcus aureus,* were investigated (Table **11**).

The standard Kirby and Bauer [23] method was used. Aliquot of each bacterial species was cultured in nutrient broth and incubated at (37^{o}C) for (14-16h) and then evenly distributed on the nutrient agar by using a sterile swab. The plates were incubated at (37^{o}C) for (30 min) and the filter paper (Whatman No.1 discs) were distributed on the agar and an amount equal to (1 mg/1 mL) or (1 mL/ 1mL) of the compound per solvent (DMSO) was added. Tetracycline and Gentamycine were used as control.

The plates were then incubated at (37^{o}C) for (18-24h). The interpretation of the results based on the diameter (mm) of the inhibition zone appeared around the disc [24]. The results were interpreted according to the report of (W.H.O.).
(A): The resistance (R) result represented the diameter of inhibition < (11) mm.
(B): The moderately sensitive (MS) result was regarded when the zone inhibition was between (12-16) mm.
(C): The sensitive (S) result was over (16) mm.

Table (1): Physical properties of diarylidene acetone (1-4)

No	Product name	X	m.p./^{o}C(Lit. value)[ref]	Yield	Colour
1	(1*E*,4*E*)-1,5-diphenyl-1,4-pentadien-3-one	H	108-110(109-110)[32]	80	yellow
2	(1*E*,4*E*)-1,5-bis(2,5-dimethoxyphenyl)-1,4-pentadien-3-one	2,5-di-OCH_3	98(105-106)[30]	82	yellow
3	(1*E*,4*E*)-1,5-bis(4-methoxyphenyl)-1,4-pentadien-3-one	4-OCH_3	110-112(119-120)[29]	72	green
4	(1*E*,4*E*)-1,5-bis(2,5-dichlorophenyl)-1,4-pentadien-3-one	2,6-di-Cl	135-138(145-146)[31]	76	Pale yellow

Table (2): Physical properties of Schiff's bases (5-8)

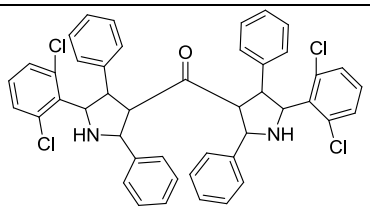
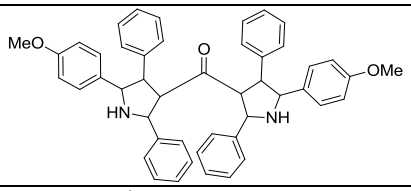
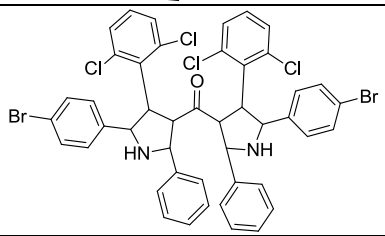
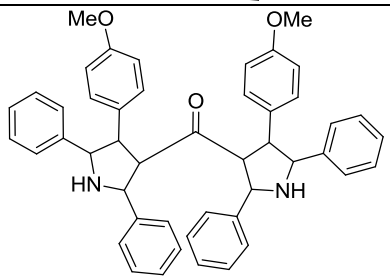
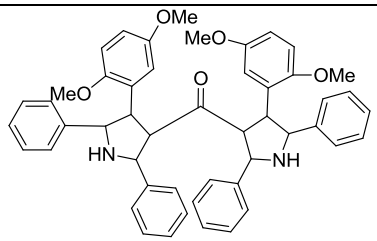
No	Product name	Y	m.p./^{o}C(Lit. value)[reference]	Yield	Colour
5	phenyl-*N*-[(*E*)-phenylmethylidene]methanamine	H	278-280(2)[bp:130-145^{o}C@0.4mmHg][33]	65	yellow
6	*N*-[(*E*)-(4-bromophenyl)methylidene](phenyl) methanamine	4-Br	38-40(43)[34]	75	yellow
7	*N*-[(*E*)-(4-methoxyphenyl)methylidene](phenyl) methanamine	4-OCH_3	203-206(bp:195-205^{o}C@2.5mmHg)[33]	83	yellow
8	*N*-[(*E*)-(2,5-dimethoxyphenyl)methylidene] (phenyl)methanamine	2,5-di-OCH_3	148-150(48)[35]	66	yellow

Table (3a): Physical properties of substituted bis-pyrrolidines (9-14)

No	Product Name	X	Y	m.p^{o}C	Yield%	Colour
9	bis[5-(2,6-dichlorophenyl)-2,4-diphenyl-3-pyrrolidinyl]methanone	2,6-di-Cl	H	102-105	75	Green
10	bis[5-(4-methoxyphenyl)-2,4-diphenyl-3-	H	4-OCH_3	105-	75	Pale yellow

	pyrrolidinyl]methanone			107		
11	bis[5-(4-bromophenyl)-4-(2,6-dichlorophenyl)-2-phenyl-3-pyrrolidinyl]methanone	2,6-di-Cl	4-Br	89-93	75	White
12	bis[4-(4-methoxyphenyl)-2,5-diphenyl-3-pyrrolidinyl]methanone	4-OCH_3	H	135-137	53	Bright brown
13	bis[4-(2,5-dimethoxyphenyl)-2,5-diphenyl-3-pyrrolidinyl]methanone	2,6-di-OCH_3	H	78-80	71	Yellowish green
14	bis[4,5-bis(2,5-dimethoxyphenyl)-2-phenyl-3-pyrrolidinyl]methanone	2,5-di-OCH_3	2,5-di-OCH_3	54-55	94	Yellowish green

Table (3b): Structures of substituted bis-pyrrolidines (9-14)

No	Structure	Name
9		bis[5-(2,6-dichlorophenyl)-2,4-diphenyl-3-pyrrolidinyl]methanone
10		bis[5-(4-methoxyphenyl)-2,4-diphenyl-3-pyrrolidinyl]methanone
11		bis[5-(4-bromophenyl)-4-(2,6-dichlorophenyl)-2-phenyl-3-pyrrolidinyl]methanone
12		bis[4-(4-methoxyphenyl)-2,5-diphenyl-3-pyrrolidinyl]methanone
13		bis[4-(2,5-dimethoxyphenyl)-2,5-diphenyl-3-pyrrolidinyl]methanone

ISSN: 0975-8585

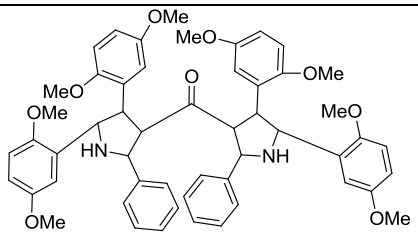
14		bis[4,5-bis(2,5-dimethoxyphenyl)-2-phenyl-3-pyrrolidinyl]methanone

Table (4): Spectral data of diarylidenes acetone (1-4)

No	UV ($CHCl_3$) λ_{max} (nm)			IR(KBr) $\nu(cm^{-1})$	
		C=O	C=C	Aromatic C=C	Others
1	312	1681	1602	1492	-----
2	390	1650	1589	1492	C-O-C = 1218
3	360	1655	1600	1508	C-O-C = 1253
4	316	1655	1606	1560	-----

Table (5): Spectral data of Schiff's bases (5-8)

No	UV ($CHCl_3$) λ_{max} (nm)		IR(KBr) $\nu(cm^{-1})$	
		C=N	Aromatic C=C	Others
5	306	1641	1600	-----
6	313	1646	1465	-----
7	320	1650	1440	C-O-C = 1245
8	328	1645	1452	C-O-C = 1235

Table (6): Spectral data of substituted bis-pyrrolidines (9-14)

No	UV ($CHCl_3$) λ_{max} (nm)		IR(KBr) $\nu(cm^{-1})$	
		C=O	N-H	Others
9	390	1705	3350	C-O-C = 1218
10	390	1703	3326	-----
11	365	1701	3399	-----
12	384	1701	3429	C-O-C = 1249
13	410	1701	3326	C-O-C = 1218
14	420	1700	3416	C-O-C = 1249

Table (7): Heat of formation and steric energy of substituted bis-pyrrolidines (9-14)

No	X	Y	Form	H.F. Kcal/mol	S.E. Kcal/mol
9	H	4-OCH_3	A	126.80111	16025.135
			B	127.61088	16125.662
			C	113.57259	17034.495
			D	131.14659	18243.066
10	2,6-di-Cl	H	-----	278.65581	26852.879
11	2,6-di-Cl	4-Br	A	449.41400	34728.680
			B	187.27365	17651.598

ISSN: 0975-8585

			C	296.58571	537.09700
			D	207.64118	117599.35
12	4-OCH_3	H	-----	127.33769	16459.190
13	2,5-di-OCH_3	H	-----	151.64306	26546.446
14	2,5-di-OCH_3	2,5-di-OCH_3	A	7.3826600	26338.026
			B	5.2691900	14864.541
			C	-119.44988	18354.610
			D	37.651410	48178.447

Table (8): ^{13}C-NMR data of compounds (1,2 and 13)

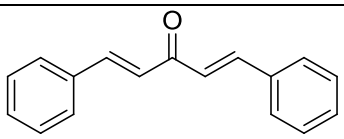
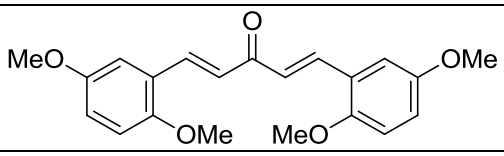
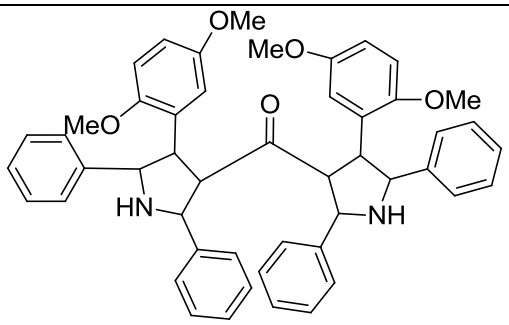
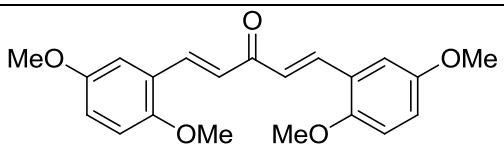
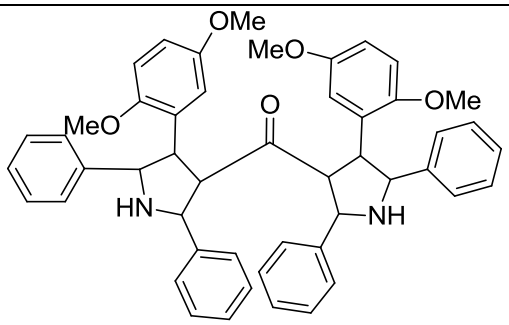
No	Structure	^{13}C-NMR ($CDCl_3$ or DMSO), δ ppm
1		125.67(C2&C4); 128.28(four ortho-position); 128.25(two para-position); 130.49(four meta position); 134.26(two carbons attached to α-β system); 142.74(C1 & C5); 188.50(C3).
2		55.82(ortho-CH_3); 56.10(meta-OCH_3); 112.43(unsubstituted ortho-position); 113.17(two para-position); 117.19(unsubstituted meta-position); 12.46(two carbons attached to α-β system); 126.30(C2 & C4).
13		58.33(two OCH_3); 62.08(C3 & C'3); 65.33(C4 & C'4); 65.80(C2 & C'2); 70.52(C5 & C'5); 116.54-181.02(aromatic carbons); 203.34(C=O).

Table (9): ^{1}H-NMR data of (2 & 13):

No	Structure	^{1}H-NMR ($CDCl_3$ or DMSO), δ ppm
2		3.82(OCH_3) at two ortho position, 6H, s; 3.89(OCH_3) at two meta position, 6H, s); 6.90 & 7.15 Ar-H, 6H, d, m; 7.37 (olefinic at C2 & C4, 2H, d; 8.03 (olefinic at C1 & C5, 2H, d).
13		2.14(N-H, 2H, s); 2.85(CH at C4 & C'4, 2H, t); 3.38(OCH_3), 6H, s; 3.83(CH at C2 & C'2, 2H, d); 3.58(CH at C5 & C'5, 2H, d); 6.43 & (6.82 – 7.49) Ar-H, 28H, d & m).

ISSN: 0975-8585

Table (10): Elemental analysis of (1, 2 and 9-14)

No	Molecular Formula		CHN analysis Calculated/Found	
		C%	H%	N%
1	$C_{17}H_{14}O$	87.17 87.19	5.97 5.92	-----
2	$C_{21}H_{22}O_5$	71.12 71.13	6.21 6.22	-----
9	$C_{47}H_{44}N_2O_3$	82.46 81.97	6.42 6.53	4.09 3.99
10	$C_{45}H_{36}Cl_4N_2O$	70.88 71.02	4.72 5.02	3.67 3.93
11	$C_{45}H_{34}Cl_4Br_2N_2O$	58.72 58.60	3.69 3.71	3.04 2.94
12	$C_{47}H_{44}N_2O_3$	82.46 82.37	6.42 6.38	4.09 4.07
13	$C_{49}H_{48}N_2O_5$	79.04 79.26	6.44 6.25	3.76 3.67
14	$C_{53}H_{56}N_2O_9$	73.62 73.63	6.47 6.56	3.24 3.19

Table (11): Inhibition effect of compounds (2, 6, 12, 14) on the growth of *Staph Aureus, Bacillus subtilis, K. Pneumonia, Proteus vulgaris & E. Coli*

No	Staph aureus	*Bacillus subtilis*	*K. Pneumonia*	*Proteus vulgaris*	*E. coli*
2	MS	MS	S	R	MS
6	R	S	R	R	R
12	S	S	S	S	MS
14	MS	S	MS	S	S
Control					
Tetracycline	30 mg/disc				
Gentamycine	10 mg/disc				

ACKNOWLEDGEMENT

The authors would like to thank Dr Abedawn I Khalaf for proofreading and revising this article.

REFERENCES

[1] Aboul-Enein MN, El-Difrawy S, Abdallah NA, Khalifa NM, Ebeid MY and Werner W. J Islamic Academy of Sciences 1990; 3 (3): 180-184.
[2] Mitchell RE and Teh KL. Org Biomo Chem 20053; 3540-3544.
[3] Yonekubo S and Fushimi N. Kissei Pharmaceutical Co Ltd. 2010, US Patent number 719201.
[4] Schwartz JC. British J Pharmacology 2011; 163(4): 713-721.
[5] Hensler ME, Bernstein G, Nizet V and Nefzi, A. Bioorganic and Medicinal Chemistry Letters 2006; 16(19): 5073-5079.
[6] Coleman RS and Kong JS. J Amer Chem Soc 1998; 120: 3538-3539.

[7] Peschke B, Bak S, Hohlweg R, Pettersson I, Refsgaard HHF, Viuff D, Rimvall K. Bioorganic & Medicinal Chemistry 2004; 12(10): 2603-2616.
[8] Eichorand T and Hauptman S. The Chemistry of Heterocyclic: reactions, synthesis and application, 2nd Ed., 2005, p.123.
[9] Vogel AI. Text Book of Practical Chemistry, 3rd Ed., 1981, Longmans, p.79
[10] Vogel AI. Practical Organic Chemistry", 2nd Ed.,1951, Longmans, p.625
[11] Popandova-Yambolieva K, Ivanov Kh. Chemica Scripta 1989; 29(3): 269-271.
[12] Williams DH and Fleming I.Spectroscopic Methods in Organic Chemistry, 2nd Ed, 1973, Mc Graw-Hill, England, pp.51-59.
[13] Silverstein RM, and Webster FX. Spectrometric Identification of Organic Compounds, 6th Ed, 1996, pp.92.
[14] Williams DH and Fleming I. Spectroscopic Methods in Organic Chemistry, 2nd Ed, 1973, Mc Graw-Hill, England, pp. 232.
[15] Williams DH and Fleming I. Spectroscopic Methods in Organic Chemistry, 2nd Ed, 1973, Mc Graw-Hill, England, pp.51-5916.
[16] Jackman LM. Structure Determination in Organic Chemistry, 1978, Butterworth and Co Publishers Ltd., pp40-44.
[17] Williams DH and Fleming I .Spectroscopic Methods in Organic Chemistry, 2nd Ed, 1973, Mc Graw-Hill, England, pp. 87.
[18] AL Hamdany AJ, 2002, Ph.D. Thesis, University of Mosul-Iraq, p194.
[19] Williams DH and Fleming I. Spectroscopic Methods in Organic Chemistry, 2nd Ed, 1973, Mc Graw-Hill, England, pp.57.
[20] Elguero J, Jacquier R, Tizane D. Bulletin de la Societe Chimique de France 1970; (3): 1129-1139.
[21] Popandova-Yambolieva K, Ivanov C. Synthetic Communications 1986; 16(1): 57-61.
[22] Ts'ao CH, Peterson KG, Galluzzo TS. American J Clinical Pathology 1979; 71(4): 379-383.
[23] Ericsson H, Tunevall G, Wickman K. Scandinavian Journal of Clinical and laboratory investigation 1960; 12: 414-22.
[24] Vandepitte J, Engbaek K, Piot P, Heuck CC. Basic Laboratory Procedure in Clinical Bacteriology, World Health Organization Geneva, 1991, pp78-85.
[25] Mikhova B, Spasov S, Popandova K, Dryanska V, Ivanov Ch, Duddeck H, Kaiser M. Journal of Molecular Structure 1987; 161: 231-235.
[26] Popandova-Yambolieva K, Dryanska V, Ivanov Kh. Izvestiya po Khimiya 1986; 19(3): 404-409.
[27] Khalaf AI, Badman CE, Ennis MP, Horspool WM, and Sultana Q. Trends in Photochemistry & Photobiology 2010; *12:* 1-5.
[28] Khalaf AI and Horspool WM. J Iraqi Chem Society 1986; *11(2*).
[29] Fairlamb IJS, Kapdi AR, Lee AF. Organic Lett 2004; 6(24): 4435-4438.
[30] Weber WM, Hunsaker LA, Abcouwer SF, Deck LM, Vander Jagt DL. Bioorganic & Medicinal Chemistry 2005; 13(11): 3811-3820
[31] Unterhalt B. Archiv der Pharmazie (Weinheim, Germany) 1978; 311(3): 262-267.
[32] Rampal JB, Satyamurthy N, Bowen JM, Purdie N, Berlin KD. J Amer Chem Soc 1981; 103(25): 7602-7609.
[33] Leftingwell JC, Reynolds RJ. Tobacco Co 1968 French patent 1544603

[34] Shoppee CW. J Chem Soc 1931; 1225-1240
[35] Rani N, Sharma JR, Manrao MR. Pesticide Research Journal 2006; 18(2): 129-132.