Synthesis and Characterization of Some New Symmetrical Bis-Isatins Derived From p-Substituted Aromatic Amines

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Abstract: A series of p-substituted isonitrosoacetanilide 2(a-g) were prepared via the condensation of p-substitutedaromatic amines (benzene-1,4-diamine,o-tolidine,4,4'-diaminodiphenylmethane, p bromoaniline p-nitroaniline, p-aminobenzoic acid and p-toluidine) with chloral hydrate and hydroxylamine hydrochloride in aqueous solution of sodium sulfate and absolute ethanol as a co-solvent under reflux conditions. The compounds 2(a-g) were cyclized in the presence of a strong acid to obtain 5-sudstituted isatin 3(a-f), which were condensed with aromatic diamines to some new bis-azomethine of isatin 4(a-h) in the presences of catalytic amounts of glacial acetic acid in EtOH. On the other hand some new bis Azomethine of isatin 5(a-d) were synthesized, from the reaction of di-isatins with aromatic monoamines (pyridin-2-amine and 5-chloropyridin-2-amine) in the presences of catalytic amounts of glacial acetic acid in DMSO. The new synthesized compounds were identified using spectroscopic techniques (IR, ^{1}H-NMR, ^{13}C-NMR, DEPT-135, Elemental analysis and Mass Spectroscopy). Finally the prepared compounds have been screened for antimicrobial activity in vitro against two types of bacteria Staphylococcus-aureus (Gr+ve) and Escherichia-coli (Gr-ve) the results showed that most of the prepared compounds are sensitive against both types of tested bacteria

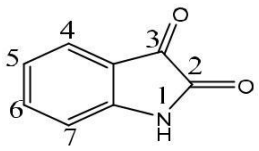
Keywords: Bis-Isonitrosoacetanilide , 5-Substituted Isatin , Bis-Isatins, Bis-Azomethine

1. Introduction

Isatin (1H-indole-2,3-dione)(1). is an aromatic heterocyclic organic compound. It has a bicyclical structure consisting of a six-membered benzene ring fused to a five-membered nitrogen containing ring. The compound's structure is based on the indoline structure but where two carbonyl groups are situated at 2- and 3-position of the five membered rings. Isatin is one of the few compounds to have been synthesized before it was discovered in nature. Isatin is found in plants of the genus Isatis (Guo & Chen, 1986) in Calanthe discolor (Yoshikawa *et al*., 1998) and in Couroupita guianensis (Bergman *et al*., 1985). It has also been found as a component of the secretion from the parotid gland of Bufo frogs (Wei *et al*., 1982) and in humans as it is a metabolic derivative of adrenaline (Ischia *et al*, 1988). The classical methods for the synthesis of isatins are Sandmeyer's method (Alam *et al*., 1989),

The Stolle procedure (Kearney *et al*, 1992), The Martinet procedure (Taylor, 1975) and Gassman procedure (Gassman *et al*, 1977), all using aniline as substrate. isatin and its derivatives are known to be associated with broad spectrum of biological activity like Anti-bacterial (Rodriguez *et al*, 2009), Anti-inflammatory (Srivastava *et al*., 1999), Analgesic (Sridhar and Ramesh, 2001), Anti-fungal (Singh and Dash, 1988), Anticancer activity (Brana & Gradillas, 2004) and Anti-HIV activity (Bal *et*

al., 2005).



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2. Experimental

Melting points were measured in an open capillary and were uncorrected. Thin Layer Chromatography was carried on silica gel glass plates as the stationary phase and mixture of toluene and ethyl acetate 7:3 as the mobile phase. Infrared spectra (IR) were obtained on a thermo-mattson IR-300 spectrophotometer using KBr disk for solid material. ^{1}H NMR ^{13}C-NMR, ^{13}C-DEPT-135 spectra were recorded on Bruke Avauce II 400MHz NMR spectrometer using deuterated DMSO and deuterated chloroform as a solvent, Tetramethylsilane serves as internal standard. Elemental analysis was done on EuroEA Elemental Analyzer. Mass spectra (ms) were recorded on 5973 Network Mass Selective Detector operating by technique at 70 eV.

3. General Procedure for Synthesis of 5-Substituted Isatin Derivatives

3.1 Synthesis of P-substituted Isonitrosoacetanilide 2(a-g)

In a 250 ml round-bottomed flask were placed chloral hydrate (30mmol, 4.96 gm) and 40ml of water. To this solution, crystallized sodium sulphate (60 gm) was added followed by a solution of an appropriate p-substituted aromatic amine (20mmol,) in 30ml of water and concentrated hydrochloric acid(4ml). After which a solution of hydroxylamine hydrochloride (40mmol, 2.78gm) in 50ml of water was added. Finally absolute ethanol (40ml) was added. The reaction mixture was refluxed so that vigorous boiling begins in about 45min, after one to two minutes of vigorous boiling the reaction was completed. During the heating period, some crystals of p-substituted isonitrosoacetanilide started separating out. On cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent. In the case, if the R group contains aromatic amine or amine group, half quantity of appropriate p-substituted aromatic (10mmol) was used. Physical data of the synthesized products are summarized in the Table (1)

3.2 Synthesis of 5-substituted Isatins 3(a-f)

Sulphuric acid (density1.84) 6ml was warmed to 50ºC in a 25ml round bottomed flask fitted with an efficient mechanical stirrer, to this, an appropriate finely powdered p-substituted isonitrosoacetanilide (15mmol) was added at such a rate so as to maintain the temperature between (60-70)ºC, applying an external cooling. After the addition of p-substituted isonitrosoacetanilide, the temperature of the solution was raised to 75ºC to complete the reaction, the mixture was maintained at this temperature for 10 minutes, then the reaction mixture was cooled to room temperature and poured onto crushed ice (0.5kg) with stirring. After standing for half an hour, the separated product was filtered, washed several times with small portions of cold water and dried. Purification of the compound was obtained by recrystallization from suitable solvent. Physical data of the synthesized

products are summarized in Table (1).

4. General Methods for Synthesis of Bis-Imines of Isatin

4.1 Use of Aromatic Monoamine 4(a-h).

5-Substituted isatin (1mmol) and aromatic di-amine (o-tolidine, 4,4'-Diaminodiphenylmethane) (0.5mmol) were dissolved in 20 ml of absolute ethanol in the presence of 2-3 drops of glacial acetic acid as catalyst. The reaction mixture was refluxed and the progress of the reaction was monitored by TLC using different solvent systems. After completion of reaction, the hot mixture was poured onto crushed ice. After standing for half an hour, the separated product was filtered. Then the crude product was purified by recrystallization using DMSO and water. Physical data of the synthesized end products are summarized in Table (2).

4.2 Use of Aromatic Primary Bis-Amines 5(A-D)

di-Isatin(0.5mmol) and aromatic monoamine (pyridin-2-amine, 5-chloropyridin-2-amine) (1mmol) were dissolved in 20 ml of DMSO in the presence of 2-3 drops of glacial acetic acid as catalyst. The reaction mixture was refluxed and the progress of the reaction was monitored by TLC using different solvent systems. After completion of reaction, the hot mixture was poured onto crushed ice. After standing for half an hour, the separated product was filtered. Then the crude product was purified by recrystallization from suitable solvent. Physical data of the synthesized end products are summarized in the Table (2).

5. Determination of Antimicrobial Activity

1- The medium of culture was Muller-Hinton that will prepare by using of nutrient agar and sterilized by autoclave, and poured in Petri dish to a depth of 4 mm.

2- Activation of the bacteria (*S*-aureus and *E*-coli) before culturing on the nutrient agar in nutrient broth which was used for dilution of bacteria and cultivation of culture isolate for 24h in 37 C°

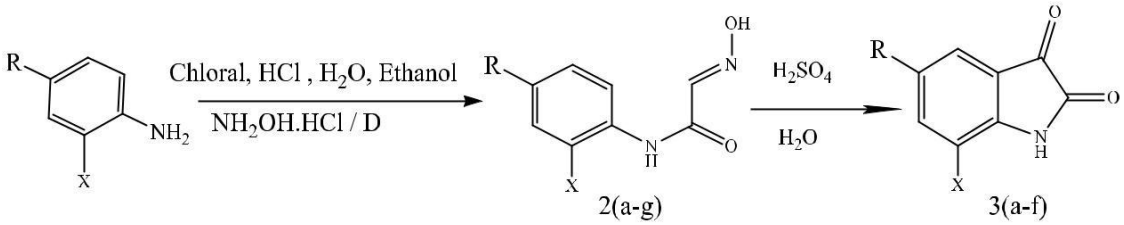
3- Incubation: the inoculated disks were incubated for 24h at 37 C°

4- Reading of zone inhibition the largest zone of inhibition represents by more (+ve) but the unaffected zone represent by (–ve) this was interpreted by national committed for clinical laboratory.

6. Results and Discussion

Reaction of chloral and hydroxylamine salt with various para substituted aromatic amines and aromatic di-amines in aqueous sodium sulphate and absolute ethanol as a co-solvent to form a para-substituted isonitrosoacetanilide, and use of concentrate (HCl) to dissolve aromatic amine. The amounts of water and sodium sulfate were varied over a considerable range, and this concentration was found to give the best yield of product of good quality. Scheme (1) , and the results of this study are summarized in table (1). Bis-imines 4(a-d), 5(a-h)of isatin, were synthesized by condensation of the keto group of isatin with different aromatic amines and di-amines in the presence of catalytic amounts of glacial acetic acid in absolute ethanol or DMSO as a solvents under reflux condition. The time of reaction out checked by TLC using different solvent systems. The results of this study are summarized in table (2). Scheme (2) and Scheme (3). The spectral data reveals that the structures of all the synthesized compounds are in good agreement with the proposed ones. The infrared spectra of synthesized compounds (2b, 3a, 3b, 4a, and 5a), figure (1, 5, 9, 11 and 14) respectively, table(3), show the absorption band at (3400)cm^{-1} as a broad band assigned to stretching vibration of O-H

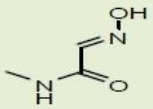
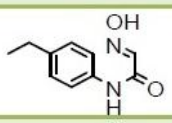
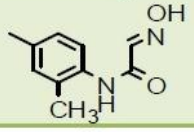
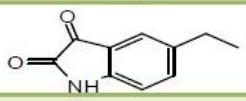
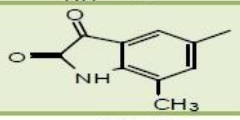
group of oxime. The NH stretching mode of amide group appeared as a single bands at about (3393-3190) cm^{-1} with its bending vibration band at about (1620-1608)cm^{-1}. The bands appeared at near (3062 and 2933) cm^{-1} were assigned to aromatic and aliphatic C-H stretching respectively. Two bands were observed at about (1740 and 1614) cm^{-1} which are assigned to C=O vibration of keto and amides groups respectively. The ^{1}H-NMR spectra of compounds (2b, 3a, 4a and 5a) in DMSO are shown in figure (2, 6, 12 and 15) respectively and peak assignments are given in table (4). The singlet peaks appeared at between δ(10.13-11.11) were assigned to chemical shift of amide proton. Chemical shifts of aromatic protons appeared as multiplet at δ(7.47-6.83). Chemical shifts of methylene protons were observed as a singlet at near δ(3.85). The singlet's at δ (2.5 and 3.3) were assigned to DMSO and its dissolved water respectively (Gottlieb *et al.*, 1997). The ^{13}C-NMR spectra of compounds (2b and 3a) in DMSO are shown in figure (3 and 7) respectively and peak assignments are given in table (8). The spectra showed peaks at δ(184-185) were attributed to carbon atoms of carbonyl group. The peaks at δ(159-160) which were assigned to carbon atom of amide group. The peaks observed at δ(112 -149) were attributed to aromatic carbons. A peak observed at δ (40) ppm due to carbon atoms of methylene group and DMSO. The mass spectra of isatin and other derivatives were examined by Ballantine and Coworkers (Ballantine *et al.*, 1971, 1968) and others (Joaquim *et al.*, 2001; Thetaz & Wentrup, 1976). It was the purpose of this study to further define the fragmentation pathways and rearrangements of Schiff base derivatives of isatin depending on the mentioned reports. The estimated molecular weight of the compound (3b) is 320 g/mol. figure (10), table(7), exhibited a peak at 320.0 with relative abundance (3.23%) which was assigned to molecular ion peak. Other characteristic peaks are observed at m/z values: 279, 264, 251, 212, 195, 180, 167, 152, 130, 115, 91, 77, 57 and 41. Suggested structural assignments of fragments are shown in scheme (4). The mass spectrum of (4b) is shown in figure (13), table (7). Exhibited a peak at 628 with relative abundance (2.16%) which was assigned to molecular ion peak $[M]^{\cdot+}$. Other Characteristic bands are observed at m/z, 419, 391, 296, 269, 235, 210, 206, 199, 155, 152, 77and 43. Suggested fragmentation pathways and structural assignments of fragments are described in Scheme (5). The mass spectrum of (5a) is shown in figure (16), table (7). Exhibited a peak at 458 with relative abundance (2.14%) which was assigned to molecular ion peak $[M]^{\cdot+}$. Other Characteristic bands are observed at m/z 412, 384, 367, 340, 306, 274, 250, 221, 150, 104, 87, 63 and 41 Suggested fragmentation pathways and structural assignments of fragments are described in Scheme (6). The ^{13}C-DEPT-135 spectra of compounds (2b and 3a) in chloroform is shown in Figure (4 and 8) and peak assignments are given in table (5). The spectra showed three positive peaks at δ(138, 124 and 112) for C-H carbon of benzene ring, A signal negative peak at δ (40) due to methylene carbon. The elemental analysis for the compounds (2b, 4a, and 4e) showed good agreement with the calculated value, as shown in the table (6). The action of some prepared p-substituted isonitrosoacetanilide, 5-substituted isatin and bis-imines of isatin on the two types of micro-organisms will be shown in the table (9). There are different effects of the compounds against *S*-aureus (Gr+ve) and *E*-coli (Gr-ve),the more active compounds against *S*-aureus (Gr+ve), were (3c, 4d, 4g and 5d) and against *E*-coli (Gr-ve), were (2d, 3b, 4a , 5b and 5c). While the ineffective compounds against both types of bacteria were (2b and 4f) and others show moderated influences. The most activity of these compounds due to their strongly believed that the specific (–C=N-) grouping (azomethine) is an important structural requirement for the bioactivity of azomethines.



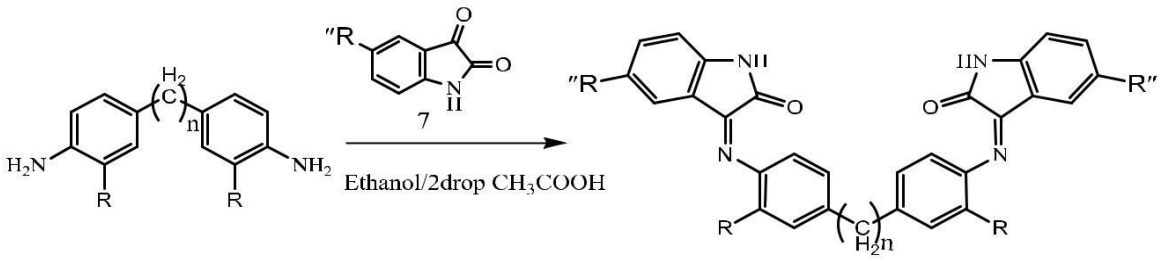
X=CH3,for compounds (2c and 3b) otherwise X= H

Scheme(1)

Table 1: Some physical properties for the synthesized compounds (2a,g) and(3a,f)

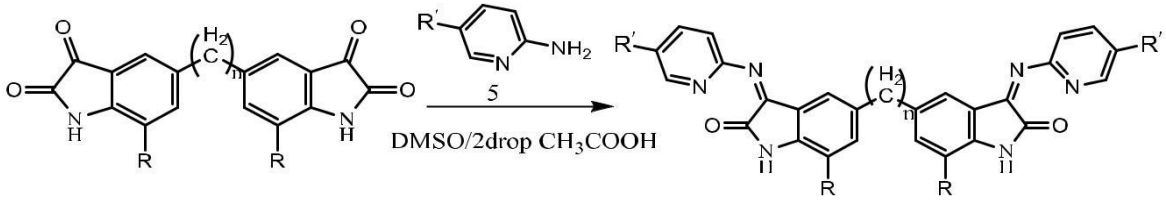
compound	R	Molecular formula	Yield %	m.p °c	R_f
2a		$C_{10}H_{10}N_4O_4$	73	238 -240	0.43
2b		$C_{17}H_{16}N_4O_4$	94	217 – 219	0.48
2c		$C_{18}H_{18}N_4O_4$	84	201 – 203	0.50
2d*	Br	$C_8H_7BrN_2O_2$	83	142 – 144	0.57
2e*	CH_3	$C_9H_{10}N_2O_2$	88	127 – 129	0.44
2f	COOH	$C_9H_8N_2O_4$	90	242 – 244	0.46
2g	NO_2	$C_8H_7N_3O_4$	69	192 -194	0.62
3a		$C_{17}H_{10}N_2O_4$	73	>320 Dec.	0.42
3b		$C_{18}H_{12}N_2O_4$	81	>320 Dec.	0.48
3c	Br	$C_8H_4BrNO_2$	67	138 – 140	0.36
3d	CH_3	$C_9H_7NO_2$	71	156 – 158	0.32
3e	COOH	$C_9H_5NO_4$	85	217 – 219	0.28
3f*	NO_2	$C_8H_4N_2O_4$	88	184 – 186	0.64

R_f : solvent systems (toluene and ethyl acetate 7:3) ,
***** Products have been synthesized before in literature (Calvery *et al.*, 1925).



R=H,CH_3 R''=CH_3,COOH,Br,NO_2 n=0,1 4(a-h)

Scheme(2)



R= H,CH_3 R'= H , Cl n=0,1 5(a-d)

Scheme(3)

Table 2: Some physical properties for the synthesized Compounds 4(a-h) and 5(a-d)

Compound	R	R'	R''	n	Molecular Formula	Reaction time/h	Yield %	m.p °c	Rf
4a	H	-	Br	1	$C_{29}H_{18}Br_2N_4O_2$	2.30	72	274 -276	0.48
4b	CH_3	-	Br	0	$C_{30}H_{20}Br_2N_4O_2$	1.30	85	297 – 299	0.30
4c	H	-	COOH	1	$C_{31}H_{20}N_4O_6$	3.00	68	302 – 304	0.43
4d	CH_3	-	COOH	0	$C_{32}H_{22}N_4O_6$	2.30	60	221 – 223	0.31
4e	H	-	CH3	1	$C_{31}H_{24}N_4O_2$	3.30	73	296 – 298	0.40
4f	CH_3	-	CH3	0	$C_{32}H_{26}N_4O_2$	3.00	65	318 – 320	0.49
4g	H	-	NO2	1	$C_{29}H_{18}N_6O_6$	2.30	74	236 – 238	0.51
4h	CH_3	-	NO2	0	$C_{30}H_{20}N_6O_6$	2.00	69	288 – 290	0.42
5a	H	H	-	1	$C_{27}H_{18}N_6O_2$	3.00	87	254 – 256	0.44
5b	CH_3	H	-	0	$C_{28}H_{20}N_6O_2$	2.30	70	› 320 Dec.	0.50
5c	H	Cl	-	1	$C_{27}H_{16}C_{l2}N_6O_2$	2.00	73	173 – 175	0.46
5d	CH_3	Cl	-	0	$C_{28}H_{18}C_{l2}N_6O_2$	2.00	72	› 320 Dec.	0.49

* R_f : solvent systems (toluene and ethyl acetate 7:3)

Table 3: Assignment of characteristic frequencies (cm^{-1}) of IR spectral data for the synthesized products

Compound	O-H str. Broad band	N-H str. Amide	C-H str. Arom.	C-H str. Aleph.	C-H str. Imine	C=O str. ketone	C=O str. Amide	N-H def. Amide	C=N str. Imine
2a	3550-2600	3381	3050	2921	2854	-	1648	1620	1543
2b	3600-2500	3393	3041	2921	2854	-	1655	1617	1559
3a	-	3282	3050	2853	-	1741	1708	1618	-
3b	-	3190	3101	2923	-	1733	1615	1615	-
4a	-	3268	3042	2914	-	1741	1637	1606	1511
4b	-	3264	3050	2918	-	1752	1651	1608	1472
4e	-	3257	3045	2912	-	1749	1644	1616	1480
4f	-	3268	3023	2919	-	1750	1649	1616	1481
5a	-	3452	3100	2921	-	1739	1672	1617	1488
5b	-	3238	3021	2911	-	1743	1647	1615	1480
5d	-	3196	3093	2919	-	1733	1682	1616	1470

Table 4: Assignment of ^{1}H-NMR spectral data of compounds (2a,b,c.f,g, 3a,b, 4a,e,f, 5a,d).

Compound	(δ) in ppm (multiplicity , intensity ,assignment)
2a	12.15 (s,2H,O-H), 10.17(s,2H,N-H), 7.65(s,6H,Ar-H and =C-H)
2b	12.16 (s,2H,O-H), 10.13(s,2H,N-H), 7.60-7.73(m,4H,Ar-H),7.3(s,2H,=C-H),3.85(s.2H,Ar-CH_2-Ar)
2c	12.20 (s,2H,O-H), 9.52(s,2H,N-H), 7.34-7.71(m,6H,Ar-H),7.0(s,2H,=C-H) ,2.29(s,6H,Ar-CH_3)
2f	12.75(s,1H.COOH),12.28(s.1H.O-H), 7.75-8.01(dd,4H,Ar-H),7.68(s,1H,=C-H),
2g	12.38(s,1H,O-H),10.73(s,1H,N-H),7.95-8.25(dd,4H,Ar-H) ,7.69(s,1H,=C-H)
3a	10.97(s,2H,N-H),6.83-7.47(m,6H,Ar-H),3.85(s,2H,Ar-CH_2-Ar)
3b	11.19(s,2H,N-H),7.75(s,2H,Ar-H), 7.63(s,2H,Ar-H),2.28(s,6H,Ar-CH_3)
4a	11.11(s,2H.N-H),6.84-7.39(m,14H,Ar-H),4.2(s,2H,Ar-CH_2-Ar)
4e	6.74-7.62(m,7H,Ar-H),6.47(s,2H,N-H),4.07(s,2H,Ar-CH_2-Ar),2.23(s,6H,Ar-CH_3)
4f	6.73-7.73(m,12H,Ar-H),6.32(s,2H,N-H),2.12(s,6H,Ar-CH_3),1.95(s,6H,Ar-CH_3)
5a	10.97((s,2H,N-H),6.36-7.42(m,14H,Ar-H), 3.86(s,2H,Ar-CH_2-Ar)
5d	7.24-8.15(m,10H,Ar-H),5.41((s,2H,N-H), 2.3(s,6H,Ar-CH_3)

Table 5: Assignment of ^{13}C-DEPT-135 spectral data of compounds (2b and 3a)

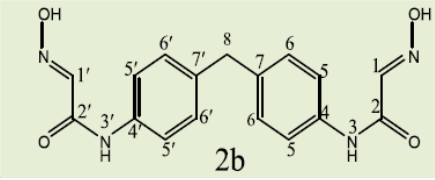
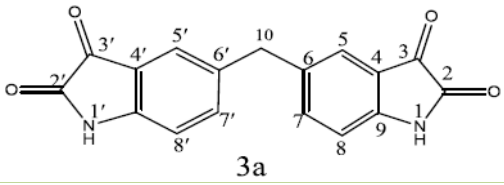
Compound	(δ) in ppm	(Positive/Negative) Peaks	Assignment
 2b	142 129 121 40	Positive (CH) Positive (CH) Positive (CH) Negative (CH2)	C1,1' C6,6' C5,5' C8
 3a	138 124 112 40	Positive (CH) Positive (CH) Positive (CH) Negative (CH2)	C7,7' C5,5' C8,8' C10

Table 6: Elemental analysis for the compounds (2a, 4a, and 4e)

compound	Chemical Formula	Calculated %			Found %		
		C	H	N	C	H	N
2a	$C_{17}H_{10}N_2O_4$	66.67	3.29	9.15	66.23	3.25	9.10
4a	$C_{29}H_{18}Br2N_4O_2$	56.70	2.95	9.12	56.36	3.09	9.56
4e	$C_{31}H_{24}N_4O_2$	76.84	4.99	11.56	76.27	5.15	11.65

Table 7: Mass fragments m/z value of synthesized compounds (3b, 4b, 4d, 4f , 4h and 5a)

compound	m/z value
3b	320, 279, 264, 251, 212, 195, 180, 167, 152, 130, 115, 91, 77, 57,41
4b	628, 419, 391, 296, 269, 235,210, 206,199, 180, 155, 152 , 77, 43
4d	560,433,368,433,314,281,239,198,176,153,130,106,77,43
4f	500,421,371,351,327,297,255,236,212,180,152,130,106,77,41
4h	560,494, 212, 180,154,130,106,77,48
5a	458,412,384,367,340,306,274,250,221,150,104,87,63,41

Table 8: Assignment of ^{13}C-NMR spectral data of compounds 2b,c, 3a,b, 4a,f and 5a)

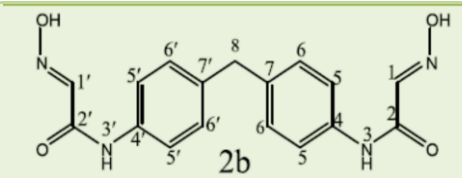
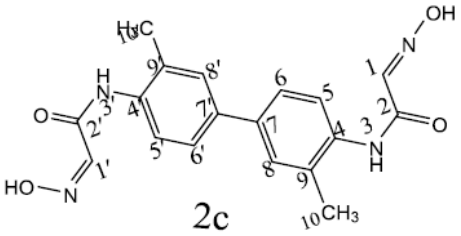
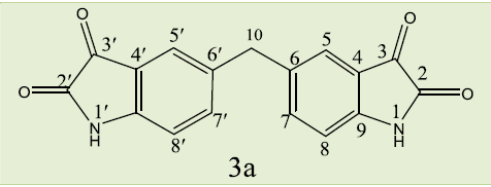
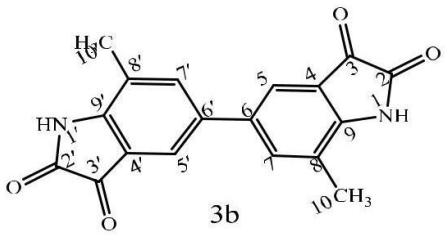
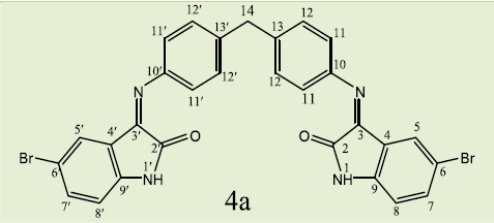
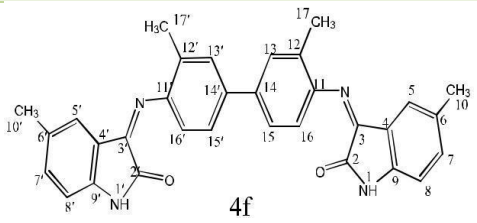
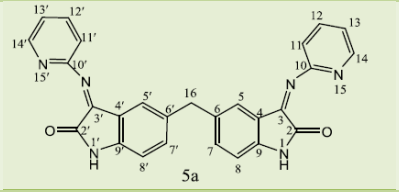
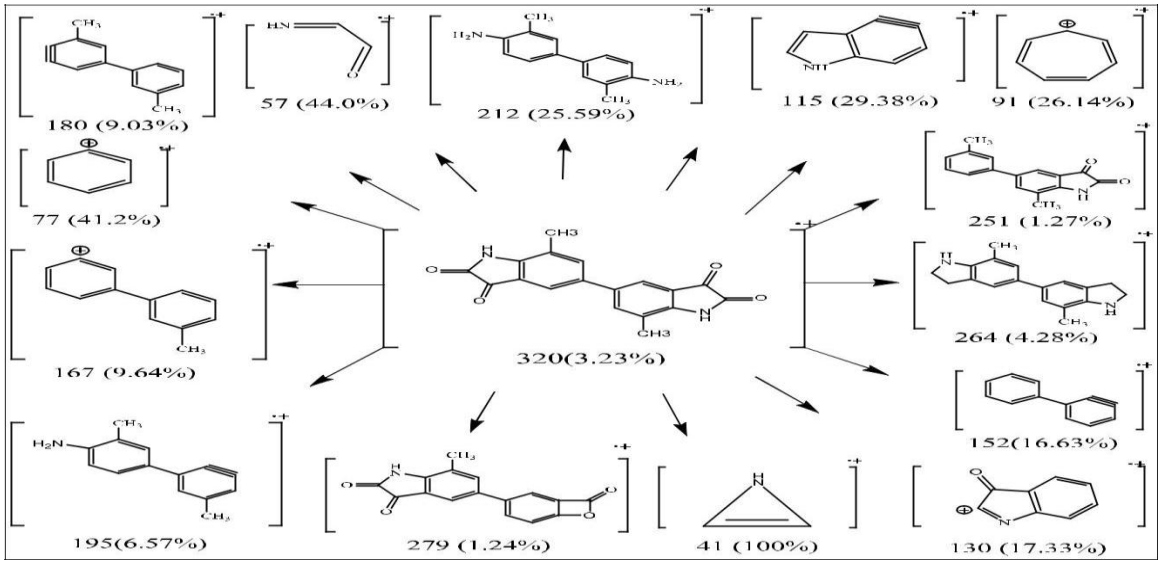
Compound	(δ) in ppm	Assignment	(δ) in ppm	Assignment
 2b	160 142 137 135 129	$C_{2,2'}$ $C_{1,1'}$ $C_{4,4'}$ $C_{7,7'}$ $C_{6,6'}$	121 40	$C_{5,5'}$ C_8
 2c	144 137 134 128 129 124	C2,2' C1,1' C4,4' C7,7' C8,8' C9,9'	125 119 18	C6,6' C5,5' C10,10'
 3a	184 159 149 138 136	$C_{3,3'}$ $C_{2,2'}$ $C_{9,9'}$ $C_{7,7'}$ $C_{6,6'}$	124 119 112 40	$C_{5,5'}$ $C_{4,4'}$ $C_{8,8'}$ C_{10}
 3b	185 160 149 137 133 122 119	C3,3' C2,2' C9,9' C7,7' C8,8' C6,6' C5,5'	118 16	C4,4' C10,10'
 4a	162 154 148 146 139 138 136 130	$C_{3,3'}$ $C_{10,10'}$ $C_{2,2'}$ $C_{9,9'}$ $C_{13,13'}$ $C_{7,7'}$ $C_{5,5'}$ $C_{12,12'}$	128 117 114 113 40	$C_{4,4'}$ $C_{11,11'}$ $C_{6,6'}$ $C_{8,8'}$ C_{14}
 4f	164 154 147 144 138 137 134 130	C3,3' C2,2' C11,11' C14,14' C9,9' C6,6' C13,13' C7,7'	127 124 117 116 115 110 20 17	C12,12' C5,5' C15,15' C16,16' C8.8' C7,7' C10,10' C17,17'
 5a	160 142 137 135 129	$C_{2,2'}$ $C_{1,1'}$ $C_{4,4'}$ $C_{7,7'}$ $C_{6,6'}$	121 40	$C_{5,5'}$ C_8

Table 9: The sensitivity of the prepared products against *E-coli (Gr-ve)* and *S-aureus (Gr+ve)* bacteria

compound	*Escherichia-coli (Gr-ve)*	Staphylococcus-aureus (Gr+ve)
2b	-	-
2c	++	+
2d	++++	+++
2f	-	++
2g	+++	++
3b	++++	-
3c	-	++++
4a	++++	-
4b	++	++
4c	-	++
4d	-	++++
4e	++	++
4f	-	-
4g	-	++++
4h	+	++
5a	+++	+++
5b	++++	++
5c	++++	++
5d	-	++++
5e	+++	++

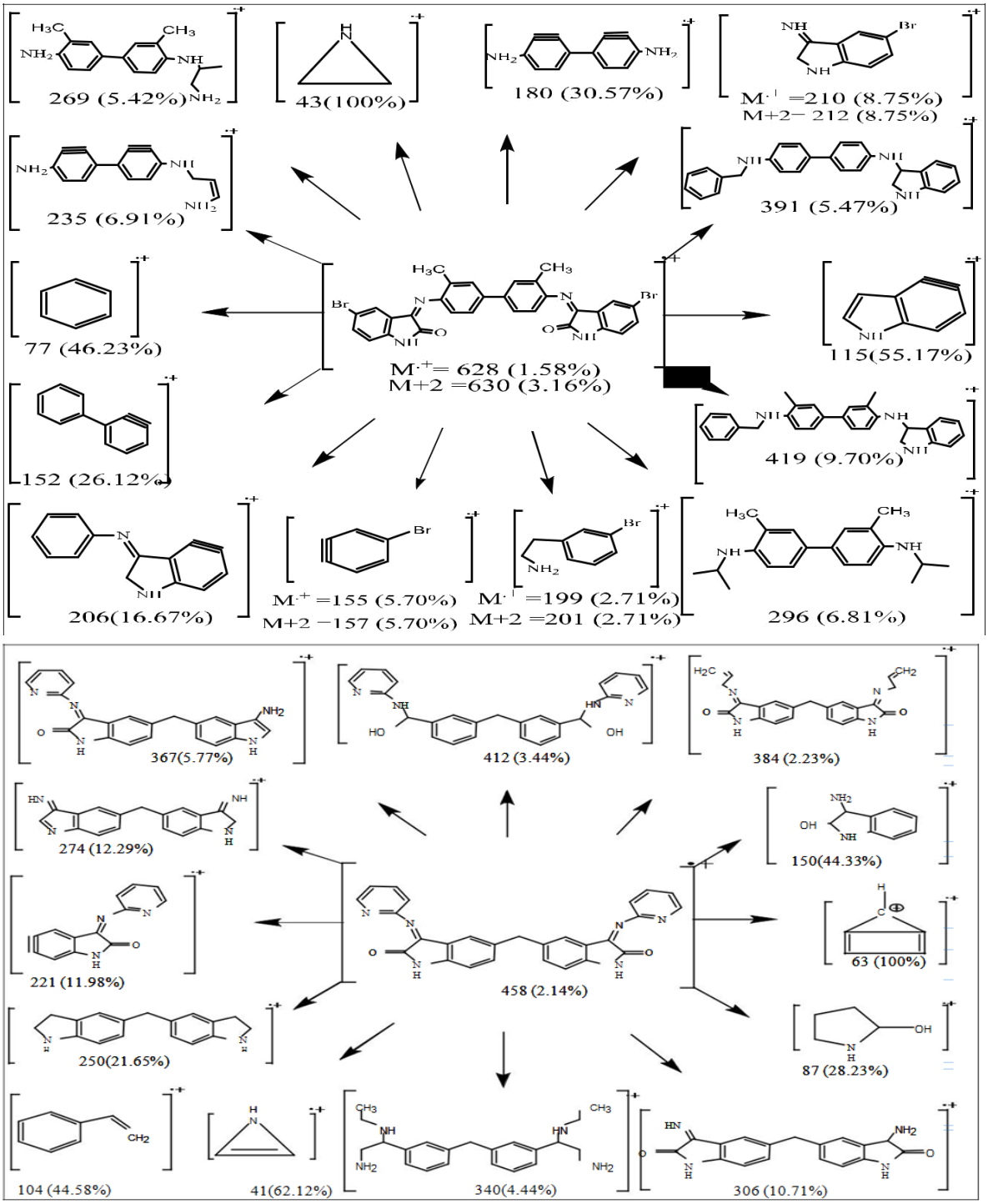
Key to the symbols

* Highly active ++++ (inhibition zone >30mm)
* Active +++ (inhibition zone 25-30mm)
* Moderately active ++ (inhibition zone 15-25mm)
* Slightly active + (inhibition zone 10-15mm)
* Inactive - (inhibition zone <10mm)



Scheme (4): Proposed mass fragmentation of (3b)

Scheme 5: Proposed mass fragmentation of compound (4b)



Scheme 6: Proposed mass fragmentation of compound (5a)

Acknowledgments

The authors deeply thank the Ishik University Research Council for financial support.

7. Spectral representation of some synthesized compounds

Figure(1): Infrared spectrum of compound (2b)

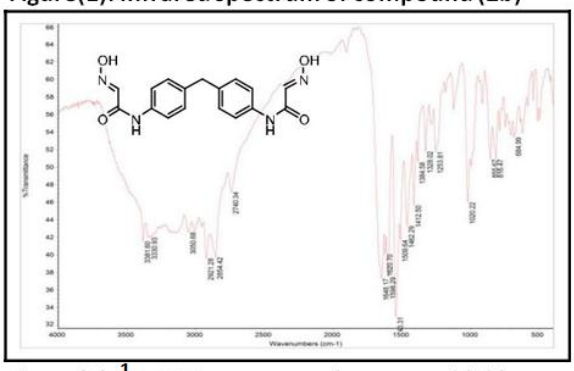


Figure (5): Infrared spectrum of compound (3a)

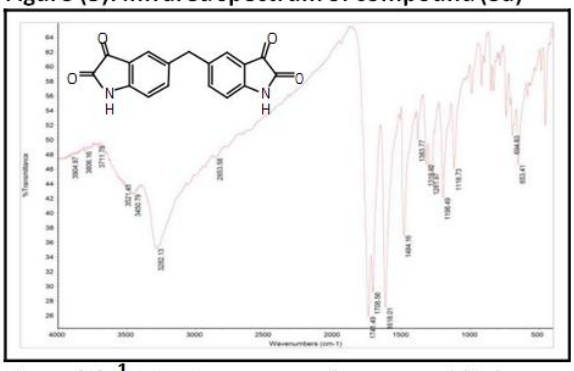


Figure (2): ^{1}H-NMR spectrum of compound (2b)

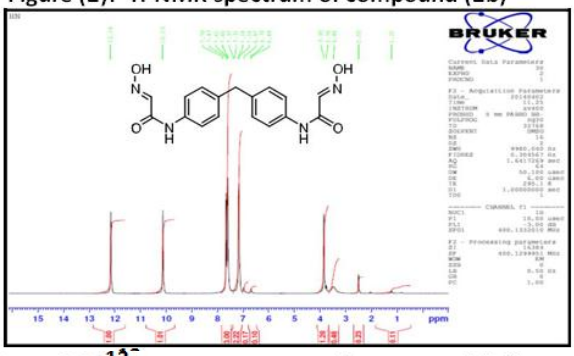


Figure (6): ^{1}H-NMR spectrum of compound (3a)

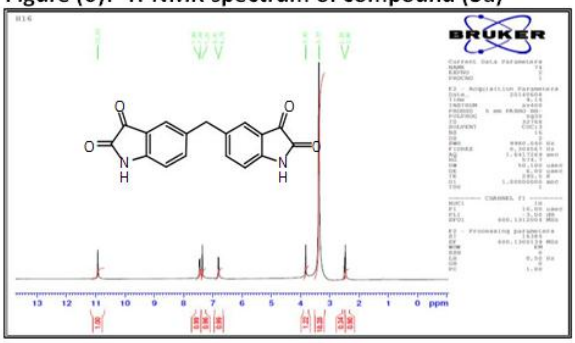


Figure (3): ^{13}C-NMR spectrum of compound (2b)

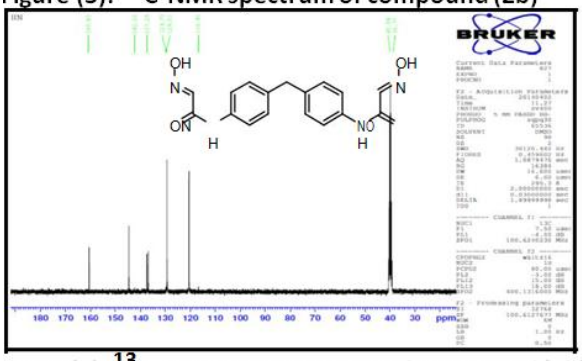


Figure (7): ^{13}C-NMR spectrum of compound (3a)

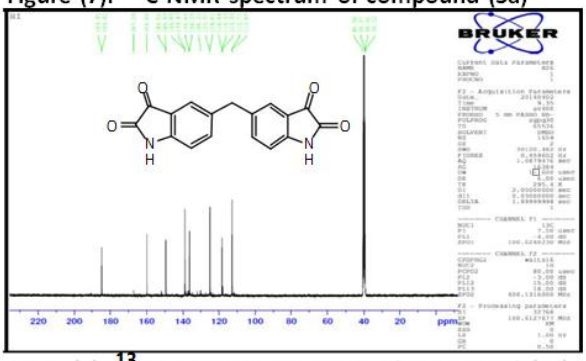


Figure (4): ^{13}C- DEPT-135 spectrum of compound (2b)

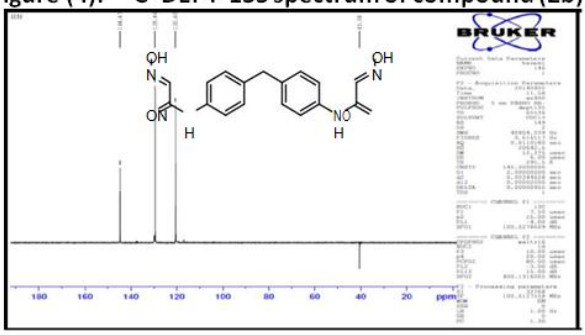


Figure (8): ^{13}C-DEPT-135 spectrum of compound (3a)

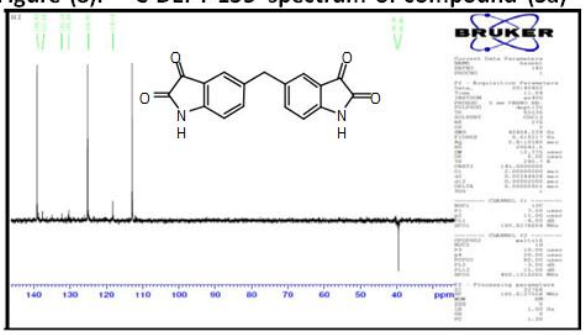


Figure (9): Infrared spectrum of compound (3b)

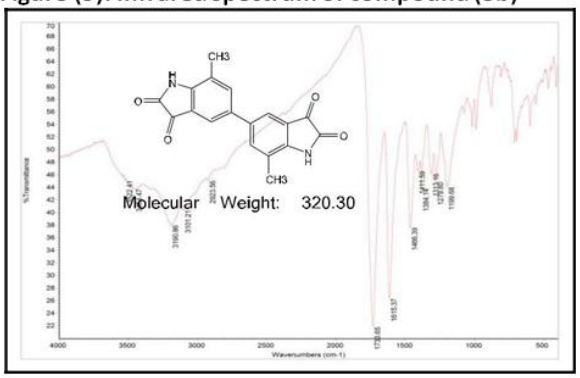


Figure (10): Mass spectrum of compound (3b)

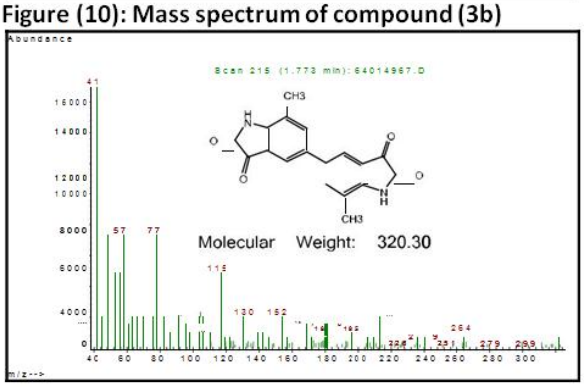


Figure (11): Infrared spectrum of compound (4a)

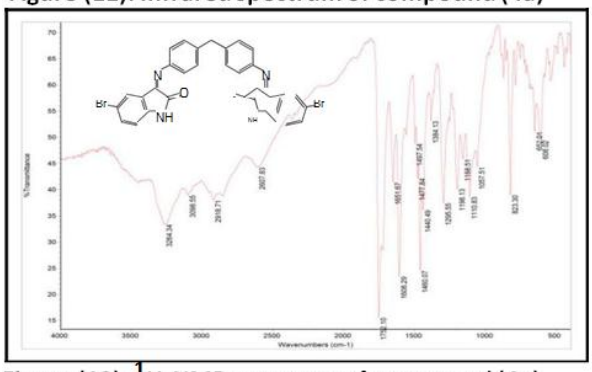


Figure (12): ^{1}H-NMR spectrum of compound (4a)

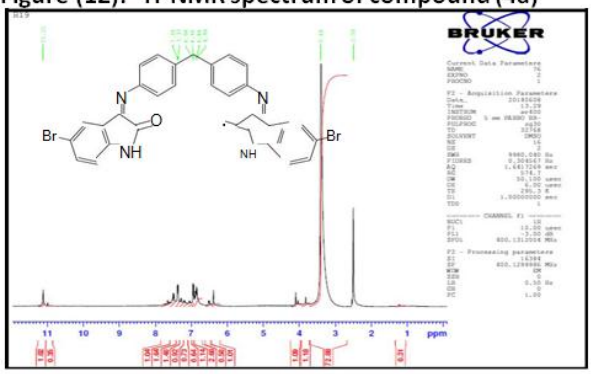


Figure (13): Mass spectrum of compound (4b)

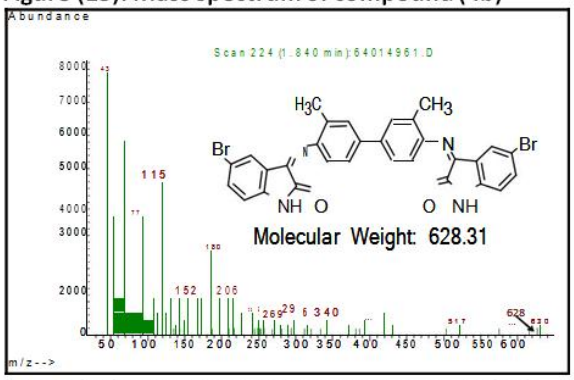


Figure (14): Infrared spectrum of compound (5a)

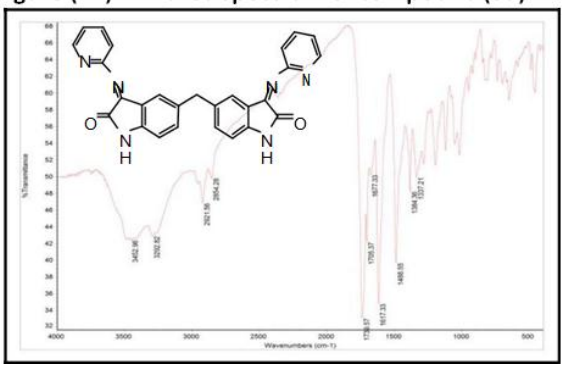


Figure (15): ^{1}H-NMR spectrum of compound (5a)

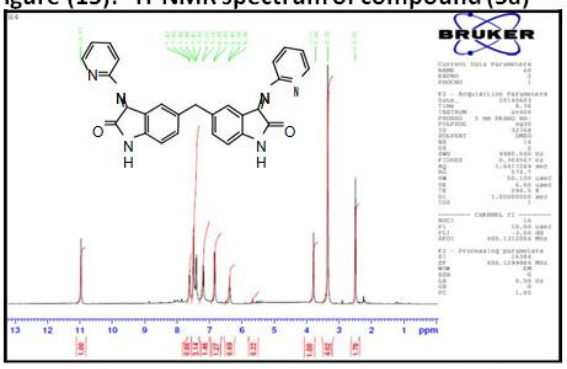
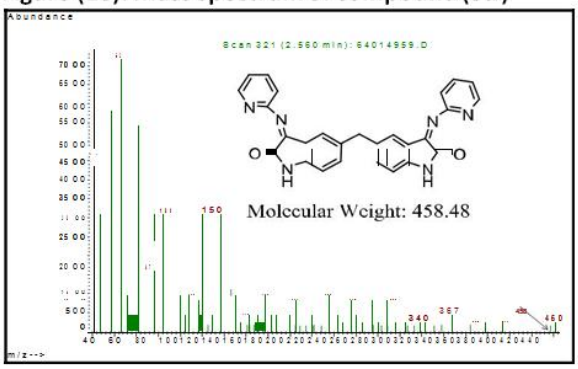


Figure (16): Mass spectrum of compound (5a)



References

Alam, M., Zafar, M., & Naeem, M., (1989). An improved synthesis of isatin, *Pak. J. Sci. Ind. Res*, 32, 246.

Bal, T., Anand, B., Yogeeswari, P., & Sriram, D., (2005). Synthesis and evaluation of anti-HIV activity of isatin beta-thiosemicarbazone derivatives. *Bioorg. Med. Chem. Lett*., 15, 4451-4455.

Ballantine, J. A., Fenwick, R. G., & Alam, M. (1968). Fragmentations in the mass spectra of 2-oxo-3-indolyl derivatives. *Organic Mass Spectrometry*, *1*(3), 467-481.

Ballantine, J. A., Fenwick, R. G., & Popp, F. D. (1971). Rearrangement and cyclization reactions in the mass spectra of a series of isatin carbonyl derivatives of medicinal interest: 2-Oxo-3-indolinylidene anils (n-arylketimines), 2-oxo-3-indolinylidene phenylhydrazones, 2-oxo-3, 3-bis-(o-diaminoaryl) indolyl deriv-atives and their 2, 3-quinoxaline heterocyclic analogues. *Organic Mass Spectrometry*, *5*(8), 1003-1014.

Bergman, J., Lindström, J., & Tilstam, U. (1985). The structure and properties of some indolic constituents in couroupita guianensis aubl. *Tetrahedron*, 41, 2879-2881.

Brana, M., & Gradillas, A. (2004). Synthesis, Biological Activity, and Quantitative Structure−Activity Relationship Study of Azanaphthalimide and Arylnaphthalimide Derivatives, *J. Med. Chem*., 47, 2236-2242.

Calvery, H. O., Noller, C. R., & Adams, R. (1925). Arsonophenyl-cinchoninic acid (arsonocinchophen) and derivatives. II. *Journal of the American Chemical Society*,*47*(12), 3058-3060.

Da Silva, J. F., Garden, S. J., & Pinto, A. C. (2001). The chemistry of isatins: a review from 1975 to 1999. *Journal of the Brazilian Chemical Society*, *12*(3), 273-324.

Gassman, P., Cue, B., & Luh, T. (1977). Indole Ring Synthesis: From Natural Products to Drug Discovery. *J. Org. Chem.*42, 1344-1348.

Gottlieb, H., Kotlyar, V., & Nudelman, A. (1997). NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities, *J.Org.Chem*. 62, 7512-7515.

Guo, Y., & Chen, F. (1986). TLC-UV-spectrophotometric and TLC-scanning determination of isatin in leaf of Isatis. *Zhongcaoyao*, 17, 8-11.

Ischia, M., Palumbo, A., & Prota, G. (1988). Adrenalin oxidation revisited. New products beyond the adrenochrome stage. *Tetrahedron*, 44, 6441-6446.

Kearney, T., Harris, P. A., Jackson, A., & Joule, J. A. (1992). Synthesis of isatin 3-oximes from 2-nitroacetanilides.*Synthesis*, *1992*(08), 769-772.

Rodríguez-Argüelles, M. C., Cao, R., García-Deibe, A. M., Pelizzi, C., Sanmartín-Matalobos, J., & Zani, F. (2009). Antibacterial and antifungal activity of metal (II) complexes of acylhydrazones of 3-isatin and 3-(N-methyl) isatin. *Polyhedron*, *28*(11), 2187-2195.

Sridhar, S., & Ramesh, A. (2001). Synthesis and pharmacological activities of hydrazones, Schiff and Mannich bases of isatin derivatives. *Biol.Bull.*, 24(10), 1149-1152.

Srivastava, S. K., Srivastava, S., & Srivastava, S.D. (1999). Synthesis of new carbazolyl-thiadiazol-2-oxoazitines: Antimicrobial, anticonvulsant andante-flammatory agents. *Indian J. Chem*., 38(B), 183-187.

Thetaz, C., & Wentrup, C. (1976). 1H-Benzazirines. Intermediates in the ring contraction of iminocyclohexadienylidenes and arylnitrenes. *Journal of the American Chemical Society*, *98*(5), 1258-1259.

Singh, W., & Dash, B. (1988). Synthesis of some new Schiff bases containing thiazole and oxazole nuclei and their fungicidal activity. *Pesticides*, 22,33.37.

Wei, L., Wang, Q., & Liu, X. (1982). Application of thin-layer chromatography in quality control of Chinese medicinal preparations. II. Qualitative analysis of some Chinese medicinal preparations of Chansu. *Yaowu Fenxi Zazhi*, *2*(5), 288-291.

Yoshikawa, M., Murakami, T., Kishi, A., & Kubo, M. (1998). Novel Indole S, O-Bisdemoside, Calanthoside, the Precursop Glycoside of Tryptanthrine, Indirubin, and Isatin. *Chem. Pharm. Bull*., 46, 886-888.