Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.7 No.12, 2015

Synthesis, Characterization and Anti-hyperglycemic Activity of some novel Formazans Derivatives

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Abstract

In the present investigation, a series of novel formazans 4[**a – h]**were synthesized by the condensation of Schiff bases**2[a-c]** and diazonium salt chloride of various substituted aromatic amines **3[a-c]** . The intermediate Schiff bases (**2a-2h)** were itself synthesized by the condensation of different aromatic amines**2**(thiocarbohydrazide , nicotinic hydrazide , carbohydrazide) with various aromatic aldehydes**1**.The completion of reactions was checked by TLC.The structures of the formazan compoundswere identified by FT- IR ,^{1}H-NMR , ^{13}C- NMR and mass spectral studies. Newly synthesized compound was screened for their anti- hyperglycemiaactivity**.**

Keywords:: Formazans, Schiff bases Anti-hyperglycemic

1.Introduction

Formazans are characterized by intense colors, ranging from cherry red to a deep purplish black and contain the characteristic chain of atoms [-N=N-C=N-NH –]. Formazans are generally solids of relatively low melting point in spite of large the size of the molecules [Senoz ,2012]. Formazans have been studied extensively because of their ready accessibility, diverse chemical reactivity, broad spectrum of biological activities and wide range of applications [Anand et al.,2009;Chavan et al.,2012].Triphenyl formazans are often particularly soluble in chloroform and acetone, while in water the solubility appears to be negligible and the solvent being colored. Their structure was first revealed by Bamberger and by von Pechmann who agreed to call them formazyl compounds. In 1933, German usage is exemplified by Beilstein, in which the compound is termed formazan. The compound which is substituted with three phenyl groups is called 1, 3, 5-triphenyl formazan [Gilroy, 2008].

Formazans have been found to possess important medical applications; the tetrazolium salts are classified as promoter of vitality formazans and heterocyclic hydrazones are known for their spectrum of biological activities such as antiviral antimicrobial, anti-inflammatory , anticancer , anti-parkinsoian activity[Khanna et al.,1990; Kumar et al.,1983; Naithani et al.,1989; Kumar et al.,1985] anti-HIV[Amarish et al.,2010] ,antifungal [Desai et al.,2005] antibacterial [Pannerselvam et al.,2009;Xiaoxiaojin et al.,2009].anti-fertility [Desai et al.,2003],etc. Formazans have been found to possess important medical applications [Marjadi et al.,2009].

Diabetes mellitus is characterized by chronic hyperglycemia and glucosuria produced by an absolute or relative insufficiency of insulin. The ailment may result into the development of further metabolic and anatomic disturbances among which is Lipemia, hypercholesterolemia, loss of weight, ketosis, arteriosclerosis, gangrene, pathologic changes in the eye, neuropathy, renal disease and coma [Andrew et al.,2009; Swanston-Flatt et al.,1990]. Management of diabetes mellitus sans side effects is still a challenge for the pharmaceutical world [Sharma et al.,2003].In the present study we have synthesized eight substituted formazan derivatives **4[a -4h**] by coupling Schiff bases prepared fromdifferent aromatic amines (thiocarbo hydrazide , nicotinic hydrazide , carbohydrazide) with various aromatic aldehydes and various aldehydes with appropriate diazonium salts chlorides in pyridine. The structures of these derivatives were assigned on the basis of, IR ,^{1}H-NMR^{13}C-NMR and mass spectral data. The synthesized compounds were screened for their anti- hyperglycemiaactivity.

2.Experimental

2.1 Chemicals

chemicals are purchased from BDH,and used without further purifications. The purity of the synthesized formazan derivative was checked by thin layer chromatography (TLC). FT-IR spectra are recorded in KBr, Shimadzuspectrophotometer in the range of 4000-200 cm^{-1}. Melting points are measured with an electrothermalsturat apparatus, model SMP30**.** ^{1}HNMR spectra are recorded on a Bruker 300 MHz spectrometer in DMSO-d_6, chemical shift in ppm relative to internal Me_4Si. ^{13}C – NMR spectra on Bruker 500 MHz spectrometer in DMSO-d_6 solvent. Mass spectra are recorded with Agilent technologies 5975 mass spectrometer.

2.2 method of synthesis of Schiff bases 2[a – h]
In general, the mono-imines **2[a – h]**were prepared by the reaction of the mixture of (0.01) mole amine with (0.01) mole aldehyde in 20 ml of methanol or chloroform and 4-6 drops of glacial acetic acid was heated in water bath at (50-60˚c), the reaction mixture was refluxed for (0.5-3) hrs. with stirring, the progress of the reaction was followed by TLC using hexane : ethyl acetate 1:3 as eluent. After completion , the solvent evaporated and then recrystallized from ethanol[Hello,2000; Krishnaswamy,2002].

2.3 General Procedure of Diazotization of amine 3[a- c]
A suitable amine(0.003 mole) dissolved in water was cooled to (0-5 ˚c) and diazotized with (0.003 mole, 0.21 g) sodium nitrite solution (10 ml) and 3M HCl (10ml). The rate of addition was adjusted so that the temperature of the solutions remains below 10 ˚c . The solution was kept in an ice bath and used immediately in the next step [Ahmed,2011].

2.4General procedure of preparation of formazans 4[a- h]
The solution of Schiff base [2a- 2h] (0.003 mole) in pyridine (20 ml) was reacted with cold diazonium chloride 3[a- c] (0.003 mole) in the presence of sodium acetate (0.3 gm) in ice bath at 0-5 ˚c for (2-3) hr. colored product obtained was filtered and washed with water till it was free from excess pyridine and recrystallized from ethanol [Ahmed,2011, Desai ,2006]. Their physical properties and analytical data are recorded in Table 1.

2.5 Evaluation of anti-hyperglycemic activity

Animals and Housing
Twenty four healthy adult male mice (Mus musculus , Balb/c) weighing (25-35 g) of a week were used in the present study. Animals were housed in the animal house of Biology Dept. College of Education, Thi-Qar University Iraq. Animals were housed in iron boxes bedded with wooden chips. During the experimental period, six animals were kept in each box and they were housed under standard laboratory conditions (12hrs light:12hrs dark photoperiod (LD)at 22 ± 2 ºC and relative humidity (45-55%)[Coskum et al.,2004]. Animals were fed on standard rat pellet and tap water *.Ad libitum.* The standard pellet contains wheat 66.6%, soya 25.6%, and sun flower oil 4.4%, lime stone 1.5%, salt 0.63%, methionine 0.158%, choline chloride 0.062% and trace elements 0.05%[Krinke,2000]**.**

Induction of Diabetes:
Diabetes was introduced experiment ally in male laboratory mice by withholding food for (12 hours) approximately by a single subcutaneous injection of (125 mg / kg B.W) of alloxan monohydrate dissolved in distilled water (D.W) immediately before injection. The controlled animals received normal saline only[Nimenibo.2003]. Alloxan treated animals were allowed to drink of D- Glucose 5% overnight to prevent the potentially massive insulin release following alloxan injection. After seven days of injection the animals show signs of extreme fatigue and frequent urination readied infected with diabetes[Alarcon,2002].

Synthesized formazan derivative administration
The amount of synthesized formazan derivative **[4e]** was calculated for each mice on body weight basis (75 mg/kg and 150 mg/kg) fatal hypoglycemia occurring as a result of respectively and prepared for each by dissolved in 3 ml of DMSO, the synthesized compound was administered to each mice orally using a syringe**.** The amount of synthesized formazan derivative **[4e]** was calculated for each mice on body weight basis (75 mg/kg and 150 mg/kg) fatal hypoglycemia occurring as a result of respectively and prepared for each by dissolved in 3 ml of DMSO, the synthesized compound was administered to each mice orally using a syringe[Tanira et al.,1996]**.** Some physical properties for synthesize formazan compounds are shown in Table 1.

3.Results and Discussion
The physical properties of novel formazan derivatives4[a- h] are presented in Table1.The compounds are quite stable and they are soluble in most organic solvents. Synthetic routes leading to target compounds are summarized in Scheme1.The structure of these compounds were proven on the basis of melting points and spectral data.

3.1Characterization of synthesized compounds 4[a -h].
Synthesis ofN-(2-(2-hydroxynaphthalen-1-yl))-carbo hydrazide formazans [4a].
IR (KBr disk. cm^{-1}) : 3255 br. (-N-H str.) , 3010w (C-H)Ar. , 1597w (C=N str.) , 1677 s (C=O str.) , 1543w (N=N) , 1488w (C=C str.) , 1327m (C-N) . ^{1}H -NMR (300 MHz, DMSO- d6) : 4.15 (2H , s, $-NH_2$) , 7.07 – 8.24 (10H, m, aromatic protons) , 9.05 , 10.94 (2H, s, -N-H), 12.22 (1H , s, -OH carboxylic acid) , 4.72 (1H , s, -OH

phenolic).^{13}C- NMR spectra (75 MHz . DMSO- *d6.* ppm): 109.43- 134..00 (phenyl group), 143.24 (C-N), 148.38 .151.18 (C=N), 156.74 .

3.2 Synthesis of N-(2-(2-hydroxynaphthalen-1-yl))– thiocarbohydrazide formazans [4b].
IR (KBr disk. cm^{-1}) :3217w (-N-H str.) , 3010w (C-H Ar.) , 1689m (C=N str.) , 1597 (C=O) , 1465w (N=N str.) , 1419w (C=C) , 1327m (C-N) .^{1}H -NMR (300 MHz, DMSO- d_6) : 7.24 -8.23 (10H , m, proton aromatic), 8.82 – 9.15 (4H , terta. , pyridyl group) , 9.47 (1H , s, -N-H) , 4.75 (1H , s, -OH phenolic , 12.31 (1H ,s, carboxylic acid).^{13}C- NMR spectra (75 MHz . DMSO- d_6. ppm): 108.53 – 135.43 (phenyl group) , 147.41 (C-N) , (148.38 ,152.58) (C=N) , (158.10 , 161.15) (2C=O).

3.3 Synthesis of (Z)-3-(4-acetamidophenyl)-5-(4-amino phenyl) -1-(hydrazine carbonyl) formazan [4d].
^{13}C- NMR spectra (75 MHz . DMSO- d_6. ppm): 24.07 (-CH_3) , 118.82- 129.30 (phenyl group) , 140.37 (C-N) , 152.04 (C=N) , 168.44 (C=O) . M^+ = 354 m/z.
IR (KBr disk. cm^{-1}) : 3212w (-N-H str.) , 3099w (C-H aromatic) , 2894w (C-H aliphatic) , 1512m (C=C str.) , 1319m (C-N str.).^{1}H -NMR (300 MHz, DMSO- d_6) : 2.09 (3H, s, -CH_3), 3.23 (1H , s, -NH) , 7.26- 8.49 (H, m, aromatic protons) , 10.06 .10.56 (2H , s, -NH).

3.4 Synthesis of (Z)-3-(4-acetamidophenyl)-5-(4-amino phenyl)-1-nicotinoylformazan [4e].
IR (KBr disk. cm^{-1}) : 3224s (-N-H str.) , 3055m (C-H aromatic) ,2846w (C-H aliphatic) , 1651s (C=N str.) , 1589m (C=O str.) , 1527s (N=N str.) , 1411m (C=C) , 1303s(C-N).
^{1}H -NMR (300 MHz, DMSO- d_6) : 2.07s (3H, s, -CH_3) , 4.79 (2H , s, -NH) , 7.47 – 7.68 (8H , m , aromatic protons) , 8.22 – 7.06 (4H , tert, pyridyl group) , 10.16 . 11.93 (2H, s, -NH). M^+ = 401 m/z.

3.5 Synthesis of (Z)-3-(4-acetamidophenyl)-5-(4-amino phenyl)-1-(hydrazine carbon thioyl)formazan [4f].
IR (KBr disk. cm^{-1}) :3420w (N-H str.) , 3100w (C-H aromatic) , 2985m (C-H aliphatic , 1535w (N=N str.) , 1512w (C=C str.) , 2300w (C=S) , 1319s (C-N str.).
^{1}H -NMR (300 MHz, DMSO- d_6) : 2.01s (3H, s, -CH_3) , 3.31 (2H, s, -NH_2) , 7.18 – 8.07 (8H , m, aromatic protons) , 10.05 , 11.33 (2H , s, -NH) . ^{13}C- NMR spectra (75 MHz . DMSO- d_6. ppm): 14.11 (-CH_3) , 118.72 – 128. 91 (phenyl group) , 140.68 (C-N) , 141.96 (C=N) , 168.60 (C=O) , 175.81 (C=S) .

3.6 Synthesis of N,N' bis-(2-(2-hydroxynaphthalen-1-yl)) -carbohydrazide formazan [4g].
IR (KBr disk. cm^{-1}) **:** 3248 br. (-NH) , 3039w (C-H aromatic) , 2899w (C-H aliphatic) , 1651s (C=N) , 1560w (C=O str.) 1558s (N=N str.) , 1465m (C=C str.) , 1319m (C-N str.).
^{1}H -NMR (300 MHz, DMSO- d_6) **:** 1.94 (3H , s, para CH_3) , 7.10 -8.31 (16H , m , aromatic protons) , 10.97 (1H , s, -NH) , 4.75 (1H , s, -OH phenolic) , 3.30 (1H , s, N=CH).**^{13}C- NMR spectra (75 MHz . DMSO- d_6. ppm):**14.49 (-CH_3) , 108.66 – 127.59 (phenyl group) 133 (C-N) , 153.15 (C=N) , 157.18 (C=O) . M^+ = 516 m/z .

3.7 Synthesis of 1-(2-(4-acetamidobenzylidene) hydrazinecarbonyl)-3- (4-acetamidophenyl)-5- (p-tolyl) formation[4h].
IR (KBr disk. cm^{-1}) :3402m (-N-H str.) , 3109m (C-H aromatic) , 2980, 2859 w (C-H aliphatic) , 1681 (C=N str.) , 1597s (C=O str.) , 1527w (N=N) , 1500w (C=C str.) , (1373, 1319)s (C-N) . ^{13}C- NMR spectra (75 MHz . DMSO- d_6. ppm): 24.09 (-CH_3) , 118.48 – 129.32 (phenyl group) , 140.39 (C-N) , 152.06 (C=N) , 168.47 (C=O) . M^+ = 498 m/z.

3.8 IR Results
The IR spectra of all compounds are recorded in the solid state using the KBr disk technique. The formation of Schiff base **2[a-h]** was indicated by their IR spectra from the appearance of azomethine CH=N stretching band at (1597-1689)cm^{-1} combined with the disappearance of IR band in region 3376 cm^{-1} and 1712 cm^{-1} corresponding to NH_2 group and C=O group of various aldehydes 1 and deferent amines**(2)[**Albayate.,2009, Issa et al .,2008]. While formazan derivatives **4[a-h]** confirmed by the appearance of IR band in the region 1465-1558 cm^{-1} due to -N=N- group and the disappearance of band at 1597-1689 cm^{-1} (-N=CH-) [Jignesh et al.,2009].

3.9 Mass Spectral Results

The Mass spectrum of (Z)-3-(4-acetamidophenyl)-5-(4-amino phenyl) -1-(hydrazine carbonyl) formazan [4d] , shows the ion peak corresponding to the particular compound = 354 m/z , and the fragmentation of [4d] showed the peaks at (339 , 311, 284, 65 , 220, 193 , 162 ,134 , 93) m/z which are attributed to the fragments of[$C_{15}H_{15}N_8O_2^+$, $C_{14}H_{15}N_8O^+$,$C_{13}H_{14}N_7O^+$, $C_5H_5^+$, $C_8H_{10}N_7O^+$, $C_7H_9N_6O^+$, $C_7H_6N_4O^+$, $C_6H_6N_4^+$] respectively.

Figure 4 the Mass spectrum ofE-1-(2-(4-acetamidobenzylidene)hydrazine carbonyl) -3-(4-acetamidophenyl)-5-(p-tolyl) formazan [4h] , shows the molecular ion peak corresponding to the particular compound 498 m/z , and the fragmentation of [4h] showed the peaks in (483 , 455 , 322, 364 , 119, 245 , 134 , 91 ,65) m/z which attributed to the fragment of [$C_{25}H_{23}N_8O_3^+$, $C_{24}H_{23}N_8O_2^+$, $C_{17}H_{16}N_5O_2^+$, $C_{18}H_{18}N_7O_2^+$, $C_7H_7N_2^+$, $C_{11}H_{11}N_5O_2^+$, $C_8H_8NO^+$, C_7H_7 , $C_5H_5^+$] respectively .

3.10 Effect of synthesized formazan derivative on blood glucose levels in normal and diabetic mice Results of determination of serum glucose concentration in males mice groups (A , B , C , D, and E) are given in Table 2and Figure 5 **.** A significant decreased can be observed among (D, E) groups as compared with group(A)after treatment (2 week) with (75 mg /kg) and (150 mg /kg)**.**respectively of formazan derivative [4e]. Also there is a significant elevation that can be observed ($P \leq 0.05$) in groups (B) as compared with group(A). The increase in blood glucoselevel is due to beta cell destruction by alloxan which lead to retardation of insulin production. glucose cannot enter into the cell leading to rise its level in the blood[Nelson et al.,2000;DeCarvalho et al.,2003]. The levels of serum glucose decreased significantly in (D and E) groups as compared to group (B) after treatment (2 weeks) with (75 mg /kg) and (150 mg /kg) from formazan compound **[**4e**]** , while groups (A , C)do not appear significant differences between them. This activity of test compounds**[**4e**]**may be due to increased utilization of glucose as indicated by decreased serum glucose levels and an increase in the activity of glycogen synthetase enzyme as evidenced by rise in liver glycogen contents in test groups

4.Conclusion

Synthesis of formazan derivatives by the condensation of Schiff bases and diazonium salts choride is the eased method to synthesis of formazanderivatives. The effect of synthesized formazan derivative on normal and diabetic mice proved that the synthesized formazan derivative exhibited better anti-hyperglycemic property. Formazan derivative was Synthesized show a highly significant decrease in blood glucose levels.

References:

Şenöz H.(2012):The Chemistry of Formazans and Tetrazolium Salts , Hacettepe. J Biol and Chem; 40(3):293-301.

Anand B, Venkatesan P and Matheswaran P(2009): E J Chem. , 6(1):438-444.

Chavan SB, Zangade SB, Archana, VIbhute Y ,and Vibhute YB(2012): J. Chem Pharm Res**.**, 3 (1):262-269.

Gilroy JP.(2008): Thesis of doctor of philosophy, University of Victoria.

Khanna R.,Saxena A.K.,Srivastava V.K. and Shanker K.(1990): Indian J Chem.,29(B), 91.

Kumar P., Nath C., Agarwal J.C.,Bhargava K.P.and Shanker K.(1983): Indian J Chem.,22(B), 955.

Naithani P K, Srivastava V K, Barthwal J P, Saxena A K, Gupta T K and ShankerK(1989):Indian J Chem., 28(B), 990.

Kumar P., Nath C.and Shanker K.(1985): Pharmazie, 40, 267.

Amarish B. Samel and Nandini R. Pai(2010) : J. Chem. Pharm. Res.,2(4):60-67.

Desai K J,and Desai K R(2005): Indian J Chem. , 44(B), 2097.

Pannerselvam p , Rather B.A , D.R..Reddy, and Kumar N.R.(2009): Eur. J.Med. Chem, 44 (5), 2328-2333**.**

XiaoxiaoJin, Jiangtao Wang, and Jie Bai(2009): Carbohydrate Res., 344(6), 825-829.

Desai J.M.,and ShahV.H. (2003): Indian J.Chem.,42(B), 631.

Marjadi S.I., Solanki J.H.and Patel A.L.(2009):E.J.Chem. **,** 63:844-848.

Andrew I.R., Belinda E. C., Helen H., Michael D. E. and Scott C. B.(2002): Journal of the Pancreas, 14: 208-210

Swanston-Flatt, S.K., Day C., Bailey C.J. and Flatt P.R.(1990): Diabetologia., 33: 462-466.

Sharma P.P. andMujundar A.M.(2003): Indian J. Tradit. Knowledge, 2:292–298.

Hello,K .M .(2000): Iraqi J .of Chem., 24 , 266 .

Krishnaswamy, D.(2002): Tetrahedron., 34 , 4567.

Ahmed M. Jasim (2011): Journal of Basrah Researches (Sciences), 37, **5.A**, 1817-2695.

Desai K.J.and Desai K.R.(2006): J. Heterocycl.Chem., 43(4): 1083-1089**.**

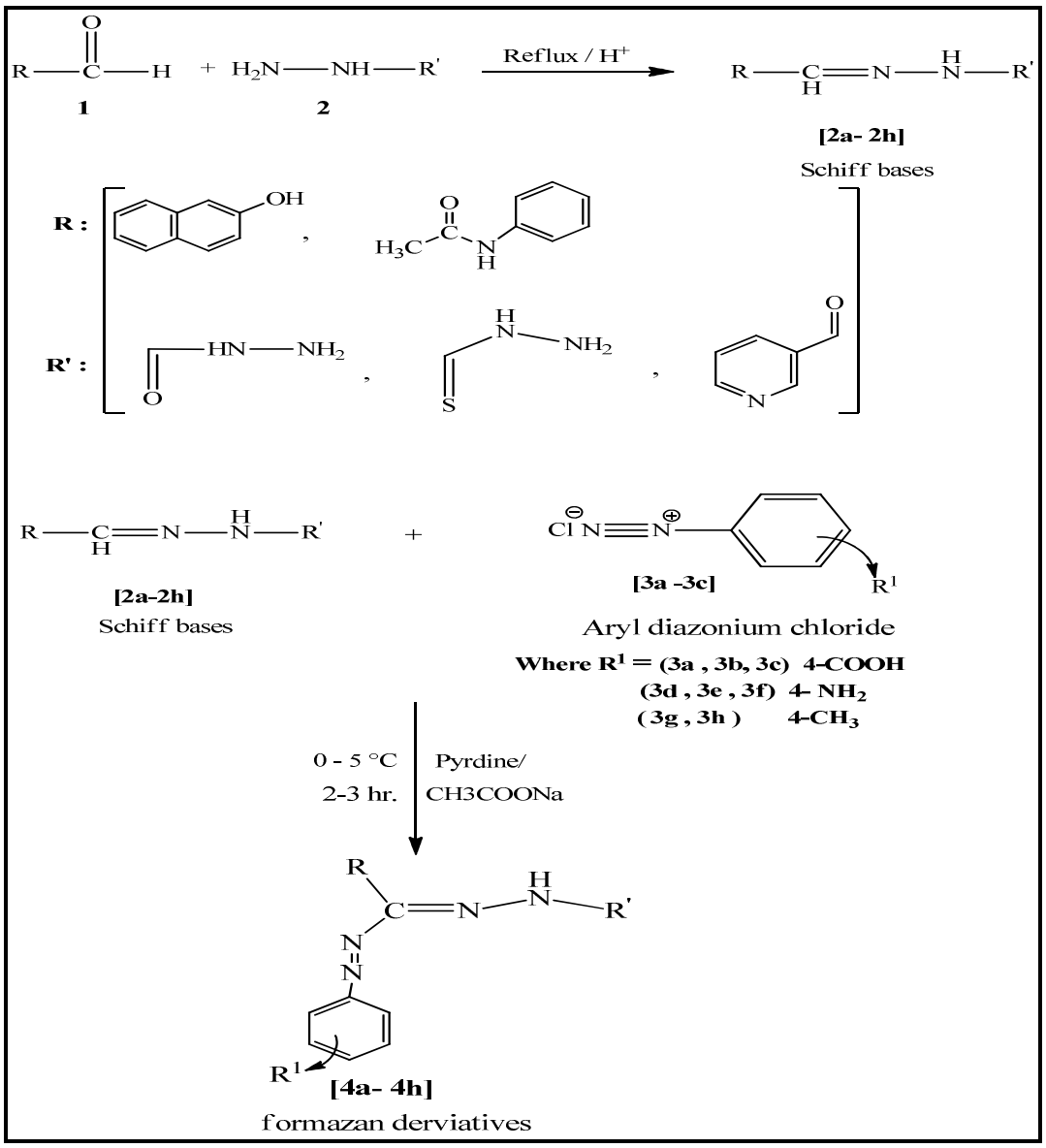
Coskun,O; Ocakci,A; BayraktarogluT.and Kanter,M; Tohoky(2004), J.Exp. Med, 203:145**.**

Krinke,G; J.A (2000):Harcourt Science and Technology company Sand Diego **.**

Nimenibo-Vadia,R.(2003) :Tropical journal of pharmaceutical Research. 2: 183-189**.**

Alarcon-Aguilara, F. J. ,Romas, , Perez-Gutierrez, S. , Aguilar-Contreras, A. ,Contreras-Weber, C.C. and Flores-Saenz, J.L.(2002): *Journal of Ethnopharmacol*. 61 (2) : 101 – 110.

Tanira M.O., Wasfi I.A., Homsi M.A. ,and Bashir A.K.(1996): *J. Pharm. Pharmacol,* **48**:1098-1102.
Albayati R.I.,Lafta S.J. and Alrakaba S.A (2009) :ALmustansiriya,J,Sci,20,2**.**
Issa R.M., A.M.Khedr and Rizk H.(2008): J Chem,Soci,55,875- 884.
Jignesh P.R., Purvesh R.P.and H.P.Nilesh: Inter J Chem,Res,13, 610-630.
Nelson, D. L. and Cox, M.M. (2000)" :Lehninger Principles of Biochemistry". 3ed ed. Worth Publishers. U.S.A.: 790-885.
DeCarvalho, E.N. , DeCarvalho, N.A.S. and Ferreiva, L.M.(2003): Experimental model of induction of diabetes mellitus in rats. *Acta. Cir. Bras* . 18 .



Scheme 1 : Synthetic route to formazan derivatives

Table 1 : Some physical properties data for the synthesized formazan compounds 4[a- h]

Compound Symbol	Molecular Formula	m.p °c	Color	Yields%	Reaction time (hr.)	Molar mass g/mole
4a	$C_{19}H_{16}N_6O_4$	280-281	Orange	62	2.5	392.12
4b	$C_{19}H_{16}N_6O_3S$	276-277	Yellow crystal	65	3	408.10
4c	$C_{24}H_{17}N_5O_4$	297-298	Orange crystal	70	2	439.42
4d	$C_{16}H_{18}N_8O_2$	270-271	Dark green	69	2	354.16
4e	$C_{21}H_{19}N_7O_2$	263-264	Brown powder	71	3	401.16
4f	$C_{16}H_{18}N_8OS$	220	Pale brown powder	67	2.5	370.13
4g	$C_{31}H_{27}N_6O_3$	278-279	Red cherry powder	71	3	516.21
4h	$C_{26}H_{26}N_8O_3$	283- 285	Red cherry crystal	65	2	498.21

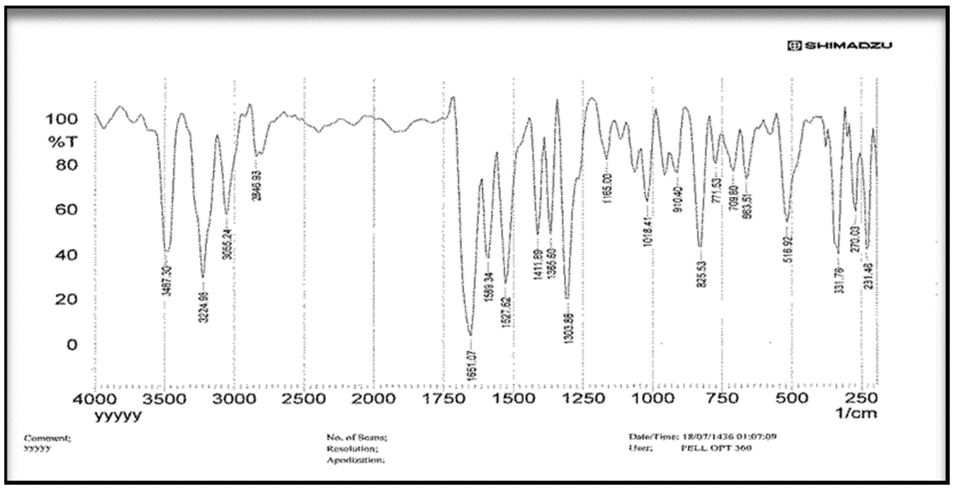


Figure 1 : IR spectrum ofcompound [4e]

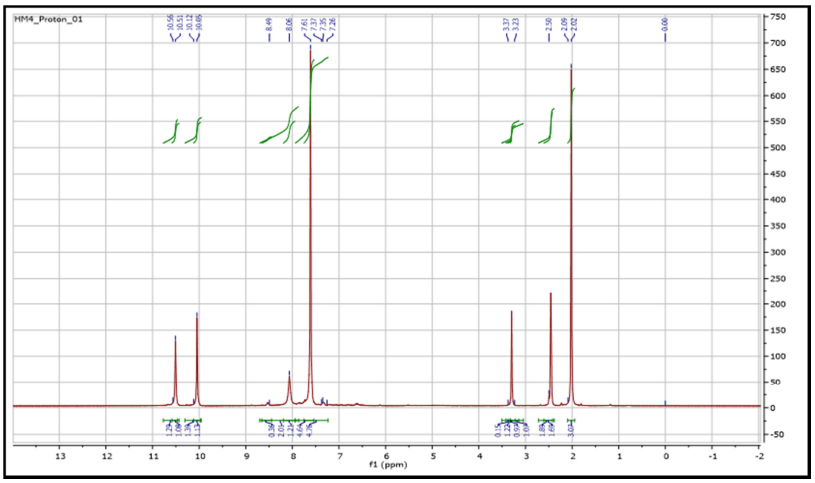


Figure 2 :^{1}H- NMR spectrum of [4d]

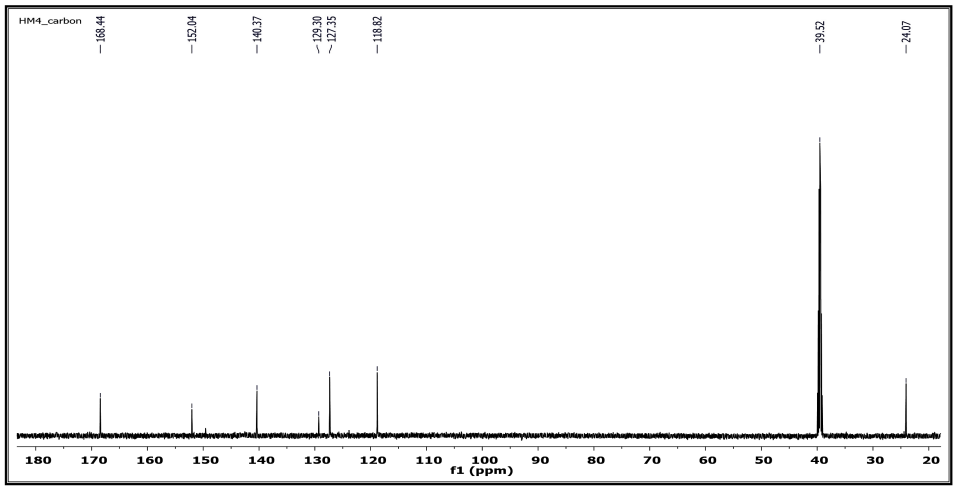


Figure 3 :^{13}C- NMR spectrum of [4d]

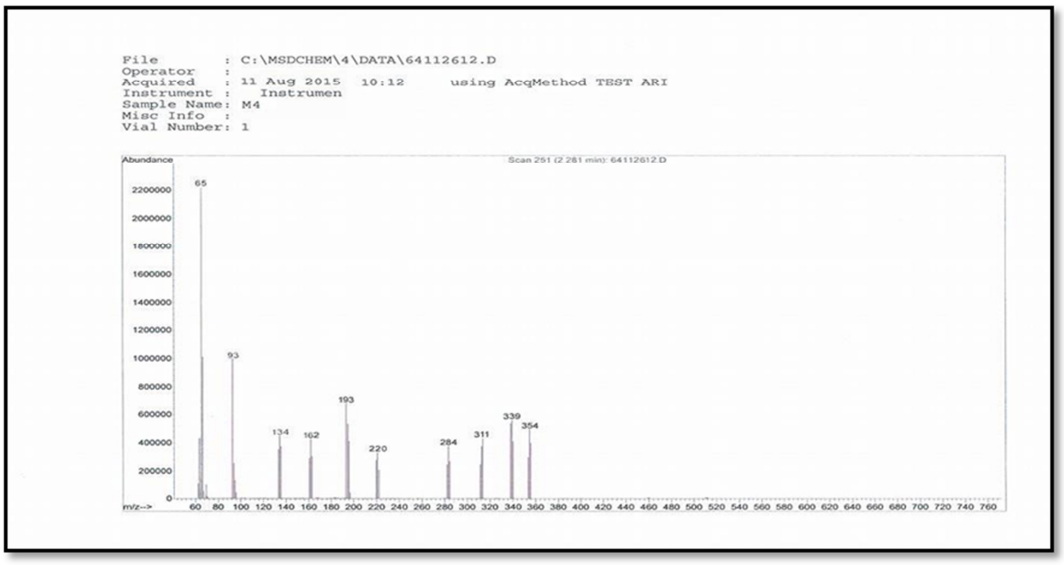


Figure 4 : Mass spectrum of [4d]

Table (2) :Serum glucose levels after treatment with formazan derivative [4e]
*** = is significant at (p≤ 0.05)**

Glucose conc. mg/dl		
Group	**No.**	**Mean±S.D**
A	**6**	**99.416**
B	**6**	**204.65**
C	**6**	**100.55**
D	**6**	**154.60***
E	**6**	**141.44***

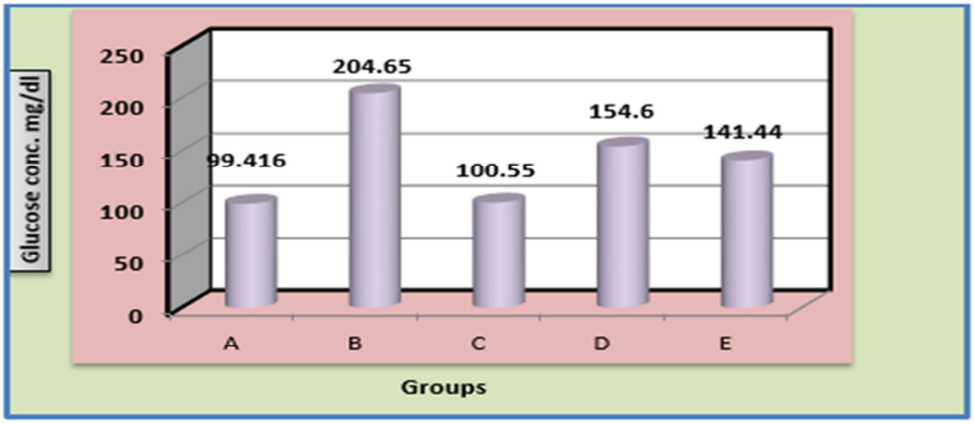


Figure 5 : Serum glucose levels in experimental groups after treatment with formazan derivative [4e]