Synthesis and Biochemical Evaluation of Some Substituted Phthalazines

Nahed F. Abd El-Ghaffar[*1], Mona A. Mohamed[2], Hala M.Ghanem[3] and Heba M. Zaki[1]

[1]Chemistry Department, [2]Biochemistry Division, Faculty of Science, Al-Azhar University, Egypt.
[3]Biochemistry Department, Faculty of Science, Ain-Shams University, Egypt.
[*]mabdelgelel@gmail.com

Abstract: The chemistry of phthalazine derivatives has been of increasing interest since many of these compounds have found chemotherapeutic applications. So this study aims to synthesize series of phthalazine derivatives, and investigate the antihyperglycemic, antihyperlipidemic and antibacterial activities of these derivatives. The influence of some synthesized phthalazine derivatives administered orally was studied in diabetic rats. Rats were divided into 5 equal groups. Group I: control rats. Group II: diabetic rats serving as a reference group for the treated groups. Groups III, IV and V: diabetic rats received a daily oral dose of 3mg/kg from each tested derivative for 15 days. At the end of the experimental period, serum levels of glucose, lipid profile and non-esterified fatty acids were assayed. Other phthalazine derivatives were tested against four pathogenic bacterial strains. The tested derivatives improved significantly serum levels of glucose, lipid profile and free fatty acids. Some phthalazine derivatives exhibited interesting high activity against Gram +ve bacteria than those of Gram -ve. Conclusion: This study reports interest findings that the tested phthalazine derivatives have antihyperglycemic and antihyperlipidemic effects at the adopted sublethal dose. The type of chemical derivatization of phthalazine confers glucose and lipid lowering activities as well as antibacterial activity.

[Nahed F. Abd El-Ghaffar, Mona A. Mohamed, Hala M.Ghanem and Heba M. Zaki. **Synthesis and Biochemical Evaluation of Some Substituted Phthalazines.** Journal of American Science 2011;7(4):771-781]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: *Phthalazinone, Chlorophthalazine, Thiophthalazine, Diabetes, Triacylglycerol, fatty acids.*

1. Introduction:

Diabetes mellitus (DM) is one of the most common chronic diseases in nearly all countries, and continues to increase in affected numbers and significance (**Shaw *et al.*, 2010**). Diabetes is not simply a disorder of glucose homeostasis but is also accompanied by various degenerative manifestations such as cardiovascular disease which is partly due to associated abnormalities of plasma lipid and lipoprotein metabolism (**Dunn, 2010**). Dyslipidemia characterized by elevated triglycerides (TG), reduced high density lipoprotein-cholesterol (HDL-C) and elevated plasma free fatty acids (FFA) levels (**Kreisberg, 1998**).

The chemistry of phthalazinone is very well known. These systems are widely used in organic chemistry as intermediates for the synthesis of numerous compounds. On the other hand; phthalazine derivatives were extensively studied as bioactive compounds. They possess remarkable biological activity, such as anticonvulsant, antimicrobial and anti-inflammatory (**Abd alla et al., 2010**). A series of 5-[4-[2-substituted phthalazinones-2(or 4yl] ethoxy] phenylmethyl] thiazolidine-2,4-diones showed the best lowering activity for plasma glucose and triacylglycerol *in vitro* and *in vivo* pharmacological studies (**Madhavan et al., 2001**) .

Consequently, this study aims to synthesize series of novel phthalazine derivatives and extends to investigate the anti-hyperglycemic and anti-hyperlipidemic activities of some of these derivatives. In addition, the antibacterial activity of these derivatives was also examined.

2. Materials and methods

All chemicals and solvents were of high analytical grade and were purchased from Sigma Chemical Company. Infrared spectra (IR) were performed by Pye Unicam SP^3-200 spectrophotometer with samples embedded in KBr discs. Varian spectrometer was utilized for NMR spectra with TMS as internal reference. Mass spectra were recorded by FINNI–Gas 3300 mass spectrometer operating at 70 ev beam energy.

a) Synthesis of substituted phthalazine derivatives:

The title compounds were synthesized according to the synthetic route in Scheme 1. The starting material 4-Biphenyl-4-yl-2H-phthalazin-1-one **(1)** [$C_{20}H_{14}N_2O$ of molecular weight (m.wt.) 298, melting point (m.p.) 252°c] was prepared according to the method reported by Soliman *et al.*, (1990).

3-(4-Biphenyl-4-yl-1-oxo-1H-phthalazin-2-yl)-propionitrile (2):

Acrylonitrile (0.01mol) was added to 0.01mol of **(1)** dissolved in 20ml pyridine and refluxed for 10 hrs. After cooling, the product was

poured onto ice/HCl mixture and the solid that separated was filtered off,washed well with water and recrystallized from benzene to give yellow crystals [$C_{23}H_{17}N_3O$, m.wt. 351, m.p. 235°c and yield 25%].

2-acetyl-4-Biphenyl-4-yl-2H-phthalazin-1-one (3):

Acetyl chloride (0.01mol) was added to 0.01mol of **(1)** dissolved in 30ml of pyridine and refluxed for 10 hrs. The solid obtained after concentration and cooling was recrystallized from benzene [$C_{22}H_{16}N_2O_2$ with m.wt. 340, m.p. 225°c and yield 25%].

(4-Biphenyl-4-yl-1-oxo-1H-phthalazin-2-yl)-acetic acid ethyl ester (4):

A mixture of 0.01mol of **(1)**, 0.04mol of ethylchloroacetate and 0.04mol of anhydrous potassium carbonate in 50ml of dry acetone was refluxed for 24 hrs. on asteam bath. The excess solvent was then removed by distillation and the reaction residue was poured onto water. The separated solid was filtered off, and recrystallized from ethanol to give white crystals [$C_{24}H_{20}N_2O_3$ with m.wt. 384, m.p. 200°c and yield 90%].

4-Biphenyl-4-yl-1-oxo-1H-phthalazin-2-yl)- acetic acid hydrazide (5):

A mixture of 0.01mol of the ester **(4)** and 0.01mol of hydrazine hydrate in 50ml of ethanol was refluxed for 10 hrs. The solid that separated after concentration and cooling was recrystallized from aceton to give white crystals [$C_{22}H_{18}N_4O_2$ with m.wt. 370, m.p. 252ºc and yield 75%].

General procedure for the synthesis of 6a-c:

To 0.01mol of **(4)** in 30ml of absolute ethanol and 0.05mol of sodium ethoxide and different aromatic aldehydes namely (salicylaldehyde, *p*-methoxy benzaldehyde and *o*-bromobenzaldehyde) were added and refluxed for 2hrs. The precipitate was filtered off on hot and recrystallised from the proper solvent to give: 2-(4-Biphenyl-4-yl-1-oxo-1H-phthalazin-2-yl)-3-(4-methoxy-phenyl) -acrylic acid ethyl ester **(6a),** 2-(4-Biphenyl-4-yl-1-oxo-1H-phthalazin-2-yl)-3-(2-hydroxy-phenyl)-acrylic acid ethyl ester**(6b) and** 2-(4-Biphenyl-4-yl-1-oxo-1H-phthalazin-2-yl)-3-(2-bromo-phenyl)-acrylic acid ethyl ester**(6c)** (Tables 4,5).

1-biphenyl-4-yl-4-chlorophthala-zine (7):

A mixture of 0.01mol of **(1)**, 0.01mol of phosphorus pentachloride and 10ml phosphorus oxychloride was refluxed for 3 hrs. on a steam-bath.

After cooling the reaction mixture was poured carefully onto ice/HCl mixture. The solid that separated was filtered off, washed well with water, dried and recrystallized from benzene to give pale yellow crystals [$C_{20}H_{13}N_2Cl$, m.wt. 316.5, m.p. 200ºc and yield 82%].

6-Biphenyl-4-yl-3-methyl-[1,2,4] triazolo [4,3-a]phthalazine (8):

A mixture of 0.01mol of **(7)** and 0.01mol of acetyl hydrazine in 40ml of n-butanol was refluxed for 48 hrs. The solid that separated after concentration and cooling was filtered off and recrystallized from ethanol as white crystals with molecular formula $C_{22}H_{16}N_4$, m.wt. 336, m.p. 235ºc and yield 60%.

1-Biphenyl-4-yl-4-methoxy phth-alazine derivative (9):

A mixture of 0.01mol of **(7)** and 0.01mol of sodium methoxide was refluxed for 8 hrs in 30 ml methanol as solvent. The solid that separated after concentration and cooling was filtered and recrystallized from ethanol as white crystals [$C_{21}H_{16}N_2O$, m.wt. 312, m.p. 180ºc and yield 70%].

General procedure for the synthesis of (10a-g):

A mixture of 0.01mol of **(7)** and 0.01mol of amines nmely ,hexamine, benzyl amine, *p*-aminobenzoic acid, *p*-chloroaniline *o*-amino-acetophenone, *p*-aminobenzophenone and/ or hydrazine hydrate was refluxed for 6 hrs. in 40ml of n-butanol. The solids that separated were recrystallized from the proper solvent to

2-(4-Biphenyl-4-yl-phthalazin-1-yl)-2H-benzo[e][1,2,3]- oxadiazine (11):

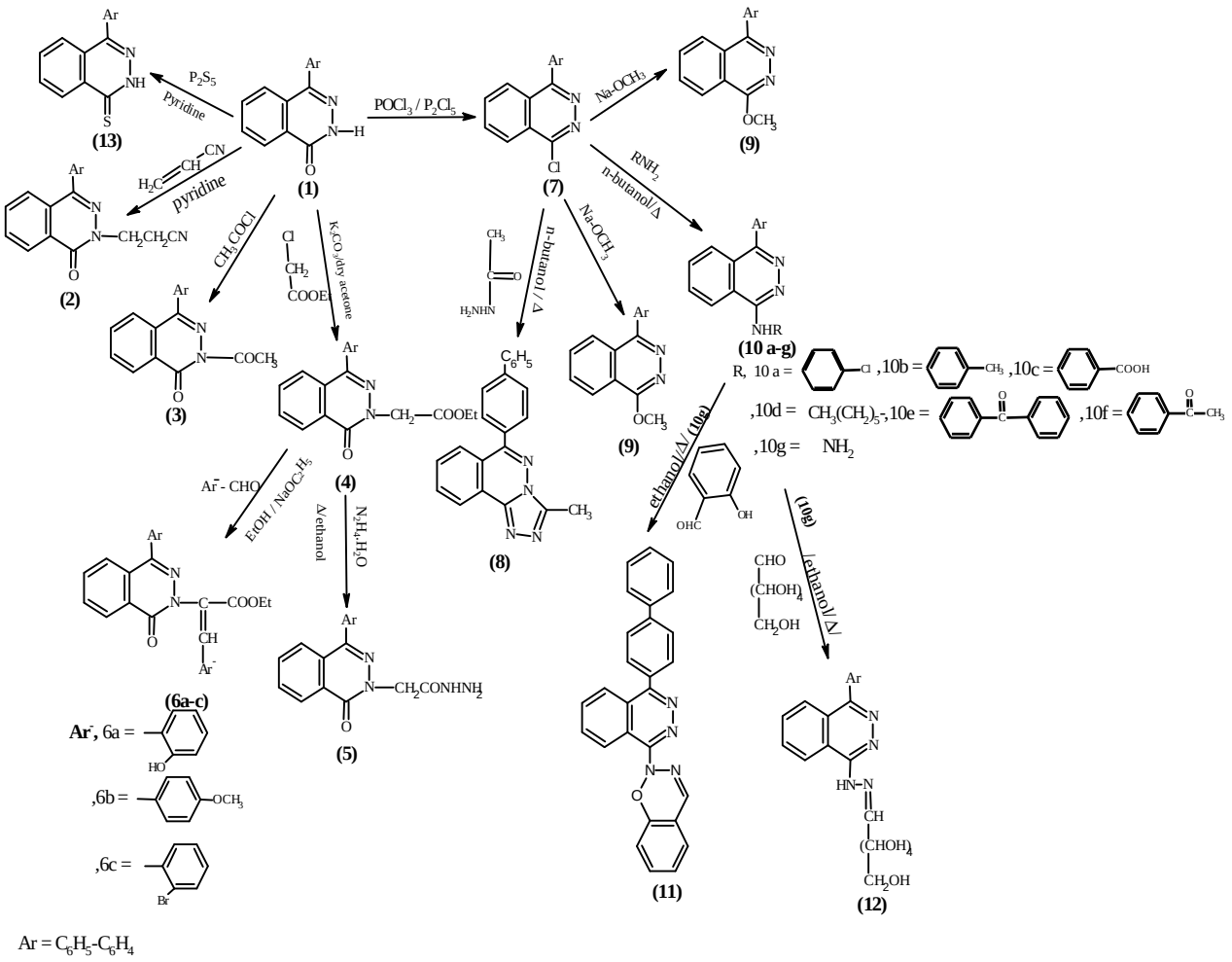
A mixture of 0.01mol of **(10g)** and 0.01mol of the salicylaldehyde in 40ml ethanol was refluxed for 6 hrs. The solid that separated, after concentration and cooling, was filtered off and recrystallized from ethanol [$C_{27}H_{17}N_4O$, m.wt. 413, m.p. 220ºc and yield 60%].

6-[(4-Biphenyl-4-yl-phthalazin-1-yl)-hydrazono]-hexane-1,2,3,4,5-pentaol (12):

To 0.01mol of **10g** a(0.01 mol) of glucose was added in 40ml ethanol and refluxed for 6 hrs. The solid that separated after cooling was filtered off and recrystallized from ethanol [$C_{26}H_{26}N_4O_5$, m.wt. 474, m.p.180ºc and yield 70%].

4-Biphenyl-4-yl-phthalazine-1-thiol (13):

A mixture of **(1)** (0.01mol) and phosphorus pentasulphide (0.02mol) in 40ml of dry pyridine was refluxed for 6 hrs. After cooling the reaction mixture was poured onto ice/HCl mixture. The solid that separated was filtered off, washed with water, dried and recrystallized from petroleum ether (b.p. 60–80ºC) to give deep yellow crystals [$C_{20}H_{14}N_2S$, m.wt. 314, m.p. 110ºC and yield 75%]



Scheme 1: Synthetic methodology for phethalazine derivatives.

b) Biochemical studies:

Induction of diabetes mellitus in rats was carried out by a single i.p. injection of freshly prepared alloxan monohydrate solution at a concentration of 120 mg/kg body weight after a fast of 12 h (**Refaie *et al*., 2005**). After 3 days of diabetes induction, treatment with the synthesized drugs was begun and lasted for 2 weeks. The tested compounds were orally administered to animals at a sublethal dose level of 3 mg/kg body weight (**Madhavan et al., 2001**). Body weight of the animals in all groups was recorded weekly until the end of the experiment. All rats were assorted into 5 equal groups (10 rats each) according to the following scheme: **Group I (NC):** comprised normal rats fed a commercial pellet diet and left intact without any treatment. **Group II (DC):** involved diabetic rats serving as a reference group for the treated groups. **Group III (D+1):** diabetic rats treated with **(1)**. **Group VI (D+11):** diabetic rats received a daily oral dose of **(11)**. **Group V (D+13):** diabetic rats treated with **(13)**.

At the end of the experimental period blood samples were taken from the retro-orbital venous plexus under light ether anesthesia after a fast of 12 hours. Serum was prepared by centrifuging blood samples at 4000 rpm for 5 min. Serum samples were aliquoted and stored at -20ºC until analysis, except for glucose which was determined on the same day without delay. Non hemolyzed sera were analyzed for liver function tests (aspartate and alanine amino transferases (AST & ALT, respectively) using assay kits (SpinReact Diagnostics, Spain), while albumin was determined by the enzymatic colorimetric method of **Doumas *et al.*, (1971)**. Kidney function tests (urea and creatinine) were assayed by methods of **Patton and Crouch (1977)**, **Heinegård and Tiderström (1973)**, respectively. Serum levels of glucose, total lipids, cholesterol and triacylglycerol were assayed by enzymatic colorimetric methods using assay kits (SpinReact Diagnostics, Spain). HDL-C was determined according to the method of **Assmann et al., (1983)**, while low and very low

density lipoprotein-cholesterol (LDL-C & VLDL-C, respectively) were calculated according to **Freidwald et al., (1972)** formulae**.** Non-esterified fatty acids were analyzed by Shimadzu GC 17-A gas chromatograph equipped with an auto-injector AOC 17 and a flame ionization detector (Shimadzu Corporation, Japan).

Anti-microbial Studies:

The anti-microbial activities were performed according to the method of **Bauer et al., (1966)** for compounds illustrated in table 5. These compounds were tested against Gram positive (G +ve) *(Staphylococcus aureus* and *Bacillis subtilis*) and Gram negative (G –ve) (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. These strains were obtained from the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Chloramphenicol was used as anti-bacterial reference standard.

Statistical analysis:

All results were expressed as mean ± SD. The data were analyzed by one-way analysis of variance (ANOVA). To compare the least significant difference among the groups, post hoc testing was performed by the LST test. The p-value less than 0.05 was considered statistically significant (**Dawson and Trapp, 2001**). Statistical analysis was performed with Statistical Package for the Social Science for Windows (SPSS, version 11.0, Chicago, IL, USA).

3. Results

The structures of the synthesized compounds were confirmed with spectral data as illustrated in tables (1-5). Fragmentations of derivatives **2, 4** and **5** were as follow: m/z 351(0.10%), at m/z 298(100%), at m/z 241 as [M+2] (27.95%), m/z 165(7.78%), m/z 88(1.19%) and m/z 76 (1.37%) for **(2)** and 368(13.58), m/z 298 (100%), m/z 221 (80.86%), m/z 144(13.73%), m/z at 70(17.16%) and m/z 77(24.87%) for **(4)**, while for **(5)** was 339(0.03%), m/z 338(0.05%), m/z 281(14.22%), m/z 297(87.07%), m/z 310(0.34%) and m/z 163(26.49%). The m/z (%) for **6b**: 502(2.08%), m/z 205(1.9%), m/z 298(39.357%), m/z 221(48.71%), m/z 167(24.36%), m/z 133(7.23%), m/z 107(25.34%), m/z 73(34.07%) and m/z 77(57.20%). Derivatives **10** showed the following fragmentation pattern in MS m/z (%): **(10a)**: 312(0.09), 297 [M+1](100), 220(4.62), 164(10.01) and 57(0.11). For **(10c)**: 133(0.33), 297(100) as [M+1], 221(5.52), 164(4.82) and 57(0.08). For **(10d)**: 145(6.73) as [M+3], 297(100) as [M+1], 221(88.53), 164(17.38) and 57(8.16) as [M+3]. For **(10f)**: 415(1.52), 119(20.99), 77(17.31), 298(100) (the second main ion peak), 221(27.71), 164(0.71) and 57(1.29). For **(10g)**: 407(75.32), 327(2.22), 298(1.18), 221(0.46), 164(3.11) and 57(0.43).

Data in table (6) showed significant elevation ($p<0.001$) in serum levels of AST and ALT activities in diabetic control, compared to normal control. Administration of compounds 11 and 13 to diabetic rats improved significantly ($p<0.01$) the enzymes activity, compared to diabetic control. Compound 13 treated rats showed non significant alteration in serum AST but a remarkable reduction ($p<0.01$) was noticed in serum ALT activity as compared to CD group. Serum albumin levels showed non significant alteration in all studied diabetic groups as compared to control group. Significant elevations were observed in serum urea ($p<0.01$) and creatinine ($p<o.o5$) in CD, compared to CN. Administration of the phthalazine derivatives improved serum levels of urea as compared to CD. Serum levels of creatinine were within the normal range in all groups, although it showed significant elevation ($p<0.05$) in rats treated with compound 13, compared to normal rats.

Table (7) demonstrated that serum level of glucose was significantly elevated ($p<0.001$) in CD and D+1 groups, compared to normal group. Oral administration of either compound 11 or 13 decreased significantly ($p<0.01$) serum glucose level as compared to CD group.

Diabetic control rats showed significant elevation in all lipid parameters except for HDL-C, which was significantly reduced ($p<0.01$), compared to normal rats. Rats treated with compounds 1 and 13 improved significantly the lipid profile except HDL-C which was not affected by the two drugs compared to CD rats. In addition, compound 1 could not affect the serum cholesterol level of the diabetic rats treated with this compound. In regard, compound 13 improved significantly all the lipid parameters as compared to control group. Serum level percent of palmitic acid was non significant in all groups. Stearic acid level was elevated significantly ($p<0.01$), while levels of oleic and linoleic were significantly decreased ($p<0.05$) in CD rats, compared to normal rats. Animals treated with compound 11 revealed normal serum levels of both stearic, oleic and linoleic acids, while serum level of α -linolenic was significantly reduced as compared to control animals. Rats treated with compound 13 showed non significant alterations in stearic and oleic acids levels and significant reduction ($p<0.01$) in serum level of α -linolenic acid, in addition to significant elevation ($p<0.01$) in linoleic acid level, compared to CD group. Administration of compound 1 improved significantly serum fatty acids levels, compared to CD group.

Data in table (8) demonstrated that the lowering effect of compound 11 on the serum levels of total lipids, cholesterol, TG, LDL-C and stearic acid was more significant than those of compounds 1 and 13. The decreasing effects of either compound 11 or 13 on the serum levels of glucose and LDL-C were more pronounced than compound 1. However, effects of compound 13 in lowering the levels of serum TG, VLDL-C and linoleic acid were more potent than the two other phthalazine derivatives.

Data in table (9) revealed that **10g** was the most potent phthalazine derivative against bacterial strains, in addition, it was as potent as chloramphenicol. Compounds 1, 5, 7 and 10d were effective in inhibiting the growth of *Bacillis subtilis*, but still less effective than standard drug. The anti-bacterial activity of compounds 3, 5, 7, 10d and g were greater than the rest compounds on *E. coli*. In regard with *Pseudomonas aeruginosa*, the tested phthalazine derivatives showed mild inhibitory activity as noticed by compounds 1, 5, 7, 10d and 10g, while the rest compounds had no activity.

Table (1): Selected mass (MS) and infrared (IR) spectral data for compounds 1-5

Comp.	MS m/z(%)	IR (υ,cm^{-1})						
		NH	NH_2	OH	C=O	C=N	C-N	CH
1	298 (100)	3377	––	3297	1650.8	1585.3	––	––
2	298 (100)	––	––	––	1663.1	1541	––	3097(Ar) 2990-2855 (Al)
3	––	––	––	––	1700 (acetyl) 1661 (phthalaz.)	1555.3	––	––
4	298 (100)	––	––	––	1659.8 (ring) 1756.1 (ester)	1553.6	––	––
5	297 (87.07)	3101.46	3157.86		1650.77 (amide)	1605.45	1261.7- 1212.04	––

Table (2): Elemental analysis (EA) (calculated and found values) and ^{1}H NMR data of compounds 1-5

Comp.	EA (Cal./ Found)			^{1}H-NMR		
	C	H	N	δ ppm	Splitting	Type of proton
1	80.54/ 80.53	4.70/ 4.68	9.40/ 9.39	7.535-8.420 12.946	M s	13 Ar-H 1 NH
2	78.63/ 78.60	4.84/ 4.82	11.97/ 11.95	-----	-----	-----
3	77.65/ 77.64	4.71/ 4.70	8.24/ 8.23	-----	-----	-----
4	75/ 75.02	5.21/ 5.20	7.29/ 7.30	7.535-8.420 1.858-3.102 3.202-3.891	M t q	13 Ar-H CH_3 CH_2
5	71.33/ 75.02	4.86/ 4.83	15.14/ 15.13	-----	-----	-----

Table (3): Selected mass, infrared spectral data and elemental analysis as well as ^{1}H NMR data.

Comp.	MS m/z (%)	IR (υ,cm^{-1})			EA (Cal./ Found)					^{1}H-NMR		
		NH	C=N	C-Cl	C	H	N	Cl	S	δ ppm	Splitting	Type of proton
7	––	––	1663.3	847.56	75.82/ 75.83	4.10/ 4.11	8.84/ 8.85	11.21/ 11.22	––	7.55- 7.93	m	13H, Ar-H
8	296 (100)	––	1680- 1602	––	78.57/ 78.55	4.76/ 4.74	16.66/ 16.67	––	––	7.12- 8.63 2.128	m s	13H, Ar-H 3H, CH_3
9	298 (100)	3374.49	1664.75	––	80.76/ 80.77	5.12/ 5.13	8.97/ 8.98	––	––	––	––	––
11	298 (100)	––	––	––	78.45/ 78.43	4.12/ 4.10	13.56/ 13.54	––	––	––	––	––
12	––	––	––	––	65.82/ 65.80	5.48/ 5.49	11.81/ 11.80	––	––	––	––	––
13	––	––	––	––	76.43/ 76.40	4.45/ 4.46	8.91/ 8.92	––	10.19/ 10.20	––	––	––

Table (4):Elemental analysis (EA) (calculated and found values) and ^{1}H NMR data of compounds 6-10

Comp.	EA (Cal./ Found)				^{1}H-NMR	
	C	H	N	Cl	δ ppm	Splitting, Type of proton
6a	76.22/ 76.20	4.91/ 4.92	5.73/ 5.74	—-	12.21 6.35-7.945 2.133-3.559	s, 1H, Ar-OH m, 17H, Ar-H d, 2H, CH=CH
6b	76.49/ 76.50	5.17/ 5.18	5.57/ 5.58	—-	——	——
6c	67.51/ 67.50	4.17/ 4.16	5.80/ 5.90	14.51/ 14.52	——	——
10a	81.88/ 81.90	7.08/ 7.10	11.02/ 11.10	——	7.485-7.935 12.92	m, 17H, Ar-H s, 1H, NH
10b	83.72/ 83.70	5.42/ 5.43	10.85/ 10.86	——	——	——
10c	76.56/ 76.54	4.41/ 4.42	10.07/ 10.10	——	——	——
10d	76.56/ 76.54	4.41/ 4.42	10.30/ 10.31	8.71/ 8.72	——	——
10e	80.96/ 80.95	5.06/ 5.10	10.12/ 10.10	——	——	——
10f	83.01/ 83.20	4.82/ 4.80	8.81/ 8.81	——	——	——
10g	76.92/ 76.90	5.12/ 5.13	17.34/ 17.35	——	——	——

Table (5): IR spectra (υ,cm^{-1}) for compounds 6 and 10.

Comp.	C-H	C=O	C=C	C=N	CH2	CH3	NH	NH2	OH
6a	2931.75- 2890.29	1678.25	1602.56	1550.01	-----	-----	-----	-----	3500
6b	2934.16- 2894.15	1649.8	1599.66	1509.99	-----	-----	-----	-----	-----
6c	2998.28- 2935.13	1650.29	1585.68	1548.56	-----	-----	-----	-----	-----
10a	3098.56	-----	-----	1553.86	1450.21	1399.1	3298.64	-----	-----
10b	2940.91	-----	-----	1662.34	-----	-----	3156.90	-----	-----
10c	2995.39	1662.34	-----	1585.20	-----	-----	3157.86	-----	-----
10d	3055.66	-----	-----	1584.24	-----	-----	3393.14	-----	-----
10e	2996.84	1665.71	-----	1591.47	-----	1356.68	3338.18	-----	-----
10g	2918.73	----	-----	1611.23	-----	-----	3159.79	3379.64	-----

Table (6): Liver and kidney function tests (Mean±SD) for the different studied groups.

Parameters	Experimental groups				
	CN	CD	D + 1	D + 11	D + 13
AST(U/L)	117.00±9.66	196.3±14.18*	132.67±32.33$^\#$	137.0±22.72$^\#$	185.67±53.81*
ALT(U/L)	50 ±8.53	111.33±39.36*	53.0±17.06$^\#$	59.83±14.25$^\#$	63.67±21.45$^\#$
Albumin(mg/dl)	2.17±0.41	1.67±0.52	1.83±0.41	1.67±0.52	1.83±0.41
Urea(mg/dl)	30.67±3.14	64.83±13.29*	49.67±17.78$^{*\#}$	37.83±3.43$^\#$	49.00±6.00$^{*\#}$
Creatinine(mg/dl)	1.44±0.27	1.74±6.12*	1.59±0.11	1.62±0.14	1.69±0.19*

* Significant vs control normal, # significant vs control diabetic.

Table (7): Serum levels of glucose and lipid profile as well as percent of free fatty acids (Mean±SD) in the different studied groups.

Parameters	Experimental groups				
	CN	CD	D + 1	D + 11	D + 13
Glucose(mg/dl)	114.67±12.52	582.83±20.40*	496.83±56.29*	304.83±176.58*#	254.33±111.33*#
Total lipid(mg/dl)	50.67±5.57	79.17±11.79*	47.00±9.67#	26.33±4.23*#	54.83±8.79#
Cholesterol(mg/dl)	52.00±12.29	88.83±27.29*	81.33±2.73*	34.33±3.83#	69.50±15.96#
TG (mg/dl)	64.17±18.35	173.67±55.86*	54.33±14.89#	86.67±18.91#	126.5±39.17*#
HDL-C(mg/dl)	41.67±2.58	20.50±5.50*	40.33±1.37#	22.33±4.89*	36.67±3.20*#
LDL-C (mg/dl)	12.17±3.66	64.50±16.18*	50.83±5.67*	33.67±17.81*#	48.00±17.29*#
VLDL–C(mg/dl)	12.83±3.67	34.73±11.17*	10.87±2.97#	17.33±3.78#	24.27±7.24*#
Palmitic(16:0)	26.00±7.67	25.50±3.83	29.50±1.64	25.50±3.83	25.5±6.02
Stearic(18:0)	10.00±1.09	19.00±2.19*	14.50±3.83*#	10.50±3.83#	20.33±2.58*
Oleic (18:1)	22.50±0.55	18.00±3.29*	19.50±1.64	24.50±4.93#	18.50±4.93
α-Linol(18:3ω3)	12.50±3.83	15.00±3.29	8.83±4.62#	7.50±2.74*#	7.33±0.82*#
Linoleic(18:2ω6)	16.50±0.55	11.50±5.92*	12.67±5.99	18.50±0.55#	20.00±1.09#

* Significant vs control normal, # significant vs control diabetic.

Table (8): Statistical comparison of the tested derivatives on different serum parameters (Mean±SD)

Parameters	Experimental groups		
	D + 1	D + 11	D + 13
Glucose (mg/dl)	496.83±56.29[a]	304.83±176.58[b]	254.33±111.33[b]
% Change vs CD	-14.76	-47.69	-56.36
Total lipid (mg/dl)	47.00±9.67[a]	26.33±4.23[b]	54.83±8.79[a]
% Change vs CD	-40.63	-66.74	-30.74
Cholesterol(mg/dl)	81.33±2.73[a]	34.33±3.83[b]	69.50±15.96[a]
% Change vs CD	-8.44	-61.35	-21.76
Triglycerides(mg/dl)	54.33±14.89[ab]	86.67±18.9[a]	126.5±39.17[b]
% Change vs CD	-68.71	-50.09	-27.16
HDL-C(mg/dl)	40.33±1.37[a]	22.33±4.89[b]	36.67±3.20[a]
% Change vs CD	96.73	8.93	78.88
LDL-C (mg/dl)	50.83±5.67[a]	33.67±17.8[b]	48.00±17.29[ab]
% Change vs CD	-21.19	-47.79	-25.58
VLDL–C(mg/dl)	10.87±2.97[ab]	17.33±3.78[a]	24.27±7.24[ac]
% Change vs CD	-68.70	-50.10	-30.12
Stearic(18:0)	14.50±3.83[a]	10.50±3.83[b]	20.33±2.58[c]
% Change vs CD	-23.68	-44.74	7.0
Oleic (18:1)	19.50±1.64[a]	24.50±4.93[b]	18.50±4.93[a]
% Change vs CD	8.33	34.21	2.78
Linoleic(18:2ω6)	12.67±5.99[a]	18.50±0.55[b]	20.00±1.09[b]
% Change vs CD	10.17	60.87	73.91

Each similar letters are non significant.

Table (9): Anti-bacterial activity of the phthalazine derivatives (5 ug/ml) against tested bacterial strains with regard to chloramphenicol (5 ug/ml) as a reference standard.

Compound	G +ve		G –ve	
	Staph. Aur.	*Bacil. Sub.*	*E. coli*	*Pseud. aer.*
1	+	++	+	+
3	+	+	++	0
4	+	+	+	0
5	+	++	++	+
7	+	++	++	+
10 d	+	++	++	+
10 g	++	++	++	+
11	+	+	+	0
12	+	+	+	0
13	+	+	+	0
chloramphenicol	++	+++	++	++

+ = 0.1-0.5cm, ++ = 0.6-1.0cm, +++ = 1.1-1.5cm and 0 = no effect.

4. Discussion:

Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes. Thus, sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications (**Jarald *et al*., 2009**).

Compound **(1)** is treated either with acrylonitrile in boiling pyridine, with acetyl chloride in the presence of few drops of pyridine or with ethyl chloroacetate in dry acetone containing anhydrous potassium carbonate to give derivatives **(2), (3)** and **(4)**, respectively. Compound **(4)** was confirmed chemically by its reaction with hydrazine hydrate in boiling ethanol to give derivative **(5)**. All these compounds were confirmed on the basis of their spectral data.

Compound **(4)** was also confirmed by its reaction with aromatic aldehydes under Claisen reaction conditions to give the arylidene derivatives **(6a-c)** which were confirmed on the basis of their spectral data.

As a point of interest, the phthalazinone derivative **(1)** exists in lactam-lactim toutomeric equilibrium. Thus, in absence of solvent or in presence of a weakly polar solvent, it reacts with electrophiles or nucleophiles in the lactim form. So, compound **(1)** was reacted with a nucleophilic reagent such as phosphrous oxychloride to give the corresponding chlorophthalazine **(7)**. Compound **(7)** was confirmed chemically by its reaction with acetylhydrazine in boiling n-butanol for 48 hrs. to give phthalazine derivative **(8)**. Also, **(7)** was confirmed chemically by its reaction with sodium methoxide under reflux to give **(9)**. These compounds were confirmed on the basis of their spectral data. The MS for derivative **(8)** was 336(0.19%) which underwent fragmentation to give ion peaks at m/z 321(0.03%) which loss C≡N molecule to give the main ion peak m/z 296 as [M+2] (100%) which underwent fragmentation to give ion peaks at m/z 220(2-35%), m/z 164(3.63%) and m/z 56(0.01%). Derivative **(9)** MS was 312(0.11%) which loss H+ to give ion peak at m/z 311(0.11%) which underwent fragmentation to give main ion peak at m/z 298(100%) which underwent fragmentation to give ion peaks at m/z 221(17.38%), m/z 164(2.5%), m/z 57(0.03%) and m/z 77(3.09%).

In addition, compound **(7)** was treated with different primary aliphatic and aromatic amines and/or hydrazine hydrate, in boiling n-butanol to give the corresponding 1-amino phthalazine derivative **(10a-g)**. Compound **(10g)** was confirmed chemically by its reaction with aromatic aldehyde or with aldohexose in boiling ethanol under reflux to give Schiff's base **(11)** or pentaol derivative **(12)** which were confirmed on the basis of their spectral data.

The presence of the lactam form was proved by the reaction of phthalazinone **(1)** with phosphorous pentasulphide in the presence of pyridine as a solvent to give the thione derivative **(13)**.

In order to assess the toxic side effects of the administered dose levels of phthalazine derivatives (**1, 11** and **13**), the change percent of body weight gain at each week point with respect to the initial body weight was recorded but non significant alterations were observed (data not shown). In addition, both the liver and kidney function tests were determined. The diabetic hyperglycemia induced by alloxan produces elevation of serum levels of ALT and AST, as well as levels of urea and creatinine in untreated diabetic rats, while albumin level was not affected. These results agree with those of **Jarald *et al.*, (2009)**. Administration of either derivative **11** or **13** improved significantly the hepatic enzymes activities. In spit, the improvement of ALT activity produced by **13**, but it did not reduce AST activity. However, the three tested derivatives improved the kidney function. This indicates that **1** and **11** are safer than **13** at the adopted dose levels.

The present results indicate the efficiency of **11** and **13** to reduce the levels of serum glucose, while **1** did not affect the serum glucose level in alloxan-induced diabetic rats. The present results are in line with **Madhavan et al., (2001)** who reported that a series of substituted phthalazinones containing thiazolidinediones showed plasma glucose and plasma triglyceride lowering activity in db/db mice.

Quinazoline, which is isomeric with phthalazine, possesses hypoglycemic and hypolipidemic effects (**Refaie et al., 2005**). These effects in both compounds could be attributed to being cyclic amidine compounds. In addition, metformin, a well known hypoglycemic drug, which acts as an inhibitor of hepatic glucose production, possesses guanidine and amidine functionalities in its molecular structure. Another class of compounds, such as triaryl imidazoles, have also an amidine moiety in a cyclic structure, displayed a significant glucagon antagonistic property (**Wu et al., 1990**). Consequently, the tested compounds produced their effects due to the amidine and guanidine part in their structure.

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease (**Pushparaj et al., 2007**). The present work illustrates significant elevation in all lipid parameters except for HDL-C, which was significantly reduced in diabetic rats, compared to normal group. Rats treated with the three tested derivatives improved significantly the serum total lipids. In spite **11** and **13** reduced significantly serum cholesterol and triacylglycerol levels, **1** reduced only the elevated triacylglycerol as compared with the diabetic reference group. These

results agree with those reported by **Madhavan *et al.*, (2001)**.

This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin, since under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridaemia (**Pushparaj *et al.*, 2007**). In addition, insulin has an inhibitory action on 3-hydroxy-3-methyl glutaryl Co A (HMG- Co A) reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles (**Murali et al., 2002**). In regard to serum lipoproteins profile, derivatives **11** and **13** reduced serum LDL-C and VLDL-C levels, while **1** reduced serum VLDL-C level, compared to their reference group. Compound **11** did not affect significantly serum HDL-C level as the other two derivatives.

These findings suggest that the reduction effect of derivatives **11** and **13** on serum cholesterol and triacylglycerol levels may be due to inhibition of HMG- Co A reductase as well as inhibition of the esterification reactions of both. However, compound 1 inhibits the synthesis of hepatic triacylglycerol since VLDL synthesized in liver and mainly contains TG.

It is known that a heterocyclic containing carbonyl group is more efficacious than a simple heterocyclic (**Watanabe *et al.*, 1994**). In regard to the tested derivatives, **11** was more potent as hypolipidemic since its percent changes were more valuable than compounds **1** and **13**, in spite **13** improved significantly all the lipid parameters as compared to control diabetic group. This activity of compound **11** may be related to its chemical structure which possesses high electron density due to the presence of four nitrogen atoms in addition to the oxygen atom, while **13** was more active than **1** due to the presence of sulfur atom with its higher number of electrons than the oxygen atom in compound **1**.

Plasma free fatty acids (FFA) play important physiological roles in skeletal muscle, heart, liver and pancreas. Fatty acids are increased in diabetic patients (**Yin *et al.*, 1997**). Elevated FFA concentrations are linked with the onset of peripheral and hepatic insulin resistance (**Boden and Shulman, 2002**).

The present work revealed that serum stearic acid level was elevated significantly, while levels of oleic and linoleic were significantly decreased in untreated diabetic rats, compared to normal rats.

These results agree to some extent to those reported by **Wang et al., (2003)**, who indicated that the incidence of diabetes was positively associated with the proportions of palmitic, palmitoleic, and dihomo-γ-linolenic acids and inversely associated with the proportion of linoleic acid in plasma cholesteryl esters. While in phospholipids, incident diabetes was positively associated with the proportions of palmitic and stearic acid. Hence authors suggested that the proportional saturated fatty acid composition of plasma is positively associated with the development of diabetes.

Animals treated with **11** revealed normal serum levels of stearic, oleic and linoleic acids, while serum level of α-linolenic was dramatically reduced by 50% as compared to control diabetic animals. Rats treated with **13** showed non significant alterations in stearic and oleic acids levels while significant reduction was observed in serum level of α -linolenic acid, in addition to significant elevation in linoleic acid level, compared to CD group. Administration of **1** improved significantly serum fatty acids levels, compared to CD group.

Laaksonen et al., (2002) reported that higher proportions of serum non-esterified and esterified linoleic acid were associated with a decreased risk of hyperglycaemia and more favorable changes in fasting insulin and glucose concentrations. Finally, evidence is discussed that FFA represent a crucial link between insulin resistance and beta-cell dysfunction and, as such, a reduction in elevated plasma FFA should be an important therapeutic target in obesity and type 2 diabetes (**Boden and Shulman, 2002**).

Antibiotic resistance is a growing problem, some of this is due to the over use of antibiotics in human, but some of it is probably due to the use of antibiotics as growth promoters in food of animals (**Johnson et al., 2006**). So, there is a growing demand for new antibiotics. Phthalazine derivatives were reported to exhibit interesting antimicrobial activity (**Singh et al., 2010**).

The results of this study showed that compound **10 g** was the most potent phthalazine derivatives against both G +ve and G -ve strains, beside it was potent as chloramphenicol. In addition, compounds **1**, **5**, **7** and **10d** were effective in inhibiting the growth of Bacillis subtilis, but less effective than the standard drug. These results agree with those of **Singh *et al.*, (2010)** who reported that phthalazine derivatives exhibited interesting high activity against their reference drugs, and all their tested derivatives exhibited relatively better antibacterial activity against G +ve bacteria, but weak against G -ve bacteria.

The antibacterial activities of tested phthalazines may be related to their ability to affect permeability of the bacterial cell wall through

interacting with the hydroxyl group of the sugar moiety (in peptidoglycan layer) as in case of **10 g** or with amino group of the side chain amino acids as compounds **1, 5, 7** and **10 d**. These interactions produce a flux of protons which induces changes in cell membrane and ultimately, cell death. The structural feature may increase the activity of the tested compound since the smaller size can facilitate their penetration through the cell wall. In addition, **10 g** possesses free amino group which is more active than both Cl in **7** and oxygen in compounds **1** and **5**, as well as the long alkyl chain of **10 d**.

The results of anti-bacterial activity of some phthalazine derivatives on Gram –ve bacteria revealed that the effects of derivatives **3, 5, 7, 10d** and **g** were greater than the rest tested compounds on *E. coli*. In regard with *Pseudomonas aeruginosa*, the tested phthalazine derivatives showed mild inhibitory activity as noticed by **1, 5, 7, 10 d** and **10 g**, while the rest compounds had no activity.

Unlike Gram +ve cell wall, the Gram -ve cell wall contains an outer membrane composed by phospholipids and lipopolysaccharides which increase the negative charge of the cell membrane and helps to stabilize the overall membrane structure (**Wang and Quinn, 2010**). Consequently, compounds **5**, **7**, **10g** and **10d** were the effective compounds against Gram -ve bacteria. Derivative **10d** is considered to be an electron deficient derivative, hence this deficiency may facilitate its penetration through the negative charges surrounding the cell membrane and reacts with the thin peptidoglycan layer to disturb the membrane structure. While the activities of compounds **5, 7** and **10g** may be related to the polarity on these derivatives. So, this polarity can disturb the electron distribution on the cell membrane. In addition, the antibacterial effects of phthalazines may also be due to their interaction with bacterial enzymes and proteins of the cell membrane.

Although the peptidoglycan layer is thinner in G –ve than Gram +ve bacteria but the effects of the tested compounds were not as potent as in G +ve. This result confirms the suggestion that the main cause of the anti bacterial effect of phthalazine derivatives was due to their interaction with the teichoic acids (not found in G -ve bacteria cell wall), in addition to their effects on the peptidoglycan layer.

5. Conclusion

Subchronic treatments of diabetic rats with the tested phthalazine derivatives (3 mg/Kg) have no significant toxic side effects. Compounds **11** and **13** possess potential anti-hyperglycemic activity, while all the tested phthalazine derivatives possess potential antihyperlipidemic activity demonstrated by the dramatic reduction in serum total lipids, triacylglycerol and most importantly VLDL-C, which is a major carrier of triacylglycerol. Type of chemical derivatization of phthalazine confers glucose and lipid-lowering activities as well as the antibacterial effects.

Corresponding author
Mona A Mohamed
Biochemistry Division, Chemistry Department **Faculty of Science**, Al-Azhar University, Egypt**.** **mabdelgelel@gmail.com**

References

Abd alla MS, Hegab MI, Abo Taleb NA, Hasabelnaby SM and Goudah A (2010): Synthesis and anti-inflammatory evaluation of some condensed [4-(3,4-dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide. Eur J Med Chem 45(4): 267-77.

Assmann G, Schriewer H, Schmitz G and Hagele EO (1983): Quantification of high density lipoprotein cholesterol by precipitation with phosphotungstic acid/MgCl2. Clin Chem 29(12):2026-2030.

Bauer AW, Kirby WM, Sherris JC and Turck M (1966): Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493-496.

Boden G and Shulman GI (2002): Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance. Eur J Clin Invest 32:14-23.

Dawson B and Trapp RG (2001): Basic and Clinical Biostatistics, 3rd ed, McGraw-Hill, USA.

Doumas B, Watson WA and Biggs HG (1971): Albumin standards and measurements of serum albumin with bromocresol green. Clin Chim Acta 31: 87-96.

Dunn FL (2010): Management of dyslipidemia in people with type 2 diabetes mellitus. Rev Endocr Metab Disord 11(1):41-51.

Friedewald WT, Levy RI and Fredrickson DS (1972): Estimation of the concentration of lowdensity lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499-502.

Heinegård D and Tiderström G (1973): Determination of serum creatinine by a direct colorimetric method. Clinica Chimica Acta, 43(3): 305-310.

Jarald E, Joshi SB and Jain DC (2009): Biochemical study on the hypoglycaemic effects of extract and fraction of Acacia catechu wild in alloxan-induced diabetic rats. Int J Diab Met 17:63-69.

Johnson J, Kuskowski M, Menard M, Gajewski A, Xercavins M and Garau J (2006): Similarity

between human and chicken *Escherichia coli* isolates in relation to ciprofloxacin resistance status. J Infect Dis 194 (1): 71–8.

Kreisberg RA (1998): Diabetic dyslipidemia. Am J Cardiol 17(82) :67U-73U.

Laaksonen DE, Lakka TA, Lakka HM, Nyyssönen K, Rissanen T, Niskanen LK and Salonen JT (2002): Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. Diabet Med 19: 456–464.

Madhavan GR, Chakrabarti R, Kumar SK, Misra P, Mamidi RN, Balraju V, Kasiram K, Babu RK, Suresh J, Lohray BB, Lohrayb VB, Iqbal J. and Rajagopalan R (2001): Novel phthalazinone and benzoxazinone containing thiazolidinediones as antidiabetic and hypolipidemic agents. Eur J Med Chem 36(7-8):627-37.

Murali B, Upadhyaya UM and Goyal RK (2002): Effect of chronic treatment with *Enicostemma littorale* in non-insulin dependent diabetic (NIDDM) rats. J Ethnopharmacol 81: 199-204.

Patton CJ and Crouch SR (1977): Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal Chem 49: 464-69.

Pushparaj PN, Low HK, Manikandan J, Tan PK, et al. (2007): Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. J Ethnopharmacol 111: 430-434.

Refaie FM, Esmat AY, Abdel Gawad SM, Ibrahim AM and Mohamed MA (2005): The antihyperlipidemic activities of 4(3H) quinazolinone and two halogenated derivatives in rats. Lipids in Health and Disease 4:22.

Shaw JE, Sicree RA and Zimmet PZ (2010): Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes research and clinical practice 87: 4-14.

Singh S, Yadav A, Meena AK, Singh U, Singh B, Gaurav A, Rao MM, Panda P and Singh R (2010): Pharmacological action and SAR of Phthalazine derivatives. Int J Chem Anal Sci 1(5):79-87.

Soliman FMA, Souka LM, Abdel-Ghaffar NF and Saleh RM (1990): Synthesis and reactions of 4(biphenyl)1(2H)phthalazinone. J Serb Chem Soc 55(2):89-96.

Wang L, Folsom AR, Zheng Z, Pankow J and Eckfeldt J (2003): Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Clin Nut 78(1): 91-98.

Wang X and Quinn JP (2010): Lipopolysaccharide: Biosynthetic pathway and structure modification. Progress in Lipid Research 49(2): 97–107.

Watanabe S, Ogawa K, Ohmo T, Yano S, Yamada H and Shirasaka T (1994): Synthesis of 4-[1-(substituted phenyl)-2-oxo-pyrrolidin-4-yl]methyloxybenzoic acids and related compounds, and their inhibitory capacities toward fatty-acid and sterol biosyntheses. Eur J Med Chem 29: 675-686.

Wu H, Johnston P, Seu W, Hollenbeck CB, Jeng CY, Goldfine ID, Chen YD and Reaven GM (1990): Effect of metformin on carbohydrate and lipoprotein metabolism in NIDDM patients. Diabetes Care 13:1-10.

Yin B, Loike JD, Kako Y, Weinstock PH, Breslow JL, Silverstein SC and Goldberg IJ (1997): Lipoprotein lipase regulates fc receptor-mediated phagocytosis by macrophages maintained in glucose-deficient medium. J Clin Invest 100: 649–657.

4/2/2011