

(2013 / 5 / 27 2013 / 3 / 19)

PAO

PAO

(p<0.05)

I

PAO

(III, II, I) PAO

0.237 0.349) (0.098 0.183 0.194) (/)

(104018 100671·141458) (69339 74512·72588)

(0.176

FAD

PAO

PAO

PAO

:

Purification of Polyamine Oxidase from Diabetic Type-I Females and Studies of the Inhibitory Effect of some Thiourea Compounds on its Activity

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ABSTRACT

The research included a determination of polyamine oxidase (PAO) activity in normal females and diabetic type I patients. It was found that the activities of PAO in red blood cells (RBC) and plasma in patients were significantly higher than that of normal ($p < 0.05$).

The partial purification of PAO from RBC of diabetic and normal females were included in this study. This was achieved by using different biochemical techniques. Three proteinous peaks with PAO activities in RBC (I, II, III) from each of normal, diabetic with specific activities ($\mu\text{mg protein}$) (0.194, 0.183 and 0.098), (0.349, 0.237 and 0.176) were isolated from ion exchange chromatography. Molecular weights (72588, 74512 and 69339), (141458, 100671 and 104018) Da respectively.

This study didn't show the existence of Cu^{2+} ion as a cofactor for any PAO isoenzyme, but indicated the existence of flavin adenine dinucleotide (FAD) as a cofactor for all PAO isoenzymes.

On the other hand, the research included a preparation of thiourea derivatives of structure analogous to sulfonylurea drugs and containing thiosemicarbazide. Thiosemicarbazide showed an inhibitory effect on partially purified PAO activity. The prepared thiourea derivatives of thiosemicarbazide showed a competitive inhibition of PAO activity.

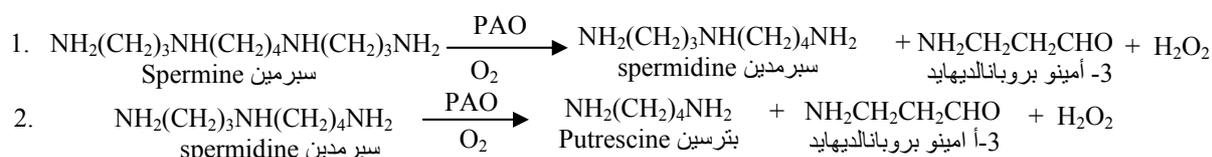
Keywords: Polyamine oxidase, diabetes mellitus, thiourea compounds.

Polyamine oxidase (PAO)

(Mendez *et al.*, 2007)

Polyamines (PA)

.(Seiler, 1987)



.....

(spm) Spermine

polyamine (PA)

(put) Putrescine

(spd) Spermidine

(Wallace *et al.*, 2003)

Spd Spm

PAO

Put

Spd

Spd

Spm

PA

.(Seiler, 1995)

DNA

PA

.(Sjoholm *et al.*, 2001)

(β)

spd

spm

-β-

spd

spm

spd

pm

.-β-

transglutaminase

.(Gimenez, 2010)

.PA

Arginase

platelet aggregation

PA L-Arg

.(Mendez and Zarzozo, 1997)

H₂O₂

lipids peroxidation

(Murray *et al.*,2009 ; Murray-

-β

.(Stewart, 2002)

Labnet

Enduro 15.10-midi-plus Gel.T.

.1

International Inc.

KBr

IR

.2

Infrared Spectrophotometer. Model Tensor 27 Bruker Co., Germany

EA-uroVector model : EA 3000 A Italy C.H.N

.3

Bruker,

¹HNMR

/ -

(DMSO-d₆)

300MHz

(40-30 °C)

80 : 1

(100 /)

)

100

.EDTA

5-4

(45-25 °C)

(10) (15000g)

%0.9 NaCl

. °4

3 (10) (15000g)

(10) (15000g)

(Rapoport *et al.*, 1979) Red Blood Cells Hemolysate (RBCH)

(Schacterle and Pollack, 1973)

RBCH

:

PAO

PAO

(spermine))

(Dahel *et al.*, 2001; Flayeh, 1988)

100mM

PAO

100mM

.pH=7.2 Na₂HPO₄-KH₂PO₄

(410)

) /

.(

PAO

PAO

:

10 : Dialysis

.1

6

10kD

.....

°4

.(Robyte and White, 1987)

- DEAE

:

.2

10

(Flayeh and Wallace, 1991)

2.5

40

-DEAE

°4

.pH=7.2

15mM

Na₂HPO₄-KH₂PO₄

(/ 68) / 1.3

. lyophilizer

PAO

:

.3

sodium dodecyl sulphate

(Robyte and White, 1987) polyacrylamide gel electrophoresis (SDS-PAGE)

480000

:

13000

23800

40000

67000

-

(0.01)

)

(0.01)

(

-

tolazamide

tolbutamide

25

(0.01)

6

.(

)

25

(9 7) .(2010)

.TLC

PAO

.Spm

PAO

Atomic absorption

.SENSAA-GBC Scintific Equipment

Spectrophotometer (Shemadzo), Double Beam

-

. (Matcheroulx, 1999) FAD

Shemadzo

RBCH

PAO

/ (200±22.5)

(P<0.05)

. / (103.32±17.75)

(P<0.05)

(1)

PAO

(15.0±4.9)

.(6.18±0.55)

PAO

:1

PAO Unit/mg protein (×10²) Mean ± SD	mg/ml	PAO Unit/ml (×10²) Mean ± SD	
a 3.62±0.35	a 28.53±11.85	a 103.32±17.75	
c 6.18±0.55	ab 32.13±3.85	c 200±22.5	
b 0.128±0.05	a 73.47±3.57	c 6.18±0.55	
c 0.15±0.06	d 100.39±3.85	b 15.0±4.9	

p<0.05

(2003)

RBC

(2001)

.....

.(Cohen *et al.*, 1976) PA RBC (2001)

PAO

.1

PAO

(3.141)

/

(0.0578)

(/

0.0184)

PAO

.(2)

(%274.46)

/

0.074)

0.033)

(2.24)

(

(%199.79)

(/

.(3)

.(Robyt and White, 1987)

:

.2

(2)

PAO

I, II, III

(1)

/

(0.0985)

(0.183)

(0.194)

(%82.18)

(5.353)

(9.945)

(10.543)

(%35.25)

(%74.13)

PAO

(2)

/

(0.176)

(0.237)

(0.215)

(3

) I

(5.36)

(7.19)

(6.51)

(%33.53)

(%22.84)

(%46.56)

(2001)

(2002)

PAO :2

%		Unit\mg protein	Unit\ml	* Unit\ml			
100	1	0.0184	7.95	0.795	429.75	10	
274.46	3.141	0.0578	21.82	2.079	377.25	10.5	Dialysis
82.18	10.543	0.194	6.534	0.108	33.56	60.5	Peak I
74.13	9.945	0.183	5.886	0.121	32.07	48.3	Peak II
35.25	5.353	0.0985	2.803	0.081	28.45	34.5	Peak III
							DEAE -

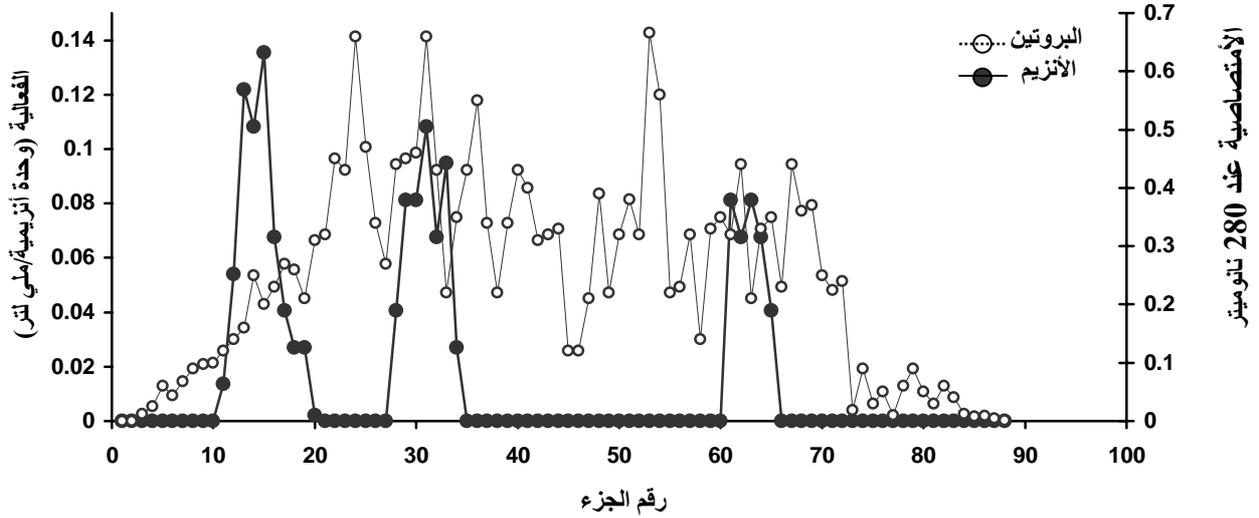
U *

Type-I PAO :3

%		Unit\mg protein	Unit\10ml	* Unit\ml			
100	1	0.033	24.16	2.416	714.75	10	
199.79	2.24	0.074	48.27	4.687	651.21	10.3	Dialysis
46.56	6.51	0.215	11.25	0.206	52.17	54.5	Peak I
22.84	7.19	0.237	5.52	0.203	23.249	27.2	Peak II
33.53	5.36	0.176	8.103	0.108	45.8	74.8	Peak III
							-DEAE

U *

.....

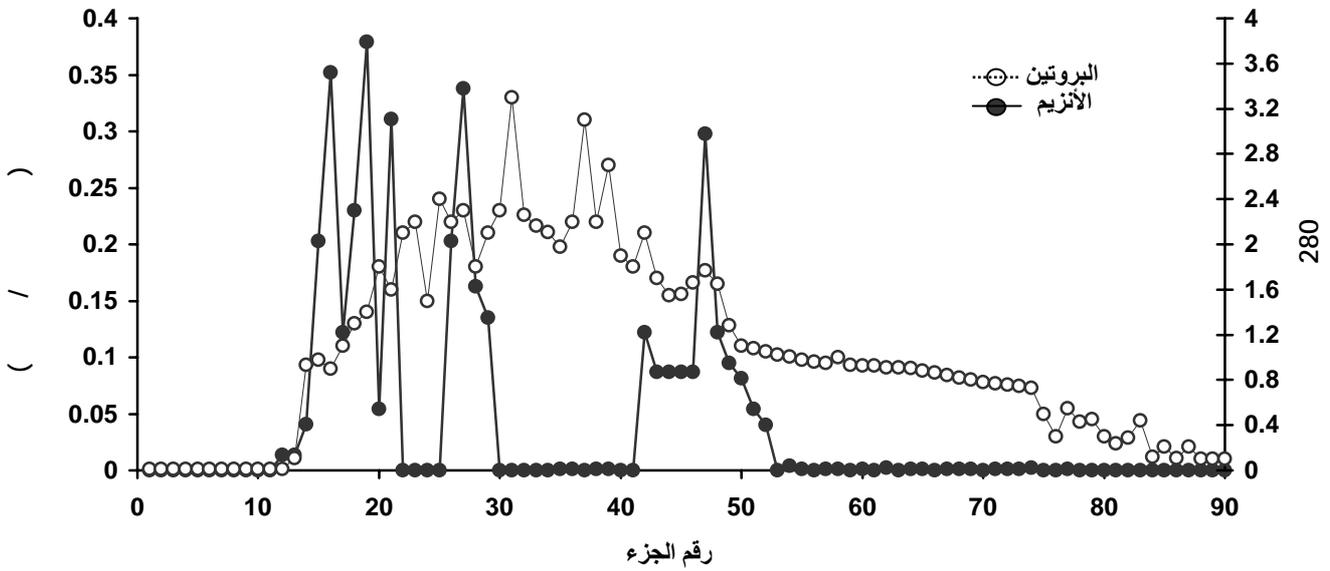


PAO

:1

-DEAE

2.5×40



PAO

:2

-DEAE

I

2.5×40

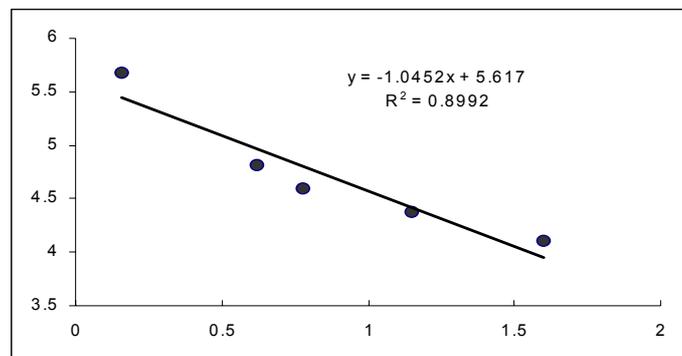
PAO
 λ_{max} - RBCH
 (4) FAD 415-360
 (CuAO) (Cu²⁺) Monoamine Oxidase
 Flavo-enzyme FAD PAO
 450-360 (Cona, 2006)
 .(Matcheroulx, 1999) FAD

600-200 FAD :4

PAO		PAO		
λ_{max}		λ_{max}		
434	0.548	426	0.063	Peak I
354	0.059	367	0.035	
414	0.844	447	0.759	PeakII
307	0.11	310	0.557	
414	0.353	436	0.394	peakIII
361	0.032	367	0.366	

SDS- PAGE
 I
 .(3)

I PAO
 .(5)

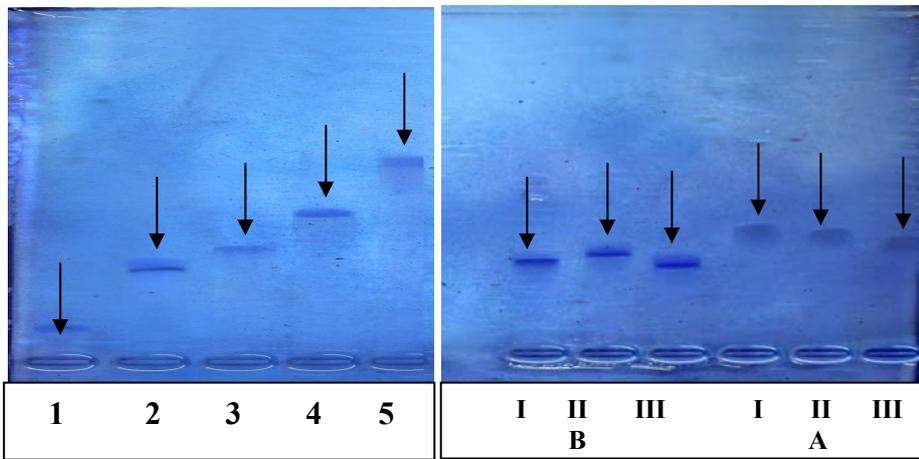


SDS-PAGE PAO :3

PAO

:5

kD		
74512	Peak I	PAO
72588	Peak II	
69339	peakIII	
100671	Peak I	PAO I
141458	Peak II	
104018	peakIII	



1-يوريز، 2-BSA، 3-بيروكسيديز، 4-تريپسين، 5-سايتوكروم اوكسيديز.
 A.متمائلات أنزيم PAO المنقاة من دم النساء السليمات.
 B. متمائلات أنزيم PAO المنقاة من دم النساء المصابات بالسكري نوع I

PAO

:1

PAO

PAO

(6)

C=S

(Jalkanen and Salmi, 2001)

.PAO

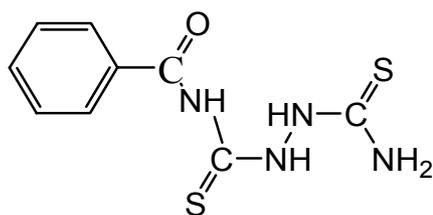
PAO

:6

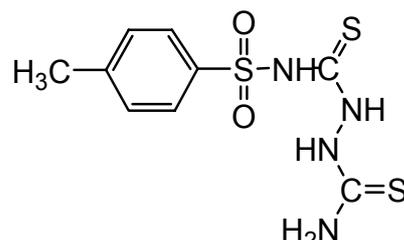
I

متمائل PAO المنقى من دم المصابات بالسكري نوع I		متمائل PAO المنقى من دم السليمات		التركيز (ملي مولار)
النسبة المئوية للتشيط %	الفعالية (وحدة أنزيمية/مل) $100 \times$	النسبة المئوية للتشيط %	الفعالية (وحدة أنزيمية/مل) $100 \times$	
0%	13.54	0%	10.8	سيطرة بدون مثبط
10	12.18	0	10.8	25
10	12.18	0	10.8	50
50	6.77	12.5	9.47	75
60	5.41	50	5.41	100
60	5.41	37.5	6.77	125
50	6.77	37.5	6.77	150
50	6.77	37.5	6.77	175

(B A)



1-(benzamido thiocarbonyl) thiosemicarbazide (A)



1-(p-toluene sulfonamido thiocarbonyl) thiosemicarbazide (B)

:7

Compound	Molecular formula	m.p °C	Yield %	Color	Rf
A	(C ₉ N ₄ S ₂ OH ₁₀)	140	45.49	yellow	0.822
B	(C ₉ N ₄ S ₃ O ₂ H ₁₂)	96	51.33	Brown	0.866

.C.H.N %

-1246 1- 3377-3160

(5.4)

A

C=S N-H

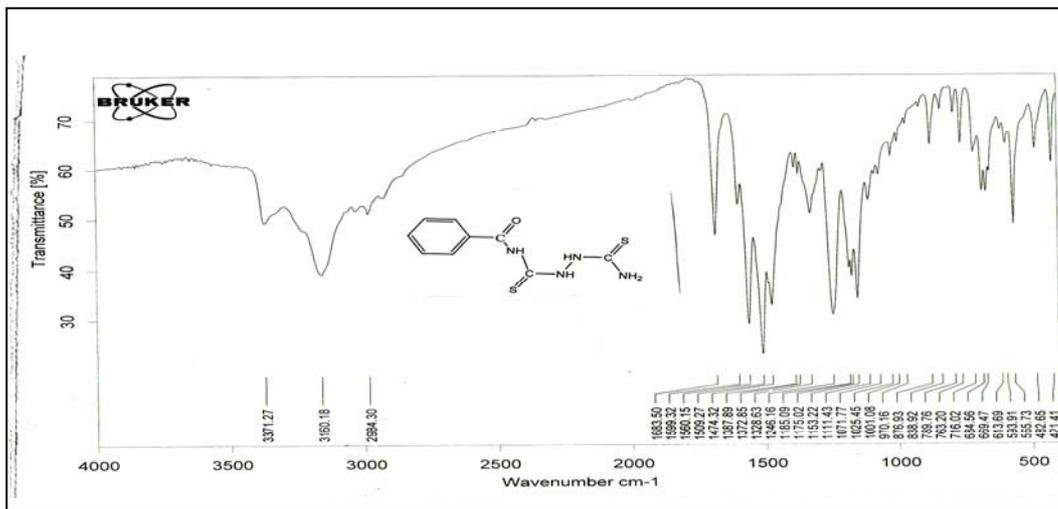
1- 1269

.....

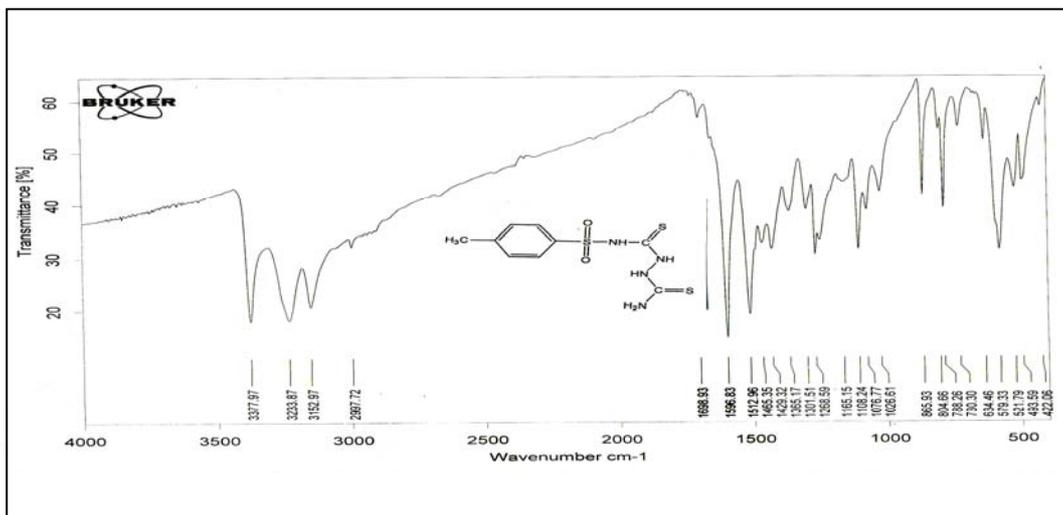
B C=O 1- 1683
 (SO₂) 1- 1365-1181
 (8)

B A :8

IR cm ⁻¹							
Compound	C—H aliphatic	C=C aromatic	C=S Stretch	<chem>O=C-NH-</chem> Amid	C—H Bending	S=O asym, sym	N—H Stretch
A	--	1599, 1560	1246	1683	763	---	3371, 3160
B	2997	1596, 1512	1269	---	804	1365, 1181	3377, 3233, 3160

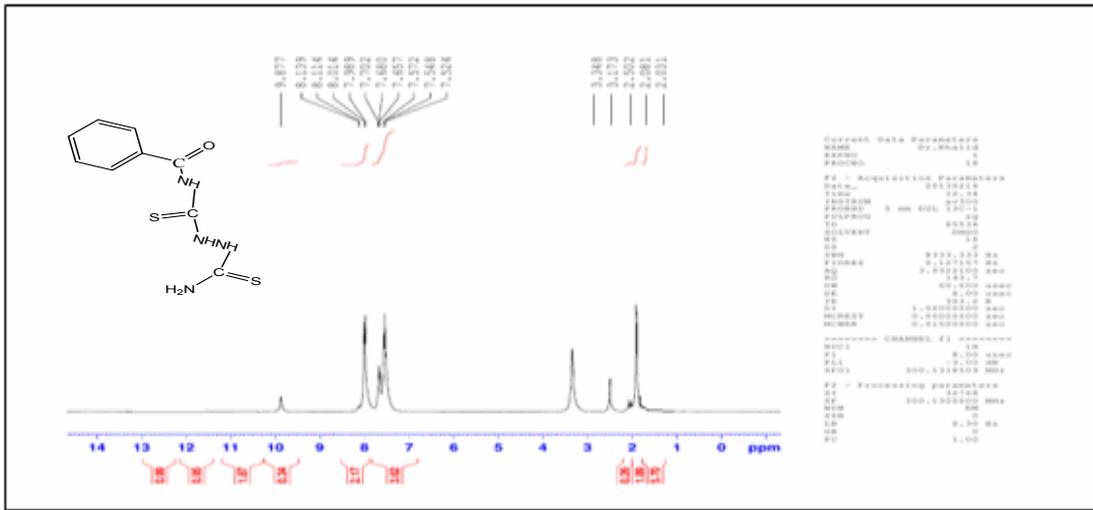


A :4

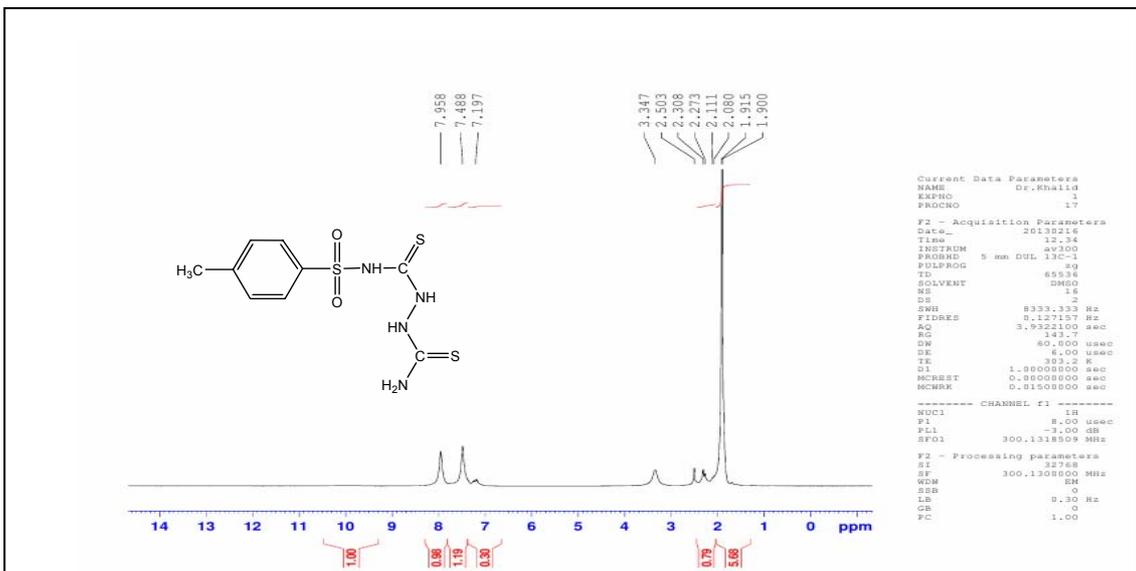


الشكل 5: طيف الأشعة تحت الحمراء للمركب B

10 ppm : A (6) ¹HNMR
 3.3 NH-NH 8.01ppm (—C(=O)-NH)
 7.5-7.7ppm . NH₂ ppm
 (7) B ¹HNMR .
 =δ NH₂ 2.2-2.5 ppm=δ NH,NHNH : δ
 7.1-=δ 2.1 ppm=δ 3H ,CH₃ 3.34 ppm
 .4H , phenyl 7.9 ppm



A ¹HNMR :6



B ¹HNMR :7

(CHN)

A

(9)

.B

C.H.N

:9

C.H.N %							
C%		H%		N%		S%	
42.51	42.58	3.93	5.47	22.04	22.35	25.19	25.69
35.52	35.78	3.94	3.46	18.42	18.49	31.57	31.85

PAO

B A

50 75

(10)

.I

RBC

B

100 125

A

I

PAO

B A

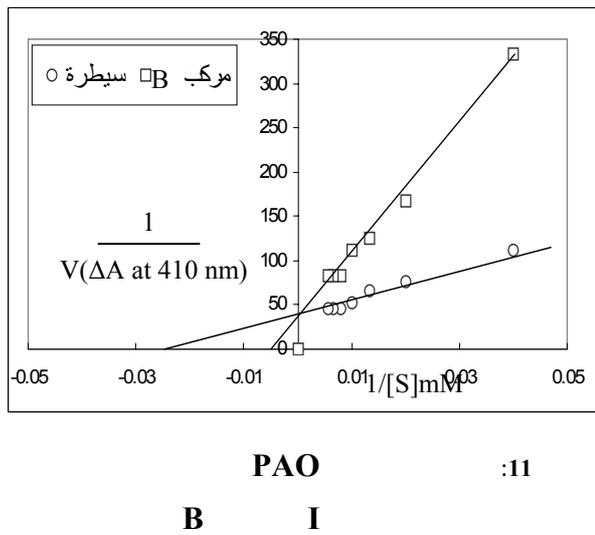
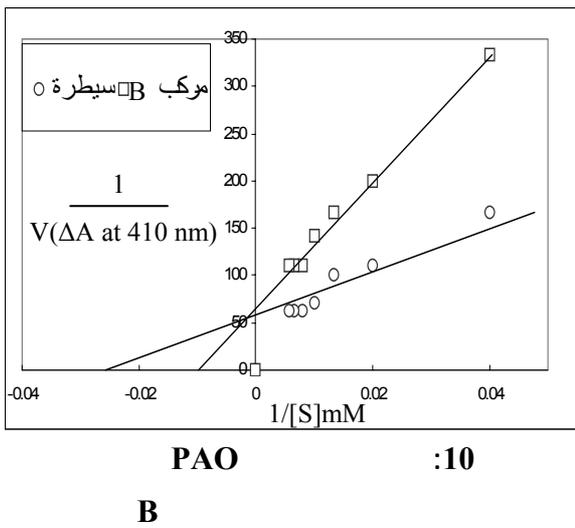
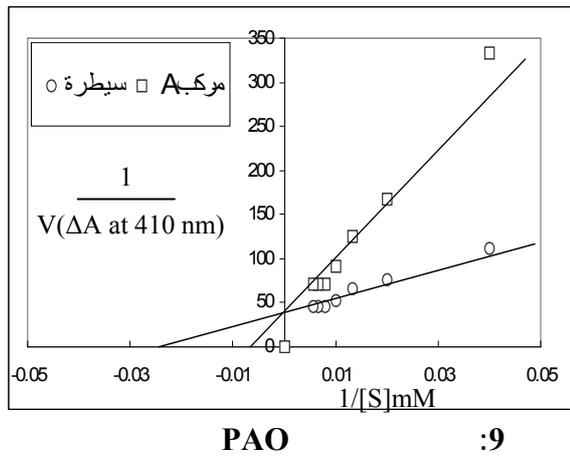
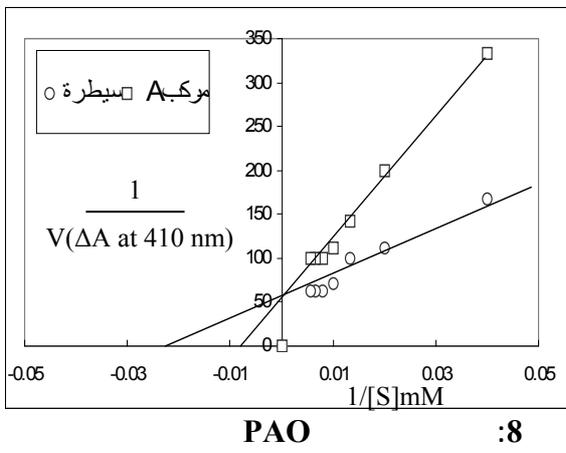
:10

I

المركب B				المركب A				
متمائل PAO المنقى من دم المصابات بالسكري نوع I		متمائل PAO المنقى من دم السليمات		متمائل PAO المنقى من دم المصابات بالسكري نوع I		متمائل PAO المنقى من دم السليمات		
النسبة المئوية للنتيبيط %	الفعالية وحدة أنزيمية/مل 100×	النسبة المئوية للنتيبيط %	الفعالية وحدة أنزيمية/مل 100×	النسبة المئوية للنتيبيط %	الفعالية وحدة أنزيمية/مل 100×	النسبة المئوية للنتيبيط %	الفعالية وحدة أنزيمية/مل 100×	التركيز ملي مولار
0%	12.18	0%	13.54	0%	13.54	0%	10.83	سيطرة بدون مثبط
22.2	9.47	40	8.12	40	8.12	37.5	6.77	25
22.2	9.47	20	10.83	60	5.41	37.5	6.77	50
11.1	13.54	10	12.18	50	6.77	50	5.41	75
55.5	5.41	40	8.12	40	8.12	37.5	6.77	100
55.5	5.41	50	6.77	40	8.12	37.5	6.77	125
44.4	6.77	40	8.12	60	5.41	37.5	6.77	150
22.2	9.47	30	9.47	30	9.47	25	8.12	175

C=S
C=S (Jalkanen and Salmi, 2001)
PAO

(8)



I PAO :11
 B,A

	(mM) Ki	V'max PAO (unit/ml)	Vmax PAO (unit/ml)	K'm (mM) PAO	Km (mM) PAO	() ()
PAO						
	24.18	0.02	0.02	142.85	45.45	(75) A
	45.81	0.02	0.02	125	45.45	(125)B
I PAO						
	10.60	0.025	0.025	200	41.66	(50) A
	16.86	0.025	0.025	250	41.66	(100)B

/ V'max / Vmax / K'm / Km
 Ki

.(2002)

C

.(2003)

.(2002)

-N

.(2010)

Rapoport, S.M.; Schewe, T.; Wiesner, R.; Halangk, W.; Ludwig, P. (1979). The lipoxygenase of Reticulocytes. *Eur. J. Biochem.*, **99**, 545-561.

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