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Synthesis, Selective Aldose Reductase Inhibitory Profile and Antihyperglycaemic Potential of Certain Parabanic Acid Derivatives.

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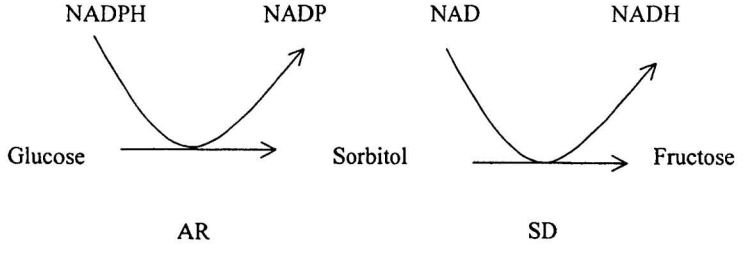
Abstract

Synthesis and aldose reductase inhibitory profile of certain parabanic acid derivatives **1a-p** is described. Also, the antihyperglycaemic potential of these compounds was studied. The most active inhibitors in this series were compounds **1g**, **1p**, and **1o** which showed inhibitory activity, 36.6, 90 and 91% respectively, at concentration 1×10^{-4}. Their IC_{50} were 2×10^{-6}, 7.5×10^{-8} and 5×10^{-8}, respectively. Compound **1o** exhibited pronounced antihyperglycaemic effect.

Keywords *: Diabetes mellitus, aldose reductase, aldose reductase inhibitors, parabanic acid derivatives.*

Introduction

Chronic diabetes leads to late-onset and long term diabetic complications which include neuropathy, nephropathy, retinopathy and cataract[(1)]. These syndromes result, at the biochemical level in hyperglycemics, from the intracellular production and accumulation of excess sorbitol and fructose due to an increased glucose flux through the polyol pathway[(2,3,4)] in tissues (nerve, kidney, retina, lens) that are insulin independent for glucose transport.



This polyol pathway, also called sorbitol pathway,is regulated by two enzymes: aldose reductase (AR; EC 1.1.1.21) which is an NADPH-linked oxidoreductase that

catalyses the conversion of glucose to sorbitol which in turn is converted to fructose by sorbitol dehydrogenase (SD; EC 1.1.1.14).

Over the past two decades, a number of structurally diverse compounds have been reported to inhibit AR (ARIs) and consequently possess potential utility for the prevention and amelioration of such diabetic complications[5,6,7] (Chart 1):

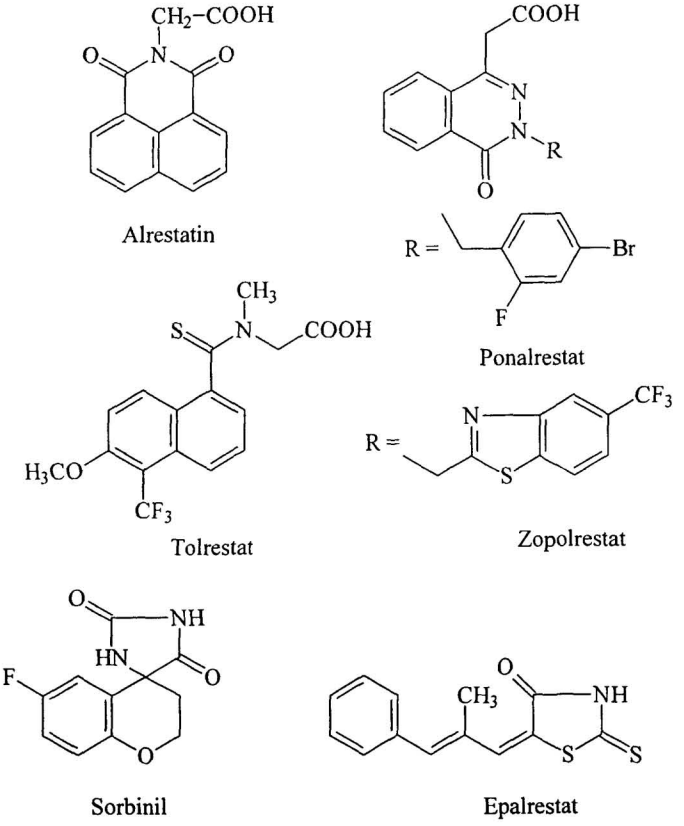
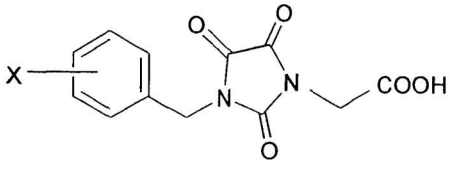


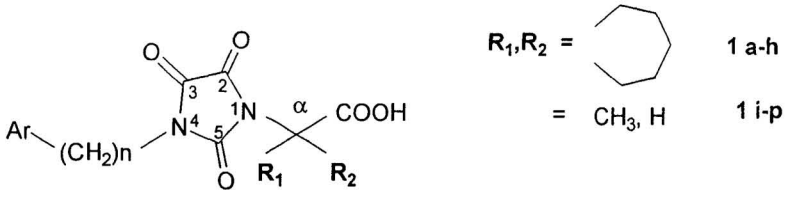
Chart 1

However, these ARIs have also been observed to inhibit aldehyde reductase (ALR; EC 1.1.1.2)[8,9], another oxidoreductase enzyme important for reduction of many aldehydes, that has useful functions such as counteraction and excretion of drugs, ascorbic acid synthesis and metabolism of lactic acid[10]. Therefore, the selective inhibition against AR seems to be important to avoid undesired adverse effects. It has been recently disclosed that certain parabanic acid derivatives namely: 3-(aralkyl)-2,4,5-trioxoimidazolidin-1-acetic acids display a high potential of

selectivity and inhibitory effect to AR[11,12,13] without significant inhibiting activity against ALR:



It is undertaken in this report the structure-based design, synthesis and **AR** inhibitory activity of a series of parabanic acids **1a-p,** in order to test the potential role of the increased lipophilicity at the α -position of the 1-acetic acid residue **(R_1,R_2)** and the AR inhibitory profile **(Tables 8 and 9).** Thus, we started with the synthesis of series **1a-h** in which **(R_1,R_2)** is a pentamethylene entity representing a high lipophilic residue, then with series **1i-p** in which **(R_1,R_2)** is one methyl group the least lipophilic moiety to be introduced.



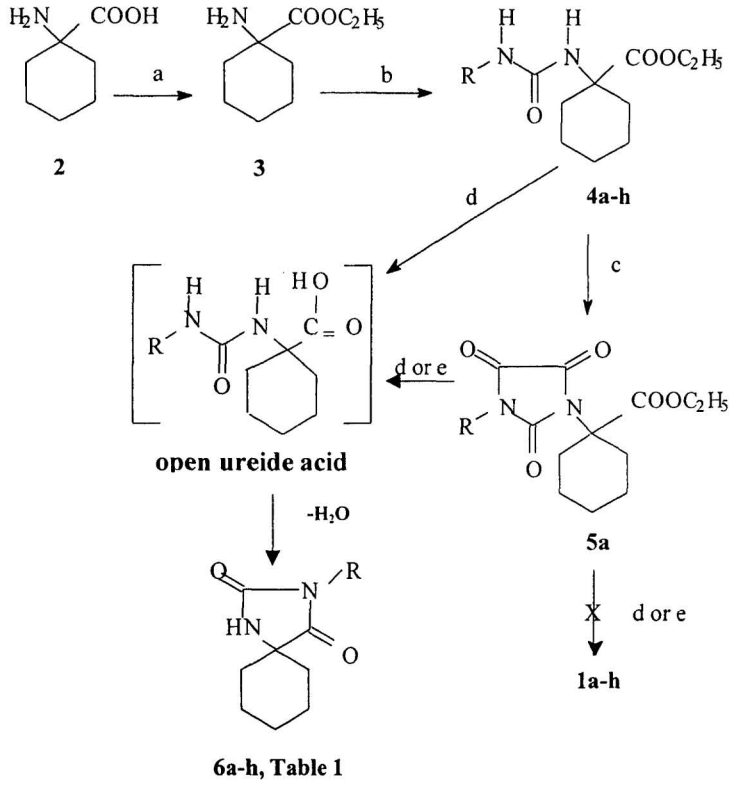
Moreover, the antihyperglycaemic potential of **1a-p** has been investigated. An approach that may lead to active species which display dual biological activities.

Chemistry

The synthetic strategy to achieve the target parabanic acids **1a-h** is outlined in **Schemes 1** and **2**. The ureidocyclohexane ethyl esters **4a-h** were obtained by the reaction of equimolar amounts of **3** with either KCNO for **4a** or with the appropriate aryl or aralkylisocyanate **14** in chloroform at O°C for compounds **4b-h**. The products **4a-h** were used as such in the next step. The trioxo-imidazolidin-1-yl cyclohexane ethyl esters **5a-h** were synthesized through the reaction of **4a-h** in THF with oxalylchloride. However, trials to reach the target compounds **1a-h** from the ethyl esters **5a-h** by adopting **Scheme 1** were not successful and instead the

respective hydantoins **6a-h** were obtained **(Table 1).** Also the same result of obtaining the corresponding hydantoins **6a-h** has been achieved when ureides **4a-h** were treated under the same conditions (**Scheme 1**). Compound **5a** was taken as a representative example to explain such reaction. Thus, hydrolysis of **5a** with either conc. HCl and AcOH under reflux[11] or LiOH[14] in tetrahydrofuran at room-temperature were not successful, and instead the respective hydantoin **6a** was obtained (**Table 1**). Also the same result of obtaining the corresponding hydantoin **6a** has been achieved when ureide **4a** was treated under the same conditions (**Scheme 1**). This result might be attributed to firstly the hydrolysis of the parabanic acid ethyl ester of the cyclohexyl series **5a** to the respective **open ureide acid** (c.f. **Scheme 1**), which is then, possibly due to the close spatial proximity of the carboxylic group to the nitrogen-3, undergoes dehydrative cyclisation to the corresponding hydantoin **6a.** These results led us to prepare the corresponding benzyl esters **8a-h** (**Scheme 2, Method A**), in order to achieve the required compounds **1a-h,** through hydrogenolysis. Consequently, the use of acid or base hydrolysis of the ethyl esters to obtain **1a-h,** and which would catalyse the hydantoin formation **6a-h** was avoided. Compound **7** as p-toluene sulphonate salt was synthesized by refluxing a mixture of 1-amino-1-cyclohexane carboxylic acid **2**[15], paratoluenesulphonic acid monohydrate and benzyl alcohol in toluene using Dean-Stark water-separator[16]. The free base of **7** was then treated with the appropriate aryl or aralkyl isocyanate **14** in chloroform firstly at 0°C then at room temperature [17] to afford the aryl- or aralkylureido benzyl esters **8a-h** in 60-97% yields (**Table 2**). The trioxoimidazolidine benzyl esters **9a-h** (**Table 3**) were obtained in 52-98% yields by stirring equimolar amounts of **8a-h** and oxalyl chloride firstly at O°C, then at ambient temperature for 3 hours. Hydrogenolysis of **9a-h** using hydrogen and Pd/C in ethylacetate gave **1a-h** in 65-85% yields (**Table 5**). The synthetic strategy for series **1i-p** is depicted in **Scheme 2, Method B**. Thus DL (±) alanine ethyl ester hydrolchloride **11** was obtained by refluxing **10** in a mixture of $SOCl_2$ and absolute ethanol[18]. The librated base of **11** was treated with the appropriate isocyanate **14** under stirring and cooling to give the ureides **12a-h** in 51-80% yield (**Table 6**). The trioxoimidazolidin propionic acid ethyl esters **13a-h (Table 4)** were obtained in 61-97% yields as illustrated under **Scheme 2** . Hydrolysis of the ethyl esters of **13 a-h** was performed by refluxing in a mixture of acetic acid and hydrochloric acid[11] to obtain **1i-p** in 34-59% yields (**Table 7**).

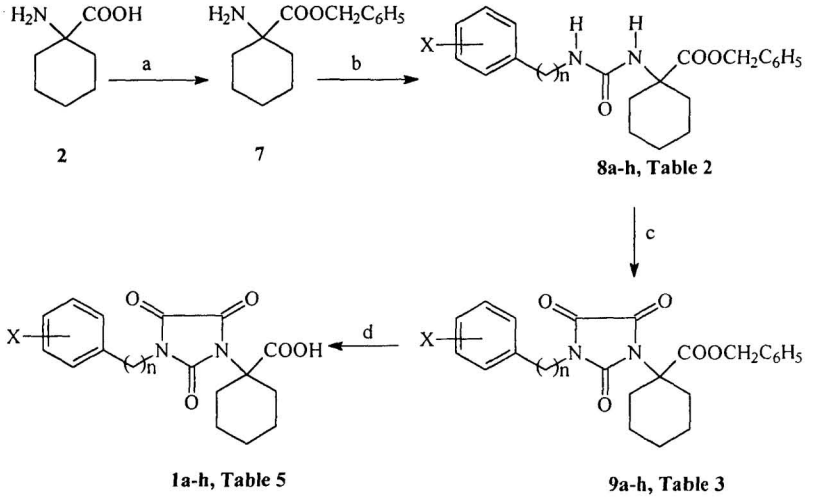
Scheme 1[a]



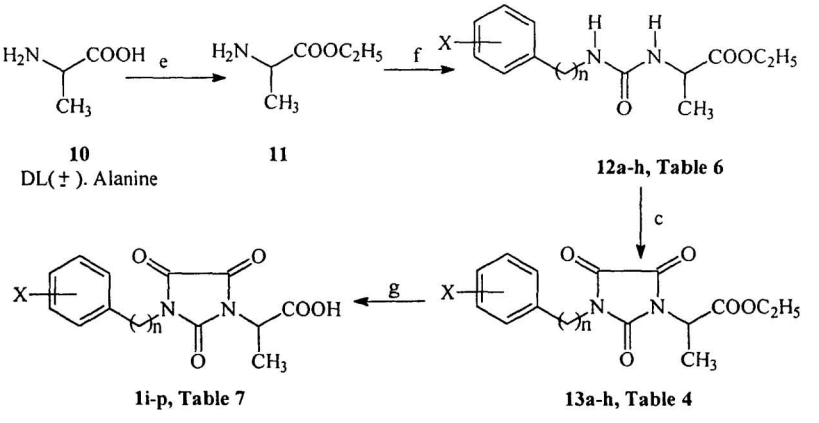
[a] **Reagents:** a) $SOCl_2$ or dry HCl gas, C_2H_5OH, m.p. of hydrochloride salt 193°C (ref. 19); b) KCNO for **4a** or the appropriate isocyanate **14 (Scheme 3)** for **4b-h**, $CHCl_3$, 0°C then r.t.3hr; c) oxalyl chloride, THF, 0°C; d) LiOH, THF; e) HCl, AcOH.

Scheme 2

Method A[a]:



Method B[a]:



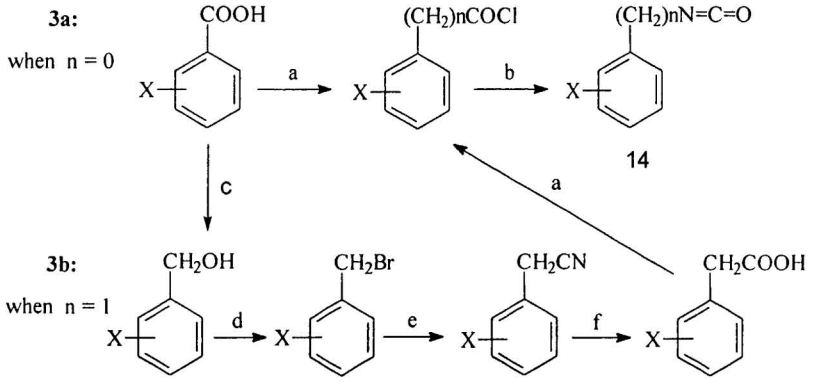
[a] **Reagents:** a) $C_6H_5CH_2OH$, 4-$CH_3C_6H_4SO_3H.H_2O$; **7** as p-toluenesulphonate (ref. 16); b) **7** (base), $CHCl_3$, appropriate isocyanate derivative **14** (**Scheme 3**), r.t.; c) oxalyl chloride, THF, 0°C then r.t.; d) H_2/Pd/C, ethylacetate; e) $SOCl_2$, ab. C_2H_5OH, reflux, **11** as hydrochloride; f) $NaHCO_3/H_2O$, appropriate isocyanate derivative **14** (**Scheme 3**), 0°C; g) AcOH, HCl, reflux.

The appropriate aryl and aralkyl isocyantes **14** were prepared using Curtius Rearrangement [17] as illustrated under **Scheme 3**:

Scheme 3a was adopted for the synthesis of aryl isocyanates. Thus, the aryl acid chloride, which was obtained by refluxing the corresponding acid with thionyl chloride, was refluxed with sodium azide in dry toluene to afford **14.**

Scheme 3b was followed for achieving aralkyl isocyanates by reacting the aralkylalchohol with PBr_3 in methylene chloride. The obtained aralkylbromide was then treated with KCN to the corresponding nitrile derivative. The latter was hydrolysed either under acidic condition or alkaline condition- in case of 4-methoxy derivative to avoid o-demethylation- to give the aralkyl acid. The latter was in turn converted to the required isocyanate **14** as described under aryl isocyanates.

Scheme 3[a]



n = 0; X = H; 4-Cl; 3,4-Cl_2; 4-Cl-3,5-$(NO_2)_2$; 3-OCH_3; 4-OCH_3.

n = 1; X = H; 4-Cl; 2,4-Cl_2; 3,4-Cl_2; 4-OCH_3; 3-NO_2.

[a] **Reagents:** a) $SOCl_2$; b) NaN_3, toluene, reflux 1h; c) $ClCOOC_2H_5$, $NaBH_4$; d) PBr_3, CH_2Cl_2; e) KCN; f) CH_3COOH, H_2SO_4 or NaOH (50%) in case of methoxy derivative.

Experimental :

Melting points were determined on Electrothermal capillary melting point apparatus and were uncorrected. Infrared spectra were recorded on a BECKMANN Infracord model 4220. ^{1}H-NMR ($CDCl_3$,DMSO-d_6-TMS) were recorded on Jeol Ex-270 MHz, NMR spectrometer. The mass spectra were performed with a Finnigan-Mat SSQ 7000 mass spectrometer,with electroenergy of 70 ev. The microanalytical data were carried out at the National Research Centre, Dokki, Cairo.The analytical results deviated maximally $\pm$ 0.4% from the theoretical values of C,H and N.

The 1-amino-1-cyclohexanecarboxylic acid **2** was synthesized according to the reported procedure[15]. The corresponding ethylester hydrochloride **3** was obtained by refluxing **2** in ethanol with thionyl chloride or dry hydrogen chloride gas [19].

1-Ureido-cyclohexanecarboxylic acid ethyl esters 4 a-h (Scheme 1):

Equimolar amounts of **3** and KCNO for **4 a** or the appropriate arylisocyanates **14** for **4b-h (Scheme 3)** were stirred in $CHCl_3$ at 0°C. The reaction mixture was left to reach room temperature and stirring was continued overnight. The organic solvent was dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to dryness. The residual oily products of **4a-h** were used as such for the next step.

1- (2,4,5- Trioxo-imidazolidin-1-yl) cyclohexanecarboxylic acid ethyl ester 5a (Scheme 1) :

Compound **5a** is taken as a representative example :

To a solution of 2.14 g (10 mmol) of **4a** in 35 ml of THF was added 1.02 ml (12 mmol) of oxalyl chloride dropwise at 0°C. The reaction mixture was left under stirring for about 3 hr to reach room temperature. The formed precipitate removed by filtration. The filtrate was concentrated to give a solid substance, which was dissolved in ethyl acetate then washed with brine. The organic layer was dried over anhydrous Na_2SO_4 evaporated under reduced pressure and crystallized from ethanol to afford **5a** mp 175-7°C (71%), I.R. (KBr,cm^{-1}) 1739 (C=O);MS m/z 268 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.01 (t3HC**H**$_3$); 1.22-1.86 (m, 10H, cyclohexyl), 3.75 (q,2H,COOC**H**$_2$), 12.11 (s, 1H,N**H**). Anal. (C_{12} H_{16} N_2 O_5) : Calc : C = 53.73,H = 6.01, N = 10.44, found : C = 53.78, H = 6.13, N = 10.51.

3-(Aralkyl and/or aryl)1,3- diaza-spiro [4,5] decan-2,4-diones 6 a-h (Scheme 1, Table 1)[20,21,22] :

Under basic conditions :

To a stirred solution of 5.5 mmol of either **4 a-h** or **5 a** in a mixture of 45 ml of THF and 34 ml of water at 0-5°C, was added 1ml of 1 M lithium hydroxide[14], while

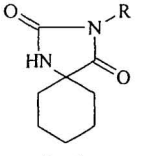
the temperature rose to ambient stirring continued for further 3 hr. On diluting the reaction mixture with water **6a-h** were precipitated and crystallized from ethanol, c.f. **Table 1**; I.R. (KBr, cm^{-1}) 3220 and 3180 (NH),1770-1730 (C=O).

Under acidic conditions :

1,3-Diaza-spiro[4.5]decan-2,4-dione **6a** was obtained by refluxing a mixture of 2.95 g (11 mmol) of **5a,** 10 ml of glacial acetic acid and 5 ml of concentrated HCl for 3 hr. The reaction mixture was concentrated under reduced pressure to give a residue, which was refluxed again with 10 ml of glacial acetic acid and 5 ml of concentrated HCl for further 2 hr. The remaining residual solid after evaporation was washed with water, followed by 10% aqueous Na_2CO_3, water, and crystallized from ethanol to give 1.57 g (85%) colourless crystals, c.f. **Table 1.**

1-Aminocyclohexanecarboxylic acid benzyl ester p-toluenesulphonate 7 (Scheme 2, Method A) was synthesized by adopting the procedure cited in ref 20 by refluxing 0.05 mol of **2**, 0.055 mol of para-toluenesulphonic acid monohydrate and 100 ml of benzyl alcohol in 150 ml of toluene using Dean-Stark water-separator.

Table 1 : 3 - (Aralkyl and/or aryl)- 1,3 - diaza - spiro [4,5] decan - 2,4 –diones **6a-h (Scheme 1)**



6a-h

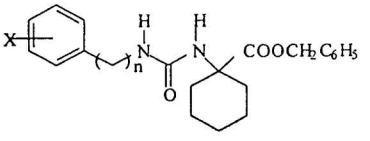
No. of comp.	R	Mp	Yield	Molecular formula mol. wt.	Analysis (%)			Ref.
					C	H	N	
6 a	H	218-20	81.85		Calc : Found:			20
6 b	C_6H_5	185-6	79		Calc : Found:			21
6 c	4-Cl-C_6H_4	245-9	82	$C_{14}H_{15}Cl\ N_2O_2$ 278.59	Calc : 60.35 Found: 60.13	5.43 5.34	10.06 10.15	
6 d	3,4-Cl_2-C_6H_3	216-8	83	$C_{14}H_{14}\ Cl_2N_2O_2$ 313.18	Calc : 53.69 Found: 53.71	4.51 4.55	8.95 8.89	
6 e	4-OCH_3-C_6H_4	186-90	80		Calc : Found:			20
6 f	$C_6H_5CH_2$	152-3 (Lit.155-6)	78		Calc : Found:			22
6 g	4-Cl-C_6H_4 CH_2	188-9 (Lit.182-3)	74		Calc : Found:			22
6 h	3,4-Cl_2-C_6H_3 CH_2	176-7	75	$C_{15}H_{16}Cl_2\ N_2O_2$ 327.21	Calc : 55.06 Found: 55.21	4.93 4.92	8.56 8.66	

General procedure for the preparation of 1- [3-(aralkyl and/or aryl)-ureido]-cyclohexanecarboxylic acid benzyl esters 8a-h (Scheme 2, Method A, Table 2) :

The free base of 1-aminocyclohexanecarboxylic acid benzyl ester p-toluenesulphonate **7** was liberated from its salt by suspending the salt in a mixture of $CHCl_3$: ice cold water (4:1), followed by addition of excess 1M $NaHCO_3$ under stirring for 15 min. The organic layer was separated, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to give the free base of **7** (97%).

A mixture of 2.33 g (10 mmol) of **7** (base) and 10 mmol of the appropriate benzyl and/or phenyl isocyanates (**Scheme 3**) in 20 ml $CHCl_3$ was stirred for 3 hr at room temperature.The solvent was removed under reduced pressure, and the residual solid substance was crystallized from ethanol-water mixture to afford **8a-h** (c.f. **Table 2**) IR (KBr, cm^{-1}) of **8a-h:** 3328-3330 (NH), 1735-1736 (C=O) ester, 1634-1636 (C=O) amidic. The following data for ^1H NMR and MS were taken as representative examples of **8a-h**: ^1H NMR (DMSO-d_6) of **8a** : δ 1.19-1.95 (m, 10H, cyclohexyl), 5.09 (s, 2H, OCH_2), 6.55 (s, 1H, NH-cyclohexyl), 6.92-7.35 (m, 10H, Ar-H), 8.43 (s, 1H, HN-phenyl). MS m/z of **8a** 352 (M^+, 37.6%); MS m/z of **8b** 386 (M^+, 11%); MS m/z of **8e** 366 (M^+, 8%);

Table 2: 1- [3-(Aralkyl and/or aryl)- ureido]- cyclohexanecarboxylic acid benzyl esters **8-a-h (Scheme 2, Method A)**



8a-h

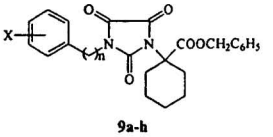
No. of * comp.	n	X	MP	Yield %	Molecular formula mol. wt.	Analysis (%) C	H	N
8a	0	H	168-70	97	$C_{21}H_{24}N_2O_3$ 352..432	Calc : 71.57 Found: 71.35	6.86 6.91	7.95 7.92
8b	0	4-Cl	250-2	60	$C_{21}H_{23}ClN_2O_3$ 386.877	Calc : 65.20 Found: 65.31	5.99 5.94	7.24 7.31
8c	0	3,4- Cl_2	174-2	67	$C_{21}H_{22}Cl_2N_2O_3$ 421.322	Calc : 59.87 Found: 59.79	5.26 5.34	6.65 6.62
8d	0	4-OCH_3	179-80	77	$C_{22}H_{26}N_2O_4$ 382.458	Calc : 69.09 Found: 69.13	6.85 6.79	7.32 7.35
8e	1	H	108-10	67	$C_{22}H_{26}N_2O_3$ 366.459	Calc : 72.11 Found: 72.44	7.15 7.20	7.64 7.61
8f	1	4-Cl	141-3	72	$C_{22}H_{23}ClN_2O_3$ 400.907	Calc : 65.91 Found: 65.99	6.29 6.31	9.48 9.52
8g	1	3,4-Cl_2	117-9	74	$C_{22}H_{24}Cl_2N_2O_3$ 435.349	Calc : 60.70 Found: 60.78	5.56 5.50	6.99 7.02
8h	1	4-OCH_3	123-5	83	$C_{23}H_{28}N_2O_4$ 396.490	Calc : 69.68 Found : 69.73	7.12 7.22	7.07 7.11

* Crystallization solvent : ethanol: water .

General procedure for the preparation of 1-[3-(aralkyl and/or aryl)-2,4,5-trioxo-imidazolidin-1-yl]-cyclohexanecarboxylic acid benzyl esters (9a-h, Scheme 2, Method A, Table 3), and 2-[3-(aralkyl and/or aryl)-2,4,5-trioxo-imidazolidin-1-yl]-propionic acid ethyl esters (13a-h, Scheme 2, Method B, Table 4):

To a solution of 10 mmol **8a-h** or **12a-h** in 50 ml of THF was added dropwise at 0°C 1.02 ml (10 mmol) of oxalyl chloride. The stirred mixture was left for 3 hr. at room temperature. The organic solvent was evaporated under vacuum and the residual semisolid substance was extracted with ethyl acetate and washed with water. the ethyl acetate layer was dried over anhydrous Na_2SO_4, evaporated under vacuum and crystallized from the appropriate solvent (c.f. **Tables 3** and **4**). IR (KBr or liquid film, cm^{-1}) of **9a-h** and **13a-h**: 1740 (C=O), 1720 (C=O). The next spectral data were taken as representative examples of **9a-h** and **13a-h** ^1H NMR ($CDCl_3$) of **9a**: δ 1.21-1.98 (m, 10H, cyclohexyl), 5.12 (s2H, OCH_2), 7.09-7.64 (m, 10H, Ar-**H**); MS m/z of **9a**: 407 (M+1, 35.2%). ^1H NMR ($CDCl_3$) of **9d**: δ 1.32-2.02 (m, 10H, cyclohexyl), 3.80 (s, 3H, OCH_3), 5.20 (s, 2H, OCH_2), 6.94, 7.18 AB system, 10 Hz integrated for 4 Ar-**H**; 7.25-7.40 (m, 5H, Ar-**H**). MS m/z of **9d**: 436 (M^+, 100%). ^1H NMR ($CDCl_3$) of **9e**: δ 1.23-2.05 (m, 10 H, cyclohexyl), 4.71 (s, 2H, NCH_2), 5.18 (s, 2H, OCH_2), 7.18-7.4 (m, 10H, Ar-**H**). MS of **9e**: 420 (M^+, 67.4%). ^1H NMR ($CDCl_3$) of **9f**: δ 1.29-2.02 (m, 10 H, cyclohexyl), 4.75 (s, 2H, NCH_2), 5.15 (s, 2H, OCH_2), 7.25-7.35 (m, 9 H, Ar-**H**,) MS m/z of **9f**: 455 (M^+, 7.8%); ^{13}C NMR ($CDCl_3$) of **9f**: δ 170.272 (COO). ^1H NMR ($CDCl_3$) of **13 a**: δ 1.30 (t, 7 Hz, 3H, CH_3 ethylester), 1.75 (d, 7.5 Hz, 3H, CH_3), 4.24 (q, 7 Hz, 2H, CH_2), 4.93 (q, 7.5 Hz, 1H, CH), 7.35-7.63 (m, 5H, Ar-**H**); MS m/z of **13a**: 290 (M^+, 83%). MS m/z of **13e**: 304 (M^+, 66%).

Table3: 1-[3-(Aralkyl and/or aryl)- 2,4, 5-trioxo- imidazolidin-1-yl]cyclohexane - carboxylic acid benzyl esters, **9 a- h (Scheme 2, Method A)**

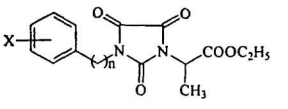


9a-h

No. of * comp.	n	X	MP	Yield %	Molecular formula mol. wt.	Analysis (%) C	H	N
9 a	0	H	90-2	86	$C_{23}H_{23}N_2O_5$	Calc : 67.97	5.46	6.89
					406.440	Found : 67.88	5.47	6.91
9 b	0	4-Cl	98-100	53	$C_{23}H_{21}ClN_2O_5$	Calc : 62.66	4.80	6.35
					440.887	Found : 62.64	4.85	6.39
9 c	0	3,4-Cl_2	99-101	63	$C_{23}H_{20}Cl_2N_2O_5$	Calc : 58.12	4.24	5.89
					475.332	Found : 58.16	4.27	5.93
9 d	0	4-OCH_3	101-3	65	$C_{24}H_{24}N_2O_6$	Calc : 66.05	5.54	6.42
					436.467	Found : 66.26	5.67	6.39
9 e	1	H	74-6	52	$C_{24}H_{24}N_2O_5$	Calc : 68.56	5.75	6.66
					420.467	Found : 68.73	5.77	6.71
9 f	1	4-Cl	98-100	89	$C_{24}H_{23}ClN_2O_5$	Calc : 63.37	5.10	6.16
					454.914	Found : 63.44	5.11	6.20
9 g	1	3,4-Cl_2	100-2	67	$C_{24}H_{22}Cl_2N_2O_5$	Calc : 58.91	4.53	5.73
					489.357	Found : 58.93	4.55	5.74
9 h	1	4-OCH_3	Oil	98	$C_{25}H_{26}N_2O_6$	Calc : 66.65	5.82	6.22
					450.493	Found : 66.66	5.90	6.24

* Crystallization solvent : 2- propanol .

Table 4: 2-[3-(Aralkyl and/or aryl)- 2,4, 5-trioxo- imidazolidin-1-yl] – propionic acid ethyl esters, **13 a- h (Scheme 2, Method B)**



13a-h

No. of * comp.	n	X	MP	Yield %	Molecular formula mol. wt.	Analysis (%) C	H	N
13 a	0	H	108-10	97	$C_{14}H_{14}N_2O_5$ 290.279	Calc : 57.93 Found: 57.88	4.84 4.87	9.65 9.63
13 b	0	4-Cl, 3,5 $(NO_2)_2$	78-80	82	$C_{14}H_{11}ClN_4O_9$ 414.722	Calc : 40.55 Found: 40.57	2.67 2.71	13.51 13.48
13 c	0	3-OCH_3	76-8	98	$C_{15}H_{16}N_2O_6$ 320.306	Calc : 56.25 Found : 56.31	5.06 5.05	8.75 8.77
13 d	0	4-OCH_3	78-80	61	$C_{15}H_{16}N_2O_6$ 320.306	Calc : 56.25 Found:56.33	5.06 5.11	8.75 8.78
13 e	1	H	102-4	85	$C_{15}H_{16}N_2O_5$ 304.306	Calc : 59.21 Found: 59.34	5.30 5.36	9.21 9.27
13 f	1	2,4-Cl_2	124-6	86	$C_{15}H_{14}Cl_2N_2O_5$ 373.196	Calc : 48.28 Found: 48.25	3.78 3.76	7.51 7.74
13 g	1	3,4-Cl_2	106-8	81	$C_{15}H_{14}Cl_2N_2O_5$ 373.196	Calc : 48.28 Found: 48.34	3.78 3.76	7.51 7.52
13 h	1	3-NO_2	Oil	93	$C_{15}H_{15}N_3O_7$ 349.305	Calc : 51.58 Found: 51.80	4.33 4.36	12.03 12.11

* Crystallization solvent : ethyl acetate: n- hexane .

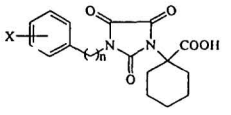
General procedure for the preparation of 1-[3-(aralkyl and/or aryl)-2,4,5-trioxo-imidazolidin-1-yl]-cyclohexanecarboxylic acids (1a-h, Scheme 2Method A, Table 5):

To a stirred solution of 5.48 mmol of **9a-h** in 20 ml of ethyl acetate, was added 0.23 g of 10% palladium on charcoal for 18 hr under hydrogen atmosphere under normal pressure and temperature. The reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was crystallized to afford **1a-h** (c.f. **Table 5**); IR (KBr, cm^{-1}) 1738-1740 (C=O), 1722 (C=O).

The following spectral data were taken as representative examples of **1a-h** : ^1H NMR ($CDCl_3$) of **1a**: δ 1.22-2.03 (m, 10 H, cyclohexyl), 7.01-7.87 (m, 5H, Ar-**H**), 13.38 (br s, 1H, COO**H**); MS m/z of **1a** 316 (M^+, 100%); MS m/z of **1b**: 350 (M^+, 38%); MS m/z of **1c**: 385 (M^+-1, 15%); MS m/z of **1d**: 346 (M^+, 18%); ^1H NMR ($CDCl_3$) of **1e**: 1:31-2.10 (m, 10H, cyclohexyl), 4.82 (s, 2H, CH_2), 7.22-7.48 (m, 5H, Ar-**H**), 13.45 (br s, 1H, COO**H**); MS m/z of **1e**: 330 (M^+, 100%); MS m/z of **1f**: 364 (M^+, 36%); ^1H NMR ($CDCl_3$) of **1g**: 1.30-2.12 (m, 10H, cyclohexyl); 4.78 (s, 2H, CH_2), 7.34-7.41 (m, 3H, Ar-**H**), 13.43 (br s, 1H, COO**H**). ^{13}C NMR ($CDCl_3$) of **1e** δ 176.910 (COOH). MS m/z of **1g**: 399 (M^+, 13%); MS m/z of **1h**: 360 (M^+, 95%).

DL (±) Alanine ethylester hydrochloride **11** was achieved according to the method cited in ref 22, by refluxing an ethanolic suspension of DL-alanine in the presence of thionyl chloride for 5 hr.

Table 5: 2-[3-(Aralkyl and/or aryl)- 2,4, 5-trioxo- imidazolidin-1-yl] – cyclohexane - carboxylic acids, **1 a- h (Scheme 2, Method A)**



1a-h

No. of * comp.	n	X	MP	Yield %	Molecular formula mol. wt.	Analysis (%) C	H	N
1 a	0	H	212.4	83	$C_{16}H_{16}N_2O_5$	Calc : 60.75	5.10	8.86
					316.317	Found: 60.72	5.13	8.84
1 b	0	4-Cl	224-6	65	$C_{16}H_{15}ClN_2O_5$	Calc : 54.79	4.31	7.99
					350.762	Found: 54.68	4.33	8.04
1 c	0	3,4-Cl_2	209-11	68	$C_{16}H_{14}Cl_2N_2O_5$	Calc : 49.89	3.66	7.27
					385.207	Found: 49.91	3.70	7.25
1 d	0	4-OCH_3	108-2	77	$C_{17}H_{18}N_2O_6$	Calc : 58.95	5.24	8.09
					346.344	Found :58.88	5.23	8.12
1 e	1	H	179-81	85	$C_{17}H_{16}N_2O_5$	Calc : 61.81	5.49	8.48
					330.344	Found: 61.83	5.51	8.47
1 f	1	4-Cl	158-60	80	$C_{17}H_{17}ClN_2O_5$	Calc : 55.97	4.70	7.68
					364.789	Found: 55.96	4.72	7.80
1 g	1	3,4-Cl_2	182-4	79	$C_{17}H_{16}Cl_2N_2O_5$	Calc : 51.15	4.04	7.02
					399.234	Found: 51.22	4.06	7.08
1 h	1	4-OCH_3	178-80	74	$C_{18}H_{20}N_2O_6$	Calc : 59.99	5.59	7.77
					360.371	Found: 59.87	5.61	7.76

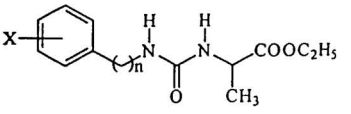
* Crystallization solvent : chloroform : n-hexane .

General procedure for the preparation of 2-[3-(aralkyl and/or aryl)-ureido] propionic acid ethyl esters (12a-h, Table 6[23]):

To a solution of 4.2 g (0.05 mol) of sodium hydrogen carbonate in 75 ml of water, was added in portions 7.3 g (0.052 mol) of **11** under stirring at 0°C, then treated with 0.054 mol of the appropriate isocyanate derivative **14** in one portion. The reaction mixture was stirred for 1hr at 0°C. The aqueous layer was extracted with chloroform (3 x 50 ml), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to afford a solid substance which was recrystallized from ethanol-water to give **12a-h** (c.f. **Table 6**); IR (KBr or liquid film, cm^{-1}) of **12a-h**: 3327-3330 (NH), 1736 (C=O), 1635-1637 (C=O). The following data for ^1H NMR and MS were taken as representative examples of **12a-h**:

^1H NMR ($CDCl_3$) of **12c**: δ 1.32 (t, 3H, J=6.8Hz, CH_2CH_3), 1.53 (d, 3H, J=7.1 Hz, $CH\text{-}CH_3$), 4.25 (q, 2H, J=6.8Hz, CH_2), 3.37 (s 3H, OCH_3) 4.75 (q, 1H, J=7.1 Hz, NCHCO), 5.85 (m, 1H, CONHC), 6.82 (m, 1H, Ar-NHCO), 7.72-7.95 (m, 4H, Ar-H) ^1H NMR ($CDCl_3$) of **12g**: δ 1.25 (t, 3H, J=6.6 Hz, CH_2CH_3), 1.42 (d, 3H, J=7Hz, $C\text{-}CH_3$), 4.18 (q, 2H, J=6.6 Hz, CH_2CH_3), 4.31 (d, 2H, J=6.1 Hz, CH_2N), 4.64 (q, 1H, J=7Hz, NCHCO), 5.87 (m, 1H, CONHC), 6.23 (t, J=6.1 Hz, CH_2NH), 7.08-7.41 (m, 3H, Ar-H); MS m/z of **12b**: 370 (M^+, 9.5%); MS m/z of **12g**: 319 (M^+, 48%).

Table 6: 2-[3-(Aralkyl and/or aryl)- ureido] propionic acid ethyl esters, **12 a- h** **(Scheme 2, Method B)**



12 a –h

No. of comp.	n	X	MP	Yield %	Molecular formula mol. Wt.	Analysis (%) C	H	N
12 a*	0	H	78-80	80	$C_{12}H_{16}N_2O_5$	Calc : 61.00	6.83	11.86
					236.273	Found 61.18	6.78	11.79
12 b	0	4-Cl, 3,5 $(NO_2)_2$	91-3	64	$C_{12}H_{13}ClN_4O_9$	Calc : 38.88	3.53	15.11
					370.716	Found. 38.78	3.54	15.15
12 c	0	3-OCH_3	Oil	75	$C_{13}H_{18}N_2O_4$	Calc : 58.63	6.81	10.52
					266.300	Found: 58.64	6.83	10.51
12 d	0	4-OCH_3	96-8	73	$C_{13}H_{18}N_2O_4$	Calc : 58.63	6.81	10.52
					266.300	Found 58.70	6.80	10.54
12 e	1	H	76-8	59	$C_{13}H_{18}N_2O_3$	Calc . 62.38	7.25	11.19
					250.300	Found 62.42	7.33	11.21
12 f	1	2,4-Cl_2	140-2	70	$C_{13}H_{14}Cl_2N_2O_3$	Calc . 48.92	5.02	8.78
					319.190	Found 48.97	5.11	8.86
12 g	1	3,4-Cl_2	130-2	72	$C_{13}H_{14}Cl_2N_2O_3$	Calc 48.92	5.02	8.78
					319.190	Found 48.95	5.07	8.90
12 h	1	3-NO_2	90-2	51	$C_{13}H_{17}N_3O_5$	Calc : 52.88	5.80	14.23
					295.299	Found 52.87	5.86	14.33

* Ref 23 , yield 60% .

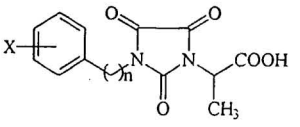
General procedure for the preparation of 2-[3-(aralkyl and/or aryl)-2,4,5-trioxo-imidazolidin-1-yl]-propionic acids (1i-p, Scheme 2, Method B, Table 7):

A mixture of 11 mmol of **13a-h**, 10 ml of glacial acetic acid and 5 ml of concentrated hydrochloric acid was heated to reflux for 2.5 hr. The reaction mixture was concentrated under reduced pressure and the residue was refluxed again with 10 ml of glacial acetic acid and 5 ml of concentrated hydrochloric acid for further 2 hr. The solid substance obtained by concentration under vacuum was dissolved in ethyl acetate, washed with water and extracted with 10% aqueous sodium carbonate. The aqueous alkaline layer was acidified with hydrochloric acid (1:1) and extracted with ethyl acetate (3x 25 ml). The organic layer was dried over anhydrous sodium sulphate and evaporated under vacuum. The remaining residue was crystallized from ethyl acetate: n-hexane to achieve **1i-p** (c.f. **Table 7**); IR (KBr, cm^{-1}) 1740 (C=O), 1715 (C=O).

The following data for ^1H, ^{13}C NMR and MS of **1i-p** were taken as representative examples:

^1H NMR (DMSO, d_6) of **1i**: δ 1:49 (d, J=8.5 Hz, 3H, CH_3), 4.64 (q, J=8.5 Hz, 1H, $NCHCO_2$), 7.00-7.64 (m, 5H, Ar-H), 13.42 (br s, 1H. COOH); ^{13}C NMR (DMSO, d_6) of **1i**; δ 170.171(COOH); MS m/z of **1i**: 262 (M^+, 48%); ^1H NMR (DMSO, d_6) of **1n**: δ 1.52 (d, J=8Hz, 3H, CH_3), 4.43 (s, 2H, CH_2, 4.70 (q, J=8 Hz, 1H, $NCHCO_2$), 7.35-7.66 (m, 3H, Ar-H), ^{13}C NMR (DMSO, d_6) of **1n** : δ 169.956(COOH); MS m/z of **1n** : 345 (M^+, 100%).

Table 7:2-[3-(Aralkyl and/or aryl)- 2,4,5-trioxo-imidazolidin-1-yl] – propionic acids, 1 i-p (Scheme 2, Method B)



1i–p

No. of comp.	n	X	MP	Yield %	Molecular formula mol. wt.	Analysis (%) C	H	N
1 i	0	H	208-10	54	$C_{12}H_{10}N_2O_5$	Calc : 54.97	3.84	10.68
					262.225	Found: 55.01	3.85	10.62
1 j	0	4-Cl, 3,5 $(NO_2)_2$	180-2	45	$C_{12}H_7ClN_4O_9$	Calc : 37.28	1.83	14.49
					386.668	Found: 37.30	1.84	14.51
1 k	0	3-OCH_3	100-2	34	$C_{13}H_{12}N_2O_6$	Calc : 53.43	4.14	9.59
					292.252	Found: 53.27	4.22	9.58
1 l	0	4-OCH_3	176-8	56	$C_{13}H_{12}N_2O_6$	Calc : 53.43	4.14	9.59
					292.250	Found:53.48	4.15	9.55
1 m	1	H	136-8	58	$C_{13}H_{12}N_2O_5$	Calc : 56.52	4.38	10.14
					276.252	Found: 56.63	4.36	10.19
1 n	1	2,4-Cl_2	228-9	59	$C_{13}H_{10}Cl_2N_2O_5$	Calc : 45.24	2.92	8.12
					345.142	Found: 45.32	2.89	8.20
1 o	1	3,4-Cl_2	167-9	53	$C_{13}H_{10}Cl_2N_2O_5$	Calc : 45.24	2.92	8.12
					345.142	Found: 45.44	2.93	8.19
1 p	1	3-NO_2	158-60	38	$C_{13}H_{11}N_3O_7$	Calc : 48.61	3.45	13.00
					321.251	Found: 48.59	3.56	13.13

General procedure for the preparation of isocyanate 14 :-

The method reported in ref. 17 was adopted . Thus, the appropriate carboxylic acid 0.1 mol was treated with thionyl chloride to afford the corresponding acid chloride. The latter was refluxed under efficient stirring with 0.11 mol of sodium azide in 100 ml of dry toluene for one hour. After cooling and quick filtration of the formed NaCl, the toluene is distilled off from the filtrate and the residual isocyanate was used as such in the next step for synthesis of **4,8** and **12** as depicted under **Schemes 1** and **2.**

Pharmacology

Materials and Methods

Pharmacological tests were conducted on adult male albino rats (150-200g) for the evaluation of the inhibitory effect of the synthesised parabanic acids **1a-p** derivatives on lens aldose reductase activity. Animals were obtained from Animal House, National Research Centre, Dokki, Cairo, Egypt.

All animals were allowed free access to water. They were kept on a constant standard laboratory diet.

DL-glyceraldehyde (Aldrich), Nicotinamide adenine dinucleotide phosphate reduced tetrasodium salt (NADPH) (Sigma), mercaptoethanol (Merk), alloxan monohydrate (Sigma), glibenclamide (Hoechst) and Biomereux Kits for estimation of serum glucose level. All other chemicals used in the experimental work are of analytical grade.

Preparation of crude aldose reductase (AR)[24]

Lenses were removed from eyes of 10 male rats weighing 150-200g and homogeneized in 2 ml cold sodium phosphate buffer (pH 6.2) containing 1 mM mercaptoethanol and 1 mM NADPH. The homogenate was centrifuged at 10.000g for 15 minutes at 4°C and the supernatant was stored at 0°C until needed.

Preparation of inhibitor solutions

Because of the poor water-solubility of parabanic acid derivatives, all test compounds were dissolved in propylene glycol. Usually a 10^{-4} M solution was prepared and diluted to the desired concentrations with propylene glycol.

Assay of aldose reductase activity[24]

Aldose reductase assays were conducted according to the procedure of Tomas-Barberan[24]. Assays were performed at room temperature in 0.1 M of phosphate buffer solution (pH 6.2), containing 0.1 mM NADPH, 10 mM DL-glyceraldehyde and 25 ul AR solution then complete to a total volume of 1.5 ml.

The reference blank, contained in all the above compounds without the substrate, was prepared in order to correct for nonspecific reduction of NADPH. The reaction was initiated by the addition of AR and the rate of NADPH oxidation was followed by recording the decrease in absorbance at 340 nm.

Determination of the AR inhibitory effect[24]

The effects of inhibitors on the enzyme activity were determined by adding to the above mentioned reaction mixture 15µl of each compound being tested.

The inhibitory activity was expressed as the rate of $A_{340\,nm}$ due to the utilization of NADPH.

Determination of IC_{50} for compounds 1a, 11 and 15

The concentration of inhibitor needed to elicit 50% inhibition (IC_{50}) was determined by the method discribed by Kador et. al.[25] for compounds 1a, 11 and 15 which proved to be the most potent AR inhibitors of the synthesized series.

Determination of antihyperglycaemic activity[26]

Fasted male albino rats (150-200g) were made diabetic by an intraperitoneal injection of alloxan monohydrate (150 mg/kg) as 10% saline solution. Rats with blood glucose levels above 200 mg/dl were used in the experiment[27].

The test compounds (25 mg/kg) as well as glibenclamide (2.5 mg/kg) as reference, were administered twice per os, first after 48 hours from alloxan administration (zero time) then after 24 hours. Three hours thereafter rats were sacrificed, blood was collected and serum glucose levels were estimated using the glucose oxidase method[26]. The decrease in blood glucose level was calculated as the percent change relative to that of the control group.

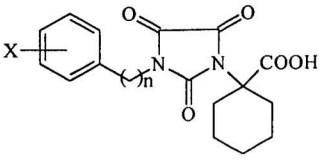
Results and Discussion :

Aldose reductase inhibitory effect (Tables 8 and 9):

In the α-pentamethylene series **1a-h**, compound **1g** (x=3,4 dichloro, n=1) is the most potent as it exhibited 86.6% aldose reductase inhibitory effect. The AR inhibitory activity in this series decrease in the following order : **1g>1c>1b>1d>1f>1h>1e>1a**. Regarding the α-methyl series **1i-p**, compound **1o** (x=3,4 Dichloro, n=1) and **1p** (x=3-Nitro, n=1) displayed the highest activity as the percentage inhibition of aldose reductase enzyme was 91 and 90%, respectively. The AR inhibitory activity of the compounds of this group is arranged in a decreasing order as follows: **1o>1p>1n>1l>1m>1i>1k>1j**. In addition, the IC_{50} for the most potent compounds **1o, 1p** and **1g** was $5x10^{-8}$, $7.5x10^{-8}$ and $2x10^{-6}$ mol/l, respectively.

Docking of compound **1a-h** to the active site of the ARI enzyme showed that the pentamethylene moiety is too large to fit to the active site easily. Such fitting would require conformational changes in the active site. This might be the reason for the weaker AR inhibitory activity of series **1a-h**. On the other hand, when the pentamethylene structure **1a-h** is replaced by the smaller CH_3 lipophilic moiety, a higher biological activity is exhibited. These results could could be deduced from **Tables 8** and 9, wher the AR inhibitory activity of **1i>1a, 1l>1d, 1m>1e** and **1o>1g.**

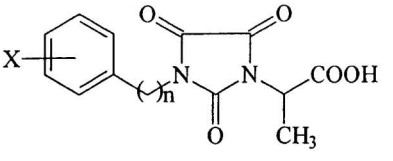
Table 8: Aldose reductase inhibitory activity of the parabanic acid derivatives, **1a-h**



1a-h

No of compound	X	N	% Inhibition 10^{-4} mol/l
1a	H	0	33.9
1b	4-Cl	0	47.2
1c	3,4-Cl_2	0	65.2
1d	4-OCH_3	0	46.3
1e	H	1	37.9
1f	4-Cl	1	43.2
1g	3,4- Cl_2	1	86.6
1h	4- OCH_3	1	40.0

Table 9 : Aldose reductase inhibitory activity of the parabanic acid derivatives, **1i-p**



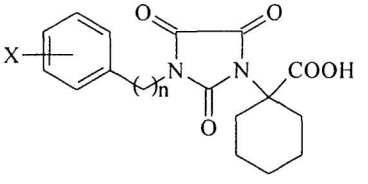
1i-p

No of compound	X	n	% Inhibition 10^{-4} mol/l
1i	H	0	47
1j	4-Cl, 3,5-$(NO_2)_2$	0	28
1k	3-OCH_3	0	35
1l	4- OCH_3	0	67
1m	H	1	60
1n	2,4-Cl_2	1	76
1o	3,4- Cl_2	1	91
1p	3-NO_2	1	90

Antihyperglycaemic effect (Tables 10 and 11) :

In the α-pentamethylene series **1a-h** compound **1h** (x=4-methoxy, n=1) was the most active one, where the percentage reduction in blood glucose level of diabetic rats was 64.3%. The anti-hyperglycaemic activity of the members of this group is arranged in decreasing order as follows: **1h> 1f> 1d> 1e> 1a> 1g> 1c> 1b.** On the other hand, compounds **1o** (x=3,4 Dichloro, n=1) and **1p** (x=3.Nitro, n=1) in series **1i-p** exhibited even higher anti-hyperglycaemic activity where the % reduction in blood glucose level in alloxan diabetic rats was 85.2 and 73.8%, respectively. The antipyperglycaemic activity of the compounds of this group is arranged in a decreasing order as follows: **1o>1p>1l>1n>1j>1m>1k>1i.**

Table 10: Antihyperglycaemic activity of the parabanic acid derivatives, **1a-h** in alloxan diabetic male rats.

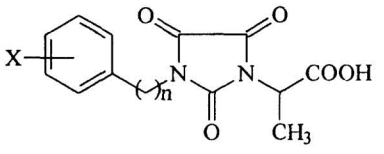


1a-h

No of compound	Dose (mg/kg)	Serum glucose level[(a)] (g/l)	% Change with respect to diabetic value
Control	propyleneglycol	$0.985^{c} \pm 0.430$	66.803
Alloxan	150	$2.967^{b} \pm 0.623$	-
Glibenclamide	2.5	$0.992^{c} \pm 0.253$	66.566
1a	25	$1.223^{c} \pm 0.438$	58.779
1b	25	$1.448^{c} \pm 0.458$	51.196
1c	25	$1.438^{c} \pm 0.444$	51.534
1d	25	$1.202^{c} \pm 0.422$	59.488
1e	25	$1.210^{c} \pm 0.440$	59.218
1f	25	$1.173^{c} \pm 0.445$	60.465
1g	25	$1.241^{c} \pm 0.452$	58.173
1h	25	$1.059^{c} \pm 0.462$	64.307

a- Each value represents the mean serum glucose level (g/l) ± s.e of the number of animals in each group (n=6).
b- Significantly different from control value at $P \leq 0.05$.
c- Significantly different from diabetic value at $P \leq 0.05$.

Table 11: Antihyperglycaemic activity of the parabanic acid derivatives, **1i-p** in alloxan diabetic male rats.



1i-p

No of compound	Dose (mg/kg)	Serum glucose level[(a)] (g/l)	% Change with respect to diabetic value
Control	propyleneglycol	$0.985^{c} \pm 0.430$	66.803
Alloxan	150	$2.967^{b} \pm 0.623$	-
Glibenclamide	2.5	$0.992^{c} \pm 0.253$	66.566
1i	25	1.770 ± 0.569	40.175
1j	25	$1.654^{c} \pm 0.624$	44.253
1k	25	$1.698^{c} \pm 0.511$	42.770
1l	25	$1.126^{c} \pm 0.410$	62.049
1m	25	$1.688^{c} \pm 0.382$	43.108
1n	25	$1.364^{c} \pm 0.619$	54.028
1o	25	$0.439^{c} \pm 0.417$	85.204
1p	25	$0.777^{c} \pm 0.339$	73.812

a- Each value represents the mean serum glucose level (g/l) $\pm$ s.e of the number of animals in each group (n=6).
b- Significantly different from control value at $P \leq 0.05$.
c- Significantly different from diabetic value at $P \leq 0.05$.

Conclusively, the present results showed that compounds **1o** and **1p** might display dual biological activities as aldose reductase inhibitors and hypoglycaemic agents.

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