Comparative anti-glycation and α-glucosidase inhibition studies of microbial transformed compounds of dydrogesterone

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Abstract: Anti-glycation and α-glucosidase inhibition activities of microbial transformed compounds of dydrogesterone (**1**); 20*R*-hydroxy-9β,10α-pregna-4,6-diene-3-one (**2**), 17β-hydroxy-9β,10α-androsta-4,6-diene-3-one (**3**) and 9β,10α-androsta-4,6-diene-3,17-dione (**4**) were evaluated. Compounds **1** and **4** showed potent α-glucosidase inhibitory activities, while **2** and **3** were found to be weak inhibitors, whereas anti-glycation activities of **1**-**4** were not observed.

Keywords: Dydrogesterone, microbial transformation, anti-glycation, α-glucosidase inhibition.

INTRODUCTION

Dydrogesterone (**1**) is similar to the progesterone. It is a synthetic sex hormone. In the hormone replacement therapy, it minimises the overgrowth of womb lining, and is generally used to treat infertility, irregular periods, premenstrual syndrome and endometriosis (Choudhary *et al*., 2008).

Glycation is a non-enzymatic process. Biomolecules (such as proteins, human DNA and lipids) are damaged in glycation by the attachment of reducing sugars (e.g. glucose) and finally leading to the formation of Advanced Glycation Endproducts (AGEs), which are highly reactive. Glycation has been associated with deleterious health effects associated with diabetes, such as retinopathy, nephropathy and neuropathy etc. Therefore efforts have now been made to discover safe and new anti-glycation agents in order to prevent the formation of AGEs (Reddy and Beyaz, 2006).

Alpha glucosidase (EC3.2.1.20) is involved in the final stages of carbohydrate metabolisms. Alpha glucosidase catalyzes the hydrolysis of disaccharides, which can inhibit the post prandial hyperglycemia (Kimura *et al*., 1997). It catalyzes the conversion of oligosaccharide to absorbable monosaccharide, glucose. Therefore, α-glucosidase inhibition is a useful intervention for the type II diabetes' management (Shim *et al*., 2003). Alpha glucosidase inhibitors are also been used as anti-obesity drugs, insect's anti-feedants, fungistatic compounds, antiviral and immune modulators, and as inhibitors of tumor metastasis (Braun *et al*., 1995).

We have already described, microbial transformation of dydrogesteone (**1**) with *Gibberella fujikuroi*, yielded 20*R*-hydroxy-9β,10α-pregna-4,6-diene-3-one (**2**), 17β-hydroxy-9β,10α-androsta-4,6-diene-3-one (**3**) and 9β,10α-androsta-4,6-diene-3,17-dione (**4**) (fig. 1) (Azizuddin and Choudhary, 2012). Now we report here a comparative anti-glycation as well as α-glucosidase inhibitory studies of compounds **1**-**4**.

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MATERIALS AND METHODS

In vitro antiglycation activity

In vitro antiglycation activity was examined by incubation of methyl glyoxal (14 mM), different concentrations of test compounds (which are prepared in 10 % final DMSO concentration), NaN_3 (30 mM) in 0.1 M buffer solution (phosphate, pH 7.4), and BSA (10 mg/mL) under aseptic conditions for 9 days at 37 °C. Each sample was observed after 9 days against sample blank for the specific fluorescence (excitation: 330 nm; emission: 440 nm) development (Choudhary *et al*., 2011; Lee *et al*., 1998). Positive control was rutin. The % inhibition for each compound (formation of AGE) versus control was determined by using the following formula:

% inhibition = (1-Fluorescence of test sample/ Fluorescence of the control group) × 100

Rutin and methylglyoxal (MG) (40 % aqueous solution) were purchased from Sigma-Aldrich (Japan), BSA (Bovine Serum Albumin) from Merck Marker (Germany), and disodium hydrogen phosphate (Na_2HPO_4), sodium azide (NaN_3) and sodium dihydrogen phosphate (NaH_2PO_4) from Scharlau Chemie, S. A. (Spain), while dimethyl sulphoxide (DMSO) was from Fischer Scientific (UK).

In vitro α-glucosidase inhibitory activity

In vitro inhibition of α-glucosidase was examined by incubation of the 0.2 U/mL enzyme in 0.1 M phosphate saline buffer of pH 6.8 with test compounds (different concentrations) for 15 minutes at 37°C. The substrate, 0.7

mM p-nitrophenyl-α-D-glucopyranoside (final) was added, then change in absorbance was monitored at 400 nm up to 30 min. 7.5 % DMSO (final) was used as control, test compound was replaced with its. The standard was 1-deoxynojirimycin (Choudhary *et al*., 2011). The change (due the hydrolysis by α-glucosidase) in absorption of PNP-G was monitored continuously at 400 nm with the spectrophotometer (SpectraMax M2, Molecular Devices, CA, USA).

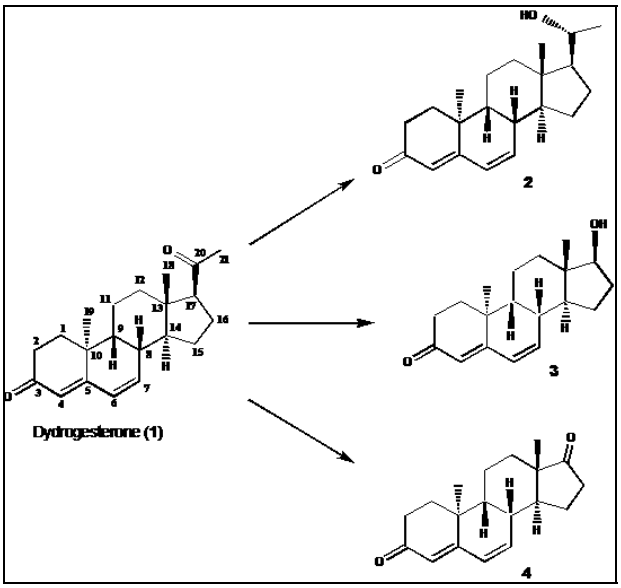

Fig. 1: Dydrogesterone (**1**) and its microbial transformed compounds **2**-**4**.

Software used/statistical analysis

All of the experiments were done in 96-well microplate reader (SpectraMax M2, Molecular Devices, CA, USA). All results were analysed by SoftMaxPro 4.8, MS-Excel and GraphPad Prism-5.0, software package. Unless mentioned above, the % inhibition was determined as:

% Inhibition = 100-(OD of test sample/OD of the control) × 100

EZ-FIT, Enzyme Kinetics Software (Perrella Scientific, Inc., USA) was used to obtain IC_{50} Values. All results are indicated in the figure legend and showed as means ± SEM (from three experiments).

RESULTS

Dydrogesterone (**1**) with its microbial transformed compounds **2**-**4** were submitted for their antiglycation activity. These compounds **1**-**4** showed lower than 50 % inhibition; therefore they were considered as inactive and were not further evaluated for their IC_{50} values. Table 1 showed all results of activities for compounds **1**-**4**.

Dydrogesterone (**1**) and its transformed compounds **2**-**4** were also submitted for their α-glucosidase inhibition activity. Table 2 showed the activities of compounds **1**-**4**. Dose dependent graphs for compounds **1**-**4** (% inhibition versus concentration) are also shown in fig. 2.

DISCUSSION

Although compounds **1**-**4** did not show any antiglycation activity as compared with rutin used as standard but compounds **3** (% inhibition = 23.85) and **4** (% inhibition = 31.37) showed higher antiglycation activity than substrate **1** (% inhibition = 15.66) (table 1).

Among all compounds, dydrogesterone (**1**) showed the most potent activity with IC_{50} value of 95.6±1.3 µM, followed by compound **4** (one of its metabolic derivative) (IC_{50} = 116.4±2.1 µM), against the α-glucosidase enzyme when compared with 1-deoxynojirimycin (standard inhibitor, IC_{50} = 440.99±0.01 µM). While compounds **2** and **3** were found to be weak inhibitors with IC_{50} values of 893.4±1.1 and 585.7±4.8 µM, respectively (table-2). Additionally these compounds **2**-**4** showed lesser potency than dydrogesterone (**1**) to inhibit the α-glucosidase enzyme.

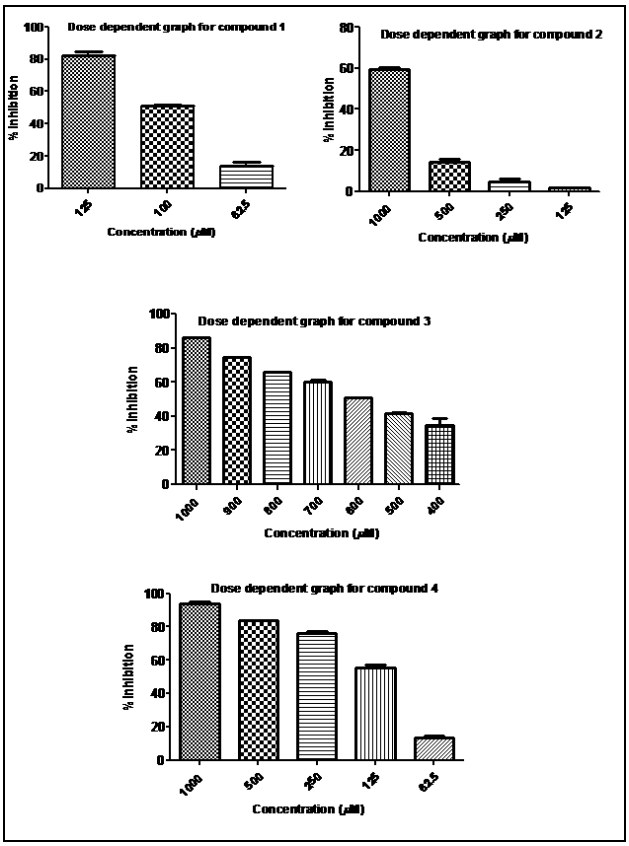

Fig. 2: Dose dependent graphs for compounds **1**-**4**.

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Table 1: Antiglycation activity by compounds **1-4**

S. Nos.	Compounds	Concentration (μM)	% Inhibition	IC_{50} (μM) ± S. E. M.
1	**1**	1000	15.66	NA
2	**2**	1000	2.44	NA
3	**3**	1000	23.85	NA
4	**4**	1000	31.37	NA
5	Rutin*	1000	86.00	294.5 ± 1.5

NA = Not active S. E. M. = Standard error of the mean of five assays * = Standard inhibitor for antiglycation bioassay

Table 2: α-Glucosidase inhibitory activity by compounds **1-4**

S. Nos.	Compounds	Concentration (μM)	% Inhibition	IC_{50} (μM) ± S. E. M.
1	**1**	125	82.2	95.6±1.3
2	**2**	1000	59.7	893.4±1.1
3	**3**	1000	86.1	585.7±4.8
4	**4**	1000	94.2	116.4±2.1
5	1-Deoxynojirimycin*	1000	70.6	440.99±0.01

S. E. M. = Standard error of the mean of five assays * = Standard inhibitor of the enzyme α-glucosidase

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