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# Science at the interface of chemistry and biology: Discoveries of α-glucosidase inhibitors and antiglycation agents*

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*Abstract*: Diseases are manifestations of complex biological processes in living systems. Through the applications of molecular biology and genetics, many diseases are now understood at the molecular level. This has provided researchers opportunities to develop lead molecules with the capacity of blocking a particular disease mechanism. Diabetes is a complex metabolic disorder, characterized by hyperglycemia. The first objective of antidiabetic chemotherapy is to achieve normal glycemic index. Recently, major discoveries have been made to understand how the disease progresses and manifests its complications. We have used this growing understanding to work toward discovery of effective α-glucosidase inhibitors and antiglycation agents of natural and synthetic origins. Reliable bench-top biochemical assays were employed, and several new molecular entities were studied with reference to their structure–activity relationships.

*Keywords*: diabetes; *Cunninghamella elegans*; *Gibberella fujikuroi*; tibolone; antiglycation agent; cytotoxicity.

Life represents the highest manifestation of chemistry. Malfunctions in the biochemical system lead to diseases and discomforts. Today, extensive efforts are being made to understand the disease mechanisms at molecular levels. A so-called reductionist approach toward health and disease advocates selecting a biomolecular target and correcting its function by a molecular lead (legend). This approach has been partly successful as several very effective enzyme inhibitors have been discovered and used in clinical practices in recent years.

Diabetes is a very common endocrine disorder, which results from an absolute or relative deficiency of insulin or insulin resistance and is characterized by hyperglycemia. Diabetes predisposes patients to chronic complications, and affects eyes (cataract), blood vessels (atherosclerosis), nerves (neuropathy), and kidney (nephropathy) and impairs wound healing. Life expectancy of diabetic patients is only two-thirds of that of the normal population. This chronic disorder affects 1–2 % of the world population. In many developing countries, up to 20 % of the population over 30 years of age suffers from IGT (impaired glucose tolerance), a problem of epidemic proportion [1].

The aim of antidiabetic therapy, both in insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus patients, is to achieve normoglycemia (normal blood glucose level). However, this goal has been only partly achieved. Better understanding of the various complex biochemical

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processes involved in the onset and progress of diabetes is now driving the antidiabetic drug development.

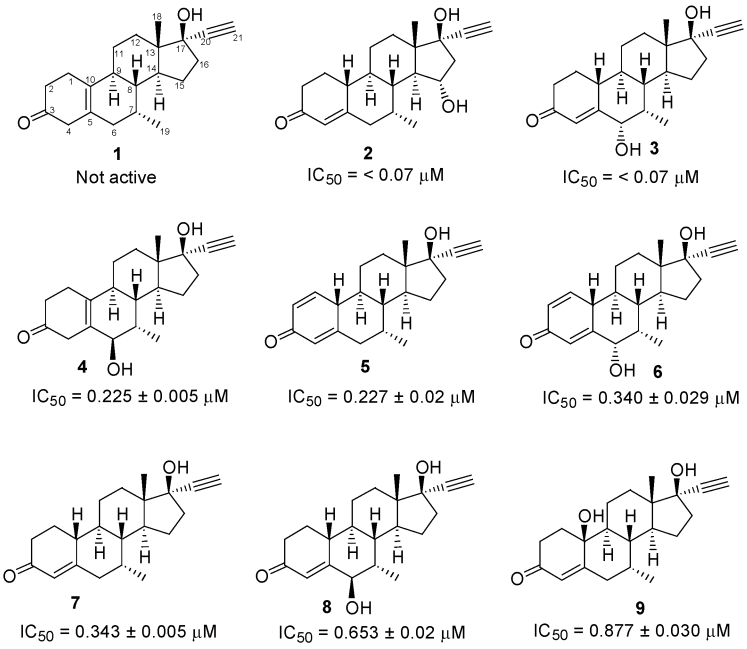
During the current study, we focused our efforts to discover effective α-glucosidase inhibitors to control post-prandial hyperglycemia as well as to identify new antiglycation agents with the objective to control the complications due to hyperglycemia. Our main approach was to screen a large number of natural products, which researchers have isolated from medicinal plants and other living sources. However, diverse classes of synthetic compounds were also screened by biochemical assays. Based on the screening studies, the most promising compounds were selected to conduct kinetic and mechanistic studies. Many of these compounds were also subjected to computational modeling studies in order to get a clear idea of the mechanism of action and binding modes of ligand molecules. Invariably, all active compounds were screened for their potential toxicity by a variety of assays. This systematic approach led to the identification of several series of active lead molecules. Some of these results are presented below.

## α-GLUCOSIDASE INHIBITORS

α-Glucosidase is a membrane-bound enzyme at the epithelium of the small intestine that catalyzes the cleavage of glucose from disaccharides. Compounds capable of inhibiting the intestinal α-glucosidase enzyme can slow the digestion and absorption of carbohydrates. They can thus control the peaks of meal-related hyperglycemia independently of insulin. During the current study, α-glucosidase type VI (Sigma 6136, Brewers yeast) was used to screen a large number of synthetic and natural compounds. Kinetic studies were conducted on the effective candidates by using the Michaelis–Menton protocol.

A series of derivatives were prepared by microbial transformations (*Cunninghamella elagans* and *Gibberilla fujikuruoi*) of tibolone (**1**) $C_{21}H_{28}O_2$. Structural transformation of bioactive compounds by pure enzyme, fungi, bacteria, and plant cell suspension culture is an activity at the interface of chemistry and biology. Biotransformation of compound **1** by the fungal cultures can yield a library of derivatives, some of which may be difficult to obtain by conventional chemical methods. Compound **1** is a synthetic derivative of the 19-nortestosterone. It possesses estrogenic and progestogenic with androgenic properties which mimic the action of a male sex hormone. Compound **1** is used for the treatment of menopausal symptoms and in the prevention of osteoporosis as a hormone replacement therapy (HRT). Recent studies show that the conjugated estrogen (like tibolone) decreases fasting glucose levels and improves the sensitivity of peripheral tissues to insulin in postmenopausal women. It also decreases the chances of developing breast or uterine cancers [2–5]. During the current study, compound **1** was found to be inactive against the α-glucosidase enzyme, however its fungal metabolites were found to be several thousand times more potent than the standard acarbose used in clinical practice (Scheme 1).

As shown, compounds with $\Delta^{4(5)}$-bond in conjugation with the 3-oxo group, and possessing either a 6α- or 15α-hydroxy group, display the highest activity in this series. In the deconjugated $\Delta^{5(10)}$-series, the presence of a 6β-hydroxy group, as in compound **4**, also imparts significant activity.




**Scheme 1** Structures and α-glucosidase inhibitory activities of tibolone derivatives.

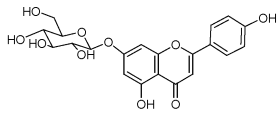
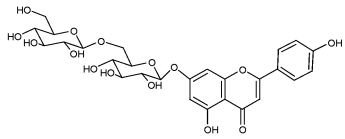
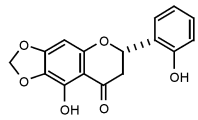
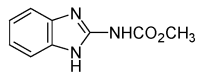
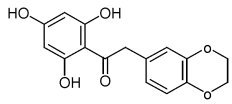
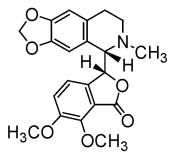
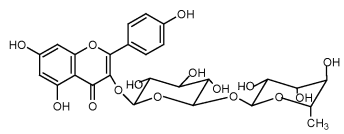
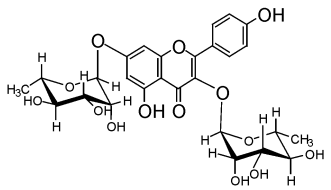
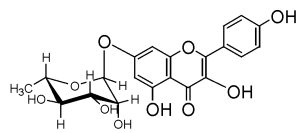
## NEW ANTIGLYCATION AGENTS

The nonenzymatic coupling of reducing sugars such as glucose with free amino residues of proteins is called protein glycation. It is a dynamic process, dependent on both concentration and time of exposure. Aldehyde or keto groups of reducing sugars, such as glucose, fructose, galactose, lactose, xylose, and deoxyribose, react mainly with α- and ε-amino groups of proteins to initially form Schiff bases and Amadori products. Further glycation of proteins causes molecular rearrangements that lead to the generation of advanced glycation end-products (AGEs). AGEs may fluoresce further produce reactive oxygen species (ROS), bind to specific cell surface receptors, and form cross-links. These products contribute to the pathophysiology of vascular diseases in diabetes [6].

Glycation of insulin contributes to development of insulin-resistance in type 2 diabetes. Glycation of proteins alters their biological activity and initiates their degradation and conversion to AGEs. This process affects eyes (cataract), blood vessels (atherosclerosis), nerves (neuropathy), and kidney (nephropathy) and causes impaired wound healing. [7]. Available strategy of influencing the development and prevention of diabetic complications involves a potentially promising antiglycation therapy. Vigorous attempts have been made to pharmacologically influence the process of glycation by preventing or slowing down the formation of AGEs. Various inhibitors have been discovered such as aminoguanidine, aspirin, rutin, antioxidants, AGE breakers, etc. [8].

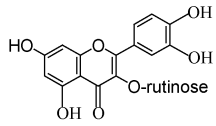
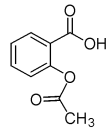
During this study, we systematically screened extracts of medicinal and edible plants, and pure natural products and synthetic compounds for their antiglycation activities through a high-throughput biochemical assay. Various proteins such as bovine and serum albumins (BSAs) and HSA were incubated with various reducing sugars at physiological pH for various durations. The extent of glycation of proteins was measured mainly by the spectrofluorimetric method. LC-MS/MS with TOF was also employed to measure the extent of glycation. As a result, several new classes of antiglycation agents were identified and their activities were compared with rutin and aspirin (Table 1). Details of this study will be published elsewhere.

**Table 1** Antiglycation activity (% inhibition and $IC_{50}$) of selected natural products.

| Source | Name of compounds | Structure | % Inhibition | $IC_{50}$ ± SM (μM) | Cytotoxicity $IC_{50}$ ± SD (μM) | Ref. no. |
|---|---|---|---|---|---|---|
| *Carum petroselinum* | Kaempferol-7-β-D-glucopyranoside (**11**) |  | 76.25 | <16 | >100 | 9 |
| *Psoralia corylifolia* | Kaempferol-7-*O*-β-D-glucopyranoside (1-6)-β-D-glucopyranoside (**12**) |  | 82.75 | 724.0 ± 14.5 | >100 | 10 |
| *Iris tenuifolia* | 5,2'-Dihydroxy-6,7 methylenedioxy – flavanone (**13**) |  | 70.4 | <16 | >100 | 11 |
| *Albatrellus dispansus* | Glycine-*N*-(1*H*-benzimidazol-2-yl)-methyl ester (**14**) |  | 80.15 | <16 | >100 | 12 |
| Commercial | 1-Ethanone, 2-(2,3-dihydro-1,4-benzodioxin-6-yl)-1-(2,4,6-trihydroxyphenyl (**15**) |  | 80.1 | <16 | 84.06 ± 1.98 | 13 |
| *Fumaria parviflora* | (3*H*)-Isobenzofuranone,6,7 dimethoxy-3-(5,6,8-Trihydro-6-methyl {1,3}dioxolo[4,5,g] isoquinoline-5-yl) (**16**) (Narkotine) |  | 76.36 | 152.66 ± 5.43 | >100 | 14 |
| *Ficaria verna* | Kaempferol-3-o-[α-L-rhamnopyranosyl-(1-2)-β-D-glycopyranoside (**17**) |  | 79.5 | <16 | – | 15 |
| *Siraitia grosvenori* | Kaempferol-3-o-α-L-rhanopyraniside-7-o-α-L-rhamnopyranoside (**18**) |  | 70 | 298.00 ± 15.01 | 4.17 ± 2.20 | 16 |
| *Rorippa indica* | Kaempferol-7-o-α-rhamnoside (**19**) |  | 65 | 458.01 ± 20.5 | 2.21 ± 1.35 | 17 |

(*continues on next page*)

**Table 1** (*Continued*).

| Source | Name of compounds | Structure | % Inhibition | $IC_{50}$ ± SM (μM) | Cytotoxicity $IC_{50}$ ± SD (μM) | Ref. no. |
|---|---|---|---|---|---|---|
| *Fagopyrum esculentum* | Rutin (**20**) |  | 82.50 | 98.01 ± 2.03 | >100 | 18 |
| *Spiraea ulmaria* | Aspirin (**21**) |  | 70.51 | 754.05 ± 9.13 | >100 | 19 |

Most of these compounds have shown good glycation inhibitory activity with $IC_{50}$ values ranging between 16–754 μM. Interestingly, most of these compounds share structural similarities with the known antiglycation agents, rutin and aspirin. The cytotoxicity of compounds was determined by using MTT assay on mouse fibroblast 3T3 cells. Cycloheximide was used as a standard with $IC_{50}$ value 0.3 μM ± 0.089. Table 1 shows the results of cytotoxicity assay in which flavonoid glycosides **18** and **19** were found to be the most toxic to fibroblast cells.

## CONCLUSION

In conclusion, the transformation of tibolone (**1**) by microorganisms has yielded a series of potent α-glucosidase inhibitors. Compound **4** was the most potent member of the series, which showed several thousand-fold more activity than the standard inhibitors, acrabase and deoxynojirimycin. Several new classes of antiglycation agents were also identified, and many of them showed good (over 70 %) glycation inhibitory activity in comparison to rutin and aspirin. The development of such effective α-glucosidase inhibitors and antiglycation agents of natural and synthetic origin may prove to be of importance in antidiabetic chemotherapy and related disorders.

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