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Tyrosinase inhibition studies of cycloartane and cucurbitane glycosides and their structure–activity relationships

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Abstract—In the present paper, tyrosinase inhibition studies and structure–activity relationship of eight cycloartane glycosides and one cucurbitane glycoside and its genin, which were isolated from *Astragalus* (Leguminoseae) and *Bryonia* (Cucurbitaceae) plants, have been discussed. The activities are compared with two reference tyrosinase inhibitors, kojic acid and L-mimosine. These studies and the SAR showed that the askendoside B which exhibited highly potent (IC_{50} = 13.95 μM) tyrosinase inhibition could be a possible lead molecule for the development of new medications of several skin diseases related with the over-expression of the enzyme tyrosinase, like hyperpigmentation. The molecule also may be interesting for the cosmetic industries as a skin whitening agent.

1. Introduction

Tyrosinase (EC 1.14.18.1) is a multifunctional copper-containing enzyme widely distributed in plants and animals. This enzyme catalyzes the oxidation of monophenols, *o*-diphenols, and *o*-quinones. Tyrosinase is known to be a key enzyme for melanin biosynthesis in plants and animals. Tyrosinase inhibitors therefore can be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation. They also find uses in cosmetics for whitening and depigmentation after sunburn. In addition, tyrosinase is known to be involved in the molting process of insect and adhesion of marine organisms.[1]

Last couple of years several potential tyrosinase inhibitors have been discovered and reported from our laboratories and several other laboratories around the globe—from natural, synthetic, and even from semi-synthetic sources. More recently we have discovered and reported some other new tyrosinase inhibitory cycloartane tri terpenoids from the methanol extract of the whole plant of *Amberboa ramosa* (Roxb.) Jafri. The structure–activity relationships (SAR) of these molecules have been discussed. Among these cycloartanes, 3β,21,22,23-tetrahydroxy-cycloart-24(31),25(26)-diene was found to be the most potent (1.32 μM) when compared with the standard tyrosinase inhibitors kojic acid (16.67 μM) and L-mimosine (3.68 μM).[2]

Recently we have reported tyrosinase inhibitory potentials of two series of variably N-substituted biperidines. We also discussed the SAR of these series of molecules. Additionally, calculations of the important QSAR molecular descriptors were done on the biperidine

Abbreviations: EC, Enzyme Commission; IC_{50}, median inhibitory concentration; MD, molecular dynamics; MMFF, molecular mechanics force field; SAR, structure–activity relationships.

Keywords: Cycloartane; Cucurbitane glycoside; *Astragalus*; *Bryonia*; Tyrosinase inhibition; Vitiligo; Melanoma.

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analogs after their 2 ps molecular dynamics (MD) simulations using molecular mechanics forcefield (MMFF) approaches. Using MD simulations potential and total energies were calculated for the energy minimized models of bipiperidine and the most active analogs.[3] We also recently reported 28 synthetic tetraketones (**1**–**28**) with variable substituents at C-7, as tyrosinase inhibitors. Among these tetraketone series remarkably compounds **25** (IC_{50} = 2.06 μM), **11** (IC_{50} = 2.09 μM), **15** (IC_{50} = 2.61 μM), and **27** (IC_{50} = 3.19 μM) were found to be the most active molecules. We hope that these findings may lead to the discovery of therapeutically potent agents against clinically very important dermatological disorders including hyperpigmentation as well as skin melanoma.[4]

In this paper, we are reporting the tyrosinase inhibitory potentials of eight cycloartane glycosides, one cucurbitane glycoside and its genin against mushroom tyrosinase enzyme (EC 1.14.18.1). We also briefly discussed the structure–activity relationships of these compounds to find out possible lead molecule for the treatment of several tyrosinase-related skin disorders.

2. Results and discussion

In the present paper, tyrosinase inhibition studies of eight cycloartane glycosides **1**–**8** and one cucurbitane glycoside (**10**) and its genin (**9**) have been discussed. Cycloartane glycosides were isolated from different species of *Astragalus* (Leguminoseae) and cucurbitane triterpenoids from the *Bryonia melanocarpa* Nab. (Cucurbitaceae).

Cycloalpioside D (**1**) $C_{35}H_{58}O_9$ is the 3-*O*-β-D-xylopyranoside of cycloalpigenin D and was isolated from overground parts of *Astragalus alopecurus* Pall.[5] The glycosides **2**–**5** contained the same genin cyclosieversigenin.

Cyclocarposide (**2**) $C_{41}H_{68}O_{13}$ is bidesmosidic glycoside, which contains D-xylose and L-rhamnose units. This glycoside was isolated from *Astragalus coluteocarpus* Pall.[6]

Cyclosieversioside F (**3**) $C_{41}H_{68}O_{14}$ is also a bioside which contains D-xylose and D-glucose. Cyclosieversioside F was isolated from roots of *Astragalus exilis* A. Kor.[7]

Askendosides B (**4**) ($C_{47}H_{76}O_{18}$) and D (**5**) ($C_{45}H_{74}O_{17}$) are bidesmosidic triosides, which contain D-xylose and L-arabinose in the ratio 2:1. These glycosides were obtained from roots of *Astragalus taschkendicus* Bunge.[8–10]

Askendoside G (**8**) $C_{46}H_{78}O_{18}$ is also bidesmosidic trioside of another genin-cycloasgenin C. This glycoside contains D-xylose, L-arabinose, and D-glucose. Glycoside **8** was isolated from roots of *Astragalus stipulosus* Boriss.[11]

Cycloorbicosides A (**6**) ($C_{35}H_{56}O_9$) and G (**7**) ($C_{41}H_{66}O_{14}$) are the derivatives of cycloorbigenin and have D-xylose, D-xylose, and D-glucose moieties, respectively. Glycosides of cycloorbigenin were found only in the aerial parts of *Astragalus orbiculatus* Ledeb.[12,13,15]

Cucurbitane triterpenoids cucurbitacin L (**9**) and bryoamaride (**10**) were isolated from underground parts of *Bryonia melanocarpa* Nab.[14] Bryoamaride is 2-*O*-β-D-glucopyranoside of cucurbitacin L (Fig. 1).

Enzyme inhibitory activities of all these compounds are summarized in Table 1. Most of the investigated compounds inhibited tyrosinase with the IC_{50} values in the range of 13.95–102.39 μM.

It is interesting to compare the tyrosinase inhibitory potentials of the glycosides **2**, **3**, **4**, and **5**. All these glycosides have same genin cyclosieversigenin and differ on the nature and numbers of sugar units. Compound **2** contains β-D-xylopyranose and α-L-rhamnopyranose located at the C-3 and C-6, respectively, has exhibited relatively less inhibition (IC_{50} = 102.39 μM).

In the case of compound **3** with β-D-glucopyranose moiety instead of α-L-rhamnopyranose at C-6 increased the inhibitory potency (IC_{50} = 95.25 μM). Glycoside **4** has three sugar units and exhibited potent inhibition (IC_{50} = 48.92 μM) against the enzyme tyrosinase. Compound **5** differs from the compound **4** only by presence of an acetyl group in the β-D-xylopyranose unit located at C-3 and has exhibited more potent inhibitory activity (IC_{50} = 13.95 μM).

Above-mentioned cycloalpioside D (**1**) is a monoxyloside of cycloalpigenin D which is differing from cyclosieversigenin only by location of secondary hydroxyl group in the B ring. Glycoside **1** has appeared inactive against the enzyme tyrosinase.

Glycoside **8** is having three monosaccharide moieties and another genin with acyclic side chain is also inactive against the enzyme tyrosinase.

Molecules **6** and **7** are belonging to another structural type of cycloartane glucosides. Glycoside **6** contains β-D-xylopyranose unit at C-3 and was inactive against tyrosinase. In contrast with this, glycoside **7** which contains β-D-xylopyranose and β-D-glucopyranose at C-3 and C-25, respectively, has exhibited potent (IC_{50} = 54.64 μM) inhibition activity against the enzyme.

Triterpenoid **9** contains no sugar unit and was inactive against the enzyme tyrosinase. Cucurbitane glycoside **10** is 2-*O*-β-D-glucopyranoside of cucurbitacin L (**9**) has exhibited very weak (IC_{50} = 85.01 μM) inhibition against the enzyme tyrosinase.

We are the first group who reported cycloartanes as potential tyrosinase inhibitors. Our previous findings from other cycloartanes from the methanol extract of the whole plant of *Amberboa ramosa* (Roxb.) Jafri, which we reported very recently,[2] are confirming the results of the present cycloartanes, although previously

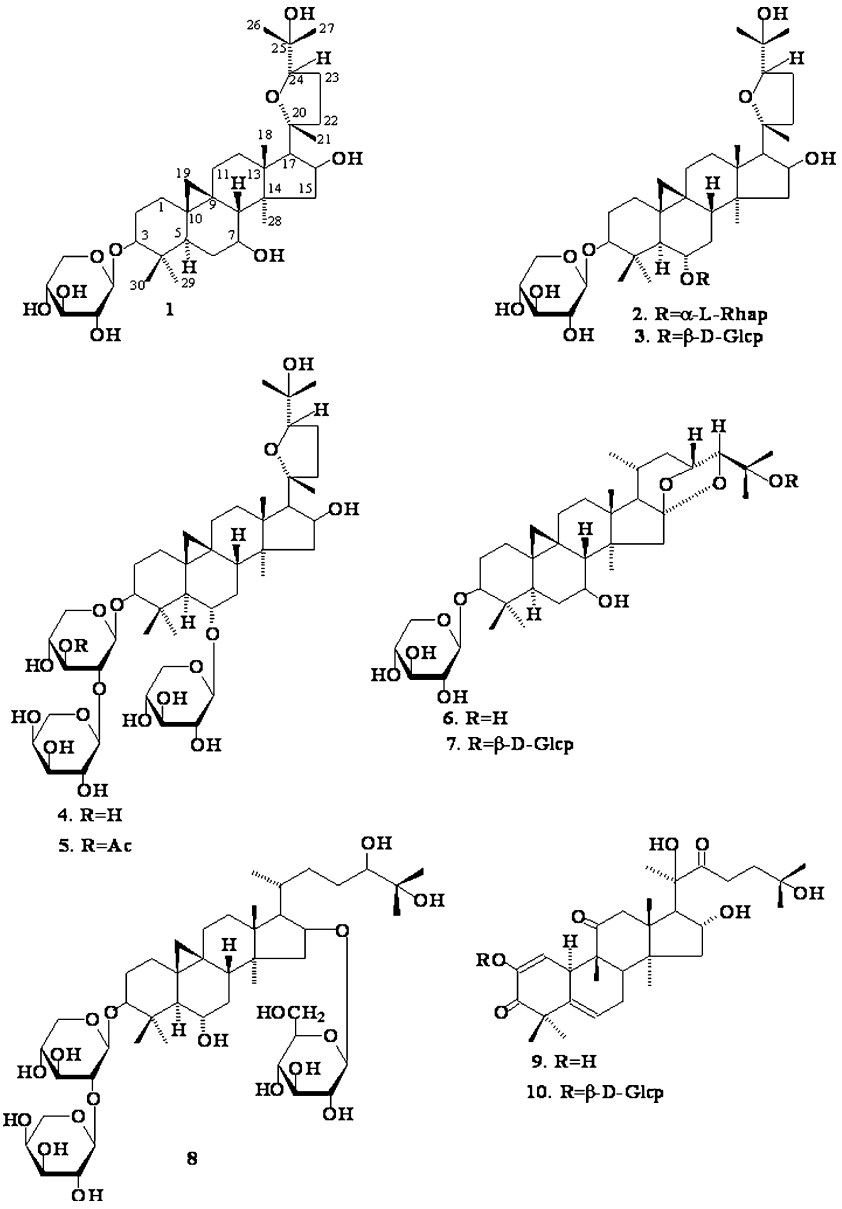

Figure 1. The structural features of the compounds **1**–**10**.

Table 1. Tyrosinase inhibitory activities of the compounds, as compared with the reference inhibitors

Compounds	IC_{50} ± SEM	Refs.
Cycloalpioside D (**1**)	NA	5
Cyclocarposide (**2**)	102.39 ± 0.26367	6
Cyclosieversioside F (**3**)	95.25 ± 0.21487	7
Askendoside D (**4**)	48.92 ± 0.08231	8,9
Askendoside B (**5**)	13.95 ± 0.56159	8,10
Cycloorbicoside A (**6**)	NA	12,15
Cycloorbicoside G (**7**)	54.64 ± 0.29424	13,15
Askendoside G (**8**)	NA	11
Cucurbitacin L (**9**)	NA	14
Bryoamaride (**10**)	85.01 ± 0.07679	14
Kojic acid (KA)[a]	16.67 ± 0.519	3
L-Mimosine (LM)[a]	3.68 ± 0.02234	4

[a] Reference inhibitors.

reported cycloartanes from *A. ramosa* were more potent than these.[2]

From this investigation it can be concluded that askendoside B (**5**) can be the potential lead molecule for the treatment of skin diseases related with tyrosinase inhibition, like hyper- and hypopigmentation of the people as well as for the animals.

3. Experimental

Tyrosinase inhibition assays were performed in 96-well microplate format using SpectraMax 340 microplate reader (Molecular Devices, CA, USA) according to the method developed by Hearing.[16] Briefly, first the

compounds were screened for the *o*-diphenolase inhibitory activity of tyrosinase using L-DOPA as substrate. All the active inhibitors from the preliminary screening were subjected to IC_{50} studies. Compounds were dissolved in methanol to a concentration of 2.5%. Thirty Units of mushroom tyrosinase (28 nM from Sigma Chemical Co., USA) first preincubated with the compounds in 50 nM Na-phosphate buffer (pH 6.8) for 10 min at 25 °C. Then the L-DOPA (0.5 mM) was added to the reaction mixture and the enzyme reaction was monitored by measuring the change in absorbance at 475 nm (at 37 °C) due to the formation of the DOPAchrome for 10 min. The percent inhibition of the enzyme was calculated as follows, by using MS Excel®™ 2000 (Microsoft Corp., USA) based program developed for this purpose:

$$\text{Percent inhibition} = [B - S]/B \times 100$$

Here B and S are the absorbance for the blank and samples, respectively. After screening the compounds, median inhibitory concentration (IC_{50}) was also calculated. All the studies have been carried out at least in triplicate and the results represent means ± SEM (standard error of the mean). Kojic acid[17] and L-mimosine[18] were used as standard inhibitors for the tyrosinase and both of them were purchased from the Sigma Chem. Co., USA.

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