European Journal of Medicinal Chemistry 93 (2015) 255–262

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Preliminary communication

Structure-based modification of 3-/4-aminoacetophenones giving a profound change of activity on tyrosinase: From potent activators to highly efficient inhibitors

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ARTICLE INFO

Article history:
Received 5 November 2014
Received in revised form
16 January 2015
Accepted 10 February 2015
Available online 12 February 2015

Keywords:
Aminophenylethylidenethiosemicarbazide compounds
Tyrosinase activator
Tyrosinase inhibitor
SARs
Inhibition mechanism
Inhibitory kinetics

ABSTRACT

In this study, we developed 3-/4-aminoacetophenones and their structure-based 3-/4-aminophenylethylidenethiosemicarbazide derivatives, respectively, as novel tyrosinase activators and inhibitors. Notably, all the obtained thiosemicarbazones displayed more potent tyrosinase inhibitory activities than kojic acid. Especially, compound **7k** was found to be the most active tyrosinase inhibitor with IC_{50} value of 0.291 μM. The structure-activity relationships (SARs) analysis showed that: (1) the amine group was absolutely necessarily for determining the tyrosinase activation activity; (2) the introduction of thiosemicarbazide group played a very vital role in transforming tyrosinase activators into tyrosinase inhibitors; (3) the phenylethylenethiosemicarbazide moiety was crucial for determining the tyrosinase inhibitory activity; (4) the type of acyl group had no obvious effect on the inhibitory activity; (5) the position of amide substituent on the phenyl ring influenced the tyrosinase inhibitory potency. Moreover, the inhibition mechanism and inhibition kinetics study revealed that compound **7k** was reversible and non-competitive inhibitor, and compound **8h** was reversible and competitive-uncompetitive mixed-II type inhibitor.

1. Introduction

Tyrosinase (EC 1.14.18.1) is a multifunctional oxygenase which is widely distributed in nature, structurally belonging to the type-3 copper protein family [1]. It is also well known that tyrosinase catalyzes two distinct reactions as the rate-limiting step of melanin biosynthesis. The first one is the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA), and the second is the oxidation of L-DOPA to *ortho*-quinone (dopaquinone). The latter was highly reactive and could further oxidized spontaneously to produce melanin, which was responsible for color of mammals-skin and -hair [2]. Moreover, tyrosinase was also found to be involved in the molting process of insects [3], the browning of fruits and vegetables [4], and the happen of Parkinson's and other neurodegenerative diseases [5].

Driven by their biologically applied power, tyrosinase activators/inhibitors have become increasingly important in agriculture and food industry, as well as in medicinal and cosmetic products. For example, tyrosinase activators could protect human skin from UV irradiation damage *via* stimulating the synthesis of melanin [6]. In contrast, tyrosinase inhibitors could be used as skin-whitening agents for the treatment of some dermatological disorders associated with melanin hyperpigmentation [7]. As a result, so far a huge number of natural and synthetic compounds acting as tyrosinase activators/inhibitors have been extensively reported [8,9]. However, most of them are not potent enough to put into practical use due to their weak individual activities or safety concerns. Undoubtedly, it is still necessary to develop new tyrosinase activators/inhibitors with improved therapeutic profiles.

Nowadays, the crystallographic structure of tyrosinase from

Abbreviations: SARs, structure-activity relationships; L-DOPA, L-3,4-dihydroxyphenylalanine; THSG, 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside; IC_{50}, the half maximal inhibitory concentration; DMSO, dimethyl sulfoxide.

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http://dx.doi.org/10.1016/j.ejmech.2015.02.013
0223-5234/

three different species [10–12] has been established, enabling a close look at its three-dimensional structure and also opening up the way for next structure-based design of new and potent tyrosinase inhibitors. Within the structure, there are two copper ions in the active center of tyrosinase and a lipophilic long-narrow gorge near to the active center. In addition, recently a wide range of thiosemicarbzone derivatives have been developed as highly potent tyrosinase inhibitors, most of which came from Chen [13] and our group's contribution [14]. Further investigation revealed that the introduction of thiosemicarbazide group was absolutely necessary for determining the tyrosinase inhibitory activity because it was able to efficiently complex the two copper ions in the active center of tyrosinase. Motivated by these, we speculated that, the introduction of both thiosemicarbazide group and a proper hydrophobic subunit to the 3-/4-aminoacetophenones scaffold, might form potential interactions with the dicopper active center and the hydrophobic pocket of tyrosinase, thus to deliver an improved compound activity.

In order to confirm the hypothesis and considering the importance of amide moiety in modern medicinal chemistry, here two series of novel 3-/4-aminophenylethylidenethiosemicarbazide derivatives were designed, synthesized and their inhibitory effects on mushroom tyrosinase were evaluated. Meanwhile, the parent 3-/4-aminoacetophenones were also investigated. Furthermore, the SARs were discussed and the inhibition mechanism and the inhibitory kinetics of selected compounds were studied. We hope that the findings can lead to the discovery of highly efficient pharmacological agents for the treatment of tyrosinase-related disorders.

2. Results and discussion

2.1. Synthesis

The synthesis of **5a-n**, **6a-i**, **7a-n** and **8a-i** was summarized in Scheme 1, and the chemical structure of the corresponding substituent at the phenyl ring was given in Tables 1 and 2. Briefly, the amide-substituted acetophenones **5a-n** and **6a-i** were synthesized smoothly through the classical amidation procedure by using **1** and **2** as the starting materials. Subsequently, the condensation of the corresponding **1**, **2**, **5a-n** and **6a-i** with thiosemicarbazide could be readily carried out in anhydrous alcohol using acetic acid as catalyst to provide the desired phenylethylidenethiosemicarbazide derivatives **3**, **4**, **7a-n** and **8a-i** in good to excellent yields.

Table 1
The structures and IC_{50} values of 1-(4-aminophenyl)ethylidenethiosemicarbazide (**3**) and 1-(4-amidophenyl)ethylidenethiosemicarbazide compounds (**7a-n**).

Entry	Amide group structure	IC_{50} (μmol/L)
3	$-NH_2$	2.62
7a	$-NHCOCH_3$	0.508
7b	$-NHCOCF_3$	1.336
7c	$-NHCOCCl_3$	0.406
7d	$-NHCOC_2H_5$	0.372
7e	$-NHCOC_3H_7^{n}$	0.644
7f	$-NHCOC_3H_7^{iso}$	0.814
7g	$-NHCOC_4H_9^{n}$	0.492
7h	$-NHCOC_5H_{11}^{n}$	0.762
7i	$-NHCOC_6H_{13}^{n}$	0.356
7j	$-NHCOOCH_3$	0.498
7k	$-NHCOC_6H_5$	0.291
7l	$-NHCOC_6H_4F$-*p*	0.610
7m	$-NHCOC_6H_4OCH_3$-*p*	1.142
7n	$-NHCOCH_2C_6H_5$	0.578
Ref.	Kojic acid	28.5

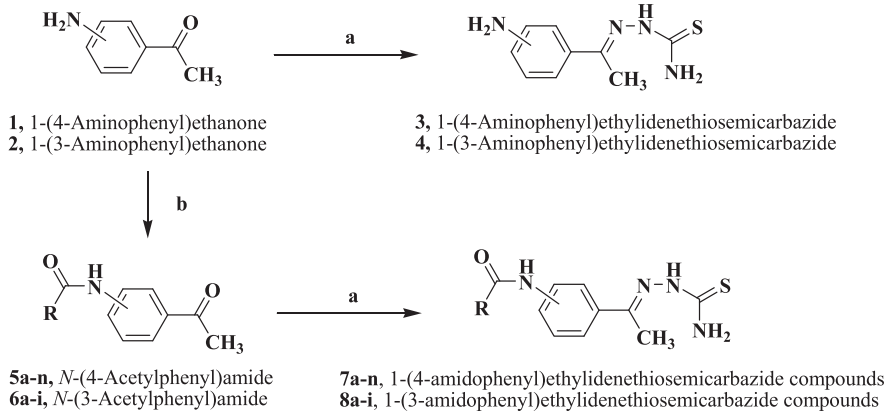
Table 2
The structures and IC_{50} values of 1-(3-aminophenyl)ethylidenethiosemicarbazide (**4**) and 1-(3-amidophenyl)ethylidenethiosemicarbazide compounds (**8a-i**).

Entry	Amido group structure	IC_{50} (μmol/L)
4	$-NH_2$	6.11
8a	$-NHCOCH_3$	4.12
8b	$-NHCOCF_3$	5.760
8c	$-NHCOC_3H_7^{iso}$	3.62
8d	$-NHCOC_4H_9^{n}$	1.54
8e	$-NHCOC_4H_9^{tert}$	4.41
8f	$-NHCOC_5H_{11}^{n}$	1.69
8g	$-NHCOC_6H_{13}^{n}$	2.202
8h	$-NHCOC_6H_5$	1.53
8i	$-NHCOCH_2C_6H_5$	1.79

2.2. Biological activity

2.2.1. Effects of *1*–*4*, *7a-n* and *8a-i* on the diphenolase activity of mushroom tyrosinase

In China, several naturally occurring tyrosinase activators are put into practical use [15]. For example, 2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside (THSG), a potent tyrosinase activator that activated tyrosinase by 12–126% at a concentration of 0.12–75.0 μg/L [15a], was originally isolated as one of the main active constituents from a traditional Chinese herb-*Radix Polygoni Multiflori*, which has been used clinically to treat hypopigmentary



Scheme 1. Synthesis of 1-(aminophenyl)ethylidenethiosemicarbazone compounds (**3** and **4**) and their derivatives (**7a**–**n** and **8a**–**i**). Reagents and reaction conditions: **a**, thiosemicarbazide, anhydrous ethanol, 50–80 °C; **b**, acyl chloride, triethylamine, anhydrous DMF, ice-bath.