Bioorganic & Medicinal Chemistry 23 (2015) 924–931

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Rational design, synthesis and structure–activity relationships of 4-alkoxy- and 4-acyloxy-phenylethylenethiosemicarbazone analogues as novel tyrosinase inhibitors

Ao You [a], Jie Zhou [a,b], Senchuan Song [a], Guoxun Zhu [a], Huacan Song [a,*], Wei Yi [a,b,*]

[a] School of Chemistry and Chemical Engineering, Sun Yat-sen University, 135 Xin Gang West Road, Guangzhou 510275, PR China
[b] VARI/SIMM Center, Center for Structure and Function of Drug Targets, CAS-Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, PR China

ARTICLE INFO

Article history:
Received 26 November 2014
Revised 15 January 2015
Accepted 17 January 2015
Available online 22 January 2015

Keywords:
4-Alkoxy- and 4-acyloxy-phenylethylenethiosemicarbazone analogues
Tyrosinase inhibitor
SARs
Inhibition mechanism
Inhibitory kinetics

ABSTRACT

In continuing our program aimed to search for potent compounds as highly efficient tyrosinase inhibitors, here a series of novel 4-alkoxy- and 4-acyloxy-phenylethylenethiosemicarbazone analogues were designed, synthesized and their biological activities on mushroom tyrosinase were evaluated. Notably, most of compounds displayed remarkable tyrosinase inhibitory activities with IC_{50} value of lower than 1.0 μM. Furthermore, the structure–activity relationships (SARs) were discussed and the inhibition mechanism and the inhibitory kinetics of selected compounds **7k** and **8d** were also investigated. Taken together, these results suggested that such compounds could serve as the promising candidates for the treatment of tyrosinase-related disorders and further development of such compounds might be of great interest.

1. Introduction

Tyrosinase (EC 1.14.18.1; polyphenol oxidase, PPO), structurally belonging to the type-3 copper protein family, is widely distributed in animals, plants and microorganisms.[1] It is also well known that the enzyme is involved in the two-step oxidation for the transformation of L-tyrosine into dopaquinone, the key products for melanin pigment biosynthesis.[2] Due to its particularly prominent role in melanogenesis, tyrosinase has emerged in the past decades as a key target for the screening and the discovery of new inhibitors as the depigmenting agents.[3] Moreover, recently tyrosinase was also reported to link to the happen of Parkinson's and other neurodegenerative diseases,[4] the molting process of insects[5] and the browning of fruits and vegetables.[6] Therefore, tyrosinase inhibitors are clinically useful for the treatment of some dermatological disorders and also should have broad applications in food industry (antibrowning), agriculture (insecticide) and cosmetics (skin-lightening) due to decreasing the excessive accumulation of pigmentation resulting from the enzyme action.

As a result, a tremendously large number of natural and synthetic compounds acting as tyrosinase inhibitors have been reported to date.[3c,7,8] However, only few of them are put into practical use, largely because of the lack of their individual activities or safety concerns. Undoubtedly, more efforts are urgently needed to discover and develop new tyrosinase inhibitors with better activities and lower side effects.

With this in mind and inspired by the pioneering work of Luo[9] and Chen,[10] recently our groups[11] have intensively developed several series of aromatic aldehydes/ketones and their thiosemicarbazone derivatives as potent tyrosinase inhibitors. The SARs analysis revealed that (1) the introduction of a proper hydrophobic group at the position-4 of the phenyl ring was beneficial to tyrosinase inhibitory activity; (2) the thiosemicarbazone moiety was crucial for their potent tyrosinase inhibitory activities; and in general, the activity of methyl ketone thiosemicarbazone compounds was better than that of the corresponding aldehyde thiosemicarbazone compounds. Afterwards the elegant work from Boumendjel and Réglier groups demonstrated that the distance between the thiosemicarbazone moiety and the aromatic nucleus played an important role in determining the tyrosinase inhibitory potency.[12] More recently, it should be emphasized that Belle and co-workers defined in detail phenylmethylene thiosemicarbazone

* Corresponding authors. Tel.: +86 20 84110918; fax: +86 20 84112245.
E-mail addresses: yjhxhc@mail.sysu.edu.cn (H. Song), yiwei2@mail2.sysu.edu.cn (W. Yi).

http://dx.doi.org/10.1016/j.bmc.2015.01.024
0968-0896/

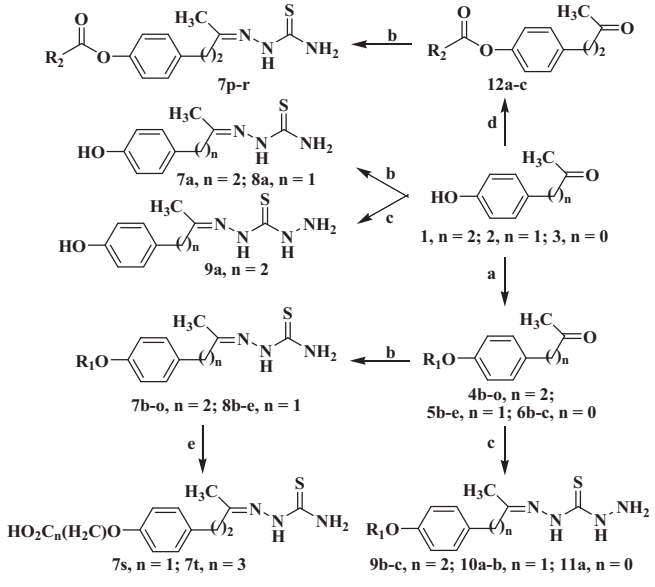
(PTSC) as tyrosinase inhibitor by combining enzymatic studies and coordination chemistry methods,[13] which opened a new avenue for the development of new and potent thiosemicarbazone-derived tyrosinase inhibitors.

Taking advantage of above information, we speculated that condensation products of thiosemicarbazide with 4-hydroxy- or 4-alkoxy-phenylketones bearing a proper length of methylene linker ($n = 0$, 1 or 2) between the phenyl ring and the ketone moiety might exhibit potent tyrosinase inhibitory activities. Therefore, in continuing our program aimed to search for potent compounds as tyrosinase inhibitors[11,14] and to better understand the structure–activity relationships (SARs) of thiosemicarbazone compounds, here a series of 4-alkoxy- and 4-acyloxy-phenylethylenethiosemicarbazone analogues were designed, synthesized and their inhibitory effects on the diphenolase activity of mushroom tyrosinase were evaluated. To the best of our knowledge, this is the first time to report the tyrosinase inhibitory effects of such compounds. Moreover, to more clearly verify the importance of thiosemicarbazone group, the corresponding thiocarbonohydrazone analogues were synthesized and investigated. Besides, the inhibition mechanism and the inhibitory kinetics of selected compounds were also studied. We hope that these findings can lead to the discovery of potential pharmacological agents for treating the tyrosinase-related disorders and also offer key and useful information for future design of highly potent tyrosinase inhibitors. The synthetic procedure was outlined in Scheme 1, and the chemical structure of the corresponding substituent at the phenyl ring was given in Tables 1 and 2.

2. Experimental

2.1. General

Melting points were determined on a WRS-1B digital instrument without correction. NMR spectra were recorded on a Varian Mercury-Plus 300 spectrometer in DMSO-d_6. All chemical shifts (δ) were quoted in parts per million and coupling constants (J) were given in Hertz. Mass spectra were obtained from VG ZAB-HS, LCMS-2010A or LCQ DECA XP spectrometer. Elemental analyses were performed with a Vario EL cube instrument. All commercially available reagents and solvents were used without further purification. Mushroom tyrosinase (specific activity of the enzyme is 6680 U/mg) and L-DOPA (L-3,4-dihydroxyphenylalanine) were purchased from Sigma Chemical Co.



Scheme 1. Synthesis of thiosemicarbazone compounds (**7a–t** and **8a–e**) and thiocarbonohydrazone compounds (**9a–c**, **10a–b** and **11a**). Reagents and conditions: (a) R_1X (X = Br or I), K_2CO_3, anhydrous acetone, rt, 4–7 h; (b) thiosemicarbazide, anhydrous ethanol, acetic acid, 50–80 °C, 3 h; (c) thiocarbonohydrazide, anhydrous ethanol, acetic acid, 50–80 °C, 3 h; (d) triethylamine, R_2COCl, rt, 1–3 h; (e) 15% NaOH, ethanol, rt, 3–8 h.

2.2. Procedure for the synthesis of targeted thiosemicarbazone compounds 7a–t, 8a–e and thiocarbonohydrazone compounds 9a–c, 10a–b, 11a

2.2.1. Synthesis of 7a, 8a and 9a

A mixture of 4-(4-hydroxyphenyl)butan-2-one (**1**) or 1-(4-hydroxyphenyl)propan-2-one (**2**) or 1-(4-hydroxyphenyl)ethanone (**3**) (5.0 mmol), thiosemicarbazide or thiocarbonohydrazide (5.0 mmol) and acetic acid (0.5 mL) was stirred in anhydrous ethanol at 50–80 °C for 3 h. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to room temperature and the precipitate solid was filtered and washed with ethanol to afford pure target compounds **7a**, **8a** and **9a**.

2.2.1.1. 4-(4-Hydroxyphenyl)butan-2-ylidenethiosemicarbazide (7a). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.90 (s, 1H), 9.09 (s, 1H), 8.01 (s, 1H), 7.41 (s, 1H), 6.98 (d, J = 7.5 Hz, 2H), 6.63 (d, J = 6.9 Hz, 2H), 2.69 (t, J = 7.4 Hz, 2H), 2.47 – 2.38 (m, 2H), 1.89 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 178.7, 156.0, 153.3, 129.9, 127.1, 115.2, 43.6, 38.7, 15.7. ESI-MS m/z = 236.1 $[M-1]^-$. It was identified with the reported data.[11a]

2.2.1.2. 1-(4-Hydroxyphenyl)propan-2-ylidenethiosemicarbazide (8a). Solid product, yield 69%, mp 155–156 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.98 (s, 1H), 9.27 (s, 1H), 8.09 (s, 1H), 7.60 (s, 1H), 7.03 (d, J = 8.2 Hz, 2H), 6.70 (d, J = 8.3 Hz, 2H), 3.39 (s, 2H), 1.79 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 178.7, 156.0, 153.3, 129.9, 127.1, 115.2, 43.6, 15.7. ESI-MS m/z = 222.1 $[M-1]^-$.

2.2.1.3. 4-(4-Hydroxyphenyl)butan-2-ylidenethiocarbonohydrazide (9a). Solid product, yield 69%, mp 157–158 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.19 (s, 2H), 9.12 (s, 1H), 6.98 (d, J = 8.4 Hz, 2H), 6.67 (d, J = 8.3 Hz, 2H), 4.74 (s, 2H), 2.58–2.45 (m, 2H), 1.65–1.49 (m, 2H), 1.10 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 172.4, 155.2, 132.1, 129.1, 128.9, 115.0, 36.8, 28.5, 20.0. ESI-MS m/z = 253.1 $[M+1]^+$.

2.2.2. Synthesis of 7b–o, 8b–e, 9b–c, 10a–b and 11a

Into 50 mL of anhydrous acetone were added 10.0 mmol of compound **1** (**2** or **3**), 13.0 mmol of the corresponding alkyl bromide or alkyl iodide and 20.0 mmol of K_2CO_3, the above mixture was stirred at room temperature for 4–7 h. After completion of the reaction as indicated by TLC, the reaction mixture was filtered and the solvent was removed by evaporation at vacuum to get crude products, followed by chromatography to provide the pure intermediates **4b–o**, **5b–e** and **6b–c**. Then, they respectively reacted with thiosemicarbazide or thiocarbonohydrazide in the presence of acetic acid (0.5 mL) at 50–80 °C for 3 h to deliver the desired products **7b–o**, **8b–e**, **9b–c**, **10a–b** and **11a**.

2.2.2.1. 4-(4-Methoxyphenyl)butan-2-ylidenethiosemicarbazide (7b). Solid product, yield 88%, mp 143–144 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.93 (s, 1H), 8.03 (s, 1H), 7.43 (s, 1H), 7.14 (d, J = 8.5 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 3.71 (s, 3H), 2.81–2.72 (m, 2H), 2.55–2.45 (m, 2H), 1.91 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 178.5, 157.4, 153.6, 133.2, 129.2, 113.6, 54.9, 39.9, 30.6, 16.6. Anal. Calcd for $C_{12}H_{17}N_3OS$: C, 57.34; H, 6.82; N,