European Journal of Medicinal Chemistry 86 (2014) 605–612

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

# Anti-tyrosinase, antioxidant, and antibacterial activities of novel 5-hydroxy-4-acetyl-2,3-dihydronaphtho[1,2-*b*]furans

Likai Xia [a], Akber Idhayadhulla [a], Yong Rok Lee [a, *], Young-Jung Wee [b], Sung Hong Kim [c]

[a] School of Chemical Engineering, Yeungnam University, Gyeongsan 712-749, Republic of Korea
[b] Department of Food Science and Technology, Yeungnam University, Gyeongsan 712-749, Republic of Korea
[c] Analysis Research Division, Daegu Center, Korea Basic Science Institute, Daegu 702-701, Republic of Korea

ARTICLE INFO

*Article history:*
Received 22 June 2014
Received in revised form
5 September 2014
Accepted 8 September 2014
Available online 9 September 2014

*Keywords:*
Dihydronaphtho[1,2-*b*]furans
CAN
Formal [3 + 2] cycloaddition
Anti-tyrosinase
Antioxidant
Antibacterial

ABSTRACT

Novel 5-hydroxy-4-acetyl-2,3-dihydronaphtho[1,2-*b*]furans (**7a**–**k**) were synthesized using ceric ammonium nitrate (CAN)-catalyzed formal [3 + 2] cycloaddition. Synthesized compounds were evaluated for their tyrosinase inhibitory, antioxidant, and antibacterial activities. A modified spectrophotometric method using L-DOPA as substrate was used to determine tyrosinase inhibitory activities, and a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was used to evaluate antioxidant properties. Antibacterial activities against gram-negative *Escherichia coli* (KCTC-1924) and gram-positive *Staphylococcus aureus* (KCTC-1916) were evaluated using the disc diffusion technique. Of the synthesized compounds, **7b** with a 4-acetyl and an electron-enriched dihydronaphthofuran ring showed the highest tyrosinase-inhibition activity ($IC_{50}$ = 8.91 μg/mL), which was comparable with that of standard kojic acid ($IC_{50}$ = 10.16 μg/mL), potent antioxidant activity ($IC_{50}$ = 3.33 μg/mL), which was comparable with that of BHT ($IC_{50}$ = 34.67 μg/mL), and excellent antibacterial activities (MICs: 0.50 μg/mL against *E. coli* and *S. aureus* strains). A mechanistic analysis of **7b** demonstrated that its tyrosinase inhibitory activity was reversible and competitive. Compounds **7c** and **7d** showed potent antioxidant activities ($IC_{50}$: 6.30 and 5.01 μg/mL), and compound **7d** also exhibited potent inhibitory activity against *E. coli* with a MIC of 0.5 μg/mL. Furthermore, compounds **7a**, **7e**, **7f**, and **7i** showed potent antibacterial activities against *S. aureus* with MICs of 0.5 μg/mL, which was comparable to that of ampicillin (MIC = 0.5 μg/mL).



## 1. Introduction

Tyrosinase is a copper-containing enzyme that is widely distributed in microorganisms, plants, and animals [1,2], and is involved in two distinct reactions required for melanin synthesis, that is, the hydroxylation of monophenols and the conversion of *o*-diphenols to corresponding *o*-quinones [3–6]. *o*-Quinones are highly active compounds that can polymerize spontaneously to form high molecular weight melanin pigments via a series of reactions with amino acids and proteins [1,2]. Melanin is one of the most important natural biopolymers responsible for pigmentation and the color patterns of mammalian skin, and protects skin from solar radiation [7]. However, the accumulation of epidermal pigmentation can cause some dermatological disorders associated with melasma, freckles, and senile lentigines [8]. Recently, melanin pigments were found in the mammalian brain, and interestingly, tyrosinase has also been linked to the neurodegenerations associated with Parkinson's disease and other degenerative diseases [9]. In addition, tyrosinase is known to be involved in the insect molting process and the adhesion of marine organisms to substrates [10–13]. In fruits and vegetables, enzymatic browning results nutritional and commercial losses [14–17]. Thus, the development of effective tyrosinase inhibitors has become an issue of importance in the agricultural, medicinal, and cosmetic industries. Numerous natural and synthetic tyrosinase inhibitors have been reported in the literature [18–26], but a few, such as, kojic acid and arbutin, are used practically as therapeutic agents or components of cosmetic products. As a result, researchers are trying to identify novel tyrosinase inhibitors with higher activities and lower side effects. Nevertheless, it has been reported that antioxidants of natural or synthetic origin could be of great importance as therapeutic agents for the treatment of several diseases caused by oxidative stress [27]. Furthermore, the use of antioxidants as preservatives by the food industry and as skin-protectives in cosmetics has also attracted much interest [28].

* Corresponding author.
*E-mail address:* yrlee@yu.ac.kr (Y.R. Lee).

http://dx.doi.org/10.1016/j.ejmech.2014.09.025
0223-5234/

The naphthofuran structural motif is frequently found in biologically active synthetic compounds and natural products [29–33]. For example, furomollugin and rubicordifolin were isolated from *Rubia cordifolia* and found to possess significant anticancer properties [34–38]. In the research field, naphthofuran containing molecules have been widely studied, for example, as hepatocyte nuclear factor 4 alpha (HNF4α) inhibitors [39], nicotinic acetylcholine receptor (nAChR) agonists [40], inhibitory kappa-β kinase (IKK-β) inhibitors [40], and nuclear transcription factor kappa-β (NF-κβ) inhibitors [41]. Naphthofuran derivatives have also been reported to possess diverse biological activities, including antidiabetic [42], anti-inflammatory [43], antimicrobial [44], and analgesic activities [45]. Recently, we have reported that mollugin and furomollugin, which possess a naphtho[1,2-*b*]pyran or naphtho[1,2-*b*]furan skeleton bearing a hydroxyl and an ester group on the ring, show potent antioxidant and antibacterial activities (Fig. 1) [46,47]. To the best of our knowledge, the tyrosinase inhibitory activities of naphthofuran derivatives have not been previously investigated. Because mollugin and furomollugin did not show potent anti-tyrosinase activities, we have designed other new compounds bearing an acyl group on the naphtha[1,2-*b*]furan ring. Thus, during our continued studies on the synthesis and biological evaluations of new naphtho[1,2-*b*]furan derivatives, we prepared various 5-hydroxy-4-acetyl-2,3-dihydronaphtho[1,2-*b*]furan derivatives and evaluated their anti-tyrosinase, antioxidant, and antibacterial activities *in vitro*.

## 2. Results and discussion

### 2.1. Chemistry

5-Hydroxy-4-acetyl-2,3-dihydronaphtho[1,2-*b*]furans (**7a**–**k**) were synthesized as shown in Scheme 1 [48]. First, the diacetylation of **1** with acetyl chloride (2.1 equiv.) in the presence of *N*,*N*-diisopropylethylamine (DIPEA, 3.0 equiv.) and 4-(dimethylamino)pyridine (DMAP, 0.05 equiv.) in $CH_2Cl_2$ at room temperature for 3 h gave naphthalene-1,4-diyl diacetate (**2**) in 95% yield. Fries rearrangement of **2** in boron trifluoride complex afforded 1-(4-ethoxy-1-hydroxynaphthalen-2-yl)ethan-1-one (**3**) in 88% yield [49]. Compound **3** was then oxidized to the corresponding naphthoquinone **4** (90% yield) using ceric ammonium nitrate (CAN, 2.0 equiv.) [50–52]. As another method, the photo Friedel–Crafts acylation of 1,4-naphthoquinone (**5**) followed by silver(I) oxide-mediated oxidation provided compound **4** in 36% yield [53]. Next, further reactions of **4** with vinyl ethers, such as, ethyl vinyl ether, *n*-propyl vinyl ether, *i*-butyl vinyl ether, and *n*-butyl vinyl ether, in the presence of 5 mol% of ceric ammonium nitrate (CAN) in acetonitrile at room temperature for 20 min provided the desired products **7a**–**d** as racemates in 81, 79, 70, and 78% yield, respectively. Compounds **7a**–**d** were easily separated by column chromatography and identified by spectroscopic analyses. For example, compound **7b** showed a strong ketone carbonyl absorption peak at 1722 $cm^{-1}$ in its IR spectra, and its $^1H$ NMR spectrum displayed a methine proton at δ 5.83 (1H, d, $J$ = 5.7 Hz) ppm attributed to a dihydronaphthofuran ring and three methyl protons at δ 2.58 ppm as a singlet due to the functional acetyl group. Furthermore, treatment of **4** with 2-methoxypropene gave compound **7e** in 38% yield, whereas treatment with ethyl 1-propenyl ether (as a 30:70 mixture of *cis* and *trans*-isomers) provided both *cis*-**7f** and *trans*-**7g** as racemates in 46 and 42% yield, respectively. The stereochemistry of the *cis*-**7f** ($J$ = 6.3 Hz) and *trans*-**7g** ($J$ = ~0 Hz) was confirmed by the coupling constants between vicinal protons on the dihydrofuran ring. Reaction of **4** with 2,3-dihydrofuran or 3,4-dihydro-2*H*-pyran in the presence of 5 mol% of CAN for 0.5 h gave *cis*-**7h** and *cis*-**7i** in 82 and 94% yield, respectively, whereas the use of α-methylstyrene or 4-methoxystyrene resulted in **7j** and **7k** in 72 and 78% yield, respectively. The stereochemistry of the *cis*-**7h** and *cis*-**7i** was confirmed by comparing with the chemical shifts and the vicinal coupling constants of the related reported *cis*-compounds [54].

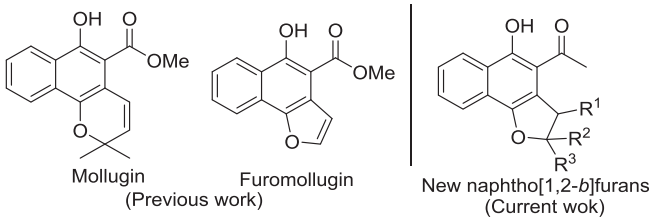


**Fig. 1.** Structures of mollugin, furomollugin, and naphtho[1,2-b]furans.

### 2.2. Biological activity

#### 2.2.1. Anti-tyrosinase activity

To evaluate tyrosinase inhibitory activities, the synthesized 5-hydroxy-4-acetyl-2,3-dihydronaphtho[1,2-*b*]furans were subjected to a tyrosinase inhibition assay using 3,4-dihydroxy-L-phenylalanine (L-DOPA) as substrate, as described previously by Bradford with slight modification [55]. Kojic acid, a well-known tyrosinase inhibitor, which is heavily used as one of the basic skin whitening ingredients in Asian countries, was selected as a reference. The results of studies conducted on the inhibitory effects of the 5-hydroxy-4-acetyl-2,3-dihydronaphtho[1,2-*b*]furans on diphenolase are summarized in Table 1. $IC_{50}$ values were determined from logarithmic concentration–inhibition curves and results are presented as the means of three independent experiments. Compound **7b** bearing a 2-*n*-propyloxy substituent showed better inhibitory activity ($IC_{50}$ = 8.91 μg/mL) than the other compounds or kojic acid ($IC_{50}$ = 10.16 μg/mL). Compounds **7a**, **7c** and **7d** exhibited good mushroom tyrosinase inhibition with $IC_{50}$ values ranging from 59.56 to 83.17 μg/mL. In particular, compounds **7e**, *cis*-**7f**, **7h**, **7j** and **7k** poorly inhibited tyrosinase ($IC_{50}$ > 100 μg/mL). Compound *cis*-**7i** had greater inhibitory activity than *cis*-**7h**, probably due to the stereoelectronic constraint. Interestingly, *trans*-**7g** demonstrated much greater inhibitory activity than the *cis*-**7f** isomer.

*2.2.1.1. Inhibitory mechanism.* The kinetic behavior of the most active compound **7b** was studied with respect to the oxidation of L-DOPA by mushroom tyrosinase at different concentrations. Initial reaction rates were determined by monitoring dopachrome formation at 475 nm. As shown in Fig. 2, Lineweaver–Burk plots of 1/V versus 1/[S] resulted in a family of straight lines with the same intercept on the vertical axis. In this figure, the abscissa 1/[L-DOPA] is the reciprocal of the L-DOPA concentrations, whereas the ordinate 1/V is the reciprocal of the reaction rate, which reflects the reciprocal of tyrosinase activity. The plots obtained indicated that compound **7b** ($K_i$ = 11.2 μM at 25 μM) is a competitive inhibitor and that its inhibitory activity decreases with increasing substrate concentration. Compound **7b** has a 2-hydroxyketone unit and kojic acid contains an α-hydroxyketone group, which suggest that inhibitory mechanism of **7b** and kojic acid is similar (Scheme 2) [56].

#### 2.2.2. Antioxidant activity

The synthesized 5-hydroxy-4-acetyl-2,3-dihydronaphtho[1,2-*b*]furans (**7a**–**k**) were also evaluated with respect to their antioxidant activities using 1,1-diphenyl-2-picrylhydrazyl (DPPH), a stable free