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Novel oxime based flavanone, naringin-oxime: Synthesis, characterization and screening for antioxidant activity

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ABSTRACT

Recent interest in polyphenolic antioxidants due to their involvement in health benefits has led to the investigation of new polyphenolic compounds with enhanced antioxidant activity. Naringin (4′,5,7-trihydroxyflavanone-7-β-L-rhamnoglucoside-(1,2)-α-D-glucopyranoside) is one of the major flavanones in citrus and grapefruit. The present study aimed to synthesize naringin oxime from naringin and to evaluate its antioxidant and anticancer potential using *in vitro* assay system. The structure of the synthesized compound, naringin oxime, was elucidated by FT-IR, ^{1}H NMR, elemental analysis and UV–vis spectroscopy. Antioxidant capacity of naringin oxime, as measured by the cupric reducing antioxidant capacity (CUPRAC) method, was found to be higher than that of the parent compound naringin. Other parameters of antioxidant activity (scavenging effects on OH·, $O_2^{\cdot-}$, and H_2O_2) of naringin and naringin oxime were also determined.

1. Introduction

Flavonoids as an important group of natural substances have variable phenolic structures, and are found in abundance in fruits, vegetables, grains, flowers, wine, and tea [1]. They possess beneficial effects against serious diseases, such as cancer, cardiovascular disease, and neurodegenerative disorders [2]. *In vitro* experimental studies show that flavonoids act as antioxidants, antimicrobials, antivirals, and antiinflammatories [3]. Naringin (4′,5,7-trihydroxyflavanone-7-β-L-rhamnoglucoside-(1,2)-α-D-glucopyranoside) is a member of the flavonoid family that shows various bioactivities on human health as antioxidant, reactive oxygen species (ROS) scavenger, antiinflammatory and antiapoptosis agent [4] having anti-carcinogenic [5] and neuro-protective effects [6], and especially a most investigated cancer preventive agent [7]. Naringin is found in abundance in citrus, grapefruit and juices [8]. When naringin is taken orally, it is metabolised by the enzymes α-rhamnosidase and β-glucosidase to its aglycone naringenine, which is in the more absorbable form [9,10], because naringin kinetically exhibits a delay in its intestinal absorption, resulting in decreased bioavailability. Naringin is a naturally available antioxidant that can be used for the synthesis of other novel antioxidants for enhancing antioxidant activity. Pereira et al. synthesized a new naringin–metal complex, naringin–Cu(II), and this compound was found to possess higher antioxidant, antiinflammatory and tumor cell cytotoxicity activities than free naringin [11].

Oximes (Ox) ($R^1R^2C{=}NOH$) constitute an important class of hydroxylamines, where R^1 is organic side chain and R^2 is either hydrogen, forming an aldoxime, or another aromatic group, forming ketoxime [12]. Oximes and their derivatives are important intermediates in organic syntheses [13]. The oxime functional group can easily be bound to such important organic groups as carbonyl, amino, nitro and cyano functions and can also serve as a convenient protective group [14].

Oximes have been used for many important pharmaceutical and synthetic chemistry applications, and often act as chemical building blocks for the synthesis of agrochemicals and pharmaceuticals [12]. Oxime-type functional groups are included in many organic medicinal agents used in the treatment of organophosphate (OP) poisoning. Inhibition of acetylcholinesterase (AChE) results from acute OP toxicity, whereas oximes, by reactivating AChE, are considered to be rather effective against OP poisoning [15,16]. Therefore, synthesis and investigation of various oximes have an important role in medicinal research. Puntel et al. investigated the capacity of butane-2,3-dionethiosemicarbazone oxime to scavenge different forms of reactive species (RS) both *in vivo* and *in vitro*, and found significant hydrogen peroxide (H_2O_2), nitric oxide (NO) and 1,1-diphenyl-2-picrylhydrazyl (DPPH·) radical scavenging activity for the oxime [16]. The antiradical and

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antioxidant activities of four biologically active *N*,*N*-diethyloaminoethyl ethers of flavanone oximes were investigated by Metodiewa et al., and these compounds were shown to act as promising antioxidants and radioprotectors comparable to rutin activities under oxidative stress conditions [17]. Naringenin oxime was investigated for its antioxidant capacity by using the cupric reducing antioxidant capacity (CUPRAC) method, where the oxime functional group significantly enhanced the antioxidant capacity of pure naringenin [18]. Because of the important advantageous uses of oximes, especially of flavanone oximes, we synthesized naringin oxime (Fig. 1) and determined its antioxidant properties by using the CUPRAC method [19]. The chromogenic oxidizing reagent of the CUPRAC assay, bis(2,9-dimethyl-1,10-phenanthroline)copper(II) (abbreviated as Cu(II)-Nc), is simple, diversely applicable to both hydrophilic and lipophilic antioxidants, stable and easily available at low cost [19]. The CUPRAC method has been successfully applied to the determination of antioxidants in food plants (apricot, herbal teas, wild edible plants, herby cheese, *etc.*), natural dyes [20], and to human serum [21]. The main CUPRAC method was modified for measuring the hydroxyl radical scavenging activities of polyphenolics [22], xanthine oxidase (XO) inhibition activity [23], hydrogen peroxide scavenging activity of polyphenolics in the presence of Cu(II) catalyst [24], and development of a CUPRAC-based antioxidant sensor on a Nafion membrane [25].

The synthesis reaction of naringin oxime compound is shown in Fig. 2. The antioxidant activities (scavenging effects on $OH^{\cdot}$, $O_2^{\cdot-}$, and H_2O_2) of naringin oxime have also been investigated. Scavenging activities against these reactive species were tested, because $OH^{\cdot}$ generated in the human body shows the strongest oxidative activity among ROS, H_2O_2 as a non-radical oxidizing species may be generated in tissues and diffuse across biological membranes, and $O_2^{\cdot-}$ has a great importance as a physiological signaling molecule while its scavengers can combat against many types of diseases [26,27]. The chelating ability toward transition metal ions is an important mechanism of secondary antioxidant action, because substitution of a C-4 (=O) group in the parent compound structure with a C-4 (=N—OH) group in the oxime derivative may enhance the antioxidant activity of the product through stronger binding of transition metal ions [18], thereby preventing Fenton-type reactions of reactive species generation. Further research is needed to show the possible pharmacological and biological activity of this newly synthesized naringin oxime compound, and this work is believed to potentiate the development of some new oxime-based antioxidants with enhanced antioxidant activity.

2. Materials and methods

2.1. Materials and apparatus

All reagents and solvents were of analytical reagent grade. Naringin (4′,5,7-trihydroxyflavanone-7-rhamnoglucoside) and neocuproine (2,9-dimethyl-1,10-phenanthroline) were purchased from Sigma Chemical Co. (Steinheim, Germany). Copper(II) chloride dihydrate, ammonium acetate (NH_4Ac), absolute ethanol (EtOH), methanol (MeOH), dimethyl sulfoxide (DMSO), hydroxylamine hydrochloride, sodium acetate trihydrate were purchased from Merck (Darmstadt, Germany). All chemicals used in reagent preparation were weighed with an accuracy of ±0.0001 g.

The spectra and absorption measurements were recorded in matched Helma quartz cuvettes using a Varian CARY Bio 100 UV–vis spectrophotometer. The elemental analyses were performed using a CHNS instrument model Carlo-Erba 1106 elemental analyzer. ^{1}H NMR spectra in DMSO were obtained on a Varian INOVA 500 MHz NMR spectrometer. The IR spectra were obtained by using KBr pellets in the spectral range 4000–400 cm^{-1} on a Perkin Elmer RXI FT-IR spectrometer. Melting points were found with a Stuart Scientific SMP3 Melting Point Apparatus.

2.2. Preparation of solutions

The standard solutions of naringin oxime and naringin were prepared in EtOH at a concentration of 1.0 mM. All standard solutions were stored at +4 °C prior to analysis. The $CuCl_2$ solution (10.0 mM) and ammonium acetate buffer solution (1 M, pH 7.0) were prepared in distilled water and neocuproine solution (7.5 mM) in absolute ethanol.

2.3. Cell culture and treatment

The canine mammary carcinoma cell line CMT-U27 (a generous gift from Assoc. Professor Eva Hellmén) was obtained from Uppsala University, Sweden. CMT-U27 cells were cultured in DMEM-F12 (Sigma Chemicals, St. Louis, USA), supplemented with 10% fetal bovine serum (Biological Industries, Israel), 100 IU mL^{-1} penicillin G, 100 μg mL^{-1} streptomycin, and 2.5 μg mL^{-1} amphotericin B (Sigma, St. Louis, USA), at 37 °C in a humidified 5% CO_2 atmosphere. Stock solution of naringin oxime was freshly prepared in DMSO and diluted with DMEM-F12 to a final concentration ⩽0.1% of DMSO. Control groups received DMSO vehicle at a concentration equal to that in naringin oxime treated cells.

2.4. Synthesis of naringin oxime

Naringin oxime was prepared by treating 0.01 mol (5.8 g) naringin with hydroxylamine hydrochloride (0.01 mol, 0.69 g) in 25 mL ethanol and sodium acetate trihydrate (0.01 mol, 1.36 g) in water. The solution was heated at reflux on a water bath for 4 h with constant stirring. On cooling to room temperature and adding 10 mL dichloromethane to solutions, a white solid precipitated out. It was filtered, washed with water, and dried over P_2O_5 under vacuum. The product yield was 60%. Melting point: 203 °C. Elemental

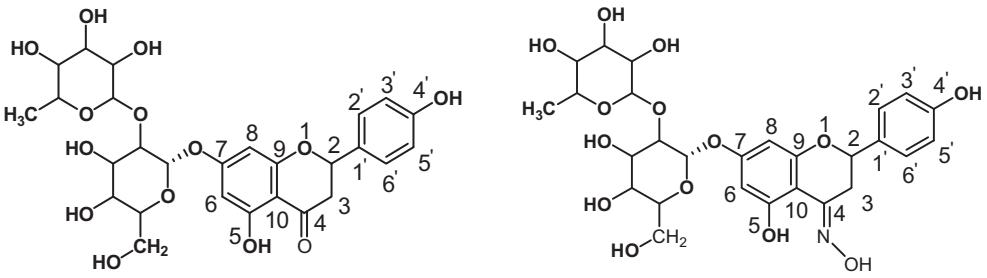

Fig. 1. The structures of naringin and naringin oxime.