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Original article

A study of cytotoxicity of novel chlorokojic acid derivatives with their antimicrobial and antiviral activities☆

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ABSTRACT

A series of 6-chloromethyl-3-hydroxy-2-substituted 4*H*-pyran-4-one derivatives were synthesized and tested for their antimicrobial and antiviral activities. Mannich base derivatives were prepared through the reaction of substituted piperazine or piperidine derivatives on chlorokojic acid and formaline. The structures of the synthesized compounds were confirmed by IR, ^{1}H and ^{13}C NMR, ESI-MS, and elemental analysis. According to the activity studies, compounds **2**–**7** (MIC: 1–2 μg/mL) were found to be highly active against *Bacillus subtilis* and *Staphylococcus aureus,* while compounds **3**, **5** and **6** showed significant activity against *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii.* Also, compounds **2**–**7** were more remarkably active against *Candida albicans* and *Candida parapsilosis* (MIC: 4–8 μg/mL). Additionally, compound **2** was the most active one against RNA virus *PI*-3.

1. Introduction

In the last few decades, the frequency and spectrum of antimicrobial-resistant infections have increased both in the hospitals and community. It is reported that certain infections that are essentially untreatable have begun to occur as epidemics both in the developing and other developed regions as a result of antimicrobial resistance. The most serious concern with antibiotic resistance is that some bacteria have become resistant to almost all of the readily available antibiotics and these bacteria can cause serious diseases. Examples include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and multidrug-resistant *Streptococcus pyogenes* (Group A Streptococcus: GAS) [1]. This is a major public health issue. On the other hand, the development of effective antiviral drugs is an important biomedical scientific achievement of the late 20th century. These are highly potent drugs against herpes viruses, human immunodeficiency virus (HIV), hepatitis B virus, influenza virus and with extension of the list to papilloma viruses, respiratory viruses, enteroviruses, and hepatitis C virus over the next 5–10 years. But this exciting background comes with the problem of drug resistance. Virally encoded drug resistance has been documented against nearly all compounds with antiviral activity, and the genetic basis of resistance is known now [2]. Many screening efforts have been made to find new antimicrobial or antiviral agents from natural or synthetic compounds that can specifically act on different molecular targets to control infections caused by various microorganisms [3–7].

Wolf and Westveer showed that 2-chloromethyl-5-hydroxy-4*H*-pyran-4-one (chlorokojic acid) contains catechol group-inhibited *Aeromonas aeruginosa, Micrococcus pyogenes* var. *aureus, Salmonella typhosa, Penicillium digitatum, Russula nigricans* and *Saccharomyces cerevisiae* [8]. Kojic acid (5-hydroxy-2-hydroxymethyl-4*H*-pyran-4-one) and its derivatives also have an inhibitory effect on the growth of *E. coli* and *S. aureus* [9,10]. Previous antimicrobial activity studies had shown that kojic acid was more active against Gram-negative bacteria than against Gram-positive ones [11]. However, some of its derivatives have shown adverse effects different from kojic acid's antibacterial activity results [12–15]. Also, chlorokojic acid and other halogen derivatives have significant antifungal activity. Moreover, their copper(II) salts' complex derivatives were prepared and found to be more active than chlorokojic acid [16]. Kojic acid and its derivatives exhibit a number of interesting bioactivities and are used in food additives [11,16,17], herbicidals [12,18], anti-speck agents [19], pesticides and insecticides [20–22], and also as a skin-whitening product in cosmetic products [11,17].

☆ The authors have declared no conflict of interest.

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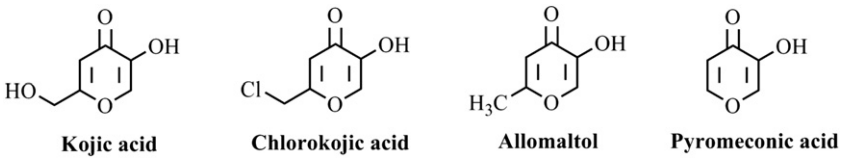


Fig. 1. Hydroxypyranone derivatives.

Previously, some of the Mannich bases of allomaltol (5-hydroxy-2-methyl-4*H*-pyran-4-one) derivatives were prepared in our laboratory for their anticonvulsant and antimicrobial activities. All these derivatives have a lower antifungal activity than chlorokojic acid [23]. Therefore, in order to continue our research program, seven novel Mannich bases of chlorokojic acid derivatives were synthesized and screened for their antimicrobial and antiviral activities.

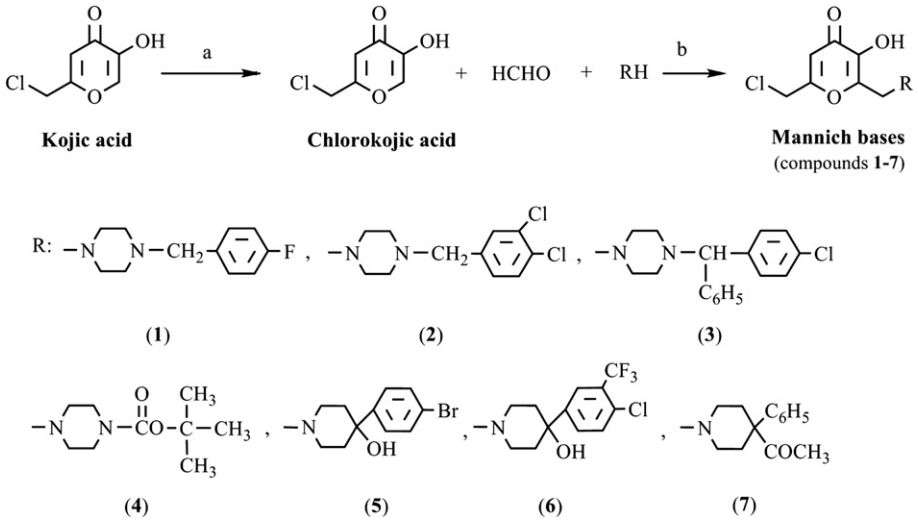
2. Results and discussion

2.1. Chemistry

Kojic acid provides a promising skeleton for development of new and more potent derivatives such as chlorokojic acid, allomaltol and pyromeconic acid (3-hydroxy-4*H*-pyran-4-one) (Fig. 1). They are good ligands for the nucleophilic and electrophilic substitution reactions [24–26]. Therefore, many researchers used them as a starting or intermediate compound for the preparation of new compounds in medicinal chemistry [13,20,23,27–31].

Chlorokojic acid was synthesized from commercially available kojic acid in a one-step reaction as suggested in previous studies [13,23,27,28]. Chlorination of the 2-hydroxymethyl moiety of kojic acid using thionyl chloride at room temperature produced chlorokojic acid, with the ring hydroxyl being unaffected. Mannich-type reactions are three-component condensation reactions involving carbonyl compounds, existing as keto–enol tautomeric forms, formaline, and a primary or secondary amine. Because of its phenol-like properties, kojic acid readily undergoes aminomethylation at room temperature during the Mannich reaction *ortho* to the enolic hydroxyl group. Woods has reported di-Mannich derivatives obtained in an acidic medium from kojic acid, formaline and aromatic amine [30]. Using dimethylamine, diethylamine, pyrrolidine, morpholine, piperidine or 4-methylpiperazine, and chlorokojic acid, only 6th-position of kojic acid was substituted by a Mannich group [27]. Additionally, 6-morpholino or piperidinomethyl chlorokojic acid was prepared via Mannich reaction [30]. In a later study, Ichimoto et al. synthesized Mannich bases of kojic acid and pyromeconic acid in either an acidic or a basic medium using aliphatic or heterocyclic secondary amines such as dimethylamine, diethylamine or morpholine, respectively [24].

Within the scope of the present study, using the methodology shown in Scheme 1, seven new 6-chloromethyl-3-hydroxy-2-substituted 4*H*-pyran-4-one derivatives were synthesized as Mannich base derivatives. The basic substituent was introduced in the 6th-position of chlorokojic acid via a Mannich-type reaction, using formaline and an appropriate substituted piperidine or piperazine derivatives in methanol at room temperature. The reaction proceeded very rapidly. Formation of the desired new Mannich base derivatives was confirmed on the basis of elemental analysis, and the structures of the compounds were supported by spectral data. The IR, ^{1}H and ^{13}C NMR, and ESI-MS were in agreement with the proposed structures. Yields and the melting points of the synthesized compounds are presented in Table 1. In the IR spectra of all compounds, the stretching (st) bands associated with C=O (pyranone), C=C and C–O were observed at 1657–1622, 1597–1456, and 1227–1198 cm^{-1}, respectively. With ^{1}H NMR spectra, characteristic singlet peaks of the 4*H*-pyran-4-one (H^5) ring proton were found in the region 6.53–6.56 ppm in accordance with literatures [13,23,28,31]. The methylene group (ClCH_2–) proton signals were displayed as singlet peaks at 4.63–4.66 ppm. The other –CH_2– group proton signals of **1**–**7** appeared also as singlet peaks at 3.43–3.62 ppm. ^{13}C NMR spectra of compounds **1**–**7** had typical peaks for 6-chloromethyl and carbonyl of pyranone ring were observed in the range of 41.33–42.10 and 173.36–174.2 ppm, respectively. Compounds **1** and **6** bearing fluoro atoms showed suitable spin–spin decoupling. All these results were found acceptable with literature [23]. The ESI-MS of all compounds showed (M^+ + H), (M^+ + H+2) and (M^+ + Na) peaks.



Scheme 1. Preparation of Mannich bases. Reagents and conditions a: $SOCl_2$; b: MeOH.