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Original article

Structural modification of a specific antimicrobial lead against *Helicobacter pylori* discovered from traditional Chinese medicine and a structure–activity relationship study

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ABSTRACT

Psoralen (**1a**) was found to be a specific and potent antimicrobial lead against *Helicobacter pylori* (*H. pylori*) from a traditional Chinese medicine (TCM) in the bioassay directed isolation. A series of structurally diverse analogues of **1a** were thus designed and synthesized to improve the antimicrobial potency, some of which showed more potent activities than the lead compound (**1a**) against *H. pylori*. Among them, compound **25a** is 16-fold stronger (MIC = 0.39 μg/mL) than **1a** (MIC = 6.25 μg/mL), and is even potent than the positive control metronidazole (MIC = 0.50 μg/mL). The *in vitro* antimicrobial activities against *H. pylori* of these structurally diverse analogues based on the scaffold of **1a** have also led to an outline of structure–activity relationship.

1. Introduction

Helicobacter pylori (*H. pylori*), a ubiquitous Gram-negative spiral bacterium, has been recognized as a vital pathogen which induces chronic gastritis, and is associated with gastroduodenal ulcer, gastric adenocarcinoma, and mucosa-associated lymphoid tissue (MALT) lymphoma [1–7]. The eradication of *H. pylori* in the infected patients has been demonstrated to be effective for the treatment of both chronic active gastritis and gastroduodenal ulcer [8–10]. Treatment regimens against *H. pylori* infection currently employed in the first-line therapy are combinations of one proton pump inhibitor and/or bismuth, and two or three antibiotics [11]. Although optimal combination regimens commonly provide high cure rates, the rising prevalence of drug resistance to the currently used antibiotic components increasingly threatens to compromise the efficacy of these treatment regimens. The high dosages of these combination therapy regimens also disrupt the natural population of commensal microorganisms in the gastrointestinal tract, leading to the undesired side effects [12–14]. Therefore, therapeutic regimens directed against *H. pylori* infection will continue to evolve, and the discovery of alternative agents with specific activity against *H. pylori* will be continuously sought among synthetic compounds and natural products.

In an effort to discover antimicrobial components against *H. pylori* from a traditional Chinese medicine (TCM), the ethanolic extract of the seeds of *Psoralea corylifolia*, which is a well-known TCM, namely "buguzhi", was found to be active [15,16]. Bioassay-directed fractionation of the ethanolic extract has led to the isolation of a class of compounds, furocoumarins **1a**–**c** (Fig. 1) with potent antimicrobial activities against *H. pylori*. Although furocoumarin analogues **1a**–**c** are known compounds, their antimicrobial activities against *H. pylori* have not been reported previously. The *in vitro* antimicrobial activities of these natural isolates **1a**–**c** against *H. pylori* (MICs of **1a**–**c** were 6.25, 25 and 25 μg/mL, respectively) suggested that the linear furocoumarin, psoralen (**1a**) as the lead compound is more favorable than the angular furocoumarin, isopsoralen (**1c**). The antimicrobial tests of **1a** against a series of microbes including Gram-positive and Gram-negative bacteria, and fungi indicated that it is a specific antimicrobial agent of *H. pylori*. Therefore, psoralen (**1a**) was served as the lead structure in the current study. Herein, we present the structural modification of psoralen **1a**, the *in vitro* antimicrobial evaluation of its structurally modified analogues, and a structure–activity relationship study.

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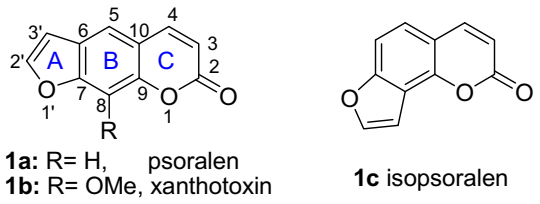


Fig. 1. The anti-*H. pylori* structures of furocoumarins **1a**–**c**.

2. Results and discussion

2.1. Chemistry

To obtain structurally diverse analogues of **1a** for more potent antimicrobial components against *H. pylori*, and to understand the structure–activity relationship of this class of compounds, a systematic modification throughout the A–C rings of linear furocoumarin **1a** was thus conducted (Schemes 1–7), and a series of structural analogues based on the scaffold of **1a** were synthesized (Tables 1–3).

2.1.1. Modification of A-ring of linear furocoumarin *1a*

Starting from psoralen **1a**, a series of 2′-substituted furocoumarin analogues have been prepared (Scheme 1). Psoralen **1a** was refluxed with NBS or NCS in CCl_4 to yield the corresponding halogenated derivatives **2a** or **2b**. Psoralen **1a** was treated with nitric acid in the presence of HOAc to produce a nitro-substituted compound **2c**. Derivative **3** was obtained by the chloromethylation of **1a** *via* a standard procedure [17] using HCHO–HCl or MOMCl–HOAc. When compound **3** was refluxed in the appropriate alcohols, such as methanol, ethanol and *n*-butanol, compounds **4a**–**c** were afforded respectively. With compound **3** at hand, furocoumarin derivatives **4d** and **4e** were also prepared readily.

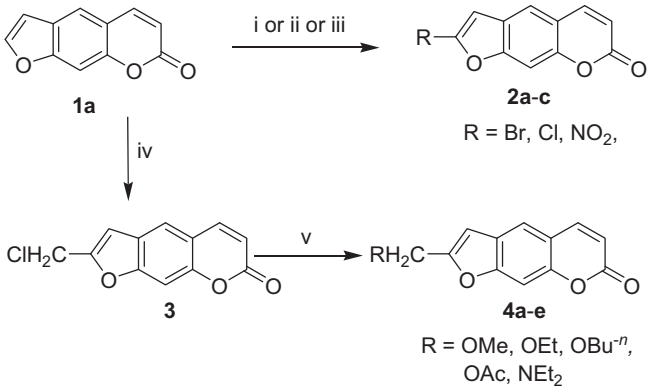
In addition, 3′-methyl-psoralen (**7**) was synthesized (Scheme 2). Starting from resorcinol and 2-hydroxysuccinic acid, 7-hydroxycoumarin (**5**) was prepared *via* Pechmann reaction, which was then converted to an α-keto ether **6** by Williamson reaction refluxing with bromoacetone at the presence of anhydrous K_2CO_3 in acetone. The desired compound **7** was finally produced by refluxing **6** in 0.5 M aqueous NaOH for 18 h [18].

2.1.2. Modification of B-ring of linear furocoumarin *1a*

Starting from xanthotoxin **1b**, a series of the central aromatic B-ring modified derivatives of linear furocoumarin **1a** have been prepared (Scheme 3). Xanthotoxin **1b** was converted to **8** by using BBr_3 as the *O*-demethylation reagent, and then compounds **9a**–**d** were facilely synthesized from **8**. The B-ring modified analogues **11a**–**g** were prepared from **1b** according to the same conditions used in the preparation of **4a**. More additional B-ring modified analogues **12a**–**c**, **13a** and **b**, **14** and **15** with electron-donating or electron-drawing groups at C-5 were also prepared (Scheme 3).

Sulfonamide or sulfonic acid ester moiety played an important role in the design and synthesis of antibiotics. Attia and coworkers [19,20] reported that introduction of sulfonamide or sulfonic acid ester moieties into furocoumarins could enhance the activities again microbes, such as *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*. A number of sulfonic acid esters and sulfonamides of xanthotoxin were thus synthesized (Scheme 4). Xanthotoxin **1b** was reacted with chlorosulfonic acid in $CHCl_3$ to yield sulfonic acid chloride **16**, which was then refluxed with the corresponding alcohols (methanol and phenyl alcohols) or amines to give sulfonic acid esters **17a**–**b** or sulfonamides **17c**–**f**, respectively.

Moreover, 5-methyl-psoralen **20** was synthesized (Scheme 5). Starting from the materials of 5-methylbenzene-1,3-diol and 2-hydroxysuccinic acid *via* Pechmann reaction, compound **18** was produced. When reacted with 3-chloropropane-1,2-diol in the presence of anhydrous K_2CO_3, **18** was converted to **19**, which was then treated with $NaIO_4$ supported on SiO_2, and the resultant was refluxed in 1 M aqueous NaOH to yield the desired compound **20**.



Scheme 1. Preparation of 2′-substituted furocoumarin analogues from psoralen 1a. Reagents and conditions: (i) NBS, CCl_4, refluxing, 9 h; (ii) NCS, CCl_4, refluxing, 9 h; (iii) HNO_3, HOAc, 0 °C–rt; (iv) MOMCl, HOAc, rt, 36 h; (v) for **4a**–**c**: ROH (R = Me, Et, *n*-Bu), refluxing; for **4d**: NaOAc, HOAc, Ac_2O, 110 °C, 3 h; for **4e**: Et_2NH, anhydrous K_2CO_3, acetone, refluxing, 18 h.

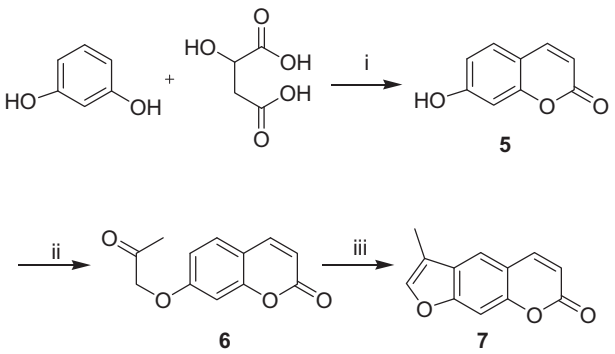
2.1.3. Modification of C-ring of linear furocoumarin *1a*

A number of C-ring modified derivatives of psoralen **1a** were prepared *via* total synthesis (Scheme 6). Starting from 2,4-dihydroxybenzaldehyde, the linear furocoumarin derivatives **25a**–**e** with different substituents at the C-4 of C-ring were prepared in a moderate to high yields [21–23]. The preparation and the spectroscopic data of intermediates **21**–**24** were described in the literature [21].

Based on the compound **25a**, C-4 substituted analogues **26**–**28** were further prepared (Scheme 7). Oxidation of compound **25a** with SeO_2 afforded an aldehyde **26** in 69% yield. Compound **26** was converted into compound **27a** in 84% yield by treating with sodium borohydride in MeOH at room temperature. With **27a** at hand, the linear furocoumarin analogues **27b** and **28** were readily prepared.

2.2. Antimicrobial evaluation

The minimum inhibitory concentrations (MICs) of lead compounds **1a**–**c** and their synthetic analogues against *H. pylori* were determined by an agar dilution method according to the standard protocol, and commercially available antibiotic metronidazole was used as the positive control. The minimum inhibitory



Scheme 2. Preparation of 3′-methyl psoralen **7**. Reagents and conditions: (i) H_2SO_4, rt, 30 min; 120–130 °C, 2.5 h; (ii) bromoacetone, anhydrous K_2CO_3, acetone, refluxing, 18 h; (iii) 0.5 M aq. NaOH, refluxing, 18 h.