http://fbe.trakya.edu.tr/tujs
Trakya Univ J Sci, 9(2):59-62, 2008
ISSN 1305–6468
DIC: 252YYET3100812080109

Araştırma Makalesi / Research Article

BENZOKROMON VE BAZI BENZOKUMARİNLERİN ANTİOKSİDANT VE ANTİMİKROBİYAL AKTİVİTELERİ

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Alınış: 20 Mayıs 2008
Kabul Ediliş: 3 Kasım 2008

Antimicrobial Activities of some Benzocoumarines and benzochromone

Abstract: *Two benzocoumarins (1, 2) and a benzochromone (3) were obtained from the reaction of 2,7 dihydroxynaphthalene and ethyl acetoacetate in the presence of sulphuric acid (95-80 %) as a catalyst. These products were screened in order to assay their antimicrobial activies against standard bacterial strains such as E. coli ATCC 29995, S. S. aureus ATCC 6538P, Mycobacterum smegmatis and the yeast C. albicans ATCC 10239. Neither 1 nor 3 exhibited the antimicrobial activies against standard bacterial strains. The only 2 showed activity against S. S. aureus ATCC 6538P. Also, the antioxidant capacities of the compounds were investigated by using the CUPRAC method.*

Keywords: *2,7-Dihydroxynapthalene, ethyl acetoacetate, benzocoumarin, benzochromone, antimicrobial activity, the CUPRAC method.*

1. Introduction

Coumarins and its derivatives are important compounds found widely in nature and more than 1000 coumarins have been reported from nature and they produced synthetically for many years for scientific and commercial purposes[1, 2]. They display a broad range of applications, as perfumery, optical brightening agents, anticoagulant agents, dyes in laser technology[1, 2], coronary vasodilator[3], hypnotic[4]. Also, they exhibit a large of biological properties such as anti-bacterial, anti-cancer, anti-HIV[5], antifungal[6], antifilarial[7], inhibition of platelet aggregation, steroid 5 α-reductase[8]. Chromones and its derivatives are also important compounds and exhibit biological properties such as antitumoral[9], anti-bacterial[10], anti- HIV[11], antifungal, antiallergenic, antiviral, antitublin, antihypertensive[12], inhibition of cal-pain[13]. In comparison, benzocoumarins and benzochromones have been less studied than coumarins and chromones. Recently, the application of benzocoumarins as non-linear optical devices and fluorescent whiteners have been reported. Two bioactive benzocoumarins have been isolated from Vismia guianensis and a benzochromone has been isolated from the roots of Sophora exigua[14].

Coumarin and its derivatives can be synthesized by various methods, which include Pechmann reaction, Perkin reaction, Reformatsky reaction and Knovenegal reaction[15]. However, Pechmann reaction is the most widely used method for the preparation of substituted coumarins. The reaction proceeds from very simple starting materials such as phenol and β-keto ester and gives good yields of various substituted coumarins. The course of the reaction depends on the substituents on the phenol, on the catalyst used and on the nature of the β-keto ester. Substituted coumarins can be prepared by using homogeneous acid catalysts such as sulfuric, hydrochloric, and Lewis acids such as zinc chloride and aluminium chloride[8] or by using heterogeneous acid catalysts such as Nafion resin/silica nanocomposi-te[2], polyaniline[16] .

We report herein evaluation of their antimicrobial and antioxidant activities of some benzocoumarins and benzochromone

2. Results and Discussion

The synthesis procedure of the target compounds is the condensation reaction of 2,7-dihydroxynapthalene and ethylacetoacetate in the presence of H_2SO_4 [17] . That reaction gave an angular benzocoumarin, a lineer benzocoumarin and a benzochromone shown Figure 1. Antimicrobial activites of the compounds have been studied against *E.coli, S.aureus, C.albicans and M.smegmatis.*. However, only compound 2 showed very weak activity against *S.aureus* with a >800 μg/mL MIC value..

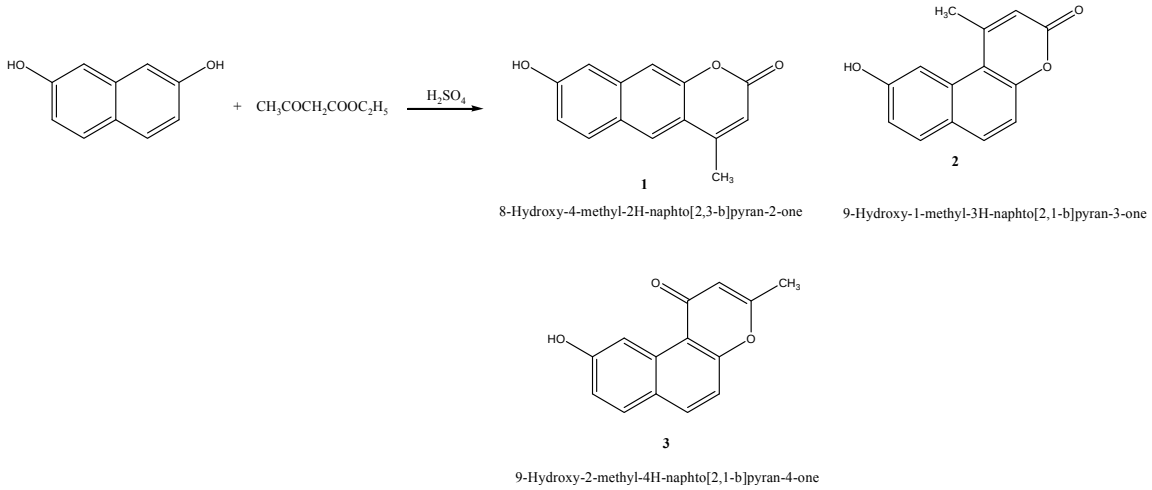

Figure 1. Synthesis of Coumarin Derivatives

Antioxidant capacities of the compounds (1-3) were also determined by using the CUPRAC method[18, 19]. The moderate antioxidant activites have been observed. The most active compound was found to be compound 1.

Table 1. Trolox equivalent antioxidant capacities of the compounds 1-3 by CUPRAC method.

Compound	$TEAC_{CUPRAC}$
1	0.32
2	0.15
3	0.15
Quercetin*	4.26
α-tocopherol*	0.85

*It is reported as 4.38 and 1.01 for quercetin and α-tocopherol, respectively in the literature[19].

3. Experimental

3.1 *Reagents and Equipments*

Ammonium acetate, copper(II) chloride and neocuproine (2,9-dimethyl-1,10-phenanthroline) were purchased from Sigma-Aldrich company. Preparation of the solutions has been reported the literatures[18, 19]. Beckman Coulter DTX 880 multimode optical specthrometer was used for the antioxidant activity assays.

3.2 *Biological Assessment*

3.2.1 ***Antibacterial, antimycobacterial and antifungal activity****:* The following compounds 1, 2 and 3 were tested against standard bacterial strains such as *E. coli* ATCC 29995*, S. S. aureus* ATCC 6538P, *Mycobacterum smegmatis and* the yeast *C. albicans* ATCC 10239 for the determination of their antimicrobial activies. The disk-diffusion method was used to determine the inhibition zones of the tested compounds against the standard bacterial strains. The compounds with inhibition zones higher than 6 mm, were selected for a tube dilution test to determine the antimicrobial activity quantitatively as minimum inhibition concentrations (MIC)[20, 21].

The tube dilution procedure[22] outlined by National Committee for Clinical Laboratory Standards was used for the determination of antimicrobial activities of the compounds. The compounds were dissolved in 10 mL of DMSO. The solution of compounds were transferred into 5 mL of Mueller Hinton Broth (Sabouraud dextrose both for the yeast *C. albicans*) to give a final concentration 200-25 μg/mL in a test tubes. Then 100 μl of 10^6 colony forming units (cfu/mL) (according to McFarland turbidity standards) of standardized microorganism suspensions were inoculated into tubes. The same test was carried out with DMSO solution as a control. End-point was determined after incubation at 37 °C for 24 h. The complete absence of growth was considered the minimum inhibitory concentration (MIC). For the positive control experiment, Gentamycin and flucanozole were used and the test procedure were described in the literature[20-22].

3.2.2 ***Total Antioxidant Capacity by CUPRAC Method***

The CUPRAC method was used for the determination of antioxidant capacity of the compounds and desribed below briefly. Fresh solutions of the compounds were prepared in DMF (1 x 10^{-3} M). 1 mL of 10^{-2} M of $CuCl_2$, 1 mL of 7.5 x 10^{-3} M neocuproine and 1 M CH_3COONH_4 solution added into the glass test tube. Then , 400 μL of freshly prepared solution added and completed the final volume (4.1 mL) with the deinozide water. This procedure repeated for the 300 μL, 400 μL, 200 μL, 100 μL and 50 μL of addition of freshly prepared solutions of the tested compounds. The prepared solutions mixed adn incubated at room temperature for 30 minutes. After the incubation 200 μL of solution from each tube transferred to the 96 whell plate. The absorbance at 450 nm was determined against a reagent blank by Beckman Coulter DTX 880 spectrometer.

The calculation of antioxidant capacity of compounds as trolox equivalents (TEAC values) by the CUPRAC method has been reported the literature. And, briefly described herein. ε_{TR} values of compounds was determined from the linear regression equation as described the literature[19].

Sample calculation:

ε_{TR}: 16700 $L.mol^{-1}.cm^{-1}$

ε_{HK1}: 5344$L.mol^{-1}.cm^{-1}$

$TEAC_{HK1} = \varepsilon_{HK1}/\varepsilon_{TR} = 5344/16700 = 0.32$

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