Bioorganic & Medicinal Chemistry 25 (2017) 4277–4284

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Stereospecific inhibition of nitric oxide production in macrophage cells by flavanonols: Synthesis and the structure-activity relationship. Part 2

Wen-Jun Jiang [a], Susumu Kitanaka, Tomoko Takamiya, Hiroshi Iijima *

School of Pharmacy, Nihon University, 7-7-1 Narashino-dai, Funabashi-shi, Chiba 274-8555, Japan

ARTICLE INFO

Article history:
Received 6 May 2017
Revised 25 May 2017
Accepted 30 May 2017
Available online 15 June 2017

Keywords:
Flavonoid
Nitric oxide
Macrophage
Structure-activity relationship
Stereoisomer

ABSTRACT

To explore the structure-activity relationships of flavanonols on the inhibition of nitric oxide (NO) production in RAW 264.7 cells, we have prepared a series of synthetic flavanonols. In our previous study, the 2′,3′-dihydroxyphenyl substructure was found to be the most potent B ring substructure among the flavanonols having 3,5,7-trihydroxychroman-4-one as the A/C ring. In this study, we examined the effect of diverse substitutions on the A ring of the 2-(2,3-dihydroxyphenyl)-3-hydroxychroman-4-one scaffold, *i.e.*, by fixing the B ring to the 2′,3′-dihydroxyphenyl substructure. Eighteen stereoisomers and 4 racemic mixtures were prepared, and their inhibitory potency on NO production in RAW 264.7 cells was tested. We observed higher inhibitory activity in the (2*R*,3*R*) stereoisomers than in the (2*S*,3*S*) stereoisomers. The presence of a hydroxy or a methoxy group at the 7-postiion enhanced the inhibitory potency, and the additional substitutions at the 6- or 8-position in the A ring increased potency and stereospecificity. A representative compound, (2*R*,3*R*)-2′,3′,7,8-tetrahydroxyflavanonol **5e**, had an IC_{50} value of 17 μM, whereas its (2*S*,3*S*) stereoisomer did not inhibit NO production at all at a concentration of 100 μM. In this study, it was necessary to determine the absolute configuration of the stereoisomers of the synthesized flavanonols that carry methoxy substitutions in the A ring. The procedure to determine their absolute configuration by the CD excitation chirality method is also discussed.

1. Introduction

Flavonoids are well known to have many biological activities, including anti-inflammatory,[1–4] anti-oxidative,[5–10] anti-allergic,[1,11] anti-microbial,[12–14] anti-viral,[15–17] anti-diarrheal,[5] anti-cancer,[2,18–21] and anti-diabetic[22] effects. We have been interested in the anti-inflammatory effects of flavonoids, especially in their effects on chronic inflammation leading to metabolic disease. Flavonoids, widely distributed in plants such as vegetables, fruits, teas, and some herbs, are considered an important dietary material that contributes to the control of chronic inflammation. The production of nitric oxide (NO) is a primary indicator of macrophage activation. NO is produced once the NF-κB complex has been activated by extracellular stimuli. Thus, chemicals that suppress NO production in macrophage cells can be expected to be useful suppressors of inflammation. We have examined the inhibitory effect of herbal materials from many sources on NO production in RAW 264.7 cells, which are stimulated by lipopolysaccharide (LPS).[23]

Indeed, many flavonoids showed inhibitory potency on NO production in RAW 264.7 cells. However, the structure-activity relationship of flavonoids on inhibitory potency against NO production remained a qualitative one. In 2011, Daikonya et al. isolated flavonoids that carry symmetrically substituted B rings from Tibetans herbal plants and determined their IC_{50} values for suppression of NO production.[24,25] These structure-activity data successfully led us to establish a quantitative structure-activity relationship (QSAR) model by comparative molecular field analysis (CoMFA).[26] This CoMFA model suggested the importance of the electrostatic feature of the B ring and prompted us to synthesize a series of 5,7-dihydroxyflavavonol derivatives that diversify in their B ring substitutions.[27] In the QSAR study, we found that (i) flavanonols that carry a 2′,3′-dihydroxyphenyl ring as the B ring show strong activity, (ii) the inhibition is stereospecific, *i.e.*, the (2*R*,3*R*) stereochemistry is relevant for the activity in macrophage RAW 264.7 cells, and (iii) the presence of a 4′-hydroxy group on the B ring of flavanonols is unfavorable for such activity. This disadvantage of the presence of the 4′-hydroxy group on the B ring is rather unexpected because in natural flavonoids, the 4′-position is

* Corresponding author.
E-mail address: iijima.hiroshi@nihon-u.ac.jp (H. Iijima).
[a] Present address: Jiangsu Yew Pharmaceutical Co., Ltd, QinXin Village, City WuXi, Province JiangSu 214199, China.

http://dx.doi.org/10.1016/j.bmc.2017.05.060
0968-0896/

often substituted by a hydroxy group due to biosynthetic destiny, and this might be one reason why it is rare to encounter a flavanonol that strongly inhibits NO production in macrophage cells.

In this study, to expand the structure-activity relationship of flavanonols on the inhibition of NO production, we synthesized flavanonols that carry a 2′,3′-dihydroxyphenyl substructure as the B ring, but which have diversity in the A ring.

2. Material and methods

2.1. General experimental procedures

Optical rotations were measured using a JASCO P-1020 polarimeter. The ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on a JEOL JNM-ECX600 spectrometer. Mass spectra were obtained on a JEOL GCMate mass spectrometer. IR spectra were recorded on a JASCO FT/IR-4200 spectrometer, UV spectra were recorded on JASCO V-730 spectrometer, and CD spectra were recorded on JASCO J-600 spectrometer. Melting points were determined by using AS-ONE ATM-02.

2.2. Separation of stereoisomers by chiral column chromatography

All of the synthesized flavanonols were further submitted to purification by a chiral column chromatograph (DAICEL, CHIRALPAK IA, 5 μm, 10 mm ϕ × 250 mm) to isolate the stereoisomers using a high-performance liquid chromatography (HPLC) system (JASCO PU-1580, UV-1575). The elution solvent was ethanol-*n*-hexane, the flow rate was 5 mL/min, and the detection wavelength was 254 nm.

2.3. NO assay

The amount of released nitrite (NO) was quantified by the Griess method.[28] RAW 264.7 cells were cultured in F-12 Ham medium (Sigma Aldrich, N4888) containing 200 mM L-glutamate (Sigma Aldrich, G7513), penicillin (100 U/mL)-streptomycin (0.1 mg/mL) (Sigma Aldrich, P4333), and immobilized fetal bovine serum (10 v/v%) (Biowest, S1780). One hundred fifty microliters of cell suspension (1.6×10^6 cells/mL culture medium) was dispensed in a well of a 96-well plate (Sumitomo Bakelite, 8096R), and 40 μL of test compound solution was added. The test compound solution was prepared by diluting the DMSO solution of the flavanonols by a ratio of 1:100 with culture medium. The cells were incubated for 2 h at 37 °C in a CO_2 incubator. Cells adhered to the culture well during this process. Ten microliters of LPS solution (Sigma Aldrich, L-2880) was then added to each well. The final concentration of LPS was 100 ng/mL. After 16 h of incubation in the CO_2 incubator, 100 μL of supernatant medium was transferred to another plate. The remaining cells were submitted to a cell viability test as described in section 2.4. Fifty microliters of 1% sulfanilamide solution (in 5 v/v% aqueous phosphoric acid) was added to each well. A few minutes later, 50 μL of 0.1% *N*-1-naphthylethylnediamine (Wako Pure Chemical Inc., 147-04141) solution was added, and the mixture was incubated at room temperature in the dark for 10 min. Absorbance at 540 nm (reference wavelength: 655 nm) was then measured using a microplate reader (BioRad Model 3550). Aminoguanidine hydrochloride (Wako Pure Chemical Inc., 328-26432) was used as a positive control. The concentrations of the test compounds were precisely determined from the ultraviolet absorption at λ_{max}.

2.4. Cell viability test

Cell viability was measured using alamarBlue® reagent (Bio-Rad AbD Serotec Ltd.). Ten microliters of alamarBlue® solution was added to the RAW 264.7 cells left in each well of the 96-well plate from the above-mentioned NO assay, followed by incubation at 37 °C for 4 h. Absorbance was measured at 570 nm (reference wavelength: 600 nm).

2.5. Synthesis of MOMO protection acetophenones **1**

NaH (1.5 equiv; depending on the number of hydroxyl groups) in dry THF was slowly added while stirring at 0–5 °C (in an ice-water bath) to a solution of hydroxyacetophenone (1 equiv) in dry DMF. When the solution was cooled to 0–5 °C, chloromethyl methyl ether (1.5 equiv; depending on the number of hydroxyl groups) was slowly added over a period of 15 min so that the temperature was maintained below 5 °C. The reaction mixture was then stirred at room temperature for another 30 min, quenched by the addition of cold distilled water, and extracted with EtOAc. The combined organic layer was washed with distilled water and brine and then dried over Na_2SO_4. The filtered organic layer was concentrated under a vacuum, and the residue was purified by silica gel column chromatography, eluting with *n*-hexane and EtOAc to give compound **1**: a colorless oil (80–95% yield).

2.6. Synthesis of bis(2-metholmethyl)benzaldehyde **2**

K_2CO_3 (10 equiv) was added while stirring at 0-5 °C (ice-water bath) to a solution of 2,3-dihydroxylbenzaldehyde (1 equiv) in dry acetone. When the solution was cooled to 0–5 °C, chloromethyl methyl ether (3.0 equiv) was slowly added over a period of 15 min to keep the temperature below 5 °C. The reaction mixture was stirred at room temperature for another 30 min, quenched by the addition of cold distilled water, and extracted with EtOAc. The combined organic layer was washed with distilled water and brine and then dried over Na_2SO_4. The filtered organic layer was concentrated under a vacuum, and the residue was purified by silica gel column chromatography, eluting with *n*-hexane and EtOAc to give compound **2**: a colorless oil (90% yield).

^{1}H NMR (600 MHz, $CDCl_3$), δ: 3.49 (3H, s, OCH_3-3), 3.56 (3H, s, OCH_3-2), 5.21 (2H, s, OCH_2-3), 5.23 (2H, s, OCH_2-2), 7.12 (1H, t, J = 7.8 Hz, H-5), 7.38 (1H, dd, J = 7.8, 1.2 Hz, H-4), 7.48 (1H, dd, J = 7.8, 1.2 Hz, H-6), 10.44 (1H, s, CHO). ^{13}C NMR (150 MHz, $CDCl_3$), δ: 56.5 (OCH_3-3), 58.1 (OCH_3-2), 95.4 (OCH_2-3), 99.9 (OCH_2-2), 121.1 (C-4), 122.4 (C-5), 124.7 (C-6), 130.8 (C-1), 150.1 (C-3), 150.3 (C-2), 190.4 (C=O).

2.7. Synthesis of chalcone **3**

KOH (3 equiv) ethanol solution was added to a solution of **1** (1 equiv) in EtOH. Then **2** (1 equiv) was added to the reaction mixture solution and stirred at room temperature for 3 h. Distilled water was added and extracted with EtOAc, and the combined organic layer was washed with distilled water and brine and dried over Na_2SO_4. The filtered organic layer was concentrated under a vacuum, and the residue was purified by silica gel column chromatography, eluting with *n*-hexane and EtOAc to give **3**: a light-yellow oil (60–90% yield).

2.8. Synthesis of epoxide **4**

H_2O_2 (30%) and aqueous 2 M NaOH were added to a methanol solution of chalcone **3**, and the mixture was stirred for 3 h at room temperature. Methanol was removed under a vacuum. Distilled water was added to the resultant aqueous suspension and extracted with EtOAc. The combined organic layer was dried over Na_2SO_4. The organic layer was concentrated under a vacuum to give compound **4**: a colorless oil (95% yield).