J Med Sci 2010;30(2):041-046
http://jms.ndmctsgh.edu.tw/3002041.pdf

Synthesis of Mannich Base Derivatives of Oroxylin A Endowed with Anti-inflammatory Activity

Chien-Ming Huang[1], An-Rong Lee[2], Pei-Hua Kang[2], and Wen-Hsin Huang[2*]

[1]*Cheng-Hsin Rehabilitation Medical Center, Taipei;*
[2]*School of Pharmacy, National Defense Medical Center, Taipei, Taiwan, Republic of China*

Synthesis of baicalein, oroxylin A and wogonin, major active constituents of *Scutellaria baicalensis* GEORGI, was successfully developed in a previous report. In this investigation of novel oroxylin A derivatives as potential anti-inflammatory agents, a series of Mannich base derivatives at C-8 in A-ring of oroxylin A were designed and synthesized. All the oroxylin A derivatives exhibited significant anti-inflammatory activity against λ-carrageenan-induced rat hind paw edema (CIPE). The results showed that the target compounds, oroxylin A (**4a**) and derivatives **4b-i** (oroxylin A bearings a 8-substituent as piperidin-1-ylmethyl, morpholin-4-ylmethyl, 4-methyl-piperazin-1-ylmethyl, 4-pyridin-2-yl-piperazin-1-ylmethyl, 4-pyrimidin-2-yl-piperazin-1-ylmethyl, 4-(2-chloro-phenyl)-piperazin-1-ylmethyl, 4-(4-nitro-phenyl)-piperazin-1-ylmethyl, 4-(4-fluoro-phenyl)-piperazin-1-ylmethyl, respectively) exerted a remarkably anti-inflammatory effect with a significant reduction in swelling. Among them, compounds **4b** (8-morpholin-4-ylmethyl oroxylin A), **4c** (8-piperidinyl-4-ylmethyl oroxylin A), and **4f** (8-[*N*-(*N*-2-pyrimidinyl)-piperazin-1-yl]methyl oroxylin A) proved to be the most potent with over 100% inhibition at 20 mg/kg dose in the first hour and potency lasted 5 hours in comparison to that of ibuprofen (as a positive control).

Key words: anti-inflammatory, Mannich base, oroxylin A, λ-carrageenan-induced rat hind paw edema

INTRODUCTION

Baicalein, oroxylin A and wogonin are the three major flavonoids of *Scutellaria baicalensis* Georgi, a traditional Chinese herb used since ancient times, characterized by possessing a very broad spectrum of biological activities, notably anti-oxidant and anti-inflammatory.[1-2]

In previous papers,[3-4] we have developed a novel, convenient method suitable for the large-scale preparation of the three major naturally-occurring polyhydroxyflavonoids of *Scutellaria baicalensis* GEORGI, i.e., bacalein, oroxylin A, wogonin, in high yields.

As for oroxylin A, one of the active polyhydroxyflavonids of *Scutellaria baicalensis* GEORGI for the treatment of allergic and inflammatory diseases in China since ancient times, a number of flavonoids have shown promising evidence as potential anti-inflammatory agents modulating pro-inflammatory gene expression.[5-6] It has also been demonstrated that oroxylin A (**4a**) possesses potent binding at the benzodiazepine site with selective antagonistic properties.[7] Furthermore, a series of 8-aminomethylated oroxylin A analogs (IC_{50} = 35~375 μM) have been synthesized as moderate potent α-glucosidase inhibitors compared to Acarbose (IC50 = 6.4 μM).[8]

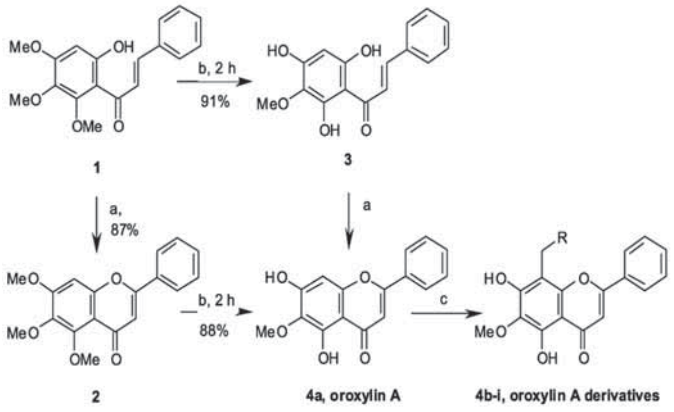
To seek new compounds related to polyhydroxyflavonoids of biologically and pharmacologically active ingredients with a view to systematically studying structure-activity relationships, we reported herein the synthesis of novel oroxylin A derivatives through Mannich reaction at C-8 in A-ring and the evaluation of potential anti-inflammatory activity of the corresponding Mannich bases. Our interests in their unique pharmacological properties prompted us to pursue a pertinent route toward the very efficient preparation of such highly prized targets.

Received: April 7, 2009; Revised: December 21, 2009; Accepted: December 29, 2009
*Corresponding author: Wen-Hsin Huang, No.161, Sec. 6, Min-Chun E. Rd, Taipei 114, Taiwan, Republic of China. Tel: +886-2-87923100 ext. 18875; Fax: +886-2-87923169; E-mail: wenhsin@ndmctsgh.edu.tw

METHODS

Chemistry

Melting points were taken in open capillary tubes on a Buchi-530 melting point apparatus and were uncorrected. UV-vis spectra were recorded on a Shimazu UV-160A



Scheme 1. (a) I_2, DMSO, 2 h; (b) 47% HBr, acetic acid, reflux; (c) $(CH_2O)_3$, 2° amines, butanol, reflux.

UV-Visible recording spectrophotometer. IR spectra were recorded on a Perkin-Elmer FTIR 1610 series infrared spectrophotometer in KBr discs. ^{1}H- and ^{13}C-NMR spectra were determined on a Varian Gemini-300 NMR instrument. Chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard, and coupling constants (*J*) were given in hertz (Hz). Fast atom bombardment (FAB) mass spectra were recorded using a Finnigan MAT 95S (GC/MS) mass spectrometer. All reactions were routinely monitored by TLC on Merck F254 silica gel plates. Merck silica gel (70-230 mesh) was used for chromatography. Elemental analyses for carbon, hydrogen and nitrogen were performed in the Instrument Center of the National Science Council at the National Taiwan University using Perkin-Elmer CHN-2400. All the solvents and reagents were obtained from commercial sources and purified before use if necessary.

Oroxylin A (**4a**) was synthesized according to our previous published procedures.[3-4]

General procedures for the synthesis of oroxylin A derivatives 4b-j.

A solution of the respective secondary amine (25 mmol) in butanol (10 ml) was added to paraformaldehyde (0.75 g, 25 mmol) and 1~2 drops H_2SO_4, the mixture was refluxed for 1 h. After adding oroxylin A (1.42 g, 5.0 mmol), the mixture was continually refluxed over 12 h. After filtration, the filtrate was evaporated in a vacuum. The crude compound was purified by silica gel column chromatography using EtOAc/CH_2Cl_2 (3:1, v/v) as eluent and recrystalized from hexane-EtOAc to afford the corresponding Mannich bases **4b-i** (oroxylin A derivatives).

5,7-Dihydroxy-6-methoxy-2-phenyl-8-(piperidin-1-ylmethyl)-chromen-4-one (4b)

Yellow crystals, 250 mg (13 % isolated yield), mp: 184-186 °C. Rf : 0.14 (EtOAc/ CH_2Cl_2 = 3:1). UV λ_{max} (MeOH) nm (log ε): 273 (4.60), 210 (4.21). IR (KBr) cm^{-1}: 3565, 3100, 2934, 1700, 1685, 1261. EI-MS *m/z* (%):296 (100), 382 (M^+, 3). ^{1}H-NMR ($CDCl_3$) δ:1.43 (2H, pent, J = 5.4 Hz, piperidinyl-H4), 1.55 (4H, pent, J = 5.4 Hz, piperidinyl-H3, H5), 2.50 (4H, t, J = 5.4 Hz, piperidinyl-H2, H6), 3.77 (2H, s, CH_2), 4.07 (3H, s, 6-OCH_3), 6.69 (1H, s, H3), 7.53 ~ 7.56 (3H, m, Ar-H3', H4', H5'), 7.96 (2H, dd, J = 5.1, 3.6 Hz, Ar-H2', H6'). ^{13}C-NMR ($CDCl_3$) δ: 23.6, 25.4, 50.1, 53.8, 60.7, 104.3, 106.5, 109.9, 115.0, 126.0, 128.6, 131.3, 133.1, 145.8, 148.1, 152.2, 164.0, 182.9.

5,7-Dihydroxy-6-methoxy-8-(morpholin-4-ylmethyl)-2-phenyl-chromen-4-one (4c)

Yellow crystals, 210 mg (11 % isolated yield), mp: 196-198°C. R_f: 0.50 (MeOH/CH_2Cl_2 = 1:9), UV λ_{max} (MeOH) nm (log ε): 270.0 (4.34), 206.0 (3.92), IR (KBr) cm^{-1} : 3689, 3649, 1700, 1654, 1360, 1262. EI-MS *m/z* (%): 297 (100), 383 (M^+, 15). ^{1}H-NMR ($CDCl_3$) δ: 2.57 (4H, t, J = 5.7 Hz, morpholinyl-H2, 6), 3.69 (4H, t, J = 5.7 Hz, morpholinyl-H3, 5), 3.81 (2H, s, 8-CH_2), 4.09 (3H, s, 6-OCH_3), 6.71 (1H, s, H3), 7.53 ~ 7.58 (3H, m, Ar-H3, H4, H5), 7.94 (2H, dd, J = 9.6, 3.2 Hz , Ar-H2, H6). ^{13}C-NMR ($CDCl_3$) δ: 49.9, 52.9, 60.8, 66.5, 104.4, 106.5, 109.0, 125.9, 128.7, 131.3, 131.4, 133.0, 145.9, 148.1, 151.9, 164.1, 182.8.

5,7-Dihydroxy-6-methoxy-8-(4-methyl-piperazin-1-ylmethyl)-2-phenyl-chromen-4-one (4d)

Yellow crystals, 130 mg (7% isolated yield), mp: 195-196 °C. R_f: 0.38 (MeOH/CH_2Cl_2 = 1:9). UV λ_{max} (MeOH) nm (log ε): 276.0 (4.59), 205.0 (4.02). IR (KBr) cm^{-1} : 3689, 3500, 1700, 1615, 1540, 1260. EI-MS *m/z* (%): 99 (100), 396 (M^+, 29). ^{1}H-NMR ($CDCl_3$) δ: 2.26 (3H, s, N-CH_3), 2.44~2.62 (8H, m, piperazinyl-H2, 3, 5, 6), 3.83 (2H, s, 8-CH_2), 4.05 (3H, s, 6-OCH_3), 6.70 (1H, s, H3), 7.52 ~ 7.56 (3H, m, Ar-H3, H4, H5), 7.94 (2H, dd, J = 8.1, 3.5 Hz, Ar-H2, H6). ^{13}C-NMR ($CDCl_3$) δ: 45.3, 49.4, 51.3, 52.3, 54.1, 54.7, 60.7, 104.3, 106.5, 109.5, 125.6, 125.9, 128.6, 131.3, 133.3, 146.1, 148.0, 152.3, 164.0, 182.8.

5,7-Dihydroxy-6-methoxy-2-phenyl-8-(4-pyridin-2-yl-piperazin-1-ylmethyl)-chromen-4-one(4e)

Yellow crystals, 460 mg (20 % isolated yield), mp: 194-196 °C. R_f: 0.75 (MeOH/CH_2Cl_2 = 1:9). UV λ_{max}

(MeOH) nm (log ε): 273.0 (4.53), 206.0 (4.21). IR (KBr) cm^{-1} : 3690, 3510, 1700, 1635, 1540, 1247. EI-MS *m/z* (%): 311 (100), 473 (M^+, 5). ^{1}H-NMR ($CDCl_3$) δ: 2.68 (4H, t, J = 4.8 Hz, piperazinyl-H2, 6), 3.51 (4H, t, J = 4.8 Hz, piperazinyl-H3, 5), 3.97 (2H, s, 8-CH_2), 4.08 (3H, s, 6-OCH_3), 6.61 (2H, td, J = 9.0, 2.1 Hz, pyridinyl-H2, 4), 6.71 (1H, s, H3), 7.42~7.51 (1H, m, pyridine-H3), 7.51 ~ 7.56 (3H, m, Ar-H3, H4, H5), 7.94 (2H, dd, J = 7.5, 1.8 Hz, Ar-H2, H6), 8.16 (1H, dd, J = 4.8, 1.8 Hz, pyridinyl-H5), 12.93 (1H, s, 5-OH). ^{13}C-NMR ($CDCl_3$) δ: 44.8, 49.7, 52.3, 60.1, 104.7, 106.5, 107.3, 109.0, 112.7, 125.9, 128.7, 131.1, 131.5, 136.2, 137.0, 147.5, 150.7, 153.2, 158.1, 159.1, 163.8, 183.0.

5,7-Dihydroxy-6-methoxy-2-phenyl-8-(4-pyrimidin-2-yl-piperazin-1-ylmethyl)-chromen-4-one (4f)

Yellow crystals, 213 mg (9 % isolated yield), mp: 195-198 °C. R_f: 0.75 ($MeOH/CH_2Cl_2$ = 1:9). UV λ_{max} (MeOH) nm (log ε) : 272.0 (4.57), 216.0 (4.13). IR (KBr) cm^{-1}: 3673, 3500, 1700, 1635, 1540, 1259. EI-MS *m/z* (%) : 311 (100), 474 (M^+, 7). ^{1}H-NMR ($CDCl_3$) δ: 2.62 (4H, t, J = 5.1 Hz, piperazinyl-H2, 6), 3.81 (4H, t, J = 5.1 Hz, piperazinyl-H3, 5), 3.96 (2H, s, 8-CH_2), 4.07 (3H, s, 6-OCH_3), 6.46 (1H, t, J = 4.8 Hz, pyrimidinyl-H4), 6.71 (1H, s, H3), 7.51 ~ 7.55 (3H, m, Ar-H3, H4, H5), 7.94 (2H, dd, J = 7.5, 2.1 Hz, Ar-H2, H6), 8.28 (2H, d, J = 4.8 Hz, pyrimidinyl-H3, 5), 12.93 (1H, s, 5-OH). ^{13}C-NMR ($CDCl_3$) δ: 43.2, 49.7, 52.3, 60.1, 104.7, 107.2, 109.3, 125.9, 128.7, 131.1, 131.5, 133.1, 136.2, 150.7, 153.2, 157.2, 158.1, 161.2, 163.8, 183.0.

8-[4-(2-Chloro-phenyl)-piperazin-1-ylmethyl]-5,7-dihydroxy-6-methoxy-2-phenyl-chromen-4-one (4g)

Yellow crystals, 270 mg (11% isolated yield), mp: 182-185 °C. R_f: 0.71 ($MeOH/CH_2Cl_2$ = 1:9). UV λ_{max} (MeOH) nm (log ε): 274.0 (4.83), 214.0 (4.32). IR (KBr) cm^{-1}: 3674, 3510, 1700, 1635, 1540, 1260. EI-MS *m/z* (%): 492 (M^+, 3). ^{1}H-NMR ($CDCl_3$) δ: 2.62 (4H, t, J = 5.1 Hz, piperazinyl-H2, 6), 3.06 (4H, t, J = 5.1 Hz, piperazinyl-H3, 5), 3.91 (2H, s, 8-CH_2), 4.11 (3H, s, 6-OCH_3), 6.72 (1H, s, H3), 6.99 (2H, dd, J = 8.1, 1.8 Hz, Ar-4',6'), 7.18 (1H, td, J = 7.8, 2.1 Hz, Ar-5'), 7.33 (1H, dd, J = 4.8, 1.8 Hz, Ar-3'), 7.54 ~ 7.57 (3H, m, Ar-H3, H4, H5), 7.96 (2H, dd, J = 7.8, 1.8 Hz, Ar-H2, H6). ^{13}C-NMR ($CDCl_3$) δ: 49.4, 50.7, 52.6, 60.7, 104.4, 106.5, 109.2, 119.9, 123.1, 126.0 (2), 127.0, 128.3, 128.7 (2), 130.1, 131.3, 131.4, 133.1, 146.1, 148.1, 148.9, 152.1, 164.1, 182.8.

5,7-Dihydroxy-6-methoxy-8-[4-(4-nitro-phenyl)-piperazin-1-ylmethyl]-2-phenyl-chromen-4-one (4h)

Yellow crystals, 480 mg (19 % isolated yield): mp: 201-205 °C. R_f: 0.75 ($MeOH/CH_2Cl_2$ = 1:9). UV λmax (MeOH) nm (log ε): 272.0 (4.62), 214.0 (4.29). IR (KBr) cm^{-1}: 3690, 3500, 1700, 1635, 1540, 1248. EI-MS *m/z* (%): 580 (M^+, 10). ^{1}H-NMR ($CDCl_3$) δ: 2.72 (4H, t, J = 4.8 Hz, piperazinyl-H2, 6), 3.40 (4H, t, J = 4.8 Hz, piperazinyl-H3, 5), 3.89 (2H, s, 8-CH_2), 4.10 (3H, s, 6-OCH_3), 6.77 (1H, s, H3), 6.79 (2H, d, J = 9.6 Hz, Ar-H2', 6'), 7.53 ~ 7.57 (3H, m, Ar-3, 4, 5), 7.93 (2H, dd, J = 8.4, 3.6 Hz, Ar-2, 6), 8.09 (2H, d, J = 9.6 Hz, Ar-3', 5'). ^{13}C-NMR ($CDCl_3$) δ: 46.5, 49.3, 51.8, 60.7, 104.5, 106.5, 108.5, 112.1, 125.4, 125.8, 128.7, 131.2, 131.5, 133.0, 138.0, 146.2, 148.0, 152.0, 154.4, 164.0, 182.7.

8-[4-(4-Fluoro-phenyl)-piperazin-1-ylmethyl]-5,7-dihydroxy-6-methoxy-2-phenyl-chromen-4-one (4i)

Yellow crystals, 950 mg (40% isolated yield), mp: 211-213 °C. R_f: 0.75 ($MeOH/CH_2Cl_2$ = 1:9). UV λ_{max} (MeOH) nm (log ε): 272.0 (4.48), 202.0 (4.27). IR (KBr) cm^{-1}: 3690, 3514, 1700, 1635, 1540, 1273. EI-MS *m/z* (%): 476 (M^+, 21). ^{1}H-NMR ($CDCl_3$) δ: 2.74 (4H, t, J = 4.8 Hz, piperazinyl-H2, 6), 3.10 (4H, t, J = 4.8 Hz, piperazinyl-H3, 5), 3.89 (2H, s, 8-CH_2), 4.10 (3H, s, 6-OCH_3), 6.72 (1H, s, H3), 6.82~6.96 (4H, m, Ar-H2', 3', 4', 5'), 7.51 ~ 7.56 (3H, m, Ar-3, 4, 5), 7.96 (2H, dd, J = 7.2, 2.4 Hz, Ar-2, 6), 12.8 (1H, s, 5-OH). ^{13}C-NMR ($CDCl_3$) δ: 49.6 and 49.7 (F effect), 50.1, 50.9, 52.4, 60.7, 104.4, 106.5, 109.1, 114.8 and 115.1 (F effect), 117.2 and 117.3 (F effect), 125.9, 128.7, 131.2, 131.4, 133.2, 146.1, 147.5 and 148.0 (F effect), 125.2, 155.2, 158.3, 164.0, 182.8.

Determination of anti-inflammatory activities by a carrageenan-induced hind paw edema test on rats.

Male albino Wistar rats (180-215 g) were housed and cared for under the guidelines of the Institutional Animal Care and Use Committee at the National Defense Medical Center, Taiwan. The rats were assigned to groups, one of them being the control. In order to induce inflammation, 50 μl of a 1% λ-carrageenan solution in normal saline was injected into the right hind paw subplantar tissue, according to the modified method of Winter *et al*.[9] The development of paw edema was measured plethysmographically (Basile 7140 plethysmometer, Ugo, Varese, Italy) and recorded prior to this administration. One hour before the λ-carrageenan challenge, a sample preparation (20 mg/kg) was injected i.p. into the rat in the test group. Normal saline was injected in the same way into animals in the control group. After the λ-carrageenan challenge, each paw volume (ml) was measured hourly up to 5 h. The percentage of paw edema and the inhibi-

tion of inflammation were calculated by the previously reported protocol.[9]

Statistical analysis.

Each experimental data value is expressed as the mean ±SEM. The statistical significance of differences was assessed with an analysis of variance (ANOVA), followed by Tukey's test or Student's T-test between two groups. Differences with *p* values of less than 0.05 are considered statistically significant.

RESULTS AND DISCUSSION

Chemistry

The oroxylin A derivatives **4** as the targets were prepared in multistep synthesis based on our previously published procedure[3-4] with further Mannich reaction in this study. Briefly, the mixture of equimolar trimethoxyphenol and cinnamoyl chloride was through Fries reaction in the presence of boron trifluride-etherate to obtain the corresponding trimethoxychalcone **1**. Further oxidation and cyclization of trimethoxylchalcone **1** by the catalytic iodine gave the trimethoxyflavone 2, followed by *O*-demethylation using hydrobromic acid and acetic acid to obtain the desired oroxylin A (**4a**). Alternatively trimethoxylchalcone 1 was *O*-demethylated by HBr/HOAc first followed by cyclization in the presence of catalytic iodine in DMSO to afford oroxylin A (**4a**) as well. The aminomethylation (Mannich reaction) of oroxylin A (**4a**) was conducted by regiochemically condensing of flavone with paraformaldehyde and secondary amines with a catalytic acid in boiling butanol. After completion, the reaction mixture was concentrated and purified by silica gel column chromatography to afford yellow target compounds.

The Mannich reaction is one of the convenient methods of chemical modification leading to the introduction into the molecule of the function of a aminomethylated base which on easy conversion into an ammonium salt improves aqueous solubility of the corresponding compound. *N*-Substituted aminoalkyl derivatives of oroxylin A (**4a**) were regiochemically carried out by the Mannich reaction. The classical Mannich reaction of the oroxylin A (**4a**) was accomplished by appropriate secondary amines and *para*-formaldehyde at electrophilic C-8 close to a hydroxyl moiety. Even though compounds **4b**, **4c** and **4f** had been prepared through Mannich reaction of oroxylin A as moderate potent α-glucosidase inhibitors compared to Acarbose by Babu et al.,[8] we can not reproduce these compounds by the described method in this literature.[8] However, a modified reaction condition of solvent used and catalytic acid added overcame the Mannich reaction of oroxylin A in our laboratory. A higher boiling point of butanol and 1~2 drops H_2SO_4 instead of ethanol, isopropanol and even propanol were used in this Mannich reaction.

All the synthetic oroxylin A derivatives **4** were fully characterized by UV, IR, NMR (^{1}H, ^{13}C), and MS. The ^{1}H-NMR spectra of compounds **4b-j** clearly indicated that the absence of the signal at δ 6.99 (1H, s, DMSO-d_6, 300 MHz) for H-8 proton of the parent compound oroxylin A (**4a**) conformed C-aminomethylated at C-8. The NMR spectrophotometric characterization of our synthetic 8-aminomethylated oroxylin A derivatives was slightly differential identification such as chemical shifts and integrals of *O*-methyl and *N*-methylene of all synthetic 8-aminomethylated oroxylin A derivatives, even the different power (mega Hz) of NMR instruments (300 *vs* 200 MHz) were used. We fully confirmed the assignment of our synthetic 8-aminomethylated oroxylin A derivatives according all spectrophotometric data.

Pharmacology

Biological significance of the 8-aminomethylated oroxylin A derivatives was established by the λ-carrageenan-induced hind paw edema test to evaluate the corresponding anti-inflammatory activity. λ-Carrageenan-induced rat paw edema, the well-established standard and most commonly used technique to screen anti-inflammatory activity, is an *in-vivo* model of acute inflammation for a variety of inflammatory and/or oxidative stress mediators with biphasic inflammatory nature.[9] A state of local acute inflammation was evoked by injecting 1% (w/v) λ-carrageenan (0.1 ml/paw) s.c. into the plantar surface of the right hind paw of the rat, with the left paw (saline treated) serving as a control. The anti-edematogenic appearance is responsible for attenuating inflammatory effects.

In the vehicle-treated control group, the mean volume of the right hind paws increased by 1.23±0.06 ml at 5 h after a λ-carrageenan challenge. All the tested flavonoids and ibuprofen (as a positive control) injected i.p. at 20 mg/kg significantly reduced the mean paw edema of the rats. The results show that the target compounds, oroxylin A (**4a**) and derivatives **4b-i**, exerted an anti-inflammatory effect with a significant reduction in swelling. The degree of *in-vivo* inhibition by the flavonoids toward the paw edema was strikingly pronounced at 1-2 h after the λ-carrageenan challenge, especially compounds **4c**, **4g** and **4i** that manifested the greatest inhibition of over

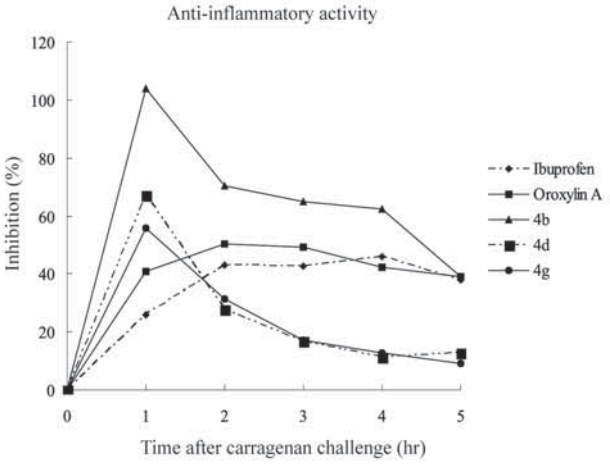
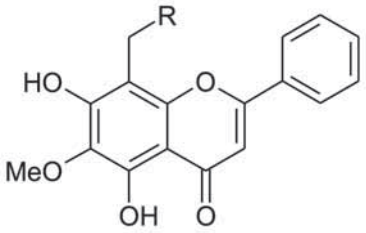


Fig. 1. Anti-inflammatory profile of representative 8-aminomethylated oroxylin A derivatives compared to ibuprofen and oroxylin A.

100% at the first hour and the potency lasted 5 hours in comparison to that of the control and ibuprofen. Most of the other compounds showed a comparable anti-inflammatory activity compared to ibuprofen.

It is noteworthy that, from the foregoing extensively structural aminomethylated modification from A-ring of oroxylin A lead to a marked difference in the potency of anti-inflammatory activities. Concerning structural features from the H-8 substitution of oroxylin A, all of the C-8 Mannich bases of oroxylin A showed more potent anti-inflammatory activity at the first hour after the carrageenan challenge than the parent compound, oroxylin A. And most of aminomethylated oroxylin A derivatives have activity lasting 5 hours except for compounds **4d** and **4g** (Fig. 1). This evidence of biological significance indicated that active Mannich bases display shorter onset of pharmaceutical significance than that of the parent compound, oroxylin A, and the positive control, ibuprofen. Alicyclic amine (morpholinyl, piperidinyl, or piperazinyl ring) substituents of oroxylin A at C-8 remarkably improve anti-inflammatory potential of the parent compound wherein morpholinylmethyl (**4c**), piperidinylmethyl (**4b**), *N*-(*N*-2-pyrimidinyl)-piperazinylmethyl (**4f**) substituents as representatives signify the best fit, respectively. It is extraordinarily noted that all of the desired Mannich base compounds except *N*-methylpiperazinylmethyl (**4d**) and *N*-(*N*-*o*-chlorophenyl)-piperazinylmethyl (**4g**) substituents have comparable duration to that of oroxylin A, and the positive control, ibuprofen.

Table 1 Preliminary screening of oroxylin A derivatives on anti-inflammatory activity.



Compounds	Substituents R	Edema rate (%) of pretreatment[a] (Corresponding inhibition%)[b] 1	3	5 (hr)
control	-	22.9±0.1	79.1±0.1	95.4±0.1
ibuprofen	-	17.0±0.1 (25.8%)*	45.3±0.2 (42.7%)*	59.3±0.1 (37.8%)*
oroxylin A (4a)		13.5±0.1 (41.0%)*,**	40.3±0.2 (49.1%)*	58.2±0.1 (39.0%)*
4b	*N*-piperidinyl	0±0.1 (104%)*,**	27.5±0.1 (65%)*,**	58.5±0.1 (39%)*
4c	*N*-morpholinyl	0.4±0.1 (98%)*,**	44.1±0.1 (44%)*	65.0±0.1 (32%)*
4d	*N*-methylpiperazinyl	7.6±0.1 (67%)*,**	70.7±0.1 (11%)*	82.7±0.2 (13%)*
4e	*N*-(*N*-2-pyridinyl)-piperazinyl	5.5±0.1 (76%)*,**	46.0±0.2 (42%)*	55.4±0.1 (42%)*
4f	*N*-(*N*-2-pyrimidinyl)-piperazinyl	0±0.1 (112%)*,**	35.1±0.2 (56%)*,**	65.3±0.1 (32%)*
4g	*N*-(*N*-*o*-chlorophenyl)-piperazinyl	10.0±0.1 (56%)*,**	65.8±0.2 (17%)*	86.8±0.1 (9.1%)*
4h	*N*-(*N*-*p*-nitrophenyl)-piperazinyl	6.6±0.1 (71%)*,**	48.5±0.2 (39%)*	62.9±0.1 (34%)*
4i	*N*-(*N*-*p*-fluorophenyl)-piperazinyl	4.2±0.1 (82%)*,**	40.7±0.2 (49%)*	61.7±0.2 (35%)*

[a]A dose 20 mg/kg ip for each SD rat is conducted. Edema rate (E %) was calculated as follows: $E\%=(V_t-V_0)/V_0\times100$; V_0: volume of hind paw before 1% **λ**-carrageenan administration; V_t: volume of hind paw after 1% carrageenan administration at t h. Percentage of inhibition (I %) was determined as follows: $I\%=(E_c-E_t)/E_c\times100$; E_c: edema rate of control group; E_t: edema rate of the respective test compound at t h. The statistical significance of differences was assessed with an analysis of variance (ANOVA), followed by Tukey's test or Student's test between two groups. Each value represents the mean±SEM, n=5, *P < 0.05 compared with the control (0.1 ml normal saline), ** P < 0.05 compared with positive control (ibuprofen), respectively.

[b]The number in parentheses indicates the percentage inhibition rate (I %).

CONCLUSION

The Mannich reaction is well documented by examples of various phenols and widely used in the organic synthesis. This reaction provides a feasible method to introduce a basic aminoalkyl side chain into hydroxyflavonoid skeleton with discrepantly versatile pharmacolog-

ical functions. The synthetic oroxylin A derivatives **4** are remarkable anti- inflammatory agents and therefore merit further study.

ACKNOWLEDGEMENTS AND FUNDING

We gratefully acknowledge the research grant supported partly from the Medical Research of NDMC (DOD98-11-01), Republic of China and from the Cheng-Hsin Rehabilitation Medical Center (CHRMC 97-63), Republic of China.

REFERENCES

1. Huang WH, Lee AR, Chien PU, Chou TC. Synthesis of baicalein derivatives as potential anti-aggregatory and anti-inflammatory agents. J Pharm Pharmacol 2005;57:219-225.
2. Huang WH, Lee AR, Yang CH. Antioxidative and anti-inflammatory activities of polyhydroxyflavonoids of Scutellaria baicalensis GEORGI. Biosci Biotechnol Biochem 2006;10:2371-2380.
3. Huang WH, Chien PY, Yang CH, Lee AR. Novel synthesis of flavonoids of Scutellaria baicalensis GEORGI. Chem Pharm Bull 2003;51:339-340.
4. Huang WH, Yang CH, Chien, PY, Lee AR. Synthesis of 5,6,7-trimethoxyflavone as key intermediate for the preparation of baicalein. Chin Pharm J 2003;55:101-107.
5. Kim HP, Son KH, Chang HW, Kang SS. Anti-inflammatory flavonoids: Modulators of proinflammatory gene expression. Nat Prod Sci 2004;10:1-10.
6. Chen YC, Yang LL, Lee TJF. Oroxylin A inhibition of lipopolysaccharide-induced iNOS and COX-2 gene expression via suppression of nuclear factor-kB activation. Biochem Pharmacol 2000;59:1445-1457.
7. Huen MSY, Leung JWC, Ng W, Lui WS, Chan MNS, Wong JTF, Xue H. 5,7-Dihydroxy-6-methoxyflavone, a benzodiazepine site ligand isolated from Scutellaria baicalensis Georgi, with selective antagonistic properties. Biochem Pharmacol 2003;66:125-132.
8. Babu T., Rao RS, Tiwari AK, Babu KS, Srinivas PV, Ali AZ, Rao JM. Synthesis and biological evaluation of novel 8-aminomethylated oroxylin A analogues as α-glucosidase inhibitors. Bioorg Med Chem Lett 2008;18:1659-1662.
9. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in the hind paw of the rats as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 1962;111:544-547.