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Anti-ulcer xanthones from the roots of *Hypericum oblongifolium* Wall

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ABSTRACT

Three new xanthones, hypericorin C (**1**), hypericorin D (**2**) and 3,4-dihydroxy-5-methoxyxanthone (**3**), along with eight known compounds; 2,3-dimethoxyxanthone (**4**), 3,4-dihydroxy-2-methoxyxanthone (**5**), 3,5-dihydroxy-1-methoxyxanthone (**6**), 3-acetylbetulinic acid (**7**), 10*H*-1,3-dioxolo[4,5-*b*]xanthen-10-one (**8**), 3-hydroxy-2-methoxyxanthone (**9**), 3,4,5-trihydroxyxanthone (**10**) and betulinic acid (**11**) were isolated from the roots of *Hypericum oblongifolium*. The structures of the new compounds **1**, **2** and **3** were deduced by spectroscopic techniques [ESI MS, ^{1}H NMR, ^{13}C NMR, and 2D NMR (HMQC, HMBC, COSY and NOESY)]. The entire series of compounds were evaluated for anti-ulcer activity.

1. Introduction

Hypericum oblongifolium Wall., which belongs to the family Guttiferae, is an erect evergreen shrub, which grows to a height of 6–12 m, that is commonly found in the Khasia Hills in India at an altitude of 5000–6000 ft and in the Himalayas [1]. In Chinese traditional herbal medicine *H. oblongifolium* has been used for the treatment of hepatitis, bacterial diseases nasal hemorrhage, and as a remedy for dog bites and bee stings [2]. In various parts of the world, the plants of genus *Hypericum* have been used in traditional medicines as a sedative, an antiseptic, and an antispasmodic, as well as for the treatment of external wounds and gastric ulcers [3].

Plants of the genus *Hypericum* are a rich source of xanthones; many of which exhibit a broad spectrum of activities. The xanthones and their derivatives, isolated from different species of *Hypericum*, exhibit potent anti-tumor, anti-fungal, cytotoxic [4], anti-microbial, anti-ulcer, anti-depressant, inhibition of lipid peroxidase [5], anti-inflammatory, anti-septic, anxiolytic, diuretic, digestive, expectorant, and vermifugal [3] activities and have received attention for the anti-viral action of hypericin and pseudohypericin on lipid enveloped and non-enveloped DNA and RNA viruses [6,7]. The most common compounds isolated from plants of this genus are xanthones [8], flavonoids [9], phloroglucinol, licinic acid derivatives [10], benzopyrans [11] and benzophenones [12].

Urease (urea amidohydrolase, EC: 3.5.1.5) occurs throughout the animal and plant kingdom. Many microorganisms use this enzyme to provide a source of nitrogen for growth, and it also plays an important role in plant nitrogen metabolism during the germination process [13,14]. The presence of urease

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activity in soils is exploited widely in agriculture. Unfortunately, excessive levels of soil urease can degrade urea in fertilizers too rapidly and result in phytopathic effects and loss of volatilized ammonia [15]. On the other hand in human and veterinary medicine, urease is a virulent factor in certain human and animal pathogens, which participate in the development of kidney stones, pyelonephritis, peptic ulcers, and other disease states [16]. The obvious remedy for treating bacterial infection with anti-microbials has often proven futile [17], and only a few combination regimes have reached clinical practice. Thus the need for alternative or novel treatments is paramount. The discovery of potent and safe urease inhibitors is a very important area of pharmaceutical research due to the involvement of ureases in different pathological conditions.

In a continuation of our study on the genus *Hypericum*, herein we report the isolation and structure elucidation of three new xanthones, hypericorin C, {(2*R*,3*R*)-*rel*-2-[(acetyloxy) methyl]-3-(3-hydroxy-4-methoxyphenyl)-2,3-dihydro-5-methoxy-7*H*-1,4-dioxino[2,3-c]xanthen-7-one}(**1**), hypericorin D, {(2*R*,3*R*)-*rel*-2-[hydroxymethyl]-3-(2,3,4-trihydroxy-5-methoxyphenyl)-2,3-dihydro-5-methoxy-7*H*-1,4-dioxino[2,3-c] xanthen-7-one} (**2**) and 3,4-dihydroxy-5-methoxyxanthone (**3**), along with four compounds previously isolated from *Hypericum*; namely 2,3-dimethoxyxanthone (**4**), 3,4-dihydroxy-2-methoxyxanthone (**5**), 3,5-dihydroxy-1-methoxy-xanthone (**6**), and 3-acetylbetulinic acid (**7**) (Fig. 1). Also isolated for the first time from *H. oblongifolium* were 10*H*-1,3-dioxolo[4,5-*b*]xanthen-10-one (**8**) [18], 3-hydroxy-2-methoxyxanthone (**9**) [19], 3,4,5-trihydroxyxanthone (**10**) [20] and betulinic acid (**11**) (Fig. 3).

2. Experimental

2.1. General

UV spectra were obtained on Optima SP3000 plus (Japan) UV–Visible spectrometer using chloroform, or methanol, as the solvent. IR spectra were recorded on a Nicolet 205 and Impact 410 FT-IR spectrometers, using KBr windows with acetone as solvent against an air background. ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a JEOL ECA-600 FT NMR spectrometer fitted with an X-H auto-tune 5 mm probe. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) and coupling constants are given in Hz. Mass spectra (ESI in either positive- or negative-ion mode) were measured on a Micromass Quattro Ultima (Triple Quad). TLC was performed on pre-coated silica gel F-254 plates (Plastic plates; F_{254}; Macherey Germany); the visualization was done at 254 nm and by spraying with ceric sulphate reagent. Column silica gel (Silica gel-60, 70-230 mesh; Material Harvest UK) and flash silica gel 230-400 mesh were used for column chromatography. Melting points were determined on a Gallenkamp apparatus and are uncorrected.

2.2. Plant material

H. oblongifolium Wall, which was authenticated by Dr. Habib Ahmad, Dean Faculty of Science, Hazara University, was collected at flowering period in June from Buner District, Khyber Pakhtunkhwa, Pakistan. A voucher specimen (HUH-002) was retained for verification purposes in the Department of Botany, Hazara University, Khyber Pakhtunkhwa, Pakistan.

2.3. Extraction and isolation

The air-dried, powdered roots (4 kg) were exhaustively successively extracted with *n*-hexane, ethyl acetate and methanol (3 × 25 L, each for 3 days) at room temperature. The extracts were concentrated in a rotary evaporator and dried under vacuum to yield gummy residues. The ethyl acetate fraction (70 g) was subjected to column chromatography over silica gel eluting with *n*-hexane–ethyl acetate and ethyl acetate–methanol in increasing order of polarity to afford 180 fractions, which were grouped according to the similarity on TLC profiles to give 21 major fractions (1–21). Fraction 4 was purified through column chromatography (*n*-hexane:chloroform; 1:1) to yield 20 mg of pure compound **7**. Fraction 5 was also subjected to column chromatography. Elution with *n*-hexane: chloroform in increasing order of polarity starting with a 80:20 mixture yielded three sub-fractions (5.1–5.3), which were further purified by preparative TLC using chloroform as eluent to give **4** (4 mg) and **8** (3 mg). Fraction 11 was also subjected to column chromatography and elution with *n*-hexane:chloroform in increasing order of polarity (started at 1:1) gave five sub-fractions (11.1–11.5). Further purification by preparative TLC using methanol:chloroform (5:95) as eluent gave **9** (3 mg) and **10** (4 mg). Fraction 12 was also subjected to preparative TLC using methanol:chloroform (5:95) as eluent and yielded pure **11** (20 mg). Fraction 17 was subjected to further column chromatography. Elution with *n*-hexane:chloroform (80:20) through to pure chloroform and then methanol:chloroform (1:99) afforded **1** (15 mg). Compound **5** was obtained from fraction 18 by preparative TLC using methanol: chloroform (7:93) as eluent. Fraction 19 was also subjected to column chromatography. Elution with *n*-hexane:chloroform (80:20 through to pure chloroform and then methanol: chloroform 1:99) gave **2** (17 mg). Finally compound **6** (6 mg) was purified from fraction 20 by preparative TLC (methanol: chloroform 7:93).

Hypericorin C (**1**): Whitish amorphous powder; $R_f = 0.6$; methanol:chloroform (1:99); mp 230–232 °C; $[\alpha]_D^{20} = +0.33°$ (0.01 acetone); IR ν_{max}(KBr) cm^{-1} 3416, 2941, 1742, 1642, 1608, 1485, 1343, 1228, 1140 and 1089; ESI $[M + 1]^+$ *m/z* 479.0 (consistent with $C_{26}H_{24}O_9$); HR-ESIMS (+): ($[M + H]^+$ *m/z* 479.1359; calcd 479.1337); UV λ_{max}(MeOH) nm (log ε): 248 (4.34), 308 (3.83), 346 (3. 82). ^{1}H (600 MHz) and ^{13}C NMR spectral data (150 MHz, $(CD_3)_2CO$): given in Table 1.

Hypericorin D (**2**): White amorphous powder; $R_f = 0.4$; methanol:chloroform (1:99); mp 250–254 °C; $[\alpha]_D^{20} = +0.58°$ (0.01 acetone); IR ν_{max}(KBr) cm^{-1} 3384, 2940, 1704, 1639, 1599, 1464, 1325, 1285, 1138 and 1089; ESI $(M - 1)^-$ *m/z* 467.0 (consistent with $C_{24}H_{20}O_{10}$); HR-ESIMS (+): ($[M + H]^+$ *m/z* 467.1359; calcd 467.1337)UV λ_{max}(MeOH) nm (log ε): 250 (4.5), 302 (4.38), 387 (3.71); ^{1}H (600 MHz) and ^{13}C NMR spectral data (150 MHz, DMSO-d_6): given in Table 1.

3,4-Dihydroxy-5-methoxyxanthone (**3**): Yellow amorphous solid; $R_f = 0.35$; chloroform:hexane (8:2); mp 230–235 °C; UV λ_{max}(MeOH) nm (log ε): 240 (4.32), 258 (4.37), 269 (4.45), 376 (3.58); IR ν_{max}(KBr): 3437, 2900, 1622, 1585, 1470, 1455, 1345, 1310, 1245, 1215 cm^{-1}; ESI $(M + H)^+$: