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Synthesis and in vitro study of novel 7-*O*-acyl derivatives of Oroxylin A as antibacterial agents

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Abstract—A series of Oroxylin A derivatives, prepared by alkylation and condensation, were fully characterized by spectroscopic methods. All the derivatives were screened for antibacterial activity against a panel of susceptible and resistant Gram-positive and Gram-negative organisms. It was observed that acylation of 7-OH group in Oroxylin A significantly enhanced the activity as compared to their parent compound (Oroxylin A).

1. Introduction

Flavanoids are a broad class of polyphenolic secondary metabolites that are abundant in plants and in various common foods such as apples, onions, tea, and red wine. Apart from their important biological roles in nitrogen fixation and chemical defense, flavanoids possess a broad range of pharmacological properties, including antioxidant, anticancer, and anti-inflammatory properties[1], and hence received considerable therapeutic importance.

Oroxylin A, a widely distributed flavanoid, has been reported to possess many biological activities, such as CoX-2 inhibition,[2,3] cytotoxic,[4] antimicrobial,[5] and antiallergic[6] activities. Furthermore, Oroxylin A has demonstrated anti-HIV[7] and lipid peroxidation inhibition[8] activities. The versatile biological activities of the Oroxylin A prompted us to prepare a new series of its derivatives and evaluate their biological significance.

In view of the above and in continuation of our earlier studies on the synthesis of new bioactive derivatives,[9–11] we illustrate the synthesis and antibacterial activities of alkoxy and 7-*O*-acyl derivatives of Oroxylin A.

2. Chemistry

Oroxylin A was isolated from the traditional Indian medicinal plant *Oroxylum indicum* in substantial yield. Alkyl derivatives of Oroxylin A were prepared by alkylation using alkyl bromide or alkyl chloride. As a result, 5,7-dialkoxy Oroxylin A and 5-hydroxy 7-alkoxy Oroxylin A were synthesized (Scheme 1). Systematic analytical investigation revealed that the substitution occurred mostly at the seventh position of Oroxylin A but very slight at its fifth position. It is due to hydrogen bonding of the 5-OH group with the carbonyl group. Acyl derivatives of Oroxylin A were synthesized by condensation using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and hydroxyl benzotriazole (HOBt) in DMF/DCM solvent[12] (Scheme 2). In this case, Oroxylin A derivatives substituted only at the seventh position were obtained but not at the fifth position. Among the alkoxy and acetoxy analogues synthesized, compounds **2a**[14], **2b**[15], **2d**[16], and **2e**[17] are previously known, and their physical and spectral characteristics are in perfect agreement with reported literature values. All the synthetic compounds were well characterized by their spectral characteristics.[18]

3. Biological activity

The minimum inhibitory concentrations (MIC) of 7-*O*-acyl derivatives of Oroxylin A were obtained against

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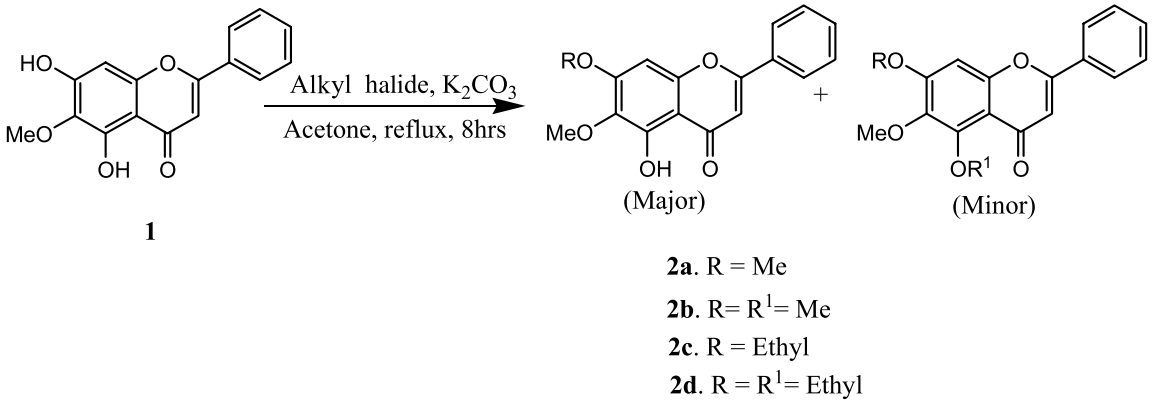
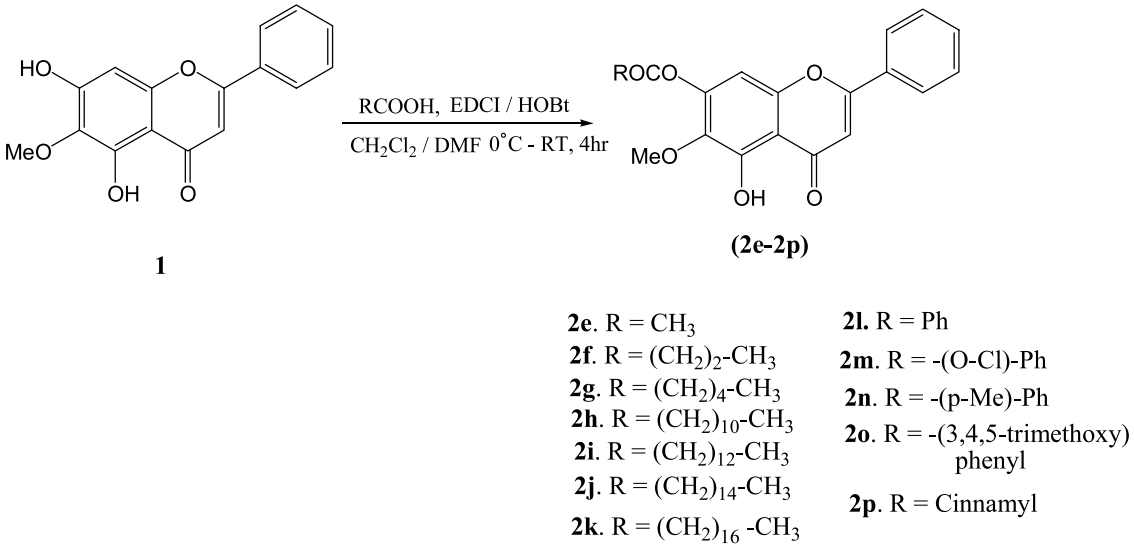


Scheme 1. Synthesis of alkoxy derivatives of Oroxylin A.



Scheme 2. Synthesis of 7-*O*-acyl derivatives of Oroxylin.

three representative Gram-positive organisms viz *Bacillus subtilis* (MTCC 441), *Bacillus sphaericus* (MTCC 11), and *Staphylococcus aureus* (MTCC 96), and three Gram-negative organisms viz *Chromobacterium violaceum* (MTCC 2656), *Klebsiella aerogenes* (MTCC 39), and *Pseudomonas aeruginosa* (MTCC 741) by the broth dilution method recommended by the National Committee for Clinical Laboratory (NCCL) standards.[13] Standard antibacterial agents, such as penicillin and streptomycin, were also screened under identical conditions for comparison. The minimum inhibitory concentrations are given in Table 1. It has been observed that the test compounds exhibited interesting biological activity however, with a degree of variation.

The alkyl substituted Oroxylin A derivatives (**2a–2d**) displayed moderate levels of antibacterial activity. However, the introduction of acyl group at the C-7 position of Oroxylin A has enhanced the inhibitory potential to a great extent. Compound (**2f**) exhibited remarkable antibacterial activity. However, by increasing the chain length of the aliphatic esters, activity did not improve.

Synthetic esters of Oroxylin A (**2e–2p**) showed varying degrees of antibacterial activity. Among the aromatic esters, compounds **2f** and **2l** showed good activity against *B. sphaericus*. Similarly, compounds **2f** and **2o** exhibited good activity against *C. violaceum*. None of the esters exhibited any activity against *P. aeruginosa*, even at a concentration of 200 μg/ml. The compounds were also inactive against the tested antifungal strains.

4. Experimental

All the melting points were uncorrected. The ^{1}H NMR spectra in $CDCl_3$ were recorded on a Bruker AV-300 spectrometer. The IR spectra were recorded on a Perkin-Elmer spectrophotometer. Schemes 1 and 2 represent a schematic sketch of the synthesis of the 7-*O*-acyl derivatives of Oroxylin A (**2a–2p**).

4.1. General procedure

4.1.1. Synthesis of alkoxy derivatives of Oroxylin A. To a solution of Oroxylin A in acetone (50 mL) under

Table 1. Minimum inhibitory concentrations (MIC, μg/ml) of 7-*O*-acyl derivatives of Oroxylin A

Compound	Microorganisms				
	Gram-positive			Gram-negative	
	B. subtilis	*B. sphaericus*	*S. aureus*	*K. aerogenes*	*C. violaceum*
Oroxylin A	50	25	50	50	25
2a	25	12.5	50	50	25
2b	50	25	25	50	25
2c	25	25	50	25	25
2d	25	12.5	25	25	25
2e	12.5	25	50	50	25
2f	25	6.25	25	25	6.25
2g	25	12.5	25	25	25
2h	25	12.5	12.5	12.5	25
2i	25	12.5	50	50	25
2j	25	25	25	12.5	25
2k	12.5	12.5	25	25	12.5
2l	12.5	6.25	25	25	12.5
2m	25	25	12.5	25	25
2n	50	25	50	25	50
2o	12.5	25	25	12.5	6.25
2p	25	12.5	12.5	12.5	25
Streptomycin	6.25	12.5	6.25	1.562	3.125
Penicillin	1.562	3.125	1.562	6.25	12.5

Negative control DMSO—no activity.

nitrogen was added K_2CO_3. After stirring for 10 min, alkyl bromide or alkyl chloride was added to the deep yellow colored solution and the mixture was refluxed for 3 h. After completion of the reaction (TLC), the reaction mixture was filtered to remove the K_2CO_3 and the filtrate was concentrated under vacuum. The residue was purified by column chromatography on silica gel (60–120 mesh) to give the 7-alkoxy Oroxylin A derivative in major yield and 5,7-dialkoxy Oroxylin A in minor yield.

4.1.2. Synthesis of 7-*O*-acyl derivatives of Oroxylin A. The corresponding acids, EDCI (0.836 mmol) and HOBt (0.69 mmol), were cooled to 0 °C and stirred in anhydrous methylene chloride (5 ml) for 15–30 min under nitrogen atmosphere. To this mixture, Oroxylin A (**1**) (0.704 mmol) in anhydrous *N*,*N*-dimethylformaldehye (3 ml) was added. The entire reaction mixture was stirred at room temperature for 4–5 h under nitrogen atmosphere. After completion of the reaction (TLC), the reaction mixture was poured into ice water and washed with methylene chloride (2×10 ml). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography on silica gel (60–120 mesh) to give the corresponding 7-*O*-acyl derivatives of Oroxylin A (**2e–2p**) in very good yield (60–80%).

5. Conclusion

In conclusion, a series of 7-*O*-acyl derivatives of Oroxylin A derivatives were synthesized and evaluated for antibacterial activity. All the compounds exhibited moderate to good activity, irrespective of their chain length and substitution. Among them, compounds (**2f**, **2l**, and **2o**) displayed the most significant activity, whereas other compounds exhibited moderate activity against both Gram-positive and Gram-negative strains.

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18. NMR data for compounds: compound (**2a**): pale yellow solid, mp 148 °C; IR (KBr) ν_{max}: 3415 (OH), 1620 (C═O) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.80 (s, 1H, OH-5), 7.84-7.86 (2H, m, H-2′, 6′), 7.46–7.54 (3H, m, H-3′, 4′, 5′), 6.64 (1H, s, H-8), 6.62 (1H, s, H-3), 3.92 (3H, s, OMe), 3.91 (3H, s, OMe). Mass (EIMS): *m/z* 298. Compound (**2b**): yellow solid, mp 162–164 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.85–7.86 (2H, m, H-2′, 6′), 7.46–7.55 (3H, m, H-3′, 4′, 5′), 6.66 (1H, s, H-8), 6.60 (1H, s, H-3), 3.98 (9H, s, 3XOMe). Mass (EIMS): *m/z* 312. Compound (**2c**): yellow solid, mp 196.2 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 12.84 (s, 1H, OH-5), 7.86–7.88 (2H, m, H-2′, 6′), 7.46–7.55 (3H, m, H-3′, 4′, 5′), 6.64 (1H, s, H-8), 6.62 (1H, s, H-3), 4.04–4.08 (m, 2H, –O–$CH_2$$CH_3$), 3.92 (3H, s, OMe), 1.37–1.39(3H, t, J = 6.5 Hz, –O–CH_2 CH_3). Mass (EIMS): *m/z* 312. Compound (**2d**): yellow solid, mp 115–118 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 7.85–7.88 (2H, m, H-2′, 6′), 7.46–7.56 (3H, m, H-3′, 4′, 5′), 6.65 (1H, s, H-8), 6.62 (1H, s, H-3), 4.08–4.10 (4H, m, –O–$CH_2$$CH_3$), 3.92 (3H, s, OMe), 1.42–1.44 (2H, t, –O–CH_2 CH_3), 1.46–1.50 (2H, t, –O–CH_2 CH_3). FABMS: 337 [M^++1]. Compound (**2e**): yellow needles, mp 128–130 °C; IR (KBr) ν_{max}: 3440 (OH), 1746 (O–C═O), 1627 (C═O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 12.82 (1H, s, OH-5), 7.84–7.88 (2H, m, H-2′, 6′), 7.48–7.56 (3H, m, H-3′, 4′, 5′), 6.66 (1H, s, H-8), 6.60 (1H, s, H-3), 3.92 (3H, s, OMe), 2.10 (3H, OCOCH_3). FABMS: 327 [M^++1]. Compound (**2f**): pale yellow solid, mp 64–66 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 12.84 (1H, s, OH-5), 7.82–7.84 (2H, m, H-2′, 6′), 7.48–7.58 (3H, m, H-3′, 4′, 5′), 6.64 (1H, s, H-8), 6.60 (1H, s, H-3), 3.92 (3H, s, OMe), 2.60 (2H, t, H-2″), 1.80–1.90 (2H, m, H-3″), 1.1 (3H, t, H-4″). FABMS: 377 [M^++Na]. Compound (**2g**): pale yellow solid, mp 115 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 12.82 (1H, s, OH-5), 7.80–7.82 (2H, m, H-2′, 6′), 7.50–7.58 (3H, m, H-3′, 4′,5′), 6.76 (1H, s, H-8), 6.70 (1H, s, H-3), 3.96 (3H, s, OMe), 2.60 (2H, t, H-2″), 2.30–2.40 (2H, m, H-3″), 1.40–1.60 (4H, br s, H-4″, H-5″), 0.86 (3H, t, H-6″). FABMS: 387 [M^++1]. Compound (**2h**): pale yellow solid, mp 101.2 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 12.82 (1H, s, OH-5), 7.88–7.92 (2H, m, H-2′, 6′), 7.50–7.56 (3H, m, H-3′, 4′, 5′), 6.70 (1H, s, H-8), 6.64 (1H, s, H-3), 3.90 (3H, s, OMe), 2.62 (2H, t, H-2″), 1.60–1.80 (2H, m, H-3″), 1.22–1.40 (16H, br s, H-4″–H-11″), 084 (3H, t, H-12″). FABMS: 467 [M^++1]. Compound (**2i**): yellow needles, mp 108.2–108.4 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 12.82 (1H, s, OH-5), 7.82–7.86 (2H, m, H-2′, 6′), 7.46–7.58 (3H, m, H-3′, 4′, 5′), 6.88 (1H, s, H-8), 6.64 (1H, s, H-3), 3.90 (3H, s, OMe), 2.60 (2H, t, H-2″), 1.78–1.84 (2H, m, H-3″), 1.20–1.45 (20H, br s, H-4″–H-13″), 0.88 (3H, t, H-14″). FABMS: 495 [M^++1]. Compound (**2j**): yellow solid, mp 105–106 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 12.42 (1H, s, OH-5), 7.92–7.96 (2H, m, H-2′, 6′), 7.52–7.60 (3H, m, H-3′, 4′, 5′), 6.78 (1H, s, H-8), 6.70 (1H, s, H-3), 3.94 (3H, s, OMe), 2.60 (2H, t, H-2″), 1.70–1.92 (2H, m, H-3″), 1.0–1.58 (24H, br s, H-4″–H-15″), 0.90 (3H, t, H-16″). FABMS: 523 [M^++1]. Compound (**2k**): pale yellow solid, mp 78.4 °C; IR (KBr) ν_{max}: 3415, 1770, 1620 cm^{-1} ^1H NMR (300 MHz, $CDCl_3$): δ 12.82 (1H, s, OH-5), 7.80–7.86 (2H, m, H-2′, 6′), 7.44–7.54 (3H, m, H-3′, 4′, 5′), 6.72 (1H, s, H-8), 6.68 (1H, s, H-3), 3.92 (3H, s, OMe), 2.60 (2H, t, H-2″), 2.36 (2H, m, H-3″), 1.78–1.82 (2H, m, H-4″), 1.58–1.68 (2H, m, H-5″), 1.20–1.50 (24H, br s, H-6″–H-17″), 0.88 (3H, t, H-18″). FABMS: 573 [M^++Na]. Compound (**2l**): yellow solid, mp 205 °C; IR (KBr) ν_{max}: 3415, 1735, 1618 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 12.84 (1H, s, OH-5), 8.22 (2H, d, J = 4 Hz, H-2″, 6″), 8.10–8.18 (2H, d, J = 2 Hz, H-3″, 5″), 7.82–7.92 (2H, m, H-2′, 6′), 7.62–7.70 (1H, m, H-4″), 7.48–7.58 (3H, m, H-3′, 4′, 5′), 6.86 (1H, s, H-8), 6.76 (1H, s, H-3), 3.92 (3H, s, OMe). FABMS: 389 [M^++1]. Compound (**2m**): yellow solid, mp 185.6 °C; IR (KBr) ν_{max}: 3415, 1744, 1632 cm^{-1}; ^1H NMR(300 MHz, $CDCl_3$): δ 12.86 (1H, s, OH-5), 8.12 (1H, d, J = 2 Hz, H-6″), 7.82–7.86 (2H, m, H-2′, 6′), 7.50–7.60 (5H, m, H-′, 4′, 5′, H-4″, 5″), 7.20–7.22 (1H, m, H-3″), 6.86 (1H, s, H-8), 6.74 (1H, s, H-3), 4.0 (3H, s, OMe). FABMS: 423 [M^++1]. Compound (**2n**): yellow solid, mp 203 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 12.82 (1H, s, OH-5), 8.46 (2H, d, J = 6 Hz, H-2″, 6″), 7.82–7.84 (2H, m, H-2′, 6′), 7.50–7.58 (3H, m, H-3′, 4′, 5′), 7.36 (2H, d, J = 6 Hz, H-3″, 5″), 6.90 (1H, s, H-8), 6.70 (1H, s, H-3), 3.96 (3H, s, OMe), 2.52 (3H, s, Ar-Me). FABMS: 429 [M^++Na]. Compound (**2o**): yellow solid, mp 180–182 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 12.84 (1H, s, OH-5), 7.82–7.88 (2H, m, H-2′, 6′), 7.48–7.58 (3H, m, H-3′, 4′, 5′), 7.42 (2H, s, H-2″, 6″), 6.84 (1H, s, H-8), 6.64 (1H, s, H-3), 3.98 (12H, 4× OMe). FABMS: 415 [M^++1]. Compound (**2p**): yellow solid, mp 169–170 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 12.40 (1H, s, OH-5), 6.60 (1H, d, J = 10 Hz, H-α), 6.62 (1H, s, H-3), 6.80 (1H, s, H-8), 7.84–7.92 (2H, m, H-2′, 6′), 7.56–7.64 (3H, m, H-3′, 4′, 5′), 7.48–7.56 (3H, m, H-2″, 6″, H-β), 7.38-7.45 (3H, m, H-3″, 4″, 5″). FABMS: 479 [M^++1].