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Synthesis and biological evaluation of novel C (7) modified chrysin analogues as antibacterial agents

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Abstract—The natural product, chrysin (5,7-dihydroxy flavone), obtained from *Oroxylum indicum*, exhibits numerous biological activities including anticancer, anti-inflammatory, and antiallergic activities. Three series of chrysin analogues were prepared, in which chrysin and heterocyclic moieties are separated by 3-carbon, 4-carbon, and 6-carbon spacers. 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 most of the derivatives displayed significant activity as compared to their parent compound (chrysin).

Polyphenolic compounds are plant products which represent common constituents of fruits and vegetables.[1] The major group of plant polyphenols is represented by flavanoids and a recent review has estimated their number as 6500 in the plant kingdom.[2] A large number of biological activities such as antioxidant, anticancer, and anti-inflammatory properties have been attributed to these compounds.[3]

Chrysin is a flavone widely distributed in plants which was reported to have many biological activities including antibacterial,[4] antioxidant,[5] anti-inflammatory,[6] antiallergic,[7] anticancer,[8] antiestrogenic,[9], and anxiolytic activities.[10] Furthermore, chrysin is also found to have tyrosinase inhibitory activity[11] and moderate aromatase inhibitory activity.[12] Chrysin can also inhibit the metabolism of the carcinogen benzo[α]pyrene by hamster embryo cells in tissue culture.[13] In fact, a number of derivatives were prepared to improve the biological activity of chrysin.[14] Such as C-isoprenylated hydrophobic derivatives are more potential P-glycoprotein modulators in tumor cells.[15] However, the efforts to date have centered mostly on the substitutions on the aromatic rings (either A or C) of the chrysin. These results prompted us to synthesize the chrysin derivatives in which chrysin and heterocyclic moieties are linked with spacers and investigate the effects to the size of the spacers and substitution patterns of the heterocyclic moieties.

In continuation of our investigations on the chemical modification of the bioactive natural flavones and their activity studies,[16] Herein, we report the synthesis of three series of analogues of chrysin[17] and their antibacterial activity studies.

Chrysin was isolated from the traditional Indian medicinal plant *Oroxylum indicum* in substantial yields. To increase the antibacterial properties of chrysin, we prepared chrysin derivatives in which chrysin ring system linked to the alkyl amines by different spacers at C-7 position with a view to enhance their lipophilicity. This was achieved in two steps, in the initial step by the alkylation of 7-hydroxy group at 7th position by the corresponding dibromo alkanes in acetone and finally coupling the secondary amines with the bromo compound. The following Scheme 1 summarizes the procedures used to prepare the three series of chrysin analogues. Compounds in series 1(**3a**–**3d**) contain 3-carbon spacer in between chrysin and heterocyclic moiety, compounds (**4a**–**4d**) separated by 4-carbon spacer, and compounds (**5a**–**5d**) contain 6-carbon spacer in between

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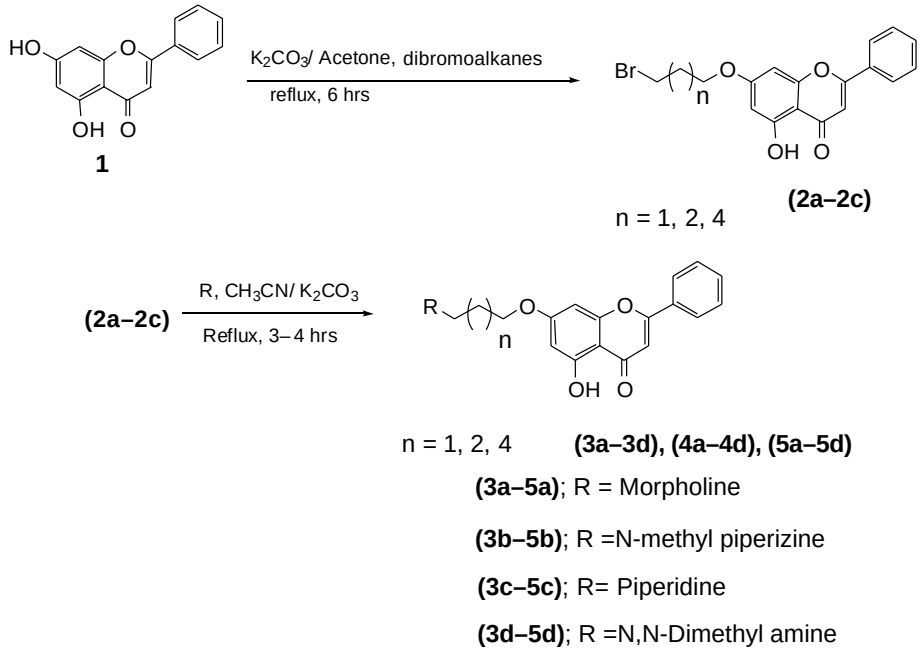

Scheme 1. Synthesis of 7-*O*-alkylamino derivatives of chrysin.

chrysin and the substituent. All the synthetic compounds were well characterized by their spectral characteristics.[18] These compounds were designated to test the importance of the substitution along with the size of the linker.

The minimum inhibitory concentrations (MIC) of 7-*O*-alkylamino derivatives of chrysin were obtained against 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 National Committee for Clinical Laboratory (NCCL) standards.[19] Standard antibacterial agents like penicillin and streptomycin were also screened under identical conditions for comparison. The minimum inhibitory concentrations are represented in Table 1. It has been observed that all the derivatives exhibited interesting biological activity however, with a degree of variation.

Compounds in series 1, which contain 3-carbon spacer, displayed good zone of inhibition for *C. violaceum* and *B. spharicus.* In this series, compounds **3a**, **3b**, **3c**, and **3d** showed good activity equal to that of streptomycin against *B. spharicus.* Similarly, compounds **3a**, **3b**, and

Table 1. Minimum inhibitory concentrations (MIC-μg/ml) of 7-*O*-alkylamino derivatives of chrysin

Compounds	Microorganisms					
	Gram positive			Gram negative		
	Bacillus subtilis	*Bacillus sphaericus*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Klebsiella aerogenes*	*Chromobacterium violaceum*
Chrysin	50	25	50	—	50	25
3a	12.5	12.5	25	—	25	12.5
3b	12.5	12.5	25	—	25	12.5
3c	25	12.5	12.5	—	25	6.25
3d	25	12.5	12.5	—	25	12.5
4a	6.25	6.25	12.5	—	12.5	6.25
4b	25	6.25	12.5	—	12.5	6.25
4c	12.5	12.5	25	—	25	6.25
4d	6.25	6.25	25	—	25	12.5
5a	25	25	25	—	25	25
5b	25	25	12.5	—	25	12.5
5c	25	12.5	25	—	25	25
5d	12.5	12.5	25	—	25	25
Streptomycin	6.25	12.5	6.25	1.562	1.562	3.125
Penicillin	1.562	3.125	1.562	6.25	6.25	12.5

Negative control DMSO, no activity.

3c displayed notable activity equal to that of penicillin and compound **3c** showed good activity against *C. violaceum*.

Similarly, compounds in series 2, which contain 4-carbon spacer, displayed good antibacterial activity against both Gram-positive and Gram-negative organisms. Compound **4a** in which heterocyclic ring (morpholine) and chrysin separated by 4-carbon chain displayed a high degree of activity against *C. violaceum* and *B. sphaericus*. Accordingly, compound **4b**, which contains 4-carbon spacer in between chrysin and piperizinyl rings, showed good activity against *C. violaceum.*

Compounds in series 3, which contain 6-carbon spacer, displayed moderate zone of inhibition against both Gram-positive and Gram-negative organisms, but displayed MIC values lower than that of the parent compound chrysin. It is important to note that all the derived compounds displayed MIC values lower than that of parent compound irrespective of the spacer. As shown in Table 1, none of the compounds exhibited any activity against *P. aeruginosa* even at the concentration of 200 μg/ml. The compounds were also inactive against the tested antifungal strains.

In conclusion, a series of chrysin derivatives were prepared containing 3, 4, and 6 carbon spacers, in between heterocyclic ring and chrysin, and were evaluated for antibacterial activity. Most of the compounds showed a moderate degree of antibacterial activity. Among them compounds in series 2, which contain 4-carbon spacer in between chrysin and heterocyclic ring, displayed good deal of activity. With this set of analogues, we are now in a position to investigate the multiple biological activities reported for chrysin.

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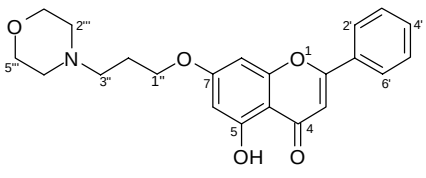
17. General procedure for the preparation of 7-*O*-alkylamino derivatives of chrysin: (i) General procedure for the preparation of 7-*O*-alkyl derivatives of chrysin (**2a**–**2c**): To a mixture of chrysin 1 (1 g, 3.93 mmol) and anhydrous potassium carbonate (0.81 g, 5.8 mmol) in 20 ml acetone, corresponding dibromoalkane (1,3-dibromo propane for **2a**, 1,4-dibromo butane for **2b**, and 1,6-dibromo hexane for **2c** were added. The mixture was refluxed under nitrogen atmosphere for 3–4 h. After completion of the reaction, potassium carbonate was filtered and washed with excess acetone (2× 50 ml). The combined acetone layers were concentrated under vacuum. The residue was purified by column chromatography on silica gel (60–120 mesh) to yield 7-*O*-bromoalkyl chrysin (**2a**, **2b**, and **2c**) in pure form. (ii) General procedure for the preparation of 7-*O*-alkyl derivatives of chrysin: To a mixture of bromoalkyl chrysin (**2a**, **2b**, and **2c**) and anhydrous potassium carbonate (2.41 g, 17.2 mmol) in 20 ml acetonitrile, the corresponding amine was added. The mixture was refluxed under nitrogen atmosphere for 3–4 h. After completion of the reaction, the reaction mixture was brought to room temperature and 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*-alkylamino derivatives of chrysin (**3a**–**3d**, **4a**–**4d**, and **5a**–**5d**) in very good yields (60–80%).
18. Physical and spectral characteristics of compound (**3a**):



Pale yellow solid, mp 138 °C, ^{1}H NMR (400 MHz, $CDCl_3$): δ 12.60 (1H, s, OH-5), 7.86–7.90 (2H, m, H-2′, 6′), 7.50–7.62 (3H, m, H-3′, 4′, 5′), 6.64 (1H, s, H-8), 6.46

(1H, s, H-3), 6.38 (1H, s, H-6), 4.18 (2H, t, H-1″), 3.82 (4H, t, H-3‴, 5‴), 2.40–2.60 (6H, m, H-2‴, 6‴, H-3″), 1.9-2.10 (2H, m, H-2″). FABMS: 382 (M^++1). Compound (3b): yellow solid, mp 128–130 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.70 (1H, s, OH-5), 7.84–7.86 (2H, m, H-2′, 6′), 7.46–7.58 (3H, m, H-3′, 4′, 5′), 6.64(1H, s, H-8), 6.52(1H, s, H-3), 6.18 (1H, s, H-6), 4.12 (2H, t, H-1″), 2.40–2.60 (10H, m, H-3″, 5‴, H-3″, and H-2‴, 6‴), 2.30 (3H, s, Me), 1.90–2.10 (2H, m, H-2″). FABMS: 395 (M^++1). Compound (4c): white solid, mp 128 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.50 (1H, s, OH-5), 7.82–7.86 (2H, m, H-2′, 6′), 7.48–7.56 (3H, m, H-3′, 4′, 5′), 6.70 (1H, s, H-8), 6.62 (1H, s, H-3), 6.42 (1H, s, H-6), 4.18 (2H, t, H-1″), 2.36–2.58 (6H, m, H-2‴, 6‴, H-4″), 1.96–2.10 (2H, m, H-3″), 1.54-1.62 (6H, m, H-2″, H-3‴, 5‴), 1.40–1.48 (2H, m, H-4‴). FABMS: 394 (M^++1). Compound (5d): pale yellow solid, mp 85 °C, ^{1}H NMR (400 MHz, $CDCl_3$)δ 12.70 (1H, s, OH-5), 7.84–7.88 (2H, m, H-2′, 6′), 7.52–7.58 (3H, m, H-3′, 4′, 5′), 6.70 (1H, s, H-8), 6.48 (1H, s, H-3), 6.38 (1H, s, H-6), 4.12 (2H, t, H-1″), 2.22–2.38 (4H, m, H-2″, 6″), 2.0 (6H, s, 2× Me), 1.78–1.82 (2H, m, H-5″), 1.44–1.58 (2H, m, H-3″), 1.38–1.42 (2H, m, H-4″). FABMS : 382 (M^++1).

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