Synthesis, Characterization and Biological Activity of Flavonoid Derivatives with Amine

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Abstract: Flavonoids have been in focus due to their nutraceutical and therapeutical significance, as they exhibit divergent biological activities. Here we have reported the synthesis of flavonoid derivatives and their characterization by elemental analysis FTIR 8201using KBr pallet method, ^{1}HNMR techniques. Furthermore, antibacterial activity of the compounds were examined. Among all the tested flavonoids derivatives compounds exhibited varying levels of antibacterial activity against all the Gram positive as well as Gram negative bacteria. . It has also been observed that Gram-positive bacteria were more susceptible towards the newly synthesized series of compounds as compared to Gram –negative bacteria.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds ubiquitous in many plants, in which they occur as the free forms, glycosides, as well as methylated derivatives. They exhibit divergent biological activities such as antioxidant, anti-inflammatory, cardioprotective, antibacterial, antitumor, hepatoprotective and antiviral activities[2-8]. Recently, there has been an enormous increase in the number of studies on flavonoids as potential antimicrobial agents[11-12]. The work of Hertog and co-workers showed the inverse correlation between flavonoids intake and coronary heart disease mortality[7].

Beta-blockers[8] have gained a remarkable place worldwide to treat several cardiovascular disorders such as hypertension, angina pectoris, cardiac arrhythmia, and open angle glaucoma[9,10]. A number of methods are available for preparing flavones, chromones, and their analogs, including the Allan Robinson synthesis, Baker-Venkataraman, and Algar-Flynn-Oyamada method[11-12]. Direct modification on the natural flavonoid scaffold is limited mostly to the phenolic o-alkylation and acylation[21]. Here we report the synthesis of flavonoid derivatives based on T_3p mechanism.

The synthesized compounds were analysed for their antibacterial activity by using Kirby-Bauer disk-diffusion method on Mueller-Hinton agar according to the guidelines of the Clinical Laboratory Standards Institute, 2007, USA[15]

EXPERIMENTAL

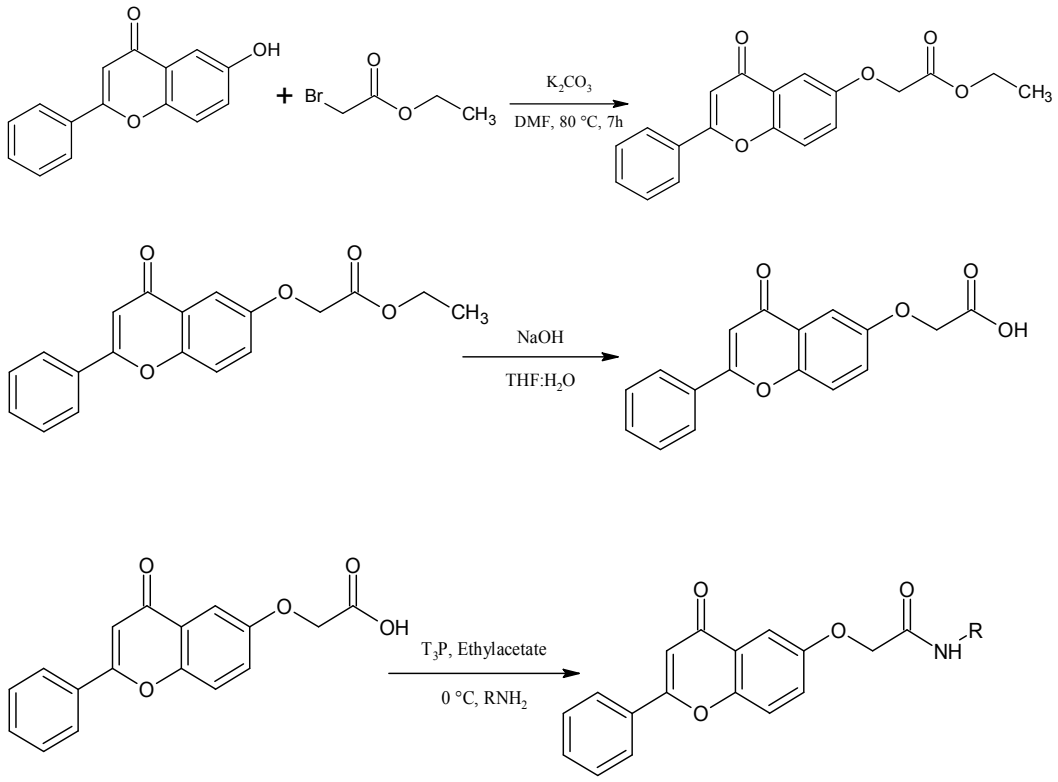
Material and Method

The chemical used for the synthesis of products were purchased from sigma-aldrich and used without further purifations. Melting points were recorded by open capillary method and are uncorrected. Infrared spectra were recorded on Shimadzu FT IR-8201 using KBr pallet method. Spectra were calibrated against the polystyrene absorption at 1610 cm-1. Mass spectra were recorded on Shimadzu GC/MSQP 2000 spectrometer operating using direct injection probe technique. ^{1}H NMR spectra were recorded on Bruker Avance 400 spectrometer by making a solution of sample in DMSO d6 and $CDCl_3$ solvents using tetramethylsilane (TMS) as the internal standard unless otherwise mentioned and are given in the δ scale. Analytical thin layer chromatography (TLC) was performed on Merck precoated silica gel-G F254aluminium plated. Visualization of the spots on TLC plates was achieved either

by exposure to iodine vapor or UV light. All evaporation of solvents was carried out under reduced pressure on Rota vapour. % yield reported are isolated yields of material judged homogeneous by TLC and before recrystallization. Elemental analysis was carried out on Elementar III Vario EL Carlo Erba 1108.

Synthesis

Compounds were synthesized following the procedure as shown in following schem 1.To a solution 6-hydroxyflavone (5g, 21mmol), ethyl-2-bromo acetate (3.9g, 24mmol) and potassium carbonate (8.6g, 63mmol) were added in DMF at 80 °C temperature and the reaction took place for 7h to form an ester. To a separating funnel, take ethyl acetate and water in 1:1 ratio. Add product (3b) and sodium hydroxide to the separating funnel and shake vigoursley for 2-3 times give acid. To a solution of acid (1 equiv) and aromatic amine (1 equiv) in triethylamine (3eq) at 0°C temperature and the reaction mixture was stirred for 10 mins. After that add propylphosphonic anhydride (0.5 ml in 50% ethylacetate) drop by drop with constant stirring, then reaction should take place for 7h. After the completion of reaction in each step as monitored by the TLC, it was then extracted with ethyl acetate and washed with water, brine solution and dried over anhydrous Na_2SO_4. The combined organic layer was concentrated under reduced pressure to give the colourless solid as the product. Similarly, we have carried this reaction for different kind of amines to give good result.



Scheme: 1

All the derivatives were derived from their parent compound. The chemical structures, spectral and elemental analysis data are for individual compounds synthesized are mentioned as follows:

FA1: N-(4-fluorophenyl)-2-[(4-oxo-2-phenyl-4*H*-chromen-6-yl)oxy]acetamide. Yield: 63% (5.14g), Creamish powder; **IR:** (KBr, cm^{-1}) 3,320, 1,684, 1,625; **^{1}H NMR ($CDCl_3$);** δ= 9.22(br. s, 1H), 8.00 (s, 1H, Ar-H), 7.95-7.93 (d, J=8, 2H, Ar-H), 7.69-7.44 (m, 5h, Ar-H), 7.03-7.02 (d, 4H, J=4, Ar-H), 6.81 (s, 1H), 4.75 (s, 2H); **Elemental analysis:** Calculated for **$C_{23}H_{15}F_2NO_4$:** C=67.81, H=3.71, F=9.33, N=3.44 Found: C=67.70, H=3.73, F=9.39, N=3.82

FA2: N-(3-fluorophenyl)-2-[(4-oxo-2-phenyl-4*H*-chromen-6-yl)oxy]acetamide Yield: 61% (4.97g); light yellow powder **IR:** (KBr, cm^{-1}) 3,751, 1,614 **^{1}H NMR ($CDCl_3$):** δ=8.33(s, 1H), 7.94-7.92 (m, 2H, Ar-H), 7.73-7.72 (d, J=4, 1H, Ar-H), 7.62 (s, 1H), 7.62-7.54 (m, 4H, Ar-H), 7.42-7.43 (d, J=4, 1H, Ar-H), 7.31 (s, 1H), 7.27-7.26

(d, J=4, 1H), 6.89-6.85 (m, 1H), 6.84 (s, 1H), 4.72 (s, 2H); **Elemental analysis:** Calculated for $C_{23}H_{15}F_2NO_4$ C=67.81, H=3.71, F=9.33, N=3.44 Found: C=67.70, H=3.73, F=9.39, N=3.82

FA3: N-benzyl-2-[(4-oxo-2-phenyl-4*H*-chromen-6-yl)oxy]acetamide. Yield: 66% (5.33g); light yellow powder ; **IR:** (KBr, cm^{-1}) 3,339, 3,057, 1,638; **^{1}H NMR (DMSO)**: δ= 8.79-8.77 (t, 2H), 8.07-8.11 (d, J=16, 1H), 7.79-7.22 (m, 10H, Ar-H), 7.03 (s, 1H), 6.98 (s, 1H), 4.71(s, 2H), 4.37 (s, 2H) ;**Elemental analysis:** Calculated for **$C_{23}H_{15}F_2NO_4$**: C=67.81, H=3.71, F=9.33, N=3.44; Found: C=67.70, H=3.73, F=9.39, N=3.82

Antibacterial Activity

All the synthesized compounds (FA1) were tested for their antibacterial activity in vitro with two gram-positive Staphylococcus aureus MTCC3160 and Bacillus cereus MTCC 430 two gram-negative bacteria E.Coli MTCC 442 and Vibrio cholera MTCC 3904. Amikacin and Gentamicin were used as standard control agents. The antibacterial activity (zone of growth inhibition) of FA series of compound were measured by Kirby-Bauer disk-diffusion method on Mueller Hinton agar according to the guideline of Clinical Laboratory Standards Institute, 2007, USA (CLSI. 2007)

RESULT AND DISCUSSION

Accordingly, we started the synthesis with commercially available 6-hydroxyflavone and ethyl-2-bromo acetate. There were treated in DMF in the presence of K_2CO_3 at 80 °C temperature for 7h to form an ester. By doing base hydrolysis, we convert ester into acid. Then by using different kind of commercially available aromatic amines, we formed different kind of amides as the product.

Antibacterial Activity

From the data table, it is clear that the substitution in aryl ring exerted significant influence on the antibacterial activity of the synthesized flavonoid derivatives. The compound (substituted by amine) FA1 was found to be more active than the other compound of the series. This compound (FA1) showed better activity profile (zone of growth inhibition 63.98 mm) against S. aureus MTCC 3160, B. cereus MTCC 430, E.coli MTCC 442 and V. cholera MTCC 3904 as compared to standard drugs Gentamycin, Amikacine. Whereas in case of S.aureusMTCC 3160, FA2 (35.23 mm) nearly same to Gentamycin (34.17 mm). In case of S. aureus MTCC 3160 and B. cereus MTCC 430, a number of compounds (FA1, FA2) displayed inhibitory activity (63.97, 37.13 mm) better than that of standard drug Gentamycin (32.0-34.0mm) and amikacin (27.0-29.0 mm)

In case of E. coli MTCC 442 and V. cholera MTCC 3904, a number of compounds (FA1, FA2) displayed inhibitory activity better than that of standard drug Gentamycin (29.01-30.11 mm) and amikacin.

Table: Antibacterial screening data for Flavonoid derivatives

Compound	Zone of Inhibition (mm)			
	Gram-Positive		Gram - Negative	
	SA[b]	BC[c]	EC[d]	VC[e]
FA1	63.29±0.56	63.97±0.06	63.98±0.30	63.14±0.06
FA2	35.23±0.11	37.13±0.11	34.12±0.9	32.37±0.20
FA3	20.83±0.21	21.11±0.21	19.45±0.12	19.21±0.7
Gentamicin[a]	34.17±0.28	32.18±0.98	30.11±0.12	29.01±0.04
Amikacin[a]	29.11±0.18	27.98±0.91	25.12±0.26	26.23±0.19

[a] Antibacterial activity of the synthesized compounds was compared with the standard antibacterial drugs Gentamicin and Amikacin. [b]SA: Staphylococcus aureus MTCC 3160; [C] BA: Bacillus cereus MTCC 430; [d]EC: Escherichia coli MTCC 442; [e]VC: Vibrio cholerae MTCC 3904

CONCLUSION

A number of flavones derivatives were synthesized as potential antibacterial agents from the method were used in this study result concluded that compounds FA1, FA2 shown good antibacterial activity against all the tested bacterial strains. The compound FA1 has shown maximum inhibitory activity among the all synthesized derivatives compared to standard antibacterial drugs Gentamycin and Amikacin against all of the Gram-positive and Gram-negative bacteria. Here it was also clear that substituents on the flavones skeleton are responsible for enhance their antibacterial activity.

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