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Antibacterial activities of Substituted 4-Iminoflavanes

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Abstract. A number of 4-iminoflavanes derivatives have been synthesized and evaluated for their antibacterial activities. Synthesis of a 4-iminoflavanes were carried out by condensation of flavanone with a primary amine without a catalysis. The chemical structures of the newly synthesized compounds were verified on the basis of spectral and elemental methods of analysis. Most of the synthesized derivatives were found to be active against *Escherichia coli*, *Bacillus subtilis*, *Listeria monocytogenesand Staphylococcus aureus*. Activity of 4-iminoflavanes was found to be higher than the initial materials flavanone. Investigated compounds having substituents like Cl, OMe and OH at the starting amine exhibited enhanced activity and the presence of electronegative groups in the studied compounds showed a direct relationship to the antibacterial activity.

Keywords: *Synthesis, Flavanone, 4 -Iminoflavan, Antibacterial*

1. Introduction

Flavonoid framework is medicinally important structural organization present in many bioactive molecules showing various activities like antiviral, antibacterial, antiprotozoal, oestrogenic, anti-inflammatory, mutagenic, antimutagenic and antineoplastic activities, and is also capable of inhibiting many types of enzymes.[1-4] This class of compounds has received much attention because of their pharmacological activities.[5-8]

The flavanones are a class of flavonoid (naturally occurring polyphenolic compounds) which are extensively distributed in vascular plants.[9] These are minor ingredients of the human diet.[10-11] Many studieshave reported that flavanone contains many pharmacological activities [12] like antioxidant effect, inhibition of HIV-1 proteinase, and anticancer [13], vasodilator, antiviral and antiallergenic[14], in addition to antimicrobial [15,16], anti-inflammatory[17] activities. The curiosity in the biological properties of flavanones has resulted extreme synthetic efforts toward the synthesis of different flavanones.[18] Moreover, amino group substituted flavone derivatives exhibit strong antitumour activity in breast cancer cells [19].

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Although, there is some work has been carried out on nitrogen containing flavones; iminoflavones have been reported to show considerable antimicrobial activities and antimalarial activities.[20,21]

It is well known that halogenated compounds are also strongly biologically active, [22,23] but to our knowledge no natural flavonoids have been reported with halogens as substituents. Additionally, we were also interested to check the effect of nitrogen atom at 4-position of flavanes on their biological activity. Therefore, in order to search for new compounds that can be used for the treatment of bacterial infections, this paper describes the synthesis of variably substituted 4-iminoflavanes and in vitro evaluation of the new and known flavanone and 4-iminoflavanes against a number of bacteria.

2. Chemistry

In view of the importance of bioactive flavanone and 4-iminoflavanes in recent years as describedabove, it was thought to replace the oxygen atom of theketone group of flavanone with asubstituted amino group to check their effect as well asthe effect of various substituents on the biological activities of flavanone.

The flavanone derivatives (7 compound) were synthesized using flavanone and aniline had been purchased from Sigma-aldrich. Tertbutylamine, *p*-chloroaniline, *m*-chloroaniline, *p*-anisidine, 1-naphthylamine and 4-aminophenol have been purchased from Fluka (Buchs, Switzerland),[24] as shown in Scheme 1.

Flavanone 1 (5 mMol) and aromatic amine (5 mMol) were refluxed in methanol (5 ml), after 3 days, the main liquid was heated to the boiling point, and an equal volume of hot water was added. Crystals were obtained upon cooling the mixture. The 4-iminoflavans were recrystallized from ethanol.[24]

A simple and one pot process for the synthesis of 4-iminoflavanes in the presence of methanol without catalysis,Under these conditions several products were found with very good yields (70–98%).

All of the compounds 2-8were fully characterized through spectroscopic and other analytical techniques, details of which are given separately in Section

3. Biological activity

Synthesized compounds were tested for their antibacterial activity by adopting agar well diffusion [23, 25] method. The following bacterial cultures were used

(i)Escherichia coli (ii)Staphylococcusaureus (ii)Pseudomonas aeruginosa (iv)Listeria monocytogenes (v)Bacillus subtilis.

The disc-diffusion method was used as a screening test for antibacterial activity. Filter paper discs impregnated with sample solutions were placed on Mueller Hinton agar plates, which have been inoculated with test organisms according to the standard protocol.The flavanone and all compounds, dissolved in dimethyl sulphoxide (DMSO), Test samples of 100µL (1 mg of test compounds) were poured into each well and the plates were incubated at 37°C for 24 h.The diameters of the inhibition zones were measured after 24 h. Filter paper discs containing DMSO without any test compound served as control and no inhibition was observed. The results of the antibacterial screening were compared with the standard antibacterial drug Ampicillin. The results of these experiments are summarized in Table 1.

4. Results and discussion

At the present work, we have employed flavanone as a naturally occurring skeleton for the synthesis of 4-iminoflavanes then screen them against representative panel of Gram-positive and Gram –

negative bacteria. The in vitro bioactivities of the synthesized compounds have shown encouraging results against various classes of bacteria as mentioned above.

The results of the screening test for the eight different flavanones derivatives against five bacteria using the disc-diffusion method are shown inTable1.

Antibacterial activity analysis of 4-iminoflavane **2-8** showed moderate activity against *Staphi* and *L.monocytogenes*but exhibited very low activity against *E.coli*and *B.cereus*. However, flavanone witch are the starting materials significant of synthesized, did not show significant activity against tree bacteria. Only one product **3**showed activity against *E.coli*, while all compounds **2-8** showed height antibacterial activity against *L.monocytogenes*. None of the eight flavanones derivatives showed activity against *P.aeruginosa*. Among the products tested, only tree **2, 3, 5**exhibitedactivity against the gram-positive bacteria *Staphi*.

The product **8**showed serious antibacterial activity with value ranging between (15.6-23.3) against tree different gram-positive bacterial.

In most cases, the presence or introduction of various functional groups in a compound does not allow to accurately explain the kind and intensity of its biological activity.[23]It is clear that antibacterial activity of flavanones derivatives increases accordingly on replacing oxygen atom with nitrogen atom at 4-position.

All 4-iminoflavanes **2-8** have more antibacterial activity as compared to flavanones. Furthermore, when 4-iminoflavanes **4, 5, 7** have methoxy, hydroxyl group and Cl atom in the amine-ring at position 4'', the linear growth inhibition against *L.monocytogenes* and *Staphi.* However, 4-iminoflavanes **4** are much more active against bacteria than **6.**Thus increase in activity is probably due to the resonance effect of aliphatic group that is more prominent than aromatic amine with give more activity against *L.monocytogenes*, Moreover, product **8** showed a very significant inhibition against 23.3.

The results show that electron-donating and electronegative groups are responsible for the antibacterial activity of flavanone and 4-iminoflavanes provided these groups are at 4''-position in amine-ring rather than any other position.

The N-(2-naphtyl) 4-iminoflavan (**8)** showed linear growth inhibition of 16.3 and 15,6 against *Staphi*and *B.cereus* respectively, whereas standard drug ampicillin showed 28 and 25, respectively.

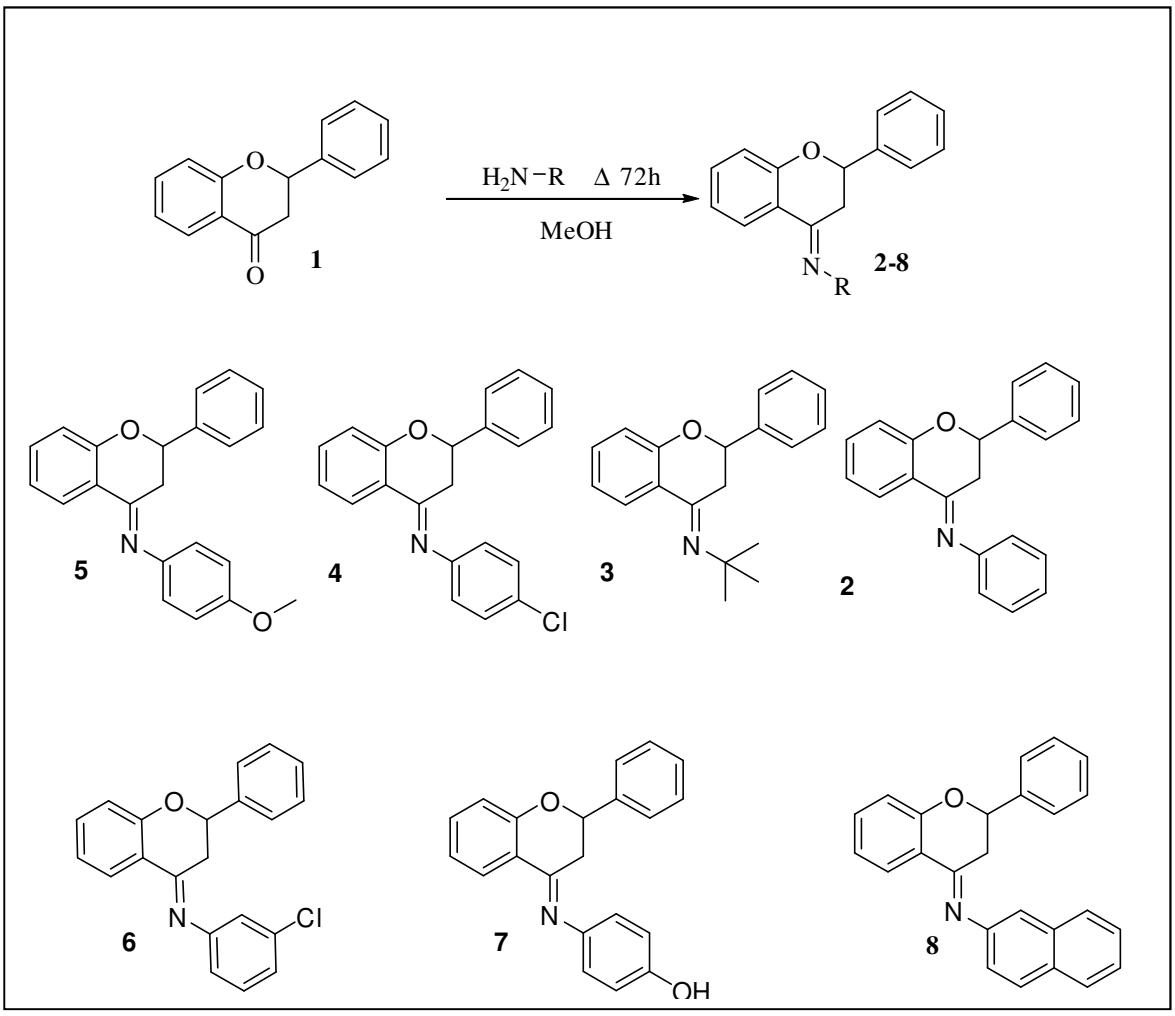
Therefore, it is concluded that a 2-naphtyl moiety in compound **8** is responsible for its enhanced antibacterial activity, while flavanone and the other 4-iminoflavanes remain inactive or less active.

Table 1. Antibacterial activities of flavanone and 4-imioflavanes compounds, relative to the standard drug Ampicillin

Compound	Bacteria				
	E.coli	*Staphi*	*P.aeruginosa*	*L.monocytogenes*	*B.cereus*
1	-	8.0	-	-	7. 3
2	-	-	-	7.3	-
3	7.0	-	-	15.0	-

4	-	7.0	-	14.0	-
5	-	-	-	7.3	7.3
6	-	7.0	-	7.3	8.3
7	-	18.0	-	20.0	18.0
8	-	16.3	-	23.3	15.6
Ampicillin	25	25	-	-	28

Inhibition zones are given in millimeter



Scheme 1:

Synthesis of 4-iminoflavanes compounds 2-8

5. Conclusion

Different compounds of flavanones derivatives have been synthesized and evaluated for their biological activities. The chemical structures of the newly synthesized compounds were verified on the basis of spectral and elemental methods of analysis. Investigation, of antibacterial activity of these compounds was determined by measuring inhibition zones for Gram-positive and Gram-negative bacteria, among the various synthesized compounds. It can further be concluded that the percentage inhibition increases as the electronegativity of the halogen atom on amine-ring increases.

6. Experimental

Chemicals used in the present study were purchased from Merck and Fluka (Germany). All solvents and reagents were obtained from Chemminova (Harboøre, Denmark) and riedel- deHaën (Sleeze, germany). Rf values were calculated by using precoated silica gel aluminium packed plates Kiesel gel 60F254Merck (Germany).Melting points were determined in open capillaries using B-540 Büchi melting point apparatus and are uncorrected. FTIR spectra, spectrophotometer usingKBr discs. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AC (400 MHz) in MeOD-d4 solution using TMS as internal standard.Purity of each compound was monitored by TLC.

The procedure for the synthesis of a representative 4-iminoflavanes **2-8** is given below. All of the compounds were synthesized according to the procedures [24] as outlined in Scheme 1. Spectroscopic as well as other physical data of the known products are given in the cited literature, while those of the new one **3, 4, 5** and **7** are given below.

N-(Tertbutyl) 4-iminoflavan (**3**): green powder, yield 83 %, F 72–74 °C, UVmax (DMF, nm): 265 (band I); 320 (band II), IR (KBr, cm−1): 3,084 (=CH arom), 2,860–2,960 (CH3), 2,919 (CH2) 1,606 (C=N), 1,547–1,498 (C=C), 1,153, 1,033 (C–O).

^{1}H NMR(400 MHz, CDCl3, ppm, *J*3, *J*2Hz): 7.87 (d, 8.02, H-5), 7.83 (m, H-7), 6.96 (d, H-6), 7.57 (d, H-2′, H-6′), 7.53 (m, H-4′), 7.31 (d, H-3′, H-5′), 6.81 (d, H-8), 5.40 (1H, dd, 3.2,2.8, H-2), 2.99 (1H, dd, 2.8, 13.6 Hz, H3b), 2.77 (1H, dd, 3.2, 13.6 Hz, H-3a), 1.30(m, 3CH3).

^{13}C NMR(400 MHz, CDCl3, ppm): 165.7 (C-4), 163.4 (C-5), 156.7 (C-7), 131.7 (C-6) 131.5 (C-8), 143.0 (C-9), 127.2 (C-10), 126.6 (C-1′), 138.7 (C-2′), 138.7 (C-6′), 128.5 (C-3′), 128.5 (C-5′) 129.6 (C-4′), 77.4 (C-2), 52.1 (C-1”), 44.6 (C-3), 30.1(3CH3).

N-(p-Chloro-Phenyl) 4-iminoflavan (**4**): dark brown powder, yield 85 %, F 72–73 °C, UVmax (DMF, nm): 265 (band I); 320 (band II), IR (KBr, cm−1): 3,084 (=CH arom), 2,860(CH2), 1,606 (C=N), 1,547–1,498 (C=C), 1,153, 1,033 (C–O).

^{1}H NMR(400 MHz, CDCl3, ppm):7.85 (d, H-5), 7.80 (dd, H-7), 7.58 (dd, 6.1, 2.3, H-2′, H-6′), 7.54 (dd, 6.2, 6.3, H-4′), 7.33 (dd, 6.1, 6.2, H-3′, H-5′), 7.01 (dd, 5.9, 6.1 H-5″), 6.69 (d, 6.1, H-2″), 6.55 (d, 2.3, H-3″), 6.43 (dd, 6.2, 2.3H-6″), 6.96 (dd, 6.1, 6.4, H-6), 6.81 (d, 6.1, H-8), 5.38 (1H, dd, *J* = 2.8, 3.2, H-2), 2.96 (1H, dd, *J* = 3.2, 13.6 Hz, H3b), 2.76 (1H, dd, *J* = 2.8, 13.6 Hz, H-3a).

^{13}C NMR(400 MHz, CDCl3, ppm): 164.6 (C-4), 163.6 (C-5), 156.3 (C-7), 132.01 (C6), 131.7 (C- 8), 143.4 (C-9), 127.6 (C-10), 126.4 (C-1′), 138.5 (C-2′), 138.5 (C-6′), 128.4 (C-3′), 128.4 (C-5′), 129.3 (C-4′), 77.8 (C-2), 44.4 (C-3), 148.17 (C-1″), 102.1 (C-4″), 159.85 (C-3″), 107.6 (C-2″), 130.4 (C-5″), 104.01 (C-6″).

N-(p-Methoxy phenyl) 4-iminoflavan (**5**): yellow powder, yield 98 % F 54-50 °C, UV max(DMF, nm): 270 (band I); 320 (band II), IR(KBr, cm−1): 3,067 (=CH ar), 2,853, 2,927 (CH2, CH3), 1,609 (C=N), 15 53, 1,491 (C=C), 1,178, 1,077 (C–O), 697 (CH ar).

^{1}H NMR(400 MHz, CDCl3, ppm):7.88 (d, H-5), 7.84 (m, H-7), 7.56 (d, H-2′, H-6′), 7.53 (m, H-4′), 7.33 (d, H-3′, H-5′), 6.21 (d, H-2″), 6.35 (d, H-3″), 7.05 (d, H-5″), 6.24d (d, H-6″), 6.96 (t, H-6), 6.83 (d, H-8), 5.33 (1H, dd, *J* = 2.8, 3.2, H-2), 2.91 (1H, dd, *J* = 3.2, 13.6 Hz, H3b), 2.77 (1H, dd, *J* = 2.8, 13.6 Hz, H-3a), 3.71 (3H, s, 4″–O–CH3).

^{13}C NMR(400 MHz, CDCl3, ppm):164.8 (C-4), 163.5 (C-5), 156.1 (C-7), 131.7 (C-6) 131.7 (C- 8), 143.7 (C-9),127.6 (C-10), 126.7 (C-1′), 138.9 (C-2′), 138.9 (C-6′), 128.4 (C-3′), 128.4 (C-5′) 129.6 (C-4′), 77.3 (C-2), 44.6 (C-3), 148.1 (C-1″), 101.0 (C-2″), 160.7 (C-3″), 107.9 (C-4″),130.0 (C-5″), 103.8 (C-6″).

N-(Hydroxyl-Phenyl) 4-iminoflavan (**7**): brown powder, yield 70 %,

F 138-139 °C, UV max(DMF, nm): 260 (band I); 314 (band II), IR(KBr, cm−1): 3,412(OH), 3,078 (=CH arom), 1,607 (C =N), 1,547–1,498 (C=C), 1,142 (C–O).

^{1}H NMR(400 MHz, CDCl3, ppm) 12.77(s, 4”-OH), 7.87(d, H-6), 7.64(m, H-6), 7.40(m, H2’, H6’), 7.19(d, H2’’, H6’’), 7.00(d, H8), 6.98(m, H-3”, H-6”), 5.41(1H, dd, *J*=2.5, 13.2Hz, H-2), 3.05(1H, dd, *J*=3.6, 2.8, H-3b), 2.85(1H, dd,*J*=3.6, 2.8, H-3a).

^{13}C NMR(400 MHz, CDCl3, ppm): 165.7 (C-4), 163.4 (C-4’’), 156.7 (C-7), 131.7 (C-6) 131.5 (C-8), 143.01 (C-9), 127.2 (C-10), 126.6 (C-1′), 138.7 (C-2′), 138.7 (C-6′), 128.5 (C-3′), 128.5 (C-5′) 129.6 (C-4′), 77.4 (C-2), 44.6 (C-3), 146.5 (C-1″), 115.0 (C-2″), 115.0 (C-6″), 129.2 (C-3″), 129.2 (C-5″).

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