Synthesis, Biological Evaluation and *In Silico* Metabolic and Toxicity Prediction of Some Flavanone Derivatives

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Flavones chemically are anthoxanthins, occur either in the free state or as glycosides associated with tannins (flavanoids). Flavanoids (derivatives of flavone) possess various pharmacological activities and due to its xanthine-oxidase enzyme inhibitory effect it also has superoxide-scavenging activities. A series of 2-phenyl-2,3-dihydrochromon-4-one derivatives (flavanone derivatives) were synthesized from chalcones by cyclization method and their activities were evaluated against some gram positive and gram-negative bacteria. IR, NMR and CHN analysis confirmed the structure of the synthesized compounds. The results of the antibacterial studies shows that compounds 2b, 2e, 2f and 2h possess activity against many bacterial strains. Among that the compound (2h) has remarkable activity against all strains *viz.* 25 μg/ml inhibitory concentration against *S. aureus*, *S. sonnei*, *E. coli*, *S. typhimurium* and *V. cholerae*. Compound 2f possess minimum inhibitory concentration of 200 μg/ml against *E. coli* and *S. typhimurium* and 25 μg/ml against *S. sonnei*, *S. dysenteriae* and *V. cholerae*. *In silico* metabolic and toxicity study of the synthesized compounds were performed and the predicted result showed that the compound having hydroxyl functional group undergo sulfate and *O*-glucuronide conjugation reaction and methoxy derivatives undergo demethylation reaction. The biologically active compounds are free of toxicity in oncogene, teratogen, sensitivity and immunotoxicity.

Key words flavanone; chalcone; bacteria; metabolism; toxicity

Flavones, which are also known as anthoxanthins, are widely distributed as yellow coloured plant pigment (flavus, latin meaning yellow) and occur either in the free state or as glycosides associated with tannins. The derivatives of phenol present as glycosidal form and chemically they are derivatives of phenyl benzo-Y-pyrone (chromone) ring.[1,2] The basic flavanoid structure is a flavone nucleus, which consists of 15 carbon atom, arranged in 2 rings labeled as A, B, C. In nature, they are available as flavone, flavonol, flavanone, isoflavone, chalcone and their derivatives. In acidic medium, it forms oxonium salts, which are very unstable in water and are hydrolyzed back to freebase.[3—5]

Natural and synthetic flavanoids and flavanones have attracted considerable attention because of their interesting biological activity, including, antioxidant, antifungal, antibacterial, anti-inflammatory, antiasthmatic, antihypertensive, antiviral, estrogenic and diuretic activity.[6—11] Flavanoids inhibit xanthine-oxidase enzyme and have superoxide-scavenging activities. Therefore they could be a promising remedy for human gout and ischemia by decreasing both uric acid and superoxide concentration in human tissues.[12]

There are number of methods available for the synthesis of flavanoids and flavanones like Kostanecki method,[13,14] Allan–Robinson method,[15] Mahal–Venkataraman method,[16] the Chalcone method, the Wheeler method and others. Almost all the methods of synthesis of flavanones and flavanoids required more amount of heating during the reaction and the yields of the products obtained was less (50—60% yield).[17—19]

The metabolic fate and toxicity of a molecule is highly dependent upon its structural elements. Exogenous chemicals entering a living system can undergo a number of chemical modifications by a wide array of enzymes, which use these chemicals as substrates. Rarely does a compound simply produce a single metabolite, in general, complex metabolic pattern of competitive and sequential reactions occur.

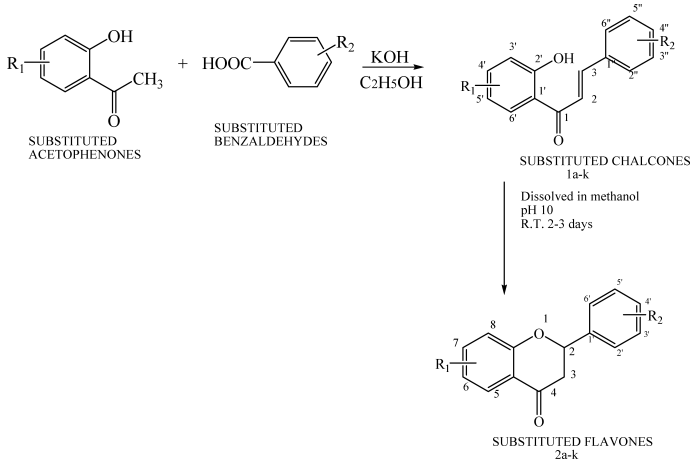

Chart 1. Synthesis of Substituted Flavones

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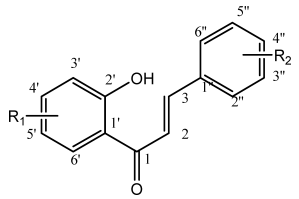
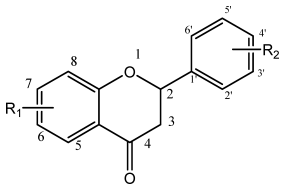
Table 1. Structure and Physicochemical Properties of Chalcone Derivatives



Comp. No	Substituents		Mol. formula (mol. wt.)	mp (°C)	% Yield	*Rf* value
	R_1	R_2				
1a	H	H	$C_{15}H_{12}O_2$ (224)	89	85	0.80
1b	H	4″-OH	$C_{15}H_{12}O_3$ (240)	164	85	0.84
1c	H	4″-OCH_3	$C_{16}H_{14}O_3$ (254)	135	85	0.82
1d	H	4″-$N(CH_3)_2$	$C_{17}H_{17}NO_2$ (267)	155	85	0.78
1e	4′-OH	6″-OH	$C_{15}H_{12}O_4$ (258)	216	90	0.85
1f	4′-OH	4″-$N(CH_3)_2$	$C_{17}H_{17}NO_3$ (283)	174	90	0.81
1g	H	6″-OH	$C_{15}H_{12}O_3$ (240)	166	85	0.86
1h	5′-OH	6″-OH	$C_{15}H_{12}O_4$ (254)	218	85	0.84
1i	5′-OH	4″-OH	$C_{15}H_{12}O_4$ (254)	208	85	0.87
1j	5′-OH	4″-OCH_3	$C_{16}H_{14}O_4$ (270)	152	85	0.79
1k	5′-OH	4″-$N(CH_3)_2$	$C_{17}H_{17}NO_3$ (283)	170	85	0.81

Table 2. Structure and Physicochemical Properties of Flavanoids Compounds



Compound	Substituents		Molecular formula (mol. weight)	mp (°C)	% Yield	*Rf* value	λ_{max}/nm
	R_1	R_2					
2a	H	H	$C_{15}H_{12}O_2$ (224)	76	75	0.82	245, 286
2b	H	4′-OH	$C_{15}H_{12}O_3$ (240)	225	75	0.84	248, 288
2c	H	4′-OCH_3	$C_{16}H_{14}O_3$ (254)	136	75	0.81	242, 282
2d	H	4′-$N(CH_3)_2$	$C_{17}H_{17}NO_2$ (267)	124	75	0.80	244, 285
2e	7-OH	6′-OH	$C_{15}H_{12}O_4$ (258)	204	80	0.82	290, 328
2f	7-OH	4′-$N(CH_3)_2$	$C_{17}H_{17}NO_3$ (283)	142	80	0.78	284, 324
2g	7-OH	6′-OH	$C_{15}H_{12}O_3$ (240)	212	75	0.81	265, 335
2h	6-OH	6′-OH	$C_{15}H_{12}O_4$ (256)	202	75	0.84	258, 303
2i	6-OH	4′-OH	$C_{15}H_{12}O_4$ (256)	191	75	0.86	260, 305
2j	6-OH	4′-OCH_3	$C_{16}H_{14}O_4$ (270)	138	75	0.83	263, 338
2k	6-OH	4′-$N(CH_3)_2$	$C_{17}H_{17}NO_2$ (283)	160	75	0.79	257, 302

Computer aided metabolic and toxicity prediction provides help to anyone who would like to gain a deeper insight in to the field of metabolism and toxicity, including medicinal chemists looking for quick information on the expected metabolic fate and toxicity of compounds still in the bottleneck stage of clinical trials.

In the present work, we undertook a milder and effective protocol for the synthesis of flavanones from the chalcones were developed via cyclization process and their metabolites and toxicities were predicted by computational method to find out structure–metabolism and structure–toxicity relationship.

Result and Discussion

The flavanones were synthesized by cyclization method as shown in Chart 1, the physicochemical characterization and the structural confirmation (UV, IR, NMR and CHN analysis) are presented in Tables 1, 2 and 3. All the synthesized compounds were obtained as crystalline needles with sharp melting points. The yields of the product were found to be remarkably higher than the other methods of synthesis of flavanones. All compounds were in conformity with the structures envisaged.

The synthesized flavanones are categorized into three classes:

1. Flavanone and 4′-substituted flavanones (**2a**—**d**)
2. 7-Hydroxy flavanone and their derivatives (**2e**—**f**)
3. 6-Hydroxy flavanone and their derivatives (**2g**—**k**).

Compounds (**1a**—**k**) gave positive test for chalcone and positive ferric chloride test indicating that the presence of hy-

Table 3. ^{1}H-NMR, IR Spectroscopic and Elemental Analysis Data of Synthesized Compounds (**1a**—**k**, **2a**—**k**)

Compound code	Spectrum data
1a	^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 1H, 2′-OH), 7.14 (dd, J=7.9, 1.8 Hz, 1H, 4″-H), 7.21 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.30 (d, J=7.9 Hz, 2H, 2″, 6″-H), 7.56 (s, 1H, –CH=CH–), 7.64 (m, J=8.3 Hz, 4H, Ar-H), 7.90 (s, 1H, –CH=CH–). IR (KBr/cm^{-1}): 3480 (–OH), 1748—1716 (–CO), 1670 (–CH=CH–), 1616, 1558 (aromatic), 754, 697 (monosubstituted benzene).
1b	^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 1H, 2′-OH), 5.1 (s, 1H, 4″-OH), 6.68 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.13 (d, J=8.0 Hz, 2H, 2″, 6″-H), 7.64—6.92 (m, J=8.3 Hz, 4H, Ar-H), 7.56 (s, 1H, –CH=CH–), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3480, 3345 (–OH), 1771, 1732 (–CO), 1682 (–CH=CH–), 1603, 1575 (aromatic), 834 (*p*-disubstituted benzene).
1c	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.73 (s, 3H, 4″-OCH_3), 5.0 (s, 1H, 2′-OH), 6.72 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.19 (d, J=7.9 Hz, 2H, 2″, 6″-H), 7.56 (s, 1H, –CH=CH–), 7.64—6.92 (m, J=8.1 Hz, 4H, Ar-H), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3480, 3446 (–OH), 1748, 1716 (–CO), 1670 (–CH=CH–), 1605, 1575 (aromatic), 834 (*p*-disubstituted benzene).
1d	^{1}H-NMR (δ/ppm in $CDCl_3$): 2.8 (s, 6H, 4″-NMe_2), 5.0 (s, 1H, 2′-OH), 6.54 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.12 (d, J=8.0 Hz, 2H, 2″, 6″-H), 7.56 (s, 1H, –CH=CH–), 7.64—6.92 (m, J=7.9 Hz, 4H, Ar-H), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3480, 3446 (–OH), 1748, 1716 (–CO), 1670 (–CH=CH–), 1621, 1558, 1521 (aromatic), 1312 (C–N stretching in Ar amines), 835 (*p*-disubstituted benzene).
1e	^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 3H, 2′, 4′, 6″-OH), 6.68 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.13 (d, J=7.9 Hz, 2H, 2″, 4″-H), 7.39 (s, 1H, –CH=CH–), 7.47—6.39 (m, J=8.2 Hz, 3H, Ar-H), 8.17 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3841 (–OH), 1732, 1698 (–CO), 1670 (–CH=CH–), 1616, 1558 (aromatic), 727, 652 (monosubstituted benzene).
1f	^{1}H-NMR (δ/ppm in $CDCl_3$): 2.85 (s, 6H, 4″-NMe_2), 5.0 (s, 2H, 2′, 4′-OH), 6.54 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.12 (d, J=7.9 Hz, 2H, 2″, 6″-H), 7.56 (s, 1H, –CH=CH–), 7.47—6.39 (m, J=8.1 Hz, 3H, Ar-H), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3480 (–OH), 1748, 1697 (–CO), 1670 (–CH=CH–), 1616, 1540 (aromatic), 1316 (C–N stretching in Ar. amines), 824 (*p*-disubstituted benzene).
1g	^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 2H, 2′, 6″-OH), 7.11—6.75 (m, J=8.2 Hz, 4H, Ar-H), 7.14 (dd, J=7.9, 1.8 Hz, 1H, 4″-H), 7.21 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.30 (s, 1H, 2″-H), 7.56 (s, 1H, –CH=CH–), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3391, 3209 (–OH), 1748, 1698 (–CO), 1653 (–CH=CH–), 1623, 1576 (aromatic), 728, 697 (monosubstituted benzene).
1h	^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 3H, 2′, 5′, 6″-OH), 6.68 (d, J=7.9 Hz, 1H, 3′-H), 6.77 (dd, J=7.9, 1.8 Hz, 1H, 6′-H), 6.97 (dd, J=7.9, 1.8 Hz, 1H, 4′-H), 7.11—6.75 (m, J=8.3 Hz, 4H, Ar-H), 7.39 (s, 1H, –CH=CH–), 8.17 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3446 (–OH), 1748, 1698 (–CO), 1670, 1652 (–CH=CH–), 1616, 1540 (aromatic), 714, 673 (monosubstituted benzene).
1i	^{1}H-NMR (δ/ppm in $CDCl_3$): 5.0 (s, 3H, 2′, 5′, 4″-OH), 6.68 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.11—6.75 (m, J=8.3 Hz, 3H, Ar-H), 7.13 (d, J=7.9 Hz, 2H, 2″, 6″-H), 7.56 (s, 1H, –CH=CH–), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3244 (–OH), 1732, 1698 (–CO), 1683 (–CH=CH–), 1646, 1557 (aromatic), 834 (*p*-disubstituted benzene).
1j	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.73 (s, 3H, 4″-OCH_3), 5.0 (s, 2H, 2′, 5′-OH), 6.72 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.11—6.75 (m, J=8.3 Hz, 3H, Ar-H), 7.19 (d, J=7.9 Hz, 2H, 2″, 6″-H), 7.56 (s, 1H, –CH=CH–), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3244 (–OH), 1732, 1716 (–CO), 1683 (–CH=CH–), 1577, 1540 (aromatic), 834 (*p*-disubstituted benzene).
1k	^{1}H-NMR (δ/ppm in $CDCl_3$): 2.85 (s, 6H, 4-NMe_2), 5.0 (s, 2H, 2′, 5′-OH), 6.54 (d, J=7.9 Hz, 2H, 3″, 5″-H), 7.11—6.75 (m, J=8.3 Hz, 3H, Ar-H), 7.12 (d, J=7.9 Hz, 2H, 2″, 6″-H), 7.56 (s, 1H, –CH=CH–), 7.90 (s, 1H, –CH=CH–), IR (KBr/cm^{-1}): 3480 (–OH), 1748, 1697 (–CO), 1670 (–CH=CH–), 1616, 1540 (aromatic), 1315 (C–N stretching in Ar. amines), 824 (*p*-disubstituted benzene).
2a	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.26—2.50 (d, J=6.2 Hz, 2H, 3-H), 5.51 (t, J=6.0 Hz, 1H, 2-H), 7.19 (s, 5H, –C_6H_5), 7.78—6.85 (m, J=7.9 Hz, 2H, 6-, 7-Ar-H), 7.78 (dd, J=7.9, 1.8 Hz, 2H, 5, 8-H), IR (KBr/cm^{-1}): 2930 (–CH_2), 1748, 1683 (–CO), 1616, 1520 (aromatic), 778, 696 (monosubstituted benzene), elemental analysis: Calcd (Found): C: 80.34 (79.18), H:5.39 (5.36).
2b	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.26—2.50 (d, J=6.2 Hz, 2H, 3-H), 5.0 (s, 1H, 4′-OH), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.66 (d, J=7.9 Hz, 2H, 3′, 5′-H), 7.02 (d, J=7.9 Hz, 2H, 2′, 6′-H), 7.78 (d, J=7.9 Hz, 1H, 5-H), 7.78—6.85 (m, J=8.3 Hz, 3H, 6-, 7-, 8-Ar-H), IR (KBr/cm^{-1}): 2925 (–CH_2), 1748, 1698 (–CO–), 1636, 1517 (aromatic), 834 (disubstituted benzene), elemental analysis: Calcd. (Found): C: 74.99 (74.97), H: 5.03 (4.94).
2c	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.26—2.50 (d, J=6.2 Hz, 2H, 3-H), 3.73 (s, 3H, 4′-OCH_3), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.70 (d, J=7.9 Hz, 2H, 3′, 5′-H), 7.08 (d, J=7.9 Hz, 2H, 2′, 6′-H), 7.78—6.85 (m, J=8.3 Hz, 3H, 6, 7, 8-H, Ar-H), 7.78 (d, J=8.0 Hz, 1H, 5-H), IR (KBr/cm^{1-}): 2840 (–CH_2), 1670, 1636 (–CO–), 1558, 1520 (aromatic), 830, 804 (disubstituted benzene), elemental analysis: Calcd (Found): C: 75.88 (75.02), H: 5.55 (5.04).
2d	^{1}H-NMR (δ/ppm in $CDCl_3$): 2.85 (s, 6H, 4′-NMe_2), 3.26—2.50 (d, J=6.2 Hz, 2H, 3-H), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.52 (d, J=7.9 Hz, 2H, 3′, 5′-H), 7.01 (d, J=7.9 Hz, 2H, 2′, 6′-H), 7.78—6.85 (m, J=8.3 Hz, 3H, 6, 7, 8-Ar-H), 7.78 (d, J=7.6 Hz, 1H, 5-H), IR (KBr/cm^{-1}): 2908, 2821 (–CH_2), 1670, 1617 (–CO–), 1558, 1521 (aromatic), 1339 (Tert. Ar. amine), 835 (disubstituted benzene), elemental analysis: Calcd (Found): C: 76.38 (75.35), H: 6.41 (6.05), N: 5.24 (5.22)
2e	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.26—2.50 (d, J=6.2 Hz, 2H, 3-H), 5.0 (s, 2H, 7, 6′-OH), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.32 (s, 1H, H-8), 6.37 (d, J=7.9 Hz, 1H, H-6), 7.1 (d, J=7.8 Hz, 1H, 2′-H), 7.61 (d, J=7.8 Hz, 1H, H-5), 7.78—6.85 (m, J=8.3 Hz, 3H, 6, 7, 8-Ar-H), IR (KBr/cm^{-1}): 3480, 3446 (–OH), 2914 (–CH_2), 1670, 1635 (–CO–), 1558, 1521 (aromatic), 754, 684 (monosubstituted benzene), elemental analysis: Calcd (Found): C: 70.31 (69.10), H: 4.72 (4.01).
2f	^{1}H-NMR (δ/ppm in $CDCl_3$): 2.85 (s, 6H, 4-NMe_2), 3.26—2.50 (d, J=6.2 Hz, 2H, 3-H), 5.0 (s, 1H, 7-OH), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.32 (s, 1H, H-8), 6.37 (d, J=7.7 Hz, 1H, H-6), 6.52 (d, J=7.7 Hz, 2H, 3′, 5′-H), 7.01 (d, J=7.9 Hz, 2H, 2′, 6′-H), 7.61 (d, J=7.9 Hz, 1H, H-5), IR (KBr/cm^{-1}): 3480, 3446 (–OH), 2926 (–CH_2), 1670, 1636 (–CO–), 1576, 1540 (aromatic), 1375, 1317 (Tert. Ar. amine), 824 (disubstituted benzene), elemental analysis: Calcd (Found): C: 72.07 (71.09), H: 6.05 (5.96), N: 4.94 (4.94).
2g	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.26—2.50 (d, J=6.3 Hz, 2H, 3-H), 5.0 (s, 1H, 6′-OH), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.68 (s, 1H, H-8), 6.80 (d, J=7.9 Hz, 1H, H-7), 7.19 (m, J=8.5 Hz, 4H, 2′, 3′, 4′, 5′, Ar-H), 7.25 (d, J=7.9 Hz, 1H, H-5), 7.61 (d, J=7.9 Hz, 1H, H-6), IR (KBr/cm^{-1}): 3237 (–OH), 2348 (–CH_2), 1669, 1635 (–CO–), 1575, 1540 (aromatic), 731, 698 (monosubstituted benzene), elemental analysis: Calcd (Found): C: 74.99 (74.98), H: 5.03 (4.92).
2h	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.26—2.59 (d, J=6.2 Hz, 2H, 3-H), 5.0 (s, 2H, 6-, 6′-OH), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.68 (d, J=7.9 Hz, 1H, H-8), 6.68 (d, J=7.5 Hz, 1H, 3′-H), 6.80 (d, J=7.9 Hz, 1H, H-7), 7.02 (q, J=8.3 Hz, 2H, 4′, 2′-H), 7.25 (d, J=7.9 Hz, 1H, H-5), 7.31 (d, J=7.9 Hz, 1H, 5′-H), IR (KBr/cm^{-1}): 3480, 3447 (–OH), 3216 (–CH_2), 1748, 1683 (–CO–), 1616, 1521 (aromatic), 760, 697 (monosubstituted benzene), elemental analysis: Calcd (Found): C: 70.31 (69.82), H: 4.72 (4.56).
2i	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.26—2.50 (d, J=6.3 Hz, 2H, 3-H), 5.0 (s, 2H, 6, 4′-OH), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.66 (d, J=7.7 Hz, 2H, 3′, 5′-H), 6.68 (bs, 1H, H-8), 6.80 (d, J=7.9 Hz, 1H, H-7), 7.02 (dd, J=7.9, 1.8 Hz, 2H, 2′, 6′-H), 7.25 (d, J=8.0 Hz, 1H, H-5), IR (KBr/cm^{-1}): 3446 (–OH), 3065 (–CH_2), 1748, 1698 (–CO–), 1617, 1540 (aromatic), 822 (disubstituted benzene), elemental analysis: Calcd (Found): C: 70.31 (69.70), H: 4.72 (4.61).

Table 3. Continued.

Compound code	Spectrum data
2j	^{1}H-NMR (δ/ppm in $CDCl_3$): 3.26—2.50 (d, J=6.2 Hz, 2H, 3-H), 3.73 (s, 3H, 4′-OCH_3), 5.0 (s, 1H, 6-OH), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.68 (bs, 1H, H-8), 6.70 (d, J=7.9 Hz, 2H, 3′, 5′-H), 6.80 (d, J=7.7 Hz, 1H, H-7), 7.08 (dd, J=7.9, 1.8 Hz, 2H, 2′, 6′-H), 7.25 (d, J=7.9 Hz, 1H, H-5), IR (KBr/cm^{-1}): 3480 (–OH), 2908 (–CH_2), 1732, 1670 (–CO–), 1593, 1540 (aromatic), 815 (disubstituted benzene), elemental analysis: Calcd (Found): C: 71.10 (70.92), H: 5.22 (4.98).
2k	^{1}H-NMR (δ/ppm in $CDCl_3$): 2.85 (s, 6H, 4′-NMe_2), 3.26—2.50 (d, J=6.3 Hz, 2H, 3-H), 5.0 (s, 1H, 6-OH), 5.51 (t, J=6.0 Hz, 1H, 2-H), 6.80 (d, J=7.9 Hz, 1H, H-7), 6.80 (bs, 1H, H-8), 6.99 (d, J=7.9 Hz, 2H, 3′, 5′-H), 7.0 (dd, J=7.9, 1.8 Hz, 2H, 2′, 6′-H), 7.25 (s, 1H, H-5), IR (KBr/cm^{-1}): 3446 (–OH), 3002, 2835 (–CH_2), 1683, 1636 (–CO–), 1575, 1540 (aromatic), 1362, 1318 (Tert. Ar. amine), 831 (disubstituted benzene), elemental analysis: Calcd (Found): C: 72.07 (71.96), H: 6.05 (5.97), N: 4.94 (4.92).

s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, bs: bis singlet, dd: double doublet.

droxyl (OH) group. With ethanolic ferric chloride, compounds (**1a**—**k**) gave red-brown colour. Similarly, compounds (**2a**—**k**) gave positive test for flavanone. Compounds (**2a**, **c**, **d**) did not give positive ferric chloride test indicating absence of hydroxyl group. Compounds (**2b**, **e**—**k**) gave positive ferric chloride test indicating the presence of hydroxylated flavanone derivatives. With ethanolic ferric chloride, compounds (**2b**, **e**—**k**) gave greenish colour.

Anti-bacterial Activity All the synthesized flavanones (**2a**—**k**) were evaluated for *in vitro* anti-bacterial activity against *Staphylococcus aureus*, *Shiegella sonnei*, *Shiegella dysenteriae*, *Salmonella typhimurium*, *Vibrio cholerae* and *Escherichia coli* at concentration of 25, 50, 100 and 200 μg/ml by agar dilution method (spot inoculation method) in sterile nutrient agar media. Norfloxacin was used as standard reference drug. The presence or absence of growth was observed visually. Compounds (**2b**), (**2e**), (**2f**) and (**2h**) were found to exhibit anti-bacterial activity.

From the table, it is clear that the compound (**2h**) was found to be the most potent against all six microorganisms used for the antibacterial study. Compound (**2f**) was found to be slightly less potent than (**2h**), followed by the compounds (**2b**) and (**2e**) respectively. Anti-bacterial activity of various synthetic flavanones in the order of their increasing potency are as follows: Compound (**2h**) > Norfloxacin > (**2f**) > (**2b**) > (**2e**).

The compound (**2h**) was the most potent against *S. aureus*, followed by the compounds (**2f**), (**2e**) and (**2b**) respectively. Compounds (**2f**) and (**2h**) were equipotent against *S. Sonnei*, followed by the compounds (**2b**) and (**2e**) respectively. The compound (**2h**) was the most potent against *E. coli*, followed by the compounds (**2b**), (**2e**) and (**2f**) respectively. The compound (**2f**) was most potent against *S. dysenteriae*, followed by the compound (**2b**), (**2h**) and (**2e**) respectively. The compound (**2h**) was the most potent against *S. typhimurium* followed by the compounds (**2b**), (**2e**) and (**2f**) respectively. Compounds (**2f**) and (**2h**) were equipotent against the *V. cholerae*, followed by the compounds (**2b**) and (**2e**) respectively.

The minimum inhibitory concentrations (MICs) of the standard drug norfloxacin and the synthetic flavanones (**2a**—**k**) against various microorganisms are tabulated (Table 4). Among the compounds **2b**, **2e**, **2f** and **2h**, **2h** needs 100 μg/ml of concentration for activity against all strains. Compound **2f** have 200 μg/ml against the *E. coli* and the *S. typhimurium* bacterium. The compound **2h** possess hydroxyl group at 6th position of the benzene ring and the 2nd position in the chromone ring. Compound **2e** have hydroxyl group at 6th position in the benzene ring and the 3rd position in the chromone ring, **2b** have hydroxyl group at 4th position of the benzene ring and **2f** have dimethylamine group at 4th position in the benzene ring and the hydroxyl group in 3rd position in the chromone ring.

The compounds having methoxy group in the benzene ring have remarkably very less activity than the hydroxyl and dimethylamine group containing compounds. From the results, it have been revealed that the compound should have hydrogen donor and acceptor group in the 4th or 6th position increases the activity with hydroxyl group in the 3rd or 4th position of the chromone ring. In compound **2f**, the dimethylamine group in the benzene ring along with hydroxyl group at 4th position of the chromone ring having antibacterial activity.

The computationally predicted metabolites and the toxicity data are given in Tables 5 and 6, the mechanism of metabolism is given in Fig. 1. The result of the computational prediction shows that the hydroxyl group present in the compounds undergoes conjugation reaction with sulfate and glucuronide. Interestingly, the compounds does not undergo any methylation or acetylation (toxic metabolites), ultimately the compounds have over all less toxicity. The compounds **2a** and **2d** have over all toxicity of 0%, unfortunately these compounds does not possess remarkable activity against the bacterial strains. Compounds **2b**, **2e**, **2f** and **2h** (most active compounds) possess 53% overall toxicity and these are have 29% mutagenicity, 53% irritability and 29% neurotoxicity.

Among the compounds (**2a**—**k**), only three compounds (**2a**, **c**, **d**) have over all less toxic than other compounds. Interestingly this compound does not undergo phase II conjugation reactions (sulfate or glucuronide). Other compounds undergo conjugation reaction. It may be concluded that the compounds undergo conjugation reaction with sulfate or glucuronide can have the possibility for mutagenicity, irritability and neurotoxicity. The compounds **2c** and **2j** possess methoxy group in the phenyl ring, while undergo metabolism it produces desmethylated product, which may cause teratogenicity (19%) of the compounds.

From the *in silico* metabolites prediction and toxicity prediction study of the synthesized compounds shows that the compounds which are producing conjugate metabolites (sulfate or glucuronide) may cause mutagenicity, irritability and neurotoxicity and the compounds undergo desmethylation may cause teratogenicity.

From the present investigation, it is concluded that we have successfully implemented the use of cyclization technique for the synthesis of substituted flavanone derivatives

Table 4. Anti-bacterial Activity of Compounds (**2a**—**k**) by spot Inoculation Test or Agar Dilution Method

Compound code	Conc. (μg/ml)	Microorganism					
		S. aureus	*S. sonnei* 2	*E. coli*	*S. dysenteriae* 1	*S. typhimurium* 74	*V. cholerae* 14033
Reference (Norfloxacin)	A	+	−	+	−	+	−
	B	+	−	−	−	+	−
	C	−	−	−	−	−	−
	D	−	−	−	−	−	−
2a	A	++	++	++	++	++	++
	B	++	++	++	++	++	++
	C	+	+	+	+	+	+
	D	+	+	+	+	+	+
2b	A	++	+	+	+	+	+
	B	+	−	−	−	−	−
	C	−	−	−	−	−	−
	D	−	−	−	−	−	−
2c	A	++	++	++	++	++	++
	B	++	++	++	++	++	++
	C	+	+	+	+	+	+
	D	+	+	+	+	+	+
2d	A	++	++	++	++	++	++
	B	++	++	++	++	++	++
	C	+	+	+	+	+	+
	D	+	+	+	+	+	+
2e	A	++	++	++	++	++	++
	B	+	+	+	+	+	+
	C	−	−	−	−	−	−
	D	−	−	−	−	−	−
2f	A	++	−	+	−	+	−
	B	−	−	+	−	+	−
	C	−	−	+	−	+	−
	D	−	−	−	−	−	−
2g	A	++	++	++	++	++	++
	B	++	++	++	++	++	++
	C	+	+	+	+	+	+
	D	+	+	+	+	+	+
2h	A	−	−	−	+	−	−
	B	−	−	−	−	−	−
	C	−	−	−	−	−	−
	D	−	−	−	−	−	−
2i	A	++	++	++	++	++	++
	B	++	++	++	++	++	++
	C	+	+	+	+	+	+
	D	+	+	+	+	+	+
2j	A	++	++	++	++	++	++
	B	++	++	++	++	++	++
	C	+	+	+	+	+	+
	D	+	+	+	+	+	+
2k	A	++	++	++	++	++	++
	B	++	++	++	++	++	++
	C	+	+	+	+	+	+
	D	+	+	+	+	+	+

A: 25 μg/ml concentration, B: 50 μg/ml concentration, C: 100 μg/ml concentration, D: 200 μg/ml concentration. ++: indicates full growth means no activity. +: indicates partial growth means no activity. −: indicates negative growth means having activity.

Table 5. *In Silico* Predicted Metabolites and Molecular Weight of the Synthesized Compounds

S. No	Metabolite	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	**2g**	**2h**	**2i**	**2j**	**2k**
1	Parent comp	224.2	240.3	254.3	267.3	256.3	283.3	240.3	256.3	256.3	270.3	283.3
2	*p*-Hydroxylation	240.3	256.3	270.3	283.3	272.3		256.3	272.3	272.3		
3	Aromatic ketone reduction	226.3	242.3	256.3	269.4	258.3	285.4	242.3	258.3	258.3	272.3	285.4
4	*m*-Hydroxylation	240.3	256.3					256.3				
5	Phenol sulfate conjugate		320.3			336.3	363.4	320.3	336.3	336.3	350.3	363.4
6	Formation of *O*-glucuronide		416.4			432.4	459.5	416.4	432.4	432.4	446.4	459.5
7	Demethylation of phenol			240.3							256.3	
8	Demethylation of amines				253.3		269.3					269.3
9	Tertiary amine oxidation				284.6		300.4					300.4

Table 6. Predicted Toxicity of Compounds

Comp. code	Toxicity	Overall toxicity	Oncogenicity	Mutagenicity	Teratogenicity	Irritability	Sensitivity	Immuno toxicity	Neuro toxicity
2a	Not probable	0	0	0	0	0	0	0	0
2b	Probable	53	0	29	0	53	0	0	29
2c	Not probable	19	0	0	19	0	0	0	0
2d	Not probable	0	0	0	0	0	0	0	0
2e	Probable	53	0	29	0	53	0	0	29
2f	Probable	53	0	29	0	53	0	0	29
2g	Probable	53	0	29	0	53	0	0	29
2h	Probable	53	0	29	0	53	0	0	29
2i	Probable	53	0	29	0	53	0	0	29
2j	Probable	53	0	29	19	53	0	0	29
2k	Probable	53	0	29	0	53	0	0	29

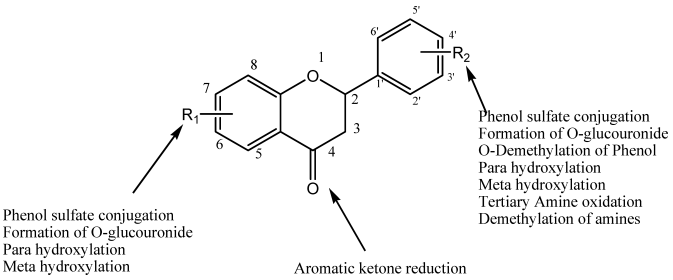


Fig. 1. *In Silico* Metabolism of the Flavanone Derivatives

from the substituted chalcones. The method developed was simple, economic and eco-friendly. The yields of the products obtained were comparable to other methods of the synthesis of flavanones. Since there is no heating during the entire course of reaction, it reduces side-reactions and decomposition of the product.

Some of the synthesized flavanones exhibited antibacterial activity against many bacterial strains. The compound possess hydrogen donor/acceptor group at 3rd position of the benzene ring and 6th position of the chromone ring have significant activity against all bacterial strains. While hydroxyl group at 4th position of the chromone ring along with hydroxyl group at 6th position of the benzene ring have less activity against all strains. The compound **2h** possess 3rd and 6th position substitution on chromone ring and benzene ring respectively.

The compound possessing hydroxyl groups in the chromone or the phenyl ring can undergo conjugation reaction and also the same group containing compounds possess more active than non-hydroxyl group containing compounds. The methoxy group containing compounds are devoid of biological activity and possess teratogenicity. But comparatively these compounds (**2b**, **e**, **f**, **h**) possess good activity against all strains of the bacteria and devoid of teratogenicity, oncogenicity, sensitivity and immunotoxicity.

Experimental

Characterization of the Compounds Melting points of the synthesized compounds were determined in open capillary tubes and are uncorrected. UV absorption spectra were recorded in methanol on Pharmaspec Shimadzu UV-1700 UV/VIS Spectrophotometer, IR absorption spectra were recorded on Jasco FT/IR-470 PLUS, KBr diffuse reflectance, ^{1}H-NMR spectra were recorded on the JEOL GSX-400, 60 MHz spectrometer in $CDCl_3$ TMS (tetramethylsilane) as an internal standard. The ^{1}H chemical shifts are reported as parts per million (ppm) downfield from TMS (Me_4Si). ^{1}H-NMR and IR spectra were consistent with the assigned structures. Purity of the compounds was checked by thin layer chromatography (TLC). The elemental analysis (CHN analysis) was done on a CHN rapid analyzer. All the compounds gave satisfactory analysis within ±0.4% of the theoretical values.

Step 1: Synthesis of Substituted Chalcones Substituted benzaldehydes (0.012 mol) were added to a mixture of substituted acetophenones (0.01 mol) in 25 ml of ethanol in a 200 ml beaker. The content of the beaker was mixed well and to that 10 ml of 10% potassium hydroxide solution was added and stirred vigorously at 25 °C until the mixture was so thick that stirring was no longer effective (3—4 h). After the completion of the stirring, the reaction mixture was kept in a refrigerator overnight. The reaction mixture was then diluted with ice-cold water (50 ml), acidified with 10% aqueous hydrochloric acid to precipitate the chalcones. The product was filtered with suction on a Buchner funnel, washed with cold water until the washings were neutral to litmus and then washed with 10 ml of ice-cold rectified spirit. The dried product was recrystallized from chloroform.

Compounds **1a**—**k** (Table 1) gave positive test for chalcone and positive ferric chloride test.[20]

Step 2: Synthesis of Flavanones from Substituted Chalcones Substituted chalcones (0.001 mol) dissolved in methanol (50 ml) in a 200 ml beaker. The resulting solution was made alkaline (pH 10.0) with potassium hydroxide pellets and was allowed to react for 2—3 h at room temperature (the reaction time for different chalcones vary from 1—3 h). The reaction mixture was then acidified (using 10% aqueous hydrochloric acid: ice-cold) to precipitate the flavanones. The product was filtered with suction on a Buchner funnel, washed with cold water until the washings were neutral to litmus and then with 5 ml of ice-cold rectified spirit. The dried product was recrystallised from 95% ethanol.

Compounds **2a**—**k** (Table 2) gave positive test for flavanone. Compounds **2a**, **2c** and **2d** gave negative ferric chloride test; whereas compounds **2b** and **2e**—**k** gave positive ferric chloride test.[20]

Biological Evaluation All the synthesized flavanones (**2a**—**k**) were evaluated for *in vitro* anti bacterial activity against *Staphylococcus aureus*, *Shiegella sonnei*, *Shiegella dysenteriae*, *Salmonella typhimurium*, *Vibrio cholerae* and *Escherichia coli* at concentration of 25, 50 100 and 200 μg/ml by agar dilution method (spot inoculation method) in sterile nutrient agar media. Norfloxacin was used as standard reference drug.[21]

Preservation of Bacterial Cultures All the strains of S*taphylococci*, *Streptococii*, *E. coli*, *Salmonella*, *Shigella* and *Vibrious* were preserved as slab-slant cultures at a temperature of 4 °C and also in freeze-dried state. Routine subculturing of the gram-positive bacteria was carried out on nutrient agar and the gram-negative strains on bromothymol blue lactose agar.

The Agar Dilution Technique (Spot Inoculation Method) for Assessment of Antibacterial Activity The minimum inhibitory concentration (MIC) of the various synthetic compounds against the bacterial strains was determined by the agar dilution technique.[22—24]

Preparation of Stock Solutions of the Synthetic Compounds Desired amount of each synthetic compounds were dissolved separately in 25% sterile dimethylsulfoxide (DMSO) to prepare the stock solutions.

Preparation of Norfloxacin Solution A stock solution of 10 μg/ml reference standard of norfloxacin was prepared with the help of sterile distilled water to prepare of 25, 50, 100 and 200 μg/ml used during agar dilution study.

Preparation of Nutrient Agar Plates Containing Different Concentration of the Synthetic Compounds Required for Determination of Minimum Inhibitory Concentrations (MIC) of the Synthetic Compounds with Respect to Different Bacteria[25,26] Measured volumes of stock solutions of the synthetic compounds individually added aseptically to molten nutrient agar (oxoid) in the following concentration (μg/ml): 0 (control), 25,

50, 100 and 200 and poured into sterile petri dishes. The pH of the media was adjusted to 7.2—7.4.

For uniform diffusion of the synthetic compound throughout the medium, the agar plates containing synthetic compounds were refrigerated overnight and subsequently dried for 2 h at 37 °C before use. Small squares were demarcated at the back of the agar containing portions of the plates with a marker to specify the actual location for each test organism.

Inoculum The inoculum for determination of the sensitivity pattern consists of one loopful of overnight grown culture of the test organism. The average size of the inoculum was about 10^5 cells contained in a 2 mm diameter standard loop.

Spot Inoculation Method (Agar Dilution Method) When the nutrient agar plates containing the synthetic compounds and also the control plates having equal volumes of solvent were made ready, the overnight grown broth culture of each test organism was spot inoculated by Checkerboard technique on the marked area of the plates. These were then incubated for 72 h at 37 °C. No growth of the organism on the test plate along with growth on the control plate was taken as an indication of antimicrobial activity of the drug. Minimum inhibitory concentration (MIC) was indicated by the lowest concentration of the synthetic drug, which inhibited the bacterial growth.

***In Silico* Metabolites and Toxicity Prediction** The metabolites and the toxicity of the compounds were predicted by computational method using Pallas version 3.1 ADME-Tox prediction software and pentium IV processor.[27)]

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